Reproducibility of Plasma and Urinary Sex Hormone Levels in Premenopausal Women over a One-Year Period[1]

Dominique S. Michaud,[2] JoAnn E. Manson, Donna Spiegelman, Robert L. Barbieri, Daniel W. Sepkovic, H. Leon Bradlow, and Susan E. Hankinson

Departments of Nutrition [D. S. M.], Epidemiology [J. E. M., D. S., S. E. H.], and Biostatistics [D. S.], Harvard School of Public Health, Boston, Massachusetts 02115; Channing Laboratory [J. E. M., S. E. H.] and the Division of Preventive Medicine [J. E. M.], Departments of Medicine and of Obstetrics & Gynecology [R. L. B.], Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115; and Strang Cancer Research Laboratory, New York, New York 10021 [D. W. S., H. L. B.]

Abstract

Although endogenous sex steroid hormones in premenopausal women may be associated with the risk of breast cancer and other illnesses, direct evidence to support this hypothesis is limited in large part by methodological issues in the conduct of relevant studies. One major unresolved issue is whether a single blood sample (such as is available in most epidemiological studies), collected in a specific phase of the menstrual cycle, reflects long-term levels in that phase. To address this issue, two sets of blood and urine samples were obtained from 87 premenopausal women over a 1-year period in both the follicular and luteal phases. Plasma estradiol, estrone, and estrone sulfate were measured in the blood samples obtained in both phases, whereas progesterone and urinary 2- and 16α-hydroxyestrone were measured in luteal-phase samples only. For all of the women combined, intraclass correlation coefficients (ICCs) ranged, with one exception, from 0.52 to 0.71 for the plasma estrogens and the urinary estrogen metabolites. The sole exception was for estradiol in the luteal phase (ICC = 0.19); inclusion of only women who were ovulatory in both cycles and who collected each sample 4–10 days before their next period resulted in a substantially higher ICC for estradiol in the luteal phase (ICC = 0.62; 95% confidence interval, 0.43–0.78). These data indicate that, for several plasma and urinary sex hormones, a single follicular- or luteal-phase measurement in premenopausal women is reasonably representative of hormone levels in that phase for at least a 1-year period.

Received 4/30/99; revised 9/16/99; accepted 9/28/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This research was supported by NIH Grants CA-67262 and CA-50385 and by a grant from the Massachusetts Breast Cancer Research Grants Program, Massachusetts Department of Public Health.

[2] To whom requests for reprints should be addressed, at Department of Nutrition, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115.

Introduction

Although several recent studies have reported an increase in breast cancer risk among postmenopausal women with high endogenous estrogen levels (1–5), there continues to be only limited evidence that endogenous hormones in premenopausal women are associated with subsequent breast cancer risk (6, 7). Because the collection and storage of blood or urine samples as well as subsequent analyses of hormone levels are both difficult and expensive, large epidemiological studies usually collect only one sample per participant. Because long-term hormone levels are generally of greatest interest in etiological studies of breast cancer, it is important to evaluate whether a single plasma hormone measurement obtained for a given individual is representative of their long-term levels.

Previous studies examining the reproducibility of plasma hormone levels over time have focused primarily on postmenopausal women. Overall, the results have indicated substantial reproducibility (8–12). In the few studies that have evaluated plasma androgens in premenopausal women, similar reproducibility has been reported, with ICCs[3] ranging from 0.6 to 0.85 (9, 12). In premenopausal women, several steroid hormones (particularly estrogens) fluctuate markedly over the menstrual cycle, and, thus, a single hormone measure cannot represent long-term levels overall. However, a single measurement from a specific phase of the menstrual cycle (*i.e.*, follicular or luteal) might be representative of long-term levels in that phase. To our knowledge, no previous study has evaluated the phase-specific reproducibility of plasma estrone, estrone sulfate, or progesterone. In the only study of plasma estradiol, poor reproducibility in the luteal phase was reported (9). In addition, only one study has evaluated the reproducibility of urinary estrogen metabolites in premenopausal women (13).

To assess the reproducibility of plasma and urinary hormone levels over approximately a 1-year period, we collected repeated blood samples in both the follicular and the luteal phase of the menstrual cycle as well as urine samples in the luteal phase. We measured estradiol, estrone, estrone sulfate, and progesterone levels in the plasma samples and the estrogen metabolites 2- and 16α-hydroxyestrone in the urine samples.

Materials and Methods

Subjects. The NHSII is a prospective cohort study of 116,671 United States registered female nurses, ages 25–42 years at entry, who responded to a baseline questionnaire in 1989. This cohort is described in detail elsewhere (14). Participants in the current study were randomly selected from those NHSII participants who: (*a*) were premenopausal

[3] The abbreviations used are: ICC, intraclass correlation coefficient; NHSII, Nurses' Health Study II; CV, coefficient(s) of variation; CI, confidence interval.

(*i.e.*, still having menstrual periods); (*b*) had not used oral contraceptives or other hormonal preparations (*e.g.*, for infertility) in the previous 6 months and had no plans to begin using them; (*c*) had not been pregnant or lactating in the previous 6 months and were not planning to become pregnant in the next year; and (*d*) had completed and returned the 1989, 1991, and 1993 NHSII questionnaires. From a sample of 746 NHSII women who were invited to participate, 438 (59%) responders were willing to collect two timed samples of blood (follicular and luteal) and one urine sample. Of these, 112 women were no longer eligible to participate (*e.g.*, had recently become pregnant); thus, sample collection kits were mailed to the 326 eligible women. Two hundred women (61% of 326) returned the kits. A second collection kit was mailed to the 121 women who had returned the first kit, remained eligible to participate, and subsequently agreed to provide a second set of samples. Eighty-seven women (72% of 121) returned the second set of samples.

Blood and Urine Collections. Each blood collection kit contained all of the supplies needed to have blood samples drawn by a local laboratory or a colleague (*e.g.*, needle, tourniquet, and blood collection tubes with sodium heparin). The participants were asked to have their first blood sample (15 ml) drawn on the 3rd, 4th, or 5th day of their menstrual cycle ("follicular blood draw"), and to have the second blood sample (30 ml) drawn 7 to 9 days before the anticipated start of their next cycle ("luteal blood draw"). Timing of the luteal sample from the estimated first day of the next menstrual cycle is generally more accurate than counting forward from day 1 of the current cycle, because the length of the follicular phase is more variable than the length of the luteal phase (15, 16). The participants were instructed to draw the blood samples, whenever possible, in the morning while fasting; 35% of the women collected blood while fasting. Participants placed their follicular blood samples in a refrigerator for 8–24 h after it was drawn; they then aliquoted the plasma into cryotubes. The plasma was kept in the participant's home freezer until the second (luteal) blood collection. A spot morning urine sample also was collected on the same day as the second blood draw. All of the samples were then returned to our laboratory by overnight mail, with a frozen water bottle to keep them cool. Upon arrival in the laboratory, the whole blood from the luteal collection was centrifuged and plasma aliquoted into labeled cryotubes, and all of the samples were then stored in liquid nitrogen freezers. In a pilot study of postmenopausal women, levels of estradiol and free estradiol (in addition to other hormones) were observed to be stable when processing was delayed for 24 to 48 h (17). In a small pilot study of 16 premenopausal women, we collected two blood samples from each woman to compare follicular hormone levels in samples processed immediately or processed exactly as described above (which included simulating transport in a cooler with a frozen water bottle). We observed similar means and standard deviations for estrone and estradiol when using the two different processing methods, and the ICCs were 0.93 and 0.80 for estradiol and estrone, respectively.

A questionnaire was sent to each subject on which to record the first day of the menstrual cycle during which the blood samples were drawn, the dates of both blood draws, and the date of urine sample collection. In addition, information was collected on the time of day of blood collection, the number of hours since last food intake prior to the two blood draws, and the participant's current weight. Finally, a postcard on which to record the first day of the next menstrual cycle was provided (95% of postcards were returned). Other data (*e.g.*, information on smoking status) were available for each participant from the NHSII questionnaires completed in 1993.

Laboratory Methods. Plasma estradiol, estrone, and progesterone (for the luteal samples only) were assayed by Quest Laboratory (San Juan Capistrano, CA), and estrone sulfate was assayed by the laboratory of Dr. Longcope (University of Massachusetts Medical Center, Worcester, MA). Because of financial constraints, samples from only 30 women were measured for estrone sulfate levels. The laboratory of one of the authors (H. L. B.) assayed the urine samples for 2- and 16α-hydroxyestrone and for creatinine. The ratio of 2-hydroxyestrone to 16α-hydroxyestrone was calculated.

Estradiol (18) and estrone (19) were assayed by organic extraction, celite chromatography and RIA. Reported values are corrected for procedural losses. This method is highly specific and is the 'gold standard' for estimating steroid levels in plasma. Estrone sulfate was assayed by RIA (of estrone) after initial extraction of estrone, enzyme hydrolysis, organic extraction, and separation by column chromatography (20). Progesterone was assayed by RIA preceded by organic extraction (21). Urine samples were assayed for 2-hydroxyestrone and 16α-hydroxyestrone by means of a new ELISA procedure, as detailed elsewhere (22). Because urine samples were not collected over a 24-h period and total urinary output was unknown, creatinine levels were measured for each sample with a Beckman manual creatinine analyzer. All of the urinary metabolite levels are thus presented as standardized values (estrogen metabolite value divided by creatinine level in each sample) to account for differences arising from variations in urine concentration.

All of the luteal and follicular samples from a single woman were assayed together in the same laboratory batch but were labeled to prevent laboratory personnel from identifying which samples were from the same woman. Intra-assay laboratory CV were obtained by sending masked replicate samples. For estradiol, estrone, and progesterone, CV were less than 10%. The CV for each of the urinary metabolites was 15%. Estrone sulfate was assayed in two batches, and the CV percentages were 15 and 7.4%, respectively.

Statistical Analysis. The natural logarithms of the plasma hormone and metabolite values were used in analyses because the transformed values were more normally distributed. Between-person and within-person variances were estimated from the two sets of hormone measurements by random effects models, as implemented by SAS (SAS Institute, Cary, NC). To assess reproducibility, ICCs were calculated by dividing the between-person variance by the sum of the between-person and within-person variances (23). CIs (95%) were calculated for the ICCs (8). Because substantial batch-to-batch variation was observed in estrone sulfate levels, we calculated the ICCs for each batch individually and then obtained a weighted average (based on the number of women in each batch) for the two batches.

Although 87 women contributed all of the requested samples of blood and urine, several provided an insufficient volume to assay all of the hormones. As a result, ICCs in this study were based on 83 women for follicular estrone, 85 women for all of the other plasma hormones, and 86 women for urinary metabolites.

Table 1 Baseline characteristics of 87 premenopausal women who donated two follicular and two luteal blood samples over approximately a 1-year period

Characteristics	Mean (SD)
At time of first blood draw	
Age (years)	41.3 (4.1)
BMI[a] (kg/m^2)	25.8 (5.7)
Time between blood sample collections (months)	11.3 (3.1)
	Percentage
From previous NHSII questionnaires	
Current cigarette smoking[b]	5.7
Age at menarche[c]	
<10 yr	14.9
11–12 yr	40.2
13–14 yr	36.8
15+ yr	8.0
Parity[b]	
Nulliparous	17.2
1	12.6
2	39.1
3–4	28.7
5+	2.3
Family history of breast cancer[c]	6.9
Personal history of benign breast disease[b]	28.7

[a] BMI, body mass index.
[b] 1993 questionnaire.
[c] 1989 questionnaire.

Table 2 Mean (SD) plasma hormone and urinary estrogen metabolite levels from 87 premenopausal women at the two sample collections[a]

	No. of women	Sample 1		Sample 2	
		Mean	(SD)	Mean	(SD)
Plasma hormone					
Follicular plasma					
Estradiol (pg/ml)	84	51.3	(31.8)	50.9	(36.2)
Estrone (pg/ml)	85	43.4	(18.7)	40.9	(17.0)
Estrone sulfate (pg/ml)	32	389	(280)	410	(309)
Luteal plasma					
Estradiol (pg/ml)	85	137	(70.8)	136	(80.8)
Estrone (pg/ml)	85	90.0	(43.7)	89.3	(41.3)
Estrone sulfate (pg/ml)	30	668	(364)	674	(308)
Progesterone (ng/dl)	85	1107	(738)	1080	(829)
Urinary metabolite					
2-hydroxyestrone[b]	86	15.5	(10.8)	15.6	(9.5)
16α-hydroxyestrone[b]	86	8.0	(4.8)	8.5	(6.6)
Ratio[c]	86	2.0	(0.9)	2.0	(0.9)

[a] Samples were collected 1 year apart, on average.
[b] Metabolite values (ng/ml) are divided by the creatinine levels (mg/ml) of each sample.
[c] 2-Hydroxyestrone:16α-hydroxyestrone.

Table 3 ICCs and 95% CIs for plasma estrogens and urinary estrogen metabolites based on two blood samples from all of the premenopausal women and from a subset of women with progesterone levels ≥300 ng/dl

Hormone	No. of pairs	ICC	95% CI
Follicular plasma			
All of the women			
Estradiol (pg/ml)	85	0.53	(0.37–0.67)
Estrone (pg/ml)	83	0.52	(0.36–0.67)
Estrone sulfate (pg/ml)	33	0.67	(0.36–0.88)
Luteal plasma			
All of the women			
Estradiol (pg/ml)	85	0.19	(0.06–0.47)
Estrone (pg/ml)	85	0.54	(0.38–0.68)
Estrone sulfate (pg/ml)	28	0.52	(0.18–0.84)
Progesterone (ng/dl)	85	0.54	(0.39–0.69)
Women with progesterone levels ≥300 ng/dl at each sample collection			
Estradiol (pg/ml)	63	0.49	(0.31–0.67)
Estrone (pg/ml)	63	0.70	(0.56–0.81)
Estrone sulfate (pg/ml)	20	0.68	(0.28–0.92)
Progesterone (ng/dl)	63	0.45	(0.27–0.65)
Women with progesterone levels ≥300 ng/dl and with blood obtained between 4 and 10 days before the beginning of next cycle at each sample collection			
Estradiol (pg/ml)	39	0.62	(0.43–0.78)
Estrone (pg/ml)	39	0.69	(0.52–0.82)
Urinary metabolite			
2-hydroxyestrone[a]	86	0.58	(0.43–0.71)
16α-hydroxyestrone[a]	86	0.56	(0.41–0.70)
Ratio[b]	86	0.71	(0.60–0.80)

[a] Metabolite values (ng/ml) are divided by the creatinine levels (mg/ml) of each sample.
[b] 2-Hydroxyestrone:16α-hydroxyestrone.

Results

The characteristics of the premenopausal women in this study are summarized in Table 1. The mean number of months between the collection of the two follicular blood samples was 11.3 months (range, 5.3–22.1 months). At the time of collection of the first blood sample, the women were 31–49 years of age, with a mean age of 41 years. The median cycle length for the women in this study was 27.5 days. The ICC for the cycle length in the first compared with the second blood collection was 0.26, increasing to 0.55 (95% CI, 0.39–0.70) when five women with 1 cycle greater than 100 days in length were removed. Baseline characteristics of participants in this study (listed in Table 1) were not substantially different from those of the women who initially met the eligibility criteria ($n = 633$), however, the participants were less likely to smoke (5.7 *versus* 11.5%) and were more likely to report a history of benign breast disease (28.7 *versus* 20.2%).

The women in this study successfully collected their follicular samples at the beginning of their menstrual cycles (0.6% on the first day, 26.8% on the second day, 42.3% on the third day, and 30.3% on the fourth day). The mean levels of follicular and luteal estrogens, progesterone, and the two urinary metabolites (measured per gram of creatinine) were similar at the two sample collections (Table 2). As expected, luteal estrogen levels were much higher than follicular estrogen levels. The mean ratio of 2-hydroxyestrone:16α-hydroxyestrone was 2.0 at both of the sample collections. Plasma levels for follicular and luteal estrogens, and for progesterone, did not vary by fasting status (data not shown).

For all of the women combined, ICCs for plasma estrogens and urinary metabolites ranged, with one exception, from 0.52 to 0.71; the sole exception was for estradiol in the luteal phase (ICC = 0.19; Table 3). The ICC for progesterone was 0.54. Correlations did not vary substantially whether the first and second blood samples were collected over a period of ≤11 months or >11 months, or if the women were ≤40 or >40 years old (data not shown). ICCs were essentially unchanged after removing women who reported being current smokers on the 1993 NHSII questionnaire ($n = 6$). In a separate analysis, we removed women ($n = 26$) who had gained or lost weight (plus or minus more than 1 body mass index kg/m^2 unit) between the two blood collections. ICCs for women with a stable weight were similar for luteal estrogens but slightly stronger for follicular

Table 4 Cross-classification of premenopausal plasma hormone and urine hormone metabolite levels: first sample by second sample

		Quartiles for sample 2				
		1	2	3	4	
Plasma follicular estradiol (n = 85)						
Quartiles for sample 1	1	**9**	4	9	1	23
	2	7	**7**	4	2	20
	3	1	8	**6**	3	18
	4	2	5	5	**12**	24
		19	24	24	18	
		Quartiles for sample 2				
		1	2	3	4	
Urinary ratio[a] (n = 86)						
Quartiles for sample 1	1	**16**	4	3	0	23
	2	2	**8**	7	3	20
	3	0	4	**9**	6	19
	4	2	7	5	**10**	24
		20	23	24	19	

[a] 2-Hydroxyestrone:16α-hydroxyestrone.

estrogens and progesterone, compared with ICCs for all of the women combined (estradiol ICC = 0.63, 95% CI = 0.47–0.77; estrone ICC = 0.57, 95% CI = 0.39–0.72; progesterone ICC = 0.70, 95% CI = 0.55–0.81).

Twenty-two women (26%) had at least one progesterone level of <300 ng/dl (which, in the luteal phase, generally indicates an anovulatory cycle); 11 (50%) of these 22 women reported having irregular cycles (overall, 65% of women with irregular cycles had a normal progesterone level). Eight of the 22 women had progesterone levels of <300 ng/dl on both blood draws and 14 on only one blood draw. Of these 22 women, 16 had missed their luteal phase altogether in at least one cycle (*i.e.,* had collected blood <4 or >13 days before the beginning of their next cycle); 3 were presumed to be anovulatory (*i.e.,* had a progesterone level of <300 ng/dl in a sample that was collected within 4–13 days of the next menses); and 3 were lacking information on the date of the blood draw and thus could not be assigned a reason for the low progesterone level. After exclusion of these women, ICCs for luteal-phase estrogens improved substantially (ICC for estradiol = 0.49; Table 3). The ICC for progesterone decreased somewhat (from 0.54 to 0.45) after restricting the analysis to women with progesterone levels ≥300ng/dl; this change could be due to a decrease in the between-person variation contributed by those women with repeated low progesterone levels.

To further investigate the reproducibility of luteal estradiol and estrone levels, we next excluded women who did not collect both blood samples during the midluteal phase of their menstrual cycle, defined here as 4 to 10 days before their next cycle. This additional exclusion substantially improved the ICCs of the plasma estrogens, compared with the estrogen ICCs for all of the women combined (r = 0.62 for estradiol and r = 0.69 for estrone; see Table 3) but did not affect the ICCs for urinary estrogen metabolites (data not shown).

In an additional analysis, we examined the reproducibility of plasma luteal estrogens among women who collected the second plasma sample at the same time during their menstrual cycle as the previous sample (within 4 days), regardless of whether that time reflected the midluteal phase. The ICCs in this analysis were similar to those obtained by the exclusion of women with progesterone levels of <300 ng/dl and those women who did not collect blood between 4 and 10 days before their next cycle (ICC = 0.71, 95% CI = 0.54–0.83 for estrone; ICC = 0.54, 95% CI = 0.33–0.73 for estradiol).

Because most studies in epidemiology will categorize continuous exposures (*i.e.,* plasma hormones) into quartiles to examine their association with disease, we have cross-classified two samples of plasma follicular estradiol and of urinary ratio (2-hydroxyestrone:16α-hydroxyestrone) to show concordance and discordance for the same women (Table 4). For follicular estradiol, 34 (40%) of 85 were perfectly classified, and 65 (77%) of 85 were off by one category or less. Similarly, for the urinary ratio, 43 (50%) of 86 were perfectly classified, and 71 (83%) of 86 were off by one category or less. Only three women (4%) for follicular estradiol and two women (2%) for the urinary ratio were extremely misclassified. These data show that one sample can classify women into the appropriate quartile of exposure relatively well.

Discussion

ICCs for levels of plasma estrogens and urinary estrogen metabolites ranged from 0.19 to 0.71 when measured 1 year apart (on average) in a sample of premenopausal women. The exclusion of women with low plasma progesterone levels from analyses substantially improved the correlations for both estradiol and estrone in the luteal phase. For example, the ICC for luteal phase estradiol increased from 0.19 to 0.49. We observed the highest ICC for plasma estradiol and estrone in a subset of women who collected both blood samples at approximately the same time in the luteal phase and were ovulatory in each cycle.

Reproducibility was high for total estradiol, estrone, and estrone sulfate measured in the follicular phase of the menstrual cycle. To our knowledge, no other study has measured the reproducibility of estrogens in the follicular phase in premenopausal women. Similarly, the two urinary estrogen metabolites measured in this study, 2-hydroxyestrone and 16α-hydroxyestrone, as well as the ratio of the two, had good reproducibility over a 1-year period when adjusted for creatinine levels. Pasagian-Macaulay *et al.* (13) reported a similar ICC for the ratio of these two metabolites over a 6-month period in a sample of 171 premenopausal women (ICC = 0.67), although the two samples were collected at random during the menstrual cycle.

Only one previous study has assessed the reproducibility over time of total estradiol in premenopausal women (9). In this study, estradiol was measured in two blood samples obtained from each of 60 premenopausal women. The samples were collected on the same day of the luteal phase (between the 20th and 24th day, counting forward) about 1 year apart. The authors observed a very low ICC for total estradiol (ICC = 0.06). This result is consistent with our data, inasmuch as we also observed poor reliability (ICC = 0.19) for total estradiol in the luteal phase before the exclusion of women who were anovulatory or missed their luteal phase.

Studies on menstrual cycle fluctuations have shown that the luteal phase is more consistent in length than the follicular phase (15, 16). For this reason, we requested that women collect luteal-phase samples 7–9 days before the first day of their next anticipated menses rather than between the 20th and 22th days of the cycle (counting forward). By subsequently obtaining postcards from the women to confirm the first day of their next cycle, we were able to remove from the analysis samples that were not collected in the luteal phase. By measuring luteal-phase progesterone levels, we also were able to exclude women who had one or more anovulatory cycles. Because the age of the women and the interval over which samples were collected in our study are similar to these variables in the study of Muti *et al.* (9), the low ICC they reported was probably due to an inability to make these important exclusions.

This study was relatively small, and only a fraction of eligible women provided two sets of samples. However, when we compared the women who participated to the entire group of eligibles, with just two exceptions, the two groups were quite similar. Participants were less likely to smoke and were more likely to report a history of benign breast disease. Adjusting for smoking status and history of benign breast disease did not alter our ICCs, and, although we had insufficient power to assess whether the ICCs varied according to these attributes (*e.g.*, ICCs in smokers *versus* nonsmokers), substantial differences would seem unlikely.

Because reproducibility studies require two samples from each participant, our sample size for the restricted (luteal) analysis was limited to the 39 women who obtained both samples 4–10 days before their next cycle and were ovulatory at each cycle. However, if one sample from each woman had been sufficient, the sample size would have been substantially larger; *i.e.*, 63% of all of the samples obtained were collected between 4 and 10 days. Because of study size, cost considerations, and concerns about participation rates, only one sample per participant can be obtained in most epidemiological studies. Therefore, it would be reasonable for researchers to collect luteal samples in a manner similar to that used in this study and to match cases and controls on the number of days the samples were collected before the next menses.

The ICCs observed in this study are comparable in magnitude to those for a number of other exposure measures commonly used in epidemiology. For example, correlations have been reported for serum cholesterol of 0.65 over 1 year (24) and 0.76 over 2 years (25) and for systolic blood pressure of 0.60 over 2 years (25). Correlations in this range result in relatively modest decreases in the estimated relative risk, although the degree of attenuation will depend on the magnitude of the relative risk (8). For example, measurement error in a variable with an ICC of 0.68 will lower a true relative risk of 2.0 and 2.5 to 1.6 and 1.9, respectively (8). Besides providing important information on the reproducibility of a variable, ICCs can be used to correct relative risk estimates for random within-person measurement error in epidemiological studies (26).

Results from this study indicate that a single sample of blood or urine collected during the follicular or midluteal phase of the menstrual cycle in premenopausal women can reasonably represent estrogen levels in blood or their metabolites in urine over at least a one-year period. Studies that assess reproducibility over a longer period are needed to further delineate long-term stability in premenopausal women.

References

1. Toniolo, P. G., Levitz, M., Zeleniuch-Jacqotte, A., Banerjee, S., Koenig, K. L., Shore, R. E., Strax, P., and Pasternack, B. S. A prospective study of endogenous estrogens and breast cancer in postmenopausal women. J. Natl. Cancer Inst., *87:* 190–197, 1995.

2. Thomas, H. V., Key, T. J., Allen, D. S., Moore, J. W., Dowsett, M., and Fentiman, I. S. A prospective study of endogenous serum hormone concentrations and breast cancer risk in postmenopausal women on the island of Guernsey. Br. J. Cancer, *76:* 401–405, 1997.

3. Hankinson, S. E., Willett, W. C., Manson, J. E., Colditz, G. A., Hunter, D. J., Spiegelman, D., Barbieri, R. L., and Speizer, F. E. Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women. J. Natl. Cancer Inst., *90:* 1292–1299, 1998.

4. Thomas, H. V., Reeves, G. K., and Key, T. J. Endogenous estrogen and postmenopausal breast cancer: a quantitative review. Cancer Causes Control, *8:* 922–928, 1997.

5. Cauley, J. A., Lucas, F. L., Kuller, L. H., Stone, K., Browner, W., and Cummings, S. R. Elevated serum estradiol and testosterone concentrations are associated with a high risk for breast cancer. Study of Osteoporotic Fractures Research Group. Ann. Intern. Med., *130:* 270–277, 1999.

6. Thomas, H. V., Key, T. J., Allen, D. S., Moore, J. W., Dowsett, M., Fentiman, I. S., and Wang, D. Y. A prospective study of endogenous serum hormone concentrations and breast cancer risk in premenopausal women on the island of Guernsey. Br. J. Cancer, *75:* 1075–1079, 1997.

7. Bernstein, L., and Ross, R. K. Endogenous hormones and breast cancer risk. Epidemiol. Rev., *15:* 48–65, 1993.

8. Hankinson, S. E., Manson, J. E., Spiegelman, D., Willett, W. C., Longcope, C., and Speizer, F. Reproducibility of plasma hormone levels in postmenopausal women over a 2–3 year period. Cancer Epidemiol., Biomark. Prev., *4:* 649–654, 1995.

9. Muti, P., Trevisan, M., Micheli, A., Krogh, V., Bolelli, G., Sciajno, R., and Berrino, F. Reliability of serum hormones in premenopausal and postmenopausal women over a one-year period. Cancer Epidemiol. Biomark. Prev., *5:* 917–922, 1996.

10. Toniolo, P., Koenig, K. L., Pasternack, B. S., Banerjee, S., Rosenberg, C., Shore, R. E., Strax, P., and Levitz, M. Reliabililty of measurements of total, protein-bound, and unbound estradiol in serum. Cancer Epidemiol. Biomark. Prev., *3:* 47–50, 1994.

11. Koenig, K. L., Toniolo, P., Bruning, P. F., Bonfrer, J. M. G., Shore, R. E., and Pasternack, B. S. Reliability of serum prolactin measurements in women. Cancer Epidemiol. Biomark. Prev., *2:* 411–414, 1993.

12. Micheli, A., Muti, P., Pisani, P., Secreto, G., Recchione, C., Totis, A., Fissi, R., Cavelleri, A., Panico, S., and Berrino, F. Repeated serum and urinary androgen measurements in premenopausal and postmenopausal women. J. Clin. Epidemiol., *44:* 1055–1061, 1991.

13. Pasagian-Macaulay, A., Meilahn, E. N., Bradlow, H. L., Sepkovic, D. W., Buhari, A. M., Simkin-Silverman, L., Wing, R. R., and Kuller, L. H. Urinary markers of estrogen metabolism 2- and 16α-hydroxylation in premenopausal women. Steroids, *61:* 461–467, 1996.

14. Rockhill, B., Willet, W. C., Hunter, D. J., Manson, J. E., Hankinson, S. E., Spiegelman, D., and Colditz, G. Physical activity and breast cancer risk in a cohort of young women. J. Natl. Cancer Inst., *90:* 1155–1160, 1998.

15. Lenton, E. A., Landgren, B. M., Sexton, L., and Harper, R. Normal variation in the length of the follicular phase of the menstrual cycle: effect of chronological age. J. Obstet. Gynecol., *91:* 681–684, 1984.

16. Lenton, E. A., Landgren, B. M., and Sexton, L. Normal variation in the length of the luteal phase of the menstrual cycle: identificaton of the short luteal phase. J. Obstet. Gynecol., *91:* 685–689, 1984.

17. Hankinson, S. E., London, S. J., Chute, C. G., Barbieri, R. L., Jones, L. A., Kaplan, L. A., Sacks, F. M., and Stampfer, M. J. Effect of transportation conditions on the stability of biochemical markers in blood. Clin. Chem., *35:* 2313–2316, 1989.

18. Mikhail, G., and Chung, H. W. RIA of plasma estrogens. Use of polymerized antibodies. *In:* F. G. Peron and B. V. Caldwell (eds.), Immunological Methods in Steroid Determination, p. 113. New York: Appleton-Century-Crofts, 1970.

19. Judd, H. L., Lucas, W. E., and Yen, S. S. C. Serum 17 β-estradiol and estrone levels in postmenopausal women with and without endometrial cancer. J. Clin. Endocrinol. Metab., *43:* 272, 1976.

20. Franz, C., Watson, D., and Longcope, C. Estrone sulfate and dehydroepiandrosterone sulfate concentrations in normal subjects and men with cirrhosis. Steroids, *34:* 563–573, 1979.

21. Kutas, M., Chung, A., Bartos, D., and Castro A. A simple progesterone radioimmunoassay without column chromatography. Steroids, *20:* 697–716, 1972.

22. Bradlow, H. L., Sepkovic, D. W., Klug, T., and Osborne, M. P. Application of an improved ELISA assay to the analysis of urinary estrogen metabolites. Steroids, *63:* 406–413, 1998.

23. Rosner, B. Fundamentals of Biostatistics, Ed. 4, p. 518. Pacific Grove, CA: Duxbury Press, 1995.

24. Shekelle, R. B., Shryrock, A. M., Paul, O., Lepper, M., Stamler, J., Liu, S., and Raynor, W. J., Jr. Diet, serum cholesterol, and death from coronary heart disease: the Western Electric study. N. Engl. J. Med., *304:* 65–70,1981.

25. Cauley, J. A., Gutai, J. P., Kuller, L. H., and Powell, J. G. Reliability and interrelations among serum sex hormones in postmenopausal women. Am. J. Epidemiol., *133:* 50–57, 1991.

26. Rosner, B., Spiegelman, D., and Willett, W. C. Correction of logistic regression relative risk estimates and CIs for random within-person measurement error. Am. J. Epidemiol., *136:* 1400–1413, 1992.

Reproducibility of Plasma and Urinary Sex Hormone Levels in Premenopausal Women over a One-Year Period

Dominique S. Michaud, JoAnn E. Manson, Donna Spiegelman, et al.

Cancer Epidemiol Biomarkers Prev 1999;8:1059-1064.