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The value of follicle-stimulating hormone
concentration and clinical findings as
markers of the late menopausal transition

John F. Randolph Jr.

Sybil L. Crawford, *University of Massachusetts Medical School*

Lorraine Dennerstein

Kevin Cain

Sioban D. Harlow

Roderick Little

Ellen S. Mitchell

Bin Nan

John Taffe

Matheos Yosef

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THE ReSTAGE PROJECT: THE VALUE OF ANNUAL SERUM FOLLICLE-STIMULATING HORMONE CONCENTRATION AS A MARKER OF THE LATE MENOPAUSAL TRANSITION IN CONJUNCTION WITH BLEEDING CHARACTERISTICS AND HOT FLASHES

John Randolph, University of Michigan

Sybil Crawford, University of Massachusetts

Lorraine Dennerstein, University of Melbourne

and The ReSTAGE Collaboration

(in alphabetical order)

Kevin Cain, University of Washington

Siobán D. Harlow, University of Michigan

Roderick Little, University of Michigan

Ellen S. Mitchell, University of Washington

Bin Nan, University of Michigan

John Taffe, Monash University

Matheos Yosef, University of Michigan

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Corresponding Author: John F. Randolph, Jr., M.D.

Reprint Requests to: John F. Randolph, Jr., M.D.
L4228 Women’s Hospital
University of Michigan Health System
1500 East Medical Center Drive
Ann Arbor, MI 48109-0276

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STRUCTURED ABSTRACT (240 words)

Context. The Stages of Reproductive Aging Workshop proposed bleeding and hormonal criteria for the menopausal transition, but operational definitions of hormone parameters were not specified. **Objective.** This paper investigates the longitudinal relationship of annual serum follicle-stimulating hormone (FSH) levels with four proposed bleeding criteria for the late menopausal transition in two cohort studies. The goal is to provide empirically-based guidance regarding application of hormonal criteria that may be optimal for widespread application in clinical and research settings for assessing menopausal stage.

Design/Setting. Prospective menstrual calendar and annual serum FSH data from two population-based cohort studies: the Melbourne Women's Midlife Health Project(MWMHP) and the Study of Women's Health Across the Nation(SWAN).

Participants: 189 MWMHP and 2256 SWAN women aged 42-57 at baseline who contributed ≥ 10 menstrual cycles and at least one annual serum FSH value.

Main Outcome Measure(s). Association between bleeding criteria for the late menopausal transition and FSH. Association of bleeding criteria, FSH, and hot flashes with the final menstrual period.

Results. A single FSH measure is an independent marker of the late menopausal transition, but FSH concentrations are less predictive of menopausal stage than any of four proposed bleeding criteria. Criterion FSH values for the late transition are similar across both studies. Experience of hot flashes adds no information in the presence of hormonal and bleeding criteria.

Conclusions. An annual serum FSH concentration of 40 IU/L could be incorporated, in

conjunction with bleeding markers, into the STRAW paradigm for markers of the late menopausal transition.

INTRODUCTION

Both clinical management and investigation of the menopausal transition have been hampered by the lack of a well-characterized and validated biomarker of the evolution of ovarian function from active reproduction to a post-reproductive state. Rigorous descriptions of this transition have relied primarily on progressive changes in menstrual bleeding patterns (1-6) to describe and relate the reproductive aging process to other endpoints of interest. Follicle-stimulating hormone, a glycoprotein dimer secreted by the anterior pituitary, has emerged as a readily and reproducibly measurable potential marker (7) but has suffered to date by a lack of precision with respect to other markers of the menopausal transition, particularly the final menstrual period (FMP) which is classically used to define menopause (8).

The need for a rigorous characterization of the menopausal transition to both standardize terminology and criteria for investigational purposes and to inform women and their care providers prompted the Stages of Reproductive Aging Workshop (STRAW) in July, 2001 (9) which resulted in a seven stage classification of female reproductive life based on the best available evidence. The proposed STRAW paradigm included bleeding, symptom and hormonal criteria, but operational definitions of hormone parameters were not included because insufficient information was available to establish definitive criteria. Consensus was reached that, of the candidate biomarkers of the transition, FSH was the best available and STRAW recommended that specific criteria be developed for incorporating FSH into the evolving system for staging reproductive aging (9). Vasomotor symptoms, including hot flashes, although

characteristic of the menopausal transition, are not experienced by all women and are not considered essential criteria within the staging system.

The ReSTAGE collaboration is conducting an empirical evaluation of proposed criteria for staging reproductive aging in the context of the natural history of the menopausal transition. In a previous paper (10) we assessed proposed bleeding criteria for the late menopausal transition using prospectively collected menstrual calendar data from four large cohort studies : TREMIN (11), the Melbourne Women's Midlife Health Project (MWMHP) (1), the Seattle Midlife Women's Health Study (SMWHS) (3), and the multi-site, multi-ethnic, Study of Women's Health Across the Nation (SWAN) (4). Although our findings supported STRAW's recommendation that amenorrhea of 60-days be used as the bleeding criterion for the late transition, a correlation between this criterion and underlying hormonal changes and symptoms has yet to be demonstrated. Annual serum FSH levels are available from MWMHP and SWAN and the assays have been cross-validated to allow comparability. This paper investigates the longitudinal relationship of annual serum FSH with four proposed bleeding markers of the late menopausal transition. Our underlying goal is to address the question: Does information on serum FSH and hot flashes improve the determination of a woman's entry into the late menopausal transition as compared to the use of bleeding criteria for the late menopausal transition alone?

EXPERIMENTAL SUBJECTS

This study has been approved by Institutional Review Boards at the Universities of Michigan, Washington, Massachusetts and Melbourne. For this paper, we conducted

secondary analyses using menstrual calendar information, annual serum FSH concentrations, and self report of hot flashes accumulated in two longitudinal cohort studies of the menopausal transition.

The *Melbourne Women's Midlife Health Project (MWMHP)* began in 1991 with a cross-sectional survey of 2001 mid-aged Australian-born women of Anglo-European heritage identified by random telephone digital dialing (response rate=71%) (12). From these women the MWMHP enrolled 438 women, aged 45 -55 years, who had menstruated in the prior 3 months and were not using hormone therapy into a longitudinal study (response rate=56%). This analysis includes records from the 189 women who maintained menstrual records through at least 10 untreated bleeding segments and provided at least one untreated serum sample for FSH measurement. (The term bleeding segment is analogous to menstrual cycle but acknowledges that menstrual calendar data cannot distinguish menstrual from nonmenstrual bleeds.)

The *Study of Women's Health Across the Nation (SWAN)* is an ongoing multi-ethnic, multi-site longitudinal community-based study of mid-aged women begun in 1995 (4). A cross-sectional survey randomly selected women aged 40-55 years from a variety of lists including a large managed health care plan, community census, utility households, and registered voters, or by random digit dialing. Each site then recruited about 450 women including Caucasian women and women from one minority group (African Americans at four sites; and Japanese, Chinese, and Hispanic women at one site each). Eligibility requirements for the longitudinal cohort included age 42-52 years, an intact

uterus, no reproductive hormones use at time of enrollment and at least one menstrual period in the previous 3 months. A total of 3302 women were enrolled. This analysis includes records of the 2256 women who maintained menstrual records through at least 10 untreated segments and provided at least one untreated serum sample for FSH measurement.

MATERIALS AND METHODS

Both studies included a menstrual calendar and asked women to record menstrual bleeding on each day bleeding occurred. Although slightly different in format, calendars identified the date of bleeding onset and days of bleeding in a comparable manner.

Ascertainments of the onset of the late transition and of the final menstrual period (FMP) are based on the menstrual calendar data.

In MWMHP, each menstrual calendar card covered one year. Participants were followed up to 10 years with annual assessments which included symptoms, measured body mass index (BMI), smoking history, hormone use, and blood sampling to measure FSH. Experience of hot flashes was obtained from the North American symptom checklist (13). Fasting morning blood samples for hormone assays were taken between days 4 and 8 of the menstrual cycle for those still cycling, or after 3 months of amenorrhea as previously described (14). FSH was measured initially by radioimmunoassay (RIA) in year one (7). 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: FSH (IMX) and RIA, 0.98, and FSH (TOSOH) with IMX 0.99. Inter- and intra-assay coefficients of variation were 5.4 % and 4.0 %, respectively.

In SWAN, women were provided monthly menstrual calendars. Assessments at baseline and annual follow-up visits included symptoms, measured BMI, smoking history, hormone use, and blood sampling to measure FSH. Blood was drawn between days 2-5 of the follicular phase of the menstrual cycle in regularly cycling women. Serum was frozen at -80 degrees Centigrade and sent on dry-ice to the SWAN Endocrine Laboratory at the University of Michigan. Hormone assays were conducted using an ACS-180 automated analyzer (Bayer Diagnostics Corp, 115 Norwood Park South, Norwood, MA. 02062-4658). Serum FSH concentrations were measured with a two-site chemiluminometric immunoassay that uses constant amounts of two monoclonal antibodies (provided by Bayer Diagnostics). Each antibody is directed to different regions on the beta subunit (one coupled to paramagnetic particles and the other labeled with DMAE) with specificity for intact FSH. Inter- and intra-assay coefficients of variation were 12.0% and 6.0 %, respectively.

Cross-validation of serum assays used in the Melbourne and SWAN Studies was conducted. Laboratories for each study received and analyzed samples from the other study. As neither measure can be defined as the gold standard, we created standardized values using an equating procedure (15) such that the standard scores (z scores) of the log

FSH distribution from each assay are equated to each other. All analyses used these standardized FSH values.

Using definitions recommended by WHO (16) and modified by ReSTAGE, a bleeding episode is a period of consecutive bleeding days; a bleeding-free interval is a period of consecutive bleeding free days; and a bleeding segment is a bleeding episode and the subsequent bleeding-free interval. In ReSTAGE, a single day of bleeding, as well as consecutive days of spotting/bleeding, were coded as bleeding episodes. Bleeding-free intervals had to consist of at least three days. One or two bleed-free days between two bleed days were considered part of the bleeding episode. Pregnancies and the first three segments post birth/abortion are considered nonmenstrual intervals and are excluded from analyses (17). For each bleeding segment, we indicate whether a woman was using hormone therapy or oral contraceptives, fertility medications, selective estrogen receptor modulators (SERMS), injectable contraceptives, contraceptive implants, or a short-course of hormones to treat a menstrual disorder. Gaps in the menstrual record are coded as missing.

This paper compares three recently proposed bleeding criteria (3, 18-20) for onset of the late menopausal transition with the current standard of 90-days of amenorrhea (5). We refer to the bleeding episode that marks when each criterion is met as the “marker event”, with age at occurrence defined as age on the first day of that bleeding episode. We calculated the following marker events: the first observed a) segment of at least 90-days(3); b) segment of at least 60-days(20); c) skipped segment, defined as a segment

that exceeded twice the median segment length of the previous 10 segments (3); and d) running range over a 10-segment sequence (18). For the running range, we identify the first day of the bleeding segment when the running range exceeds 42 days. Menopause is defined as the date of the FMP recorded in the menstrual calendars established retrospectively after 12 months of amenorrhea were observed.

We created a dichotomous variable based on whether the woman reported experiencing any hot flashes (yes/no). In MWMHP, women were asked whether they had been bothered by hot flashes in the last two weeks. Originally scored on a 4 point scale (0=not present, 1=causes minor irritation, 2=interferes with normal life, 3=debilitating), we defined scores of 1-3 as having hot flashes. SWAN inquired about the presence of hot flashes in the past two weeks, with frequency originally indicated on a five point scale (0=not at all to 4=everyday). We defined scores of 1-4 as having hot flashes. Smoking status (current, former, never) is based on annual questionnaires. Body mass index (BMI) is based on a linear interpolation of annual height and weight measures, and categorized as <25, 25-29.9, 30-35, and >35.

ANALYSIS

Women were censored at hysterectomy, bilateral oophrectomy, initiation of hormonal birth control, hormone therapy or chemotherapy (Table 1). Segments during which a short-course of hormonal medication, including fertility medication, was used to treat a menstrual disorder were treated as gaps in the menstrual record.

Ideally, we would have a gold standard against which to evaluate bleeding, FSH, and symptom criteria for onset of the late menopausal transition. In the absence of such a standard, we focus on how well bleeding criteria are associated with FSH and on how well each criterion alone versus bleeding criteria, FSH, and hot flashes predict FMP. Significance was defined by a p-value <0.05 in all analyses.

We examined the association of FSH with each bleeding marker by estimating a binomial logistic regression model for each marker (has occurred, yes/no) as a function of log-transformed FSH, before and after adjusting for concurrent chronologic age, baseline body mass index and baseline smoking status, and ethnicity in SWAN. Within-woman correlation from multiple observations per woman was accounted for by using generalized estimating equations (21). We tested interactions between log FSH and each covariates to determine whether associations between FSH and bleeding markers varied by subgroup. From these models, we estimated the probability that a marker had occurred for various values of FSH. In addition, we computed odds ratios for marker occurrence in terms of categorized serum FSH. Estimated probabilities of marker occurrence, done separately for each study, reflect not only the role of FSH but also the age distribution of each study. In contrast, the odds ratios indicate the contribution of FSH apart from age.

We used a Cox model to assess how well bleeding markers, serum FSH concentrations, and hot flash experience predict FMP. We started with a varying coefficient Cox model (22) where the marker effect on FMP varies with age at the marker

event (23). Estimation proceeded using the regression spline method. Because the log hazard as a function of age at the marker event is approximately linear, the final regressions model the log hazard of the marker to FMP as a linear function of age at marker. These models were compared with corresponding models excluding FSH or hot flashes to assess the additional contributions of FSH and hot flashes in predicting the FMP. Potential confounding and interactions by smoking status, BMI and, within SWAN, by ethnicity, were examined.

RESULTS

Baseline characteristics of each study sample are summarized in Table 2. Reflecting eligibility criteria, MWMHP participants were older than SWAN participants. SWAN participants were heavier and MWMHP participants were less likely to have smoked.

Serum FSH was strongly related to each of the bleeding markers in both studies (Figure 1). The steepest increase in the marker probability occurred with an increase in FSH from 10 to 20 IU/L. The curves for the 10-segment running range and a 60-day segment were similar to each other, particularly in the MWMHP dataset. At any given FSH value, estimated probabilities for a skipped segment and a 90-day segment were lower. Upon adjustment for age, the increase in the marker probability with an increase in FSH was less steep, but FSH remained significantly positively associated with each marker. Further adjustment for covariates resulted in little additional change in the FSH

associations with markers, and interactions of FSH with these covariates were not statistically significant (data not shown). Associations were similar for SWAN and MWMHP.

Consistent with Figure 1, an increase in FSH was more strongly related to the 10-segment running range and the 60-day segment than to the skipped segment, as seen in the larger odds ratios (Table 3). The odds ratio was larger still for the 90-day segment. For all markers, odds ratios were highest for FSH ≥ 40 IU/L (versus < 10 IU/L). Adjustment for age reduced the magnitude of the FSH odds ratios such that only an FSH level greater than 40 IU/L was consistently associated with a statistically significant increased odds of experiencing each bleeding marker. Results were similar for SWAN and MWMHP.

Table 4 presents the relative hazard of FMP for serum FSH concentrations and hot flashes.. Cutpoints of 20-39 IU/L and ≥ 40 IU/L were selected based on Figure 1 and the published standards (23, 24). The likelihood of reaching the FMP increases with age, thus, the log-relative hazard of menopause for those women who have experienced a bleeding marker as compared with those who have not declines with age (20, 23). When considered alone, serum FSH concentrations greater than 20-39 IU/L were associated with a 2 to 4-fold increase in the log relative hazard of FMP, while values of 40 IU/L and above were associated with a 6-10 fold increase, although the confidence intervals are wide for both cutpoints and in both cohorts. However, when serum FSH concentrations were considered jointly with bleeding markers, the effect of FSH is markedly attenuated, reflecting the associations of FSH with the bleeding markers seen in Figure 1 and Table

3. In the MWMHP, the relative hazard of FMP was increased 4-fold when serum FSH concentrations were 20 IU/L or higher. In SWAN, which includes younger women and a shorter period of follow-up, a similar magnitude of effect was seen when serum FSH concentrations were 40 IU/L or higher. The adjusted association between FSH and FMP varied little across models including different bleeding marker of the late transition. Annual serum FSH concentration provided additional information about which women were closest to menopause.

Hot flashes, considered alone, were only weakly associated with the log relative hazard, and this association disappeared after adjustment for occurrence of a bleeding marker and serum FSH concentration.

DISCUSSION

This paper demonstrates that a single annual early follicular serum follicle-stimulating hormone level is a marker of the late menopausal transition, but FSH concentrations are less predictive than any of the proposed bleeding criteria for the late menopausal transition (10). Moreover, when bleeding markers and FSH concentrations are considered together, the contribution of FSH is attenuated. Experience of hot flashes, while also a marker of the late menopausal transition in the absence of other hormonal or bleeding criteria, adds no information in the presence of these criteria. This paper also demonstrates that FSH is associated with both the bleeding criterion of 90-days of amenorrhea and of bleeding criteria based on shorter durations of amenorrhea, adding further evidence to support the STRAW recommendation that 60-days of amenorrhea be used as the criterion marker of the late menopausal transition.

Single annual serum follicle-stimulating hormone concentrations have been associated with the menopausal transition (25) as an indirect measure of the change in ovarian function that underpins reproductive aging, but they have not been rigorously related to well-defined bleeding markers of menopausal stage (5). FSH levels were qualitatively included in the STRAW model for reproductive aging (9), but specific cutpoints were not specified given the absence of a well-defined relationship between FSH concentrations and onset of the transition.

Specification of cutpoints for annual serum FSH levels that might serve as criteria for onset of the menopausal transition has been problematic due to the variable pattern of FSH secretion, the variation in available assays (26), and the independent effects of age, body size and other variables (27). Cutpoints in this analysis reflect clinically utilized values but should not be construed as having specific intrinsic biological implications. We found that an annual serum FSH concentration of 40 IU/L is informative across both studies and could clearly be incorporated in conjunction with bleeding markers into the STRAW paradigm. In MWMHP there was no difference between the cutpoints 20 IU/L and 40 IU/L. In SWAN, 20 IU/L was less predictive than 40 IU/L. SWAN includes younger women and women who have transitioned earlier suggesting that the higher FSH values may be most predictive of women who transition faster or at a younger age. MWMHP includes older women who have all transitioned, suggesting that FSH over 20 IU/L may be more informative for older women whereas an FSH level over 40 IU/L may

be more informative for younger women. Future analyses will evaluate whether algorithms for predicting the FMP should employ age-specific FSH cutpoints.

Bleeding markers are most informative of a woman being in the late menopausal transition (20, 22, 23), but several competing criterion are currently proposed (20, 23). FSH levels are less informative but do add to the predictability of time to FMP. The additional information provided by serum FSH concentrations is similar regardless of the choice of bleeding criteria, suggesting that the ovarian biology underlying these changes in menstrual bleeding is the same for all of the proposed bleeding criteria for the late menopausal transition. Thus, selection of an optimal bleeding marker should be based on considerations such as frequency of occurrence, association with time to FMP, and ease of measurement.

Follicle-stimulating hormone stimulates folliculogenesis and estradiol (E2) production in the ovary, and is regulated primarily in a classic negative feedback loop by the inhibins A and B secreted by the corpus luteum and follicle, respectively (14, 25, 28, 29), and to a lesser extent by E2. As oocyte competence and the total number of ovarian follicles declines with age, negative feedback decreases as reflected in a progressive rise in FSH initially in the early phase of the menstrual cycle (25, 30). In both cross-sectional (7, 27) and longitudinal (25, 31, 32) studies of the menopausal transition, single measures of these biomarkers have substantial variability but increases in Inhibin B and FSH are observed before decreases in E2 (14, 25, 32). Widespread use of the Inhibin B measure has been precluded by cost and sensitivity of the assay (33). Assays of FSH vary by as

much as two-fold (26), making comparisons between studies problematic and the establishment of criteria concentrations as reliable markers of transition stages difficult. These difficulties have been overcome in the current analysis by the similarities of the study design and subject populations, and by cross-validation of the FSH assay in the respective laboratories (15).

The association of FSH with the bleeding markers is expected since bleeding markers are the clinical reflection of the underlying utero-ovarian biology. Given the variability of annual serum measures of FSH, bleeding markers may be most informative in the late menopausal transition as they reflect the cumulative impact of a changing hormonal environment including the most biologically significant changes in FSH. As such, they remain the most clinically significant markers of the late transition. Serum FSH may be a more important marker for the early menopausal transition when bleeding markers are less predictive of FMP, and future analyses of the ReSTAGE collaboration will address this question (34).

Self report of hot flashes has also been associated with the menopausal transition (5, 35, 36) and serum FSH levels (37), and is independently informative in the absence of any other marker of the late transition, albeit only modestly. However, when either FSH level or any bleeding marker is available, reported hot flashes do not add additional information regarding the likelihood of being in the late transition.

This analysis has some limitations. Both studies set a lower age limit for enrollment and excluded women who were not still menstruating, so we can not evaluate whether FSH concentrations are more or less relevant for women with an earlier onset of the late menopausal transition. Moreover, SWAN is still in progress and not all women have completed their reproductive transition. Nonetheless, the results are notably robust, and support recommendations regarding the modification of the STRAW paradigm to include an annual serum FSH concentration of ≥ 40 IU/L as an additional criterion for the late menopausal transition.

In summary, we have shown that that a single annual early follicular serum follicle-stimulating hormone level is positively associated with four bleeding markers of the late menopausal transition, and is an independent but less robust marker of the late transition. Hot flashes are similarly positively associated with the late transition, but are much less robust and are no longer significant when considered together with FSH and bleeding markers. Thus, serum FSH levels should be incorporated into classification criteria for the late menopausal transition.

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REFERENCES

1. **Dennerstein, L., E. C. Dudley, J. L. Hopper, J. R. Guthrie, and H. G. Burger** 2000. A prospective population-based study of menopausal symptoms. *Obstet. Gynecol.* **96**:351-358.
2. **Dudley, E. C., J. L. Hopper, J. Taffe, J. R. Guthrie, H. Burger, and L. Dennerstein** 1998. Using Longitudinal Data to Define the Perimenopause by Menstrual Cycle Characteristics. *Climacteric.* **1**:18-25.
3. **Mitchell, E. S., N. F. Woods, and A. Mariella** 2000. Three stages of the menopausal transition from the Seattle Midlife Women's Health Study: Toward a more precise definition. *Menopause.* **7**:334-349.
4. **Sowers, M. F., S. Crawford, B. Sternfeld, D. Morgenstein, E. Gold, G. Greendale, D. Evans, R. Neer, K. Matthews, s. Sherman, A. Lo, G. Weiss, and J. Kelsey** 2000. Design, Survey Sampling and Recruitment Methods of SWAN: A Multi-center, Multi-ethnic, Community-based Cohort Study of Women and the Menopausal Transition. *In* J. Wren, R. A. Lobo, J. Kelsey, and R. Marcus (eds), *Menopause: Biology and Pathobiology*, vol. 32. Academic Press.
5. **Brambilla, D. J., J. B. McKinlay, and C. B. Johannes** 1994. Defining the Perimenopause for Application in Epidemiologic Investigations. *American Journal Epidemiology.* **140**:1091-1095.
6. **Johannes, C. B., S. Crawford, C. Longcope, and J. B. McKinlay** 1996. Bleeding Patterns and Changes in the Perimenopause: A Longitudinal Characterization of Menstrual Cycles. *Clinical Consultations in Obstetrics and Gynecology.* **8**:9-20.
7. **Burger, H. G., E. C. Dudley, J. L. Hopper, J. M. Shelley, A. Green, A. Smith, L. Dennerstein, and C. Morse** 1995. The Endocrinology of the Menopausal Transition - a Cross-Sectional Study of a Population-Based Sample. *J Clin. Endocrinol. Metab.* **80**:3537-3545.
8. **World Health Organization** 1996. Research on Menopause in the 1990's. WHO Technical Report Series. World Health Organization.
9. **Soules, M. R., S. Sherman, E. Parrott, R. W. Rebar, N. Santoro, W. Utian, and N. Woods** 2001. Executive summary: Stages of reproductive aging workshop (STRAW) *Fertil. Steril.* **76**:874-878.
10. **Harlow SD, Cain K, Crawford S, Dennerstein L, Little R, Mitchell ES, Nan B, Randolph J, Taffe J, Yosef M** 2006. The ReSTAGE Project: Evaluation of Four Proposed Bleeding Criteria for the Onset of Late Menopausal Transition (submitted)

11. **Treloar, A. E., R. E. Boynton, B. G. Behn, and B. W. Brown** 1967. Variation of Human Menstrual Cycle through Reproductive Life. *International J Fertil.* **12**:77- .
12. **Taffe, J., L. Dennerstein, and A. MacLennan** 2001. Menstrual Diary Data and the Menopausal Transition: Methodological Issues. *Acta Obstet. Gynecol. Scand.* **81**:588-594.
13. Menopausal Symptoms in Women of Various Ages. *Psychosomatic Medicine.* **27(3)**:266-273.
14. **Burger, H. G., N. Cahir, D. M. Robertson, N. P. Groome, E. Dudley, A. Green, and L. Dennerstein** 1998. Serum Inhibins A and B fall differentially as FSH rises in perimenopausal women. *Clin Endocrinol* **48(6)**:809-13.
15. **Holland, P.W. and Rubin,D.B.** (1982, eds.) *Test equating.* New York: Academic Press.
16. **Rodriguez, G., A. Faundes-Latham, L.E. Atkinson** 1976. An approach to the analysis of menstrual patterns in the critical evaluation of contraceptives. *Studies in Family Planning*, **7**: p. 42-51
17. **Campbell OMR, Gray RH** 1992. Characteristics and determinants of postpartum ovarian function in women in the United States. *Am J Obstet Gynecol* **169**:55-60.
18. **Taffe, J., and L. Dennerstein** 2002. Menstrual Patterns Leading to the Final Menstrual Period. *Menopause.* **9**:32.40.
19. **Taffe, J.,L. Dennerstein,** Time to the Final Menstrual Period. *Fertility and Sterility*, 2002. **78**:397-403.
20. **Lisabeth L, Harlow SD, Gillespie B, Lin X, Sowers MF** 2004. Staging Reproductive Aging: A Comparison of Proposed Bleeding Criteria for the Menopausal Transition. *MENOPAUSE* **11(2)**:186-197.
21. **Diggle PJ, Liang K-Y, Zeger SL.** *Analysis of Longitudinal Data.* Oxford: Clarendon Press, 1994.
22. **Nan, B., X. Lin, L.D. Lisabeth, S.D. Harlow** 2006. A Varying Coefficient Cox Model for the Effect of Age at a Marker Event on Age at Menopause. *BIOMETRICS* **61**:576-583.
23. **Taffe, J., and L. Dennerstein** 2002. Time to the Final Menstrual Period. *Fertil. Steril.* **78**:397-403.

24. **Metcalf, M. G., and J. H. Livesey** 1985. Gonadotropin Excretion in Fertile Women - Effect of Age and the Onset of the Menopausal Transition J. Endocrinol. **105**:357-362.
25. **Burger, H. G., E. C. Dudley, J. L. Hopper, N. Groome, J. R. Guthrie, A. Green, and L. Dennerstein** 1999. 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. **84**:4025-4030.
26. **McConnell, D.S.**, unpublished data
27. **Randolph, J.F., Sowers, M.F., Gold, E., Mohr, B. Luborsky, J., Santoro, N., McConnell, D., Finkelstein, J., Korenman, S., Matthews, K., Sternfeld, B., Lasley, B** 2003. Reproductive Hormones in the Early Menopausal Transition: Associations with ethnicity, body size, and menopausal status. J Clin Endocrinol Metab **88** (4) 1516-1522.
28. **Burger, H., E. Dudley, P. Mamers, N. Groome, and D. M. Robertson** 2000. Early Follicular Phase Serum FSH as a Function of Age: The Roles of Inhibin B, Inhibin A and Estradiol. Climacteric. **3**:17-24.
29. **Klein, N. A., P. J. Illingworth, N. P. Groome, A. S. McNeilly, D. E. Battaglia, and M. R. Soules** 1996. Decreased inhibin B secretion is associated with the monotropic FSH rise in older, ovulatory women: A study of serum and follicular fluid levels of dimeric inhibin A and B in spontaneous menstrual cycles J. Clin. Endocrinol. Metab. **81**:2742-2745.
30. **Randolph, J.F., Ginsburg, K.A., Leach, R.E., Blacker, C.M., Moghissi, K.S., Diamond, M.P., Reame, N.E.** 2003. Elevated Early Follicular Gonadotropin Levels in Women with Unexplained Infertility: Lack of Evidence for Disordered Gonadotropin Releasing Hormone Secretion as Assessed by Lutenizing Hormone Pulse Characteristics. Fertil Steril **80** (2) 320-327.
31. **Metcalf, M. G., R. A. Donald, and J. H. Livesey** 1982. Pituitary-Ovarian Function before, During and after the Menopause - a Longitudinal-Study Clin. Endocrinol. **17**:489-494.
32. **Randolph, J.F., Sowers, MF, Bondarenko, I.V., Harlow, S.D., Luborsky, J.L., Little, R.J.** 2004. Change in Estradiol and Follicle Stimulating Hormone Across The Early Menopausal Transition: Effects of Ethnicity and Age. J. Clin Endocrinol Metab **89** (4) 1555-61.

33. **Welt CK**, 2002 The physiology and pathophysiology of inhibin, activin and follistatin in female reproduction. *Curr. Opin. Obstet. Gynecol.* **14**: 317-323.
34. **Stellato R, Crawford S, McKinlay S, Longcope C.** 1998 Can follicular stimulating hormone be used to define menopause? *Endocrine Practice* **4**:137-141.
35. **Guthrie JR, Dennerstein L, Hopper JL, Burger HG** 1996 Hot flushes, menstrual status, and hormone levels in a population-based sample of midlife women. *Obstet Gynecol* **88**:437-42.
36. **Dennerstein L, Dudley EC, Hopper JL, Guthrie JR, Burger HG** 2000 A prospective population-based study of menopausal symptoms. *Obstet Gynecol* **96**(3):351-8.
37. **Randolph JF, Sowers MF, Bondarenko I, Gold EB, Greendale GA, Bromberger JT, Brockwell SE, Matthews KA** 2005. The Relationship of Longitudinal Change in Reproductive Hormones and Vasomotor Symptoms during the Menopausal Transition. *J Clin Endocrinol Metab* **90** (11): 6106-6112.

Table 1: Distribution of women by final menstrual period (FMP), and reasons for censoring in SWAN and MWMHP.

	MWMHP		SWAN+	
	(n=189)		(n=2256)	
Final Status	<u>N</u>	<u>%</u>	<u>N</u>	<u>%</u>
FMP observed	45	23.8%	159	7.1%
Hysterectomy	11	5.8%	42	1.9%
Still menstruating	57	30.2%	1532	67.9%
Stopped maintaining calendar although still menstruating	51	27.0%	271	12.0%
Endometrial Ablation	3	1.6%	0	0

Began Using	22	11.6%	252	11.2%
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Hormone Therapy

+ Analysis to date of the SWAN calendar is based on the first 6 years of menstrual calendar data.

Table 2: Characteristics of participants in the analytic sample.

Characteristic	SWAN (N=2256)		MWMHP (N=189)	
	% (N)	Mean (SD)	% (N)	Mean (SD)
Age at entry		46.0 (2.5)		48.8 (2.0)
Ethnicity:				
African American	23.4 (527)		0.0 (0)	
Caucasian	50.7 (1144)		100.0 (189)	
Chinese	9.3 (210)		0.0 (0)	
Hispanic	6.1 (137)		0.0 (0)	
Japanese	10.6 (238)		0.0 (0)	
Hormone use / fertility meds during study:				
No	76.0 (1714)		81.5 (154)	
Yes	24.0 (542)		18.5 (35)	
Body mass index (kg/m ²) at study entry:		27.5 (6.9)		25.7 (4.7)
< 25	43.6 (984)		54.0 (102)	
25 – 29.9	25.1 (566)		30.7 (58)	
30 – 35	14.2 (321)		9.5 (18)	
> 35	13.9 (313)		5.8 (11)	
Unknown	3.2 (72)		0.0 (0)	
Smoking status at study entry:				
Never	59.8 (1350)		63.0 (119)	
Past	13.8 (311)		19.1 (36)	
Current	25.6 (577)		18.0 (34)	
Unknown	0.8 (18)		0.0 (0)	

Table 3: Estimated associations of serum FSH with occurrence of late-stage bleeding markers from repeated measures logistic regression, after adjustment for age.

Marker	Age-adjusted Odds Ratio (95% CI) for occurrence of marker	
	SWAN	MWMHP
RR10:		
FSH < 10 IU/L	Reference	Reference
10 – 19.9 IU/L	1.01 (0.90, 1.15)	0.99 (0.59, 1.67)
20 – 39.9 IU/L	1.39 (1.21, 1.61)	1.36 (0.76, 2.42)
40+ IU/L	2.89 (2.45, 3.40)	3.65 (2.07, 6.44)
VLS60:		
FSH < 10 IU/L	Reference	Reference
10 – 19.9 IU/L	0.96 (0.85, 1.08)	0.99 (0.59, 1.66)
20 – 39.9 IU/L	1.37 (1.19, 1.57)	1.26 (0.72, 2.19)
40+ IU/L	2.81 (2.40, 3.31)	3.41 (1.89, 6.13)
SS:		
FSH < 10 IU/L	Reference	Reference
10 – 19.9 IU/L	1.05 (0.92, 1.19)	0.63 (0.39, 1.02)
20 – 39.9 IU/L	1.34 (1.16, 1.56)	0.88 (0.50, 1.55)
40+ IU/L	2.10 (1.78, 2.49)	2.17 (1.23, 3.83)
VLS90:		
FSH < 10 IU/L	Reference	Reference
10 – 19.9 IU/L	0.94 (0.79, 1.11)	1.11 (0.62, 1.98)
20 – 39.9 IU/L	1.48 (1.23, 1.78)	1.70 (0.99, 2.92)
40+ IU/L	4.10 (3.36, 5.01)	3.49 (1.97, 6.19)

All associations of categorized serum FSH with marker occurrence, before and after adjustment for age, are statistically significant ($p < 0.0001$)

Table 4: Relative Hazard of reaching FMP by serum FSH concentration and hot flash, unadjusted and adjusted for occurrence of bleeding markers of the late transition and BMI.

Models	Relative Hazard	SWAN		MWMHP	
		95 % Confidence Limits		Relative Hazard	95%Confidence Limits
FSH 20-39	2.43	1.38, 4.25		4.44	1.53, 12.90
FSH 40+	10.66	6.56, 17.31		6.76	2.53, 18.1
Hot flash	1.91	1.35, 2.71		1.62	0.78, 3.34
Adjusted Model*					
With >=60-day segment					
FSH 20-39 IU/L	1.59	0.90	2.80	5.01	1.66, 15.10
FSH 40+ IU/L	3.44	2.09	5.66	2.80	1.03, 7.57
Hot flash	1.24	0.86	1.78	0.92	0.51, 2.57
With >=90-day segment					
FSH 20-39 IU/L	1.54	0.87, 2.71		4.09	1.39, 12.10
FSH 40+ IU/L	2.83	1.69, 4.74		3.51	1.28, 9.65
Hot flash	1.22	0.82, 1.75		1.37	0.65, 2.89
With skipped segment					
FSH 20-39 IU/L	1.88	1.08, 3.28		4.82	1.65, 14.06
FSH 40+ IU/L	5.51	3.36, 9.02		4.29	1.56, 11.82
Hot flash	1.42	0.99, 2.03		1.49	0.70, 3.18
With 10-segment running range					
FSH 20-39 IU/L	1.60	0.91, 2.80		4.47	1.51,13.27
FSH 40+ IU/L	3.63	2.21, 5.94		3.36	1.24, 9.13
Hot flash	1.21	0.84, 1.73		1.22	0.56, 2.64

* adjusted for BMI and age at marker

Figure 1: Estimated unadjusted associations between bleeding markers and annual serum FSH concentrations.

