HHS Public Access

Author manuscript

Cancer Causes Control. Author manuscript; available in PMC 2018 December 01.

Published in final edited form as:

Cancer Causes Control. 2017 December ; 28(12): 1441–1452. doi:10.1007/s10552-017-0971-2.

Menstrual Cycle Characteristics and Steroid Hormone, Prolactin, and Growth Factor Levels in Premenopausal Women

Leslie V Farland[4,†], **Fan Mu**[1,†], **A. Heather Eliassen**[1,2], **Susan E. Hankinson**[1,2,3], **Shelley S. Tworoger**[1,2], **Robert L. Barbieri**[4], **Mitch Dowsett**[5], **Michael N. Pollak**[6], and **Stacey A. Missmer**[1,2,4,7]

[1]Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts

[2]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts

[3]Division of Biostatistics and Epidemiology, School of Public Health and Health Sciences, University of Massachusetts, Amherst, Massachusetts

[4]Department of Obstetrics, Gynecology, and Reproductive Biology; Brigham and Women's Hospital and Harvard Medical School; Boston, Massachusetts

[5]Academic Department of Biochemistry, The Royal Marsden Hospital, London, United Kingdom

[6]Department of Oncology, McGill University and Lady Davis Research Institute, Montreal, Quebec, Canada

[7]Division of Adolescent Medicine, Boston Children's Hospital and Harvard Medical School, Boston, MA

Abstract

Purpose—Menstrual cycle characteristics are markers of endocrine milieu. However, associations between age at menarche and adulthood sex steroid hormones levels have been inconsistent, and data on menstrual characteristics and non-sex steroid hormones are sparse.

Methods—We assessed the relations of menstrual characteristics with premenopausal plasma sex steroid hormones, sex hormone binding globulin(SHBG), prolactin, and growth factors among 2,745 premenopausal women (age 32–52) from the Nurses' Health Study II. Geometric means and tests for trend were calculated using multivariable general linear models.

Results—Early age at menarche was associated with higher premenopausal early-follicular free estradiol(percent difference<12 vs >13 years=11%), early-follicular estrone(7%), luteal estrone(7%), and free testosterone(8%)(All P_{trend}<0.05). Short menstrual cycle length at age 18–22 was associated with higher early-follicular total(<26 vs >39 days=18%) and free estradiol(16%), early-follicular estrone(9%), SHBG(7%), lower luteal free estradiol(−14%),

Corresponding author and person to whom reprint requests should be addressed: Leslie V. Farland, ScD., Ob/Gyn Epidemiology Center, Brigham and Women's Hospital and Harvard Medical School, 221 Longwood Ave, Boston, MA 02115, Phone: 617--732-8496, Fax: 617-525-2008, lfarland@mail.harvard.edu.

[†]Denotes equal contribution as first author

Disclosure statement: The authors have nothing to disclose.

total(−6%) and free testosterone(−15%)(All P_{trend}<0.05). Short adult menstrual length was associated with higher early-follicular total estradiol(<26 vs >31 days=14%), SHBG(10%), lower luteal estrone(−8%), progesterone(−9%), total(−11%) and free testosterone(−25%), and androstenedione(−14%)(All P_{trend}<0.05). Irregularity of menses at 18–22 was associated with lower early-follicular total(irregular vs very regular=−14%) and free estradiol(−14%), and early-follicular estrone(−8%)(All P_{trend}<0.05). Irregularity of adult menstrual cycle was associated with lower luteal total estradiol(irregular vs. very regular= −8%), SHBG(−3%), higher total(8%) and free testosterone(11%)(All P_{trend}<0.05).

Conclusions—Early-life and adulthood menstrual characteristics are moderately associated with mid-to-late reproductive year's hormone concentrations. These relations of menstrual characteristics with endogenous hormones levels could partially account for associations between menstrual characteristics and reproductive cancers or other chronic diseases.

Keywords

steroid hormones; prolactin; growth factors; menstrual cycle; premenopausal

INTRODUCTION

Menstrual cycle characteristics define and reflect a woman's endogenous endocrine environment. Scientists have long used menstrual cycle characteristics as crude markers of endocrine status and have observed associations with chronic diseases such as breast, ovarian, and endometrial cancers, as well as endometriosis, and cardiovascular disease[1–4]. It has been hypothesized that these relations are mediated through the hormone milieu. For example, early age at menarche may be associated with higher risk of breast cancer through longer exposure to higher levels of estrogen[5, 6]. However, studies have investigated age at menarche and sex steroid hormones levels with inconsistent results[7–9]. Moreover, data are sparse on menstrual cycle characteristics and hormones other than sex steroid hormones, such as prolactin and growth factors.

Therefore, we examined the relations between age at menarche, menstrual cycle length and regularity at age 18–22 and in adulthood with plasma concentrations of estradiol, free estradiol, estrone, estrone sulfate, progesterone, testosterone, free testosterone, androstenedione, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), sex hormone binding globulin (SHBG), prolactin, growth hormone (GH), insulin-like growth factor-I (IGF-I), and IGF binding protein-3 (IGF-BP3). Using data from the prospective cohort study, the Nurses' Health Study II (NHSII) we were able to assess the prospective relationship between self-reported menstrual characteristics and hormones in 2,745 premenopausal women, ages 32–52 years old.

METHODS AND MATERIALS

Study population

The NHSII was established in 1989, when 116,430 female registered nurses, aged 25–42 who resided in one of 14 states in the USA, completed a questionnaire. The cohort has been

followed biennially since inception to update exposure variables and ascertain newly diagnosed disease.

Between 1996 and 1999, 29,611 cohort members, aged 32–52 years, provided a blood sample (described in detail previously[10]). Premenopausal women who had not taken hormones, been pregnant, or breastfed within six months (n=18,521) answered a short questionnaire and provided timed blood samples on the 3rd-5th day of their menstrual cycle (early-follicular) and 7–9 days before the anticipated start of their next cycle (mid-luteal). All other women (n=11,090) provided a single untimed blood sample.

Women who participated in the blood collection signed an IRB approved informed consent regarding risks and benefits of providing the blood sample. Participants in this study were controls from nested case-control studies of breast cancer (n=1,291), endometriosis (n=602), ovarian cancer (n=91), rheumatoid arthritis (n=153) and benign breast diseases (n=499) [10], and also a subset of women who provided timed follicular and luteal samples for a biomarker stability study (n=109, baseline samples)[11]. We restricted these analyses to premenopausal women, defined as providing timed samples, reporting that her menstrual cycles had not ceased, or had a hysterectomy but had at least one ovary remaining and was ≤47 (for nonsmokers) or ≤45 (for smokers). The study was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital.

Exposure and covariate data

Information on exposures and covariates was obtained from biennial questionnaires and a questionnaire completed at blood collection. On the baseline questionnaire in 1989, detailed information was collected on age at menarche, usual menstrual cycle length and regularity at age 18–22. Adult menstrual cycle length and regularity were assessed in 1993, which was 3–6 years prior to blood draw. For menstrual cycle length, women were asked "what is the current usual length of your menstrual cycle (interval from first day of period to first day of next period)?" and were provided categorical response options "<21 days, 21–25 days, 26–31 days, 32–39 days, 40–50 days, 51+ days or too irregular to estimate." For the analyses, categories were collapsed for ages 18–22 (<26, 26–31, 32–39 >39) and adult (<26, 26–32, >32). Similarly, for menstrual cycle regularity, women were asked "what is the current usual pattern of your menstrual cycles (when not pregnant or lactating)?" with response options of "extremely regular (no more than 1–2 days before or after expected), very regular (within 3–4 days), regular (within 5–7 days), usually irregular, always irregular, and no periods." At age 18–22 and adulthood categories were collapsed into "very regular (extremely regular and very regular), regular, and irregular (usually irregular and always irregular).

Data on covariates were collected before blood draw. Body size at age 10 was recalled in 1989 using a 9-level figure drawing (1). Birthdates, weight at age 18, and height were reported on the baseline questionnaire. Adult body mass index (BMI), oral contraceptive use, smoking history, parity and alcohol consumption were obtained in 1995. Physical activity was collected in the 1997 questionnaire. In addition, weight at blood draw and details regarding blood collection date, time, and fasting status were reported on the blood collection questionnaire. First day of the next menstrual cycle following the blood collection was reported on a pre-paid postcard. Whether a sample was collected during an ovulatory

cycle was determined by the luteal progesterone value; cycles with level of progesterone≤400 ng/dL were defined as anovulatory. Luteal days were calculated as date of the first day of the next menstrual period minus luteal blood draw date. If participants did not return the postcard, their samples were not considered timed (3%).

Laboratory assays—Hormone assay methods for estrogens, androgens, progesterone, and prolactin have been described previously (2, 3). Briefly, plasma levels were assayed in up to nine batches. Estrone, estradiol, and estrone sulfate were assayed in follicular and luteal timed samples. Testosterone, androstenedione, SHBG and prolactin were assayed in follicular and/or luteal timed samples as well as untimed samples. Progesterone was measured in luteal timed samples, and DHEA, DHEAS, GH, IGF-1 and IGFBP-3 were measured in luteal or untimed samples.

Estrogens (3 batches), progesterone (1 batch), testosterone (5 batches), and androstenedione (2 batches) were assayed at Quest Diagnostics (San Juan Capistrano, CA). Estrogens and testosterone were assayed by RIA following extraction and celite column chromatography. After extraction of estrone, enzyme hydrolysis, and column chromatography, estrone sulfate was assayed by RIA. Androstenedione was also assayed by RIA. Progesterone was assayed by RIA preceded by organic extraction. Four batches of estrogens and testosterone were assayed at Mayo Medical Laboratories using liquid chromatography-tandem mass spectrometry. The correlation between the RIA and mass spectrometry assays was >0.90 and the intraclass correlation coefficient between RIA and mass spectrometry was >0.81 for all hormones. Two batches of DHEA and androstenedione, and four batches of DHEAS, SHBG, and progesterone were assayed at the Royal Marsden Hospital. Androstenedione was assayed by RIA and DHEAS, sex-hormone binding globulin (SHBG), and progesterone were assayed by chemiluminescent immunoassay. The remaining batch of DHEAS was assayed at Mayo Medical Laboratories by chemiluminescent enzyme immunoassay. One batch of progesterone (RIA) and three batches of SHBG (chemiluminescent enzyme immunometric assay) were assayed at Massachusetts General Hospital (Boston MA) and one batch of SHBG and progesterone were assayed at the Children’s Hospital Boston. Prolactin was measured using a microparticle enzyme immunoassay at the Massachusetts General Hospital, by the AxSYM Immunoassay system. IGF-1 and IGFBP-3 levels were assayed in eight batches for luteal and untimed samples. GH was assayed in two batches for luteal and untimed samples. IGF-1, IGFBP-3, and GH were assayed by ELISA after acid extraction at the Department of Medicine and Oncology at McGill University, using reagents from Diagnostic Systems Laboratory (Webster, TX, USA).

We included 10% blinded replicates in each batch to assess laboratory precision. The overall inter-assay coefficients of variations were between 1 and 36% for all analytes, with mean (SD) 11% (7%) and 10–90th percentile range 4–19%.

Free estradiol and free testosterone were calculated using the methods of Sodergard (4). When calculating follicular free values, concentrations of SHBG or testosterone from luteal or untimed samples were used if follicular concentrations were missing. Follicular free estradiol calculated with luteal SHBG and testosterone are highly correlated with

calculations done using the timed follicular SHBG and testosterone (correlation coefficient from a subset of our data with both values (n=603) is 0.97).

Statistical Analysis

For each analyte, participants with missing values related to assay difficulties or low volume were excluded. We also excluded a small number of values (n≤ 6 per hormone) that were statistical outliers, as identified with the generalized extreme Studentized deviate many-outlier detection method[13]. To adjust for laboratory drift, we adjusted hormone levels for batch according to methods by Rosner et al[14], as we have utilized in prior publications(2). Specifically we recalibrated measures across batches to an average batch by regressing hormone levels on batch, adjusting for age and BMI, as well as luteal day of collection and ovulatory status in the cycle of collection for luteal samples, and follicular day of collection for follicular samples that may have differed between batches. Predicted levels are then outputted and subtracted from the average study effect to give batch-adjusted values.The associations with follicular and luteal estrogens were examined separately. Average values of follicular and luteal phases (whenever both available) were used for testosterone, free testosterone, androstenedione, prolactin, and SHBG, because levels did not vary substantially between the phases[15, 16]. Values from luteal or untimed samples of DHEA, DHEAS, IGF-I, IGFBP-3, and GH were used. We calculated spearman correlations among all hormones under study (Supplementary Table 1s).

We log-transformed hormone concentrations to improve normality and used general linear models to calculate adjusted geometric means for each hormone by category of exposure. Exposures consisted of age at menarche (<12, 12, 13, >13 years), menstrual cycle length (<26, 26–31, 32–39, >39 days) and regularity (very regular, regular and irregular) at age 18–22, menstrual cycle length (<26, 26–31, >31 days) and regularity (very regular, regular and irregular) in adulthood. We calculated the percent difference in the geometric means for the top versus bottom category as $(e^{\beta}-1)\times 100$. Tests for trend (P_{trend}) were conducted using the Wald statistic[17]. For all exposures we conducted secondary analyses excluding anovulatory cycles (progesterone level≤400 ng/dL were defined as anovulatory), irregular cycles (not for adulthood menstrual regularity) and cycles with extreme luteal days (<3 or >21 days). Additionally, analyses excluding women with menstrual length >40 days were conducted for menstrual length analyses. We additionally reported results using Bonferroni correction for multiple comparison.

Multivariable models were adjusted for age at blood draw, fasting status, time of the day, and month of blood draw, luteal days, and ovulatory status of the cycle to increase precision in the effect estimates. We also adjusted a priori for body size at age 10, BMI at age 18, BMI at 18 squared, adult BMI, adult BMI squared, duration of past oral contraceptive use, physical activity (metabolic equivalents (METS) from recreational and leisure-time activities), smoking status and alcohol intake. Sensitivity analyses were conducted where selected BMI variables were not adjusted in the models, e.g., BMI at age 18 and in adulthood were not adjusted in age at menarche analyses). All analyses were conducted using SAS software, version 9.2; all p-values were two-sided.

RESULTS

2,745 women comprised the study population. The mean age at blood draw was 43 years and mean BMI at blood collection was 26 kg/m^2 (Table 1). The majority of women were 12 or 13 years old at menarche. The most common menstrual length was 26–31 days, both at age 18–22 and in adulthood. 77% of women at age 18–22 and 94% of women in adulthood reported regular cycles. The mean time between the return of the 1993 questionnaire and blood collection was 51 months. All hormones were in the expected ranges for premenopausal women [18].

Early age at menarche was associated with higher early-follicular free estradiol (percent difference >13 years vs <12 years=−11%, P_{trend}=0.02), early-follicular estrone (−7%, P_{trend}=0.04), luteal estrone (−7%, P_{trend}=0.05) and free testosterone (−8%, P_{trend}=0.04). Early age at menarche also was suggestively associated with higher early-follicular total estradiol (−9%, P_{trend}=0.05), luteal total estradiol (−8%, P_{trend}=0.06) and luteal free estradiol (−9%, P_{trend}=0.05). Similar trends also were observed for early-follicular (−13%, P_{trend}=0.16) and luteal estrone sulfate (−16%, P_{trend}=0.11), despite sample size (Table 2). After exclusion of anovulatory, irregular cycles and cycles with extreme luteal days, results for estrogens were slightly attenuated while results for free testosterone was unchanged (Table 2 and Table 5). When BMI at age 18 and adult BMI were not adjusted, the association with free testosterone was stronger (−14%, P_{trend}<0.001) and earlier age at menarche was also associated with lower SHBG levels (13%, P_{trend} <0.001) (data not shown).

Short menstrual cycle length at age 18–22 was associated with higher levels of early-follicular total (>39 vs. <26 days=−18%, P_{trend}=0.001) and follicular free estradiol (−16%, P_{trend}=0.004), and early-follicular estrone (−9%, P_{trend}=0.03) (Table 3). In contrast, short menstrual cycle length at 18–22 was associated with lower luteal free estradiol levels (14%, P_{trend}=0.02). Moreover, short menstrual length at 18–22 was associated with lower progesterone levels (14%, P_{trend}=0.05), total (6%, P_{trend}=0.04) and free testosterone (15%, P_{trend}=0.001) but higher SHBG levels (−7%, P_{trend}=0.02) (Table 3). After the exclusion of anovulatory, irregular cycles and cycles with extreme luteal days, all associations remained unchanged. However, when excluding women with menstrual cycle length at 18–22 >40 days, results for estrogens were slightly attenuated (data not shown). Results remained largely unchanged when adulthood BMI or all the BMI variables (including body size at age 10, BMI at 18–22 and adulthood BMI) were not adjusted (data not shown).

The results observed for adult menstrual cycle length were very similar to that of ages 18–22 (Table 3). For example, short adult menstrual cycle length was associated with higher early-follicular total estradiol (>31 days vs. <26 days=−14%, P_{trend}=0.04) and lower luteal estrone (8%, P_{trend}=0.04). After the exclusion of anovulatory, irregular cycles and cycles with extreme luteal days, results for luteal estrone, total testosterone and androstenedione were attenuated (Table 3). Results remained largely unchanged when not adjusting for any BMI variables or when excluding women with adult menstrual cycle length >40 days (data not shown).

Menstrual cycle irregularity at age 18–22 was associated with lower levels of early-follicular total estradiol (irregular vs very regular=−14%; P_{trend}<0.001), early-follicular free estradiol (−14%, P_{trend}<0.001) and early-follicular estrone (−8%, P_{trend}=0.004) but higher testosterone (4%, P_{trend}=0.05) (Table 4). After the exclusion of anovulatory cycles, irregular cycles, and cycles with extreme luteal days all associations remained unchanged (Table 4). Results were similar when adulthood BMI or all the BMI variables were not adjusted (data not shown).

Adulthood menstrual cycle irregularity was associated with lower luteal total estradiol (irregular vs very regular=−8%; P_{trend}=0.01). Those with irregular cycles as adults had higher total (8%, P_{trend}=0.01) and free testosterone (11%, P_{trend}<0.001) but lower SHBG (−3%, P_{trend}=0.02) (Table 4). After the exclusion of anovulatory cycles and cycles with extreme luteal days, adult menstrual cycle irregularity additionally was associated with lower early-follicular total (−14%, P_{trend}=0.002) and free estradiol (−13%, P_{trend}=0.01) and luteal estrone (−4%, P_{trend}=0.04), whereas the result for total testosterone was attenuated (Table 4). Results remained essentially unchanged when not adjusting for any BMI variables (data not shown).

We evaluated the statistical significance after adjustment for multiple comparisons. For each menstrual cycle characteristic, we conducted 19 Wald tests corresponding to P_{trend} for each hormone. Therefore, using the conservative Bonferroni correction with 19 tests, the adjusted α level was 0.05/19=0.003. With significance level α=0.003, associations that remained statistically significant were: menstrual cycle length at age 18–22 with early-follicular total estradiol and free testosterone levels, adult menstrual cycle length with total and free testosterone levels, cycle regularity at age 18–22 with early-follicular total and free estradiol levels, and cycle regularity in adulthood with free testosterone levels.

DISCUSSION

In this study of 2,745 premenopausal women, we observed that early age at menarche was associated with higher estrogens and free testosterone in adulthood; short menstrual cycle length at age 18–22 and in adulthood was associated with higher early-follicular estrogens and SHBG, but lower luteal estrogens, progesterone and androgens; menstrual cycle irregularity at age 18–22 and in adulthood was associated with lower estrogens but higher androgens.

Many previous studies investigated age at menarche with estrogen levels. Two studies found that early age at menarche was associated with higher estradiol or estrone in adolescence[5, 19], however, studies among women over 20 years old had inconsistent results[5–9, 20–22]. With two timed samples per cycle and the largest sample size to date, we observed that early age at menarche was consistently positively associated with each of the estrogens measured, though the associations were modest or borderline significant. Studies that found higher estrogen levels with earlier age at menarche tended to have multiple samples timed across a menstrual cycle[6, 19, 22], whereas studies with null findings tended to have two or fewer samples[8, 9]. Estrogen levels are highly variable across the cycle, thus more samples collected during a cycle may enhance the chance to detect the difference of estrogen levels

across age at menarche categories. It is also possible that as time since menarche increases, the strength of the relation with estrogen levels is diminished.

It is possible that higher estrogen levels are part of the physiologic trigger of menarche resulting in earlier onset, with perimenarchal estrogen levels positively correlated with adulthood estrogen levels [6]. Apter et al. found that girls with early menarche had a larger increase in serum estradiol at about 10 years old, and after that age the concentrations remained higher than in girls having later menarche [23]. Common genetic background may be another factor underlying the association; some studies found that the variant allele A2 of CYP17 is associated with higher estrogen levels[24, 25] and earlier age at menarche[26, 27]. Perimenarchal BMI is one of the important factors provoking menarche[28], however, whether adjusting for body size at age 10 did not have an impact on the results.

In the present study we also noted that early age at menarche was associated with higher free testosterone levels, which is likely driven by lower SHBG level. Findings on the relation of age at menarche with SHBG have been mixed [6, 8, 9, 21, 29, 30], but the studies varied greatly in sample size, temporality, and analytic models. Similar to our finding, Moore et al. demonstrated that late menarche was associated with increased SHBG, and BMI adjustment reduced the magnitude of the association [30]. Earlier age at menarche predicts higher adulthood BMI [31], and BMI has been shown to be negatively associated with SHBG [32–34]. Therefore, the association between age at menarche and SHBG levels is likely to be mediated through BMI.

We observed that short and regular menstrual cycles, correlated in our study population, were associated with higher plasma early-follicular estrogens. Regular menstrual cycles also were associated with higher luteal estrogens, whereas short cycles were associated with lower luteal estrogens, although most trends were not statistically significant. These findings are consistent with previous studies[35, 36]. For example, Mumford et al. found women with short cycles (<26 days) had higher serum follicular estradiol but lower serum luteal estradiol[35]. It is possible that the biologic mechanism underlying the common variation of short cycles and regular cycles is the status of follicular growth and development. Most of the variation in menstrual cycle length is observed in follicular phase [37]. Short and regular cycle may be associated with advanced follicle recruitment and steady follicle growth with good quality and rhythm[38, 39]. This may lead to higher estrogen concentrations during follicular phase as estrogen is produced by the granulosa cells of the pre-ovulatory follicle. The Harlow et al. study supports this hypothesis as they found that the most common estrogen pattern in long follicular phases is consistent with delayed emergence of a dominant follicle and another was a pattern consistent with demise and replacement of a dominant follicle[39]

We noted that long menstrual cycle length and irregular cycles at two time points were associated with higher androgens and lower SHBG levels. These findings are consistent with previous studies of plasma or salivary hormones in cycling women[40, 41]. This hormonal pattern is also seen in polycystic ovary syndrome (PCOS). PCOS is characterized by anovulation or oligoovulation (cycles of ≥36 days or <8 cycles a year), plus hyperandrogenism and hyperinsulinemia[42]. Moreover, there is an approximately 50%

reduction in circulating SHBG, a response to the increased testosterone, in anovulatory women with PCOS, and in women with hyperinsulinemia, due to a direct insulin effect on the liver[42]. As noted by Anders et al., this hormonal pattern seen in a clinical population can be extended to healthy adult women[41]. It is possible that higher androgen and lower SHBG levels among women with long and irregular menstrual cycles is driven by underlying or preclinical PCOS. That the total testosterone and androstenedione associations were attenuated after the exclusion of anovulatory cycles supports this hypothesis. Unfortunately, we were unable to validly classify women as having PCOS based on self-report, however future studies should investigate this subgroup."

Short menstrual cycle length in early and later adulthood were associated with lower progesterone in later adulthood, especially after the exclusion of anovulatory, irregular cycles and cycles with extreme luteal days, despite relatively low within-person intraclass correlation coefficient (ICC) of progesterone over 3 years (0.29)[43]. Consistent with our study, Mumford et al. also found that women with short cycles had marginally lower progesterone levels[35]. Windham et al. noted that short follicular phase length was associated with elevation of progesterone whereas short luteal phase length was associated with lower progesterone[22], indicating the association between cycle length and progesterone might have been driven by luteal phase length. It is possible luteal phase length reflects the development and growth of the corpus luteum that has a primary function of producing progesterone.

Few associations were observed for estrone sulfate, progesterone, androstenedione, DHEA, DHEAS, prolactin, GH, IGF-1 and IGFBP-3. Previous studies of age at menarche and progesterone had mixed results, as Westhoff et al. observed a greater progesterone level among those with a later age at menarche[20], while Verkasalo et al. found no association[9]. Consistent with our study, Dorgan et al. found no associations between age at menarche and estrone sulfate, androstenedione, and DHEAS[29]. Findings on relations of age at menarche with IGF-1 and IGFBP-3 were not consistent[44][45]. However, a recent pooled analysis of 17 prospective studies found that IGF-1 and IGFBP-3, adjusted for age, were inversely associated with age at menarche with small magnitude[46].

Our study represents the largest on menstrual cycle characteristics and endogenous steroid hormone, SHBG, prolactin, and growth factor levels among premenopausal women. Notably, the blood draw samples are timed within the menstrual cycle, allowing relatively accurate assessment of estrogen concentrations during the early-follicular and mid-luteal phases. We were able to adjust for many potential confounders and minimal confounding was observed. The results for menstrual cycle length and irregularity at age 18–22 and 3–6 years prior to blood draw were similar. While age at menarche, menstrual cycle length and regularity at age 18–22 were recalled at baseline, it is unlikely that the recall was associated with hormone levels. Thus any potential misclassification due to recall would be non-differential with respect to hormone levels and lead to attenuation of overall associations. Although we are limited to samples collected during one menstrual cycle, a prior reproducibility study provides evidence of reasonable stability across a three-year period [43].

Our study provides evidence of modest associations between age at menarche and estrogens and testosterone levels, and evidence of associations between menstrual cycle length and irregularity in early and later adulthood and estrogen, testosterone, progesterone and SHBG levels, among mid to late reproductive age women.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We gratefully acknowledge the Nurses' Health Study II participants for their continuing contributions.

Grant support: This study was supported by NIH grants UM1 CA176726, CA67262, CA138580, and CA50385.

References

1. Rockhill B, Moorman PG, Newman B. Age at menarche, time to regular cycling, and breast cancer (North Carolina, United States). Cancer Causes Control. 1998; 9(4):447–53. [PubMed: 9794178]
2. Missmer SA, Hankinson SE, Spiegelman D, Barbieri RL, Malspeis S, Willett WC, et al. Reproductive history and endometriosis among premenopausal women. Obstetrics and gynecology. 2004; 104(5 Pt 1):965–74. [PubMed: 15516386]
3. Lakshman R, Forouhi NG, Sharp SJ, Luben R, Bingham SA, Khaw KT, et al. Early age at menarche associated with cardiovascular disease and mortality. The Journal of clinical endocrinology and metabolism. 2009; 94(12):4953–60. [PubMed: 19880785]
4. Gong TT, Wu QJ, Vogtmann E, Lin B, Wang YL. Age at menarche and risk of ovarian cancer: a meta-analysis of epidemiological studies. International journal of cancer. Journal international du cancer. 2013; 132(12):2894–900. [PubMed: 23175139]
5. MacMahon B, Trichopoulos D, Brown J, Andersen AP, Cole, deWaard F, et al. Age at menarche, urine estrogens and breast cancer risk. International journal of cancer. Journal international du cancer. 1982; 30(4):427–31. [PubMed: 7141738]
6. Apter DRM, Vihko R. Some endocrine characteristics of early menarche, a risk factor for breast cancer, are preserved into adulthood. Int J Cancer. 1989; 44(5):783–787. [PubMed: 2511157]
7. Bernstein LPM, Ross RK, Henderson BE. Age at menarche and estrogen concentrations of adult women. Cancer causes & control : CCC. 1991; 2(4):221–5. [PubMed: 1873451]
8. Ingram DM, Nottage EM, Willcox DL, Roberts A. Oestrogen binding and risk factors for breast cancer. British journal of cancer. 1990; 61(2):303–7. [PubMed: 2310682]
9. Verkasalo PK, Thomas HV, Appleby PN, Davey GK, Key TJ. Circulating levels of sex hormones and their relation to risk factors for breast cancer: a cross-sectional study in 1092 pre- and postmenopausal women (United Kingdom). Cancer causes & control : CCC. 2001; 12(1):47–59. [PubMed: 11227925]
10. Tworoger SS, Sluss, Hankinson SE. Association between plasma prolactin concentrations and risk of breast cancer among predominately premenopausal women. Cancer Res. 2006; 66(4):2476–82. [PubMed: 16489055]
11. Missmer SA, Spiegelman D, Bertone-Johnson ER, Barbieri RL, Pollak MN, Hankinson SE. Reproducibility of Plasma Steroid Hormones, Prolactin, and Insulin-like Growth Factor Levels among Premenopausal Women over a 2- to 3-Year Period. Cancer Epidemiol Biomarkers Prev. 2006; 15(5):972–8. [PubMed: 16702379]
12. Stunkard AJ, Sorensen T, Schulsinger F. Use of the Danish Adoption Register for the study of obesity and thinness. Research publications - Association for Research in Nervous and Mental Disease. 1983; 60:115–20. [PubMed: 6823524]
13. Rosner B. Percentage points for a generalized ESD many-outlier procedure. Technometrics. 1983; 25:165–172.

14. Rosner B, Cook N, Portman R, Daniels S, Falkner B. Determination of blood pressure percentiles in normal-weight children: some methodological issues. American journal of epidemiology. 2008; 167(6):653–66. [PubMed: 18230679]
15. Eliassen AH, Missmer SA, Tworoger SS, Spiegelman D, Barbieri RL, Dowsett M, et al. Endogenous steroid hormone concentrations and risk of breast cancer among premenopausal women. Journal of the National Cancer Institute. 2006; 98(19):1406–15. [PubMed: 17018787]
16. Tworoger SS, Sluss, Hankinson SE. Association between plasma prolactin concentrations and risk of breast cancer among predominately premenopausal women. Cancer research. 2006; 66(4): 2476–82. [PubMed: 16489055]
17. Hosmer, DW., Lemeshow, S. Applied Logistic Regression. New York: John Wiley & Sons; 1989.
18. Yen, SSC., Jaffe, RB. Reproductive Endocrinology. 3. Philadelphia: W.B. Saunders Company; 1991. Vol
19. Apter D, Bolton NJ, Hammond GL, Vihko R. Serum sex hormone-binding globulin during puberty in girls and in different types of adolescent menstrual cycles. Acta endocrinologica. 1984; 107(3): 413–9. [PubMed: 6391059]
20. Westhoff C, Gentile G, Lee J, Zacur H, Helbig D. Predictors of ovarian steroid secretion in reproductive-age women. American journal of epidemiology. 1996; 144(4):381–8. [PubMed: 8712195]
21. Moore JW, Key TJ, Wang DY, Bulbrook RD, Hayward JL, Takatani O. Blood concentrations of estradiol and sex hormone-binding globulin in relation to age at menarche in premenopausal British and Japanese women. Breast cancer research and treatment. 1991; (18 Suppl 1):S47–50. [PubMed: 1873557]
22. Windham GC, Elkin E, Fenster L, Waller K, Anderson M, Mitchell PR, et al. Ovarian hormones in premenopausal women: variation by demographic, reproductive and menstrual cycle characteristics. Epidemiology. 2002; 13(6):675–84. [PubMed: 12410009]
23. Apter DVR. Premenarcheal endocrine changes in relation to age at menarche. Clin Endocrinol (Oxf). 1985; 22(6):753–60. [PubMed: 3160508]
24. Feigelson HS, Shames LS, Pike MC, Coetzee GA, Stanczyk FZ, Henderson BE. Cytochrome P450c17alpha gene (CYP17) polymorphism is associated with serum estrogen and progesterone concentrations. Cancer research. 1998; 58(4):585–7. [PubMed: 9485002]
25. Garcia-Closas M, Herbstman J, Schiffman M, Glass A, Dorgan JF. Relationship between serum hormone concentrations, reproductive history, alcohol consumption and genetic polymorphisms in pre-menopausal women. International journal of cancer. Journal international du cancer. 2002; 102(2):172–8. [PubMed: 12385014]
26. Lai J, Vesprini D, Chu W, Jernstrom H, Narod SA. CYP gene polymorphisms and early menarche. Molecular genetics and metabolism. 2001; 74(4):449–57. [PubMed: 11749050]
27. Ambrosone CB, Moysich KB, Furberg H, Freudenheim JL, Bowman ED, Ahmed S, et al. CYP17 genetic polymorphism, breast cancer, and breast cancer risk factors. Breast cancer research : BCR. 2003; 5(2):R45–51. [PubMed: 12631398]
28. Wronka I. Association between BMI and age at menarche in girls from different socio-economic groups. Anthropologischer Anzeiger; Bericht uber die biologisch-anthropologische Literatur. 2010; 68(1):43–52. [PubMed: 20954455]
29. Dorgan JF, Reichman ME, Judd JT, Brown C, Longcope C, Schatzkin A, et al. Relationships of age and reproductive characteristics with plasma estrogens and androgens in premenopausal women. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 1995; 4(4):381–6.
30. Moore JW, Key TJ, Clark GM, Hoare SA, Allen DS, Wang DY. Sex-hormone-binding globulin and breast cancer risk. Anticancer research. 1987; 7(5B):1039–47. [PubMed: 3324935]
31. Pierce MB, Leon DA. Age at menarche and adult BMI in the Aberdeen children of the 1950s cohort study. The American journal of clinical nutrition. 2005; 82(4):733–9. [PubMed: 16210700]
32. Tchernof A, Despres JP. Sex steroid hormones, sex hormone-binding globulin, and obesity in men and women. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme. 2000; 32(11–12):526–36. [PubMed: 11246820]

33. Tchernof A, Toth MJ, Poehlman ET. Sex hormone-binding globulin levels in middle-aged premenopausal women. Associations with visceral obesity and metabolic profile. Diabetes care. 1999; 22(11):1875–81. [PubMed: 10546023]
34. Pasquali R, Vicennati V, Bertazzo D, Casimirri F, Pascal G, Tortelli O, et al. Determinants of sex hormone-binding globulin blood concentrations in premenopausal and postmenopausal women with different estrogen status. Virgilio-Menopause-Health Group. Metabolism: clinical and experimental. 1997; 46(1):5–9.
35. Mumford SL, Steiner AZ, Pollack AZ, Perkins NJ, Filiberto AC, Albert PS, et al. The utility of menstrual cycle length as an indicator of cumulative hormonal exposure. The Journal of clinical endocrinology and metabolism. 2012; 97(10):E1871–9. [PubMed: 22837188]
36. Fortner RT, Hankinson SE, Schairer C, Xu X, Ziegler RG, Eliassen AH. Association between reproductive factors and urinary estrogens and estrogen metabolites in premenopausal women. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2012; 21(6):959–68.
37. Waller K, Swan SH, Windham GC, Fenster L, Elkin EP, Lasley BL. Use of urine biomarkers to evaluate menstrual function in healthy premenopausal women. American journal of epidemiology. 1998; 147(11):1071–80. [PubMed: 9620051]
38. Speroff LAFM. Clinical Gynecologic Endocrinology and Infertility. 2005:223–224.
39. Harlow SD, Baird DD, Weinberg CR, Wilcox AJ. Urinary oestrogen patterns in long follicular phases. Human reproduction. 2000; 15(1):11–6.
40. Wei S, Jones G, Thomson R, Otahal, Dwyer T, Venn A. Menstrual irregularity and bone mass in premenopausal women: cross-sectional associations with testosterone and SHBG. BMC musculoskeletal disorders. 2010; 11:288. [PubMed: 21190585]
41. Van Anders SM, Watson NV. Menstrual cycle irregularities are associated with testosterone levels in healthy premenopausal women. American journal of human biology : the official journal of the Human Biology Council. 2006; 18(6):841–4. [PubMed: 17039468]
42. Speroff LAFM. Clinical Gynecologic Endocrinology and Infertility. 2005:465.
43. Missmer SA, Spiegelman D, Bertone-Johnson ER, Barbieri RL, Pollak MN, Hankinson SE. Reproducibility of plasma steroid hormones, prolactin, and insulin-like growth factor levels among premenopausal women over a 2- to 3-year period. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2006; 15(5):972–8.
44. Lukanova A, Toniolo, Akhmedkhanov A, Hunt K, Rinaldi S, Zeleniuch-Jacquotte A, et al. A cross-sectional study of IGF-I determinants in women. European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation. 2001; 10(5):443–52.
45. Johansson H, Baglietto L, Guerrieri-Gonzaga A, Bonanni B, Mariette F, Macis D, et al. Factors associated with circulating levels of insulin-like growth factor-I and insulin-like growth factor binding protein-3 in 740 women at risk for breast cancer. Breast cancer research and treatment. 2004; 88(1):63–73. [PubMed: 15538047]
46. Key TJ, Appleby PN, Reeves GK, Roddam AW. Insulin-like growth factor 1 (IGF1), IGF binding protein 3 (IGFBP3), and breast cancer risk: pooled individual data analysis of 17 prospective studies. The lancet oncology. 2010; 11(6):530–42. [PubMed: 20472501]

Table 1

Characteristics of premenopausal women in the Nurses' Health Study II (n = 2,745).

	Mean (SD) or %
Age at blood draw (yr)	42.7 (4.0)
BMI[1] at age 18 (kg/m^2)	21.1 (3.1)
BMI at blood draw (kg/m^2)	25.6 (5.8)
Parous, %	80.9
Parity (among parous women)	2.3 (0.9)
Past oral contraceptive use, %	84.6
Ever smoker, %	30.6
Alcohol intake (grams/day)	3.6 (7.0)
Physical activity[2] (METs/week)	19.1 (22.8)
Time between return of 1993 questionnaire and blood draw (months)	51.4 (9.5)
Age at menarche (yr), %	
≤11	22.2
12	29.7
13	28.6
≥14	19.5
Menstrual cycle length when age 18–22 (days), %	
≤25	10.3
26–31	65.3
32–39	16.6
≥40 or irregular	7.9
Menstrual cycle regularity when age 18–22, %	
Very regular	42.3
Regular	34.8
Irregular	22.9
Menstrual cycle length in 1993 (days), %	
≤25	17.0
26–31	70.7
≥32	12.3
Menstrual cycle regularity in 1993, %	
Very regular	68.6
Regular	25.0
Irregular	6.5
	Median (10th – 90th percentile)
Estradiol, pg/mL	
Follicular phase	46.5 (22.2 −101.1)
Luteal phase	133 (72 – 236)
Free Estradiol, pg/mL	
Follicular phase	0.6 (0.3 – 1.2)

	Mean (SD) or %
Luteal phase	1.7 (0.9 – 2.8)
Estrone, pg/mL	
Follicular phase	40.6 (25.0 – 67.6)
Luteal phase	84.2 (51.0 – 143.1)
Estrone Sulfate, pg/mL	
Follicular phase	662 (299 – 1528)
Luteal phase	1462 (574 – 3300)
Progesterone, ng/dL	1404 (252 – 2748)
Testosterone[3], ng/dL	23.5 (14.3 – 36.7)
Free testosterone[3], ng/dL	0.2 (0.1 – 0.4)
Androstenedione[3], ng/dL	100 (61 – 162)
DHEA, ng/dL	615 (346 – 1130)
DHEAS, ug/dL	87.9 (40.0 – 164.2)
SHBG[3], nmol/L	64.6 (32.0 – 116.5)
Prolactin[3], ng/mL	14.3 (8.1 – 27.4)
GH, ng/mL	0.2 (0.1 – 4.0)
IGF-1, ng/mL	240 (153 – 344)
IGFBP-3, ng/mL	4813 (3823–5972)

[1] Among parous women only (n = 2213).

[2] Abbreviations: BMI = body mass index; DHEA = dehydroepiandrosterone; DHEAS = dehydroepiandrosterone sulfate; SHBG = sex hormone binding globulin; GH = growth hormone; IGF = insulin-like growth factor; BP = binding protein

[3] Average of follicular and luteal phase levels when available

Table 2

Geometric mean levels[1] of sex steroid hormones, SHBG, prolactin, and growth factors by category of age at menarche in premenopausal women.

	Age at menarche (yr)					
	n	**<12**	**12**	**13**	**>13**	**p-trend[2]**
Estradiol, Follicular, pg/mL	1424	50	47	46	46	0.05
Estradiol, Luteal , pg/mL	1555	137	131	133	126	0.06
Free Estradiol, Follicular, pg/mL	1388	0.63	0.58	0.58	0.56	0.02
Free Estradiol, Luteal , pg/mL	1539	1.73	1.65	1.69	1.58	0.05
Estrone, Follicular, pg/mL	1443	43	41	40	40	0.04
Estrone, Luteal , pg/mL	1603	89	84	85	82	0.05
Estrone Sulfate, Follicular, pg/mL	447	706	694	673	616	0.16
Estrone Sulfate, Luteal , pg/mL	452	1580	1380	1342	1333	0.11
Progesterone, ng/dL	1620	1083	1080	1057	1082	0.80
Testosterone, ng/dL	2004	24	23	23	23	0.20
Free Testosterone, ng/dL	1632	0.20	0.19	0.19	0.19	0.04
Androstenedione, ng/dL	624	102	102	97	99	0.27
DHEA[3], ng/dL	479	603	596	648	611	0.44
DHEAS, ug/dL	1252	84	85	86	84	0.97
SHBG, nmol/L	1963	62	64	62	65	0.32
Prolactin, ng/mL	944	14	15	15	15	0.81
GH, ng/mL	484	0.58	0.51	0.39	0.50	0.14
IGF-1, ng/mL	1787	233	238	231	233	0.60
IGFBP-3, ng/mL	1786	4803	4849	4741	4771	0.25
Excluding anovulatory and irregular cycles, and women reporting extreme luteal days[3]						
Free Estradiol, Follicular, pg/mL	1118	0.64	0.60	0.59	0.58	0.03
Estrone, Follicular	1163	41	41	41	40	0.23
Estrone, Luteal	1290	88	85	84	84	0.16
Free testosterone	1320	0.20	0.19	0.19	0.18	0.06

[1]Adjusted for assay batch, age at blood draw, fasting status , time of day of blood draw , month of blood draw, luteal days, anovulatory status (defined by progesterone >400 ng/dL), body size at age 10, BMI at age 18, adult BMI, duration of past oral contraceptive use, physical activity, smoking history and alcohol intake.

[2] Trend across category medians of age at menarche, using the Wald test estimated by multivariate linear regression.

[3] Results displayed for those hormones with statistically significant trends among all women and hormones that became statistically significant after the exclusion. Exclusions defined as anovulatory - plasma progesterone <=400 ng/dL, irregular cycles - variation from cycle to cycle of greater than +/− 7 days, extreme luteal day - calculated by date of the first day of the next menstrual period minus luteal blood draw date.

Table 3

Geometric mean levels[1] of sex steroid hormones, SHBG, prolactin, and growth factors by typical menstrual cycle length at age 18–22 and in adulthood among premenopausal women.

	Cycle length at age 18–22					Cycle length in adulthood			
	<26	26–31	32–39	>39	p-trend[2]	<26	26–31	>31	p-trend[2]
Estradiol, Follicular, pg/mL	49	49	44	41	0.001	48	48	42	0.04
Estradiol, Luteal , pg/mL	126	133	130	138	0.28	128	132	134	0.31
Free Estradiol, Follicular, pg/mL	0.62	0.60	0.56	0.52	0.004	0.59	0.59	0.53	0.13
Free Estradiol, Luteal , pg/mL	1.58	1.66	1.69	1.80	0.02	1.59	1.67	1.72	0.08
Estrone, Follicular, pg/mL	43	41	40	39	0.03	41	41	40	0.81
Estrone, Luteal , pg/mL	82	86	85	84	0.84	81	85	88	0.04
Estrone Sulfate, Follicular, pg/mL	645	686	639	697	0.93	639	678	687	0.47
Estrone Sulfate, Luteal , pg/mL	1472	1409	1199	1601	0.98	1261	1437	1453	0.19
Progesterone, ng/dL	964	1081	1100	1102	0.05	962	1097	1049	0.02
Testosterone, ng/dL	23	23	24	25	0.04	22	23	25	0.002
Free Testosterone, ng/dL	0.18	0.19	0.20	0.21	0.001	0.18	0.19	0.22	<0.001
Androstenedione, ng/dL	97	100	102	98	0.76	94	101	107	0.02
DHEA[3], ng/dL	611	607	635	649	0.33	584	624	667	0.09
DHEAS, ug/dL	85	84	83	89	0.62	82	85	88	0.21
SHBG, nmol/L	66	63	60	61	0.02	65	63	59	0.01
Prolactin, ng/mL	15	15	15	15	0.54	15	15	15	0.93
GH, ng/mL	0.52	0.52	0.39	0.43	0.19	0.51	0.52	0.41	0.47
IGF-1, ng/mL	225	235	232	235	0.58	235	235	227	0.26
IGFBP-3, ng/mL	4710	4799	4809	4796	0.42	4804	4789	4758	0.57
Excluding anovulatory and irregular cycles, and women reporting extreme luteal days[4]									
Estradiol, Follicular	53	50	45	41	<0.001	52	49	40	0.001
Free estradiol, Follicular	0.67	0.61	0.56	0.52	0.001	0.63	0.60	0.50	0.01
Free estradiol, Luteal	1.64	1.69	1.79	1.82	0.02				
Estrone, Follicular	43	41	40	38	0.01				
Estrone, Luteal						83	85	87	0.32

Cancer Causes Control. Author manuscript; available in PMC 2018 December 01.

	Cycle length at age 18–22					Cycle length in adulthood			
	<26	**26–31**	**32–39**	**>39**	**p-trend[2]**	**<26**	**26–31**	**>31**	**p-trend[2]**
Progesterone	1322	1568	1532	1639	0.01	1358	1568	1565	0.001
Testosterone	23	23	23	24	0.11	22	23	23	0.11
Free testosterone	0.18	0.19	0.20	0.21	0.001	0.17	0.19	0.21	<0.001
Androstenedione						94	102	102	0.10
SHBG	69	65	61	63	0.01	68	65	58	0.003

[1] Total sample size for hormones constant across exposures. Reported in table 2. Models were adjusted for assay batch, age at blood draw, fasting status , time of day of blood draw , month of blood draw, luteal days, anovulatory status (defined by progesterone >400 ng/dL), body size at age 10, BMI at age 18, adult BMI, duration of past oral contraceptive use, physical activity, smoking history and alcohol intake.

[2] Trend across category medians of menstrual cycle length at age 18–22 or 3–6 years prior to blood draw, using the Wald test estimated by multivariate linear regression.

[3] Results displayed for those hormones with statistically significant trends among all women and hormones that became statistically significant after the exclusion. Exclusions defined as anovulatory - plasma progesterone <=400 ng/dL, irregular cycles - variation from cycle to cycle of greater than +/− 7 days, extreme luteal day - calculated by date of the first day of the next menstrual period minus luteal blood draw date.

Table 4

Geometric mean levels[1] of sex steroid hormones, SHBG, prolactin, and growth factors by typical menstrual cycle pattern at age 18–22 and in adulthood in premenopausal women.

	Menstrual cycle pattern at age 18–22				Menstrual cycle pattern in adulthood			
	Very regular	Regular	Irregular	p trend[2]	Very regular	Regular	Irregular	p trend[2]
Estradiol, Follicular, pg/mL	50	47	43	<0.001	48	45	45	0.05
Estradiol, Luteal , pg/mL	134	133	128	0.21	134	127	123	0.01
Free Estradiol, Follicular, pg/mL	0.62	0.59	0.53	<0.001	0.59	0.56	0.56	0.16
Free Estradiol, Luteal , pg/mL	1.67	1.69	1.62	0.53	1.68	1.64	1.57	0.17
Estrone, Follicular, pg/mL	42	41	39	0.004	41	40	42	0.85
Estrone, Luteal , pg/mL	86	86	81	0.09	86	82	83	0.07
Estrone Sulfate, Follicular, pg/mL	642	700	690	0.31	665	664	780	0.39
Estrone Sulfate, Luteal , pg/mL	1390	1378	1391	0.99	1416	1336	1581	0.97
Progesterone, ng/dL	1041	1083	1090	0.14	1083	1058	1004	0.16
Testosterone, ng/dL	23	23	24	0.05	23	24	25	0.01
Free Testosterone, ng/dL	0.19	0.19	0.20	0.17	0.19	0.21	0.21	<0.001
Androstenedione, ng/dL	99	99	101	0.60	100	102	101	0.58
DHEA[3], ng/dL	600	605	656	0.13	607	691	555	0.36
DHEAS, ug/dL	85	83	85	0.82	86	85	84	0.74
SHBG, nmol/L	64	62	63	0.52	64	60	62	0.02
Prolactin, ng/mL	15	14	14	0.45	14	16	14	0.29
GH, ng/mL	0.49	0.57	0.38	0.25	0.51	0.49	0.50	0.88
IGF-1, ng/mL	234	234	234	1.00	234	237	226	0.69
IGFBP-3, ng/mL	4776	4811	4795	0.63	4806	4756	4691	0.12
Excluding anovulatory and women reporting extreme luteal days[3]								
Estradiol, Follicular	52	47	44	<0.001	50	45	43	0.002
Estradiol, Luteal					139	132	131	0.04
Free estradiol, Follicular	0.64	0.59	0.54	<0.001	0.61	0.56	0.53	0.01
Estrone, Follicular	42	40	39	0.004				
Estrone, Luteal					87	82	83	0.04

	Menstrual cycle pattern at age 18–22				Menstrual cycle pattern in adulthood			
	Very regular	Regular	Irregular	p trend[2]	Very regular	Regular	Irregular	p trend[2]
Testosterone	23	23	24	0.14	23	23	24	0.14
Free testosterone					0.18	0.20	0.20	0.01
SHBG					66	61	65	0.01

[1] Total sample size for hormones constant across exposures. Reported in table 2. Models were adjusted for assay batch, age at blood draw, fasting status , time of day of blood draw , month of blood draw, luteal days, anovulatory status (defined by progesterone >400 ng/dL), body size at age 10, BMI at age 18, adult BMI, duration of past oral contraceptive use, physical activity, smoking history and alcohol intake.

[2] Trend across categories of menstrual cycle pattern at age 18–22 or 3–6 years prior to blood draw, using the Wald test estimated by multivariate linear regression.

[3] Results displayed for those hormones with statistically significant trends among all women and hormones that became statistically significant after the exclusion. Exclusions defined as anovulatory - plasma progesterone <=400 ng/dL, irregular cycles - variation from cycle to cycle of greater than +/− 7 days, extreme luteal day - calculated by date of the first day of the next menstrual period minus luteal blood draw date.

Table 5

Summary of statistically significant associations between menstrual cycle characteristics and endogenous hormones in 2,745 premenopausal women from the Nurses' Health Study II, age 32–52 years at the time of plasma collection[1]

	Age at menarche	Cycle length at age 18–22	Cycle length in adulthood	Cycle irregularity at age 18–22	Cycle irregularity in adulthood
Follicular Estrogens	−	−	−	−	_[2]
Luteal Estrogens	−	+	+		−
Androgens	−	+	+	+	+
Progesterone		+	+[2]		
SHBG3	+[4]	−	−		−

[1] "-" indicates an inverse association and "+" indicates a positive association. Hormones for which no statistically significant associations were found are not listed in this table; including prolactin, growth hormone, insulin-like growth factor-1, and insulin-like growth factor binding protein-3.

[2] Became statistically significant after exclusion of anovulatory (defined by plasma progesterone <=400 ng/dL) and irregular cycles (variation from cycle to cycle of greater than +/− 7 days), and cycles with blood samples collected on an extreme luteal day (calculated by date of the first day of the next menstrual period minus luteal blood draw date).

[3] SHBG = sex hormone binding globulin

[4] Statistically significant when not adjusted for body mass index at age 18 and in adulthood