Original Research

Bone Turnover Response to Acute Exercise with Varying Impact Levels: A preliminary investigation

AMY L. MORGAN^{‡1}, JENNIFER WEISS^{*2}, and EDWARD T. KELLEY^{*1}

¹School of Human Movement, Sport and Leisure Studies, Exercise Science Program, Bowling Green State University, Bowling Green, OH, USA; ²Orthopedic Physician Associates, Seattle, WA, USA

^{*}Denotes professional author, ^{*}Denotes undergraduate student author

ABSTRACT

International Journal of Exercise Science 8(2): 154-163, 2015. The purpose of this investigation was to determine if there are changes in metabolic markers of bone turnover within the 24-hour period following an acute bout of jogging or water exercise. Ten healthy females (22-30 yr.) underwent three trials with different ground impact forces: jogging, water aerobics and control. For the jogging and water aerobics trials, subjects exercised for 30 min at 60-70% of predicted maximal heart rate in addition to a 5 min warm up and cool down. For the control trial, subjects rested for 40 min, i.e., the total exercise time of the other trials. For each trial, blood samples were collected pre, immediately post, 1 hr. post, and 24 hr. post exercise. All samples were analyzed for levels of osteocalcin, bone specific alkaline phosphatase and cross-linked N telopeptides. A repeated-measure ANOVA was used to determine if there were differences between impact levels or over time. There were no significant differences over time, or between impact levels of exercise. Therefore these results indicate that healthy young women demonstrate no changes in bone turnover within 24 hr. of a single bout of exercise. Due to biochemical markers of bone turnover responding in a similar manner regardless of activity type, these findings suggest that water aerobics can be seen as a beneficial form of exercise for maintenance of bone density.

KEY WORDS: Mechanical loading, remodeling, bone density, water aerobics

INTRODUCTION

Osteoporosis is a life altering disease characterized by low bone mass and microarchitectural deterioration of bone tissue. Osteoporosis is associated with an increased risk of fracture, particularly at the vertebrae and proximal femur (7, 10). Of those individuals affected, approximately 80% are women. The risk of osteoporosis increases progressively with age, often resulting in disabling injuries or death. For example, individuals suffering hip fractures have a mortality rate of greater than 24% within one year (23). In 2005, approximately two million osteoporosisrelated fractures in the United States resulted in expenditures of \$19 billion (23), indicating that osteoporosis is not only a physically disabling disease but also a socio-economic burden. By 2025, experts predict that these costs will rise to over \$25 billion (23).

Two major contributing factors that lead to the development of osteoporosis are the level of peak bone mass (PBM) developed during childhood and adolescence (2, 29)

and the rate at which bone is lost across the lifespan. Peak bone mass is the maximal bone density that a person can achieve; women reach peak bone mass by their mid-20's, while bone loss begins around age 30. Maximizing PBM during adolescence may provide the skeleton with protection against the age-related reduction in bone density (29), i.e., when a greater PBM is achieved, a larger amount of bone must be before individual lost an becomes osteoporotic. Unfortunately, the importance of maximizing bone loss early in life is often not realized until it is too late (12).

Bone mineral density (BMD) is the product of a continuous turnover process consisting of bone formation and resorption. Within the bone matrix, several types of cells are responsible for remodeling and rebuilding damaged or aged bone. During active bone turnover, markers specific to either bone formation (e.g., osteocalcin, bone-specific alkaline phosphatase) or resorption (e.g., cross-linked N-telopeptides of Type I collagen) are released into the blood. As a result, immunoassays can be utilized to measure the rate of bone formation and resorption, making it possible to monitor acute changes in bone turnover.

Physical activities with moderate ground impact forces have been found to stimulate osteoblasts to lay down more fibers within the bone matrix, giving rise to a stronger and denser bone mass (6,14,33). Individuals who participate in non-impact activities or in reduced gravity environments, such as swimming, have been shown to have lower bone densities than their counterparts (6, 8,30). To date, it is known only that impact exercise can attenuate bone loss (10, 12, 18, 37). However, the minimal amount of impact necessary to stimulate bone formation has yet to be determined.

Shallow water aerobics, though conducted in a reduced gravity environment, may have potential for a greater osteogenic stimulus than swimming because the activity involves ground contact in combination with resistance. Water aerobics can be a valuable form of exercise for many individuals suffering from ailments such as arthritis, allowing for increased mobility and movements that the individual could not withstand on land. However, water aerobics has not been examined to determine if it is a beneficial form of exercise to stimulate bone formation. If shown to provide an osteogenic stimulus, water aerobics could be an ideal form of exercise for individuals who are unable to tolerate jogging or other higher impact activities.

The majority of studies examining the impact of exercise on bone health target long-term changes in bone turnover (5, 12, 19, 22). These studies have demonstrated that both impact and resistance exercise provide benefit to the skeleton by stimulating osteogenic factors that result in the maintenance of strong, healthy bones. However, few studies have examined the effects of acute bouts of exercise on bone turnover (35). Of these studies, even fewer have examined acute bouts of cardiovascular endurance exercise (27), while resistance training (28), plyometrics (26), and comparisons of exercise intensities in both fed and fasted states (27) have been By defining the immediate examined. timeline in which an acute bout of exercise changes rates of bone turnover, it may be possible to determine if jogging and/or water aerobics provide an osteogenic

stimulus. Therefore, the purpose of this study was to compare changes in metabolic markers of bone turnover in physically active women within the 24-hour period following different modes of exercise, specifically jogging and water aerobics.

METHODS

Participants

Ten regularly active, healthy females aged 22 - 30 yr. were recruited from the student population of а large Midwestern university (See Table 1). To be eligible, all women had to participate regularly (2-3 days per week) in moderate physical activity, have regular menstrual cycles (10+ per year), and be in good health as assessed by a health history questionnaire. All completed volunteers questionnaires pertaining to their current health history, physical activity level, eating behavior (EAT-26; to assess for eating disorders) (15), and food frequency intake (to measure Individuals calcium). dietary were excluded for the following reasons: diabetes mellitus, metabolic bone disease, renal disease, liver or thyroid dysfunction, severe obesity (BMI > 30), eating disorder as defined as an EAT-26 score > 25, high alcohol consumption (15+ drinks per week), current smoker, or currently taking a medication affect known to bone taking metabolism. Participants oral contraceptives were not excluded, as they have not been shown to influence markers of bone metabolism (6). The University's Institutional Review Board approved the investigation and written informed consent was obtained from each volunteer prior to testing.

The food frequency questionnaire was analyzed by Diet System Computer

software (National Cancer Institute, Bethesda, MD) to calculate participants average daily calcium intake. Percent body fat was estimated from three skinfolds (triceps, suprailiac, thigh) (17) taken by a trained technician following the third experimental trial.

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Characteristic	Mean ± SD	Range
Age (yr.)	24.4 ± 2.76	22-30
Height (m)	1.68 ± 0.04	1.60-1.74
Weight (kg)	65.06 ± 7.5	56.5-78.0
BMI	23.08 ± 2.36	20.1-26.9
Body fat (%)	25.5 ± 4.3	17.6-33.7
Calcium intake (mg)*	1161 ± 657.5	487-2920
Caloric intake (kcal)*	1872 ± 424.1	1139-2673

BMI, body mass index. *Average daily intake.

Protocol

Subjects were asked to participate in three separate trials: no exercise control (CON), high-impact jogging (HIGH), and lowimpact water aerobics (LOW). Nine of the subjects completed all trials; one individual chose not to participate in the LOW trial. All participants were asked to refrain from exercise for a minimum of 2 days prior to each testing session as well as throughout each trial period. Experimental trials were separated by at least one week.

Each trial lasted two days. On day 1, the subject underwent the exercise session and three blood samples were collected. On day 2, one additional blood sample was collected. Blood samples were collected at the following time points for each experimental trial: immediately prior to the exercise session (Pre), immediately following the exercise session (Post), 1 hour following the exercise session (1H), and 24hours following the exercise session (24H) (36). These four time points were selected according to previous investigations of

similar nature (25, 26). Blood samples were approximately 6.5 ml, collected by venipuncture. After sampling, blood was stored on ice for no more than 1.5 hours, and then centrifuged at 2500 rpm for 10 minutes. Serum was pipetted from the sample and stored at -70°C until analysis.

During the high impact trial (HIGH), participants jogged on an indoor track at 60 - 70% of age predicted maximal heart rate (HR). This workload was selected to be below anaerobic threshold, thus preventing hormonal fluctuations (1, 3). The activity consisted of a 5-min warm-up, 30-min of continuous jogging within the target HR zone, and a 5-min cool down. Participants were stagger started and instructed to from socializing with other refrain participants during the exercise to minimize any effect on pace or heart rate. HR (bpm; Polar Electro Inc., Woodbury, NY) was recorded every 5 minutes throughout the exercise bout to assure that each participant's HR was within the specified zone. The POST blood sample was taken immediately after exercise; subjects rested quietly until the 1H blood sampling. The 24H blood sample was collected 24 hours after completion of each trial.

The protocol for the water aerobics, or low impact (LOW) trial, was similar to that for the HIGH trial. Subjects participated in 40 minutes of water aerobics taught by a certified instructor, which included 5 min warm-up and cool down segments. During the exercise, props such as buoyant resistive barbells and foam 'noodles' were used to increase resistance. Exercise was conducted in 3.5 feet of water; due to hydrostatic forces of the water, special consideration was made for HR in the water, i.e., 10 beats per minute less than 70% of age predicted maximal HR. HR was taken manually and recorded every 5 minutes throughout the LOW exercise session. As with the HIGH trial, blood samples were collected at POST, 1H and 24 H.

For the control trial (CON), blood samples were collected at the same time points throughout two days, but the subjects completed no exercise. During the time that the exercise session would have occurred, subjects sat quietly.

Bone turnover was assessed by three separate enzyme-linked immunosorbent assays (ELISA) to measure markers of bone formation and resorption. For each subject, serum samples for all trials were analyzed on the same plate. All samples were analyzed in duplicate with strict adherence to the manufacturers' instructions.

Resorption was measured by examining serum levels of cross-linked-N-telopeptides of Type I collagen (NTx) (Osteomark, Ostex International). Osteomark NTx assay is a competitive-inhibition enzyme linked immunosorbent assay where absorbance is determined spectrophotometrically and concentration is calculated utilizing a 4-parameter logistic curve. The intra-assay variation was \leq 7.3%.

Bone formation was measured by two separate markers, osteocalcin (OC) (Quidel Corporation) and bone-specific alkaline phosphatase (BALP) (Quidel Corporation). OC fluctuates on a diurnal cycle, making timing and consistency of blood collection very important. To control for this confounding variable, all trials were completed at the same time each week. For OC, the intra-assay coefficient of variation was \leq 4.8%; for BALP the intra-assay coefficient of variation was \leq 5.8%.

Difference in bone formation and resorption between trials was assessed by determining an 'uncoupling index'. The index was calculated by subtracting the *z*-score of the resorption values (NTx) from the *z*-score of the formation values (OC and BALP) (11).

Statistical Analysis

A repeated measures analysis of variance (ANOVA) was used to determine if there were differences between groups or over time. A Pearson product moment correlation was computed to compare OC to BALP as well as the uncoupling index for OC and BALP. Statistical significance was determined *a priori* as $p \le 0.05$. Data is reported as the mean \pm SD.

RESULTS

On average, subjects consumed the recommended amount of calcium (1000 mg/day) (www.nal.usda.gov) and had a BMI that fell within the accepted normal range (18.5 - 24.9) (13).

There were no significant differences between trials or over time as compared to the baseline measurements for OC, BALP, or NTx (See Figure 1). Values for each of these markers fell within the accepted reference range.

An uncoupling index can be calculated to quantify, or standardize the rate of bone turnover (11), although it is not universally done. In this way, biochemical markers for bone formation and resorption may be combined to determine overall bone turnover. An uncoupling index (UI) was



Figure 1. Responses of markers of bone formation (OC, BALP) and resorption (NTx) following acute exercise. (Mean values shown). No significant differences within or between trials were observed.

calculated for both markers of formation to determine if bone turnover was occurring at a positive or negative rate during the 24hr post exercise. Neither UI (i.e., for BALP or OC) was significant at any time point (p ≥ 0.05) (See Table 3).

Correlations between BALP and OC were inconsistent (Table 2). However, the majority of the correlations between the UI were significant and fairly strong (Table 3).

Time	PRE	POST	1H	24H
	.87	.70	.65	.58
Control	p=0.001	p=0.036	p=0.041	p=0.081
	.52	.28	.67	.67
Water Aerobics	p=0.153	p=0.458	p=0.048	p=0.048
	.65	.54	.44	.33
Jogging	p=0.041	p=0.109	p=0.202	p=0.353

Table 2. Correlation between OC and BALP

DISCUSSION

The current investigation indicates that healthy young women demonstrate no changes in bone turnover within 24-hours of a single, 30-min bout of exercise below anaerobic threshold. Because markers of responded similarly turnover bone following water aerobics and jogging, these findings suggest that water aerobics provides enough stimuli to maintain bone turnover in the 24-hours following an acute bout of exercise. Our data were only collected at three finite time points following exercise (i.e., 0, 1, and 24-hr post), therefore, it is possible that changes in bone turnover do occur during the 24-hours following an acute bout of exercise, but that these changes are noticeable at a time that we did not measure. It is also possible that our time frame was too short to measure biochemical changes in turnover that result from 30-min of acute exercise.

For example, Welsh et al. (35) studied young men who were not regularly active. They noted increases in markers of bone resorption (urinary crosslinks of pyridinium) and no change in markers of formation (OC and BALP) 32-hours after a 30-min bout of treadmill walking at 60% of each individual's age predicted maximal heart rate. In contrast, Whipple et al. (36) decreases observed in markers of resorption (NTx) and no change in formation (BALP, PICP) at 1, 8, and 48 hours after a bout of resistance training in inactive men.

Other modes of exercise have also been examined. Following a Wingate cycle test in male athletes, Kristoffersson et al. (21) noted no immediate (1 hr.) changes in formation (OC, PICP) or resorption (ICTP). Other work by this group has indicated increases in markers of formation (PICP) at 24 and 72 hours and increases in resorption (ICTP) at 72 hours following a 90-min walk at 50% of VO₂max in sedentary, older (e.g. age) women (32) and increases in both formation (PICP) and resorption (ICTP) markers at 24 and 72 hours after a 45-min run in inactive, healthy young women (31).

Time	PRE	POST	1H	24H	
	.87	.84	.73	.66	
Control	p=0.001	p=0.003	p=0.016	p=0.038	
	.75	.66	.61	.80	
Water Aerobics	p=0.016	p=0.037	p=0.053	p=0.006	
	.74	.66	.69	.78	
Jogging	p=0.015	p=0.039	p=0.028	p=0.008	

Table 3. Correlations between uncoupling indexes (UI) for OC and BALP (n=9).

International Journal of Exercise Science

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Clearly, the interactions of a number of variables make solid conclusions difficult. For instance, the mode, duration, and intensity of the exercise bout, the time following exercise at which samples are taken, and the age, sex, and activity levels of the individuals tested to date vary between investigations. Regardless of these limitations, most groups conclude that favorable changes in overall bone turnover occur in response to exercise. The mechanisms by which turnover occurs is either by stimulating osteoblasts and triggering bone remodeling (35) or by decreasing osteoclast activity, resulting in less bone loss or greater potential for bone (36). This divergence in gain the mechanisms by which bone remodeling occurs has led some to utilize an indicator of uncoupling.

In the current investigation, both uncoupling indexes (for BAP and OC) indicated that bone accrued at a positive rate at the end of the exercise trials; however, the changes were not significant. Differences in the uncoupling indexes were minimal both between trials and over time: possibly because the markers measured reflect total body turnover rather than sitespecific responses. In addition, large standard deviations and a small sample size may have contributed to these low values. Regardless, in the current investigation, water aerobics did not differ from jogging, which is known to promote long term bone formation, nor was it different from the control trial. Therefore, the current findings indicate that water aerobics cannot be ruled out as an osteogenic mode of exercise over a longer period of time.

The majority of research conducted on bone turnover concludes that moderate to highimpact activities such as running and jumping provide the greatest osteogenic stimulus and result in large improvements in BMD (4, 6, 14, 16, 22, 37). Jogging is also considered to be an osteogenic mode of exercise (12). However, the current data do not indicate that jogging had an osteogenic impact, because all values were similar to the control trial at all time points. Because the last sample was measured 24 hours after exercise; it is possible that the time frame was too short to measure biochemical changes in turnover that result from acute exercise or those changes occurred at a time point that was not measured within the 24hr trial.

While high-impact exercises like jogging are known to positively affect bone, lowerimpact activities that focus on aerobic activity and resistive exercise have also shown a positive effect on bone formation. Vincent and Braith (34) suggested that both high and low intensity resistive exercise significantly changed biochemical markers (i.e., OC and BALP) promoting bone formation, while Whipple et al. (36) showed decreases in markers of resorption following a bout of moderate resistance activity.

It also has been reported that aerobic activities increase muscle mass, stimulating bone growth. For example, Doyle, Brown and Lachance (9) noted a significant correlation between bone mass and associated muscle at autopsy, suggesting that activity that increases muscle mass will have a positive benefit on bone. Similarly, Kohrt et al. (20) demonstrate strong relationships between muscle mass and bone mineral content, providing support for the concept that muscle forces load and stimulate the skeleton. In the current investigation, one bout of exercise may not have been sufficient to induce bone formation, since muscle mass does not increase within 24-hours as a result of one bout of exercise. In addition, since the current participants were already active, the intensity of the exercise bout they performed for this investigation may not have been sufficient to stimulate muscle, and therefore bone, growth.

Swimmers typically have a high percentage of fat-free body mass (i.e., muscle mass), yet examinations of bone turnover have demonstrated that swimming provides little osteogenic stimulus (6, 30) and has been linked to decreased rate of bone formation (6). With the combination of running, jumping, and the use of props (e.g., barbells and noodles), water aerobics has both resistive and ground-impact components making it different from the no ground-impact activity of swimming. Therefore, water aerobics may provide enough resistance and strain on the bones to be a beneficial form of exercise to promote bone formation, or to attenuate bone loss. This investigation demonstrated that bone marker levels following water aerobics were similar to jogging, suggesting that water aerobics has enough stimuli to maintain bone turnover over a 24-hour period.

Due to the subjects' age, special attention was focused on the intensity of the exercise trials. Evidence exists that, for bone, there may be an 'ideal' range of intensity that is age-specific for women (pre- or postmenopause). Investigations including postmenopausal women have suggested that the intensity of exercise must be above anaerobic threshold to increase BMD (4, 16, 37), although the findings of Thorsen et al. (32) contradict this. In contrast, studies with younger women suggest exercise should be restricted to below anaerobic threshold to prevent estrogen levels from decreasing as a result of high-intensity exercise (1, 3).

Finally, it should be noted that differences in basal and post-exercise levels of some markers of bone metabolism have been noted. Specifically, Nishiyama et al. (24) observed higher baseline levels of OC in active male subjects when compared to their inactive counterparts. These findings suggest that active individuals have higher rates of turnover on a daily basis. Activity level is not a concern in the current investigation, however, as all of the subjects reported similar activity levels, i.e., physically active 2-3 days per week.

While the current results are promising, questions remain. To elucidate further on this investigation, it is necessary to complete an investigation with additional participants, and perhaps to control for the use of oral contraceptives. In addition, the time points at which samples are collected should be expanded both within and beyond the 24-hour time frame utilized in this investigation. Finally, a training intervention, perhaps including both preand post-menopausal women, would shed additional light on the response of metabolic markers of bone to different modes of exercise, and allow further examination of water aerobics as a low impact alternative.

To summarize, the current results indicate that either 1) one bout of aerobic exercise below anaerobic threshold may not be sufficient to acutely increase bone turnover in young women or 2) that bone turnover may occur at a different time point than was measured in this investigation.

In conclusion, no significant changes in bone turnover were detected over a 24-hour period after moderate exercise in healthy, regularly active young women. Biochemical markers of bone formation and resorption responded in a similar manner regardless of the mode of activity when compared to the control trial. Although the current findings do not directly support water aerobics as a mode of exercise to maintain bone density, they do not contradict this. Thus, water exercise cannot be ruled out as a beneficial form of exercise for the maintenance of bone density.

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International Journal of Exercise Science

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BONE TURNOVER RESPONSE TO ACUTE EXERCISE

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International Journal of Exercise Science