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High prevalence of subtle and severe menstrual disturbances in exercising women: confirmation using daily hormone measures

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BACKGROUND: The identification of subtle menstrual cycle disturbances requires daily hormone assessments. In contrast, the identification of severe menstrual disturbances, such as amenorrhea and oligomenorrhea, can be established by clinical observation. The primary purpose of this study was to determine the frequency of subtle menstrual disturbances, defined as luteal phase defects (LPD) or anovulation, in exercising women, with menstrual cycles of 26–35 days, who engage in a variety of sports, both recreational and competitive. Secondly, the prevalence of oligomenorrhea and amenorrhea was also determined via measurement of daily urinary ovarian steroids rather than self report alone.

METHODS: Menstrual status was documented by daily measurements of estrone and pregnanediol glucuronide and luteinizing hormone across two to three consecutive cycles and subsequently categorized as ovulatory (Ovul), LPD, anovulatory (Anov), oligomenorrheic (Oligo) and amenorrheic (Amen) in sedentary (Sed) and exercising (Ex) women.

RESULTS: Sed ($n = 20$) and Ex women ($n = 67$) were of similar ($P > 0.05$) age (26.3 ± 0.8 years), weight (59.3 ± 1.8 kg), body mass index (22.0 ± 0.6 kg/m^2), age of menarche (12.8 ± 0.3 years) and gynecological maturity (13.4 ± 0.9 years). The Sed group exercised less ($P < 0.001$) (96.7 ± 39.1 versus 457.1 ± 30.5 min/week) and had a lower peak oxygen uptake (34.4 ± 1.4 versus 44.3 ± 0.6 ml/kg/min) than the Ex group. Among the menstrual cycles studied in the Sed group, the prevalence of subtle menstrual disturbances was only 4.2% (2/48); 95.8% (46/48) of the observed menstrual cycles were ovulatory. This finding stands in stark contrast to that observed in the Ex group where only 50% (60/120) of the observed menstrual cycles were ovulatory and as many as 50% (60/120) were abnormal. Of the abnormal cycles in the Ex group, 29.2% (35/120) were classified as LPD (short, inadequate or both) and 20.8% (25/120) were classified as Anov. Among the cycles of Ex women with severe menstrual disturbances, 3.5% (3/86) of the cycles were Oligo and 33.7% (29/86) were Amen. No cycles of Sed women (0/20) displayed either Oligo or Amen.

CONCLUSIONS: This study suggests that approximately half of exercising women experience subtle menstrual disturbances, i.e. LPD and anovulation, and that one third of exercising women may be amenorrheic. Estimates of the prevalence of subtle menstrual disturbances in exercising women determined by the presence or absence of short or long cycles does not identify these disturbances. In light of known clinical consequences of menstrual disturbances, these findings underscore the lack of reliability of normal menstrual intervals and self report to infer menstrual status.

Key words: exercise / menstrual disturbances / prevalence / anovulation / luteal phase defects

Introduction

Menstrual cycle disturbances are often observed among exercising women (De Souza *et al.*, 1998). The etiology of these menstrual disturbances in exercising women has been casually linked to a chronic energy deficiency arising from conditions where energy intake is inadequate to compensate for energy expenditure (De Souza and Williams, 2004). The suppression of reproductive function during conditions of chronic energy deficiency is well-recognized to be an energy conserving response to preserve fuel for more vital bodily processes, such as cellular maintenance and locomotion, and to avoid the costly functions of gestation and lactation (Wade *et al*., 1996; De Souza and Williams, 2004).

The prevalence of oligomenorrhea and amenorrhea in athletes has been frequently reported in the literature (De Souza and Williams, 2004). These more severe presentations of menstrual disturbances have been reported to range from 6 to 43% in runners (Feicht *et al*., 1978; Dale *et al*., 1979; Sanborn *et al*., 1982; Shangold and Levine, 1982; Glass *et al*., 1987; Cobb *et al*., 2003) and 1–31% in both high school (Nichols *et al*., 2007) and collegiate athletes (Beals and Manore, 2002; Beals and Hill, 2006) from a variety of different sports. These reported prevalence rates exceed that observed in the general population of non-athletic, college-aged women (Pettersson *et al*., 1973; Singh, 1981; Bachmann and Kemmann, 1982). To date, however, ovarian steroid documentation of these cycle irregularities has been absent, and reports have relied solely on self-report. The method of self-report is only able to capture those disturbances clearly recognized by women as irregularities in menstrual cycle length (oligomenorrhea) or the absence of regular periods (amenorrhea). Therefore, recent publications (Torstveit and Sundgot-Borgen, 2005a, b) reporting the prevalence of menstrual disturbances as a component of the Female Athlete Triad are likely to have underestimated the percentage of women at risk for the Triad, by overlooking those with apparently regular yet abnormal cycles. Data remain scarce on the prevalence of subtle menstrual disturbances, such as luteal phase defects (LPD) and anovulation, in either competitive athletes or recreationally physically active women. This type of information is necessary in order for scientists to define the scope of the potential problem, i.e. the number of women affected, and the depth or magnitude of concern warranted, i.e. the health risks associated with these disturbances. Indeed, menstrual disturbances in athletes have been associated with an array of clinical consequences including decreased bone mineral density (De Souza and Williams, 2004; Nattiv, 2007), an increased prevalence of stress fractures (De Souza and Williams, 2004; Nattiv, 2007), and endothelial dysfunction (O'Donnell *et al*., 2007, 2009) In general, menstrual disturbances are also associated with infertility and poor cycle fecundity (McNeely and Soules, 1988).

In 1998, we published a detailed prospective observational study (De Souza *et al*., 1998) to document the frequency of LPD and anovulatory disturbances, verified with daily hormone measurements, in recreationally active runners during consecutive, asymptomatic, regular menstrual cycles of normal length. In that study, we evaluated ~100 menstrual cycles in 24 exercising women and 11 sedentary controls, and reported a sample prevalence of 48% and sample incidence of 79% of subtle menstrual disturbances (LPD and anovulation) among the recreational runners. Since then, no other prospective studies employing daily hormone measures have been published to explore this issue further, likely because the daily measures of luteinizing hormone (LH) and the ovarian steroids required to adequately quantify and identify these types of menstrual disturbances are both time consuming and costly (McNeely and Soules, 1988; Jordan *et al*., 1994).

The primary purpose of this study was to re-evaluate the prevalence of menstrual disturbances in exercising women in a larger sample by combining two data sets, one represented in our earlier publication (De Souza *et al*., 1998) and one from a more recent study conducted at the University of Toronto. In the latter study, we did not limit inclusion to only those women having regular menses (26–35 days) as in our earlier study. Thus, the current study also includes data on the prevalence of oligomenorrhea and amenorrhea from our more recent data set. Moreover, the current study includes exercising women from a wider variety of sports/physical activity modes. Menstrual status of subjects with both subtle (LPD and anovulation) and severe (oligomenorrhea and amenorrhea) menstrual disturbances was experimentally observed and verified by daily measurements of urinary ovarian steroids and LH across several consecutive menstrual cycles. We hypothesize that exercising women will exhibit a greater prevalence of subtle and severe menstrual disturbances compared with sedentary women.

Materials and Methods

Experimental design

In this study, we present data collected at two different sites, Connecticut, USA and Toronto, Canada. The data from Connecticut (De Souza *et al*., 1998) were previously published and are now combined with a dataset from Toronto to further explore the prevalence of subtle menstrual disturbances (LPD and anovulation) in a larger dataset with greater statistical power than the first publication. Other than our 1998 publication (De Souza *et al*., 1998), these data represent the only data published that have evaluated the prevalence of LPD and anovulation in exercising women across several consecutive cycles via daily measurement of ovarian steroid excretion. For the purpose of determining the prevalence of oligomenorrhea and amenorrhea, only the women who participated at the Toronto site were used since the inclusion criteria accommodated for irregular and absent menstrual cycles in addition to cycles of 26–35 days. This study is the first to describe the prevalence of oligomenorrhea and amenorrhea in exercising women by direct quantification of daily urinary ovarian steroid excretion, as previous reports have relied exclusively on self-reported menstrual cycle characteristics.

This study was an observational prospective study designed to determine the prevalence of LPD and anovulatory disturbances (48 exercising women) as well as amenorrhea and oligomenorrhea (43 exercising women) among women participating in a wide array of physical activities at either the recreational or competitive level. A control group of 20 age-matched sedentary women were also studied. We prospectively assessed daily urinary ovarian hormone excretion over one to three consecutive menstrual cycles of subjects whose health status, menstrual history, aerobic fitness, and past and current training history were also documented.

Medical screening

Eligibility criteria for the study were strictly adhered to and included, (i) aged 18–35 years; (ii) good health as determined by a medical exam; (iii) no chronic illness, including hyperprolactinemia and thyroid disease; (iv) stable menstrual status over the preceding 3 months if menstruating (both study sites) or irregular or absent menses without a history of polycystic ovarian syndrome (PCOS) (Toronto site only); (v) non-smoker; (vi) not currently dieting and weight stable for the preceding 3 months, as determined by self-report; (vii) not taking any form of hormonal therapy for at least 12 months; (viii) no history of an eating disorder within the past 3 years or no current clinical diagnosis of an eating disorder; (ix) performing regular, purposeful exercise [heart rate (HR) greater than 55% of maximal HR] for >2 h/week, as documented in exercise logs (De Souza *et al*., 1998) if physically active or <2 h/week of purposeful exercise (HR greater than 55% of maximal HR) if sedentary; (x) no history of PCOS; and (xi) no other contraindications that would preclude participation in the

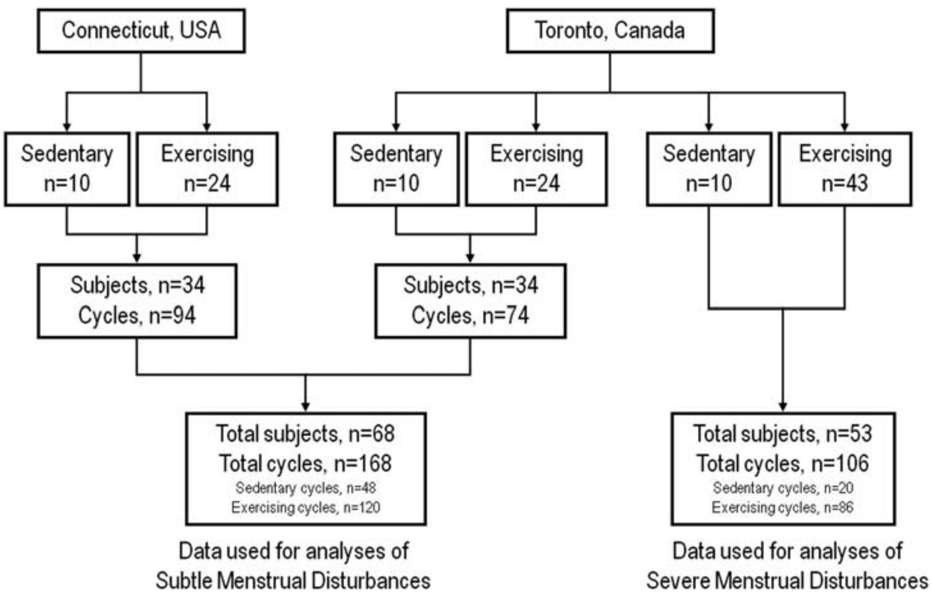

Figure 1 Study design and the number of subjects and cycles contributed by the two study sites to separately determine the prevalence of subtle menstrual disturbances (LPD and anovulation) and severe menstrual disturbances (oligomenorrhea and amenorrhea).

The Connecticut site contributed a total of 34 subjects (10 Sed and 24 Ex) and 94 cycles to determine the prevalence of subtle menstrual disturbances. Likewise, the Toronto site contributed a total of 34 subjects (10 Sed and 24 Ex) and 74 cycles to these subtle menstrual disturbance analyses, resulting in a total sample size of 68 subjects and 168 cycles. The prevalence of severe menstrual disturbances was determined by data collected only at the Toronto site which contributed 53 subjects (10 Sed and 43 Ex) and 106 cycles to these analyses. LPD, Luteal phase defect; Sed, Sedentary; Ex, Exercising.

study. The study was approved by the Ethics Review Board at the University of Connecticut Health Center and the University of Toronto, and all volunteers signed an approved Informed Consent document.

Volunteers

A flow chart is presented (Fig. 1) to describe the design of the study and the contribution of subjects and cycles from each study site to assess the prevalence of subtle menstrual disturbances (LPD and anovulation) and severe menstrual disturbances (oligomenorrhea and amenorrhea). For the evaluation of the prevalence of subtle menstrual disturbances (LPD and anovulation) in exercising women, 68 volunteers were recruited on a rolling basis over several years at the Toronto and Connecticut (De Souza *et al*., 1998) sites. At the Connecticut site, a total of 10 sedentary women and 24 exercising women were studied, contributing a total of 94 menstrual cycles. At the Toronto site, a total of 10 sedentary women and 24 exercising women were studied, contributing a total of 74 menstrual cycles (a combined total of 48 cycles in sedentary women and 120 cycles in exercising women). For the evaluation of the prevalence of severe menstrual disturbances (oligomenorrhea and amenorrhea) in exercising women, 53 women from the Toronto site were evaluated, including 10 sedentary and 43 exercising women, contributing a total of 106 cycles (20 cycles in sedentary women and 86 cycles in exercising women).

The exercising women at the Connecticut site were predominantly recreational runners (De Souza *et al*., 1998). In an effort to expand the types of exercising women included in the study, the women at the Toronto site represented a range of exercising women to include competitive athletes across multiple sport activities. The range of physical activities of the exercising women at the Toronto site included indoor and outdoor running, elliptical running, stationary cycling, aerobic classes, mountain biking and other sport activities including field hockey, tennis, soccer, rugby, volleyball and dance. A total of 54% of the exercising women reported participation in weight lifting.

Recruitment and dropouts

Volunteers were recruited by posters targeting both sedentary and physically active women. At the Connecticut and Toronto sites, women with a history of regular cycles of 26–35 days were recruited; however, at the Toronto site women with oligomenorrhea and amenorrhea were also recruited. Screening procedures included questionnaires on exercise, eating, menstrual and medical health history. At the Connecticut site, six women failed screening (two thyroid disease, one smoker, one current diagnosis of eating disorders, two irregular menses), four declined study participation and five women dropped out due to time constraints. At the Toronto site, 20 failed screening (10 used oral contraceptives, three were smokers, one had a current clinical diagnosis of an eating disorder, one hyperprolactinemia, one thyroid disease, four PCOS), 12 declined participation and three dropped out due to time constraints.

Observational time periods

Volunteers were monitored for one to three menstrual cycles. Forty-four of the women were studied for two menstrual cycles (eight sedentary and 36 exercising woman), 35 of the women were studied for three menstrual cycles (10 sedentary and 25 exercising woman) and eight of the women were monitored for only one menstrual cycle (two sedentary and six exercising woman).

Volunteer grouping categories

Volunteers were defined as 'exercising' or 'sedentary'. If purposeful exercise exceeded 2 h/week (documented in exercise logs), the volunteer was defined as 'exercising'; whereas, if purposeful exercise was <2 h/week (documented in exercise logs), the volunteer was defined as 'sedentary' (De Souza *et al.*, 1998). In conjunction with the hours of exercise activity criterion, we also utilized a VO_2 max of <40 ml/kg/min to reflect sedentary status and ≥40 ml/kg/min to reflect exercising status consistent with published data of this parameter (Saltin and Åstrand, 1967).

For the subtle menstrual disturbances study, each individual menstruating volunteer was also retrospectively categorized by their overall menstrual cycle category (ovulatory or abnormal) during the 1–3 month observation period, and when abnormal cycles presented, by an abnormal cycle subcategory of either LPD (short and/or inadequate) or anovulatory. In order to place an individual woman into a single menstrual category, we categorized the volunteer consistent with the predominant presentation of each of the one or three menstrual cycles studied. For example, if two of two or two of three cycles monitored were ovulatory, that volunteer was classified as ovulatory. For individuals that were inconsistent, i.e. one ovulatory cycle and one anovulatory cycle, the abnormal menstrual category was utilized that was consistent with the most severe menstrual disturbance observed. We also compared the ovarian steroid and menstrual cycle characteristics of each menstrual cycle evaluated in this study. For these descriptive purposes, we subsequently categorized abnormal cycles as a short LPD cycle, inadequate LPD cycle, both short and inadequate LPD cycle or an anovulatory cycle, and cross-referenced these categorizations to exercise status (sedentary or exercising).

For the severe menstrual disturbances study, oligomenorrhea was defined as menses of >35 days but <90 days and amenorrhea as no menses for at least 90 days (De Souza and Williams, 2004).

To determine the relative proportion of sedentary and exercising subjects displaying consistent and inconsistent ovulatory cycles and subtle menstrual disturbances (LPD and anovulation), we assessed the consistency of a given menstrual category for each study volunteer. We categorized the volunteer's menstrual cycles as (i) consistently ovulatory when all of the observed cycles for a given volunteer were ovulatory, for example, if two of two or three of three cycles monitored were ovulatory, or (ii) consistently abnormal when all of the observed cycles for a given volunteer were abnormal, for example, if two of two or three of three cycles monitored were LPD or anovulatory and (iii) inconsistently abnormal when there were different categorizations of cycles from the same individual, for example if one cycle was LPD and one ovulatory.

Each menstrual cycle or monitoring period categorization was established by the daily urinary ovarian steroids and LH assessments, as described below. All menstrual categorizations were then cross-referenced to exercise status (sedentary or exercising).

Menstrual cycle characteristics

To allow for a clinical interpretation of our data, we determined menstrual characteristics in all volunteers, defined in this study by classifying menstrual cycles by length of the intermenstrual interval, length of follicular and luteal phases, the presence of menses, and by ovulatory status, i.e. ovulatory, anovulatory, etc. Volunteers were considered eumenorrheic if menses occurred at regular intervals of 26–35 days, oligomenorrheic if menses occurred at intervals of 36–90 days and amenorrheic if menses occurred at intervals >90 days (De Souza and Williams, 2004). Menstrual cycle length was defined as the number of days from Day 1 of menses to the day before the first day of the next menses. The follicular phase length was defined as the number of days from the first day of menses up to and including the day of the LH surge (De Souza *et al.*, 1998). Daily urine samples were assayed for LH, estrone-1-glucoronide (E1G) and pregnanediol glucoronide (PdG) to assess ovulatory status, and estrogen and progesterone exposure, as assessed by E1G and PdG area under the curve (AUC). Ovulatory status was determined by day of the urinary LH surge, identified as an LH peak on the day of or day after the midcycle E1G peak (De Souza *et al.*, 1998). Specific hormonal criteria for detecting ovulation included a LH surge concentration above 25 mIU/ml, the E1G peak concentration above 35 ng/ml and the peak PdG concentration above 5 μg/ml during the luteal phase (Kesner *et al.*, 1992; Santoro *et al.*, 2003; De Souza *et al.*, 2008). An anovulatory cycle was defined as a cycle in which minimal increases in E1G was observed concomitantly with a failure of LH to rise at midcycle, or when a luteal phase exhibited no increase in PdG concentration from a 5-day follicular phase baseline or when the peak PdG value was below 2.49 μg/ml (De Souza *et al.*, 1998; Santoro *et al.*, 2003; De Souza *et al.*, 2008). LPD was defined as short when the luteal phase length was less than 10 days, or inadequate when the sum of the 3-day midluteal peak PdG (sum of midluteal peak PdG ± 1 day) was less than 10 μg/ml and when the PdG peak concentration was below 5 μg/ml (De Souza *et al.*, 1998; Santoro *et al.*, 2003). To determine estrogen and progesterone exposure, E1G and PdG urinary metabolites were compared among the groups of menstrual cycles using the trapezoidal integrated AUC and mean levels of E1G and PdG during the follicular and luteal phase, and across the entire cycle.

Anthropometric testing

Total body mass was measured to the nearest 0.1 kg on a scale weekly, and the mean of these measurements is presented. Height was measured to the nearest 1.0 cm at the beginning of the study. Body mass index (BMI) was calculated from height and mean weight (kg/m^2).

Peak aerobic capacity

VO_2 peak was measured once during a progressive treadmill test to volitional exhaustion using open-circuit spirometry (Connecticut: Medical Graphics Exercise System 2000, Medical Graphics, St. Paul, MN and Toronto: Moxus Modular VO_2 System, Applied Electrochemistry). Detailed methods are published elsewhere (De Souza *et al.*, 2008).

Urinary measurement of E1G and PDG

The validity of the urinary technique as representative of the 24-h pattern of E1G and PdG excretion has been reported by other investigators (Beitins *et al.*, 1991; Munro *et al.*, 1991) and the secretion of these metabolites in the urine parallels serum concentrations of the parent hormones. Microtiter plate competitive enzyme immunoassays were used to measure the urinary metabolites E1G and PdG, respectively, using the techniques of Munro *et al.* (1991). The E1G (R522-2) and PdG (R13904) assays utilized a polyclonal capture antibody supplied by Coralie Munro at the University of California (Davis, CA, USA). The competitors for these assays are E1G or PdG conjugated to horseradish peroxidase (E1G-HRP and PdG-HRP, respectively). An end-point substrate color reaction is developed with azino-*bis*-ethylbenzthiazoline sulfonic acid and peroxidase. E1G and PdG standards (Sigma) were used for the standard curve, and high and low internal controls from in-house samples were used. The inter-assay coefficients of variation for high and low internal controls for the E1G assay were 14.7 and 13.1%, respectively, for the Connecticut samples and 14.2 and 13.7% for the Toronto samples. The inter-assay coefficients of variation for high and low internal controls for the PdG assay were 15.6 and 12.9%, respectively. The PdG intra and inter-assay variability was determined in-house as 15.6 and 12.9%, respectively, for the Connecticut samples and 15.6 and 17.7%, respectively, for the Toronto samples. The sensitivity of the E1G and PdG assays were 7.8 ng/ml and 0.15 μg/ml, respectively. Values below the sensitivity were reported at the minimum detection limits. All urine samples were corrected for creatinine (Connecticut samples) or specific gravity (Toronto samples) (NSG Precision Cells, Inc., Farmingdale, NY, USA) to account for hydration status. Specific gravity has been reported to perform as well as creatinine correction for adjusting urinary hormone concentrations (Miller *et al.*, 2004). Specific gravity measurements were taken with a hand-held urine specific gravity refractometer (Atago Uricon-PN; NSA Precision Cells, Inc., Framingham, NY).

Urinary LH was determined by double antibody radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA, USA). The sensitivity of the LH assay is 0.6 mIU/l. The intra-assay and inter-assay coefficients of variation were 1.6 and 7.1%, respectively.

Statistical analyses

The data presented were obtained at two different locations for a period of 3 years at each site. To explore whether the two cohorts (sedentary Connecticut versus sedentary Toronto, exercising Connecticut versus exercising Toronto, and all subjects Connecticut versus all subjects Toronto) could be combined, we investigated whether they were similar with respect to key demographic characteristics using independent sample Student's *t* tests. The two cohorts were similar ($P > 0.05$) with respect to age, gynecological age, body weight, BMI and menstrual cycle length (menstruating women only). On the basis of these data, the two data sets were combined and grouped according to their exercise (sedentary or exercising) status.

Data screening was conducted prior to statistical analysis in order to identify whether the data met the assumptions required by specific statistical techniques. Data screening involved outlier detection and examination of variable distributions within each of the two groups for normality. Since all of the distributions were normal, parametric analyses were utilized for inferential analyses.

Demographic characteristics of the subjects were analyzed using an independent samples Student's *t*-test to compare means between sedentary and exercising women; a one-way ANOVA was utilized to find between group differences when the subjects were grouped according to exercise and menstrual status. When a significant difference was observed between groups, multiple comparisons utilizing the LSD method were performed to determine which groups presented significantly different means.

To determine the prevalence of ovulatory versus abnormal menstrual cycles, volunteers' menstrual cycles were categorized based on specific ovarian and gonadotrophin hormone criteria described below. For further comparisons, abnormal cycles were subsequently categorized as luteal phase deficient cycles or anovulatory cycles. For these purposes, volunteers were categorized into a single menstrual classification according to the predominant presentation of each menstrual cycle. For example, if two of two or two of three cycles monitored were ovulatory, that subject was classified as ovulatory. For individuals that were inconsistent, i.e. one ovulatory and one anovulatory, abnormal menstrual classification was consistent with the most severe menstrual disturbance observed. Contingency tests (χ^2) on the categorical menstrual cycle variable were then performed to determine if the presentation of abnormal, oligomenorrheic or amenorrheic cycles significantly differed between sedentary and exercising women.

Other research questions of primary interest were investigated based on the categorization of each menstrual cycle (ovulatory, luteal phase deficient, anovulatory, oligomenorrheic or amenorrheic); therefore, each menstrual cycle was treated as a separate observation in these analyses. On the basis of the cross classification of volunteers discussed above, each menstrual cycle was grouped according to both exercise status (sedentary or exercising) and specific menstrual cycle presentation (ovulatory, LPD, anovulatory, oligomenorrheic or amenorrheic) into one of five groups for the subtle menstrual disturbances study [sedentary ovulatory (SedOvul), sedentary LPD (SedLPD), exercising ovulatory (ExOvul), exercising LPD (ExLPD) or exercising anovulatory (ExAnov)] or into one of two groups for the severe menstrual disturbances study [exercising oligomenorrheic (ExOligo) and exercising amenorrheic (ExAmen)]. Since multiple cycles were observed from the same individual, these data were considered to have a nested nature, and linear mixed model ANOVA was used to account for multiple cycles from individuals in a single experimental category. All menstrual cycle characteristics (cycle and phase length) as well as ovarian steroid and gonadotrophin (E1G, PdG and LH) data were analyzed via a linear mixed model ANOVA. When a significant main (fixed) effect was observed, Bonferroni pair-wise comparisons were performed to identify significantly different groupings.

A significance level of 0.05 was used to detect differences for all statistical procedures. All data are presented as means ± SEM, unless otherwise indicated. All data were analyzed by using SPSS for Windows (version 12.0; Chicago, IL, USA).

As previously reported (De Souza *et al*., 1998) a sample size of 35 women and 97 cycles was large enough to provide more than 99.95% probability to detect differences larger than 2 standard deviations (SD) and 80% probability of detecting differences larger than 0.8 SD with a tolerable type I error rate of 5%. Since the sample size used in this study (69 women, 166 cycles) is larger than De Souza *et al*. (1998), it was determined that our current study would provide adequate power to detect differences among the groups with respect to the key outcomes of interest.

Results

Demographic characteristics

The demographic characteristics of the study volunteers are presented in Tables I and II. Eighty-seven women participated in this prospective evaluation of menstrual status; 68 women and 53 women were included in the analyses of subtle and severe menstrual disturbances, respectively. Twenty women were sedentary and 67 women were categorized as exercising, consisting of predominately recreationally physically active women, although some competitive athletes were included. As presented in Table I, the sedentary and exercising women were similar ($P > 0.050$) with respect to age, height, weight and BMI. There were no significant differences in gynecological age ($P = 0.183$) and age of menarche ($P = 0.622$) between the groups. As expected, exercising women had a significantly higher VO_2 peak ($P < 0.001$) and exercised more ($P < 0.001$) than the sedentary group.

The demographics of the subjects categorized according to exercise and menstrual status are presented in Table II. The groups were similar ($P > 0.050$) with respect to height, weight, BMI and age of menarche. ExAnov and ExAmen groups were younger ($P = 0.010$) than the SedOvul and ExOvul groups, and the ExAmen group also had a lower gynecological age ($P = 0.003$) than the SedOvul and ExOvul groups. The gynecological age of the ExAnov and ExAmen groups were significantly younger than the ExOvul and ExLPD groups, respectively.

Prevalence of ovulatory versus abnormal cycles: comparisons by subject

To determine the prevalence of subtle menstrual disturbances, each woman was categorized according to the predominant type of menstrual cycle category observed, that is, as either ovulatory or abnormal, and abnormal cycles were further categorized as LPD or anovulatory; these data are presented in Fig. 2. Among the sedentary women, 95.0% (19/20) were categorized as ovulatory and 5.0% (1/20) were categorized as abnormal, of which, the woman with abnormal cycles presented with LPD in all cycles observed. Among the exercising women, 47.9% (23/48) were categorized as ovulatory; whereas, 52.1% (25/48) were categorized as abnormal, consisting of 27% of the exercising women (13/48) with LPD cycles (short and/or inadequate luteal phase), and 25% of the exercising women (12/48) with anovulatory cycles. The prevalence of abnormal cycles was much greater ($\chi^2 = 13.25$, $P < 0.001$) in the exercising women than in the sedentary women.

Table I **Demographic characteristics of study subjects categorized by exercise (sedentary or exercise) status**

	Sed women (*n* = 20)	Ex women (*n* = 67)	Probability
Demographic Characteristics			
Age (year)	27.2 ± 1.0	25.3 ± 0.6	0.154
Height (cm)	162.5 ± 1.1	165.3 ± 0.7	0.054
Weight (kg)	60.2 ± 2.8	58.4 ± 0.8	0.531
BMI (kg/m^2)	22.7 ± 0.9	21.3 ± 0.2	0.138
Reproductive characteristics			
Age of menarche (year)	12.9 ± 0.4	12.7 ± 0.2	0.622
Gynecological age (year)	14.2 ± 1.1	12.5 ± 0.7	0.183
Training characteristics			
VO_2 peak (ml/kg/min)	34.4 ± 1.4	44.3 ± 0.6	<0.001
Physical activity (min/week)	96.7 ± 39.1	457.1 ± 30.5	<0.001

Values are the mean ± SEM.
Sed, sedentary; Ex, exercising.

Prevalence of oligomenorrheic and amenorrheic cycles: comparisons by subject

To determine the prevalence of severe menstrual disturbances, the women at the Toronto site who fulfilled the criteria of either oligomenorrhea or amenorrhea were categorized accordingly. The remainder of the subjects were either ovulatory or displayed a subtle menstrual disturbance. As shown in Table III, 7.0% (3/43) of the exercising women were oligomenorrheic, but 37.2% (16/43) of the exercising women displayed the most severe form of menstrual disturbance, amenorrhea. None of the sedentary women displayed either oligomenorrhea or amenorrhea. The prevalence of women with amenorrheic cycles was significantly greater ($\chi^2 = 5.330$, $P < 0.023$) among the exercising women when compared with the sedentary women.

Consistency of cycles

For the women with apparently regular cycles, the percentages of sedentary and exercising subjects presenting with consistently ovulatory, consistently abnormal or inconsistently abnormal cycles are displayed in Table IV. Among the sedentary women who were monitored for multiple cycles ($n = 18$), 94.4% (17 women) were consistently ovulatory and 5.6% (1 woman) were inconsistently abnormal.

Table II **Demographic characteristics of subjects categorized by exercise and menstrual status**

	SedOvul* (*n* = 19)	ExOvul (*n* = 23)	ExLPD (*n* = 13)	ExAnov (*n* = 12)	ExOligo (*n* = 3)	ExAmen (*n* = 16)	Probability
Demographic characteristics							
Age (year)	27.5 ± 1.1	27.9 ± 1.1	26.1 ± 1.6	23.7 ± 1.6[a]	22.0 ± 1.5	22.9 ± 0.9[a]	0.010
Height (cm)	162.3 ± 1.1	165.2 ± 1.4	164.0 ± 1.9	166.1 ± 1.5	163.7 ± 2.2	166.1 ± 1.1	0.375
Weight (kg)	60.2 ± 3.0	57.9 ± 1.4	57.8 ± 1.7	61.4 ± 6.2	58.7 ± 6.0	57.2 ± 1.7	0.742
BMI (kg/m^2)	22.8 ± 0.9	21.2 ± 0.4	21.4 ± 0.4	22.2 ± 0.5	21.5 ± 1.4	20.7 ± 0.6	0.238
Reproductive characteristics							
Age of menarche (year)	12.9 ± 0.4	12.4 ± 0.3	12.8 ± 0.3	12.5 ± 0.4	14.3 ± 1.3	13.2 ± 0.4	0.313
Gynecological age (year)	14.6 ± 1.1	15.0 ± 1.0	13.7 ± 1.6	11.1 ± 1.7[d]	6.8 ± 3.3[b]	9.3 ± 0.9[b,c]	0.003
Training characteristics							
VO_2 peak (ml/kg/min)	34.3 ± 1.5	44.5 ± 1.3[e]	41.6 ± 1.2[e]	45.3 ± 1.3[e]	47.8 ± 0.6[e]	45.3 ± 0.9[e]	<0.001
Physical activity (min/week)***	100.1 ± 41.2	395.3 ± 36.7[f]	386.5 ± 51.4[f]	442.2 ± 62.0[f]	336.0**	650.5 ± 86.1[f,g]	<0.001

Values are the mean ± SEM.
SedOvul, sedentary ovulatory; ExOvul, exercising ovulatory; ExLPD, exercising luteal phase deficient; ExAnov, exercising anovulatory; ExOligo, exercising oligomenorrheic; ExAmen, exercising amenorrheic.
*SedLPD ($n = 1$) not included.
**SEM not applicable due to missing data for two subjects.
***Multiple comparisons could not be performed due to missing data in ExOligo group.
[a]SedOvul and ExOvul versus ExAnov and ExAmen.
[b]SedOvul and ExOvul versus ExOligo and ExAmen.
[c]ExLPD versus ExAmen.
[d]ExOvul versus ExAnov.
[e]SedOvul versus ExOvul, ExLPD, ExAnov, ExOligo, ExAmen.
[f]SedOvul versus ExOvul, ExLPD, ExAnov, ExAmen.
[g]ExOvul, ExLPD, ExAnov versus ExAmen.

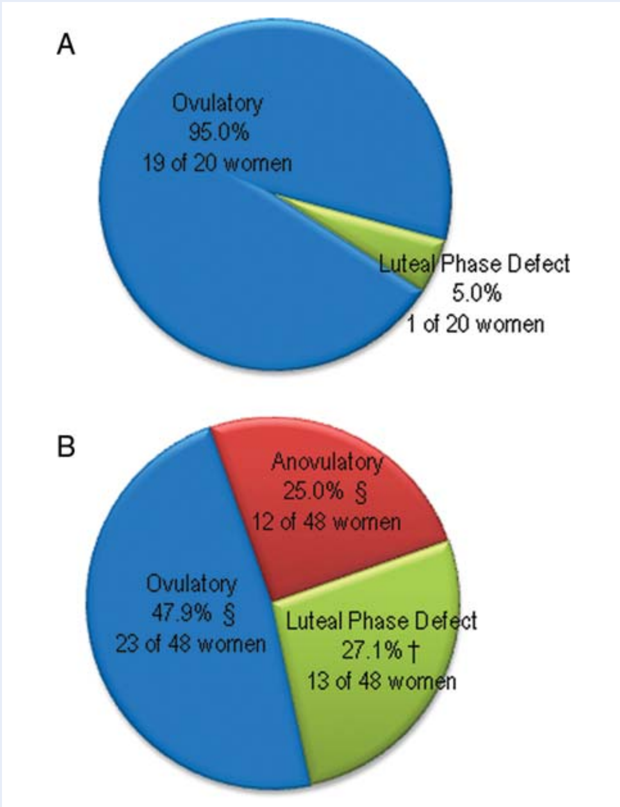

Figure 2 Prevalence of subtle menstrual disturbances among sedentary and exercising subjects.

(**A**) Demonstrates the proportion of sedentary women ($n = 20$) categorized as having ovulatory or LPD cycles. Ninety-five percent (19/20) of the sedentary women had mostly ovulatory menstrual cycles, and only 5.0% (1/20) of the sedentary subjects were characterized as having LPD cycles. (**B**) Illustrates the proportion of exercising women ($n = 48$) categorized as having ovulatory, anovulatory or LPD cycles. Only 48% (23/48) of the exercising women had ovulatory cycles. Twenty-seven percent (13/48) were categorized as having LPD cycles, and 25% (12/48) were categorized as having anovulatory cycles. Fewer exercising versus sedentary women displayed ovulatory cycles ($P < 0.001$, $\chi^2 = 13.252$). A significantly greater proportion of exercising women displayed anovulatory cycles ($P < 0.001$, $\chi^2 = 6.071$) when compared with sedentary women. There was a trend ($P = 0.050$, $\chi^2 = 4.211$) toward a greater proportion of exercising women categorized as LPD when compared with sedentary women. † Indicates $P = 0.050$; § indicates $P < 0.001$. LPD, Luteal phase defect.

Table III By subject comparisons of University of Toronto subjects grouped by menstrual category of oligomenorrheic and amenorrheic

	Sed women (n = 10)	Ex women (n = 43)	Probability
Oligomenorrheic	0% (0/10)	7.0% (3/43)	1.000 ($\chi^2 = 0.740$)
Amenorrheic	0% (0/10)	37.2% (16/43)	0.023 ($\chi^2 = 5.330$)

Values are presented as percentages. Data in parenthesis is the number of subjects in the respective menstrual group over the total number of subjects in the respective exercise group.
Sed, sedentary; Ex, exercising.

Two women in the sedentary group were monitored for only one cycle. Among the exercising women monitored for multiple cycles ($n = 47$), 31.9% (15 women) were consistently ovulatory, 31.9% (15 women) were consistently abnormal, and 36.2% (17 women) displayed inconsistently abnormal cycles. One exercising subject was monitored for only one menstrual cycle. There were fewer consistently ovulatory cycles in the exercising women ($\chi^2 = 20.361$, $p < 0.001$), more consistently abnormal cycles in the exercising women ($\chi^2 = 7.468$, $p = 0.006$) and more inconsistently abnormal cycles in the exercising women ($\chi^2 = 6.092$, $p = 0.014$).

Clinical menstrual cycle characteristics

Menstrual cycle characteristics for the menstrual cycles grouped by exercise status (sedentary or exercising) and menstrual status (ovulatory, LPD or anovulatory) are presented in Table V. The intermenstrual interval (overall menstrual cycle length) of the SedOvul, ExOvul, ExLPD and ExAnov groups of cycles were similar ($P = 0.574$). The ExLPD cycles had significantly longer follicular phases (18.70 ± 0.6 days) than the ExOvul (15.12 ± 0.46 days) and SedOvul (16.13 ± 0.52 days) cycles. As anticipated, luteal phase length was significantly shorter in the ExLPD cycles (9.91 ± 0.38 days) compared with the ExOvul (12.64 ± 0.29 days) and SedOvul (12.76 ± 0.33 days) cycles. The ExAnov cycles were excluded from the statistical analyses on luteal and follicular phase lengths.

Prevalence of ovulatory versus abnormal cycles: comparisons by menstrual cycles

The prevalence of subtle menstrual disturbances, determined when menstrual cycles were grouped by menstrual cycle category of ovulatory or abnormal, or when abnormal is further categorized as a short LPD cycle, an inadequate LPD cycle, a short and inadequate LPD cycle or an anovulatory cycle, is shown in Fig. 3. Among the sedentary women, 46 of 48 (96%) menstrual cycles monitored were ovulatory; whereas among the exercising women, only 60 of 120 (50%) of the menstrual cycles monitored were ovulatory. Only two cycles from one sedentary woman had inadequate luteal phases (low progesterone concentrations). Among the exercising women, 50% of the 120 menstrual cycles monitored demonstrated either LPD cycles (29.2%, $n = 35$) or anovulation (20.8%, $n = 25$). Among the LPD menstrual cycles, 11.7% ($n = 14$) had characteristic short luteal phases, 8.3% ($n = 10$) were inadequate and 9.2% ($n = 11$) were short and inadequate. Figure 4 displays the cumulative composite graphs of the characteristic menstrual cycles when categorized by SedOvul, ExOvul, ExLPD and ExAnov.

Prevalence of oligomenorrheic and amenorrheic cycles: comparisons of menstrual cycles

The prevalence of severe menstrual disturbances, as determined by analysis of the cycles collected at the Toronto site, is shown in Table VI. Among the cycles of exercising women, 3.5% (3/86) fulfilled the criteria of oligomenorrhea; whereas, 33.7% (29/86) were characteristic of amenorrhea. Neither oligomenorrheic nor amenorrheic cycles were observed among the cycles of sedentary women. The prevalence of amenorrheic cycles was significantly greater ($\chi^2 = 9.284$, $P < 0.001$) among cycles of exercising women than those of

Table IV Proportion of sedentary and exercising subjects displaying consistent and inconsistent ovulatory cycles and subtle menstrual disturbances (LPD and anovulation)

	Sed women (n = 18)	Ex women (n = 47)	Probability
Consistently ovulatory	94.4% (17/18)	31.9% (15/47)	<0.001 ($\chi^2 = 20.361$)
Consistently LPD/anovulatory	0% (0/18)	31.9% (15/47)	0.006 ($\chi^2 = 7.468$)
Inconsistently LPD/anovulatory	5.6% (1/18)	36.2% (17/47)	0.014 ($\chi^2 = 6.092$)

Values are presented as percentages. Data in parenthesis is the number of subjects with consistent or inconsistent cycles over the total number of subjects in the respective exercise group.
Sed, sedentary; Ex, exercising.

Table V Menstrual cycle parameters and LH for the menstrual cycles grouped by exercise status (sedentary or exercising) and menstrual status (ovulatory, luteal phase deficient or anovulatory)

	SedOvul (n = 48)	ExOvul (n=60)	ExLPD (n=35)	ExAnov (n=25)	Probability
Menstrual cycle length (days)	28.89 ± 0.63	27.78 ± 0.55	28.64 ± 0.73	28.42 ± 0.82	0.574
Follicular phase length (days)	16.13 ± 0.52	15.12 ± 0.46	18.70 ± 0.6[a]	N/A	<0.001
Luteal phase length (days)	12.76 ± 0.33	12.64 ± 0.29	9.91 ± 0.38[a]	N/A	<0.001
Peak LH (mIU/l)	109.19 ± 6.49	89.24 ± 5.76	84.01 ± 7.57[b]	N/A	0.022

Values are the mean ± SEM.
SedOvul, sedentary ovulatory; ExOvul, exercising ovulatory; ExLPD, exercising luteal phase deficient; ExAnov, exercising anovulatory.
[a]SedOvul and ExOvul versus ExLPD.
[b]SedOvul versus ExLPD.

sedentary women. Figure 5 displays a characteristic ovulatory and a characteristic oligomenorrheic cycle of a subject who inconsistently presents with oligomenorrhea as well as two 28-day monitoring periods of a consistently amenorrheic, exercising subject.

Ovarian steroid characteristics of menstrual cycles studied

Estrogen excretion

E1G excretion patterns for the menstrual cycles grouped by exercise status (sedentary or exercising) and menstrual status (ovulatory, LPD or anovulatory) are presented in Table VII. There were no significant differences observed between groups with respect to peak concentration of the E1G excretion; however, the day of the E1G peak was significantly later ($P = 0.001$) in the ExLPD group (17.39 ± 0.66) compared with the ExOvul group (14.02 ± 0.50). The ExAnov cycles demonstrated significant suppression ($P = 0.018$) of E1G excretion during the early follicular phase (Days 2–5 of the menstrual cycle) compared with the SedOvul cycles. During Days 6–12, E1G excretion was significantly less ($P = 0.001$) in the ExAnov cycles than in the SedOvul and ExOvul cycles. Over the entire follicular phase, E1G secretion did not differ among the SedOvul, ExOvul or ExLPD cycles, but less E1G was excreted during the ExAnov cycles ($P = 0.001$). For the ExAnov cycles, the follicular phase represented Days 1–14 of the 28-day intermenstrual interval. The AUC for E1G during the follicular phase was lower ($P = 0.001$) in the ExAnov cycles than in the SedOvul and ExOvul cycles. During the luteal phase, E1G AUC was lower ($P = 0.002$) in the ExLPD and ExAnov cycles in comparison to the SedOvul cycles.

Progesterone excretion

PdG excretion patterns for the menstrual cycles grouped by exercise status (sedentary or exercising) and menstrual status (ovulatory, LPD or anovulatory) are presented in Table VIII. Peak PdG excretion was significantly less ($P < 0.001$) in the ExAnov and ExLPD cycles than in the SedOvul and ExOvul cycles. During the luteal phase, PdG excretion was significantly less ($P < 0.001$) in the ExLPD and ExAnov cycles compared with the SedOvul and ExOvul cycles, with the lowest PdG excretion presenting in the ExAnov cycles as assessed by PdG AUC and mean luteal phase PdG (truncated luteal phase of 0.5/intermenstrual interval, as a true luteal phase could obviously not be discerned).

Gonadotrophin excretion characteristics

LH excretion patterns for the menstrual cycles grouped by exercise status (sedentary or exercising) and menstrual status (ovulatory, LPD or anovulatory) are presented in Table V. Peak urinary LH was significantly lower ($P = 0.022$) in the ExLPD cycles than in the SedOvul cycles.

Discussion

This study explored the frequency of luteal and ovulatory disturbances in consecutive, asymptomatic, regular menstrual cycles of normal length in physically active women. Secondly, the frequency of oligomenorrhea and amenorrhea among a subset of exercising women was also determined. The physically active cohort in this study represented a cross-section of recreational and competitive athletes across a wide array of physical activities. This issue is important given the well-

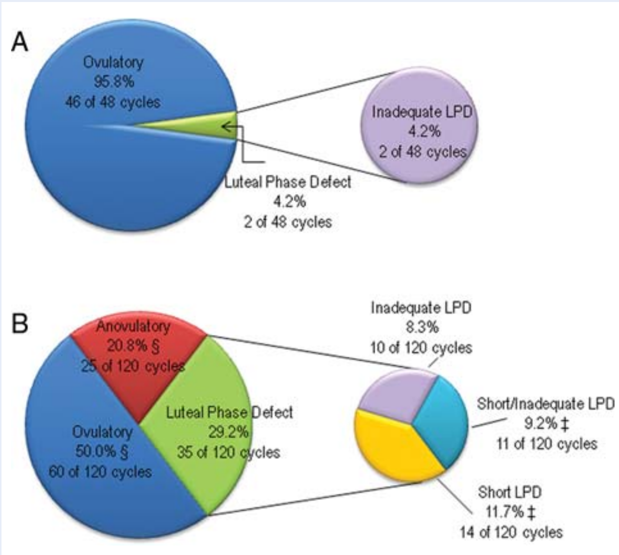

Figure 3 Prevalence of subtle menstrual disturbances when all individual cycles are examined independent of the subjects.

(**A**) Demonstrates the proportion of cycles displaying subtle menstrual disturbances among sedentary women ($n = 48$). Cycles of sedentary women displayed a 95.8% (46/48) prevalence of ovulatory cycles and a 4.2% (2/48) prevalence of abnormal cycles, all of which were characterized by an inadequate LPD. (**B**) Demonstrates the proportion of cycles displaying subtle menstrual disturbances among exercising women ($n = 120$). Cycles of exercising women displayed a 50.0% (60/120) prevalence of ovulatory cycles, 29.2% (35/120) prevalence of LPD, and 20.8% (25/120) prevalence of anovulation. Further analysis revealed that 11.7% (14/120) were short LPD, 8.3% (10/120) were inadequate LPD, and 9.2% (11/120) were both short and inadequate LPD. Cycles of exercising women displayed a significantly lower proportion of ovulatory cycles ($P < 0.001$, $\chi^2 = 30.931$) but a significantly higher proportion of short LPD cycles ($P = 0.001$, $\chi^2 = 6.109$), short/inadequate LPD cycles ($P = 0.035$, $\chi^2 = 4.708$), and anovulatory cycles ($P < 0.001$, $\chi^2 = 11.748$) than cycles of sedentary women. ‡ Indicates $P < 0.050$; § indicates $P < 0.001$. LPD, Luteal phase defect.

described wide array of clinical consequences associated with menstrual disturbances in exercising women, including decreased bone mineral density, increased risk of stress fractures, endothelial dysfunction and poor cycle fecundity (McNeely and Soules, 1988; De Souza and Williams, 2004; Nattiv, 2007, O'Donnell *et al*., 2007, 2009).

We clearly demonstrate the high prevalence of subtle menstrual disturbances in presumed normal cycles of 26–35 days in physically active women, confirming our earlier report (De Souza *et al*., 1998). We report herein that the 2–3 month sample prevalence of abnormal ovarian function, including LPD and anovulation, in our physically active women was 52%, compared with only 5% among the sedentary age-matched women. These perturbations in ovarian function occurred in the face of remarkably consistent intermenstrual intervals of ∼28 days supporting our previous assessment (De Souza *et al*., 1998) that menstrual cycle length is not an accurate marker of ovarian function in this population. Thus, in spite of the women in this study presenting with repeatable and consistent intermenstrual intervals of normal length, their ovarian function was highly variable and frequently abnormal. For this reason, the percentage of both physically active women and female athletes at risk for the menstrual disturbance component of the Female Athlete Triad may actually be greater than that which has been previously published (Torstveit and Sundgot-Borgen, 2005a, b) due to the inability of menstrual history questionnaires to capture individuals with subtle menstrual disturbances. Again, given the clinical sequelae associated with the Triad, physicians need to be aware of the limitations associated with solely relying on menstrual cycle length as an indicator of 'normal' menstrual function.

In fact, detailed analysis of the data reveals that as many as 50% of the 120 cycles evaluated from the exercising women were abnormal (LPD or anovulation); whereas, only 4% of the 48 cycles evaluated from the sedentary women were abnormal. The previous percentage is sobering if we consider that at least half of the menstrual cycles of recreationally active women and athletes may be characterized by a menstrual disturbance without any clinical demonstration or symptom of the abnormality. Because these abnormalities are unobtrusive, both clinicians and active women are unlikely to take action to reverse these disturbances and restore normal, ovulatory cycles. The frequency of subtle menstrual disturbances reported in this article agrees with our previous findings in which 10% of 31 cycles from sedentary women and 55% of 66 cycles from exercising women were considered abnormal (De Souza *et al*., 1998). Although the previously reported percentages of abnormal cycles in both the exercising and sedentary women are slightly greater (De Souza *et al*., 1998) than those reported herein, the frequency of abnormal cycles between sedentary and exercising women remains starkly and significantly different. Neither the previously reported data (De Souza *et al*., 1998) nor the frequencies reported herein revealed any anovulatory cycles in sedentary women; all abnormal cycles in this group were characterized by a LPD. Small non-significant differences were apparent when comparing the type of abnormalities observed in the cycles of exercising women. The data published in 1998 (De Souza *et al*., 1998) revealed that 43 and 12% of the cycles of the exercising women were LPD and anovulatory, respectively. The current dataset demonstrated a similar distribution between these two main types of subtle disturbances; 30% of the cycles of exercising women were characteristic of a LPD; whereas, 20% were considered anovulatory. The aforementioned differences in these frequencies, however, were not of statistical significance. The frequency of anovulatory cycles increased with a rise in the number of cycles analyzed, suggesting that perhaps the more severe subtle menstrual disturbance, anovulation, may be more prevalent among exercising women than originally thought.

In our analysis of 43 exercising women, 37% were considered amenorrheic. Among 86 28-day monitoring periods from these subjects, 34% were considered amenorrheic. This study represents the first published data documenting the prevalence of amenorrhea in exercising women by assessments of ovarian steroids in daily urine samples. Although two studies (Feicht *et al*., 1978; Hoch *et al*., 2007) reported the frequency of amenorrhea to be slightly greater than that reported here (40–43 versus 37%), the majority of studies that have analyzed the prevalence of self-reported amenorrhea have presented percentages smaller than that observed herein. For example, Feicht *et al*. (1978) reported a variable prevalence of amenorrhea of 6–43% which was dependent on running mileage among collegiate cross country and track and field teams. In a sample of female club triathlon team members, Hoch *et al*. (2007) reported that 40% of the women had a history of either primary or secondary amenorrhea and, as such, may represent an inflated prevalence rate due to the inclusion

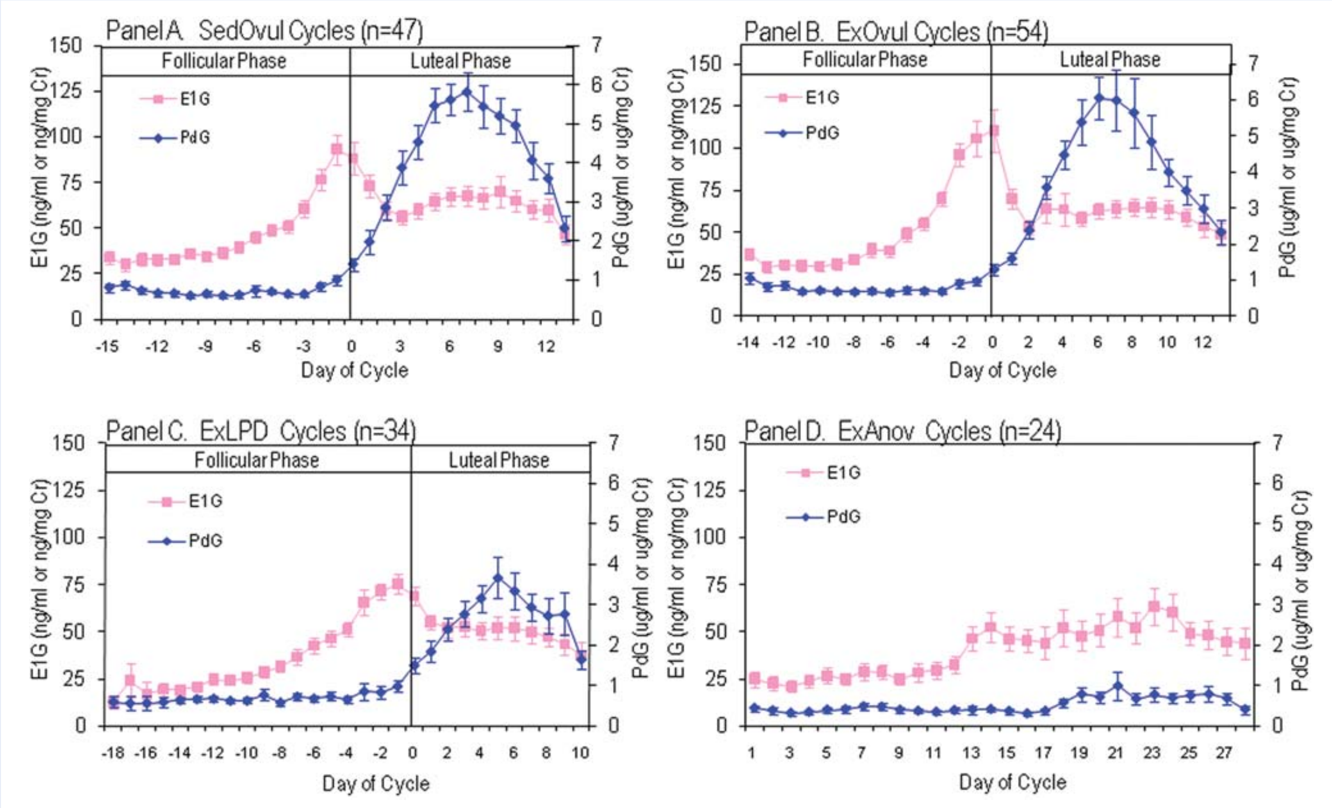

Figure 4 Composite graphs of daily urinary reproductive excretion for ovulatory and abnormal cycles when categorized by exercise and menstrual status. (**A**–**D**) Demonstrate the mean daily E1G and PdG concentrations among all SedOvul, ExOvul, ExLPD and ExAnov cycles, respectively. SedOvul, Sedentary ovulatory; ExOvul, Exercising ovulatory; ExLPD, Exercising luteal phase deficient; ExAnov, Exercising anovulatory; E1G, estrone-1-glucoronide; PdG, pregnanediol glucoronide.

Table VI Individual comparisons of University of Toronto AWS menstrual cycles grouped by menstrual category of oligomenorrheic and amenorrheic

	Sed cycles (n = 20)	Ex cycles (n = 86)	Probability
Oligomenorrheic cycles	0% (0/20)	3.5% (3/86)	1.000 ($\chi^2 = 0.718$)
Amenorrheic 28 day monitoring periods	0% (0/20)	33.7% (29/86)	0.001 ($\chi^2 = 9.284$)

Values are presented as percentages. Data in parenthesis is the number of cycles in the respective menstrual group over the total number of cycles in the respective exercise group. Sed, sedentary; Ex, exercising.

of those with primary amenorrhea. The frequency of self-reported amenorrhea among exercising women described by other studies (Dale et al., 1979; Sanborn et al., 1982; Shangold and Levine, 1982; Brooks-Gunn et al., 1987; Glass et al., 1987; Beals and Manore, 2002; Cobb et al., 2003; Beals and Hill, 2006; Nichols et al., 2007) ranges from 1 to 26%; however, it is imperative to note that the populations of athletes studied as well as the definitions of amenorrhea differ considerably among studies. This must be taken into account when comparing our reported frequency with those presented by others. That said, reliance on the assessment of daily urinary ovarian steroid concentrations to determine the prevalence of severe and clinically observable menstrual disturbances such as oligomenorrhea and amenorrhea renders a greater frequency of amenorrhea among exercising women than many studies presenting self-reported data. Thus, it appears that self-report of amenorrhea may not accurately capture the prevalence of this health problem among exercising females.

A higher frequency of amenorrhea is displayed among our exercising women when compared with the general population. Utilizing a definition of amenorrhea similar to ours, Bachmann and Kemmann (1982) reported that 2.6% of college students had no menses for at least 3 months. In our study of severe menstrual disturbances, no sedentary women displayed cycles indicative of amenorrhea; however, it is logical that a survey of the general population will result in a slightly higher prevalence presumably attributable to the inclusion of both sedentary and exercising women.

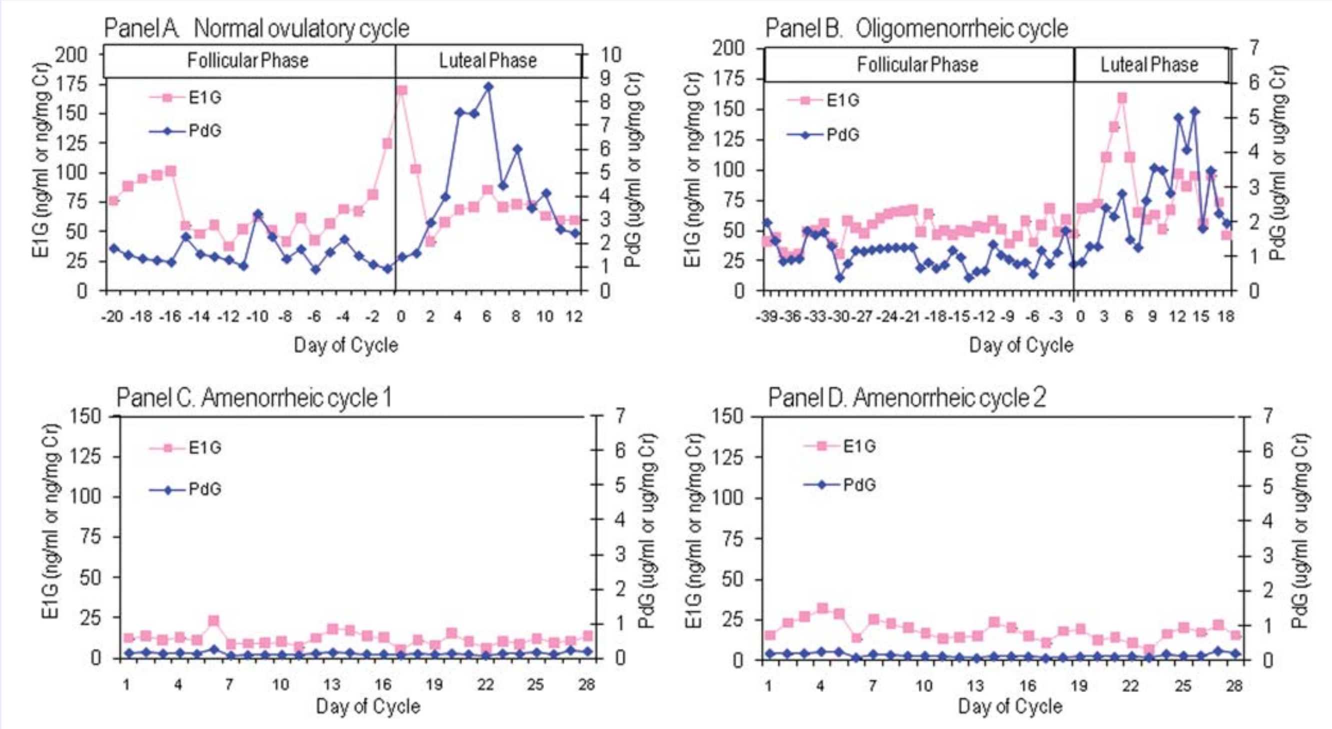

Figure 5 Two consecutive cycles from an exercising subject who inconsistently presented with oligomenorrhea (**A**, ovulatory cycle and **B**, oligomenorrheic cycle) and two consecutive 28-day monitoring periods from an exercising subject who is consistently amenorrheic (**C** and **D**).

Table VII Estrogen excretion characteristics for the cycles grouped by exercise status (sedentary or exercising) and menstrual status (ovulatory, luteal phase deficient or anovulatory)

	SedOvul (*n*=48)	ExOvul (*n*=60)	ExLPD (*n*=35)	ExAnov (*n*=25)	Probability
Estrogen excretion					
E1G peak (ng/ml)	112.77 ± 11.26	118.83 ± 10.0	99.80 ± 13.15	N/A	0.540
E1G peak day	15.24 ± 0.57	14.02 ± 0.50	17.39 ± 0.66[a]	N/A	0.001
E1G Days 2–5	30.55 ± 2.26	28.12 ± 2.00	23.24 ± 2.61	19.97 ± 2.94[b]	0.018
E1G Days 6–12	44.02 ± 2.94	41.11 ± 2.66	31.83 ± 3.67	27.00 ± 3.87[c]	0.001
E1G follicular phase*	46.55 ± 3.05	49.18 ± 2.67	46.46 ± 3.59	29.64 ± 3.92[d]	0.001
E1G luteal phase*	63.09 ± 4.94	60.24 ± 4.39	59.65 ± 5.77	48.88 ± 6.50	0.364
Area under the curve analysis					
E1G follicular phase AUC	665.22 ± 49.88	723.42 ± 44.21	612.00 ± 60.41	405.99 ± 66.17[c]	0.001
E1G luteal phase AUC	770.08 ± 51.56	661.54 ± 46.22	507.47 ± 62.12[e]	506.77 ± 67.83[e]	0.002

Values are the mean ± SEM.
SedOvul, sedentary ovulatory; ExOvul, exercising ovulatory; ExLPD, exercising luteal phase deficient; ExAnov, exercising anovulatory; AUC, area under the curve.
*Follicular and luteal phase values for ExAnov group represent Days 1–13 (follicular) and Day 14 to last day of cycle (luteal).
[a]ExOvul versus ExLPD.
[b]SedOvul versus ExAnov.
[c]SedOvul and ExOvul versus ExAnov.
[d]SedOvul, ExOvul and ExLPD versus ExAnov.
[e]SedOvul versus ExLPD, ExAnov.

In this data set, the prevalence of oligomenorrhea or irregular cycles among exercising women appears to be low compared with other studies. On the basis of self-report of menstrual disturbances, other investigators have observed the frequency of oligomenorrhea to range between 9.8 and 40% among exercising women (Dale, 1979; Shangold and Levine, 1982; Brooks-Gunn *et al*., 1987; Glass *et al*., 1987; Beals and Manore, 2002; Cobb *et al*., 2003; Nichols *et al*., 2007) and between 5.6 and 11.3% in the general population of

Table VIII Progesterone excretion characteristics for the cycles grouped by exercise status (sedentary or exercising) and menstrual status (ovulatory, luteal phase deficient or anovulatory)

	SedOvul ($n=48$)	ExOvul ($n=60$)	ExLPD ($n=35$)	ExAnov ($n=25$)	Probability
Progesterone excretion					
Peak PdG	7.93 ± 0.67	8.31 ± 0.59	4.51 ± 0.78[a]	1.75 ± 0.90[a]	<0.001
Peak PdG day	23.42 ± 0.55	21.83 ± 0.48	23.50 ± 0.65	23.38 ± 0.75	0.076
PdG luteal phase*	4.43 ± 0.33	4.25 ± 0.29	2.68 ± 0.39[a]	1.12 ± 0.43[a,b]	<0.001
Sum of 3-day midluteal PdG	19.28 ± 1.61	19.56 ± 1.42	11.03 ± 1.88[a]	3.59 ± 2.16[a]	<0.001
Area under the curve analysis					
PdG luteal phase AUC	52.54 ± 3.24	47.51 ± 2.90	23.71 ± 3.90[a]	8.73 ± 4.26[a]	<0.001

Values are the mean ± SEM.
SedOvul, sedentary ovulatory; ExOvul, exercising ovulatory; ExLPD, exercising luteal phase deficient; ExAnov, exercising anovulatory; AUC, area under the curve.
*Luteal phase PdG values for ExAnov group represent Days 14 to last day of menstrual cycle.
[a]SedOvul and ExOvul versus ExLPD and ExAnov.
[b]ExLPD versus ExAnov.

college students (Singh, 1981; Bachmann and Kemmann, 1982). Our reported prevalence of 7 and 0% among exercising and sedentary women, respectively, may be an underestimation due to the long duration oligomenorrheic cycles and, consequently, the difficulty of collecting daily urine samples from a single woman for extended periods. It is also possible that since we excluded all women with a current or past history of PCOS we are therefore reporting a conservative but more accurate estimate of oligomenorrhea and possibly amenorrhea in exercising women. Of course, there are no data published to date that have utilized daily ovarian steroid assessments of oligomenorrhea nor carefully excluded cases of PCOS by detailed biochemical evaluations, and as such, self-reported cases may reflect an overestimation, especially if women with PCOS were not excluded. Some investigators have suggested that oligomenorrhea in exercising women may be due to a distinctly different hormonal milieu than the energy deficiency-related mechanisms observed in exercising, amenorrheic women (Rickenlund *et al*., 2003). Specifically, it is proposed that hyperandrogenism is, more often than not, the primary mechanism underlying oligomenorrhea in exercising women (Rickenlund *et al*., 2003).

Clinicians seek to restore normal menstruation in females that present with symptoms of menstrual dysfunction due to the known consequences associated with suppressed ovarian activity. Such clinical consequences include infertility (McNeely and Soules, 1988), impaired vascular function and an unfavorable lipid profile (Rickenlund *et al*., 2005; O'Donnell *et al*., 2009), and the loss of bone mass (De Souza and Williams, 2004; Nattiv, 2007). The ovarian steroids, estrogen and progesterone, play a role in achieving optimal reproductive, cardiovascular, and bone health (McNeely and Soules, 1988; Mendelsohn and Karas, 1999; Compston, 2001); therefore, the suppressed E1G and PdG concentrations reported herein among exercising women with exercise-associated menstrual disturbances may contribute to decreased cycle fecundity and an increased risk of cardiovascular disease and osteoporosis among these women. Appropriate clinical assessment of menstrual status in exercising women with a focus on adequate energy intake is essential for these women to fully experience the benefits of exercise.

In conclusion, the 2–3 month sample prevalence of subtle menstrual disturbances, determined by the analysis of daily urinary ovarian steroids, suggests that approximately half of all exercising women experience abnormal menstrual cycles, i.e. LPD and anovulation, which are associated with decreased cycle fecundity and habitual abortion (McNeely and Soules, 1988). Since these subtle menstrual disturbances are not detected by changes in intermenstrual interval, these disturbances remain largely undiagnosed. Estimates of the prevalence of subtle menstrual disturbances in exercising women determined by the presence or absence of short or long cycles does not identify these disturbances. Furthermore, more than one third of exercising women may present with amenorrhea, a severe menstrual disturbance known to compromise bone health (De Souza and Williams, 2005; De Souza *et al*., 2008). Due to the possible health consequences associated with menstrual dysfunction and its impact on fertility, determining appropriate and feasible measures that can more critically evaluate menstrual disturbances in the clinical setting is warranted. Heightened awareness among physically active women and raising awareness among athletic trainers, coaches, physical therapists and other healthcare professionals about the Female Athlete Triad is essential to promote healthy exercise practices among girls and women.

Authors' Roles

M.J.D. was responsible for designing the study, data collection and interpretation and writing the manuscript. R.J.T. participated in data analyses, data interpretation and writing the manuscript. J.L.S., E.O.D. and S.L.W. participated in data collection, data analyses and writing the manuscript. N.I.W. participated in designing the study, data interpretation and writing the manuscript.

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