Effects of a single bout of low intensity KAATSU resistance training on markers of bone turnover in young men

D.A. Bemben, I.J. Palmer, T. Abe, Y. Sato, M.G. Bemben

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Correspondence to:
Dr. Debra A. Bemben,
Department of Health and
Exercise Science, University of
Oklahoma, 1401 Asp Avenue,
Norman OK 73019, USA
dbemben@ou.edu

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See end of article for
authors' affiliations

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Traditional high intensity resistance exercise programs have been shown to have positive effects on bone metabolism. KAATSU resistance training, which combines low intensity resistance exercise with vascular restriction, accelerates muscle hypertrophy, however, the benefits of this type of training on bone have not been established. PURPOSE: To investigate the effects of acute (1 bout) KAATSU training for knee extensors and knee flexors on serum bone biomarkers in young men, 18-30 years of age. METHODS: Nine males performed two test sessions, KAATSU (vascular restriction + low intensity resistance exercise) and control (low intensity resistance exercise only) 48 hours apart in random order. The exercise protocol consisted of 1 set of 30 reps followed by 3 sets of 15 reps with 30 seconds rest between sets at 20% 1-RM for both muscle groups. On both days, fasting blood draws were obtained immediately prior to exercise, immediately post exercise and 30 minutes post exercise for the measurement of the bone formation (bone-specific alkaline phosphatase, BAP) and bone resorption (cross-linked N-telopeptide of type I collagen, NTx) markers. Hematocrit was measured at each sample time to estimate plasma volume changes. Serum samples were aliquoted and frozen at -70°C until the BAP (Metra BAP EIA kit, Quidel Corporation) and NTx (Osteomark® NTx Serum, Wampole Laboratories) assays were performed. RESULTS: KAATSU training resulted in greater plasma volume decreases ($p < .05$) immediate post exercise compared to the control session. There was a significant ($p < .05$) training x time effect for NTx levels. 30 minute post exercise NTx levels (21.4±3.4 nM BCE) significantly ($p < .05$) decreased from baseline (24.9±4.3 nM BCE) after KAATSU training but not in response to the control training. After correcting for plasma volume shifts, significant decreases in serum NTx were observed for both KAATSU post exercise samples. There were no significant ($p > .05$) training or time effects for BAP. CONCLUSION: A single bout of KAATSU training resulted in decreases in the bone resorption marker (NTx) but had no effect on the bone formation marker (BAP). The NTx response to KAATSU was not mediated by shifts in plasma volume.
Key words: Bone biomarkers, Resistance exercise, Blood flow restriction

INTRODUCTION

There is growing interest in finding effective exercise interventions for improving bone health in a variety of populations (Kohrt et al., 2004). Current recommendations for bone loading include moderate to high intensity weight-bearing or resistance exercise with short duration (Kohrt et al., 2004; Turner, 1998). Novel exercise also is important as bone adapts to customary mechanical loading, resulting in the bone cells becoming less responsive to routine loading signals (Turner, 1998; Turner and Robling, 2003).

High intensity resistance training has been shown to be an effective stimulus for increasing bone mineral density (BMD) in a variety of populations (Nelson et al., 1994; Kerr et al., 1996; Maddalozzo and Snow, 2000). Few investigations, however, have focused on the bone biomarker responses to resistance exercise. In contrast to BMD measured by

densitometry, serum or urine bone biomarkers provide information about bone remodeling rates and are useful for monitoring the effectiveness of therapies within short time intervals (Camacho and Kleerekoper, 2006). This information has proven beneficial for assessment of osteoporosis risk, as bone markers have been predictors of fracture risk independent of BMD (Garnero et al., 1996). Bone markers levels are affected by diet, circadian rhythms, and assay/sample method (e.g. serum versus urine); thus, these variables must be carefully controlled in order to avoid large intra- and inter-individual variability in these assays (Warnick, 2001; Camacho and Kleerekoper, 2006).

There is increasing interest in the use of bone biomarkers to indicate bone metabolism changes with exercise (Fujimura et al., 1997; Wallace et al., 2000; Maimoun et al., 2006; Whipple et al., 2004). An

acute bout of high intensity resistance exercise significantly increased resting serum levels of the bone formation marker, bone-specific alkaline phosphatase (BAP), 2-3 days after the exercise session, while the bone resorption marker (urinary deoxypyridinoline) transiently increased (Ashizawa et al., 1998). In young men, chronic resistance training was associated with significantly elevated BAP after 1 month, suggesting osteoblastic activity was increased in response to the high intensity resistance exercise (Fujimura et al., 1997). Other studies have found no change in bone formation or bone resorption markers with chronic resistance training (Nelson, et al., 1994; Bemben et al., 2000).

Recently, a novel form of low intensity (20% 1-RM) resistance training with vascular restriction, termed KAATSU training, was shown to elicit positive muscle adaptations and endocrine responses (Abe et al., 2005; Takano et al., 2005). KAATSU training utilizes pressure cuffs on each thigh, which when inflated, causes reduced femoral artery blood flow and pooling of venous blood in the legs (Iida et al., 2005). These temporary alterations in leg circulation have implications for bone physiology. There is evidence suggesting that capillaries exist in the bone structural units (BSU) and that vascular endothelial cells may play a role in the coupling of bone resorption and formation (Parfitt, 2000) by secreting substances which inhibit osteoclast activity (Chikatsu et al., 2002) and increase osteoblast recruitment (Parfitt, 2000). Therefore, KAATSU resistance training, even at a low load (20% 1 repetition maximum) may influence bone metabolism by altering vascular endothelial cell function. Blood flow restriction during walk training was associated with significant increases in resting levels of serum BAP (Beekley et al., 2005). However, there is no information to date whether serum markers of bone turnover are affected by KAATSU resistance training. The purpose of this study was to examine the effects of a single bout of low intensity resistance exercise with vascular restriction on serum bone markers in young, recreationally trained men. We hypothesized that the bone formation marker, BAP, would be significantly elevated after the KAATSU protocol.

MATERIALS AND METHODS

Subjects

Nine men, 18-30 years of age, volunteered for the study. Subjects were recreationally active in aerobic or resistance exercise. This study was approved by the Institutional Review Board of the University of Oklahoma, Norman, OK. Table 1 shows the physical characteristics of the participants.

Research Design

This study employed a repeated measures randomized crossover design, where each subject performed both the restricted blood flow (KAATSU) condition and resistance exercise control condition in random order. Subjects were required to participate in three test sessions. During the initial session, the men completed forms and questionnaires (informed consent, PAR-Q), strength testing for knee flexors/extensors, and body composition assessment. Subjects were then required to come to the Neuromuscular Laboratory in the morning (7-8 am) after an overnight fast for the KAATSU and control sessions which were performed approximately 48 hours apart.

Body Composition Assessment

Dual Energy X-Ray Absorptiometry (DXA) (GE Lunar Prodigy, enCORE software version 8.80.001) was used to measure the BMD (g/cm²) and body composition of the total body. Bone scans were analyzed by a single technician. Body composition variables (% body fat, bone-free lean tissue mass, fat mass) were obtained from the total body scan analysis. In vivo precision (CV%) in the Bone Density Laboratory is 1% for total body BMD, 1.5% for lean body mass (LBM) and 2% for fat mass (FM).

Muscular Strength

Subjects had the strength of their quadriceps and hamstrings muscle groups assessed by 1-repetition maximum (1-RM) testing supervised by trained personnel. The tests were performed using the seated knee extension and seated knee flexion isotonic machines (Cybex, Medway MA). Subjects began with a 5 minute warm-up on a Monark cycle ergometer, followed by static stretches (5-10 seconds, 3 repetitions) for the calf and thigh of each leg. After a brief rest period, subjects were familiarized with the

Table 1. Subject Characteristics (n=9)

Age (yrs)	Height (cm)	Weight (kg)	% Fat	Bone-Free LBM (kg)	Fat Mass (kg)	Total Body BMD (g/cm^2)
24.9 ± 2.5	178.1 ± 6.6	81.1 ± 7.6	15.4 ± 7.6	65.3 ± 8.5	12.4 ± 5.9	1.322 ± 0.094

Values are Mean ± SD

two resistance exercise machines by performing 5-10 repetitions at approximately 50% of the estimated 1-RM. After 1 minute rest, the subjects lifted a load of approximately 80% of estimated 1-RM through the full range of motion. After each successful lift, the weight was increased until a failed attempt occurred which was recorded as the maximum load. All 1-RMs were achieved within 5 attempts, and each attempt was separated by 1 minute rest.

KAATSU and Control Session Protocols

During each session, subjects were seated either in the knee extension or knee flexion Cybex isotonic resistance machines in random order. There was a warm-up of 30 repetitions (cadence 1.5 seconds up-1.5 seconds down) at 20% 1-RM, then a 30 second rest followed by 3 sets of 15 repetitions at 20% 1-RM, with each set separated by 30 seconds of rest. Once one muscle group was completed, there was another 30 second rest period before exercising the second muscle group.

The KAATSU cuffs (KAATSU-Master, Sato Sports Plaza, Tokyo, Japan) are specially designed elastic belts which are placed around the upper thigh of each leg simultaneously. The belt contains a pneumatic bag which is connected to an electronic air pressure control system that monitors the pressure. Generally, the pressure used to restrict blood flow to the lower extremities is based upon a subject's resting systolic blood pressure (SBP). Normal resting SBP of the legs is about 20% higher than when measured at the upper arm and the pressure used to restrict blood flow (KAATSU) is about another 20% higher than the blood pressure of the legs. For example, with a normal resting systolic blood pressure measured in the upper arm of 120 mmHg, the SBP for the legs would then be estimated to be 20% higher (about 144 mmHg) and the cuff pressure would then be set at a level 20% higher (about 170-180 mmHg).

The initial restrictive pressures in the KAATSU belts were set at 40-60 mmHg. In order for the subjects to become accustomed to the restrictive exercise pressures, the pressure was increased from resting pressure to 120 mmHg for a 30 second period, then released to the initial pressure for 10 seconds. This procedure was repeated for 140 and 160 mmHg before reaching the final testing pressure of 180 mmHg. The total exercise time with vascular restriction lasted approximately 10 minutes.

Blood Chemistry

Fasting venous blood samples were obtained between 7-9 am by a phlebotomist at rest, immediately after the exercise (post 1) and at 30 minutes post exercise (post 2) for each resistance exercise session. Hematocrits (Hct) were measured in duplicate at each sample time to estimate plasma

volume (PV) changes using the formula: % (PV = (100/[100 - Hct pre]) * 100([Hct pre - Hct post]/Hct post) (Van Beaumont, 1972). Bone marker concentrations were corrected for PV changes using the following formula: Corrected Concentration = (uncorrected concentration) * ((100 + % (PV) /100). Blood samples were centrifuged and the serum was aliquoted into microtubes and frozen at -84°C until the bone marker assays were performed. Enzyme-Linked Immunoassay kits were used to measure serum levels of BAP, a marker of bone formation (Quidel Corporation, Santa Clara CA) and cross-linked N-telopeptide of type I collagen (NTx), a marker of bone resorption (Wampole Laboratories, Princeton NJ). The BAP concentrations are expressed in Units per liter (U/L) and NTx concentrations are measured in nanomoles Bone Collagen Equivalents per liter (nM BCE). All samples were assayed in duplicate. The intra-assay coefficients of variation ranged from 0.9% to 11.5% for BAP and from 2.5% to 8.3% for NTx. Inter-assay coefficients of variation for both bone markers were < 9%.

Statistical Analyses

All data are reported as means ± standard deviation (SD). SPSS 11.5 was used to execute all statistical analyses. Two-way (condition x time) repeated measures ANOVA was used to compare the acute bone marker responses between the KAATSU and control test sessions. The Bonferroni post hoc procedure was used when a significant time effect was found. Paired t-tests were used as post hoc tests for significant condition x time interaction effects. The level of significance was set at $p \leq 0.05$.

RESULTS

Mean knee extensor and flexor strengths were 1185 ± 80 N and 1000 ± 49 N, respectively. The mean 20% 1-RM loads were 236 ± 18 N for knee extension exercise and 200 ± 13 N for knee flexion exercise.

Table 2 shows the PV and BAP responses to the KAATSU and control trials. There were significant time (p=.003) and condition x time (p=.006) effects for percent PV changes. KAATSU resistance training resulted in significantly greater PV decreases immediate post exercise compared to the control session. The hemoconcentration resolved by 30 minutes post exercise, thus, there was no difference in PV change between conditions for the post 2 sample time.

The bone resorption marker, NTx, was affected significantly by the KAATSU condition (Figure 1). There was a significant ($p < 0.05$) condition x time effect for uncorrected NTx levels, with the 30 minute post exercise NTx being significantly lower than the baseline (p<0.05) and post 1 (p < 0.05) values for the KAATSU condition and significantly lower than both

Table 2. Plasma Volume (PV) and Uncorrected Bone-specific Alkaline Phosphatase (BAP) Responses for the KAATSU and Control Conditions

Variable	KAATSU			CONTROL		
	Pre	Post 1	Post 2	Pre	Post 1	Post 2
PV (%Δ)		-11.5 ± 6.2[*#]	3.3 ± 6.3		-5.6 ± 7.2	-1.6 ± 6.7
BAP (U/L)	33.2 ± 7.4	31.8 ± 6.5	33.8 ± 6.3	30.3 ± 5.7	30.4 ± 8.6	31.4 ± 6.3

Values are Mean ± SD; Post 1 – immediately after exercise; Post 2 – 30 minutes after exercise

[*]p < 0.01 significant vs. Post 2; [#]p<0.05 significant vs. both control Post 1 and 2 samples

control post samples (p < 0.05). The uncorrected post 1 NTx was not significantly different (p > 0.05) from the baseline for the KAATSU session nor was there a significant (p > 0.05) NTx response for control session. The post 2 NTx decrease (13.3%±3.4) was greater than the corresponding %PV change. After correcting for PV shifts, the significant (p < 0.05) condition x time effect remained, however, significant decreases in serum NTx occurred for both KAATSU post 1 and post 2 exercise samples. There were no significant (p > 0.05) condition, time, or condition x time effects for the bone formation marker, BAP.
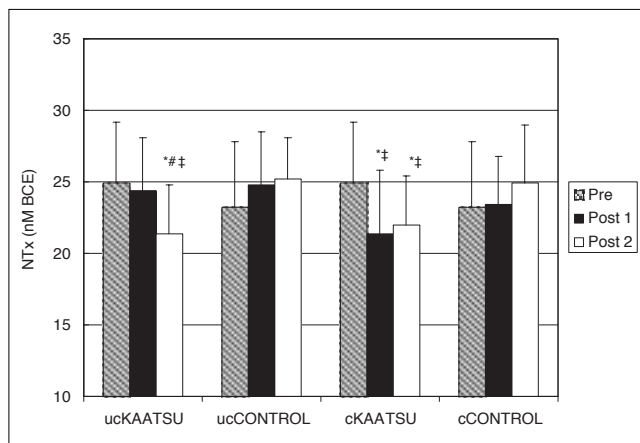
DISCUSSION

To our knowledge, this is the first study to examine

Figure 1. Serum Cross-linked N-Telopeptide of Type I Collagen (NTx) Responses to KAATSU and Control Conditions (Mean ± SD)

ucKAATSU – uncorrected concentrations for KAATSU condition; ucCONTROL – uncorrected concentrations for CONTROL condition; cKAATSU – concentrations corrected for plasma volume changes for KAATSU condition; cCONTROL - concentrations corrected for plasma volume changes for CONTROL condition; Post 1 – immediately after exercise; Post 2 – 30 minutes after exercise

* p < 0.05 significant vs. pre; [#] p < 0.05 significant vs. Control Post 1 ; [‡] p<0.05 significant vs. Control Post 2

bone marker responses to KAATSU resistance training. We documented that a single bout of KAATSU resistance training resulted in a significant decrease in the bone resorption marker, serum NTx, by 30 minutes post exercise. Our serum NTx findings are similar to those of Whipple et al. (2004) who reported this bone marker significantly decreased 1 and 8 hours after an acute bout of high intensity resistance exercise. The lack of BAP response did not support our hypothesis which was based on previous studies which reported significant increases in BAP to acute aerobic (Wallace et al., 2000; Rudberg et al., 2000; Maimoun et al., 2006), acute KAATSU walk training (Beekley et al., 2005), and acute resistance exercise (Ashizawa et al., 1998).

The physiology of bone circulation and its role in bone mineralization and fracture repair are important considerations for bone health. There are blood vessels located in the BSUs which may serve several important roles in the coupling of the bone remodeling process (Parfitt, 2000). Recent evidence supports an active role for blood vessels in osteogenesis via the release of vasoconstrictor and vasodilator substances and local regulatory factors (e.g., interleukin-6, endothelin-1, nitric oxide) which modulate bone activity (McCarthy, 2006; Parfitt, 2000). These factors may signal osteoblast recruitment and directly inhibit osteoclast activity (Parfitt, 2000; Chikatsu et al., 2002). Recently, interleukin-6 was found to play a role in the early stages of fracture healing as delayed mineralization and callus maturation occurred in interleukin-6 knockout mice (Yang et al., 2007). Since KAATSU restricts blood flow in the lower limbs, the vascular endothelial cell secretory functions may be affected causing a disruption in the coupling process between bone resorption and formation. KAATSU resistance exercise also reduces muscle oxygenation and increases blood lactate levels (Tanimoto et al., 2005); and changes in pH and hypoxia are physiological conditions which regulate osteoclast activity (McCarthy, 2006).

There are several mechanisms which could explain the NTx responses. First, hemoconcentration during exercise increases the concentrations of substances

(e.g. hormones, proteins) circulating in the blood. It is well documented that plasma volume generally decreases with acute resistance exercise as the plasma shifts from the intravascular to extravascular spaces (Ahmadizad et al., 2006). Although hemoconcentration did occur immediately post exercise for the KAATSU condition, correcting the NTx concentrations for PV changes did not attenuate the NTx response. In fact, both post exercise NTx levels for the KAATSU condition significantly decreased compared to baseline after the correction for hemoconcentration.

This finding suggests that there are other factors influencing the bone marker responses. It is possible that the hypoxic or pH stimuli induced by the KAATSU resistance training initiate a change in the rate of the bone remodeling cycle. Since the resorption phase precedes formation phase in the remodeling cycle, the bone resorption marker would respond more quickly to a stimulus than a formation marker. This response pattern is well-documented in the literature, as resorption markers tend to respond more quickly to drug treatments, followed by changes in the bone formation markers (Camacho and Kleerekoper, 2006). It is unlikely that these responses were mediated by circadian rhythms in the bone markers as all testing was completed within a 1.5 hour time period, at the same time of day (7-9 am).

In conclusion, an acute bout of low intensity KAATSU training affected bone metabolism by decreasing NTx. Future research is needed to assess bone marker responses 2-3 days after acute exercise and responses to chronic KAATSU resistance training.

ACKNOWLEGEMENTS

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Authors' affiliations

Debra. A. Bemben, I. J. Palmer, Michael G. Bemben, Department of Health and Exercise Science, University of Oklahoma, Norman, OK 73019, USA

Takashi Abe, Graduate School of Frontier Science, University of Tokyo, Kashiwa, Chiba, Japan

Yoshiaki Sato, Department of Ischemic Circulatory Physiology, University of Tokyo, Hongo, Bunkyo-ku, Tokyo, Japan