A Prospective Exploration of Cognitive Dietary Restraint, Subclinical Ovulatory Disturbances, Cortisol, and Change in Bone Density over Two Years in Healthy Young Women

Jennifer L. Bedford, Jerilynn C. Prior, and Susan I. Barr

Human Nutrition (J.L.B., S.I.B.) and Centre for Menstrual Cycle and Ovarian Research and Department of Medicine, Division of Endocrinology (J.C.P.), The University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4

Context: Cross-sectional studies have found associations among elevated cognitive dietary restraint (CDR), increased ovulatory disturbances, and lower bone mass, possibly mediated by cortisol.

Objective: To determine whether healthy young women with higher CDR have more menstrual cycles with subclinical ovulatory disturbances (SOD), elevated 24-h urinary free cortisol (UFC), and less positive 2-yr areal bone mineral density change (Δ-aBMD).

Design, Setting, and Participants: We conducted a 2-yr longitudinal study of 123 healthy, community-dwelling, nonobese, regularly menstruating women aged 19–35 yr.

Main Outcome Measures: Key variables were Three Factor Eating Questionnaire Restraint score, percent of cycles with anovulation and/or luteal phase length <10 d (%SOD), UFC, and Δ-aBMD at the lumbar spine (L1–L4), total hip, and whole body. Anthropometrics, general stress, physical activity, and energy intake were measured. Adjusting for potential confounders, differences were examined by general linear modeling using median split of CDR score and %SOD.

Results: Women with higher CDR had higher %SOD (56 vs. 34%, P < 0.001) and higher UFC (28.0 vs. 24.0 µg/d, P = 0.021). Δ-aBMD did not differ by CDR. Women with higher %SOD had less positive Δ-aBMD at L1–L4 (0.7 vs. 1.9%, P = 0.034) and hip (−0.6 vs. 0.9%, P = 0.001), and higher CDR score (8.7 vs. 7.1, P = 0.04). Physical activity, general stress, body mass index, and energy intake did not explain differences by CDR or %SOD. UFC was not associated with %SOD or Δ-aBMD.

Conclusion: Women with more frequent SOD reported higher CDR and experienced less positive Δ-aBMD. Although women with higher CDR had higher UFC, the mechanism linking CDR, SOD, and Δ-aBMD is not clear. (J Clin Endocrinol Metab 95: 3291–3299, 2010)

Cognitive dietary restraint (CDR) reflects the perception of limiting food intake in an effort to achieve/maintain a perceived ideal body weight (1). Different from dieting, a behavior where energy intake is limited to lose weight, CDR is a cognitive construct reflecting habitual monitoring of food intake and body weight preoccupation. CDR’s perceptual nature is illustrated by the lack of clear evidence that energy intake, relative body mass, or weight change differs by restraint level (e.g., 2–4).

Evidence suggests that the experience of higher CDR may detrimentally affect ovarian function and bone. Young women with higher restraint scores are more likely...
to report menstrual cycle irregularities (2, 5) and to un- knowingly experience subclinical ovulatory disturbances (SOD) (6–8). These disturbances, including short luteal phase length (LPL) and anovulation, indicate reproductive hormone inadequacies and may influence bone (9). It is well established that overt ovarian disturbances such as amenorrhea detrimentally affect bone (10). Whether SOD are associated with lower bone mineral density (BMD) or increased bone loss is controversial (11–17). A direct cross-sectional relationship between higher restraint and reduced bone has been reported in some (5, 18–21), but not all (7, 22) studies. In the only prospective study to date, CDR was not associated with SOD or BMD change, although BMD change was lower in women with more SOD (17).

The relationship among CDR, ovulatory function, and bone may be mediated by the physiological stress response. Constant monitoring and attempts to control food intake may activate the hypothalamic-pituitary-adrenal (HPA) axis. Stress activation of the HPA axis triggers a cascade of events resulting in increased cortisol and concurrent inhibition of the hypothalamic-pituitary-gonadal axis leading to disturbed menstrual cycles and ovulatory function (Fig. 1) (23). Reports of higher cortisol among women with higher restraint (24–28) suggest restraint may be a chronic stressor resulting in modest but persistent elevations in cortisol within the physiological range. Cortisol has well-established direct effects on bone, and clinical hypercortisolism is consistently associated with reduced BMD (23). Whether cortisol elevations within the normal range influence bone in young healthy women is unclear (5, 7, 18–22, 29). Therefore, we hypothesized that women with higher CDR would have increased 24-h urinary free cortisol (UFC), more frequent SOD, and less positive 2-yr BMD change (Fig. 1).

**Participants and Methods**

**Participants**

Participants were recruited from University of British Columbia classes and the community. Recruitment materials did not refer to eating/body attitudes. Interested women were interviewed by telephone to determine eligibility including age 19–35 yr, no pregnancy/breastfeeding currently or within 12 months, regular menses (self-reported menses every 21–35 d in the previous 6 months or longer), nonobese (self-reported body mass index (BMI) 18–30 kg/m²), consistent sleep patterns (arise and retire at approximately the same time most days), and absence of medical conditions (current or previous diagnosis of eating disorder, polycystic ovarian syndrome, Cushing’s syndrome, inflammatory conditions, hyperthyroidism, or hirsutism) or use of medications (oral contraceptives, progesterone, or glucocorticoids currently or within the past 6 months) that could affect study variables. Of 148 women assessed, 142 were eligible, 137 completed baseline data collection, and 123 (for whom data are reported) completed the study (Fig. 2). The university’s Clinical Research Ethics Board approved the study protocol.

**Data collection**

Data collection occurred at baseline and two follow-ups at 6–12 (mean = 7) months and 1.5–2.5 (mean = 2) years after baseline (Fig. 2). At each of the three data collections, participants met with an investigator to complete anthropometric measurements and for orientation to study procedures. After each meeting, participants completed a questionnaire package, food frequency questionnaire, and 24-h urine collection at home. Every day during the 2-yr study, participants were asked to record their basal temperature in a provided temperature calendar. Dual-energy x-ray absorptiometry (DXA) scans were conducted at Vancouver General Hospital at baseline and 2-yr follow-up.

**Questionnaires**

The questionnaire package (completed at baseline and both follow-ups) included validated questionnaires and questions to elicit demographic and health information. The well-validated Three-Factor Eating Questionnaire Restraint subscale was used to assess CDR (1). To evaluate general psychosocial stress, the Perceived Stress Scale was completed to determine stress perception over the previous month (30), and the Daily Stress Inventory was completed after each 24-h urine collection to determine the
frequency and impact of stressful events (31). The Baecke Questionnaire of Habitual Physical Activity (32) measured participants’ usual activity at work, in sport, and during leisure. The Diet History Questionnaire (version 1; National Cancer Institute, 2002) was completed and analyzed using a Canadian version of the program (33). At the final follow-up, the Eating Disorder Examination Questionnaire (EDE-Q) (34) was completed to confirm continued absence of clinical eating disorders, and any reproductive hormone use was documented.

Ovulatory function

Participants were asked to record their temperature daily throughout the study, immediately upon waking, using a digital thermometer (Becton Dickinson, Franklin Lakes, NJ; product no. 524052). Wake time, menstrual flow status, and any illness were recorded, and sleep quality was rated. Completed temperature records were returned at each follow-up. Temperatures collected during hormonal contraceptive use were not analyzed.

Least-squares quantitative basal temperature (LS-QBT) analysis was conducted to determine evidence of luteal activity (35). This method has been validated against the LH serum peak (35) and urinary progesterone rise (36), established ovulation markers. Cycles were not analyzed if exogenous hormones were used, if febrile illness occurred for 5 or more days or at any point midcycle, or if 33% or more of the temperatures or 3 or more days at midcycle were missing (35).

Cycles are classified as having evidence of luteal activity or ovulatory if there is a significant mean temperature difference between two phases (35). If no temperature increase occurs, the cycle is classified as anovulatory. LPL, the number of days from the day of significant temperature rise until the day before menstrual flow begins, is classified as short if is less than 10 d or normal if 10 d or longer (35). The percentage of cycles with SOD (%SOD) was calculated, and participants were classified with a higher or lower percentage of disturbed ovulation by median split.

Urine analysis

Within several weeks of meeting with investigators at each data collection, participants chose a normal day free of any unusual physical or mental stresses to complete the 24-h urine collection. Participants discarded their first void, recorded the time, and then collected all subsequent voids for 24 h including a void at the recorded time the following morning. UFC (micrograms per 24 h) was analyzed by high-throughput chromatography and tandem mass spectrometry (37). Six participants completed two urine collections, and 117 completed three.

Physical measurements

Height and weight were measured at each data collection. At baseline and final follow-up (1.95 ± 0.14 yr after baseline), DXA scans of the lumbar spine (L1–L4), both total hips, and whole body were completed. Total-body bone-free lean body mass (LBm; kilograms), fat mass (kilograms), percent body fat, and areal BMD (aBMD; grams per square centimeter) were measured on a Lunar Prodigy machine with enCORE software (GE Healthcare, Madison, WI). Daily quality assurance tests were conducted using a spine phantom scan and densitometric calibration. The in-house coefficient of variation with repositioning for aBMD at L1–L4 averaged 0.94% (0.82–1.10%), and the coefficient of variation for total proximal femur averaged 0.70% (0.65–0.76%).

Statistics

Data were coded, verified, entered into SPSS software (version 17, SPSS Inc., Chicago, IL) and cross-checked for accuracy. Physiological variables were examined for outliers (mean ± SD), and none were present.

Repeated-measures general linear model with least significant difference post hoc analysis was used to examine changes over time. Because reported nutrient intakes, questionnaire scores (including CDR), UFC, and urine volume did not change, averages

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**FIG. 2.** Flow diagram depicting study recruitment, participation, and data collection at baseline and first and final follow-up.
were calculated and used in analyses. For all physical measurements, the 2-yr percent change (Δ) was calculated.

A general stress Z-score was calculated from the average Perceived Stress Scale and Daily Stress Inventory Impact and Frequency scores [(participant score – mean score)/SD]. Questionnaire Z-scores were summed and divided by three.

Descriptive statistics were used to characterize the sample. Pearson’s correlations were conducted to identify potentially confounding covariates of study outcome variables (CDR score, UFC, %SOD, and ΔaBMD). Women were classified by median split for SOD (≥38.8 vs. <38.8% of cycles) and for CDR score (≥7.7 vs. <7.7). Group comparisons were completed using independent t tests, χ², and general linear model. Because steroid metabolism may differ between Asians and Caucasians (38), interactions between ethnicity and CDR were examined for UFC, %SOD, and ΔaBMD. The significance level for all analyses was P ≤ 0.05, and cases were excluded pairwise.

Results

Sample

Consistent with the University of British Columbia student body, the sample included Asians (63%) and Caucasians, who did not differ in study outcome variables (data not shown). Mean baseline age was 22.1 ± 3.3 yr, and gynecological age was 9.7 ± 3.7 yr. Almost all had completed some postsecondary education (96%) and were single (92%), nonsmokers (98%), and nulliparous (98%). During the study, 18 women took oral contraceptives for 1–22 months (mean ± SD 6 ± 4.1 months), two used hormonal intruterine systems for 8–18 months, and two used progesterone cream for 0.3–3 months. Participants who used hormones did not differ in outcome variables vs. nonusers (data not shown), and therefore all were included. EDE-Q scores (data not shown) were lower than published norms (39, 40).

Questionnaires and urine analysis

Table 1 shows average values for CDR, general stress, physical activity, energy intake, urine volume, and UFC. CDR (adjusted for BMI) was not correlated with these variables.

Urine volume was correlated with UFC (r = 0.34; P < 0.001) and was therefore included as a covariate. Only general stress Z-score was associated with UFC. UFC was not correlated with baseline or Δ-anthropometrics (data not shown).

Ovulatory function

One hundred fourteen women provided one to 28 cycles (mean ± SD = 13.6 ± 7.0) sufficient for analysis. There were no differences in demographics or outcome variables between participants providing 10 or fewer (n = 42), five or fewer (n = 17), and three or fewer (n = 6) cycles vs. those providing more. The number of cycles analyzed was not correlated with %SOD. Therefore, all 114 participants were included.

Average study cycle length was 30.8 ± 4.1 d, with 14 women experiencing oligomenorrhea (36–90 d between cycles) and one experiencing amenorrhea (>180 d between cycles). Cycle length was inversely associated with age, gynecological age, height, weight, and physical activity (r = −0.19 to −0.32; P < 0.05). Cycle length was not associated with BMI, Δ- anthropometrics, energy intake, number of cycles analyzed, or %SOD, and there were no differences in study outcome variables between those with irregular vs. normal cycle length (data not shown).

Mean %SOD was 43.7 ± 32.0%. Sixty-one percent of women had at least one anovulatory cycle, and 82% had at least one cycle with short LPL. %SOD was associated with age (r = −0.25; P = 0.008), gynecological age (r = −0.29; P = 0.002), and BMI (r = 0.20; P = 0.031) but not other anthropometrics (baseline or Δ; data not shown). After adjustment for baseline gynecological age and BMI, %SOD correlated positively with CDR score (r = 0.22; P = 0.018) but not with physical activity, energy intake, or UFC (urine volume as additional covariate; data not shown).

<table>
<thead>
<tr>
<th>TABLE 1. Mean questionnaire scores, energy intake, urine volume, and UFC and adjusted correlates of CDR and UFC in healthy premenopausal women (n = 123)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rp</strong></td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>CDR</td>
</tr>
<tr>
<td>General stress</td>
</tr>
<tr>
<td>Physical activity</td>
</tr>
<tr>
<td>Occupational</td>
</tr>
<tr>
<td>Sport</td>
</tr>
<tr>
<td>Leisure</td>
</tr>
<tr>
<td>Energy intake (kcal)</td>
</tr>
<tr>
<td>Urine volume (liters/24 h)</td>
</tr>
<tr>
<td>UFC (μg/24 h)</td>
</tr>
</tbody>
</table>

Data are reported as mean ± SD. Values reported are averages from assessments at baseline and both follow-ups because values did not change over time by repeated-measures general linear model. Rp, Partial correlation.

a Adjusted for BMI (kilograms per square meter).

b Adjusted for urine volume (liters/24 h).

c Three Factor Eating Questionnaire Restraint subscale; possible score 0–21.

d Z-score of the Perceived Stress Scale and Daily Stress Inventory Impact and Frequency subscales assessed on the days of urine collection.

e Baecke Habitual Physical Activity Questionnaire; possible scores for subscales 1–5 and total 3–15.

f P = 0.009.
TABLE 2. Physical measurements at baseline and 2-yr follow-up in healthy premenopausal women (n = 123)

<table>
<thead>
<tr>
<th>Physical measurement</th>
<th>Baseline</th>
<th>2 yr</th>
<th>2-yr % changea</th>
<th>P valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>163.0 ± 7.2</td>
<td>163.1 ± 7.2</td>
<td>0.1 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>57.9 ± 8.8</td>
<td>58.4 ± 9.0</td>
<td>1.2 ± 5.5</td>
<td>0.036</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.8 ± 2.5</td>
<td>21.9 ± 2.6</td>
<td>0.7 ± 5.6</td>
<td>0.198</td>
</tr>
<tr>
<td>LBM (kg)</td>
<td>37.8 ± 5.0</td>
<td>38.0 ± 5.1</td>
<td>0.7 ± 3.7</td>
<td>0.051</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>16.8 ± 5.6</td>
<td>17.3 ± 5.7</td>
<td>4.1 ± 17.7</td>
<td>0.053</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>30.3 ± 6.6</td>
<td>30.7 ± 6.5</td>
<td>2.2 ± 12.8</td>
<td>0.169</td>
</tr>
<tr>
<td>Total-body aBMD (g/cm²)</td>
<td>1.136 ± 0.077</td>
<td>1.147 ± 0.078</td>
<td>1.1 ± 1.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lumbar spine aBMD (g/cm²)</td>
<td>1.183 ± 0.121</td>
<td>1.196 ± 0.122</td>
<td>1.2 ± 2.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hip aBMD (g/cm²)</td>
<td>1.025 ± 0.120</td>
<td>1.027 ± 0.122</td>
<td>0.2 ± 2.2</td>
<td>0.380</td>
</tr>
</tbody>
</table>

Data are presented as mean ± sd.

a Two-year measurements were conducted 1.95 ± 0.14 yr after baseline. Measurements before or after the 2-yr time point were corrected to 2-yr percent change.

b Level of significance of differences between baseline and 2-yr values by repeated-measures general linear model.

**Physical measurements**

Table 2 describes participants’ baseline, 2-yr, and percent change in physical measurements. Height, weight, and total-body and L1–L4 aBMD increased during the study. Baseline height, weight, and LBM were not associated with Δ-aBMD, and L1–L4 Δ-aBMD was not associated with Δ- anthropometrics (data not shown). Hip Δ-aBMD correlated inversely with Δ-fat mass (r = −0.18; P = 0.047) and Δ-percent body fat (r = −0.20; P = 0.026) and positively with baseline BMI (r = 0.23; P = 0.012), fat mass (r = 0.23; P = 0.01), and percent body fat (r = 0.19; P = 0.038). Total-body Δ-aBMD was positively associated with Δ-weight (r = 0.21; P = 0.018), Δ-BMI (r = 0.18; P = 0.049), and Δ-LBM (r = 0.18; P = 0.048). Δ-aBMD was not associated with BMI-adjusted CDR or volume-adjusted UFC (data not shown). Adjusted for Δ-LBM, baseline gynecological age, and BMI, only hip Δ-aBMD was significantly associated with %SOD (r = −0.29; P = 0.002).

**Differences by CDR median split**

Study hormone use, number of cycles analyzed, and Δ- anthropometrics did not differ by CDR (data not shown). As shown in Table 3, women with higher CDR had higher baseline weight, BMI, fat mass and percent body fat, and BMI-adjusted energy intakes. Physical activity and general stress did not differ. After adjusting for baseline BMI and gynecological age, %SOD was higher in women with higher CDR. The ethnicity effect and the ethnicity-by-CDR interaction were not significant.

Women with higher CDR had significantly higher UFC (Table 3). There was no effect of ethnicity, but there was a significant ethnicity-by-CDR interaction: Caucasians but not Asians with higher CDR had higher UFC, and among Caucasians, CDR and UFC tended to correlate (r = 0.29; P = 0.056). For Δ-aBMD, there were no main effects of CDR or ethnicity and no interaction.

**Discussion**

The purpose of this study was to examine whether %SOD and UFC differed by level of CDR, and if these variables affected two-year Δ-aBMD in healthy young women. We confirmed previous reports of increased %SOD and higher UFC among women with higher CDR (Table 3). We also confirmed that less positive Δ-aBMD occurred in women with more frequent SOD (Table 4). However, UFC did not differ by %SOD (Table 4), and there was no difference in Δ-aBMD by CDR level (Table 3). Additionally, UFC was not associated with Δ-aBMD. Consequently, whether cortisol mediates the relationship among CDR, SOD, and aBMD (Fig. 1) still remains to be established.

The most noteworthy finding was the confirmation of less positive lumbar spine Δ-aBMD among women with more frequent SOD (12–15, 17). We also found less pos-
TABLE 3. Differences between healthy premenopausal women with higher and lower CDR (by median split) in baseline anthropometrics, questionnaire scores, energy intakes, SOD, UFC, and Δ-aBMD (n = 123)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Higher CDR&lt;sup&gt;a&lt;/sup&gt; (n = 60)</th>
<th>Lower CDR&lt;sup&gt;b&lt;/sup&gt; (n = 63)</th>
<th>P value&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>59.5 ± 1.2</td>
<td>56.3 ± 1.0</td>
<td>0.041</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.4 ± 0.3</td>
<td>21.2 ± 0.3</td>
<td>0.008</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>18.0 ± 0.8</td>
<td>15.7 ± 0.6</td>
<td>0.025</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>31.6 ± 0.9</td>
<td>29.1 ± 0.8</td>
<td>0.033</td>
</tr>
<tr>
<td>Total physical activity&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.9 ± 0.2</td>
<td>7.7 ± 0.2</td>
<td>0.478</td>
</tr>
<tr>
<td>Sport activity&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.6 ± 0.1</td>
<td>2.5 ± 0.1</td>
<td>0.388</td>
</tr>
<tr>
<td>General stress&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.04 ± 0.1</td>
<td>−0.05 ± 0.1</td>
<td>0.536</td>
</tr>
<tr>
<td>Energy intake (kcal)</td>
<td>1676 ± 61</td>
<td>1443 ± 60</td>
<td>0.009</td>
</tr>
<tr>
<td>Cycle length&lt;sup&gt;f&lt;/sup&gt; (d)</td>
<td>31.5 ± 0.5</td>
<td>30.1 ± 0.5</td>
<td>0.060</td>
</tr>
<tr>
<td>SOD&lt;sup&gt;d&lt;/sup&gt; (% of cycles)</td>
<td>55.8 ± 4.0</td>
<td>34.1 ± 3.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Caucasian</td>
<td>64.5 ± 6.5</td>
<td>33.3 ± 5.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Asian</td>
<td>47.1 ± 4.7</td>
<td>34.9 ± 4.9</td>
<td>0.001</td>
</tr>
<tr>
<td>UFC&lt;sup&gt;c&lt;/sup&gt; (µg/24 h)</td>
<td>28.0 ± 1.7</td>
<td>24.0 ± 1.7</td>
<td>0.021</td>
</tr>
<tr>
<td>Caucasian</td>
<td>32.0 ± 2.1</td>
<td>22.6 ± 1.7</td>
<td>0.021</td>
</tr>
<tr>
<td>Asian</td>
<td>25.8 ± 1.4</td>
<td>25.4 ± 1.8</td>
<td>0.021</td>
</tr>
<tr>
<td>Total-body Δ-aBMD&lt;sup&gt;d&lt;/sup&gt; (%)</td>
<td>0.9 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>0.424</td>
</tr>
<tr>
<td>L1–L4 Δ-aBMD&lt;sup&gt;d&lt;/sup&gt; (%)</td>
<td>1.0 ± 0.4</td>
<td>1.5 ± 0.4</td>
<td>0.323</td>
</tr>
<tr>
<td>Hip Δ-aBMD&lt;sup&gt;d&lt;/sup&gt; (%)</td>
<td>−0.1 ± 0.2</td>
<td>0.4 ± 0.3</td>
<td>0.292</td>
</tr>
</tbody>
</table>

Data are presented as mean ± se. Questionnaire scores, energy intake, and UFC values are averages from assessments at baseline and both follow-ups because values did not change over time by repeated-measures general linear model.

<sup>a</sup> Women with Three Factor Eating Questionnaire Restraint score of 7.7 or higher (median).

<sup>b</sup> Women with Three Factor Eating Questionnaire Restraint score less than 7.7 (median).

<sup>c</sup> Level of significance of difference between women with higher and lower CDR by independent t-test or general linear model adjusted for covariates.

<sup>d</sup> Baecke Habitual Physical Activity Questionnaire; possible scores for sport 1–5 and total 3–15.

<sup>e</sup> Z-score of the Perceived Stress Scale and Daily Stress Inventory Impact and Frequency subscales assessed on the days of urine collection.

<sup>f</sup> Adjusted for BMI (kilograms per square meter).

<sup>g</sup> n = 114; adjusted for weight (kilograms) and gynecological age.

<sup>h</sup> n = 114; adjusted for baseline gynecological age and BMI (kilograms per square meter). Interactive effect of ethnicity-by-CDR: F = 3.103; P = 0.038. Main effect of ethnicity: F = 1.930; P = 0.168.

<sup>i</sup> Adjusted for baseline gynecological age and BMI (kilograms per square meter). Interactive effect of ethnicity-by-CDR: F = 4.586; P = 0.034. Main effect of ethnicity: F = 0.218; P = 0.641.

Table 1: Differences between healthy premenopausal women with higher and lower CDR (by median split) in baseline anthropometrics, questionnaire scores, energy intakes, SOD, UFC, and Δ-aBMD (n = 123)

TABLE 4. Differences between healthy premenopausal women with higher and lower %SOD (by median split) in age, anthropometrics, menstrual cycle length, questionnaire scores, UFC and Δ-aBMD (n = 114)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Higher SOD&lt;sup&gt;a&lt;/sup&gt; (n = 57)</th>
<th>Lower SOD&lt;sup&gt;b&lt;/sup&gt; (n = 57)</th>
<th>P value&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>21.4 ± 0.4</td>
<td>22.9 ± 0.5</td>
<td>0.011</td>
</tr>
<tr>
<td>Gynecological age (yr)</td>
<td>8.5 ± 0.4</td>
<td>10.8 ± 0.5</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.2 ± 0.3</td>
<td>21.4 ± 0.3</td>
<td>0.085</td>
</tr>
<tr>
<td>Change in lean mass (%)</td>
<td>1.6 ± 0.5</td>
<td>0.2 ± 0.4</td>
<td>0.018</td>
</tr>
<tr>
<td>Number of cycles</td>
<td>12.8 ± 0.9</td>
<td>14.5 ± 0.9</td>
<td>0.193</td>
</tr>
<tr>
<td>Cycle length (d)</td>
<td>30.9 ± 0.5</td>
<td>30.7 ± 0.6</td>
<td>0.754</td>
</tr>
<tr>
<td>Total physical activity&lt;sup&gt;d,e&lt;/sup&gt;</td>
<td>7.9 ± 0.2</td>
<td>7.7 ± 0.2</td>
<td>0.621</td>
</tr>
<tr>
<td>Sport activity&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.5 ± 0.1</td>
<td>2.5 ± 0.1</td>
<td>0.963</td>
</tr>
<tr>
<td>General stress&lt;sup&gt;e&lt;/sup&gt;</td>
<td>8.7 ± 0.5</td>
<td>7.1 ± 0.5</td>
<td>0.040</td>
</tr>
<tr>
<td>UFC&lt;sup&gt;c&lt;/sup&gt; (µg/24 h)</td>
<td>25.8 ± 1.3</td>
<td>25.6 ± 1.3</td>
<td>0.894</td>
</tr>
<tr>
<td>Total-body Δ-aBMD&lt;sup&gt;d&lt;/sup&gt; (%)</td>
<td>1.0 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>0.775</td>
</tr>
<tr>
<td>L1–L4 Δ-aBMD&lt;sup&gt;d&lt;/sup&gt; (%)</td>
<td>0.7 ± 0.4</td>
<td>1.9 ± 0.4</td>
<td>0.034</td>
</tr>
<tr>
<td>Hip Δ-aBMD&lt;sup&gt;d&lt;/sup&gt; (%)</td>
<td>−0.6 ± 0.3</td>
<td>0.9 ± 0.3</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean ± se. Questionnaire scores and UFC values are averages from assessments at baseline and both follow-ups because values did not change over time by repeated-measures general linear model.

<sup>a</sup> Menstrual cycles were anovulatory and/or had a luteal phase length less than 10 d by LS-QBT analysis at least 38.8% of the time.

<sup>b</sup> Menstrual cycles were anovulatory and/or had a luteal phase length less than 10 d by LS-QBT analysis less than 38.8% of the time.

<sup>c</sup> Level of significance of difference between women with higher and lower percentage of cycles with SOD by independent t-test or general linear model adjusted for covariates.

<sup>d</sup> Baecke Habitual Physical Activity Questionnaire; possible scores for sport 1–5 and total 3–15.

<sup>e</sup> Adjusted for baseline gynecological age and BMI (kilograms per square meter).

<sup>f</sup> Three Factor Eating Questionnaire Restraint subscale; possible score 0–21.

<sup>g</sup> Z-score of the Perceived Stress Scale and Daily Stress Inventory Impact and Frequency subscales assessed on the days of urine collection.

<sup>h</sup> Adjusted for urine volume (liters/24 h), baseline gynecological age, and BMI (kilograms per square meter).

<sup>i</sup> Adjusted for Δ-LBM (kilograms), baseline gynecological age, and BMI (kilograms per square meter).

tive hip Δ-aBMD in women with higher %SOD. It is well established that overt menstrual cycle abnormalities lead to bone loss (10). Yet whether SOD are associated with bone loss remains controversial (9). Our findings suggest that they are, although their impact is modest. One potential reason for conflicting findings regarding bone and SOD could be the duration of ovulatory observations. Because ovarian function is highly variable (41), long-term monitoring is critical to correctly identify women with SOD. The studies that did not observe associations between ovulatory disturbances and bone monitored two to four cycles (11, 16), whereas our study and most others detected a relationship monitored nine to 14 cycles (13, 15, 17).

Our results corroborate that SOD are more common among women reporting higher CDR (6–8). The only other prospective study of CDR and ovulatory function did not find a difference by CDR level in the proportion of women with three or more SOD cycles (17). The null relationship between CDR and SOD in that study is most likely due to the very low proportion of women with three or more SOD cycles (~7%), making detection of a differ-
ence more difficult. The authors did not describe why they classified women on that basis, but the low SOD prevalence may be related to their sample’s greater gynecological maturity, such that psychosocial stress would be less likely to affect cycles. Furthermore, the definition of short LPL in that study (<10 d by urinary LH surge detection) may underestimate the prevalence of cycles with SOD. Urine LH peaks before follicular collapse by ultrasound (42), whereas the significant rise in basal temperature detected with LS-QBT occurs approximately 2 d after the LH peak (35). To equate the two methods, the criterion for short LPL based on urinary LH would be less than 11–12 d rather than less than 10 d used with LS-QBT.

It has been suggested that normal- or underweight women with higher CDR experience more frequent SOD due to caloric restriction and other dieting behaviors (17). However, examination of data from studies that observed relationships between SOD and CDR suggest that mechanism is unlikely (6–8). In our current sample, for example, physical activity did not differ by CDR level, and women with higher CDR actually had higher BMI and energy intake. Furthermore, we used the EDE-Q to establish that participants did not exhibit clinical eating disorders. Therefore, an energy deficit in women with higher CDR was an unlikely cause of SOD. Finally, although various life stresses are associated with SOD (43), among our participants, general stress was not associated with SOD and did not differ by CDR. In fact, the only measured variables differing by SOD level were CDR score and Δ-aBMD.

We did not find that cortisol mediated the relationships among CDR, SOD, and Δ-aBMD. Although UFC was higher among women with higher CDR, as previously reported (24–28), it was not correlated with CDR score in the entire group and did not differ by %SOD level. It is generally accepted that stress-induced HPA axis activation is related to ovarian disturbances (43). CRH alters pulsatile GnRH release (Fig. 1), leading to impaired reproductive hormone secretion and a spectrum of disturbances of decreasing severity from amenorrhea to oligomenorrhea to regular cycles with SOD (23). However, it could be that eating- and body-related stress impacts ovulatory function via neuroendocrine pathways that do not involve the HPA axis. GnRH secretion can be affected by numerous neurotransmitters and neuropeptides of which several relate to appetite control (43). This may be relevant to CDR in which women attempt to override physiological hunger cues.

Furthermore, we found that UFC was elevated in Caucasians with higher CDR (and that UFC and CDR tended to correlate in Caucasians), but UFC did not differ by CDR level among Asians. In a study of healthy, regularly menstruating women, Asian women had 6-β-hydroxycortisol to cortisol ratios that were two to three times lower than Caucasians (38). This is significant because the 6-β-hydroxycortisol to cortisol ratio indirectly indicates cytochrome P450 3A4 activity, an enzyme involved in steroid metabolism (38). However, as reported elsewhere (44, 45), we did not see a difference in UFC by ethnicity. Moreover, Asians with higher CDR, despite having similar UFC as Asians with lower CDR, had higher %SOD. Taken together, this suggests that cortisol may not mediate the association between CDR and SOD.

That UFC differed by CDR level among Caucasians but not Asians is an interesting finding. There were no differences in CDR, general stress, %SOD, UFC, or Δ-aBMD by ethnicity (data not shown). If cortisol is metabolized more rapidly by Asians (38), we would still expect to see the same pattern of difference by CDR, although lower absolute levels. It could be that despite similar CDR scores, the qualitative experience of eating/body-related stress differs between Asians and Caucasians. The influence of ethnicity on the experience of CDR as a stressor has not yet been explored.

We also did not find an association between UFC and Δ-aBMD. Although reduced BMD is observed in hypercortisolism (23), it is less clear whether this occurs in healthy young women, when cortisol is elevated yet remains within the normal range (5, 7, 18–22, 29). Estrogen may mediate the relationship between cortisol and bone; women who continue to menstruate, such as our participants, would likely have normal estrogen levels. A major negative effect of cortisol on bone may be prevented by estrogen’s antiresorptive effects (46). This may explain why studies including women with oligomenorrhea found an association between higher restraint and lower aBMD (5, 19). It could also be that the association of Δ-aBMD with elevated cortisol within the normal range is subtle and difficult to detect over 2 yr. In fact, we observed a modest inverse association between UFC and aBMD at baseline in this sample (29). Although differences in the rate of bone loss between those with slightly elevated vs. lower cortisol may be relatively small, over time, the accumulated effects could impact aBMD and fracture risk.

This study was not without limitations. Our sample was relatively homogeneous, and our findings generalize only to those with similar characteristics. We did not account for osteoporosis family history or physical activity during adolescence. Both may be associated with aBMD in healthy premenopausal women. Twenty-two women used hormonal contraceptives during the study; however, these did not influence Δ-aBMD. Moreover, in our study, women initiated hormone use for contraception, not because of menstrual abnormalities. Although we
screened for polycystic ovarian syndrome based on clinical symptoms, androgen levels were not measured. Our method of observing ovulatory function is not as accurate as cyclic determinations of reproductive hormones; however, LS-QBT has been validated (35, 36), is inexpensive, and is acceptable to women. Furthermore, given within-person variability in ovulatory function (41), accurate characterization requires monitoring cycles over a long period. This method allowed us to accomplish this.

The use of DXA to measure aBMD may also be a limitation. DXA assesses bone mass rather than bone strength. Additional prospective studies examining eating attitudes, ovulatory function, and bone would be improved by using quantitative computed tomography, which differentiates between cortical and trabecular bone, or other measures that can document bone microarchitecture.

Our study contributes to the emerging field of research linking psychosocial and physiological health, highlighting the importance of both attitudes and behaviors in defining well-being. We confirmed that healthy premenopausal women with higher CDR experienced more frequent SOD and that a higher occurrence of SOD resulted in less positive Δ-aBMD over 2 yr. Although the magnitude of the effect on bone was modest, SOD may have a persistent negative influence on bone in young women (15). Contrary to our hypothesis, although UFC was higher in women with greater CDR, we did not confirm that cortisol played a role in mediating associations among CDR, SOD, and Δ-aBMD. Future studies would be improved by examining other potential mechanisms including neuropeptides. The high variability in BMD and ovulatory function indicate the need for a large sample with longer follow-up to firmly establish whether young women’s eating attitudes can affect bone.

Acknowledgments

We thank our participants, Amandeep Ghuman (data entry), Romy Chan and her staff (urinalysis), Vancouver General Hospital Nuclear Medicine technologists (DXA), and Dr. Christine Hitchcock (analysis/interpretation of temperature records).

Address all correspondence and requests for reprints to: Dr. Susan Barr, 2205 East Mall, Vancouver, British Columbia, Canada V6T 1Z4. E-mail: susan.barr@ubc.ca.

This work was supported by Canadian Institute for Health Research (CIHR) Operating Grant 79563. J.B. was supported by a CIHR Doctoral Award and was a Michael Smith Foundation for Health Research Trainee.

Disclosure Summary: The authors have nothing to disclose.

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