Int J Behav Med. Author manuscript; available in PMC 2010 September 01.

Published in final edited form as:
Int J Behav Med. 2010 September ; 17(3): 207–215. doi:10.1007/s12529-009-9064-2.

The relationship between 24-hr urinary cortisol and bone in healthy young women

Jennifer L. Bedford, BSNH, PhD Candidate and **Susan I. Barr, PhD, RD***
Human Nutrition, The University of British Columbia, 2205 East Mall, Vancouver, British Columbia, V6T 1Z4, CANADA

Abstract

Background—Cortisol within the normal range has been associated with reduced bone density in the elderly, but little is known about this relationship in healthy young women.

Purpose—To assess whether 24-hr urinary free cortisol excretion (UFC) is related to bone density in 132 healthy, non-obese, regularly-menstruating women, aged 19–35.

Method—Participants completed questionnaires (food frequency, demographics, physical activity, dietary restraint, perceived and daily stress) and a 24-hr urine collection. UFC was determined by high-throughput liquid chromatography and tandem mass spectrometry. Anthropometrics were completed and a dual energy x-ray absorptiometry scan measured a real bone mineral density (aBMD, g/cm^2) and bone mineral content (BMC, g) at the lumbar spine (L1-4), hip and total body (TB) as well as total body lean (LBM) and fat mass.

Results—aBMD and BMC were significantly positively associated with height, LBM, physical activity, calcium intake and duration of previous oral contraceptive use (except L1-4) and negatively with perceived stress. UFC was not correlated with any measured variables except urine volume ($r=0.17$, $p=0.046$). After adjusting for urine volume, height, LBM, ethnicity and prior oral contraceptive use, UFC was significantly inversely associated with TB BMC ($r=-0.30$, $p<0.001$) and aBMD ($r=-0.27$, $p=0.003$), L1-4 aBMD ($r=-0.19$, $p=0.035$) and BMC ($r=-0.18$, $p=0.049$) and hip BMC ($r=-0.23$, $p=0.011$). Further adjustment for sport activity, calcium intake and perceived stress did not change these relationships meaningfully except that L1-4 became nonsignificant ($p<0.07$).

Conclusion—Cortisol within the normal range appears to have a minor negative influence on bone density in healthy young women.

Key terms

Cortisol; bone density; physical activity; perceived stress; women; dietary restraint

*Corresponding Author: Dr. Susan Barr, 2205 East Mall, Vancouver, BC, V6T 1Z4, CANADA. susan.barr@ubc.ca, Telephone: 604-822-6766, Fax: 604-822-5143.

Disclosure statement: The authors have nothing to disclose.

Ethical Approval: The procedures of this study received ethics approval from the University of British Columbia's Clinical Research Ethics Board on May 11, 2006 (reference number: C05-0257), and approval was renewed annually.

Introduction

Stress, whether inflammatory, traumatic or psychological, activates the hypothalamic-pituitary-adrenal (HPA) axis triggering an increase in cortisol, a glucocorticoid stress hormone, which over time may have implications for various body systems [1]. Cortisol may negatively affect bone density by altering bone turnover, impairing intestinal absorption and renal reabsorption of calcium, and, in premenopausal women, by inhibiting reproductive hormones [2]. A recent review strongly suggests that patients with Cushing's syndrome, a condition of hypercortisolism, have reduced bone formation, lower bone density and an increased incidence of osteoporosis and fractures [2]. Subclinical hypercortisolism, as found with adrenal adenoma, shows similar patterns [2]. Whether variation in cortisol within the normal range can also have adverse effects on bone density is less clear.

Studies among healthy older adults report an inverse association between cortisol and bone density [3–6] and a positive association between cortisol and fracture risk [7]. Among premenopausal women, evidence suggestive of an inverse relationship between cortisol and bone density comes from clinical samples of patients with major depression and eating disorders [8–13]. However, the findings are not consistent [14–16], possibly due to the presence of other bone-related factors in these disorders such as amenorrhea, immune dysfunction and medication side effects. Only three cross-sectional studies have assessed whether cortisol is related to bone density in healthy young women, all of which included women with high versus low levels of cognitive dietary restraint [17–19]. Dietary restraint represents the perception that one is constantly monitoring and attempting to limit food intake in an effort to achieve a perceived ideal body weight, and may be a subtle but chronic stressor, as women with high restraint have been reported to have higher salivary or 24-hr urinary cortisol than women with low restraint [17, 20–22]. However, due to small sample sizes, inconsistent findings, and selection of women with particular eating attitudes, the findings related to bone density and cortisol from these studies are inconclusive [17–19]. Thus, the purpose of the current study was to assess the relationship between 24-hr urinary free cortisol excretion and bone density in healthy young women.

Materials and Methods

Participants

From August to December 2006, participants were recruited using announcements in University of British Columbia classes and poster advertisements in the community (Vancouver, British Columbia, CANADA). Women were asked to participate if they were: 19–35 years of age, regularly-menstruating (menses every 21–35 days), non-obese (self-reported body mass index (BMI) of 18–30 kg/m^2), and in general good health. Women were excluded if they reported any medical conditions (eating disorder, polycystic ovarian syndrome, Cushing's syndrome, inflammatory conditions and thyroid disorders) or use of any medications, currently or within the previous six months, that would affect the HPA axis or bone density (oral contraceptives, progesterone, glucocorticoids). After 148 interested women were screened for eligibility by phone, 142 were eligible: women were ineligible as a result of oral contraceptive use (n=3), shift work (n=1), BMI of <17 kg/m^2 (n=1) and glucocorticoid use (n=1). Two women did not attend their study orientation and were

unavailable to reschedule. Therefore, 140 participants provided written informed consent and met with an investigator (JB) to have anthropometric measurements made and to receive materials and instructions on completion of a questionnaire package, food frequency questionnaire (FFQ), 24-hr urine collection and a dual energy x-ray absorptiometry (DXA) scan. Data collection occurred from August 2006 to February 2007. The study protocol was approved by the university's Clinical Research Ethics Board.

Questionnaires

Participants completed a questionnaire package which included questions to elicit information about some variables known or thought to be associated with bone density including the following: demographics (e.g. age, ethnicity), cigarette use, age of menarche, pregnancies and previous use of progesterone and oral contraceptives. To assess the influence of physical activity on bone density, the widely used *Baecke Questionnaire of Habitual Physical Activity* was used to measure occupational, sport, non-sport leisure and total physical activity [23]. The 16 items are scored on a five-point Likert scale. Higher scores reflect higher physical activity levels, with possible scores of 1–5 for each domain and 3–15 for total score. To evaluate the psychosocial aspects of stress we used the following well-validated and widely used questionnaires. The *Perceived Stress Scale* (PSS) includes 14 items, each scored on a five-point Likert scale, to determine the perception of stress in the previous month [24]. Possible scores range from 0–56 with higher scores indicating elevated stress perception. The 21 item Restraint subscale of the *Three Factor Eating Questionnaire* (TFEQ) assesses the level of cognitive dietary restraint. Possible scores range from 0 to 21, and higher scores suggest increased awareness and concern with weight, shape and eating [25]. As well, participants answered the *Daily Stress Inventor*y (DSI) to assess the potential impact of 58 everyday minor stressful events which may have occurred during the 24-hr urine collection. Events that did not occur are scored as zero, and those that occurred are scored on a scale of 1 (not at all stressful) to 7 (caused me to panic) [26]. Accordingly, higher scores indicate increased general stress for the day of the urine collection.

Dietary Intake

The FFQ used in this study was the Diet History Questionnaire (DHQ, Version 1.0 National Institutes of Health, Applied Research Program, National Cancer Institute, 2002). The DHQ has been adapted for use with the Canadian Nutrient File [27]. Energy intakes of <600 or >3500 kcal/d are deemed 'biologically implausible' and removed [27], resulting in removal of three questionnaires >3500 kcal. Reported nutrient intakes are from food and supplements.

Urinalysis

At home, participants completed a 24-hr urine collection on a 'normal day' avoiding any unusual physical or mental stresses. On the day of collection, participants discarded their first urine void, recorded the time this occurred and then collected all subsequent voids for 24 hours (including a void at the recorded time the following morning). Completed samples were delivered to the Vancouver General Hospital (VGH) Laboratory by courier. Volume was measured and aliquots were frozen and stored prior to analysis. Twenty-four hour

urinary free cortisol excretion (UFC; μg) was measured by high-throughput liquid chromatography and tandem mass spectrometry [28].

Anthropometrics and Body Composition

Anthropometric measurements were made at study entry. Waist circumference was measured at the narrowest point between the iliac crest and lowest rib to the nearest 0.1 cm using an inelastic, flexible measuring tape. Height without shoes was measured to the nearest 0.1 cm at full inspiration using a stadiometer (model 214; Seca, Hamburg, Germany). While wearing light indoor clothing without shoes, weight was measured to the nearest 0.1 kg using an electronic scale. From these data, body mass index (BMI; kg/m^2) was calculated. Measurements were made in duplicate. If differences occurred, a third measurement was made and the two closest measurements were averaged.

At VGH, areal bone mineral density (aBMD, g/cm^2), bone mineral content (BMC, g) and bone area (cm) at the lumbar spine (L1-4), both hips and total body were measured using DXA (Lunar Prodigy, enCORE software; GE Healthcare, Madison, WI). As well, total body bone-free lean and fat mass (kg) and percent body fat were determined. Daily quality assurance tests were conducted using a spine phantom scan and densitometric calibration. Repeat aBMD measurements fall within ±0.01 g/cm^2 for L1-4 and ±0.012 g/cm^2 for the proximal femur according to the manufacturer. The in-house coefficient of variation (CV) for aBMD at the lumbar spine averaged 0.94% (range 0.82 – 1.10%) and the CV for total proximal femur averaged 0.70% (range 0.65–0.76%).

Statistical Analysis

Data were coded, verified and entered into SPSS (v.15) software (SPSS Inc., 2006) and crosschecked for accuracy. Data were examined for outliers. Descriptive statistics were used to characterize the sample. Pearson's correlations were used to identify variables associated with aBMD, BMC, bone area, UFC and PSS score. Relationships between UFC and aBMD, BMC and bone area were then examined using partial correlations to adjust for potentially confounding affects of urine volume, ethnicity, height, lean mass and duration of previous oral contraceptive use as well as lifestyle variables (physical activity, perceived stress, calcium intake). For calcium intake, correlations were examined as calcium intake per unit of energy (mg/kcal). When independent variables were highly inter-correlated (e.g., weight and lean mass; total activity and sport activity), the variable with the highest univariate correlation with aBMD and BMC was included in partial correlations. Differences between Caucasians and Asians were examined by independent sample t-tests and general linear model adjusting for covariates. For all analyses, cases were excluded pairwise. As this was an exploratory study, P values ≤0.10 are reported.

Results

Characteristics of the Participants

Of the 140 women who were oriented to the study, 137 completed a DXA scan and 135 returned completed questionnaire packages. A urine collection was completed by 139 participants and UFC data were available for 134 as the lab lost four samples and one

extreme outlier (7.4 SD above the mean) was removed from all analyses. The data presented in this paper are from the 132 women for whom UFC and DXA measurements were available. Participants were 22.3 ± 3.6 years of age; almost all were students (94%) and single (92%); 41% were Caucasian and 59% were Asian; and 3% currently smoked cigarettes. Mean age at menarche was 12.6 ± 1.4 years, 98.5% were nulliparous, 32% reported previous use of oral contraceptives and 8% reported previous progesterone use. Table 1 provides descriptive statistics of physical activity and questionnaire scores, intake of bone-related nutrients, urinalysis, anthropometrics and DXA measurements. Asians were significantly younger, smaller (height, weight, waist circumference, lean mass, fat mass, BMI), less physically active (sport, leisure and total physical activity) and had lower intakes of calcium/kcal and alcohol/kcal (data not shown). Asians were less likely to report previous progesterone or oral contraceptive use and among those who did, for a significantly shorter period of time (data not shown). However, dietary restraint, age of menarche, percent body fat and intake of energy, vitamin D/kcal or caffeine/kcal did not differ by ethnicity (data not shown).

Associations with aBMD, BMC and Bone Area

Table 2 shows Pearson's correlation coefficients for the variables that were significantly associated with bone parameters (aBMD, BMC and bone area). All bone parameters were positively associated with height, weight, waist circumference, lean mass, physical activity, sport activity, leisure activity (except total hip aBMD) and energy-adjusted calcium intake (for total body aBMD, $p=0.055$). All bone parameters were negatively associated with PSS score except L1-4 area. Fat mass was related to total body aBMD and BMC, L1-4 BMC and total hip and L1-4 area. Total body and total hip parameters were positively correlated with duration of previous oral contraceptive use. Bone parameters did not differ by ethnicity after controlling for height, lean mass, sport activity, prior oral contraceptive use and calcium/kcal intake. No other variables (including age or age of menarche) or nutrients of relevance to bone health were correlated with bone parameters.

Associations with 24-hr Urinary Free Cortisol Excretion

The volume of urine collected was related to UFC ($r=0.174$, $p=0.046$). Urine volume was positively associated with height, weight, LBM, BMI, waist circumference, reported energy intake and sport activity ($r=0.18$ to 0.37, $p<0.05$) but not with fat mass or scores of dietary restraint, PSS or DSI. Controlling for weight and/or height did not change the relationship between UFC and urine volume and therefore, urine volume was included as a covariate in all further analyses. In univariate and partial correlation adjusting for urine volume, UFC was not correlated with age, any questionnaire scores or anthropometric measurements including waist circumference, fat and lean mass (data not shown). UFC did not differ by ethnicity before or after adjusting for urine volume (data not shown). As dietary restraint was positively correlated with BMI ($r=0.32$, $p<0.001$), the analysis was repeated with BMI as an additional covariate however; the relationship between restraint and UFC remained nonsignificant.

Associations with PSS Score

PSS score was negatively correlated with physical activity ($r = -0.37$, $p<0.001$), sport activity ($r= -0.39$, $p<0.001$), non-sport leisure activity ($r= -0.17$, $p=0.048$), weight ($r= -0.27$, $p=0.002$), lean mass ($r= -0.30$, $p<0.001$), BMI ($r= -0.24$, $p=0.006$) and waist circumference ($r= -0.23$, $p=0.006$). An inverse relationship between PSS score and age ($r= -0.17$, $p=0.056$) approached significance. Positive associations were observed between PSS and DSI scores for the day of urine collection ($r=0.24$, $p=0.006$).

Associations Between 24-hr Urinary Free Cortisol Excretion and aBMD, BMC and Bone Area

As shown in Table 2, UFC was negatively correlated to total body aBMD and approached significance with total body BMC ($p=0.057$) and L1-4 aBMD ($p=0.074$) in univariate analyses. Urine volume was positively related to BMC and area at all sites and hip aBMD. Partial correlations between UFC and bone parameters are shown in Table 3. Adjusting for urine volume, UFC was negatively correlated to total body aBMD and BMC, L1-4 aBMD and total hip BMC. The inverse relationship between UFC and L1-4 BMC approached significance ($p=0.061$). After controlling for variables identified as being related to bone parameters in univariate analyses (urine volume, ethnicity, height, lean mass, duration of previous oral contraceptive use), significant inverse associations were observed between UFC and total body BMC and aBMD, total hip BMC, and L1-4 aBMD and BMC. The relationships between UFC and total hip aBMD ($p=0.076$) and area ($p=0.092$) approached significance. These relationships between UFC and bone parameters did not change meaningfully after additional adjustment for lifestyle variables (calcium/kcal intake, sport activity and PSS score) except that L1-4 aBMD ($p=0.059$) and BMC ($p=0.070$) became nonsignificant. The association between UFC and total hip area ($p=0.09$) also did not reach significance in the final adjusted model.

Discussion

Our findings suggest that cortisol within the normal range is negatively associated with bone density in healthy young women, consistent with reports from samples of healthy older adults [3–6]. We found that 24-hr urinary free cortisol excretion (UFC) was modestly associated with total body and lumbar spine aBMD and BMC and total hip BMC.

Previously, the majority of data concerning cortisol and bone density in young women came from studies using samples with clinical depression and eating disorders. While some observed negative associations between cortisol and bone density [8–13], others did not [14–16]. These discrepancies are perhaps related to disease conditions and/or treatments which may have a greater impact on bone density. The authors are aware of only three studies that examined associations between cortisol and bone density in healthy young women, all of which included only women with high and low dietary restraint, and found no significant relationships between aBMD and fasting serum, salivary or 24-hr urinary cortisol [17–19]. The small sample sizes (n=62–78) in these studies and recruitment of women with particular eating attitudes may have limited their power to detect a potential association between

cortisol and bone density. As well, the method of determining cortisol may be important when assessing this relationship.

As cortisol secretion is characterized by marked diurnal variation, fasting single measurements, overnight sampling or sampling during hospitalization may not reflect usual cortisol levels. Using a 24-hr urine collection captures all daily cortisol excretion and for the most part participants are able to go about their normal activities. This method may therefore more accurately reflect usual cortisol levels resulting from stress-induced activation of the HPA axis. Nevertheless, we acknowledge that our method of assessing cortisol also has limitations. Though detailed instruction and support were provided, the collection of urine for 24 hours may be imprecise if, for example, the collection period is not exactly 24 hours or if some voids are not collected. As well, we did not account for participants' menstrual cycle phase, and the diurnal rhythm of cortisol appears to differ during the follicular and luteal phases of the menstrual cycle [29–33]. However, 24-hr quantitative measures do not differ between phases [29–33], so this is unlikely to have affected our results.

In addition to cortisol, our indicator of HPA axis activity, we examined psychosocial indicators of stress. Interestingly, UFC was not related to perceived stress over the previous month (PSS score), the amount of stress encountered on the day of the UFC measurement (DSI score), or to concern related to eating and body image (as assessed by dietary restraint). Several other groups report no relationship between salivary cortisol and PSS scores among young women leaving the welfare system [34], undergraduate students [21, 35], white-collared male workers [36–37], post-menopausal women [20] or healthy middle-aged [38] and older adults [39]. The effect of perceived stress on cortisol may be mediated by other psychosocial variables, such as mood [37, 39], which were beyond the scope of this study.

Furthermore, UFC was not associated with lean or fat mass, % body fat or waist circumference. Obesity, particularly the accumulation of visceral fat, is a well-known characteristic of hypercortisolism and some evidence suggests the potential for cortisol to lead to positive energy balance and abdominal obesity among healthy individuals [1]. There is little evidence of an association between cortisol and indicators of body fat in healthy, normal-weight women and therefore prospective data are required to determine if cortisol is associated with gains in visceral fat or overall weight over time in these women.

We had speculated that dietary restraint may be a stressor for young women sufficient to increase cortisol, which over time may cause adverse affects on health including bone density. However, dietary restraint was not correlated with PSS score, UFC or aBMD and BMC at any site in the current study before or after adjustment for confounders. Our previous work where UFC and BMC in young women [17] and UFC in post-menopausal women [20] differed by dietary restraint level involved prescreening women for either high (TFEQ Restraint subscale score ≥13) or low (score ≤5) dietary restraint. It is possible that there may be a threshold for an effect of dietary restraint, such that in the current study we did not have enough women with very high scores (n=23 with high restraint) to detect an association between restraint and UFC or bone density. Studies similar to ours in sample size and characteristics that examined bone density over a spectrum of dietary restraint scores also reported no relationship with aBMD [40–41], although one study noted lower total body

BMC with higher restraint in three out of four body weight groups [41]. In addition, two studies of teens and young women with high activity levels reported lower aBMD among those with high versus low dietary restraint [42, 43], although the inclusion of women with oligo-amenorrhea in those studies complicates interpretation. Thus, whether dietary restraint has an independent effect on bone density remains to be established.

One of the strengths of the present study is that we assessed and accounted for other variables thought to affect bone density in young women including ethnicity, calcium intake, previous oral contraceptive use and physical activity. Consistent with the literature, we found that bone density did not differ between Asians and Caucasians after adjustment for differences in body size [45] and we observed modest positive associations between aBMD and BMC and energy-adjusted calcium intake [45] and the duration of previous oral contraceptive use [46]. Physical activity is highly important in maintaining bone density [47] and we observed correlations between physical activity, particularly sport activity, and aBMD, BMC and bone area at all sites. This is consistent with findings of greater differences in bone density between athletes and controls than between physically-active and normally-active women [48].

Interestingly, PSS score was negatively associated with aBMD and BMC at all sites, though the correlations were no longer significant after controlling for physical activity (data not shown). Stress relief is promoted as a benefit of physical activity and a relationship between higher physical activity and lower perceived stress has been reported in the literature [49–53], and was also observed in our sample. However, neither physical activity nor PSS score was associated with our indicator of HPA axis activity, UFC. Intense exercise is a stress condition in which cortisol secretion is elevated and there is much research on cortisol during acute exercise [54]. Few studies however have examined the relationships between cortisol, perceived stress and usual physical activity. A few small intervention trials have observed a reduction in cortisol after an 8-week jogging program in 49 young women with mild depression [55] and after an 18-week Tai Chi program in 9 young adults [56]. Additionally, PSS scores were reduced among 10 older adults from an assisted living community after a 10-week exercise program, though serum cortisol levels did not change significantly over time or differ from 10 controls [52]. It could be that in addition to the well-established mechanical role, physical activity may help to maintain bone density by limiting stress-induced elevations in cortisol. Further investigation as to whether stress reduction programs that incorporate physical activity can reduce cortisol levels among young healthy women with high levels of perceived stress may be warranted.

In summary, study findings suggest that endogenous cortisol within the normal range is negatively associated with bone density in young women with characteristics similar to the study sample. As such study findings are generalizable to other healthy, regularly-menstruating, non-obese, non-smoking women as only 3% were current smokers compared to smoking rates of 8% among women aged 20–24 in the health region of the sample population [57]. We are not suggesting that cortisol within the normal range is a major determinant of bone density, but rather that cortisol may be one of the many factors that have a small, but persistent influence on bone density over time. Longitudinal data examining the

relationship between cortisol and changes in aBMD are needed to further establish this relationship in healthy young women.

Acknowledgments

We are extremely grateful to our participants who enthusiastically committed their time to this study. We extend our gratitude to Amandeep Ghuman for her assistance in data entry. We thank Romy Chan and her staff at VGH for the urinalysis and the VGH Nuclear Medicine technologists for the DXA measurements.

Funded by: Canadian Institute for Health Research (CIHR) operating grant 79563. JB was supported by a CIHR Doctoral Award and was a Michael Smith Foundation for Health Research Trainee.

References

1. McEwen BS. Central effects of stress hormones in health and disease: understanding the protective and damaging effects of stress and stress mediators. Eur J Pharmacol. 2008; 583:174–185. [PubMed: 18282566]
2. Chiodini I, Torlontano M, Carnevale V, Trischitta V, Scillitani A. Skeletal involvement in adult patients with endogenous hypercortisolism. J Endocrinol Invest. 2008; 31:267–276. [PubMed: 18401211]
3. Cetin A, Gokce-Kutsal Y, Celiker R. Predictors of bone mineral density in healthy males. Rheumatol Int. 2001; 21:85–88. [PubMed: 11765226]
4. Dennison E, Hindmarsh P, Fall C, Kellingray S, Barker D, Phillips D, et al. Profiles of endogenous circulating cortisol and bone mineral density in healthy elderly men. J Clin Endocrinol Metab. 1999; 84:3058–3063. [PubMed: 10487665]
5. Raff H, Raff JL, Duthie EH, Wilson CR, Sasse EA, Rudman I, et al. Elevated salivary cortisol in the evening in healthy elderly men and women: correlation with bone mineral density. J Gerontol A Biol Sci Med Sci. 1999; 54:M479–M483. [PubMed: 10536652]
6. Reynolds RM, Dennison EM, Walker BR, Syddall HE, Wood PJ, Andrew R, et al. Cortisol secretion and rate of bone loss in a population-based cohort of elderly men and women. Calcif Tissue Int. 2005; 77:134–138. [PubMed: 16151676]
7. Greendale GA, Unger JB, Rowe JW, Seeman TE. The relation between cortisol excretion and fractures in healthy older people: results from the MacArthur studies. J Am Geriatr Soc. 1999; 47:799–803. [PubMed: 10404922]
8. Altindag O, Altindag A, Asoglu M, Gunes M, Soran N, Deveci Z. Relation of cortisol levels and bone mineral density among premenopausal women with major depression. Int J Clin Pract. 2007; 61:416–420. [PubMed: 17313608]
9. Biller BM, Saxe V, Herzog DB, Rosenthal DI, Holzman S, Klibanski A. Mechanisms of osteoporosis in adult and adolescent women with anorexia nervosa. J Clin Endocrinol Metab. 1989; 68:548–554. [PubMed: 2493036]
10. Kahl KG, Rudolf S, Stoeckelhuber BM, Dibbelt L, Gehl HB, Markhof K, et al. Bone mineral density, markers of bone turnover, and cytokines in young women with borderline personality disorder with and without comorbid major depressive disorder. Am J Psychiatry. 2005; 162:168–174. [PubMed: 15625216]
11. Legroux-Gerot I, Vignau J, D'Herbomez M, Collier F, Marchandise X, Duquesnoy B, et al. Evaluation of bone loss and its mechanisms in anorexia nervosa. Calcif Tissue Int. 2007; 81:174–182. [PubMed: 17668143]
12. Misra M, Prabhakaran R, Miller KK, Goldstein MA, Mickley D, Clauss L, et al. Prognostic indicators of changes in bone density measures in adolescent girls with anorexia nervosa-II. J Clin Endocrinol Metab. 2008; 93:1292–1297. [PubMed: 18089697]
13. Naessen S, Carlstrom K, Glant R, Jacobsson H, Hirschberg AL. Bone mineral density in bulimic women--influence of endocrine factors and previous anorexia. Eur J Endocrinol. 2006; 155:245–251. [PubMed: 16868137]

14. Eskandari F, Martinez PE, Torvik S, Phillips TM, Sternberg EM, Mistry S, et al. Low bone mass in premenopausal women with depression. Arch Intern Med. 2007; 167:2329–2336. [PubMed: 18039992]
15. Petronijevic M, Petronijevic N, Ivkovic M, Stefanovic D, Radonjic N, Glisic B, et al. Low bone mineral density and high bone metabolism turnover in premenopausal women with unipolar depression. Bone. 2008; 42:582–590. [PubMed: 18226593]
16. Yazici AE, Bagis S, Tot S, Sahin G, Yazici K, Erdogan C. Bone mineral density in premenopausal women with major depression. Joint Bone Spine. 2005; 72:540–543. [PubMed: 16046174]
17. McLean JA, Barr SI, Prior JC. Dietary restraint, exercise, and bone density in young women: are they related? Med Sci Sports Exerc. 2001; 33:1292–1296. [PubMed: 11474329]
18. Bacon L, Stern JS, Keim NL, Van Loan MD. Low bone mass in premenopausal chronic dieting obese women. Eur J Clin Nutr. 2004; 58:966–971. [PubMed: 15164118]
19. Nickols-Richardson SM, Beiseigel JM, Gwazdauskas FC. Eating restraint is negatively associated with biomarkers of bone turnover but not measurements of bone mineral density in young women. J Am Diet Assoc. 2006; 106:1095–1101. [PubMed: 16815126]
20. Rideout CA, Linden W, Barr SI. High cognitive dietary restraint is associated with increased cortisol excretion in postmenopausal women. J Gerontol A Biol Sci Med Sci. 2006; 61:628–633. [PubMed: 16799147]
21. Putterman E, Linden W. Cognitive dietary restraint and cortisol: importance of pervasive concerns with appearance. Appetite. 2006; 47:64–76. [PubMed: 16624445]
22. Anderson DA, Shapiro JR, Lundgren JD, Spataro LE, Frye CA. Self-reported dietary restraint is associated with elevated levels of salivary cortisol. Appetite. 2002; 38:13–17. [PubMed: 11883913]
23. Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J Clin Nutr. 1982; 36:936–942. [PubMed: 7137077]
24. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. J Health Soc Behav. 1983; 24:385–396. [PubMed: 6668417]
25. Stunkard AJ, Messick S. The Three-Factor Eating Questionnaire to measure dietary restraint, disinhibition and hunger. J Psychosom Res. 1985; 29:71–83. [PubMed: 3981480]
26. Brantley PJ, Waggoner CD, Jones GN, Rappaport NB. A daily stress inventory: development, reliability, and validity. J Behav Med. 1987; 10:61–74. [PubMed: 3586002]
27. Csizmadi I, Kahle L, Ullman R, Dawe U, Zimmerman TP, Friedenreich CM, et al. Adaptation and evaluation of the National Cancer Institute's Diet History Questionnaire and nutrient database for Canadian populations. Public Health Nutr. 2007; 10:88–96. [PubMed: 17212847]
28. Taylor RL, Machacek D, Singh RJ. Validation of a high-throughput liquid chromatography-tandem mass spectrometry method for urinary cortisol and cortisone. Clin Chem. 2002; 48:1511–1519. [PubMed: 12194928]
29. Parry BL, Javeed S, Laughlin GA, Hauger R, Clopton P. Cortisol circadian rhythms during the menstrual cycle and with sleep deprivation in premenstrual dysphoric disorder and normal control subjects. Biol Psychiatry. 2000; 1(48):920–931.
30. Abplanalp JM, Livingston L, Rose RM, Sandwisch D. Cortisol and growth hormone responses to psychological stress during the menstrual cycle. Psychosom Med. 1977; 39:158–177. [PubMed: 866540]
31. Bao AM, Ji YF, Van Someren EJ, Hofman MA, Liu RY, Zhou JN. Diurnal rhythms of free estradiol and cortisol during the normal menstrual cycle in women with major depression. Horm Behav. 2004; 45:93–102. [PubMed: 15019795]
32. Kudielka BM, Kirschbaum C. Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase. Psychoneuroendocrinology. 2003; 28:35–47. [PubMed: 12445835]
33. Aedo AR, Landgren BM, Diczfalusy E. Studies on ovarian and adrenal steroids at different phases of the menstrual cycle: II. A comparative assessment of the circadian variation in steroid and lutropin levels during the follicular, periovulatory and luteal phases. Contraception. 1981; 23:407–424. [PubMed: 7273761]

34. Kneipp SM, Welch DP, Wood CE, Yucha CB, Yarandi H. Psychosocial and physiological stress among women leaving welfare. West J Nurs Res. 2007; 29:864–895. [PubMed: 17630386]
35. Knowles SR, Nelson EA, Palombo EA. Investigating the role of perceived stress on bacterial flora activity and salivary cortisol secretion: a possible mechanism underlying susceptibility to illness. Biol Psychol. 2008; 77:132–137. [PubMed: 18023961]
36. Ebrecht M, Hextall J, Kirtley LG, Taylor A, Dyson M, Weinman J. Perceived stress and cortisol levels predict speed of wound healing in healthy male adults. Psychoneuroendocrinology. 2004; 29:798–809. [PubMed: 15110929]
37. van Eck M, Berkhof H, Nicolson N, Sulon J. The effects of perceived stress, traits, mood states, and stressful daily events on salivary cortisol. Psychosom Med. 1996; 58:447–458. [PubMed: 8902896]
38. Lasikiewicz N, Hendrickx H, Talbot D, Dye L. Exploration of basal diurnal salivary cortisol profiles in middle-aged adults: associations with sleep quality and metabolic parameters. Psychoneuroendocrinology. 2008; 33:143–151. [PubMed: 18155362]
39. Simpson EE, McConville C, Rae G, O'Connor JM, Stewart-Knox BJ, Coudray C, et al. Salivary cortisol, stress and mood in healthy older adults: the Zenith study. Biol Psychol. 2008; 78:1–9. [PubMed: 18243482]
40. Waugh EJ, Polivy J, Ridout R, Hawker GA. A prospective investigation of the relations among cognitive dietary restraint, subclinical ovulatory disturbances, physical activity, and bone mass in healthy young women. Am J Clin Nutr. 2007; 86:1791–1801. [PubMed: 18065600]
41. Van Loan MD, Keim NL. Influence of cognitive eating restraint on total-body measurements of bone mineral density and bone mineral content in premenopausal women aged 18–45 y: a cross-sectional study. Am J Clin Nutr. 2000; 72:837–843. [PubMed: 10966907]
42. Vescovi JD, Scheid JL, Hontscharuk R, De Souza MJ. Cognitive dietary restraint: impact on bone, menstrual and metabolic status in young women. Physiol Behav. 2008; 95:48–55. [PubMed: 18508099]
43. Barrack MT, Rauh MJ, Barkai HS, Nichols JF. Dietary restraint and low bone mass in female adolescent endurance runners. Am J Clin Nutr. 2008; 87:36–43. [PubMed: 18175735]
44. Walker MD, Novotny R, Bilezikian JP, Weaver CM. Race and diet interactions in the acquisition, maintenance, and loss of bone. J Nutr. 2008; 138:1256S–1260S. [PubMed: 18492866]
45. Heaney RP. Calcium, dairy products and osteoporosis. J Am Coll Nutr. 2000; 19:83S–99S. [PubMed: 10759135]
46. Liu SL, Lebrun CM. Effect of oral contraceptives and hormone replacement therapy on bone mineral density in premenopausal and perimenopausal women: a systematic review. Br J Sports Med. 2006; 40:11–24. [PubMed: 16371485]
47. Kohrt WM, Bloomfield SA, Little KD, Nelson ME, Yingling VR. American College of Sports Medicine. American College of Sports Medicine Position Stand: physical activity and bone health. Med Sci Sports Exerc. 2004; 36:1985–1996. [PubMed: 15514517]
48. Nichols DL, Sanborn CF, Essery EV. Bone density and young athletic women. An update. Sports Med. 2007; 37:1001–1014. [PubMed: 17953469]
49. Aldana SG, Sutton LD, Jacobson BH, Quirk MG. Relationships between leisure time physical activity and perceived stress. Percept Mot Skills. 1996; 82:315–321. [PubMed: 8668498]
50. Brown JD. Staying fit and staying well: physical fitness as a moderator of life stress. J Pers Soc Psychol. 1991; 60:555–561. [PubMed: 2037967]
51. Iwasaki Y, Zuzanek J, Mannell RC. The effects of physically active leisure on stress-health relationships. Can J Public Health. 2001; 92:214–218. [PubMed: 11496634]
52. Starkweather AR. The effects of exercise on perceived stress and IL-6 levels among older adults. Biol Res Nurs. 2007; 8:186–194. [PubMed: 17172317]
53. Taylor-Piliae RE, Haskell WL, Waters CM, Froelicher ES. Change in perceived psychosocial status following a 12-week Tai Chi exercise programme. J Adv Nurs. 2006; 54:313–329. [PubMed: 16629916]
54. Mastorakos G, Pavlatou M, Diamanti-Kandarakis E, Chrousos GP. Exercise and the stress system. Hormones (Athens). 2005; 4:73–89. [PubMed: 16613809]

55. Nabkasorn C, Miyai N, Sootmongkol A, Junprasert S, Yamamoto H, Arita M, et al. Effects of physical exercise on depression, neuroendocrine stress hormones and physiological fitness in adolescent females with depressive symptoms. Eur J Public Health. 2006; 16:179–184. [PubMed: 16126743]

56. Esch T, Duckstein J, Welke J, Braun V. Mind/body techniques for physiological and psychological stress reduction: stress management via Tai Chi training - a pilot study. Med Sci Monit. 2007; 13:CR488–497. [PubMed: 17968296]

57. British Columbia (BC) Stats. Summary of smoking rates for BC: September 2006 to August 2007 [Internet]. Victoria (BC): Provincial Government, BC Stats; 2007 Oct 23. [cited 2009 July 1]. Available from: http://www.bcstats.gov.bc.ca/data/ssa/reports/tobacco/smoke07060707.pdf

Table 1

Physical activity and questionnaire scores, reported nutrient intakes, 24-hr urinary free cortisol excretion, anthropometrics and DXA measurements of 132 participants

	Mean ± SD
Physical activity scores[a]	
Occupational activity	2.4 ± 0.6
Sport activity	2.7 ± 1.3
Non-sport leisure activity	3.0 ± 0.7
Total physical activity	8.0 ± 1.8
Questionnaire scores	
Perceived stress scale[b]	27.0 ± 6.5
Cognitive dietary restraint[c]	8.1 ± 4.5
Daily stress inventory[d]	31.1 ± 18.0
Nutrient intakes[e]	
Energy (kcal)	1648 ± 525
Calcium (mg)	862 ± 409
Vitamin D (μg)	90.4 ± 143
Caffeine (mg)	143 ± 195
Alcohol (g)	3.5 ± 5.3
Urinalysis	
24-hr urinary free cortisol (μg/d)[f]	27.3 ± 13.6
24-hr urine volume (L)	1.8 ± 0.9
Anthropometric measurements	
Height (cm)	163.3 ± 7.2
Weight (kg)	58.1 ± 8.5
Body mass index (kg/m^2)	21.7 ± 2.4
Waist circumference (cm)	65.2 ± 5.8
DXA measurements	
Total lean mass (kg)	38.1 ± 5.0
Fat mass (kg)	16.8 ± 5.4
Body fat (%)	30.1 ± 6.5
Total body aBMD (g/cm^2)	1.136 ± 0.075
Total body BMC (g)	2392 ± 365
Lumbar spine aBMD (g/cm^2)	1.184 ± 0.123
Lumbar spine BMC (g)	62.00± 11.33
Lumbar spine area (cm^2)	52.11 ± 5.25
Total hip aBMD (g/cm^2)	1.026 ± 0.120
Total hip BMC (g)	30.24 ± 5.04
Total hip area (cm^2)	29.38 ± 2.45

aBMD, areal bone mineral density; BMC, bone mineral content.

Data are presented as mean ± standard deviation

[a] Baecke Questionnaire of Habitual Physical Activity [23], range 1–5 for subscales and 3–15 for total score

[b] Perceived Stress Scale [24], possible score range 0–56

[c] Cognitive Dietary Restraint subscale of the Three-Factor Eating Questionnaire [25], possible score range 0–21

[d] Daily Stress Inventory [26], possible score range 0–406

[e] n=129 for reported dietary intake, includes intake from food and supplements

[f] To convert from metric (μg/d) to SI units (nmol/d), multiply by 2.759

Table 2

and bone area with anthropometrics, perceived stress, physical activity, duration of previous oral contraceptive use, calcium/
e cortisol excretion and 24-hr urine volume.

Weight (kg)	Lean Mass (kg)	Fat Mass (kg)	Waist Circ (cm)	PSS score	Total Activity score	Sport Activity score	Leisure Activity score	Prior OC use[a]	Calcium (mg/kcal)	UFC (μg/d)	Urine Volume (L)
0.52***	0.58***	0.19*	0.35***	−0.27**	0.34***	0.40***	0.18*	0.18*	0.17^{t}	−0.19*	0.13
0.79***	0.79***	0.42***	0.51***	−0.28***	0.36***	0.43***	0.17*	0.20*	0.23**	−0.17^{t}	0.22*
0.42***	0.47***	0.16^{t}	0.24**	−0.21*	0.27**	0.30***	0.19*	0.13	0.18*	−0.16^{t}	0.11
0.57***	0.67***	0.21*	0.30***	−0.21*	0.32***	0.35***	0.23**	0.15^{t}	0.21**	−0.13	0.19*
0.58***	0.70***	0.23**	0.30***	−0.15^{t}	0.29***	0.30***	0.21*	0.15^{t}	0.19*	−0.06	0.23**
0.39***	0.50***	0.09	0.25**	−0.27**	0.32***	0.40***	0.13	0.21*	0.19*	−0.10	0.19*
0.60***	0.76***	0.17^{t}	0.35***	−0.30***	0.42***	0.50***	0.18*	0.28***	0.25**	−0.12	0.26**
0.62***	0.78***	0.20*	0.33***	−0.19*	0.37***	0.42***	0.18*	0.27**	0.21*	−0.09	0.27**

t point between lowest rib and iliac crest; PSS, Perceived Stress Scale; OC, oral contraceptive; UFC, 24-hr urinary free cortisol excretion; aBMD, areal bone mineral
ontent (g); area, bone area (cm^2). Data are presented as Pearson's (*R*) coefficients. Exact *n* varied by comparison as cases were excluded *pairwise*.

ith p<0.10. Correlation is significant at:

or to the study (non user = 0)

Int J Behav Med. Author manuscript; available in PMC 2010 September 01.

Table 3

Partial correlation models of the relationship between aBMD, BMC and bone area and 24-hr urinary free cortisol excretion

	UFC Model 1	UFC Model 2	UFC Model 3
Total body aBMD	−0.21 *	−0.27 **	−0.25 **
Total body BMC	−0.21 *	−0.30 ***	−0.28 **
Lumbar spine aBMD	−0.18 *	−0.19 *	−0.17 t
Lumbar spine BMC	−0.16 t	−0.18 *	−0.17 t
Lumbar spine area	−0.10	−0.07	−0.08
Total hip aBMD	−0.13	−0.16 t	−0.14
Total hip BMC	−0.18 *	−0.23 *	−0.21 *
Total hip area	−0.15 t	−0.15 t	−0.16 t

UFC, 24-hr urinary free cortisol excretion (μg); aBMD, areal bone mineral density (g/cm^2); BMC, bone mineral content (g); area, bone area (cm^2). Data are presented as Pearson's (*R*) and partial (*Rp*) coefficients adjusting for variables identified as cofounders in univariate analyses. Exact *n* varied by comparison as cases were excluded *pairwise*.

t Correlation approaches significance at p<0.10. Correlation is significant at:

* p<0.05,

** p<0.01,

*** p<0.001.

Model 1: Adjusted for 24-hr urine volume (L). Model 2: Partial correlations adjusting for 24-hr urine volume, height, lean mass, ethnicity and previous duration of oral contraceptive use (zero for non-users). Model 3: Partial correlations adjusting for 24-hr urine volume, height, lean mass, ethnicity, previous duration of oral contraceptive use, sport activity, calcium/kcal intake and Perceived Stress Scale score.