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Acute Bone Marker Responses to Whole-Body Vibration and Resistance Exercise in Young Women

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Abstract

Whole-body vibration (WBV) augments the musculoskeletal effects of resistance exercise (RE). However, its acute effects on bone turnover markers (BTM) have not been determined. This study examined BTM responses to acute high intensity RE and high intensity RE with WBV (WBV +RE) in young women (n=10) taking oral contraceptives in a randomized, cross-over repeated measures design. WBV+RE exposed subjects to 5 one-minute bouts of vibration (20 Hz, 3.38 peak–peak displacement, separated by 1 minute of rest) prior to RE. Fasting blood samples were obtained before (Pre), immediately post WBV (PostVib), immediately post RE (IP), and 30 minutes post RE (P30). Bone ALP did not change at any time point. TRAP5b significantly ($p<0.05$) increased from the Pre to PostVib, then decreased from IP to P30 for both conditions. CTX significantly decreased ($p<0.05$) from Pre to PostVib and from Pre to P30 only for WBV +RE. WBV+RE showed a greater decrease in CTX than RE ($-12.6 \pm 4.7\%$ vs. $-1.13 \pm 3.5\%$). In conclusion, WBV was associated with acute decreases in CTX levels not elicited with resistance exercise alone in young women.

Keywords

Vibration; resistance exercise; bone turnover

Introduction

Whole-body vibration (WBV) mechanically stimulates the musculoskeletal system to augment improvements in body composition and muscular strength typically attained with resistance exercise (RE) alone (Fjeldstad et al. 2009; Bemben et al. 2010). Use of WBV, alone or in conjunction with resistance exercise, to increase bone mineral density (BMD) in humans has yielded mixed results (Verschuere et al. 2004; Gilanz et al. 2006; Bemben et

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al. 2010). A recent meta-analysis for the effects of WBV found that BMD was not altered, but leg strength and muscular performance did improve compared to controls (Lau et al. 2011). In contrast, WBV has been shown to attenuate bed rest-induced bone loss (Armbrrecht et al. 2010).

Bone turnover markers (BTM) provide useful information about bone responses to treatments since they respond more rapidly than DXA measurements and may exhibit greater changes than BMD (Janckila and Yam 2009). Given the large variability in serum BTM levels, it is important to control for time of day, food intake, and previous bouts of exercise when assessing BTM responses to WBV and/or exercise (Szulc and Delmas 2008). Longitudinal intervention studies have not documented significant changes in resting BTM levels in response to WBV alone or in combination with resistance training (Verschuere et al. 2004; Bembien et al. 2010), however, single bouts of weight-bearing or high impact exercise have elicited significant acute BTM responses in men (Ashizawa et al. 1998; Whipple et al. 2004; Bembien et al. 2007; Rogers et al. 2011). The clinical significance of transient changes in BTM levels is not clear, but resting levels are predictive of bone loss and fractures (Szulc and Delmas 2008). In vitro evidence suggests that low magnitude, high frequency vibration stimulates osteocyte responses that result in reduced osteoclast formation and activity (Lau et al. 2010). These findings have implications for in vivo studies, such as bone marker responses to WBV and to exercise in humans. No studies to date have examined BTM responses to acute resistance exercise combined with WBV, therefore, the purpose of this study was to determine bone formation and bone resorption marker responses to combined WBV + RE and to RE alone in untrained young women, taking oral contraceptives. We hypothesized that the bone formation marker, Bone-specific Alkaline Phosphatase (Bone ALP), would significantly increase after each exercise bout, with greater increases occurring in the WBV+RE session; and that the bone resorption markers, C-terminal Telopeptide of Type I Collagen (CTX) and Tartrate-Resistant Acid Phosphatase 5b (TRAP5b), would decrease after the RE session, and decrease even further after the WBV+RE session.

Methods

Subjects

Ten healthy, recreationally active women, ages 20-30 years, taking oral contraceptives for at least 6 months prior to the study and not resistance- or endurance-trained within the previous 12 months, participated in this study. Since BTM serum levels have been reported to vary depending on menstrual cycle phase (Gass et al. 2008), we recruited oral contraceptive users to minimize the influence of menstrual phase variations in endogenous sex hormones on BTM levels. The exclusion criteria were: 1. Current smokers; 2. Women with irregular menstrual cycles prior to OC use; 3. Women using other forms of hormonal contraception; 4. Medications that affect bone metabolism; and 5. Contraindications to whole-body vibration (e.g. epilepsy, fresh bone fractures, bone cancers, open wounds on feet or legs, recent surgery, acute thrombosis). The University of Oklahoma Institutional Review Board for Human Subjects approved this study.

Research Design

This study utilized a randomized repeated measures cross-over design where participants completed two exercise protocols in random order: 1. whole-body vibration plus resistance exercise (WBV+RE); and 2. resistance exercise only (RE). Participants first completed the informed consent form, and questionnaires for medical screening (Physical Activity Readiness Questionnaire (PAR-Q); health status); menstrual history; calcium intake (Musgrave et al. 1989); and physical activity (Bone Specific Physical Activity (BPAQ) questionnaire (Weeks and Beck 2008)). Bone densities were measured, then the women were familiarized with the weight training equipment and the vibration platform. Muscular strength was assessed during the second visit to determine intensity for the main exercise protocols (visits 3 and 4).

Bone Mineral Density

Dual-energy X-Ray Absorptiometry (DXA) (GE Lunar Prodigy, Prodigy enCORE software version 13.31.016, Madison, WI) measured areal BMD (g/cm^2) and BMC (kg for total body; g for other sites) of the total body, anteroposterior (AP) lumbar spine (L1-L4), and the dual proximal femur (femoral neck, trochanter, and total hip). Scan speeds were determined by the measured thickness of the subject at the navel (Thick = >25 cm; Standard = 13 – 25 cm; and Thin = <13 cm). Dual femur scans were performed using the detail setting. The in vivo precision (%CV) was less than 1% for all BMD sites (total body – 0.6%, AP spine – 0.9%, dual proximal femur – 0.4 to 0.8%) and all DXA scans were performed and analyzed by the same technician.

Muscular Strength Testing

Muscular strength was assessed by 1 repetition maximum tests (1RM) for 4 lower body (supine two leg press, hip extension (right leg), hip abduction, hip adduction (right leg)) and 2 upper body resistance exercises (seated military press, seated row) using Cybex® isotonic weight training equipment (Ronkonkoma, NY). Following a 5 minute warm up on a stationary cycle, subjects completed one set of 10 repetitions at about 50% of their estimated 1RM. Within 5 attempts with 2 minute rest periods, the weight was progressively increased until the participant could not complete a single attempt with proper form.

Exercise Protocols

Participants completed both exercise protocols 2 weeks apart to eliminate potential 48-72 hour last-bout effects on BTM responses (Ashizawa et al. 1998). Also, the exercise sessions were not performed during their oral contraceptive placebo week. The RE only protocol consisted of 3 sets of 10 repetitions of each exercise at 80% 1RM, with two minutes of rest between sets.

The WBV + RE protocol included the RE protocol listed above, but was preceded by 5 - 1 minute intermittent exposures of WBV at 20 Hz with 3.38 mm peak-to-peak displacement using a Vibraflex Vibration Platform (Orthometrix, Inc., Naples, FL). This gave a load stimulus of approximately 2.7 g, based on the following equation: $G\text{-Force} = (A(2\pi f)^2)/9.81$, where A is amplitude of the displacement (meters), and f is frequency (Hz). There was a 1-minute rest period between WBV exposures. Participants stood bare foot on the vibration

platform with the second toe in line with the dot midway between foot positions 1 and 2 with knees bent at a 30 degree angle to minimize the transmission of the vibration stimulus to the neck and head for safety reasons (Rittweger 2010).

Blood Sampling and Biochemical Assays

Four blood samples were obtained by venipuncture of the antecubital vein during the WBV +RE test: at rest before WBV (Pre); immediately post WBV exposure (PostVib); immediately post RE (IP), and 30 minutes post (P30) RE. The PostVib blood draw was 20 minutes after the Pre draw and the IP sample was obtained 60 minutes after the Pre draw for this session. We were not able to obtain a PostVib blood draw for one subject, so n=9 for that time point. Three blood samples were obtained for the RE protocol: at rest before the exercise (Pre); immediately post RE (IP); and 30 minutes post (P30) RE. The RE IP sample was obtained 40 minutes after the Pre blood draw. All samples were obtained in the morning, following an 8-hour overnight fast and both protocols were conducted at the same time of day, beginning at 7 am and ending about 10 am.

Hematocrit was measured in duplicate using a microhematocrit centrifuge (StatSpin, Norwood, MA), and a digital reader (StatSpin, Norwood, MA). Lactate was measured at Pre and IP using a Lactate Plus Portable Lactate Analyzer (Nova Biomedical, Waltham MA). Percent change in plasma volume (% PV) was determined with the following equation: $\% \text{ PV} = (100 / (100 - \text{Hct Pre})) * 100((\text{Hct Pre} - \text{Hct Post}) / \text{Hct Post})$ (Van Beaumont, 1972). Serum was frozen at -80° C, and thawed only once prior to performing the BTM assays.

Serum levels of Bone-specific Alkaline Phosphatase (Bone ALP) were assessed in duplicate using a Metra BAP Enzyme ImmunoAssay (EIA) kit (Quidel Corporation, Mountain View, CA). Inter assay CV% for Bone ALP were 5.2-6.8%, and intra assay CV% were 4.5-13.1%. Serum levels of C-terminal Telopeptide of Type I Collagen (CTX), and Tartrate-Resistant Acid Phosphatase (TRAP5b) were measured in duplicate using commercial ELISA kits (Immunodiagnosics Systems, Inc). Inter assay CV% ranged from 1.4-5.1% and 7.5-10.2% for CTX and TRAP5b, respectively. Intra assay CV% ranged from 4.1-8.4% and 0.7-8.7% for CTX and TRAP5b, respectively. BTM values were adjusted for plasma volume changes using the following formula: Corrected value = Uncorrected value*((100 + PV%)/100). Bone marker ratios were calculated for the bone formation marker, Bone ALP vs. each resorption marker. Also, the CTX:TRAP5b ratio was calculated as a bone resorption index since CTX is an indicator of osteoclast activity and TRAP5b is an indicator of osteoclast number (Rissanen et al., 2008).

Data Analyses

Data are reported as means ± standard error (SE). SPSS for Windows version PASW 18 was used for all statistical procedures. Descriptive statistics were computed for all dependent variables for each condition and time point. All dependent variables were determined to be normally distributed using the Kolmogorov-Smirnov procedure. Two (condition) × three (time) repeated measures ANOVA were used to detect bone marker and hematocrit changes. Two (condition) × two (time) repeated measures ANOVA was used to detect changes in lactate concentrations and percent changes in plasma volume and BTM. Paired t-tests were

used to determine the effect of vibration in the WBV +RE bout by comparing Pre and PostVib and as a post-hoc procedure when significant condition $\times$ time interactions were found. Pearson's correlation coefficients were used to determine the relationship between bone marker responses and BMD variables. The level of significance was set at $p = 0.05$.

Results

Subject Characteristics

Table 1 shows the subject characteristics, and bone density data. All participants had Z-scores in the normal range according to ISCD guidelines (Baim et al. 2008). The descriptive data for muscular strength for each resistance exercise are presented in Table 2.

Blood Lactate, Hematocrit and BTM Responses

Table 3 presents the biochemical and BTM responses for the two exercise conditions. Blood lactate and hematocrit significantly increased ($p < 0.01$) IP and there were no significant differences between exercise conditions. Plasma volume during the RE session was $4.5 \pm 2.8\%$ lower at IP and $2.3 \pm 1.9\%$ higher at P30 compared to Pre. During the WBV+RE session, plasma volume decreased $5.0 \pm 2.2\%$ from Pre to PostVib; decreased $9.7 \pm 3.3\%$ from Pre to IP; and increased $2.3 \pm 3.1\%$ from Pre to P30. Percent plasma volume changes were not different ($p > 0.05$) between conditions.

There were no significant differences between conditions for the Pre concentrations for any BTM (Table 3). Both bone resorption markers showed a significant response immediately post the acute WBV exposure, although TRAP5b significantly ($p < 0.05$) increased from Pre to PostVib and CTX significantly ($p < 0.05$) decreased even after correcting for plasma volume changes (Table 4).

Bone ALP concentrations were not significantly altered by either exercise condition (Table 3). Also, there were no significant time, condition, or time $\times$ condition interaction effects for the Bone ALP:CTX, Bone ALP:TRAP5b ratios. There was a significant ($p < 0.01$) time effect for TRAP5b, which decreased from IP to P30 for both exercise conditions. After correcting for plasma volume changes, TRAP5b changes were no longer significant ($p > 0.05$). CTX responses showed a significant ($p < 0.05$) time $\times$ condition interaction effect as it decreased from Pre to P30 for the WBV+RE condition. However, significant CTX decreases ($p < 0.05$) were found for Pre vs. IP and Pre vs. P30 for the WBV+RE after correcting for plasma volume shifts. Also, relative decreases in uncorrected and corrected CTX levels from Pre to IP and Pre to P30 time were significantly greater ($p < 0.05$) for WBV+RE compared to RE (Fig. 1). There was a significant condition $\times$ time interaction effect ($p < 0.05$) for the CTX:TRAP5b ratio, however, none of the pairwise post hoc comparisons within each condition were significantly different (Table 3).

Generally, BTM responses were not related to the BMD measures. Total body and lumbar spine BMD were positively correlated ($r = 0.64$ and 0.71 , respectively, $p < 0.05$) to % change in TRAP5b.

Discussion

To our knowledge, this is first study to examine immediate bone marker responses to a WBV stimulus performed prior to resistance exercise. We found acute changes post vibration and immediately post resistance exercise in the bone resorption markers, TRAP5b and CTX, although the responses were in opposite directions. Also, the magnitude of relative change was greater for CTX than TRAP5b. We did not find significant changes in the bone formation marker for either exercise condition.

We demonstrated that the mechanical signal from WBV alone stimulated responses in the bone resorption markers, although correcting the concentrations for plasma volume shifts eliminated the significant TRAP5b increase. Recent in vitro evidence documented that osteocytes respond to low magnitude, high frequency vibration resulting in a significant reduction in osteoclast formation and activity and in a decrease in RANKL 30 minutes after the vibration stimulus (Lau et al. 2010). These findings may partly explain the decreases in CTX levels that occurred after the WBV stimulus in our subjects. However, the TRAP5b response seems to be the result of hemoconcentration during WBV, rather than an increase in osteoclast cell number. TRAP5b is more of an indicator of osteoclast number than osteoclast activity (Rissanen et al. 2008); and it is improbable that osteoclast number would change in the short time period of the vibration exposures (< 15 minutes). According to Judex and Rubin (2010), there are several ways in which WBV-induced mechanical signals can be transmitted to bone, including being directly sensed by the bone cells, and increased muscle activation causing greater forces exerted on the bone during muscle contraction. There is a growing body of evidence showing WBV training enhances neuromuscular responses (Marin and Rhea 2010) and can acutely increase force of muscle contraction during resistance exercise (Rønnestad 2009).

Bone marker responses to acute bouts of exercise vary depending on the type of exercise (e.g. aerobic versus resistance), subject characteristics, and timing of the blood sampling (Banfi et al. 2010). There have been only a handful of acute resistance exercise studies and they have been conducted in men (Ashizawa et al. 1998; Whipple et al. 2004; Bemben et al. 2007; Rogers et al. 2011). Our CTX findings agree with previous studies that observed decreases in bone resorption markers, but differ in that we found this response only for the WBV+RE trial and not for RE alone. Whipple et al. (2004) reported the resorption marker, N-terminal Telopeptide of Type I Collagen (NTX), decreased 1 hour post resistance exercise in young men. In a previous study, we also found a decrease in NTX after low intensity resistance exercise with blood flow restriction in young men (Bemben et al. 2007). In contrast, Rogers et al. (2011) reported TRAP5b declined at 15 minutes after a fasted resistance exercise session, whereas CTX levels did not change significantly after the exercise in fed men. The decrease in CTX we observed may be accounted for in part by its diurnal variation as serum CTX levels are highest at night then decrease during the day, although the magnitude of this variation is reduced in fasting subjects (Christgau et al. 2000). We cannot confirm this effect since we did not have a control trial in our study, however, the percent CTX decline we observed was larger than the diurnal variation of 8.8% reported by Christgau et al. (2000). Rogers et al. (2011) did have a fasting no-exercise control trial in their study and did not find a significant time effect for CTX. It should be

noted that their 2 hour post mean CTX was about 24% lower than the mean pre CTX for the control trial. Plasma volume shifts also can contribute to acute bone marker responses. We found plasma volume decreased approximately 5% and 10% for the post vibration and immediately post exercise, respectively, for the WBV+RE session, which accounted for the increase in TRAP5b levels. However, correcting for plasma volume changes resulted in a greater relative decrease in CTX.

Our lack of Bone ALP response to either exercise condition was similar to the findings of our previous study in men (Bemben et al. 2007) and to those of Whipple et al. (2004). Recently, Rogers et al. (2011) reported that their lowest mean Bone ALP levels occurred 1 hour post resistance exercise, however, they also documented a significant decrease in this marker during the fasted no-exercise control trial, suggesting that this was a diurnal effect.

There are several limitations to our study. We tested women taking oral contraceptives to control for the effects of menstrual cycle phase on BTM levels, thus, our findings cannot be applied to women not taking oral contraceptives. Previous reports that OC use causes a reduction in resting BTM concentrations (Hermann and Seibel 2010), raise the possibility that it also may affect BTM responses to acute exercise. Currently, there is no evidence to support this postulation as there are no comparison studies in premenopausal women not taking OC. In this study, we controlled for menstrual phase variation, circadian rhythm, pre-exercise food intake, last exercise bout effect, and physical activity status to reduce the variability in serum BTM levels. We did not standardize dietary intakes or ensure that our participants were in energy balance the day before the exercise sessions. The effects of dietary/energy intake on the assessment of BTM levels is unclear as some studies found that energy restricted diets altered BTM levels at rest (Ihle et al. 2004) and during an acute bout of endurance exercise (Zanker et al. 2000), whereas Rogers et al. (2011) found no difference in BTM responses to acute resistance exercise in fasted or fed conditions.

In conclusion, whole-body vibration was an effective stimulus for altering serum bone resorption markers in young women taking oral contraceptives. The lack of bone formation marker response agrees with previous acute resistance exercise studies conducted in men. More research on women, including non-oral contraceptive users, is needed to elucidate the impact of these bone marker responses to whole-body vibration on bone health.

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Fig.1.

Percent changes in uncorrected (Uncor) and plasma volume shift corrected (Cor) CTX levels from rest (Pre) to immediate post (IP) and 30 minutes post (P30) resistance exercise (IP) for WBV+RE and RE exercise conditions * $p < 0.05$ between exercise conditions

Table 1
Participant Characteristics (n=10)

Variable	Mean $\pm$ SE
Age (years)	20.7 $\pm$ 0.2
Weight (kg)	67.8 $\pm$ 5.6
Height (cm)	165.4 $\pm$ 2.0
% Body Fat	37.3 $\pm$ 2.6
Fat Mass (kg)	26.1 $\pm$ 3.9
Bone Free LBM (kg)	38.4 $\pm$ 1.8
Calcium Intake (mg/day)	923 $\pm$ 88
BPAQ Scores	
Current	2.4 $\pm$ 0.7
Past	131.9 $\pm$ 26.7
BMD (g/cm ²)	
Total Body	1.152 $\pm$ 0.027
Lumbar Spine	1.207 $\pm$ 0.042
Left Total Hip	1.088 $\pm$ 0.048
Left Femoral Neck	1.095 $\pm$ 0.047
Left Trochanter	0.858 $\pm$ 0.046

LBM: Lean Body Mass; BPAQ: Bone-specific Physical Activity Questionnaire

Muscular Strength (n=10)**Table 2**

Resistance Exercise	Mean $\pm$ SE
Leg Press (kg)	111.8 $\pm$ 7.5
Hip Extension (kg)	80.9 $\pm$ 7.0
Hip Abduction (kg)	45.2 $\pm$ 3.4
Hip Adduction (kg)	55.1 $\pm$ 6.1
Low Row (kg)	41.4 $\pm$ 3.4
Shoulder Press (kg)	30.9 $\pm$ 1.8

kg - kilograms

Table 3

Hematocrit, Lactate, and Bone Turnover Marker Responses[#] Before (Pre), Immediate Post Resistance Exercise (IP), and 30 Minutes Post Resistance Exercise (P30) (n = 10; Mean ± SE).

Variable	RE	WBV+RE
Hematocrit (%) ^{**} (IP>Pre)		
Pre	41.2 ± 1.0	40.4 ± 0.9
IP	42.4 ± 0.9	43.0 ± 0.8
P30	40.7 ± 0.9	40.3 ± 1.0
Lactate (mmol/L) [*] (IP>Pre)		
Pre	1.1 ± 0.3	1.7 ± 0.9
IP	5.1 ± 0.6	5.1 ± 0.6
Bone ALP (U/L)		
Pre	36.45 ± 4.40	34.72 ± 3.54
IP	36.37 ± 3.97	35.97 ± 3.70
P30	35.79 ± 4.58	33.24 ± 3.26
CTX (ng/mL) [†] (WBV+RE Pre > P30)		
Pre	0.477 ± 0.087	0.521 ± 0.070
IP	0.474 ± 0.087	0.445 ± 0.067
P30	0.470 ± 0.084	0.444 ± 0.063
TRAP5b (U/L) ^{**} (IP>P30)		
Pre	3.74 ± 2.66	2.66 ± 0.20
IP	4.06 ± 2.74	2.76 ± 0.22
P30	3.75 ± 2.60	2.58 ± 0.20
Bone ALP:CTX Ratio		
Pre	93.0 ± 13.4	76.6 ± 11.1
IP	96.1 ± 13.3	88.3 ± 8.6
P30	91.0 ± 11.8	82.9 ± 9.4
Bone ALP:TRAP5b Ratio		
Pre	13.8 ± 1.1	13.4 ± 1.3
IP	13.8 ± 1.4	13.4 ± 1.3
P30	14.1 ± 1.3	13.3 ± 1.2
CTX:TRAP5b Ratio [†]		
Pre	0.171 ± 0.022	0.191 ± 0.018
IP	0.166 ± 0.023	0.160 ± 0.020
P30	0.174 ± 0.023	0.170 ± 0.018

[#] uncorrected concentrations

^{*} p<0.01

^{**} p<0.05 Significant time effect;

[†] p<0.05 significant time × condition interaction effect.

RE: Resistance Exercise; WBV: Whole-body Vibration; Bone ALP: Bone-Specific Alkaline Phosphatase; CTX: C-Terminal Telopeptides of Type I Collagen; TRAP5b: Tartrate-Resistant Acid Phosphatase.

Table 4

Lactate, Hematocrit, and Bone Turnover Marker Values Before (Pre) and Immediate Post-Vibration Exposures (PostVib) (n = 9; Mean $\pm$ SE).

Variable	Pre	PostVib	% Change
Hematocrit (%)	40.5 $\pm$ 1.0	41.8 $\pm$ 0.8*	3.4 $\pm$ 1.4
Lactate (mmol/L)	0.96 $\pm$ 0.11	1.33 $\pm$ 0.26	49.9 $\pm$ 30.0
Bone ALP (U/L)			
Uncorrected	34.58 $\pm$ 3.95	35.39 $\pm$ 4.12	2.3 $\pm$ 2.4
Corrected [#]		33.56 $\pm$ 4.03	-3.0 $\pm$ 2.4
CTX (ng/mL)			
Uncorrected	0.493 $\pm$ 0.072	0.444 $\pm$ 0.065*	-8.5 $\pm$ 3.4
Corrected [#]		0.416 $\pm$ 0.059**	-13.1 $\pm$ 4.0
TRAP5b (U/L)			
Uncorrected	2.55 $\pm$ 0.18	2.67 $\pm$ 0.20*	4.6 $\pm$ 1.7
Corrected [#]		2.52 $\pm$ 0.18	-0.7 $\pm$ 2.7

* p<0.05 significant vs. Pre;

** p<0.01 significant vs. Pre;

BAP: Bone-Specific Alkaline Phosphatase; CTX: C-Terminal Telopeptides of Type I Collagen; TRAP5b: Tartrate-Resistant Acid Phosphatase.

[#] Corrected for plasma volume shifts