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Are the Cardiovascular Risk Factors of Women Approaching Menopause associated with Menstrual Cycle Characteristics and Reproductive Hormones in the Follicular and Luteal Phase?: Study of Women's Health Across the Nation Daily Hormone Study

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ABSTRACT

Context. Menstrual cycle characteristics may be associated with risk for cardiovascular disease.

Objective. To describe the relationships between menstrual cycle characteristics and daily reproductive hormone measures with cardiovascular risk factors in a multiethnic sample of middle-aged menstruating women.

Design and Setting. Cross-sectional analysis based on the Study of Women's Health across the Nation-Daily Hormone Study. Associations were examined between cardiovascular risk factors and urinary luteinizing hormone, follicle stimulating hormone, estrone conjugates (E1c), and pregnanediol glucuronide (Pdg) measured across one menstrual cycle or 50 days.

Participants. 500 menstruating women who were free from diabetes or past stroke or heart attack who met other eligibility criteria, including risk factor measurement within 90 days of hormone measurement.

Main Outcome Measures. Cardiovascular risk factors, including body mass index (BMI), blood pressure, hemostatic factors, and metabolic factors.

Results. 84% of cycles showed evidence of luteal activity (ELA), presumably evidence of ovulation. There were few differences in risk factors between women with ELA and those with no evidence of luteal activity. Among ELA women, the more elevated the cardiovascular risk factors the longer cycle length, but associations adjusting for age and BMI were reduced substantially. Those with lower E1c and PdG averaged across the follicular phase had higher waist circumference, triglycerides, insulin, PAI-1, tPA-ag, and Factor VIIc levels in age- and BMI-adjusted analyses, ps < .05.

Conclusions. In mid-life menstruating women, a longer cycle length was related to cardiovascular risk factors, in large part through their common association with BMI. More

favorable levels of metabolic and hemostatic factors were associated with higher levels of follicular-phase estrogen, a pattern consistent with a more competent ovary, and higher levels of follicular-phase PdG, perhaps of adrenal origin. These findings suggest that metabolic and hemostatic factors may sensitive to hormonal variation during the early perimenopausal transition.

INTRODUCTION

It has long been recognized that women are protected from coronary heart disease (CHD) prior to the menopause (1). The extent to which that protection is due to reproductive hormones is not known. One approach to addressing this issue is to evaluate the magnitude of change in established CHD risk factors during the perimenopause, compared to another time period, or statistically adjusting for chronological aging. A series of cohort studies that followed this strategy suggested that lipid levels change during the perimenopause but that there are no consistent increases in blood pressure or weight that are not accounted for by aging (see 2 for review). Nonetheless, these studies had significant limitations. Most participants were European Americans, despite the fact that risk factor levels vary by ethnicity (3). More recently identified risk factors, e.g. hemostatic factors, were not measured. Only one study measured hormones at one point during the menstrual cycle (4), which limited characterization of ovarian function across the menstrual cycle. The importance of the latter is supported by a literature review (5) concluding that heightened CVD risk is associated with menstrual cycle irregularity and pregnancy losses and suggesting that abnormal ovarian function during the premenopausal years may accelerate risk for CHD (6, 7).

The present study examined associations between ovarian function during the menstrual cycle and cardiovascular risk factors in the Daily Hormone Study (DHS) from the Study of Women's Health across the Nation (SWAN), a multi-ethnic cohort study of pre- and early peri-menopausal women. Urinary levels of gonadotropins and sex steroid metabolites were assessed daily during one complete menstrual cycle or up to 50 consecutive days, whichever was shorter. Blood pressure, weight and waist circumference, metabolic factors, lipids and lipoproteins, and

hemostatic and inflammatory markers were measured within a 3-month window of the hormone collection.

A priori categorization of women's menstrual cycles allowed us to address a number of hypotheses. First, we anticipated that women who had evidence of luteal activity (ELA), presumably representing an ovulatory cycle, would have lower levels of CHD risk factors than women who experienced presumably anovulatory cycles (NELA). Second, given that a longer cycle length occurs as women approach the perimenopause, as well as in mild reproductive disorders, we anticipated that women with longer menstrual cycles, even if ovulatory, would have elevated risk factors. Third, we expected an inverse association between CHD risk factors and high estrogen in the follicular phase and high progesterone in the luteal phase, both being indicative of a reproductively competent ovary. We anticipated the findings to be stronger for metabolic and perhaps hemostatic factors, but not for blood pressure or weight gain, given the results of menopause cohort studies.

METHODS

Participants

The sample consisted of 500 (153 Caucasian, 107 African American, 36 Hispanic, 92 Chinese, and 112 Japanese) DHS volunteers whose menstrual cycle urine collection occurred within 90 days of cardiovascular risk factor assessment (mean number of days = 37.2, SD = 26.9) and whose health history was negative for diabetes, heart attack, or stroke. They were identified from the approximately 3200 women enrolled at one of the seven clinical sites. SWAN eligibility criteria included: being from a designated minority for the given site or Caucasian; aged 42-52 years; intact uterus and at least one ovary; not currently pregnant or breast-feeding; no use in the previous three months of exogenous hormone preparations, which

could affect ovarian or pituitary function; and at least one menstrual period within the past three months. Sites used a number of sampling strategies and sampling frames to enroll women in SWAN (8). Informed consent was obtained, and all the relevant institutional review boards approved the study.

The DHS began the first year following baseline evaluation (12). With the exception of age, criteria were as above. The four African American sites targeted recruiting approximately 90 participants, with half African Americans. The Japanese and Hispanic sites targeted 180 and 90 participants, respectively, the designated minority, and, the Chinese site targeted 238 recruiting proportionately more Chinese than Caucasians. A total of 867 women (259 Caucasian, 181 African American, 95 Hispanic, 155 Chinese, and 177 Japanese women) completed a daily specimen collection across the menstrual cycle or 50 days. Of these women, 840 had sufficient data to allow determination of whether there was evidence of luteal activity (see definition below). Comparisons of the 840 eligible participants, 247 eligible women who did not participate, and 113 eligible women with incomplete collections showed no differences by pre vs. perimenopausal status, smoking history, or body mass index (BMI) or serum hormones other than follicular stimulating hormone (FSH). DHS participants were slightly younger (by ½ year), of Japanese and Chinese origin, less educated, and had lower serum FHS levels, relative to the others. .

Specimen Collection

Women collected specimens beginning with the first day of menstrual bleeding, if possible, and ended on the first day of bleeding of the subsequent cycle or after 50 days, whichever occurred first. They stored their samples in a non-frost-free freezer. When collection

was completed, urine specimens were transported on ice to the CLASS Laboratory at the University of Michigan for analysis (9 for details of the protocol).

Hormone Measurement

Specimens were glycerol-preserved to permit measurement of uLH and uFSH over long storage periods (10) and to prevent interference with the estrone conjugates (E1c), and pregnanediol glucuronide (Pdg) assays (11, 12). Samples were normalized for the amount of creatinine in each specimen and expressed per milligram creatinine. uLH, uFSH, E1c, and Pdg were assayed using newly adapted chemiluminescent assays configured to be compatible with the ACS-180 Autoanalyzer (CIBA-Corning) (9). For uFSH, the inter- and intra-assay coefficients of variations (CVs) were 11.4% and 3.8%, respectively. For uLH, the inter- and intra-assay CVs were 10.9% and 4.6%, respectively. For E1c, the minimum detectable concentration was 0.1 ng/ml, and the inter- and intra- assay CVs for the E1c were 11.5% and 8.1%, respectively. For Pdg, the minimum detectable concentration was 0.0001 ng/ml, and the inter- and intra-assay CVs were 17.8% and 7.7%, respectively.

Serum hormone levels were collected at the annual examinations within the early follicular phase days 2 to 7 (13). . Serum estradiol (E2) concentrations were measured with a modified, off-line ACS:180 (E_2-6) immunoassay. Inter- and intra-assay coefficients of variation averaged 10.6% and 6.4%, respectively, and the lower limit of detection was 1 pg/ml. Testosterone concentrations were evaluated with the ACS:180 total testosterone assay modified to increase precision in the low ranges. Inter- and intra-assay coefficients of variation were 10.5% and 8.5%, respectively, and the lower limit of detection was 2 ng/dl. Serum dehydroepiandrosterone sulfate (DHEAS) concentrations involved competitive binding of dimethyl-acridinium ester with to a commercially available anti-DHEAS antibody and a solid

phase conjugated to paramagnetic particles. Inter- and intra-assay coefficients of variation for DHEAS were 11.3% and 7.6%, respectively, and the lower limit of detection was 2 ug/dl.

Measurement of Menstrual Cycle Characteristics

Women were categorized in the following groups: women who had evidence of luteal activity (ELA) and women who had no evidence of luteal activity (NELA), with the latter group further divided into those who ended the cycle collection with a menstrual bleed (NELA-bleed) vs. no bleed (NELA-no bleed). The dichotomous determination of luteal activity was based on having the algorithm developed by Kassam et al. (14) and modified for use in SWAN (9). The algorithm defined a cycle-specific baseline of Pdg using a 5-day moving average and considered a three-fold increment over the nadir 5-day average Pdg that was sustained for a minimum of 3 days to be consistent with ovulation. The Kassam algorithm was validated against weekly serum progesterone determination from a test sample of midreproductive, regularly cycling women. The probable day of the luteal transition in ELA cycles was assessed using a modified version (9) of previously developed algorithms of Waller et al. and Baird et al. (15, 16) and was based on an increase in the ratio of E1c/Pdg followed by an immediate decrease in the ratio. These assessments allowed us to divide cycles into follicular (all days prior to the day of the luteal transition, day 0) and luteal (all days after the day of the luteal transition) phases. The modified Kassam et al. algorithm had 98% sensitivity and 96% specificity for the detection of ELA status, when compared to a panel of 6 expert raters, and the algorithmic-derived day of luteal transition agreed with the experts to within 3 days in 92% of the ELA collections (9).

97% of women provided at least 80% of all possible cycle days and were considered to have adequate data for characterization of their menstrual cycle. Daily hormone concentrations were averaged within the follicular and luteal phases, using the trapezoidal rule. Cycles missing

hormones for two or more consecutive days were omitted from the analyses of integrated hormones. Integrated hormones were calculable for 93.0% of ELA cycles. Among ELA women, cycle length was categorized into ≤24 days; 25-32 days; and ≥ 33 days.

Cardiovascular Risk Factor Measurement

Risk factors were measured if they were predictors of coronary events in women or if available evidence suggested that they were influenced by hormonal factors Because of the DHS began one year after SWAN baseline examination, risk factor data were from the first annual follow-up for all but 3 women. The blood draw for risk factor assays occurred after a minimum 10-hour fast between day 2-5of the follicular phase to allow for a reasonable standardized hormonal milieu among women still menstruating. Samples were maintained at 4º C until separated, frozen at -80º C, and shipped on dry ice to the central laboratory (Medical Research Laboratories, Highland Heights, Kentucky, USA), which is certified by the Centers for Disease Control Lipid Standardization Part III program (17). All lipid and lipoprotein fractions were analyzed on EDTA treated plasma. Total cholesterol and triglycerides were analyzed by enzymatic methods and HDL-C was isolated using heparin-2M manganese chloride (18). Serum insulin was measured using RIA (DPC Coat-a-count, Los Angeles, CA) procedure and monitored as part of the monthly quality assurance program by the Diabetes Diagnostic Laboratory at the University of Missouri. Glucose was measured using a hexokinase-coupled reaction on a Hitachi 747-200 (Boehringer Mannheim Diagnostics, Indianapolis, IN). Lp(a) was quantified by competitive ELISA (19). Fibrinogen and Factor VII were measured in frozen citrated plasma using a clot-based turbidometric detection system, with Factor VII assay using Factor VII deficient plasma in preparing the standard curve. tPA-ag was measured in plasma using a double antibody in an enzyme-linked immunosorbant assay (American Diagnostica,

Greenwich, CT), with a human single chain tPA-ag as a standard calibrated against an international standard (Hertfordshire, England). PAI-1 was measured using a solid phased monoclonal antibody and an enzyme labeled goal second antiserum for detection (American Diagnostica, Greenwich, CT). CRP-hs was measured using an ultra-sensitive rate immunoephelometry (Dade-Behring, Marburg, Germany).

Waist circumference (narrowest part of torso) was measured over undergarments. Two blood pressure measurements were taken using a standard mercury column when seated after a minimum of five minutes of rest and were averaged. The technicians were certified for their performance and compliance with standard SWAN protocol before collecting the physical measures. Current smoking status (yes/no) was assessed by self-report. Height in centimeters and weight in kilograms were measured and body mass index (BMI) was calculated by dividing weight by the square of height in meters.

Statistical Analyses

Skewed variables were log transformed prior to analyses, i.e. integrated hormones, triglycerides, Lp (a), glucose, insulin, PAI-1, and CRP-hs. Tables present untransformed medians and interquartile ranges for logged transformed variables and means and standard deviations for the remaining risk factors. Women who were on lipid lowering medications (N=5) were excluded from the lipid analyses, and women on hypertensive medication (N=51) were excluded from the blood pressure analyses. Study hypotheses were analyzed with a series of t-tests and ANOVAs for continuous variables, and chi square tests for categorical variables comparing the cardiovascular risk factors of ELA and NELA women; the NELA-bleed and NELA-no bleed women; and three cycle length categories among ELA women. Age-adjusted partial correlation coefficients described the associations between the daily integrated hormone

measures and risk factors. Additional partial correlations were adjusted for age, BMI, and smoking; the 17 women without BMI data were excluded from this analysis. The interval between the interval between measurements of menstrual cycle characteristics and risk factors was not associated with any cardiovascular risk factors and is not presented. To test whether the significant age- and BMI-adjusted associations between risk factors and menstrual cycle characteristics and hormones varied by ethnic group, we also conducted regression analyses including the interaction of ethnicity and the predictor variable in the model along with age, BMI, ethnicity, and predictor variable. Unless noted otherwise, the results of the full covariate analysis and that of age and BMI were the same and associations did not vary by ethnic group. P-values $\leq$.05 were considered statistically significant.

RESULTS

Table 1 shows the characteristics of the study sample. By design women were from diverse ethnic backgrounds. Most women had more than a high school education, were early perimenopausal (bleeding within the last 3 months but some self-reported irregularity in the past year), and had ELA cycles.

Cardiovascular Risk Factors according to Evidence of Luteal Activity

The proportions of women in each ethnic group did not vary between ELA and NELA status, p = .31. Women classified as having ELA cycles had lower BMI, waist circumference, diastolic blood pressure, and fibrinogen, compared to women classified as having NELA cycles (Table 2; age-adjusted). Further adjustment for BMI did not alter the results for diastolic blood pressure and fibrinogen.

Cardiovascular Risk Factors according to Length of Menstrual Cycle

Among women who with ELA cycles, 65 had short cycles, 51 had long cycles, and 304 had intermediate length. Cycle length varied by ethnicity, with more Hispanics having long cycles relative to Chinese or Japanese (30.0% vs.6.3% and 7.1% respectively), with African Americans and Caucasians intermediate (16.7 % and 12.6% respectively). Longer cycle length was associated with higher BMI, waist circumference, blood pressure, triglycerides, glucose, insulin, fibrinogen, tPA-ag, Factor VII, and CRP-hs levels and lower HDL-C levels, after age-adjustments (Table 3). When adjusted for BMI, triglycerides and CRP-hs remained elevated among the long cyclers. Length of the luteal phase was positively associated with only fibrinogen ($r = .11$, $p = .03$).

Cardiovascular Risk Factors according to Integrated Hormones during the Follicular and Luteal Phases

E1c Concentrations: Among women classified as having an ELA cycle, higher E1c concentrations during the follicular phase were associated with lower BMI, waist circumference, apoB, triglycerides, Lp(a), glucose, insulin, PAI-1, tPA-ag, and CRP-hs, with the associations remaining significant for waist circumference, triglycerides, Lp(a), insulin, and PAI-1 in the age- and BMI-adjusted analyses (Table 4). The age- and BMI-adjusted associations between integrated E1c concentrations during the follicular phase and Lp(a), and PAI-1 varied by ethnicity. E1c concentrations were inversely associated with Lp(a) among whites and Chinese, but positively associated among Japanese women. E1c concentrations were inversely associated with PAI-1 among Chinese and whites, but positively associated among African Americans.

The daily E1C concentrations averaged within the luteal phase were only associated with lower PAI-1 in the age- and BMI-adjusted analyses.

PdG Concentrations: As expected, higher daily Pdg concentrations averaged within the luteal phase were associated with lower BMI, waist circumference, blood pressure, triglycerides, Lp(a), insulin, PAI-1, tPA-ag, Factor VII, and CRP-hs in age-adjusted analyses. The association with systolic blood pressure was strongest for Hispanic women. However, higher daily Pdg concentrations were only associated with lower tPA-ag in the age- and BMI-adjusted analyses.

Unexpectedly, lower Pdg concentrations averaged within the follicular phase were associated with all but one risk factor. Age- and BMI-adjusted analyses showed that higher Pdg measured during the follicular phase was associated with lower waist circumference, triglycerides, insulin, PAI-1, tPA-ag, and Factor VII (Table 4).

Other Urinary Hormones: Women with lower risk factor levels had higher uFSH and uLH concentrations measured during either phase. However, following age- and BMI-adjustments, few risk factors were associated with uFSH and uLH concentrations measured in both phases.

Other Relevant Data

Because of the unexpected association between cardiovascular risk factors and urinary Pdg concentrations during the follicular phase, we examined the associations among the urinary hormone levels by phase. Among women who had ELA cycles, there were strong associations between each of the urinary concentrations across phases, rs $\geq$ .57. Urinary E1c concentrations in both the follicular and luteal phase were similarly correlated with serum DHEAS (rs = .17 and .15, ps < .002, respectively), but were only associated with serum E2 when measured in the follicular phase (r = .17, p < .001). Pdg concentrations were more strongly associated with serum DHEAS when measured during the follicular than luteal phase (r = .31, p < .001 vs r = .09, p = .09). Pdg concentrations during the luteal phase were associated negatively with serum

testosterone (r = -.14, p = .005). Age-and BMI-adjusted correlations showed that follicular phase serum E2 was associated significantly with higher HDL-C (r=.13, p = .004) and fibrinogen (r=.14, p = .003) and lower fasting glucose (r = -.09, p = .05); testosterone with higher HDL-C (r=.11, p = .02) and tPA (r=.10, p < .05); and DHEAS with higher fasting glucose (r = .13, p = .004).

DISCUSSION

Our study tested the hypotheses that higher levels of risk factors would be apparent among women classified as having a NELA cycle, relative to women classified as having an ELA cycle, and among women with a longer cycle length among women classified as having an ELA cycle. In contrast to expectations, women who were classified as having an ELA vs. NELA cycle exhibited few differences in cardiovascular risk factors and the associations between longer cycle length and increased cardiovascular risk factors among ELA women were primarily accounted for by BMI differences by cycle length. In a larger sample of ELA women, we reported longer cycle length and lower whole cycle uLH, uFSH, and Pdg excretion in obese and overweight women, compared to normal weight women (20).

There was partial support for a third hypothesis. Pdg concentrations were inversely associated with elevated risk factor levels, but unexpectedly in both the luteal and follicular phase. Age- and BMI- adjusted associations were statistically significant for more risk factors when PdG was measured in the follicular than in the luteal phase (6 vs 1 risk factor associations). The source of Pdg concentrations during the follicular phase is likely to be adrenal rather than ovarian (21). In support of this interpretation, our results showed that urinary Pdg concentrations were more related to the adrenal hormones in the serum measured during the follicular than the luteal phase. Alternatively, Pdg in the very early follicular phase is the result

of delayed excretion from the prior luteal phase. Thus, high rates of progesterone production in the prior luteal phase or a foreshortened follicular phase could increase the average follicular phase Pdg levels.

As anticipated, higher daily E1c concentrations during the follicular phase were associated with lower levels of BMI, adjusted for age, and waist circumference, triglycerides, Lp(a), insulin, and PAI-1, adjusted for BMI and age. The findings imply that women who have a reproductively competent ovary in midlife, as evidenced by high levels of E1c during the follicular phase and evidence of luteal activity, are at lower risk for cardiovascular disease, primarily through lower levels of metabolic and hemostatic factors.

An advantage of the daily assessment of urinary hormone metabolites is its better approximation of women's overall exposure to hormones during a menstrual cycle, in contrast to a single blood draw during the very early follicular phase, per SWAN core protocol, when estrogens and progesterone are known to be particularly low and have less variability than later in the cycle. Our results showed stronger and more theoretically consistent associations with urinary hormone metabolites than with serum hormone measures, although the serum hormones were measured concurrently with the cardiovascular risk factors. It should be noted that an index of free circulating testosterone (not measured in DHS) was also associated with elevated risk factors, especially the metabolic factors in the full SWAN cohort (22-23).

One can posit that obesity in mid-life can lead to longer cycles and suppressed reproductive hormones, even when cycles are presumed to be ovulatory, which, in turn, lead to alterations in other CHD risk factors. Adipose tissue secretes a number of adipocytokines, such as leptin, which are known to influence hypothalamic-pituitary-ovarian function (24). Thus, increased adiposity per se may have an adverse effect on reproductive hormones and menstrual

cycles. Studies demonstrating improvement in fertility and reproductive hormone secretion with weight loss support this concept (25, 26).

Another possibility is that lower reproductive hormone levels in midlife lead to increasing levels of risk factors, including weight, such that obesity is not the initiating event. Arguments against this model are that the perimenopausal transition is not associated with weight gain, beyond the effect of chronological aging (2), and that weight reduction interventions lead to improvement in other cardiovascular risk factors (27) and menstrual cyclicity (25, 26).

Finally, obesity in mid-life may influence the reproductive and metabolic systems independently. The fact that low E1c levels observed during the follicular phase were associated with metabolic risk factors and some hemostatic factors, independent of BMI, is consistent with this possibility. Longitudinal measures of hormones, cardiovascular risk factors, and menstrual cycle characteristics will permit us to support or refute these models as we continue to follow the enrolled women.

The study has a number of limitations and strengths. One single menstrual cycle may not be representative of cycles in the months surrounding the time of cardiovascular risk factor assessment and may account for the few differences between ELA and NELA women's risk factors. On the other hand, characterizing a complete cycle has considerable advantage over a single annual blood draw, even when the annual blood draw is timed very carefully with bleeding patterns. Second, while the modified Kassam algorithm permitted the determination of cycles that were presumed to be ovulatory, the cycles classified as having no evidence of clear luteal activity were a somewhat heterogeneous mixture of ovulatory and anovulatory menstrual cycles. We did, however, compare the risk factors of women who had no evidence of clear luteal

activity according to whether their specimen collections ended with bleeding or not and this distinction was not related to cardiovascular risk factors. Among the strengths of the study are the large number of cycles that were characterized, the large number of established and emerging cardiovascular risk factors measured, and the multi-ethnic nature of the sample.

In sum, evidence of ovulation in a single cycle had minimal association with cardiovascular risk factors levels in pre- and peri-menopausal women. A longer cycle length was related to cardiovascular risk factors, in large part through their common association with BMI. Estrone urinary metabolites and Pdg concentrations during the follicular phase were associated with metabolic and hemostatic factors, independent of age and BMI, suggesting that metabolic and hemostatic factors may be most sensitive to change during the perimenopausal transition. Characterization of hormonal exposure across the menstrual cycle phases provides unique information about risk factors associated with later cardiovascular disease.

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Table 1. Demographic and menstrual cycle characteristics of the sample.

% (N) Ethnicity	
African American	21.4 (107)
Caucasian	30.6 (153)
Chinese	18.4 (92)
Hispanic	7.2 (36)
Japanese	22.4 (112)
% (N) Education	
≤ High school	23.1 (113)
> High school	76.9 (377)
% (N) Married women	71.3 (356)
Mean (SD) age at hormone collection	47.1 (2.5)
% (N) Site	
Detroit area, MI	12.4 (62)
Boston, MA	13.0 (65)
Chicago, IL	9.2 (46)
Oakland, CA	26.0 (130)
Los Angeles, CA	22.4 (112)
Newark, NJ	7.2 (36)
Pittsburgh, PA	9.8 (49)
% (N) Current smoker	11.0 (55)
% (N) Menopausal status at risk factor assessment	

Pre-menopause	28.6 (142)
Early perimenopause	71.4 (355)
% (N) women with ELA cycles[1]	84.0 (420)
Mean (SD) Menstrual cycle length (days) among women with ELA cycles	28.3 (5.1)

[1] ELA refers to evidence of luteal phase activity.

Table 2. Unadjusted mean (standard deviation) or **median (interquartile range)** of cardiovascular risk factors according to evidence of luteal phase activity (ELA) versus no evidence of luteal phase activity (NELA)

	Menstrual Cycle Status		Age-adjusted	Age- and BMI-adjusted
Cardiovascular Risk Factor	ELA	NELA	P - value	P - value
Body composition				
BMI, kg/m^2	26.3 ± 6.1	28.2 ± 7.1	.02	-
Waist circumference, cm	82.7 ± 14.3	87.3 ± 15.2	.02	.21
Blood pressure				
Systolic, mm Hg	111.6 ± 13.3	116.5 ±15.3	.07	.20
Diastolic, mm Hg	72.5 ± 9.7	76.3 ± 10.7	.02	.04
Lipids/lipoproteins				
LDL-C, mg/dL	112.0 ± 29.6	113.2 ± 28.8	.98	.70
HDL-C, mg/dL	59.4 ± 14.4	60.0 ± 15.7	.95	.46
Triglycerides, mg/dL	**96.0 (73.0–133.0)**	**99.0 (80.0–137.0)**	.93	.91
Apo-B, mg/dL	103.8 ± 23.2	105.3 ± 23.1	.86	.86
Lp(a), mg/dL	**14.0 (4.0-35.0)**	**13.0 (4.0-32.0)**	.88	.62
Glucose metabolism				
Glucose, mg/dL	**90.0 (85.0-96.0)**	**90.0 (85.0-98.0)**	.89	.59
Insulin, μlU/mL	**8.0 (6.3-10.6)**	**7.9 (5.8-11.1)**	.52	.32

Inflammatory/hemostatic				
Fibrinogen, md/dL	266.4 ± 49.8	283.2 ± 57.8	.02	.03
PAI-1, ng/mL	**16.5 (9.0-28.2)**	**20.2 (11.0-35.2)**	.06	.20
tPA-ag, ng/mL	8.0 ± 3.6	8.7 ± 3.9	.30	.55
Factor VIIc, %	113.0 ± 23.5	116.2 ± 25.5	.52	.81
CRP-hs, mg/L	**1.0 (0.4-3.1)**	**1.2 (0.5-4.0)**	.09	.70

Table 3. Unadjusted mean (standard deviation) or **median (interquartile range)** of cardiovascular risk factors according to cycle length among women with ELA cycles.

Cardiovascular Risk Factors	Cycle Length ≤ 24 days n = 65	25-32 days n = 304	≥ 33 days n = 51	Age-adjusted p-value	Age- and BMI-adjusted p-value
Body composition					
BMI, kg/m^2	24.5 (± 5.0)	26.3 (± 6.0)	29.1 (± 7.2)	.001	-
Waist circumference, cm	79.5 (± 13.3)	82.5 (± 13.7)	88.0 (± 17.3)	.007	.15
Blood pressure					
Systolic, mm Hg	108.9 (± 15.0)	111.6 (± 13.1)	115.3 (± 11.5)	.04	.23
Diastolic, mm Hg	69.9 (± 10.1)	72.9 (± 9.6)	73.4 (± 9.8)	.05	.09
Lipids/lipoproteins					
LDL-C, mg/dL	107.4 (± 31.6)	113.1 (± 28.9)	110.9 (± 31.2)	.36	.35
HDL-C, mg/dL	61.9 (± 13.8)	59.8 (± 14.3)	53.8 (± 14.4)	.01	.19
Triglycerides, mg/dL	**82.0 (66.0-133.0)**	**96.0 (73.0-131.0)**	**116.5 (89.0-182.0)**	< .0001	.005
Apo-B, mg/dL	99.0 (± 23.6)	104.0 (± 22.3)	108.2 (± 27.7)	.09	.48

Lp(a), mg/dL	10.0 (4.0-24.0)	15.0 (5.0-35.0)	16.0 (4.0-43.0)	.46	.95
Glucose metabolism					
Glucose, mg/dL	**88.5 (83.0-93.5)**	**90.5 (85.0-96.0)**	**90.0 (86.0-98.0)**	.003	.16
Insulin, μlU/mL	**7.6 (6.2-10.4)**	**7.8 (6.2-10.4)**	**9.0 (6.8-14.2)**	.01	.52
Inflammatory/ hemostatic					
Fibrinogen, mg/dL	265.4 (± 55.7)	263.7 (± 47.2)	284.8 (± 54.4)	.03	.33
PAI-1, ng/mL	**16.5 (8.0-27.0)**	**16.3 (9.0-26.7)**	**18.9 (12.0-34.6)**	.12	.67
tPA-ag, ng/mL	8.1 (± 3.4)	7.7 (± 3.5)	9.8 (± 4.0)	.003	.12
Factor VIIc, %	108.2 (± 20.4)	112.7 (± 22.1)	120.8 (± 32.9)	.02	.08
CRP-hs, mg/L	**0.9 (0.3-3.2)**	**0.9 (0.4-2.5)**	**2.5 (1.0-8.1)**	<.0001	.01

Table 4. Partial correlation coefficients (adjusted for age) between cardiovascular risk factors at year 01 and integrated hormone levels within the follicular and luteal phases among women with ELA cycles.

	uFSH		uLH		Pdg		Estrone	
Cardiovascular Risk Factor	Follicular	Luteal	Follicular	Luteal	Follicular	Luteal	Follicular	Luteal
Body composition								
BMI	-0.11*	-0.20*	-0.22*	-0.23*	-0.34*	-0.38*	-0.15*	-0.07
Waist circumference	-0.15*	-0.18*	-0.27*	-0.25*	-0.36*†	-0.37*	-0.18*†	-0.11*
Blood pressure								
Systolic	0.04	-0.11*	-0.04	-0.16*	-0.15*	-0.11*	-0.05	-0.03
Diastolic	0.04	-0.08	0.01	-0.07	-0.13*	-0.09	-0.05	-0.01
Lipids/lipoproteins								
LDL-C	-0.09	-0.15*†	-0.10	-0.14*	-0.10*	-0.04	-0.06	-0.01
HDL-C	0.12*	0.16*	0.14*	0.15*	0.15*	0.05	0.08	0.03
Triglycerides	-0.08	-0.12*	-0.15*	-0.16*	-0.28*†	-0.14*	-0.24*†	-0.11*
Apo-B	-0.01	-0.08	-0.06	-0.09	-0.14*	-0.07	-0.12*	-0.06
Lp(a)	-0.08	-0.12*	-0.08	-0.06	-0.11*	-0.11*	-0.15*†	-0.09
Glucose metabolism								
Glucose	0.01	-0.06	-0.12*	-0.14*	-0.11*	-0.04	-0.11*	0.00
Insulin	-0.12*	-0.17*	-0.23*†	-0.19*	-0.35*†	-0.28*	-0.19*†	-0.09
Inflammatory/								

hemostatic								
Fibrinogen	-0.15*†	-0.06	-0.18*†	-0.09	-0.08	-0.10	0.02	0.01
PAI-1	-0.10	-0.09	-0.22*†	-0.22*†	-0.23*†	-0.20*	-0.14*†	-0.16*†
tPA-ag	-0.02	-0.02	-0.10	-0.06	-0.24*†	-0.25*†	-0.13*	-0.07
Factor VIIc	-0.08	-0.09	-0.07	-0.07	-0.19*†	-0.11*	-0.08	-0.10
CRP-hs	-0.09	-0.14*	-0.19*	-0.15*	-0.28*	-0.22*	-0.10*	-0.05

* $p < .0.05$ adjusted for age. † $p < 0.05$ adjusted for age and BMI