New findings from non-linear longitudinal modelling of menopausal hormone changes

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Changes in FSH and estradiol (E₂) across the menopausal transition are clearly not linear. The present study utilizes data from 204 women who completed the 13-year prospective Melbourne Women's Midlife Health Project. E₂, FSH, symptoms, self-rated health, mood, sexual function and coronary heart disease (CHD) risk were measured longitudinally. We presumed an s-shaped curve for each hormone and estimated five parameters for each hormone curve for each woman: baseline, final value, range, slope at inflexion point and age at inflexion point. These parameters were found to adequately estimate the curve for each hormone. The median age of transition observed for E₂ occurs 1 year later than the median age of transition observed for FSH. FSH parameters did not affect any of the health outcomes analysed. Hot flushes, night sweats, sleeping problems, vaginal dryness and to a lesser extent self-rated health were highly significantly associated with E₂ range and slope. Sexual response and CHD risk were highly significantly associated with final E₂ level (post-menopausally). These findings have clinical relevance in identifying which symptoms will be triggered by steep transitions of E₂ such as sudden withdrawal and which health parameters may require a maintenance level of E₂.

Keywords: hormone levels; symptoms; menopause; sexuality; coronary heart disease risk.

Introduction

The menopausal transition encompasses a phase of change in the hypothalamic–pituitary–ovarian axis. In order to study these endocrine changes and any effects on health outcomes a number of cohort studies were established in the USA (Avis et al., 2000; Mitchell et al., 2000; Randolph et al., 2005; Freeman et al., 2006), Australia (Guthrie et al., 2004a; Dennerstein et al., 2005a; Burger et al., 2007) and Sweden (Landgren et al., 2004). These longitudinal or prospective studies involve the repeated collection of data or measures from the same individuals followed over time and contrast with cross-sectional studies where the sample is assessed only once.

The analysis of longitudinal studies is complex as a new temporal dimension is added to other components of the study. Many statistical approaches have been used to deal with the complex data generated and these were the subject of a previous review (Lehert and Dennerstein, 2002). Four main categories of techniques were found. Cross-sectional reduction of data was used by 56% of papers reviewed. This method, while useful for preliminary analysis, pools the data from all observations regarding each as independent when they are often highly correlated, and may generate erroneous results (Dennerstein et al., 1999a, 2005a).

A second technique is to study the change between two data points before and after an event of interest, such as final menstrual period (Dennerstein et al., 2000). The major shortcoming of this technique is that some aspects of the information obtained cannot be utilized as the data collected across a number of time points are averaged. Repeated measurement multivariate analysis of variance overcomes this problem and allows for a number of estimations of various effects such as that of adjusting for age, baseline level of variables and the effects of various covariates (Dennerstein et al., 1999b). Structural equation modelling acknowledges the possible interactions between various variables measured in a study and allows the best model to be selected that describes these powerful effects and interactions (Dennerstein et al., 2007).

A major limitation in modelling the effects of hormonal changes of the menopausal transition on health outcomes is the recognition that reproductive hormone change during the menopausal transition is clearly not linear (Burger et al., 1999, 2007). Examples of possible individual curves for FSH and estradiol (E₂) are provided in Fig. 1. FSH starts from a small value in the late reproductive/early menopausal transition, then increases from early through late menopausal transition and converges to a stable
The following analysis uses data accumulated in the 13-year prospective Melbourne Women’s Midlife Health Project which is described below.

**Materials and Methods**

**Design**

The Melbourne Women’s Midlife Health Project is a prospective, population-based study of Australian-born women assessed annually for most of 13 years as they passed through the menopausal transition (Guthrie et al., 2004a). The study began in 1991 with population sampling of 2001 Australian-born women aged 45–55 years (Dennerstein et al., 1993). All those women at baseline who had not experienced menopause in the prior 3 months, and who were not taking any oral contraceptive pill (OCP) or hormone therapy, were invited to participate in a longitudinal study (n = 779). Of those eligible, 438 (56%) accepted, and all participants were Caucasian. They took part in annual interviews conducted in participants’ homes by trained field workers. Fasting blood samples were taken while the women were between days 4–8 of the menstrual cycle if still menstruating, or on any day after 3 months of amenorrhoea. Measures were collected annually in years 1–8 of follow-up and then in years 11–13 of follow-up.

The study was approved by the Human Research Ethics Committee of the University of Melbourne and the procedures followed were in accordance with the ethical standards of the National Health & Medical Research Council. All subjects provided written informed consent for their participation in the study.

**Sample**

A total of 438 women entered the longitudinal study cohort. At baseline, comparing the 438 participants with 341 non-participants, participants more often reported better self-rated health, current employment, >12 years of education, exercising at least once a week and to having had a Pap test smear (Burger et al., 1995). The following women were excluded from this analysis: (i) 56 women who dropped out for various unrelated reasons; (ii) women who at baseline were found to already be in the late menopausal transition (n = 39); (iii) women who were not observed to reach late menopausal transition during the 13 years of observations (n = 4); (iv) women with frequent missing data (missing >3 values for hormones, n = 32); (v) women using the OCP (n = 5); (vi) women who had surgical menopause before reaching final menstrual period, (n = 37); hormone therapy users: excluding all women with one hormone therapy period would result in many excluded women. To avoid this exclusion, a hormone measurement corresponding to hormone therapy use was considered as missing. In conformity with the above missing rate, we excluded 61 women who were hormone therapy treated for >3 years. The final analysis sample comprised 204 women. Of these, 145 never took hormone therapy.

**Menopausal status**

Reproductive and menopausal status was determined from change in menstrual status asked at each annual interview for those women...
who were not taking hormone therapy. Adapting the recommendations of the Staging Reproductive Aging Conference (Soules et al., 2001) to those already in use in our project: late reproductive status was assigned to those women who reported regular menstrual cycles; early menopausal transition status was assigned to women who had menstruated in the prior 3 months but reported change in menstrual frequency; late menopausal transition status was assigned when women reported at least 3 months of amenorrhea but <12 months amenorrhea. Women were deemed to be post-menopausal when there had been amenorrhea for at least 12 months. Reports of three or more months of amenorrhea were verified by fieldworkers from prospectively kept daily menstrual diaries. Women who experienced a hysterectomy plus/minus oophorectomy or an endometrial ablation were classified as having a surgical (or induced) menopause. Women using hormone therapy were categorized separately as hormone therapy users for those observations during which they used hormones.

Measures

Menopausal status and health outcome variables were measured at baseline and at each follow-up visit annually for years 1–8 and then years 11 and 13 of follow-up. Health outcomes include: CHD risk was calculated using the PROCAM scoring system (Assmann et al., 2001; Dennerstein et al., 1997b, 2001a; Dennerstein and Lehert, 2004). The questionnaire is handed to each woman annually at the time of interview. The woman completes the questionnaire and hands it back to the fieldworker. Thus, only one PEQ is completed each year. Items are measured within each short PEQ (SPEQ) domain: sexual response: enjoyment of sexual activities; frequency of arousal, frequency of orgasm, frequency of sexual thoughts; frequency of sexual activities. Dyspareunia and vaginal dryness recorded on the SPEQ are not analysed in the present study as this latter variable was also measured in the symptom list.

Symptoms

The most important menopause-specific symptoms described in our previous paper were: hot flushes; night sweats; sore breasts, dry vagina and trouble sleeping (Dennerstein et al., 1993, 2000). However, the symptom of sore breasts was not included in the first 3 years of study. We, therefore excluded this symptom from analysis. The four symptoms (hot flushes; night sweats; dryness of vagina and trouble sleeping) were separately analysed, whereas all the others were grouped into an ‘other symptom’ category. The symptoms were documented as presence/absence, intensity (1, minor; 2, interfering with normal life and 3, debilitating) and frequency (number of days in the last two weeks). To describe severity of the first four symptoms, we computed intensity, I, and frequency, F, as [(I x F)/4.2] to produce a scale between 0 and 10. For the category ‘other symptoms’ to keep values between 0 and 10, we used the calculation (I x F)/39.2 (392 = 14 x 28 is the maximum). There are 28 symptoms and 14 possible days. Using this scale a value of 10 would be a continuous debilitating state caused by a symptom and 0 would mean total absence of the symptom.

Hormone measures

Fasting morning blood samples for hormone radioimmunoasays (RIA) were taken between days 4 and 8 of the menstrual cycle for those still cycling, or after 3 months of amenorrhea as previously described (Dennerstein et al., 2002b). Blood samples for hormone assays were collected the same day as interviews and questionnaires. Only FSH and E₂ were chosen for analysis in the present study as previous analyses of the Melbourne data indicate that neither testosterone nor DHEAS have been shown to vary with the menopausal transition (Burger et al., 2000, 2007), and neither had any discernible effect thus far on mood or sexual function (Dennerstein et al., 2002b). FSH was measured initially by RIA as described previously, for year one (Burger et al., 1995). Two subsequent changes in method were used, the automated microparticulate enzyme immunoassay (Abbott Diagnostics IMX Analyser Chicago, IL, USA) (years 2 and 3) and for all samples from year 4 onwards, the TOSOH AIA1200 automated enzyme immunoassay. Correlation coefficients were as follows: FSH (IMX) and RIA, 0.98 and FSH (TOSOH) with IMX 0.99. E₂ was measured using the double antibody RIA kit (Diagnostic Products Corporation, Los Angeles, USA). Women <39 years of age sampled between the fourth and seventh days of the cycle had serum FSH levels ranging from 2.2–8.3 IU/l. E₂ from 150–500 pmol/l. Post-menopausal women have serum FSH levels >22 IU/l.

Statistical analysis

The visual observation of E₂ and FSH series suggest that change in time of these hormones during menopause follows a sigmoid or S-curve. This curve is mathematically defined by four independent parameters b, r, a, and s, where b is the baseline value (corresponding
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The table reports for E2 and FSH, the means and 95% confidence intervals (CI) for each estimated parameter: r, a, b, f and s. The units correspond to measurement units for r, f and b; median age is in years, and slope s is unitless. However, s = 1 means that the tangent at median age point is 45 degrees. The higher the slope the faster the transition.

**Interrelationships between parameters**

**FSH parameters**

There are no significant correlations between the parameters. Thus, the initial baseline level is not associated with the final post-menopausal FSH level. The slope is not associated with the initial value, and median age is not correlated with any other value. A small correlation was found between FSHs and FSHr (r = 0.08, P = 0.05) where large ranges are associated with increased speed of transition (large FSHs values).

**E2 parameters**

A significant but modest correlation is found between age and slope, which suggests that women passing through the transition at a later age may experience a more sudden transition (r = 0.24, P = 0.01). The other parameters are independent.

**Mutual effects between E2 and FSH**

Low correlations were found between parameters (P > 0.01).

**Median age compared with mean transitional ages.**

The mean age at transition defined as between early menopause transition (MT) and late MT was compared with the median estimated ages for E2r and FSHr. E2r and mean age at transition are very close and not significantly different while FSHr occurs significantly earlier (Tukey HSD, P < 0.001).

**Effects on health outcomes**

All the outcomes that might be associated with hormones and for which we had sufficient data were separately studied [symptoms, CHD risk estimated by Procam score, sexual function by SPEQ domains (sexual response, frequency of sexual activities), positive and negative mood and wellbeing scores and self-rated health].

For each analysed outcome, we calculated the mean value corresponding to post-menopausal periods and we carried out a stepwise regression analysis in considering systematically, the associated premenopausal or baseline value (recorded in year 1 of the study), and the s parameters for FSH and E2. It should be noted that all health outcomes were significantly associated with their level at baseline. No association was found for FSH parameters and any of the health outcomes. The results for E2 parameters and health outcomes are shown in Table 2.

**Symptoms**

Hormone parameters were only associated with the following symptoms: hot flushes, night sweats, sleeping problems and vaginal dryness. For each of these symptoms, premenopausal or baseline state was an important variable with proportional effect on post-menopausal value of symptoms. However, using the hormone parameters, the most important associated variables were the E2 range (change in E2), for which higher range was associated with higher current severity of these symptoms, and E2 slope, where fast change was associated with more severe symptoms. Another variable where there were some associations

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**Goodness-of-fit of model**

For each subject, the s parameters for FSH and E2 were estimated. The mean correlations between estimates and observed values were 0.74 for E2 and 0.84 for FSH. The correlation was observed to be larger for women who did not use hormone therapy and decreased with the number of hormone therapy periods.

**Hormone parameters**

Table 1 reports the non-linear components estimates of FSH IU/l and E2 pmol/l.

<table>
<thead>
<tr>
<th></th>
<th>E2 (mean and 95% CI)</th>
<th>FSH (mean and 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>−272.5 (−294.9, −250.1)</td>
<td>90 (86.8, 94.1)</td>
</tr>
<tr>
<td>a</td>
<td>52.71 (52.57, 52.85)</td>
<td>50.75 (50.49, 51.01)</td>
</tr>
<tr>
<td>b</td>
<td>329.37 (300.26, 358.48)</td>
<td>13.75 (13.07, 14.44)</td>
</tr>
<tr>
<td>f</td>
<td>48.21 (41.02, 55.40)</td>
<td>104.06 (100.38, 107.74)</td>
</tr>
<tr>
<td>s</td>
<td>1.31 (1.19, 1.44)</td>
<td>1.03 (0.95, 1.12)</td>
</tr>
</tbody>
</table>

r, range for hormone; a, median age at which inflexion in hormone slope is reached; b, baseline or initial value of hormone in the first year of the study; f, final value of hormone in the 13th year of the study; s, slope of hormone.
with E₂ is self-rated health, which mimics the pattern shown for symptoms but with lower statistical significance ($P < 0.05$).

**CHD risk**

The most important variable was the baseline CHD risk status (CHD risk when women were premenopausal). The final value of E₂ was the next most important associated factor. Contrary to symptoms, E₂ range and slope were not found to be associated with CHD risk. A very similar model was found for sexual response.

**Other variables**

Positive and negative mood and wellbeing were not associated with hormone parameters. Frequency of sexual activities was associated with the baseline state, whereas sexual response was associated with the final level of E₂.

**Discussion**

We have proposed, and described the application of the S-curve, as a relevant model for E₂ and FSH during this stage of reproductive ageing and that the parameters of this curve can be used in longitudinal modelling to enhance understanding of hormonal changes across the menopausal transition and the effects of these on various health outcomes.

Few correlations were found between parameters. FSH parameters were not associated with any of the perceived health outcomes. As FSH and E₂ parameters are not correlated, the lack of associations with FSH parameters cannot be explained by existence of E₂ parameters in the model.

The median age of the inflexion in E₂ and FSH curves (parameters $E_2a$ and $FSHa$) was not associated with the health outcomes measured, indicating that the health outcomes measured are not associated with the age of the woman at transition. We also found that the median age of E₂ accurately coincided with the mean age of transition observed between the early menopausal transition and the late menopausal transition, and the median age of E₂ occurs 1 year later than median age of FSH.

This study confirms our previous findings of the health outcomes influenced by hormones. In a previous analysis, using longitudinal structural equation modelling and the first 9 years of data, we depicted associations of E₂ with a small number of variables (Dennerstein et al., 2007). The present study confirms the association of E₂ with certain symptoms, CHD risk and sexual response. Self-rated health was associated with the same parameters of E₂ which were associated with symptoms. Longitudinal modelling using structural equations indicates that self-rated health is directly associated with symptom experience (Dennerstein et al., 2007). Significantly, the present analysis identifies which parameters of E₂ are important for each of these health outcomes. This has clinical utility.

It should be noted that recent trials of exogenous hormone therapy in women many years post-menopausal do not support a reduction in CHD (Hulley et al., 1998; Rossouw et al., 2002). However, more recent data indicate a trend for a reduction in cardiovascular risk in women initiating hormone therapy <10 years after menopause (Rossouw et al., 2007). For example, there was a statistically significant reduction in the composite end point of myocardial infarction, coronary artery revascularization and coronary death in women aged 50–59 years, randomized to estrogen therapy alone in the Women’s Health Initiative Trial (Hsia et al., 2006). Further research is needed on the issue of timing of initiation of hormone therapy relative to menopause as well as on different hormone formulations (Manson et al., 2006).

On the basis of Table 2, the outcomes can be classified into three groups:

(i) Outcomes associated with E₂ range and slope: hot flushes, night sweats, sleep problems, vaginal dryness and self-rated health.

(ii) Outcomes associated with final value of E₂: CHD risk and sexual response. Sexual response was not corrected for vaginal dryness and dyspareunia as our previous modelling had indicated that these variables did not significantly impact sexual response during and immediately after the menopausal transition. (Dennerstein et al. 2001c,2005a,b; Dennerstein and Lehert, 2004).

(iii) Not associated with hormones: mood/wellbeing, frequency of sexual activities.

Using hot flush experiences as an example of Group 1 health outcomes, it is evident that hot flush experience early in the transition remains an important variable associated with the level experienced post-menopausally. The most important variable for post-menopausal hot flushes is the E₂ range or the difference between final and initial values. The severity of the symptoms is more strongly associated with the relative variation of E₂ than the final value, and the additional association of the slope must be mentioned: the faster the change, the more severe was symptom experience. Thus, sudden withdrawal of hormone therapy might be expected to trigger hot flushes. Another possible clinical
consequence would be that a larger dose of E2 as replacement may be necessary for symptom relief for women in conditions of fast transition or high initial E2 values. As these symptoms are more strongly associated with relative changes, these symptoms should be limited in time, and reduce once hormone levels stabilize. Our results differ from that of Randolph et al. (2005) who found that FSH annual levels were a better predictor of vasomotor symptom prevalence than were annual E2 levels. The differing results may reflect the short follow-up in the Randolph et al. study or the different statistical analytic techniques used.

Using sexual response as an example of the Group 2 health outcomes it is evident again that sexual response in the post-menopause is associated with baseline sexual response early in the transition, as well as the final E2 value. There is no association with the range of E2 during the transition or the E2 slope. Thus, a clinical implication is that maintaining sexual function will require continuation of E2 administration which is not the case for symptoms in Group 1.

A comparison was made between the 438 women who entered the longitudinal study and the analysis subset of 204 women. There were few differences. Women included in the present analysis reported better perceived health and less stress than those in the initial sample. Excluding these differences, the two subsamples were reasonably homogeneous. However, the real problem comes from the reduced sample size, with a consequence of a reduction of statistical power. Thus, if relationships are not confirmed in this analysis, this may simply originate from an underpowered analysis. By using linear regression on log-transformed hormone values, we estimated that the final sample size (n = 202) provided a reasonable power of 0.75 to detect an effect size, ES = 0.5 (considered as the lower limit for clinical relevance), for all the considered end points, given an assumed correlation, r = 0.5, between final and baseline values. In order to maintain sample size, we included some subjects who had a small number of observations coinciding with hormone therapy use. These particular observations were not used in the analysis and were considered as missing. When the analysis was restricted to non-hormone therapy users an even better goodness-of-fit was obtained.

This non-linear analysis faces the same limitations we have experienced earlier in terms of assay sensitivity. In fitting individual curves, unreliable measures may harm the estimations. However, our primary results based on multiple regression tests were confirmed by using bootstrapping. The obtained non-linear results are stable and not influenced by abnormal values.

Other hormones (DHEAS, inhibin and testosterone) were measured annually in the Melbourne study. The present analysis has been limited to E2 and FSH as the reliability of testosterone assays has been contentious. As well we have not found any change in either total testosterone or DHEAS with the menopausal transition (Burger et al., 2000, 2007). Inhibin was not included as levels of inhibin were not measured annually once levels had dropped below the level of sensitivity of the assay.

Conclusions

Changes in E2 across the menopausal transition can be illustrated by a limited number of parameters. E2 range and slope are associated with women’s experience of certain symptoms. The final level of E2 in the post-menopause is associated with women’s maintenance of sexual response and to CHD risk.

References


Non-linear modelling of menopausal hormone changes


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