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

Lorraine Dennerstein[1,4], Philippe Lehert[2], Henry G. Burger[3] and Janet R. Guthrie[1]

[1]Office for Gender and Health, Department of Psychiatry, Royal Melbourne Hospital Melbourne, The University of Melbourne, Victoria 3050, Australia; [2]Statistical Department, Faculty of Economics, University of Mons, Belgium; [3]Prince Henry's Institute of Medical Research, Clayton, Victoria 3168, Australia

[4]Correspondence address. Tel: +613 8344 4520; Fax: +613 9347 4127; E-mail: ldenn@unimelb.edu.au

Changes in FSH and estradiol (E_2) 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. E_2, 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 E_2 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 E_2 range and slope. Sexual response and CHD risk were highly significantly associated with final E_2 level (post-menopausally). These findings have clinical relevance in identifying which symptoms will be triggered by steep transitions of E_2 such as sudden withdrawal and which health parameters may require a maintenance level of E_2.

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 (E_2) 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

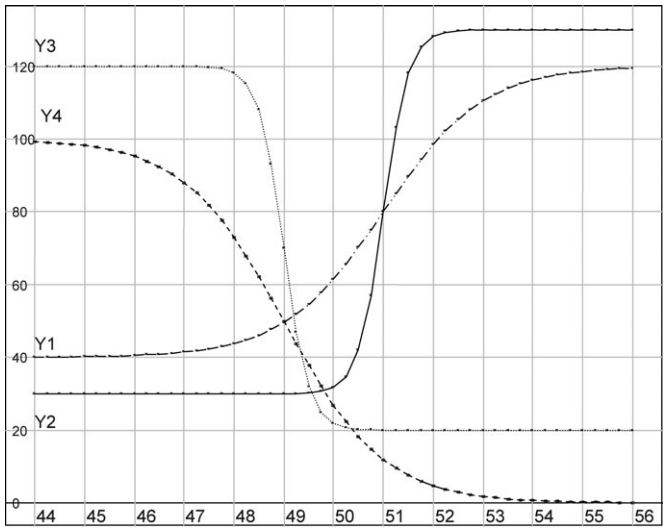

Figure 1: Examples of sigmoid curves for FSH and E_2 across age range 44–56 years
This example set of curves do not illustrate the overall finding that the median age of transition observed for E_2 occurs >1 year later than the mean age of transition observed for FSH. X axis, years of age; Y axis, hormone levels Y1, 2, examples of curves for FSH; Y3, 4, examples of curves for E_2

higher limit at post-menopause (Fig. 1, curves Y1, Y2). Thus, FSH cannot be linear in time, moreover, the speed of increase during the transition period may vary among women. E_2 has the opposite shape in time (Fig. 1, curve Y3, Y4), starting from high values before the menopausal transition and reaching very small values during the post-menopause. This type of change in time cannot be fitted by a simple line, but other mathematical models are available.

This paper is based on our hypotheses that individual women will show different patterns of hormonal change across the menopausal transition and that some characteristics of these changes may be linked to certain health outcomes such as experience of hot flushes. Our objective in the present study is to find an adequate mathematical model to describe for each woman changes in E_2 and FSH across the menopausal transition so that these can be used to more accurately study the relationship between hormones and health variables.

We have previously assessed the effects of hormone levels on different health outcomes, such as bothersome symptoms (Dennerstein *et al*., 2000), mood (Dennerstein *et al*., 1999b, 2001b, 2002b, 2004), sexual function (Dennerstein *et al*., 2002b, 2005b), self-rated health (Dennerstein *et al*., 2003) and coronary heart disease (CHD) risk (Guthrie *et al*., 2004b). Some associations were found, but the exact relationship between any particular health outcome and the hormonal change was not identified. For example, hot flush frequency is known to increase with the late menopausal transition and to be directly associated with E_2 (Guthrie *et al*., 2005). The simplest concept is that hot flush frequency is influenced by the E_2 level at the same time, but many other alternatives may exist: hot flush frequency may also be associated with the earlier E_2 level when women were at the beginning of the transition, or by the magnitude of the difference between levels from early transition to post-menopause, or by the speed of the transition and not by the magnitude. Thus, being able to describe hormones by parameters which identify the shape of the hormone curve may be very useful in discussing and interpreting results. These concepts were developed on the basis of our own and other investigators' studies of the menopausal transition, as reviewed in the accompanying manuscript (Burger *et al*., 2007).

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 experienced menses in the prior 3 months, and who were not taking the 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 Papanicolaou 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 amenorrhoea but <12 months amenorrhoea. Women were deemed to be post-menopausal when there had been amenorrhoea for at least 12 months. Reports of three or more months of amenorrhoea 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 (Guthrie *et al*., 2004b), self-rated health (Smith *et al*., 1994; Dennerstein *et al*., 2003), mood (positive, negative and overall well-being (Dennerstein *et al*., 1994, 1997a, 1999b, 2001b, 2002a,b), sexual function (Dennerstein *et al*., 2005a) and bothersome symptoms (Dennerstein *et al*., 1993, 2000, 2007; Guthrie *et al*., 2005).

Hormone levels were measured each year of follow-up, for years 1–8 and then years 11 and 13, and included FSH, E_2, inhibin, sex hormone-binding globulin, testosterone and dehydroepiandrosterone sulphate (DHEAS).

CHD risk was calculated using the PROCAM scoring system (Assmann *et al*., 2002; Guthrie *et al*., 2004b) from variables measured each year including age, low density lipid cholesterol, cigarette smoking, high density lipid cholesterol, systolic blood pressure, family history of premature myocardial infarction, diabetes mellitus and triglycerides. The variables are listed in their order of importance.

Self-rated health was asked at each examination 0, bad or worse than others; 1, reasonable or same as others; 2, good or better than others (Dennerstein *et al*., 2003).

Mood

Mood scores are derived from the Affectometer-2 (Kammann and Flett, 1983), administered at annual interviews by the fieldworker. There are both positive and negative (or depressed) mood subscales. Each scale contains ten adjectives with the score being the mean of the responses. Women are asked whether they had felt that way most of the time, often, sometimes or hardly ever (Dennerstein *et al*., 1997a). This differs from Kammann and Flett (1983) who described five possible responses to each question. However, distributional characteristics and degree of intercorrelation among the scores were similar to those published Dennerstein *et al*. (1997a). The negative mood subscale correlates 0.83 with the beck depression inventory (short form). Kammann and Flett (1983) report high reliability with an alpha of 0.95.

Sexual function

The personal experiences questionnaire (PEQ) was based on the McCoy female sexuality questionnaire (McCoy and Matyas, 1996) with modifications as already described (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 meaned 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 $[(I \times F)/4.2]$ to produce a scale between 0 and 10. For the category 'other symptoms' to keep values between 0 and 10, we used the calculation $(I \times F)/39.2$ ($392 = 14 \times 28$ 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 radioimmunoassays (RIA) were taken between days 4 and 8 of the menstrual cycle for those still cycling, or after 3 months of amenorrhoea as previously described (Dennerstein *et al*., 2002b). Blood samples for hormone assays were collected the same day as interviews and questionnaires. Only FSH and E_2 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. E_2 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, E_2 from 150–500 pmol/l. Post-menopausal women have serum FSH levels >22 IU/l.

Statistical analysis

The visual observation of E_2 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

here to values measured when women were very early in the menopausal transition), *r* is the range or signed difference between the final and the baseline values, *a* is the median age based on the age at the inflexion point and *s* is the slope and determines the speed of the change at inflexion point. The final value as a dependent fifth parameter is directly found as $f = b + r$. Fig. 1 illustrates these parameters. It can be seen that Y3 is associated with a sharp and sudden decrease around 49.5 years, whereas Y4 shows a much slower decrease centred at the same age with a final value *f* close to 0.

In this analysis, we estimate for each individual woman the change in hormone levels of E_2 and FSH as the parameters of the *S*-curve, and study these parameters instead of the yearly values.

As this model is intrinsically non-linear and cannot be estimated by a simple linear regression, the estimates were found via non-linear egression, using the best known technique for estimation of non-linear regression by successive approximations, the Marquardt algorithm (Marquardt, 1963). Separate non-linear *S*-curve regressions were carried out for each woman. Cooks or Jacknife iterations were used to study the stability. The goodness-of-fit was calculated as the Pearson correlation co-efficient for the regression of observed on non-linear predicted values.

The major objective was to assess the relationship of hormone changes on the following health outcomes in the post-menopause: mood and overall wellbeing, symptoms, self-rated health, CHD risk and sexual fuion. Once goodness-of-fit was demonstrated, we were able to use the non-linear estimate parameters for each individual in a classical model. We carried out a stepwise linear regression analysis considering the following: the premenopausal value (found to have the strongest association from previous studies), the baseline *b*, range *r*, median age *a*, slope *s* and the final value *f* (calculated as $f = b + r$) associated with FSH and E_2 (thus 10 parameters).

Results

Goodness-of-fit of model

For each subject, the *s* parameters for FSH and E_2 were estimated. The mean correlations between estimates and observed values) were 0.74 for E_2 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 for E_2 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.

Table 1: Mean values of the non-linear components estimates of FSH IU/l and E_2 pmol/l

	E_2 (mean and 95% CI)	FSH (mean and 95% CI)
r	−272.5 (−294.9, −250.1)	90 (86.8, 94.1)
a	52.71 (52.57, 52.85)	50.75 (50.49, 51.01)
b	329.37 (300.26, 358.48)	13.75 (13.07, 14.44)
f	48.21 (41.02, 55.40)	104.06 (100.38, 107.74)
s	1.31 (1.19, 1.44)	1.03 (0.95, 1.12)

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.

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 FSH*s* and FSH*r* ($r = 0.08$, $P = 0.05$) where large ranges are associated with increased speed of transition (large FSH*s* values).

E_2 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 E_2 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 E_2a and FSH*a*. E_2a and mean age at transition are very close and not significantly different while FSH*a* 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 E_2. 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 E_2 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 E_2 range (change in E_2), for which higher range was associated with higher current severity of these symptoms, and E_2 slope, where fast change was associated with more severe symptoms. Another variable where there were some associations

Table 2: Summary table of relationship of E_2 parameters to health outcomes showing model coefficients (95% CI) are provided

End point	Baseline (*b*)	E final value (*f*) pmol/l	Range (*r*) pmol/l	Slope (*s*)	R^2
Hot flushes	0.306 (0.118, 0.494)**		−0.004 (−0.006, −0.002)***	0.460 (0.134, 0.786)**	0.196
Night sweats	0.09 (−0.193, 0.372)**		−0.007 (−0.008, −0.005)***	0.340 (0.110, 0.57)**	0.402
Sleep problems	0.415 (0.285, 0.546)**		−0.002 (−0.002, −0.001)***	0.109 (0.031, 0.187)**	0.391
Vaginal dryness	0.190 (0.071, 0.381)*		−0.003 (−0.004, −0.002)***	0.134 (0.009, 0.259)**	0.341
CHD risk	0.735 (0.619, 0.851)***	−0.004 (−0.006, −0.002)***			0.486
Self-rated health	0.270 (0.151, 0.391)***		0.001 (0.000, 0.002)*	−0.005 (−0.062, −0.052)*	0.510
Sexual response	0.484 (0.373, 0.595)***	0.005 (0.002, 0.008)***			0.368
Positive mood	0.452 (0.348, 0.555)***				0.231
Negative mood	0.326 (0.237, 0.414)***				0.145
Wellbeing	0.350 (0.254, 0.445)***				0.134

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; CHD, coronary heart disease.

with E_2 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 E_2 was the next most important associated factor. Contrary to symptoms, E_2 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 E_2.

Discussion

We have proposed, and described the application of the *S*-curve, as a relevant model for E_2 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 E_2 parameters are not correlated, the lack of associations with FSH parameters cannot be explained by existence of E_2 parameters in the model.

The median age of the inflexion in E_2 and FSH curves (parameters E_2a and FSH*a*) 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 E_2 accurately coincided with the mean age of transition observed between the early menopausal transition and the late menopausal transition, and the median age of E_2 occurs gt;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 E_2 with a small number of variables (Dennerstein *et al*., 2007). The present study confirms the association of E_2 with certain symptoms, CHD risk and sexual response. Self-rated health was associated with the same parameters of E_2 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 E_2 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 E_2 range and slope: hot flushes, night sweats, sleep problems, vaginal dryness and self-rated health.

(ii) Outcomes associated with final value of E_2: 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 E_2 range or the difference between final and initial values. The severity of the symptoms is more strongly associated with the relative variation of E_2 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 E_2 as replacement may be necessary for symptom relief for women in conditions of fast transition or high initial E_2 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 E_2 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 E_2 value. There is no association with the range of E_2 during the transition or the E_2 slope. Thus, a clinical implication is that maintaining sexual function will require continuation of E_2 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 E_2 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 E_2 across the menopausal transition can be illustrated by a limited number of parameters. E_2 range and slope are associated with women's experience of certain symptoms. The final level of E_2 in the post-menopause is associated with women's maintenance of sexual response and to CHD risk.

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