Some Sex Hormone Profiles are Consistent over Time in Normal Menstruating Women: Implications for Sports Injury Epidemiology

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Abstract:

Purpose-It is unclear whether sex hormone profiles obtained in two consecutive months are consistent within women. Month-to-month consistency in daily, nadir, peak and mean hormone concentrations during the early follicular and luteal phases in recreationally active, young eumenorrheic women was prospectively examined.

Methods-60 healthy, non-smoking women who reported normal and consistent menstrual cycles lasting 26–32 days for the past 6 months were followed prospectively to obtain serum samples for the first 6 days of menses and for 8 days after a positive ovulation test over two consecutive months. Month-to-month consistency of daily concentrations of oestradiol (pg/ml), progesterone (ng/ml), testosterone (ng/dl), sex hormone-binding globulin (nmol/l) and free androgen index were determined using linear mixed models. Month-to-month consistency in nadir, peak and mean concentrations were then assessed using intraclass correlation coefficients and SEM to more precisely examine intraindividual consistency.

Results-Linear mixed models revealed stable hormone concentrations across cycles and cycles by day. Reliability estimates for nadir, peak, mean menses and mean postovulatory concentrations range from 0.56 to 0.86 for oestradiol, 0.44 to 0.91 for progesterone, 0.60 to 0.86 for testosterone, 0.88 to 0.97 for sex hormone-binding globulin and 0.78 to 0.91 for free androgen index.

Conclusions-Hormone profiles were reproducible over two consecutive months. To reduce month-to-month intraindividual variations and improve measurement consistency, it is recommended that multiple samples be taken over consecutive days as opposed to a single sample.

Article:

Variations in sex hormone concentrations in young, physically active women may be associated with the risk of non-contact anterior cruciate ligament (ACL) injury. Studies report a greater number of injuries than expected during the perimenstrual[1–3] and periovulatory[4 5] days, whereas others generally identified the follicular phase as being the phase of higher risk.[6 7] These studies collected a single sample (blood or urine) shortly after the injury (range 2 h[7] to 72 h[2]), making it difficult to identify the specific time in a particular phase when injury occurred (ie, whether hormone levels were rising, falling or near their peak). Other work suggests a time delay

between when hormone concentration change and when soft tissues change (eg, laxity),[8] emphasising the importance of documenting hormone profiles in the days preceding the injury. Because ACL injuries occur infrequently, retrospective studies are the most practical research design to comprehensively examine hormone profiles associated with injury risk. For retrospective studies to be valid, establishing consistency of hormone profiles month to month is both necessary and paramount as a first step. The application of the present data may also be useful in a research setting when projecting hormonal phases for future data collections.

Although the typical hormone profile of a 28-day cycle is well established, women have substantial variations from this typical profile in their cycle length (both follicular and luteal phases), the timing of changes in one hormone relative to another, the day of ovulation and absolute change in hormone concentrations.[8–12] Although this variability is substantially greater between women than within a woman from one month to the next, some variations within a woman also occur.[13] Therefore, it is important to quantify the magnitude of these intraindividual variations to determine how consistent an individual's hormone profile will be from one month to the next. We examined the month-to-month consistency in daily, nadir, peak and mean hormone profiles during the first 6 days of the early follicular phase and the first 8 days of the early luteal phase for two consecutive cycles in young, normal menstruating women. Our expectation was that sex hormone profiles would be highly reproducible from one month to the next.

METHODS

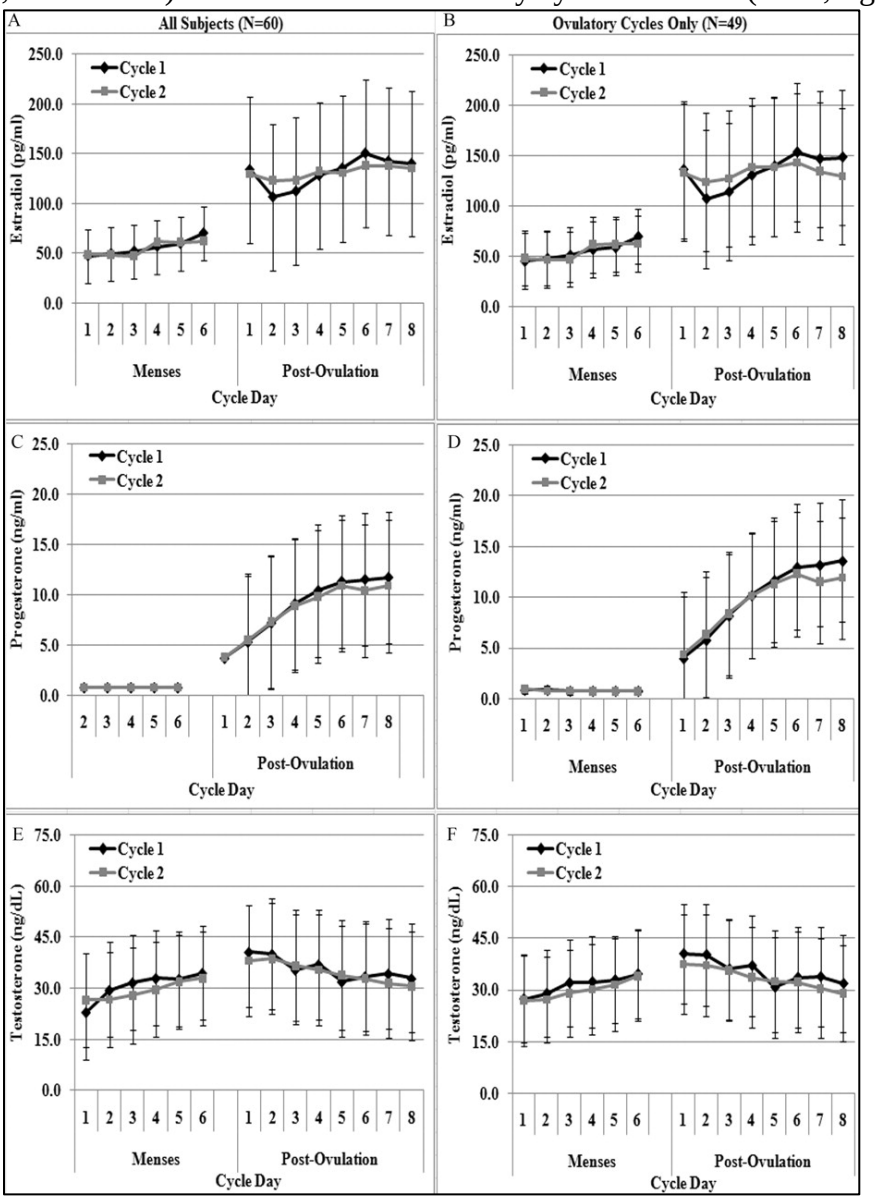
Serum samples were obtained prospectively from 60 women (age 21.7 (2.6) years, height 163.9 (6.5) cm and weight 60.3 (8.6) kg), participating in a larger project examining the effects of sex hormone-mediated changes in knee laxity on knee joint function. Subjects were included if they self-reported regular physical activity between 2.5 and 10 h per week for the past 3 months, normal menstrual cycles lasting 26–32 days with consistent cycle lengths varying no more than ±1 day each month for the past 6 months, no use of oral contraceptives or other hormone-stimulating medications for the past 6 months, and no history of pregnancy. Subjects with a body mass index >30 (BMI; weight/height^2), a previous history of knee ligament injury, or who smoked were excluded. At the time of this study, 67 female subjects had been enrolled in the larger study. However, seven were excluded because of incomplete data (four voluntarily withdrew; three lacked a positive ovulation test). All participants were informed of the study procedures and signed a consent form approved by the university's Institutional Review Board for the Protection of Human Subjects.

Procedures

Serum (10 cc) was collected daily using standard venipuncture procedures during the first 6 days of the early follicular phase (day 1 identified as the day immediately after the onset of menses per self-report; labelled M1–M6) and for the first 8 days of the early luteal phase (day 1 identified as the first day after evidence of ovulation; labelled L1–L8) for two consecutive months. To control for diurnal fluctuations in hormone concentrations, all samples were obtained in the morning hours (07:00–09:00, usually within ±30 min for each participant) before physical activity. To estimate the day of ovulation, participants used a commercially available ovulation kit (CVS One Step Ovulation Predictor (sensitivity 20 mIU ml luteinizing hormone, accuracy 99%; CVS Corporation, Woonsocket, Rhode Island, USA) starting with day 8 of their menstrual cycle. Participants were instructed to maintain normal activity patterns and avoid excessive

physical activity for 2 days before any testing and to defer their normal activity until their serum was collected on each test day. Subjects were also instructed to abstain for alcohol consumption for 24 h prior and throughout each testing block. Participants completed a daily questionnaire to ensure study compliance.

Figure 1: Daily mean early follicular (M1–M6) and early luteal (L1–L8) oestradiol (A, B), progesterone (C, D) and testosterone (E, F) concentrations over two consecutive months for all subjects (N=60, left column) and those with anovulatory cycles removed (n=49, right column).

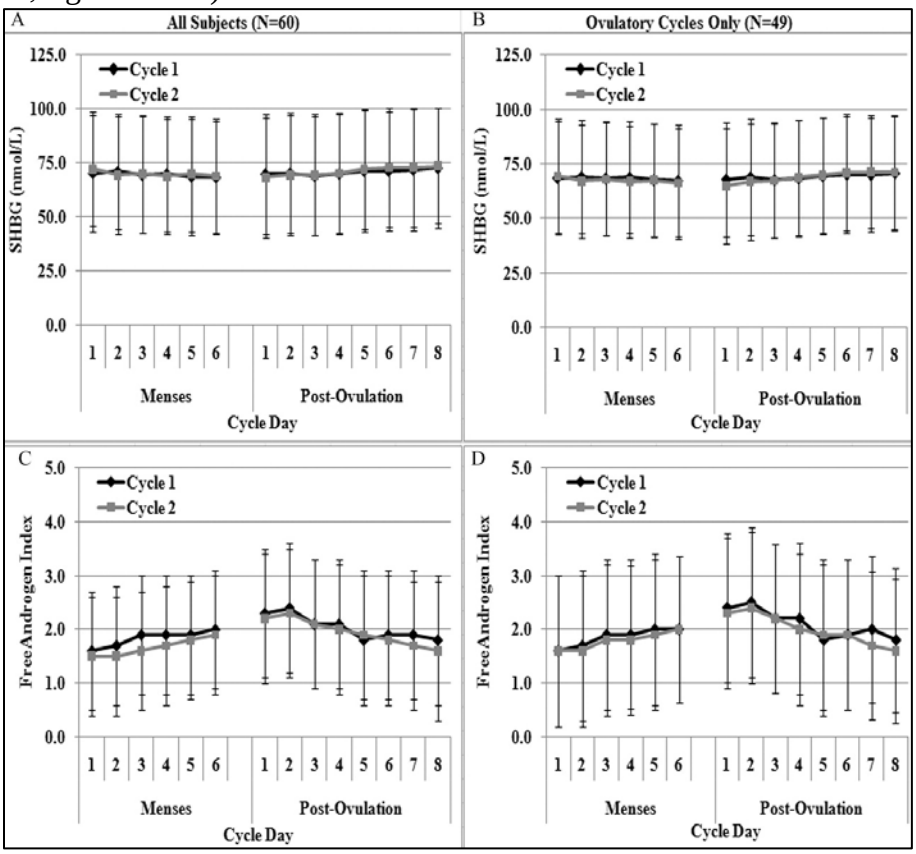


Assays

Blood samples were separated and stored at −80°C and shipped to a Ligand Assay and Analysis Core Laboratory to assay serum levels of oestradiol, progesterone, testosterone and sex hormone-binding globulin (SHBG). All samples for a given subject were analysed within the same assay test kit. Oestradiol was assayed using a double-antibody radioimmunoassay assay (DSL-4400;

Beckman Coulter, Webster, Texas, USA), and progesterone and testosterone concentrations were assayed using Coat-A-Count radioimmunoassay assays (TKPG-2 and TKTT-2; Siemens Medical Solutions Diagnostics, Los Angeles, California, USA). SHBG was assayed using the Immulite chemiluminescent technology (LKSH5; Siemens Medical Solutions Diagnostics). Free androgen index (FAI) was calculated based on testosterone and SHBG levels (FAI=total testosterone (nmol/l)/SHBG (nmol/l)). Mean percent intra-assay and inter-assay coefficient of variations (%CV), respectively, were 5.2% and 10.6% for oestradiol, 4.1% and 6.4% for progesterone, 3.4% and 8.1% for testosterone, and 2.4% and 5.8% for SHBG. Assay sensitivities were 10 pg/ml (oestradiol), 0.1 ng/ml (progesterone), 10 ng dl (testosterone) and 0.2 nmol/l (SHBG).

Figure 2: Daily mean early follicular (M1–M6) and early luteal (L1–L8) serum hormone-binding globulin (SHBG) (A, B) and free androgen index (C, D) concentrations over two consecutive months for all subjects (N=60, left column) and those with anovulatory cycles removed (n=49, right column).



Although oestradiol, progesterone and, to a lesser extent, testosterone have been the primary hormones of interest when describing the hormone profile of a particular woman, the capacity of these sex steroids to exert their effect on soft tissues is dependent on the amount of each sex hormone that is freely circulating (ie, biologically active).[14 15] SHBG is considered to be the major regulator of plasma free concentrations for oestradiol and testosterone because it has a high binding affinity for these hormones. Although research suggests that little if any change in SHBG concentrations occur across the menstrual cycle[16] (thus the freely circulating concentration of oestradiol and testosterone), we felt it was important to confirm the extent to which SHBG concentrations (and similarly a measure of the biologically available fraction of a

hormone, ie, FAI in this study) vary from cycle to cycle because this would have further implications on the ability to reliably predict hormone concentrations retrospectively.

Data analysis

To examine the general consistency of daily changes in hormone concentrations during the 6 menses days (M1–M6) and the 8 postovulatory days (L1–L8) from one month to the next, we ran separate linear mixed models (Proc Mixed, Statistical Analysis System V.9.1.2; SAS Institute, Cary, North Carolina, USA) for each of the hormones, with the hormone as the dependent variable and cycle, day, and the cycle-by-day interaction as the independent variables. Significance was set a priori at $p<0.05$. To more closely examine the intraindividual month-to-month consistency of hormone profiles, intraclass correlation coefficient formula 2,1 ($ICC_{2,1}$)[17] compared cycles 1 and 2 on absolute nadir and peak hormone concentrations obtained during M1–M6 and during L1–L8, respectively. We then used ICC formula 2,k[17] to compare cycles 1 and 2 on mean concentrations obtained across M1–M6 and L1–L8. For each reliability estimate, the SEM was calculated ($SD\sqrt{(1-ICC)}$).[18]

Once the serum was assayed, 11 women (18%) were found to have anovulatory cycles in one (n=8) or both (n=3) months, defined as a luteal phase progesterone level that did not exceed 3 ng/ml.[19 20] Therefore, we ran all our analyses on both the entire data set (N=60) and then limited analyses to those with two confirmed ovulatory cycles (n=49) to determine the effect of anovulatory cycles on measurement consistency. Our rationale to include anovulatory cycles is that it may not always be possible to confirm the presence or absence of anovulatory cycles in retrospective studies.

RESULTS

Comparison of daily hormone concentrations between cycles. The daily means and SD of the five hormone concentrations during M1–M6 and L1–L8 over two cycles are shown in figs 1 and 2. Table 1 reports the linear mixed model results comparing hormone concentrations between cycles, days and cycle by days. During M1–M6, all hormone concentrations differed between individual days, except for SHBG, which remained stable ($p>0.117$). In all cases, daily changes in hormone concentrations were generally consistent between cycles because no significant differences in hormone concentrations by cycle or cycle by day were observed. When examining L1–L8, all hormone concentrations differed between individual days (all $p<0.004$), and these differences were also consistent across cycle and cycle by day. Results did not change when anovulatory cycles were removed.

Consistency of nadir, peak and mean hormone concentrations. Table 2 lists the means, SD and reliability estimates examining the month-to-month consistency in absolute nadir and peak hormone concentrations. ICCs for the entire sample ranged from 0.58 to 0.88 for nadir and 0.44 to 0.89 for peak concentrations. Although ICC values were consistently high for SHBG and FAI (0.78–0.89), estimates were lower for oestradiol, progesterone and testosterone levels (0.44–0.71). Analyses of the sources of variance and SEM values for these hormones indicate that the lower ICCs were primarily due to random error rather than systematic differences in concentrations between cycles. When anovulatory cycles were removed, ICC values were similar, except for nadir and peak oestradiol levels, where the ICC values decreased somewhat but the SEMs stayed relatively unchanged.

Table 1: ANOVA results for sex steroid hormones when all subjects were included in the analysis and when analysis included only subjects with two ovulatory cycles

		p Value	
	Effect	**All subjects (N=60)**	**Ovulatory cycles (n=49)**
Oestradiol			
Menses	Cycle	0.691	0.880
	Day	<0.001*	<0.001*
	Cycle×day	0.201	0.335
Postovulatory	Cycle	0.932	0.885
	Day	0.004*	0.003*
	Cycle×day	0.353	0.145
Progesterone			
Menses	Cycle	0.480	0.391
	Day	<0.001*	<0.001*
	Cycle×day	0.395	0.341
Postovulatory	Cycle	0.657	0.561
	Day	<0.001*	<0.001*
	Cycle×day	0.842	0.247
Testosterone			
Menses	Cycle	0.058	0.243
	Day	<0.001*	<0.001*
	Cycle×day	0.597	0.807
Postovulatory	Cycle	0.387	0.077
	Day	<0.001*	<0.001*
	Cycle×day	0.498	0.380
SHBG			
Menses	Cycle	0.749	0.518
	Day	0.117	0.374
	Cycle×day	0.321	0.696
Postovulatory	Cycle	0.820	0.874
	Day	<0.001*	<0.001*
	Cycle×day	0.761	0.416
FAI			
Menses	Cycle	0.052	0.313
	Day	<0.001*	<0.001*
	Cycle×day	0.760	0.834
Postovulatory	Cycle	0.222	0.182
	Day	<0.001*	<.0001*
	Cycle×day	0.485	0.415

ANOVA, analysis of variance; FAI, free androgen index; SHBG, sex hormone-binding globulin.
Analysis was separated by phase of the menstrual cycle (menses and postovulatory).
* p<0.05.

Table 3 lists the means, SD and reliability estimates examining the month-to-month consistency in the mean hormone concentrations collapsed across M1–M6 and across L1–L8. These analyses revealed substantially stronger ICCs and improved precision (SEMs) compared with those obtained for peak and nadir concentrations. ICC values ranged from 0.81 to 0.97 (and were similar with and without anovulatory cycles), with the exception of mean postovulatory progesterone levels (0.54–0.59), which were more variable month to month.

Table 2: Mean, SD, ICC and SEM comparing month-to-month consistency in absolute nadir (from the 6 days of menses) and peak (from the 8 postovulatory days) sex hormone concentrations for all subjects and with anovulatory cycles removed

	Cycle 1	Cycle 2		
	Mean (SD)	**Mean (SD)**	**ICC**	**SEM**
All subjects (N=60)				
Nadir (menses) values				
Oestradiol (pg/ml)	37.5 (14.0)	35.4 (15.2)	0.58	9.8
Progesterone (ng/ml)	0.6 (0.3)	0.6 (0.3)	0.67	0.2
Testosterone (ng/dl)	22.6 (11.4)	20.2 (10.2)	0.60	7.2
SHBG (nmol/l)	60.8 (24.2)	62.9 (23.6)	0.88	8.4
FAI	1.3 (0.9)	1.1 (0.8)	0.78	0.4
Peak (postovulatory) values				
Oestradiol (pg/ml)	208.7 (92.5)	209.0 (111.2)	0.56	73.6
Progesterone (ng/ml)	14.6 (7.8)	13.9 (7.9)	0.44	5.9
Testosterone (ng/dl)	49.2 (17.4)	48.7 (19.6)	0.71	10.5
SHBG (nmol/l)	80.0 (30.1)	80.9 (33.8)	0.89	11.4
FAI	2.9 (1.6)	2.8 (1.5)	0.82	0.7
Ovulatory cycles only (n=49)				
Nadir (menses) values				
Oestradiol (pg/ml)	36.0 (12.7)	34.3 (14.7)	0.48	10.6
Progesterone (ng/ml)	0.6 (0.3)	0.6 (0.2)	0.66	0.2
Testosterone (ng/dl)	22.6 (10.9)	21.0 (9.7)	0.60	6.9
SHBG (nmol/l)	59.5 (23.6)	60.7 (22.2)	0.86	8.7
FAI	1.3 (0.9)	1.2 (0.8)	0.82	0.4
Peak (postovulatory) values				
Oestradiol (pg/ml)	214.1 (83.1)	211.1 (95.3)	0.38	74.8
Progesterone (ng/ml)	16.5 (6.7)	15.7 (6.5)	0.41	5.1
Testosterone (ng/dl)	48.5 (16.7)	46.6 (15.6)	0.73	8.6
SHBG (nmol/l)	77.7 (28.4)	78.0 (31.5)	0.87	11.3
FAI	3.0 (1.7)	2.9 (1.6)	0.85	0.7

FAI, free androgen index; ICC, intraclass correlation coefficient; SEM, standard error of measurement; SHBG, sex hormone-binding globulin.

Table 3: Mean, SD, ICC (2,k) and SEM examining the consistency of mean sex hormone concentrations during menses (days 1–6) and postovulation (days 1–8) across two menstrual cycles for all subjects and with anovulatory cycles removed

	Cycle 1	Cycle 2		
	Mean (SD)	**Mean (SD)**	**ICC**	**SEM**
All subjects (N=60)				
Mean menses values				
Oestradiol (pg/ml)	55.6 (19.7)	53.7 (18.7)	0.86	7.5
Progesterone (ng/ml)	0.8 (0.3)	0.8 (0.3)	0.91	0.1
Testosterone (ng/dl)	31.6 (13.0)	29.3 (11.4)	0.81	5.7
SHBG (nmol/l)	69.4 (26.7)	69.8 (25.8)	0.97	4.5
FAI	1.8 (1.1)	1.7 (1.0)	0.90	0.3
Mean postovulatory values				
Oestradiol (pg/ml)	131.0 (46.3)	131.3 (55.0)	0.82	23.2
Progesterone (ng/ml)	8.7 (5.0)	8.4 (5.6)	0.59	3.7
Testosterone (ng/dl)	35.8 (12.9)	34.7 (14.4)	0.86	5.3
SHBG (nmol/l)	61.4 (21.0)	71.0 (27.9)	0.90	8.8
FAI	2.1 (1.2)	2.0 (1.0)	0.91	0.4
Ovulatory cycles only (n=49)				
Mean menses values				
Oestradiol (pg/ml)	55.0 (19.2)	53.2 (17.8)	0.83	8.0
Progesterone (ng/ml)	0.8 (0.3)	0.8 (0.3)	0.90	0.1
Testosterone (ng/dl)	31.3 (11.7)	30.0 (10.9)	0.81	5.1
SHBG (nmol/l)	68.3 (25.9)	67.5 (24.1)	0.97	4.5
FAI	1.9 (1.1)	1.8 (1.0)	0.93	0.3
Mean postovulatory values				
Oestradiol (pg/ml)	134.4 (40.8)	133.6 (50.4)	0.81	22.2
Progesterone (ng/ml)	10.0 (4.4)	9.5 (4.8)	0.54	3.3
Testosterone (ng/dl)	35.5 (12.5)	33.6 (11.5)	0.88	4.1
SHBG (nmol/l)	59.9 (19.5)	68.9 (26.6)	0.90	8.6
FAI	2.1 (1.3)	2.0 (1.1)	0.94	0.3

FAI, free androgen index; ICC, intraclass correlation coefficient; SEM, standard error of measurement; SHBG, sex hormone-binding globulin.

For descriptive purposes only, table 4 lists the means and SD comparing nadir, peak and mean hormone concentrations between women with two ovulatory cycles (OVUL, n=49), one ovulatory and one anovulatory cycle (OVUL/ANOV, n=8) and two anovulatory cycles (ANOV, n=3). Although difficult to examine statistically given the small number of women with anovulatory cycles, oestradiol levels appear to vary considerably when women were stratified based on the consistency of ovulatory versus anovulatory cycles. Specifically, women with anovulatory cycles had substantially lower luteal phase oestradiol levels, with the lowest levels observed in those with consistent anovulatory cycles. SHBG concentrations also appear to vary somewhat, with higher concentrations observed in women with inconsistent ovulatory cycles and somewhat lower concentrations in those who had consistent anovulatory cycles. Related to these differences in SHBG, proportional changes in FAI were observed, suggesting that the free fraction concentrations of these hormones may also differ between these groups.

Table 4: Means and SDs comparing nadir, peak and mean hormone concentrations between women with two ovulatory cycles (OVUL, n=49), one ovulatory and one anovulatory cycle (OVUL/ANOV, n=8) and two anovulatory cycles (ANOV, n=3)

		OVUL/ANOV (n=8)		
	OVUL (n=49)	**OVUL cycle**	**ANOV cycle**	**ANOV (n=3)**
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Nadir and peak values				
Nadir (M1–M6)				
Oestradiol (pg/ml)	36.0 (12.7)	50.9 (60.2)	43.4 (11.1)	29.4 (18.9)
Progesterone (ng/ml)	0.6 (0.3)	0.7 (0.4)	0.6 (0.2)	0.5 (0.2)
Testosterone (ng/dl)	22.6 (10.9)	24.2 (11.9)	16.6 (10.3)	17.5 (18.2)
SHBG (nmol/l)	59.5 (23.6	76.6 (33.0)	75.2 (26.5)	53.4 (12.2)
FAI	1.3 (0.9)	1.0 (0.3)	0.8 (0.5)	1.0 (1.1)
Peak (L1–L8)				
Oestradiol (pg/ml)	214.1 (83.1)	261.8 (181.0)	197.1 (137.9)	92.5 (18.2)
Progesterone (ng/ml)	16.5 (6.7)	14.8 (7.4)	1.4 (0.5)	1.4 (0.9)
Testosterone (ng/dl)	48.5 (16.7)	59.0 (22.0)	60.8 (33.8)	42.1 (17.1)
SHBG (nmol/l)	77.7 (28.4)	105.3 (40.2)	98.0 (41.0)	61.8 (18.2)
FAI	3.0 (1.7)	2.6 (1.0)	2.4 (0.8)	2.8 (1.2)
Mean values				
Menses (M1–M6)				
Oestradiol (pg/ml)	55.0 (19.2)	64.5 (22.3)	62.3 (17.3)	39.8 (22.1)
Progesterone (ng/ml)	0.8 (0.3)	0.9 (0.5)	0.8 (0.3)	0.7 (0.3)
Testosterone (ng/dl)	31.3 (11.7)	35.0 (14.6)	26.7 (13.4)	26.1 (22.1)
SHBG (nmol/l)	68.3 (25.9)	85.5 (35.4)	83.6 (31.3)	57.8 (13.2)
FAI	1.9 (1.1)	1.5 (0.3)	1.1 (0.4)	1.6 (1.5)
Postovulatory (L1–L8)				
Oestradiol (pg/ml)	134.4 (40.8)	162.1 (74.1)	117.1 (59.2)	61.6 (14.4)
Progesterone (ng/ml)	10.0 (4.4)	8.0 (5.8)	1.0 (0.5)	0.9 (0.5)
Testosterone (ng/dl)	35.5 (12.5)	40.5 (14.8)	43.8 (24.2)	29.3 (16.4)
SHBG (nmol/l)	59.9 (19.5)	85.6 (32.1)	79.4 (29.9)	52.4 (13.0)
FAI	2.1 (1.3)	1.6 (0.4)	1.8 (0.8)	1.9 (1.1)

FAI, free androgen index; SHBG, sex hormone-binding globulin.

DISCUSSION

Much of the literature on hormone repeatability focuses on older premenopausal women (typically 35–50 years of age) to determine whether a single hormone measure can reliably predict hormone exposure over time, thus future disease risk.[21–25] However, in the case of ACL injury where acute hormone exposure is of interest, there is a need to know if hormone profiles obtained postinjury can adequately reflect the hormone profiles just before injury. As an initial step towards this effort, we quantified the consistency in hormone profiles in young, physically

active and normal menstruating women. Our findings in a group of young, recreationally active eumenorrheic women revealed that daily mean hormone concentrations measured during M1–M6 and L1–L8 varied by day as expected, but were generally stable from one month to the next. However, when examining intraindividual month-to-month consistency in nadir, peak and mean hormone concentrations, reliability estimates varied across the five hormones tested. With the exception of nadir and peak oestradiol (0.38–0.48 with anovulatory cycles removed) and peak progesterone levels (0.41 and 0.44 with and without anovulatory cycles), reliability estimates for all other nadir and peak hormone values ranged from 0.56 to 0.89. These estimates appear to represent highly reliable measures based on what has been reported in the hormone literature.[22 24 25] This is particularly true of SHBG and FAI, suggesting that the proportion of each hormone concentration that is biologically available is very stable from month-to-month within a woman. The lower reliability we observed for nadir and peak oestradiol and progesterone levels is consistent with previous studies in premenopausal women where a single measure of oestradiol and progesterone was obtained (oestradiol (0.38–0.53 for follicular measures, 0.06–0.45 for luteal phase measures),[13 21 22 24 25] progesterone (0.29–0.54 for luteal phase measures)),[22 24] suggesting there is substantial month-to-month variations in absolute daily levels of oestradiol and progesterone within a woman, particularly during the postovulatory days. However, when concentrations are averaged over multiple days, reliability estimates improved considerably (table 2 vs 3). Hence, it may be necessary to take multiple samples to gain an adequate representation of a woman's hormone profile, particularly when examining oestradiol and progesterone.

To fully appreciate the magnitude of this variability, the SEM provides a unit value of measurement precision that is based on the distribution of measurement error.[26] Specifically, there is a 68% and 95% chance that the participant's true hormone value will fall within ±1 or ±2 SEMs, respectively, of the value obtained from a subsequent month. In some cases, particularly for peak oestradiol, progesterone and testosterone levels, the SEMs seem rather large and suggest considerable measurement error. When these error variances are compared against the overall deviation and range in concentrations obtained in this cohort, SEM values generally represented <15% of the total range in concentrations obtained for nadir (7.0–14.5%) and peak (8.2–16.3%), and <10% of the total range in mean menses (3.6–7.5%) and mean luteal (6.2–11.7%) values. The only exception was peak progesterone levels, where the SEM represented 18–20% of the range in values for nadir and peak levels, and 10–19% of the range in values for mean menses and luteal values. As epidemiological studies often reduce hormone values to quartiles when classifying the association of a particular hormone with disease,[22] the precision of these values may be acceptable. The improved measurement precision when using mean values (table 3) again indicates the importance of taking multiple samples to enhance the accuracy of determining hormone profiles in a subsequent month.

Our findings also revealed that measurement consistency and precision were relatively robust to the presence of occasional anovulatory cycles. When women with one or both anovulatory cycles were removed (18% of the sample), the ICC and SEM values remained relatively unchanged except for nadir and peak oestradiol values (table 2). As noted in table 4, women with anovulatory cycles had substantially lower oestradiol levels, particularly during the luteal phase. Hence, the lower ICC values for oestradiol values when anovulatory cycles were removed may result from a lower proportion of between-subject variance. Perhaps most important is that the

SEM values for luteal-phase oestradiol and progesterone did not improve appreciably when the eight subjects with inconsistent ovulatory cycles (thus inconsistent oestradiol and progesterone levels) were removed. This further speaks to the inherent intraindividual variation in oestradiol and progesterone levels, even in those with consistent ovulatory cycles.

In summary, sex hormone profiles are in large part reproducible over two consecutive months in young, recreationally active eumonorrheic women. Although intraindividual variations exist, they are substantially smaller than between-subject variations. To reduce month-to-month intraindividual variations and improve measurement accuracy, it is recommended that multiple samples be taken over consecutive days as opposed to a single sample to represent a given phase. Furthermore, the inherent stability of SHBG values within a woman from one month to the next suggest that although total hormone concentrations may vary somewhat within a woman from one cycle to the next, the proportion of these concentrations that are biologically active should change very little within a woman from one cycle to the next. Although these findings support the feasibility of retrospectively examining relationships between hormone profiles and injury risk, there are important limitations to the current work. Specifically, these findings are limited to healthy physically active women who, by nature of the parent project, were uninjured and reported normal menstrual cycles lasting 26–32 days for the past 6 months and maintained a consistent level of activity throughout the study. Before we can study hormone profiles retrospectively in an injured population, the impact of the trauma and acute changes in exercise status as a result of the injury on hormone profile reproducibility needs to be investigated. For example, one study observed a relationship between physical trauma and surgical stress with irregular cycles postinjury[27]; however, these findings were not based on a young physically active population undergoing surgery for ligament trauma. In regards to exercise, previous research has shown that the intensity of physical activity may also influence menstrual cycle characteristics,[28] and therefore a substantial modification in exercise due to injury may affect hormone profiles in some athletes.

These findings are also limited to the reproducibility of sex steroid hormone concentrations. As one report noted a greater than expected risk of ACL injury near ovulation,[5] and suggested that it may be important to also account for the gonadatropins that control ovulation and sex steroid secretion (follicle-stimulating hormone or luteinizing hormone) when examining injury susceptibility, studies examining the reproducibility of these gonadatropins should also be considered. Finally, it is well accepted that many competitive athletes experience irregular cycles, and we are not aware of studies that have examined hormone profile consistency in oligomenorrheic or amenorrheic female athletes. Hence, this study represents only a first step in understanding the feasibility of examining hormone profiles retrospectively. It is our hope that by first quantifying the natural intraindividual variations in hormone concentrations with these factors controlled, future work can better quantify any additional variability associated with these other factors. Specifically, there is a need to examine the immediate (eg, subsequent month) and longer term (eg, 3–6 months later) effects of musculoskeletal trauma and acute exercise changes on hormone reproducibility to determine the best postinjury time frame to obtain an accurate representation of a woman's typical hormone profile. Examining these effects in populations at greatest risk for ligament trauma (eg, basketball and soccer) is also important.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Although there is a growing consensus that the risk of ACL injury varies across the menstrual cycle, the hormonal profile associated with a greater likelihood of injury remains unclear. Because ACL injuries occur infrequently, retrospective studies offer the most practical research design to examine hormone profiles associated with injury risk.

WHAT THIS STUDY ADDS

To initially examine the feasibility of retrospectively examining hormone profiles, month-to-month consistency in hormone concentrations was examined in young, recreationally active eumonorrheic women. Results indicate that sex hormone profiles are in large part reproducible over two consecutive months, particularly when multiple samples are taken over consecutive days.

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