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The effect of exercise and estrogen on osteoprotegerin in premenopausal women ☆

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ABSTRACT

Background: The benefits of exercise are widely recognized, however physically active women can develop exercise associated menstrual cycle disturbances such as amenorrhea (i.e., estrogen deficiency) secondary to a chronic energy deficiency.
Objective: To assess the effects of exercise status and estrogen deficiency on osteoprotegerin (OPG) and its relationship to bone resorption in premenopausal exercising women.
Design: Cross-sectional study of serum OPG, urinary c-telopeptides (uCTX), urinary estrone 3-glucuronide (E1G), urinary pregnanediol 3-glucuronide (PdG) and bone mineral density (BMD) measured on multiple occasions in 67 women. Volunteers were retrospectively grouped: 1) sedentary menstruating group (SedMen $n=8$), 2) exercising menstruating group (ExMen, $n=36$), and 3) exercising amenorrheic group (ExAmen, $n=23$). One-way ANOVAs were performed, and LSD post-hoc tests were performed when differences were detected.
Results: Subjects were similar with respect to age (24.2±1.0 years), weight (57.8±1.7 kg), and height (164.3±1.3 cm) ($p>0.05$). ExMen and ExAmen groups were more aerobically fit ($p=0.003$) and had less body fat ($p=0.002$) than the SedMen group. Resting energy expenditure/fat free mass was lowest ($p=0.001$) in the ExAmen groups. Mean E1G across the measurement period ($p<0.001$) and overall E1G exposure as assessed by E1G area under the curve (AUC) ($p<0.001$) were lower in the ExAmen group vs. the SedMen and ExMen groups. U-CTX-I was elevated ($p=0.033$) in the ExAmen group (281.8±40.3 μg/L/mmCr), compared with the SedMen and ExMen groups (184.5±22.4, 197.2±14.7 μg/L/mmCr, respectively). OPG was suppressed ($p=0.005$) in the ExAmen group (4.6±0.2 pmol/L) vs. ExMen group (5.2±0.2 pmol/L), and OPG was lower in the SedMen group (4.1±0.3 pmol/L) compared with the ExMen group. Findings were translated to BMD; the ExAmen group had suppressed total body BMD ($p=0.014$) and L2–L4 BMD ($p=0.015$) vs. the ExMen group.
Conclusions: Our results suggest that OPG responds to the bone loading effect of exercise, and that suppressed OPG may play a role in the etiology of increased bone resorption observed in exercising women with chronic estrogen deficiency secondary to hypothalamic amenorrhea.

Introduction

The osteogenic benefits of exercise on the skeleton are widely recognized, particularly exercise that involves high loading forces [1–4]. Biomechanical strain produced by the contraction of muscle during exercise is a predominant contributor to bone mass accretion [5,6]. The importance of biomechanical strain on bone is evidenced by literature that demonstrates negative alterations in bone health caused by disuse [7–12], while exercise intervention studies demonstrate that physical activity improves bone mineral density (BMD) [13,14].

Estrogen, a steroid with direct effects on bone remodeling [15], is another important contributor to bone health in women. Functional hypothalamic amenorrhea is a menstrual cycle disturbance characterized by complete suppression of estrogen and this suppression is often cited as a primary cause of reduced bone mass in amenorrheic women [16,17] resulting in low BMD, particularly at the spine [18–24]. Alterations in bone remodeling (i.e., bone markers of formation and resorption) have also been demonstrated in amenorrheic women; we recently reported an increase in bone resorption, as indicated by elevated levels of U-CTX-I, and suppression of bone formation, as indicated by suppressed PINP, in exercising women who were estrogen and energy deficient [25]. We demonstrated that estrogen deficiency, in the presence of an energy deficient environment, leads to significant perturbations in bone health [25].

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Bone remodeling is a highly coordinated process involving osteoblasts, osteoclasts, and various cytokines including receptor activator of nuclear factor kβ ligand (RANKL) and osteoprotegerin (OPG) [26,27]. The binding of RANKL to RANK stimulates the formation, differentiation, and activation of osteoclasts and bone resorption [15,26]. OPG functions as a decoy receptor [15,26–28], and aids in regulating bone loss by blocking the actions of the RANKL/RANK interaction [15,26,27]. OPG is proving to be important in the pathogenesis of bone loss in postmenopausal women [27,29–33] and underscores the importance of investigating this cytokine in a premenopausal cohort.

Estrogen and exercise both impact bone metabolism and OPG. In vivo, the absence of estrogen enhances the ratio of RANKL to OPG, promoting osteoclastogenesis and consequently the acceleration of bone loss [27]. Data in women with a history of anorexia nervosa, who have comparable endocrine alterations to amenorrheic women [34,35], demonstrate an elevation in bone resorption and concomitant suppression of OPG compared with controls [36], however results remain equivocal [36–39]. Regarding the effect of exercise on OPG, Ziegler et al. [40] reported elevated OPG levels immediately after long distance running in middle aged men and women, while mechanical loading and various types of stress and strain increases OPG levels [41–44]. However, investigations on the effects of exercise on OPG in premenopausal women are limited; there is only one study to date that has investigated OPG in premenopausal exercising women, however, estrogen status was not assessed. In that study, although Herrmann and Herrmann [45] reported that athletes exhibited a higher level of bone resorption when compared with controls, OPG levels reported were comparable between the groups.

To date, there are no investigations of OPG in premenopausal exercising women that carefully quantifies the impact of the estrogen environment (by the assessment of daily measures of urinary estrogen metabolites), while considering energy status, on circulating serum OPG and bone resorption. The primary purpose of this study was to evaluate circulating serum levels of OPG in a cross-sectional study of premenopausal women categorized by exercise (sedentary vs. exercising) and menstrual (eumenorrheic vs. amenorrheic) status. Since bone loading exercise is associated with positive skeletal effects, and a positive effect on OPG levels [41–44], we hypothesized that sedentary menstruating women (SedMen) would have lower levels of OPG compared to exercising menstruating women (ExMen). Secondly, since we recently demonstrated that bone resorption was elevated in amenorrheic women [25], and because OPG is tightly linked to bone resorption, we hypothesized that serum OPG levels would be lower in exercising amenorrheic women who are chronically estrogen deficient compared to estrogen replete menstruating women independent of exercise status.

Materials and methods

Experimental design

We conducted an observational study in 67 premenopausal women over 2–3 months to determine the associations between exercise, estrogen status and OPG. The two primary grouping variables were exercise status and menstrual (i.e., estrogen) status. Exercise status was determined by self-reported physical activity records. We categorized women as exercising (Ex) if they participated in greater than 2.5 h of purposeful physical activity per week at a heart rate of >55% max heart rate while women who participated in less than 2.5 h/week of purposeful physical activity at a heart rate of >55% max heart rate were categorized as sedentary (Sed) [46].

Menstrual status was determined by self-report and corroborated by measurement of estrone and pregnanediol glucuronides (E1G and PdG) in daily urine samples. Women were categorized into two groups based on menstrual status: those with hypothalamic amenorrhea (Amen), and menstruating women (Men). These categorizations are described in more detail below. Volunteers were then retrospectively categorized into 3 groups according to their exercise and their menstrual status as follows: 1) sedentary menstruating group (SedMen, $n=8$), 2) exercising menstruating group (ExMen, $n=36$), and 3) exercising amenorrheic group (ExAmen, $n=23$).

The cytokine OPG, and bone resorption marker U-CTX-I were the dependent variables of primary interest in this study. Demographic and anthropometric data were collected, which included measurements of body mass, height, BMD, body composition, dietary characteristics, resting energy expenditure (REE) and peak aerobic capacity.

Volunteers

Volunteers were recruited by posters targeting both sedentary and physically active women for a study on women's health. Screening procedures included questionnaires on exercise, eating, menstrual, and self-reported medical health history. Eligibility criteria for the study included, 1) aged 18–35 years; 2) good health as determined by a medical exam; 3) no chronic illness, including hyperprolactinemia and thyroid disease; 4) stable menstrual status over preceding 3 months; 5) non-smoker; 6) not currently dieting and weight stable (i.e. no gain or loss of >2 kg) for the preceding 3 months, as determined by self-report; 7) not taking any form of hormonal therapy for at least 12 months; 8) no current clinical diagnosis of eating disorders and 9) no other contraindications that would preclude participation in the study. The study was approved by the Ethics Review Board at the University of Toronto and all subjects signed an approved Informed Consent document.

Observational time periods

Eumenorrheic women were monitored for 2 to 3 consecutive menstrual cycles, oligomenorrheic women, for no more than 90 days and amenorrheic women, for 2 to 3 consecutive 30-day monitoring periods. All data presented in this study represent the mean of the 2–3 repeated menstrual cycles (eumenorrheic), 30 (amenorrheic) or 30+ (oligomenorrheic) day study periods monitored. Some volunteers were monitored for only one cycle or one 30-day monitoring period, including 11 amenorrheic women, and 6 eumenorrheic women. Repeated measurements of REE, dietary intake, and blood assessments were conducted on days 2–6 of each menstrual interval for the menstruating volunteers and during the first 6-days of each 30-day monitoring period for the amenorrheic volunteers. All data presented represent the mean of these 2–3 repeated measurements.

Determination of exercise status

Exercise status was determined by review of exercise logs, where exercising women were defined as participating in purposeful exercise (HR greater than 55% of maximal HR) for ≥2.5 h/week, while sedentary women were defined as participating in purposeful exercise (HR greater than 55% of maximal HR) for <2.5 h/week.

Determination of menstrual status and menstrual characteristics

We determined menstrual characteristics in all volunteers by classifying menstrual cycles by length and presence or absence of menses, i.e., amenorrheic, eumenorrheic or oligomenorrheic. Volunteers were considered amenorrheic if they failed to menstruate for a minimum of 3 consecutive months, eumenorrheic if menses occurred at regular intervals of 26–35 days, and oligomenorrheic if menses occurred at irregular intervals of 36–90 days [16], and these