

**The Relationship Between Insulin-Like Growth Factor-1 and Bone Mineral Density in  
Osteopenic Men Following a 12-Month Osteogenic Exercise Intervention**

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The undersigned, appointed by the dean of the Graduate School, have examined the thesis or dissertation entitled

THE RELATIONSHIP BETWEEN INSULIN-LIKE GROWTH FACTOR-1 AND  
BONE MINERAL DENSITY IN OSTEOPENIC MEN FOLLOWING A 12-MONTH  
OSTEOGENIC EXERCISE INTERVENTION

presented by Timothy Sinak,

a candidate for the degree of master of science,

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**ABSTRACT:** Research suggests that weight-bearing exercise can have beneficial effects on bone mineral density [BMD]. Specifically, plyometric [PLY] and resistance training [RT] have been successfully used to improve BMD in both men and women. Often, the hormonal response to exercise training is an important mediator of exercise-induced adaptations. However, there is a paucity of research in men with osteopenia regarding the effects of osteogenic exercise interventions on BMD and how the hormonal response to exercise training is related any changes in BMD. **PURPOSE:** To test the following hypotheses 1) 12 months of either RT or PLY will maintain or improve BMD of the whole body [WB], lumbar spine [LS], hip, and femoral neck [FN], 2) IGF-1 concentrations will increase in the RT group, but not the PLY group, and 3) changes in IGF-1 will be positively related with the aforementioned changes in BMD in the RT group, but not the PLY group. **METHODS:** Twenty healthy [25-60 yrs], recreationally active males [ $\geq 4$  hrs/wk] with osteopenia were randomly assigned to either of two, 12 month exercise interventions: RT [n=10] or PLY [n=10]. RT subjects completed two training sessions/wk on non-consecutive days for 12 months, consisting of three sets/exercise, which varied in intensity based on their one-repetition maximum. PLY subjects completed three training sessions/wk on non-consecutive days for 12 months, accumulating a maximum of 100 jumps. Participants in both groups completed seven-day diet and physical activity logs at 0 and 12 month time points to monitor changes in either during the duration of the study. Dual energy X-ray absorptiometry [DXA] was used to measure bone area and BMD of the LS, WB, Hip, and FN at 0 and 12-mo time points. IGF-1 concentrations were assessed at 0 and 12-mo time points with immunoassays. Repeated measures ANOVA was used to assess changes in BMD and IGF-1, and Pearson-product moment correlations were used to assess relationships between percent changes in IGF-1 and percent changes in BMD. **RESULTS:** Subjects in both groups experienced increases in

WB BMD [time,  $p=0.025$ ] and LS BMD [time,  $p=0.047$ ]. Hip BMD was improved only in the RT group, while the PLY remained unchanged [group x time,  $p = 0.068$ ]. FN BMD did not change significantly in either group. IGF-1 concentrations were increased in both groups, with the RT group increasing significantly more than the PLY group [group x time,  $p=0.000$ ]. No significant relationships were found between percent change in IGF-1 concentration and percent change in BMD at any site. **CONCLUSION:** The results of the present study suggest that RT and PLY may be effective interventions to maintain or improve BMD in osteopenic men. However, changes in circulating IGF-1 concentration are not related to changes in BMD during a 12-mo exercise intervention in men with osteopenia.



## **INTRODUCTION**

### **Osteoporosis in Men**

Osteoporosis is a nationally recognized health problem in the United States (154). The World Health Organization states that osteoporosis is “a systematic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with consequent increase in bone fragility and susceptibility to fracture (154).” They define osteoporosis as a bone mineral density [BMD] of the lumbar spine or femoral neck that is -2.5 standard deviations below the mean for a young adult woman. Osteopenia, which is the precursor to osteoporosis, is characterized by a BMD between -2.5 and -1.0 standard deviations below the young adult mean. Since bone strength is defined as its resistance to fracture, and lower bone is related to increased fracture risk, both of these conditions can increase an individual’s fracture risk (11, 154).

The prevalence of osteoporosis in men is significant (104, 105). In the United States alone, osteoporosis affects more than 2 million males, while almost 12 million more have osteopenia (105). Research from Khosla et al suggests that as the aging population increases, the number of men who suffer a hip fracture worldwide may reach 1.8 million by 2050 (104, 105). This is important because men who sustain an osteoporotic fracture have an increased mortality risk (104, 105). The 12-month mortality rate in men following a hip fracture is 15-32% greater than in women (2, 109). In addition, the cost of treating osteoporosis and the related fractures, including loss of productivity, is around \$12-18 million (104). In addition, current research suggests that approximately 60-80% of all hip fractures and 70-90% of all spine fractures in men may be attributed to low BMD (143). Thus, research in men with low BMD is needed to

determine effective treatments to increase BMD and reduce fracture risk in this population.

### **Exercise and BMD**

Physical inactivity is a modifiable risk factor for male osteoporosis (11). Previous research suggests that activity that is weight bearing in nature is most effective at increasing and/or maintaining BMD (153, 170). Specifically, activities involving high ground reaction forces [GRF] such as running or jumping have been shown to increase both whole body and lumbar spine BMD to a greater extent than low impact activities such as swimming and cycling (170, 209, 241). The term GRF is used to describe the force incurred on the skeleton during human locomotion (245). Weeks et al reported that the landing after performing a squat jump induces GRF equal to that of 3.8-times bodyweight (234). In addition, evidence from studies in both men and women suggests that resistance training [RT] may be a viable treatment to increase BMD (39, 43, 96, 144, 170, 174, 214, 231).

There is a paucity of research done specifically in men looking at the effects of exercise interventions on BMD. A meta-analysis by Kelley et al reviewed studies of exercise and BMD in men published between 1966 and 1998 (102). The authors concluded from the available data that exercise has positive effects on BMD, especially in older adults [ $>31$  yrs], and that these effects are site-specific (102). However, the authors found only 8 prospective studies done in men that had included a control group and a training program that lasted at least 16 wks (102). Furthermore, only 3 of these studies included RT as a treatment (25, 62, 144, 153). Additionally, no research to this

author's knowledge has investigated the effects of osteogenic exercise interventions specifically in men with low BMD.

### ***Importance of Weight Bearing Activity***

Research suggests that weight bearing activity may be more effective than non-weight bearing activity at improving bone health (153, 170, 209, 241). Several studies have reported low lumbar spine BMD among elite road cyclists (188, 203, 204, 232). Nichols et al found that male cyclists had 10% lower spine and hip BMD compared to age matched sedentary controls (170). Similarly, Stewart et al compared BMD of cyclists and runners, finding lower BMD amongst the cyclists (203, 204). The authors concluded that a lack of weight bearing exercise is harmful to bone health, and that high GRFs are an important contributor to the osteogenic response to exercise in men (204). Interestingly, when the BMD of road cyclists was compared to that of mountain cyclists and controls, the mountain cyclists had significantly higher BMD at all sites (232). It is important to note that mountain cycling involves challenging terrain including drops and jumps while cycling down a mountain, thus incurring GRFs during cycling (232). These data further support the hypothesis that activity that incurs GRFs is important for deriving an osteogenic response from exercise, and that a lack of weight bearing activity may be detrimental to bone health.

Additionally, studies that have compared athletes in high impact and low impact sports have found that those athletes in low impact sports have lower BMD than those in high impact sports (40, 60, 232). Specifically, Creighton et al reported that female athletes who participated in basketball and volleyball [high impact] displayed a greater BMD at weight-bearing sites than those who participated in soccer and track and field

[medium impact], as well as swimmers and controls [low impact] (40). Thus, frequent participation in high GRF inducing activities appears to have an osteogenic effect.

### ***Plyometrics and BMD***

Plyometrics [PLY] is a form of exercise designed to maximize the effect of the stretch-shortening cycle on muscle contraction, resulting in an increased force of muscle contraction (32). Thus, PLY allows for a greater strain on the bone via muscle contraction that is normally experienced without the stretch-shortening cycle (10, 70, 152, 179). Plyometric exercise also offers the additional benefit of incurring high GRFs upon landing, which may increase its potential to induce osteogenesis (234). However, few studies investigating the effects of PLY training on bone have been performed in men. Rantalainen et al studied the effects of a 12-wk bilateral hopping program in elderly men [69-77yrs] (168). Researchers divided participants into a control group [n=12] or a treatment group [n=13] who performed 5 -7 bouts of 10-second, continuous jumping on the balls of the feet. Bone-specific alkaline phosphatase [BAP] and C-telopeptide of collagen crosslinks [CTX] concentrations were measured to assess changes in bone formation and resorption, respectively. The authors reported no significant changes in any markers of bone turnover, suggesting that the intervention was ineffective in promoting osteogenesis (168). However, the authors postulated that the minimal impact through the heel upon landing as well as the lack of rest between individual jumps may explain the lack of osteogenic response to the protocol (168). Guadalupe-Grau et al studied the effects of a 9wks combined RT and PLY training program in college-aged [18-23yrs] men (70). Subjects performed 3 sessions/wk of 4-5 sets of 5-10 jump exercises [depth jumps and hurdles] followed by 3 sets of the following exercises: leg

press, half squat, leg extension, and leg curl (70). The authors reported 45% increases in serum osteocalcin, a marker of bone formation, as well as significant increases in whole body BMD following the 9wk training protocol (70). However, the specific effects of PLY exercise on these outcomes cannot be determined due to the combined RT and PLY training protocol. Similarly, Erickson et al investigated the effects of adding a periodized jumping program to an RT program for 8 weeks in college-aged men [18-23yrs] (50). Researchers divided 21 subjects into 3 groups: a group that jumped once per day, and group that jumped twice per day, separated by 6 hrs, and a control group that only participated in their normal strength training routines. Subjects in the jumping groups performed maximal vertical jumps against resistance in the Plyo Press© resisted jumping apparatus. Resistance began as 80% of bodyweight, and progressed to 100% of bodyweight over the course of the 8wk study. Both jumping groups performed the same total number of jumps per day, which were divided equally over the two training sessions in the group that jumped twice per day. Bone-specific alkaline phosphatase [BAP] and C-telopeptide of collagen crosslinks [CTX] concentrations were measured to assess changes in bone metabolism. The authors reported significant increases in BAP and reductions in CTX, and that these changes were enhanced in the group that jumped twice per day (50). The previous studies were limited by both the duration [8-12wks] and intervention design [combined PLY and RT], leaving a void in the research regarding long-term [ $\geq 6$  mo] PLY training and bone health in men. This is important, as valid changes in BMD are typically not detectable via DXA for 6-12 months due to the lengthy process of bone remodeling (35). In addition to the aforementioned studies, which specifically examined

PLY exercise in men, Welsh et al reported that a 12-mo intervention of high-impact step aerobics improved or maintained BMD in men aged 50-75 yrs (236). Subjects experienced a significant 2.21% increase in greater trochanter BMD, a non-significant increase in femoral neck BMD, and no change in whole body BMD (236). However, subjects in the control group experienced 1.9% and 0.72% decreases in femoral neck and whole body BMD, respectively (236). Thus, the authors concluded that at the very least, high-impact exercise may slow the loss of BMD in older men (236).

The only human intervention studies specifically examining the impact of long-term, exclusive PLY training on bone have been done in women. Bailey et al conducted a 6-month intervention study in which 21 premenopausal women did 50 plyometric jumps on one leg, 2-7 times per week, with the opposite leg serving as the control (10). The BMD of the FN in the active leg increased by an average of 1.09%, compared to no change in the control leg (10, 153). Thus, in PLY, bone undergoes increased strain both from increased muscle contraction when jumping and increased GRFs upon landing (10, 32). These data suggest that PLY may be effective at improving BMD. However, no studies to date have examined the effect of an exclusive PLY protocol in osteopenic men.

### ***Resistance Training and BMD***

As previously mentioned, resistance training [RT] may be a viable treatment to increase BMD (39, 43, 96, 144, 170, 174, 214, 231). Some studies that examined the effects of RT on BMD found no effect of RT on BMD (43, 237). Whiteford et al examined the effects of RT on BMD in men during a 1-yr intervention (237). He studied 148 older men [age 55-80 yrs] who were randomized into either an RT group [3d/wk RT]

or an active control [30min walking 3d/wk] (237). Subjects in the RT group performed 3d/wk of supervised RT for one year, consisting of hip extension, hip flexion, hip abduction, and hip adduction performed on a cable pulley machine. These exercises were chosen to load the muscles that act at the hip. The exercises were loaded with >15RM loads for 8 weeks, and progressed to 8RM loads for the remaining duration of the study. The active control group was advised to walk 30min/3d/wk for the 1-yr duration of the study. Despite an increase in muscle mass and strength in the RT group, the results showed no benefit of RT on BMD over walking in older men (237).

By contrast, the majority of both prospective and retrospective studies that have investigated BMD in men who performed RT have suggested a positive effect of RT on BMD and markers of bone formation and resorption in men (39, 62, 96, 144, 214, 215, 231). Tsuzuku et al did a cross-sectional study comparing the BMD of male powerlifters and sedentary controls (214). The powerlifters had been training the bench press, squat, and deadlift for at least the 12 months prior to the study, while the controls had not participated in more than 2 hr/wk of purposeful physical activity, and had not participated in RT for the same time period. They found a significantly higher BMD of the whole body, lumbar spine, arm, leg, and hip in the powerlifters than in sedentary controls after adjusting for body weight and percent body fat (214). They also regressed the performance [1RM] in the squat, deadlift, and bench press on BMD at the aforementioned sites to determine if a site specific relationship existed between any of the lifts and BMD at any sites. They found a strong positive correlation [ $r > 0.70$ ] between squat and deadlift, but not bench press performance and lumbar spine BMD (214). The authors concluded that training with

high-intensity loads that compress the spine may be effective for improving BMD of the spine (214). Similarly, several studies of junior male weightlifters [age $\leq$ 18 y] have shown higher BMD at all sites as compared to age matched athletes in different sports (39, 96, 231).

Very few prospective studies have been done examining the effects of RT on BMD in men. Menkes et al looked at the effects of 16 wks of RT on BMD in untrained, middle age and older men [age 50-70y]. The subjects performed 3 days/wk of RT including the chest press, horizontal row, lat pulldown, overhead press, leg press, leg curl, leg extension, triceps extension, modified sit-ups, and arm curls on pneumatic resistance machines and dumbbells. Training weights were set to a 5RM at the beginning of the exercise, and lowered as the subject reached fatigue to allow for the completion of 15 repetitions. After 16 wks, subjects in the RT group experienced a mean of 2% increase in lumbar spine BMD and a 3.8% increase in femoral neck BMD, while the control group experienced no significant changes in BMD at any sites (144). Fujimura et al studied the effects of a 4-mo RT program in 17 young oriental men [age 23-31y] on BMD (62). Subjects performed 3d/wk of RT consisting of the leg extension, leg curl, bench press, lat pulldown, sit up, back extension, wrist curl, and leg lunge for the first and last workout of the week. The second workout of the week consisted of the half squat, bench press, lat pulldown, shoulder press, sit up, back extension, arm curl, and wrist curl. After 4-mo, there were no significant changes in BMD at any sites, but there was a significant increase in the ratio of markers of bone formation [osteocalcin] to bone resorption [DPYR] (62). Braith et al studied the effects of an RT intervention in post-operation rehabilitation for male heart transplant patients (25). Subjects [n=16, age 42-62



y] were randomly assigned to either a 6-mo RT intervention or a control group that did not perform RT. The RT group performed back extensions, chest press, knee extension, knee flexion, triceps extension, biceps flexion, shoulder press, and abdominal crunches. After the 6-mo post-operation RT intervention, subjects in the RT group had restored whole body, femoral neck, and lumbar spine BMD to within 1%, 1.9%, and 3.6% of pre-operation values, respectively. BMD for subjects in the control group did not change significantly from pre- to post-operation (25). Almstedt et al investigated the effects of a 6-mo RT program consisting of bench press, squats, and deadlifts on changes in BMD in college-age men [n=12] and women [n=12] (6). Subjects performed the training protocol on 3 non-consecutive days per week. Loads were gradually progressed from 67% of 1RM to 95% of 1RM over the course of 4wk microcycles, at which point the 1RMs were reassessed. The authors reported that BMD of the lumbar spine and femoral neck increased by an average of 3.7% and 4.5%, respectively, over the course of the intervention in the men, while changes were not significant in the women (6). Additionally, our lab recently reported that an acute bout of either RT or PLY in men was effective at increasing markers of bone formation [bone-specific alkaline phosphatase] and decreasing markers of bone resorption [tartrate-resistant acid phosphatase isoform-b], suggesting that both RT and PLY may be effective modes of exercise for inducing osteogenesis (179).

The majority of the aforementioned studies were done in men with normal bone density, and no studies to this author's knowledge have examined the effects of RT in healthy, physically active men with low BMD. Furthermore, no studies have looked at

the effects of an RT program specifically designed to be osteogenic and to maximally load clinically significant BMD sites [lumbar spine, hip], such as the squat, military press, and Romanian deadlift in men with low BMD.

### **Mechanisms By Which Osteogenic Exercise Increases BMD**

Exercise may influence BMD through both mechanical and hormonal mediators. Thus, it is important to understand both the mechanical and hormonal mechanisms by which exercise may influence BMD in order to maximize the effectiveness of exercise interventions for osteopenic men. Furthermore, investigation of the contribution of these mechanisms to changes in BMD during an exercise intervention is warranted.

#### ***Muscle Forces***

One proposed mechanism by which RT increases BMD is through the strain the muscle contraction places on the bone. Frost's mechanostat states that bone adapts to external loading in order to prevent fracture and maintain structure within a normal physiologic range of strain (60). As the muscle contracts, the osteocytes at the attachment site are strained (61). These osteocytes sense the mechanical forces exerted on the bone, and convey them through the bone tissue via gap junctions, which stimulates an increase in bone remodeling that favors bone formation (157).

This process of bone remodeling results in an increase in bone mass in the areas that are strained (61).

Due to the bipedal nature of human locomotion, the arm provides an excellent skeletal site at which to study the effects of muscle contraction on bone while virtually eliminating the effects of weight bearing and gait. Research by Hasegawa et al investigating the relationship between grip strength and bone architecture parameters of

the radius has shown a strong, positive correlation between grip strength and radius BMD (81). Another in vivo model for studying the effects of muscle forces on BMD is in newborns with intra-uterine onset neuromuscular paralysis. These newborns typically exhibit normal bone length, but impaired bone density and strength, resulting in multiple fractures upon birth (177). Given the aqueous, and virtually “weightless” environment of the uterus, impact forces are unlikely to contribute to bone loading in these infants. Thus, deficiencies in BMD can likely be attributed to a lack of muscle forces acting on the bones of these infants in-utero. At the very least, these data support the importance of muscle forces on bone mass and BMD in the developing skeleton.

Animal studies have also provided important insight into the effects of muscle contraction on bone. Umemura et al developed a rat model in which to study the effects of muscle contraction on bone without the influence of ground reaction forces (219). To accomplish this, rats were trained to jump to a platform at a height such that the rats would have to catch the edge and climb to reach the top. This model serves to minimize the GRF that would normally be incurred in response to the landing portion of them jump. Several studies using this model have reported improved BMD with variations of the jumping protocol (152, 219, 221). These results suggest that purely muscle contractile forces can significantly influence the properties of bone. Furthermore, several studies of RT have also demonstrated a site-specific

effect of muscle contraction on BMD (39, 96, 174, 214, 231). Thus, by imposing a challenge on the bone via muscle contraction during strength training, BMD may be improved particularly in the areas in which these muscles insert (60).

### ***Impact Forces***

One mechanism by which exercise may exert an osteogenic effect on the skeleton is through impact forces, or ground reaction forces [GRF]. GRFs are a measure of the amount of force incurred by the ground upon the skeleton during human locomotion (110). Weeks et al determined that simple jumping exercises can exert GRFs on the skeleton equal to 3.8-times bodyweight (234). Research has yet to elucidate the direct contribution of GRFs to exercise-induced osteogenesis, as it is impossible to separate the effects of muscle contraction on bone in vivo (19, 42, 91). However, the importance of gravitational loading in bone health is evident in studies of skeletal unloading, such as bed rest, spinal cord injury, or space flight. Studies of astronauts have consistently reported reductions in BMD of weight bearing sites, such as the hip, spine, and greater trochanter as great as 23.4% with space flights lasting 1-6mo (21, 37, 229). Cross-sectional studies of patients recovering from spinal cord injuries, which severely limit weight bearing activity, have reported loss of bone mass in the femur as great as 22% just 3 mo post-injury (64). More recently, it was observed that out of 41 patients with spinal cord injuries, 61% met the World Health Organization criteria for osteoporosis, and an additional 19.5% were osteopenic (123). Additionally, studies of long term [12-17wks] bed rest patients have reported significant decreases in lumbar spine, femoral neck, greater trochanter, and calcaneus – up to 10.4% (124, 228). However, the aforementioned studies are not without limitations. Specifically, the observation of

reduced BMD in patients with spinal cord injuries and/or bed rest does not necessarily take into account the simultaneous reduction of muscle contractile forces seen in these patients (79, 124). Still, these studies suggest that gravitational forces are at least a large contributor to bone health, especially in weight bearing portions of the skeleton.

### ***IGF-1***

IGF-1 is produced mainly in the liver in response to signaling from growth hormone [GH] (98). IGF-1 has been shown to positively affect the development of bone mass both in vitro and in vivo, although the exact mechanisms are yet to be elucidated (8, 17, 28, 119, 133, 201). IGF-1 has been shown to stimulate proliferation of osteoblasts as well as inhibit collagen degradation in cell cultures, suggesting that IGF-1 may have both an anabolic and anti-catabolic effect on bone mass (17, 28). Rydziel et al found that treatment of culture osteoblasts with IGF-1 decreased mRNA expression of the gene for collagenase 3 – a potent collagen degrading protein found in bone (187). Thus, IGF-1 may have anti-catabolic effects on bone through the inhibition of collagenase 3 formation. Furthermore, in vivo studies in rats and mice have shown that IGF-1 administered systemically can induce bone formation and repair (8, 133, 201). Spencer et al infused adult female rats with IGF-1 for 14 days, and reported an increase in both trabecular and cortical bone formation (201). These data suggest that an increase in systemic IGF-1 concentrations can have a positive effect on bone mass, and that this effect may be both anabolic and anti-catabolic in nature.

### ***Animal Data***

Several recent reviews of both human and animal data suggest that IGF-1 is a key regulator of bone mass (100, 128, 138, 156). There are three main sources of IGF-1 that

act on bone: liver-derived IGF-1, muscle-derived IGF-1, and bone-derived IGF-1 (100, 156, 197). The majority of circulating IGF-1 is produced in the liver, accounting for close to 80% of serum IGF-1 (197). Animal research suggests a positive relationship between serum IGF-1 and bone strength (196, 243). Yakar et al bred mice to have low serum IGF-1 [70% less than wild type controls], and compared their bone growth to wild type control mice (243). The researchers found reduced length and density of the femurs of IGF-1 deficient mice compared to control mice, especially in the cortical bone, which is a strong predictor of bone strength (243). Additionally, research from Govoni et al suggests that both liver and bone derived IGF-1 are important for development of peak bone mass (67, 68). To examine the impact of bone derived IGF-1 on bone development, researchers bred osteoblast-specific IGF-1 knockout mice. The researchers reported reduced bone mass in the knockout mice compared to wild type controls, suggesting that both liver and bone derived IGF-1 are required for the acquisition of normal peak bone mass during development (67, 68). Additionally, several studies of osteoporotic mice have reported increased or restored trabecular and cortical bone volume in response to systemic IGF-1 treatment (8, 133, 151). Collectively, data from animal research suggest that increases in systemic IGF-1 can have a therapeutic effect on BMD in rats with low BMD.

#### *Human Data*

Human data on the role of IGF-1 on BMD stems from patients with GH or IGF-1 deficiencies. In patients with GH deficiency, treatment with GH results in increased serum IGF-1 that is associated with increased BMD (100, 156). Similarly, patients with low serum IGF-1 due to defective IGF-1 gene expression frequently display osteopenia,

further supporting a role for IGF-1 in adult bone metabolism (100, 156). However, human data on the relationship between IGF-1 and BMD are conflicting, with some studies showing an association (14, 65, 88, 120, 207), and some showing no association (31, 38, 184). Specifically, the Framingham heart study and Rancho Bernardo Study found a positive association between serum IGF-1 and BMD in women but not men (14, 120). Conversely, the Rotterdam Study reported a positive association between serum IGF-1 and BMD in men but not women (88). The only study done specifically in men that investigated the relationship between IGF-1 concentration and bone health in men was the Osteoporotic Fractures in Men Sweden [MrOS] study (156). The MrOS study involved approximately 2900 men, with a mean age of 75 years. Serum IGF-1 was measured at baseline, and post-measurement fractures were recorded and validated for 3 years. After adjusting for age, researchers found a modest, significant relationship between serum IGF-1 concentration and femoral neck [ $r=0.60$ ,  $p<.01$ ] and lumbar spine [ $r=0.70$ ,  $p<.01$ ] BMD. Additionally, researchers found an inverse relationship for baseline IGF-1 concentration and fracture incidence in these men, suggesting that serum IGF-1 concentration may be an important predictor of fracture risk in men (156).

#### *IGF Binding Protein-3 [IGFBP-3] and Free IGF-1*

Similar to IGF-1, the vast majority of circulating IGFBP-3 is produced in the liver (98). IGFBP-3 binds to IGF-1 in circulation, preventing it from attaching to IGF receptors on the bone and initiating its anabolic/anti-catabolic effects, but also prolonging its circulation in the blood stream (167). Evidence for an inhibitory effect of IGFBP-3 on bone stems from transgenic mouse models (167, 195). Mice that were bred to overexpress IGFBP-3 suffered both pre- and post-natal bone growth retardation (167).

Additionally, Silha et al reported increased osteoclast number, impaired osteoblast proliferation, and impaired bone formation in similarly bred mice (195). Given the evidence that IGFbps inhibit the actions of IGF-1 by converting free IGF-1 to bound IGF-1, Yakar et al hypothesized that specifically increasing concentration of free IGF-1 would lead to enhanced IGF-1 action on bone (49). Researchers used a genetic knock-in method to cause mice to produce IGF-1 that had a low affinity for IGFbps, which resulted in increased free IGF-1 (49). The authors reported significantly increased cortical bone mass in the knock-in mice as compared to wild-type controls (49). Thus, IGFBP-3 may play an important role in mediating the effects of IGF-1 on bone by reducing the concentration of free IGF-1 in circulation. However, no studies to this date have examined the relationship between IGFBP-3 or free IGF-1 concentrations and bone health in men. Thus, further research is needed to elucidate these effects.

### ***Nutrition and IGF-1***

The main nutrition-related regulators of IGF-1 concentrations appear to be energy balance and protein content of the diet (210). The animal literature suggests that long-term caloric restriction can lower IGF-1 concentrations by as much as 40% (55). However, there is a discrepancy in the human data related to the duration of the nutritional intervention. IGF-1 concentration have been reported to decrease by as much as 20% following short-term [6-10 days] of caloric or protein restriction (200, 210). However, long-term [1-6 yrs] caloric restriction has been reported to have no effect on circulating IGF-1 concentration (56, 145, 165). Racette et al reported that IGF-1 concentration were unchanged following 1-yr of energy restriction in subjects who reduced their energy intake by approx. 300 kcal/day, but maintained the same protein



intake (165). Fontana et al hypothesized that this discrepancy may be due to inadequate protein intake in previous studies that report decreased IGF-1 in response to caloric restriction. To investigate this hypothesis, 6 subjects from a previous long-term caloric restriction study were followed during a 3-wk reduction of protein intake from 1.76g/kg to 0.75g/kg (56). This resulted in a 24% decrease in serum IGF-1 concentration in these subjects, suggesting that the protein content of the diet is an important determinant of the effects of energy balance on IGF-1 concentration. Collectively, these data suggest that a combination of energy balance and protein content of the diet may be important regulators of IGF-1 concentration in humans.

### ***RT and IGF-1 Concentration***

It is well documented that exercise involving moderate volumes, high intensities, and short or absent rest periods has the most pronounced effect on IGF-1 concentration (112, 164). Research regarding the impact of RT on circulating IGF-1 suggests that long term RT leads to increased circulating IGF-1 concentration (24, 111, 137). Borst et al reported a significant increase in IGF-1 concentration after just 13 weeks of a 25 week RT program in both men and women (24). Similarly, Marx et al reported a significant increase in resting IGF-1 concentration in women following 6 months of RT. In a retrospective analysis, Rubin et al reported higher IGF-1 concentration in resistance trained men than untrained men (183), suggesting that IGF-1 concentration increase in response to long term RT in men. However, no longitudinal, long term studies to date have investigated the effects of RT on IGF-1 concentration in men with low BMD.

### ***PLY and IGF-1***

An extensive search of the literature revealed no studies that examined the impact of PLY on IGF-1. Thus, research is desperately needed to fill this void in the literature. However, the type of exercise that has been shown in the literature to have a profound effect on circulating IGF-1 concentration has typically been RT, utilizing moderate loads, high volumes, and short or absent rest periods (112, 166). The design of the PLY intervention of this study was to maximize the power of each jump, so as to induce higher muscle contractile forces and GRFs upon landing, which necessitates rest periods between individual jump repetitions as well as between jumping exercises. Thus, we hypothesized that IGF-1 concentration would not increase significantly in the PLY group.

### *Gaps in the Literature*

There is a paucity of research surrounding exercise and BMD specifically in men. Research in women has suggested that RT and/or PLY may be effective exercise modalities to incur beneficial effects on bone (99). Despite this evidence, only a handful of studies have investigated the effects of RT and/or PLY on BMD in men. Only one study to date has included PLY in an exercise intervention in men, and all subjects completed both the RT and PLY protocols, making it impossible to examine the effects of PLY only exercise on these subjects (70). Of the studies that have investigated the effects of RT on bone specifically in men, only two were of sufficient duration [ $\geq 6$ mo] to notice valid, appreciable changes on BMD, and reported conflicting results (25, 237). Additionally, despite the data that suggest that IGF-1 is a key regulator of bone metabolism, no studies to the author's knowledge have investigated the association between plasma IGF-1 concentration and BMD specifically in men with low BMD. Furthermore, despite the data that suggest that IGF-1 concentration may increase with

chronic exercise training, no studies to the author's knowledge have investigated the association between changes in IGF-1 concentration with changes in BMD during an exercise intervention designed to improve BMD in men with low BMD.

## **PURPOSE**

Little is known as to how RT or PLY affects BMD in men with low BMD. Even less is known as to how changes in circulating IGF-1 are related to changes in BMD in men with low BMD. Thus, the specific aims of this study were:

**Specific Aim 1:** To determine if RT and/or PLY are effective at improving BMD of the whole body, lumbar spine, and hip of osteopenic men.

**Specific Aim 2:** To determine if IGF-1 concentrations increase with participation in PLY or RT

**Specific Aim 3:** To determine if changes in BMD of the whole body, lumbar spine, and hip are related to changes in IGF-1.

## ***Hypothesis***

The hypotheses of this study were:

**Hypothesis 1:** We expected that both RT and PLY would result in positive changes [i.e. maintenance or increase] in BMD at all clinically significant sites.

**Hypothesis 2:** Based on the aforementioned research on RT and IGF-1 concentration, and the dissimilarity between PLY and exercise that has been shown to have effects on IGF-1 concentration, we expected to see a significant increase in IGF-1 in the RT group, but not the PLY group.

**Hypothesis 3:** We expected to see an increase in IGF-1 concentrations only in the RT group. Thus, we expected to see a strong, positive correlation between changes in IGF-1 concentrations and changes in BMD in RT group, but not the PLY group.

## **DESIGN**

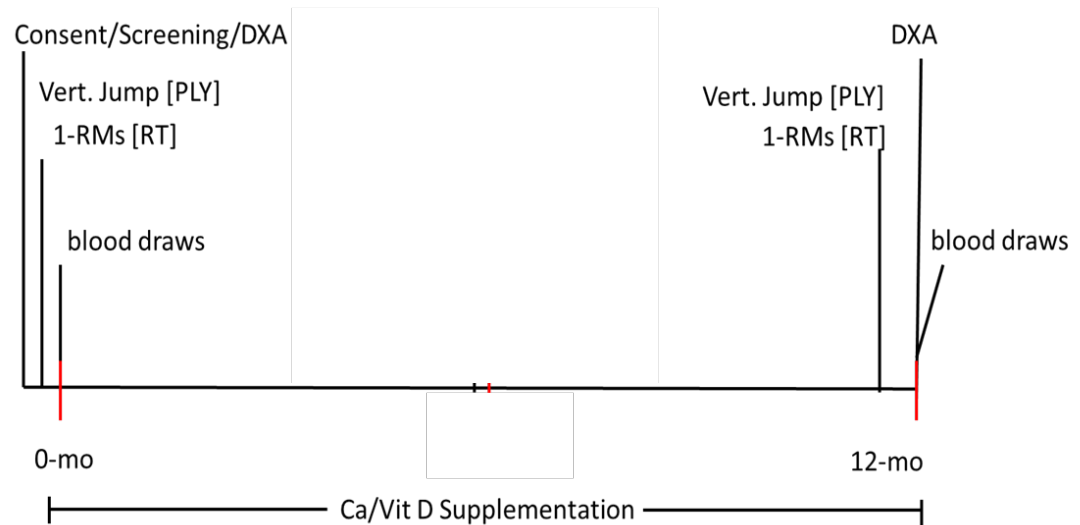
A longitudinal intervention was used to determine the effects of a 12-mo RT or PLY exercise program on BMD in physically active men with low BMD.

### ***Subjects***

Male subjects [n=20] were recruited from the Columbia, MO region through the University of Missouri Info email, bulletin board advertisements, local track and athletic clubs, and local sporting goods stores. To be eligible for the study, participants had to be participating in at least 4 hours of moderate intensity physical activity per week, be between the ages of 25-65 y, and have osteopenia of the lumbar spine or hip, which is defined as a T-score between 1.0 and 2.5 standard deviations less than average for a healthy adult. Exclusion criteria included a current or previous medical condition affecting bone health, currently taking any medications that affect bone metabolism or prevent exercise and/or currently taking anti-inflammatory steroids, current participation in either RT or PLY exercise, excessive alcohol consumption [ $>3$  drinks/day or 21 drinks/wk], and cigarette smoking. Before initial screening, all participants were informed of any risks associated with this study and give written informed consent to participate. Following written consent, subjects provided anthropometric measures, medical and sports history questionnaires [to determine previous bone loading scores],

and dual energy X-ray absorptiometry [DXA] scans of the whole body, lumbar spine, and hip to determine both BMD and BMC. Approval for the study was obtained from the University of Missouri-Columbia Health Sciences Institutional Research Board.

### ***Study Timeline***



**Fig 1 – Study timeline for 12-mo exercise intervention.**

### ***Anthropometric data***

Participants' age, body weight, height, body mass index [BMI], and percent body fat were measured. Body height was measured to the nearest 0.5 cm and body mass was measured to the nearest 0.05 kg. These measures were used to determine BMI [kg/m<sup>2</sup>]. Body composition was measured using a whole body DXA scan. These measures were taken at the 0-mo and 12-mo time points.

### ***Bone Density***

Area and BMD of the lumbar spine [L1-L4], total left hip, femoral neck, and whole body were measured using DXA [Hologic QDR 4500, Waltham, MA]. Participants were categorized using the World Health Organization's criteria as having normal BMD [ $>-1.0$  standard deviations [SD]], osteopenia [ $<-1.0$  SD,  $>-2.5$  SD], or

osteoporosis [ $\leq -2.5$  SD] of the lumbar spine [L1-L4] or hip as established by the manufacture's means for a young, adult population. Areal BMD [ $\text{g} \cdot \text{cm}^{-2}$ ] was calculated from bone area [ $\text{cm}^2$ ] and BMC in grams [g] by the software supplied with the DXA scanner. These data were collected at 0-mo and 12-mo time points.

### ***PLY Protocol***

Prior to beginning the PLY program, subjects received 2 familiarizations during which they were instructed on proper form for each jump. This served to orient the subjects to the jumping exercises. Following the familiarizations and prior to beginning the first 6-week cycle, subjects in the PLY group performed vertical jump testing. This was done using a Vertimax vertical jump measuring device [Perform Better, California, USA]. After a 10 min general warm-up, subjects were given three attempts at a maximal vertical jump, and the highest value was recorded. This testing was repeated at the beginning of each 6-week cycle. The PLY group came to the lab 3 times a week, each week, for the 12-month study, with a minimum of 8 hours between workouts. Each cycle was comprised of 6 wks of exercise followed by a rest week. The first two weeks of the cycle were light weeks, where each participant did 10 repetitions of 4 different jumps, for a total of 40 repetitions. The third and fourth weeks of the cycle were the moderate weeks, where each subject did 10 repetitions of 8 different jumps, for a total of 80 repetitions. The fifth and sixth weeks of the cycle were the heavy weeks, where each participant did 10 repetitions of 10 different jumps, for a total of 100 repetitions. The plyometric jumps included the squat jump, forward hop, split squat, lateral box push-off, bounding, lateral bounding, box jump, lateral hurdle, zig-zag, single-leg lateral hurdle, jump off the box, and depth jump. "Jump off the box" and "depth jump" are the high-intensity jumps and

were only done as the last two jumps during the heavy weeks [Table 1]. In between each jump was a 10 second rest period, with the exception of the bounding jumps. The bounding jumps were performed in two sets of 5, in which the first 5 were done consecutively, and then the subject rested for 30 seconds, and then the last 5 were completed. Each workout began with a 10 minute warm-up ended with a 5-minute cool down. Subjects were instructed on stretches for the back, shoulders, chest, hamstrings, and quadriceps which were to be performed post workout. Additionally, subjects performed 2 sets of 15 repetitions of back extensions and abdominal crunches at the conclusion of each workout.

**Table 1.** Progression of the number of jumps per week for the plyometric group.

	Weeks 1-2 Light	Weeks 3-4 Moderate	Weeks 5-6 Heavy	Week 7 Rest
Touches [Jumps]	40	80	100	--

***RT Protocol***

Subjects performed linear progressive RT 2d/wk for 56 wks. The squat, military press, modified deadlift, bent over row, barbell lunge, and calf raise were chosen in order to maximally load the spine and hip, as well as work most major muscle groups. Prior to beginning the RT program, each subject completed 2 familiarizations, during which the subjects were instructed on proper form for each of the aforementioned exercises. These served to orient the subjects to the exercises and prepare them for the movements. Each cycle included 6 wks of RT, followed by one week of rest during which repetition max [RM] testing was performed. Each cycle was divided into 3 microcycles: 2 wks of light

[3 sets of 10 reps at 50% RM], 2 wks of moderate [2 sets of 10 reps at 60%RM, 1 set of 6-8 reps at 70-75% RM], and 2 wks of heavy RT [2 sets of 10 at 60% RM, 1 set of 3-5 reps at 80-90% RM] [Table 2]. During each week, all exercises began with a warm up set consisting of 10 reps at 20% RM. During testing sessions, subjects were given three attempts at a RM after two warm up sets. A 1RM was collected for the squat, deadlift, and military press, and a 10RM was collected for the lunge, row, and calf raise. Each workout began with a general cardiovascular warm up of 5-10 min, and end with a cool down of the same length. Subjects were instructed on stretches for the back, shoulders, chest, hamstrings, and quadriceps which were to be performed post workout. Additionally, subjects performed 2 sets of 15 repetitions of back extensions and abdominal crunches at the conclusion of each workout.

**Table 2.** Progression for 1 cycle of RT

	Weeks 1-2 Light	Weeks 3-4 Moderate	Weeks 5-6 Heavy	Week 7 Rest/Testing
Set 1	10 @ 20% 1RM	10 @ 20% 1RM	10 @ 20% 1RM	--
Set 2	10 @ 50% 1RM	10 @ 60% 1RM	10 @ 60% 1RM	--
Set 3	10 @ 50% 1RM	10 @ 60% 1RM	10 @ 60% 1RM	--
Set 4	10 @ 50% 1RM	6-8 @ 70/75% 1RM	3-5 @ 80/90% 1RM	--

### ***Calcium and Vitamin D Supplementation***

It has been determined that calcium and vitamin D intake in the general population and the athletic population is sub-optimal (239). In order to ensure all subjects in this study received adequate amounts of these nutrients, they were supplied with *Nature Made* calcium and vitamin D supplements containing 1200 mg calcium and 400 I.U. of vitamin D, split into two daily doses. Each subject was instructed to take one tablet in the morning and one in the evening, every day during the intervention duration



[Fig 1]. Logs were kept for each subject to ensure compliance with the supplement protocol.

### ***Diet and Physical Activity***

Research suggests that changes in energy and/or protein status may affect IGF-1 concentrations (56, 165, 210). Thus, in order to evaluate and account for any changes in habitual energy expenditure and intake, 1-week diet and physical activity records were reported by subjects at 0- and 12-mo time points [**Appendices G and H**]. No significant changes were noted found from 0- to 12-mo time points in either group [**Appendix I**].

### ***IGF-1 Analysis***

Blood samples were collected at 0- and 12-mo time points from an antecubital vein by a trained phlebotomist. At each time point, the blood sample was taken after an overnight fast (at least 10 hours) and a control meal 2 hours prior consisting of 2 meal replacement shakes [Wal-Mart, Arkansas, USA]. The purpose of these shakes was to minimize the discomfort of exercise on an empty stomach while still controlling for the caloric and macronutrient content of the meal between subjects and across time points. Blood was drawn between the hours of 6:00 and 8:00am to control for diurnal variation. The blood was dispensed into plasma separator tubes containing EDTA, where it sat for 20 minutes. The blood was then centrifuged at 2000 rpm for 15 minutes at 0 °C. The plasma was removed from the EDTA tube and immediately frozen at -80 °C.

### ***Statistical Analysis***

Descriptive statistics were used on demographic and anthropometric data. Normality was checked for each variable using the Kolmogorov-Smirnov test, and visually checking histograms and Q-Q plots to ensure a bell-shaped curve and a linear line, respectively. Significant differences in baseline characteristics, as well as significant percent changes in strength [RT], vertical jump [PLY], and BMD were analyzed using the student's t-test. Pearson product-moment correlation tables were used to determine significant covariates for inclusion in the RMANOVA analyses, such as height, weight, and age. None of the covariates were significant [**Appendix J**]. A two-way [group and time] repeated measures ANOVA was performed to compare the effects of PLY versus RT on changes in BMD of the whole body, LS, and hip. Significant main [ $p \leq 0.05$ ] or interactive [ $p \leq 0.1$ ] effects in the repeated measures ANOVA were followed-up with paired samples T-tests. Relationships between percent changes in IGF-1 and BMD at all sites were assessed with one-tailed Pearson product-moment correlations, with  $p \leq 0.05$  considered significant. In order to assure the validity of the changes in BMD, RMANOVAs were performed to assess changes in bone area from 0 to 12-mo time points. No significant changes in bone area were observed [**Appendix H**]. Due to an error performing the 0-mo Hip BMD scan on subject from the RT group was excluded from any analyses involving Hip or FN BMD.

## **RESULTS**

### ***Subjects***

Subjects in the RT versus PLY group differed only in body weight and BMI, such that the RT group was approximately 16.1 kg heavier and had a BMI approximately 5 kg/m<sup>2</sup> higher than the PLY group [Table 2]. Subjects in the RT group experienced increased strength [1-RM] in the squat, modified deadlift, and military press, and subjects

in the PLY group experienced increases in vertical jump height [Table 3]. There were no significant changes in diet or physical activity during the intervention in either group [Appendix J].

**Table 3.** Subject characteristics.

	<b>RT</b>		<b>PLY</b>	
	<b>0-mo</b>	<b>12-mo</b>	<b>0-mo</b>	<b>12-mo</b>
<b>Age (y)</b>	42.1 ± 9.5	N/A	41.9 ± 10.4	N/A
<b>Height (m)</b>	1.78 ± 0.06	N/A	1.77 ± 0.74	N/A
<b>Weight (kg)</b>	88.9 ± 5.0	86.8 ± 4.7	72.8 ± 3.2*	72.6 ± 3.5*
<b>Body Mass Index (kg/m<sup>2</sup>)</b>	28.1 ± 2.8	27.2 ± 2.3	23.1 ± 3.3*	22.9 ± 3.2*
<b>Lean Body Mass (kg)</b>	64.8 ± 3.0	65.4 ± 3.2	58.3 ± 1.8*	58.2 ± 1.7*
<b>Body Fat (%)</b>	20.6 ± 4.8	19.2 ± 3.2	20.6 ± 5.4	20.2 ± 4.3

**Table 4.** Percent changes in strength and vertical jump.

	<b>% Δ</b>	<b>p-value</b>
<b>Vertical Jump</b>	20 ± 2	p=0.021*
<b>Squat 1-RM</b>	147 ± 15	p=0.002*
<b>Deadlift 1-RM</b>	139 ± 15	p=0.005*
<b>Military Press 1-RM</b>	124 ± 11	p=0.008*

\*Significant change from baseline [ $p \leq 0.05$ ]. Data are means ± S.E.

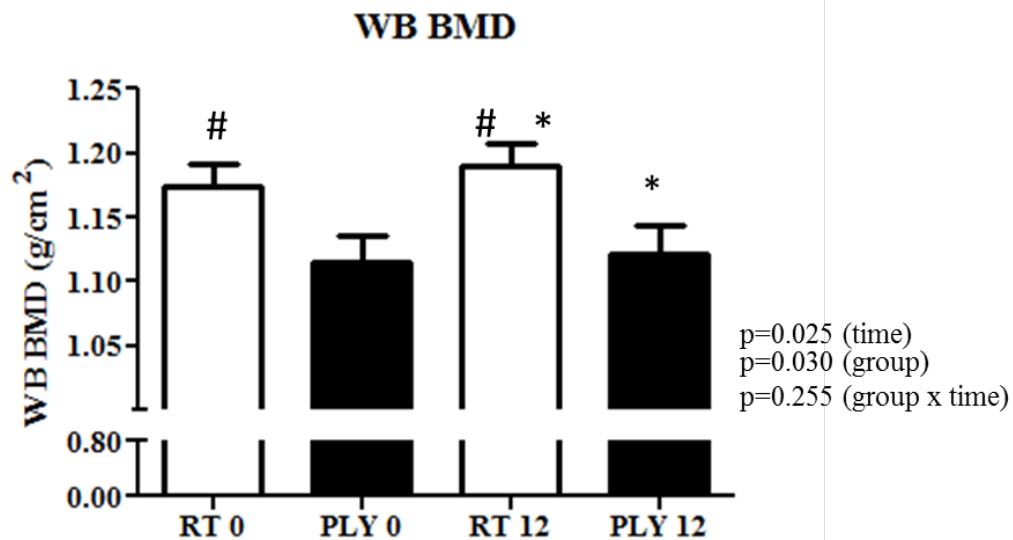
**BMD**

Subjects in both the RT and PLY groups experienced gains in WB BMD [main effect for time,  $p=0.025$ , Fig 2]. Similarly, both RT and PLY groups experienced significant increases in LS BMD during the 12-mo intervention [main effect for time,  $p=0.047$ , Fig 3]. The RT group significantly increased Hip BMD while the PLY group remained unchanged [group x time,  $p=0.068$ , Fig 4]. FN BMD did not change significantly in either group [Fig. 5].

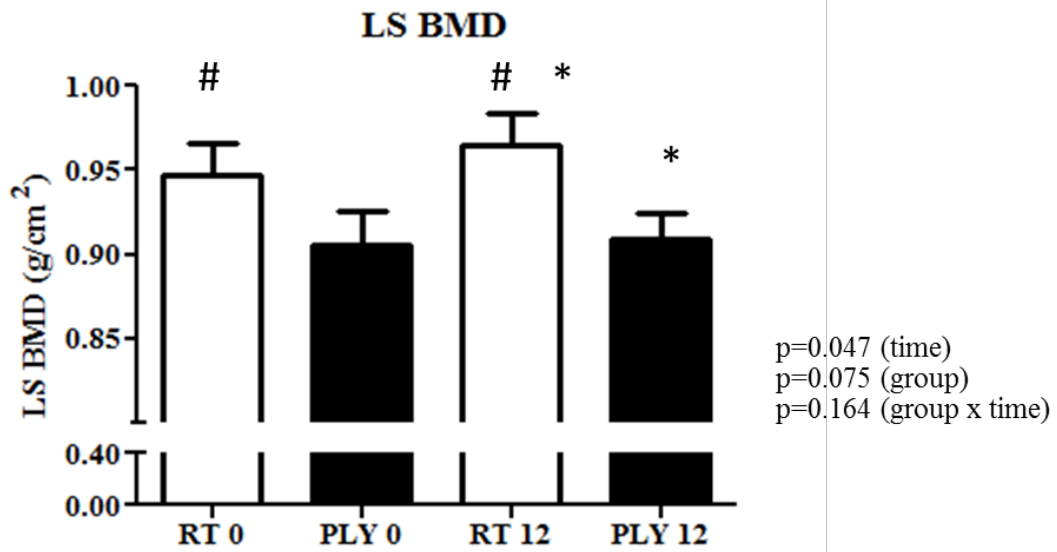
**Table 5.** Percent changes in BMD at various sites.

	<b>%Δ WB BMD</b>	<b>%Δ LS BMD</b>	<b>%Δ FN BMD</b>	<b>%Δ Hip BMD</b>
<b>RT</b>	$1.45 \pm 2.17^*$ (n=10)	$1.77 \pm 2.45^*$ (n=10)	$0.26 \pm 3.23$ (n=9)	$1.46 \pm 2.59^*$ (n=9)
<b>PLY</b>	$0.50 \pm 1.41^*$ (n=10)	$0.46 \pm 2.10^*$ (n=10)	$0.52 \pm 3.10$ (n=10)	$-0.17 \pm 2.71$ (n=10)

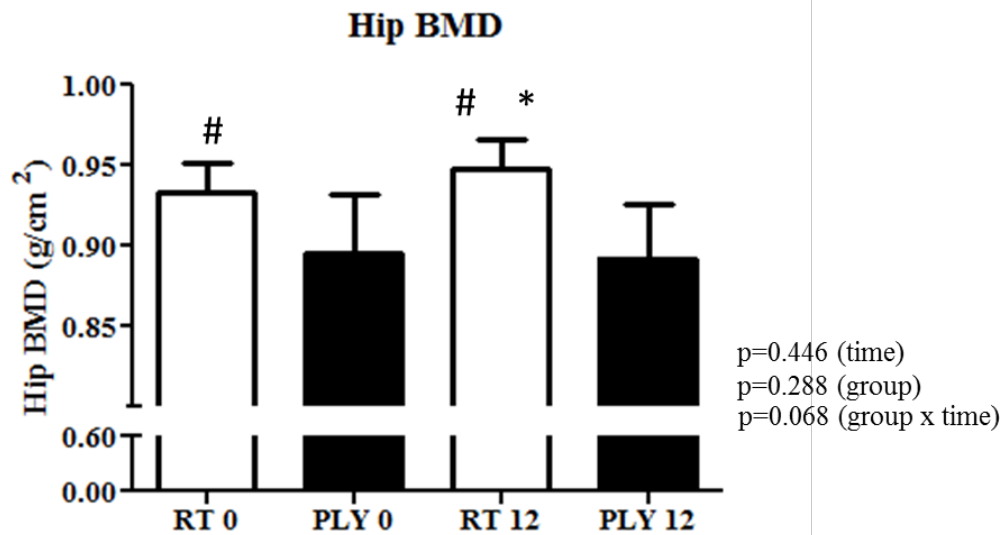
\*Significant change from baseline,  $p \leq 0.05$ . Data are means + S.E.



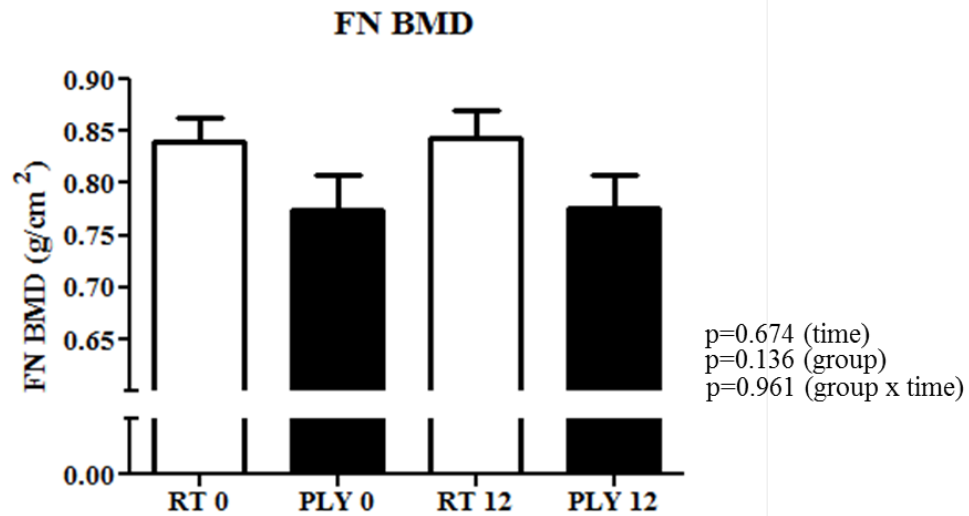
**Fig 2 -** \* Significantly different from 0-mo within treatment group. # Significantly different from PLY within time point,  $p \leq 0.05$ . Data are means  $\pm$  S.E.



**Fig 3** –\* Significantly different from 0-mo within treatment group. # Significantly different from PLY within time point,  $p \leq 0.05$ . Data are means  $\pm$  S.E.



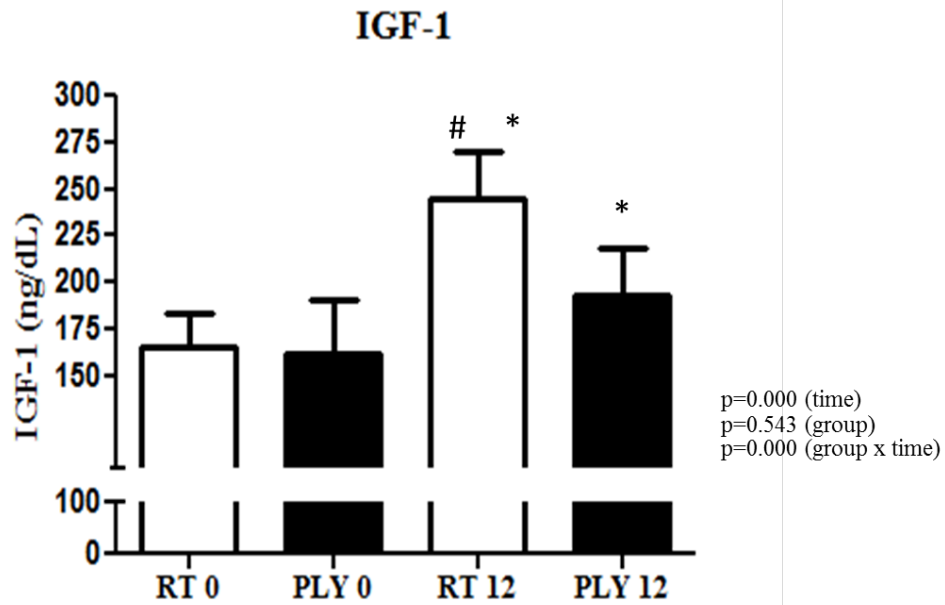
**Fig 4** –\* Significantly different from 0-mo within treatment group. # Significantly different from PLY within time point,  $p \leq 0.05$ . Data are means  $\pm$  S.E.



**Fig 5 –\* Significantly different from 0-mo within treatment group. # Significantly different from PLY within time point,  $p \leq 0.05$ . Data are means  $\pm$  S.E.**

### ***IGF-1***

Baseline IGF-1 values for both groups were within the normal range based on gender and age [110-230 ng/dL] (58). Both groups significantly increased IGF-1 over the 12-mo intervention, and the RT group increased more than the PLY group [group x time,  $p < 0.001$ , Fig 6]. Specifically, the RT and PLY groups experienced a 49% and 10% increase in IGF-1 concentration, respectively.



**Fig 6** –\* Significantly different from 0-mo within treatment group. # Significantly different from PLY within time point,  $p \leq 0.05$ . Data are means  $\pm$  S.D.

*Relationships between changes in IGF-1 and changes in BMD at various sites*

There were no significant correlations between percent change in IGF-1 and percent changes in BMD in either group at any of the investigated sites [Table 6, Fig 7-10].

**Table 6. Relationship between percent change in IGF-1 and percent change in BMD at various sites.**

		% $\Delta$ WB BMD	% $\Delta$ LS BMD	% $\Delta$ FN BMD	% $\Delta$ Hip BMD
% $\Delta$ IGF-1	RT	$r = 0.433$ $p = 0.105$ ( $n = 10$ )	$r = -0.012$ $p = 0.487$ ( $n = 10$ )	$r = -0.518$ $p = 0.077$ ( $n = 9$ )	$r = 0.144$ $p = 0.356$ ( $n = 9$ )
	PLY	$r = -0.14$ $p = 0.35$ ( $n = 10$ )	$r = -0.213$ $p = 0.278$ ( $n = 10$ )	$r = -0.412$ $p = 0.088$ ( $n = 10$ )	$r = -0.75$ $p = 0.149$ ( $n = 10$ )

WB=whole body, LS=lumbar spine, FN=femoral neck, BMD=bone mineral density. No significant correlations were found [one-tailed,  $p \leq 0.05$ ].

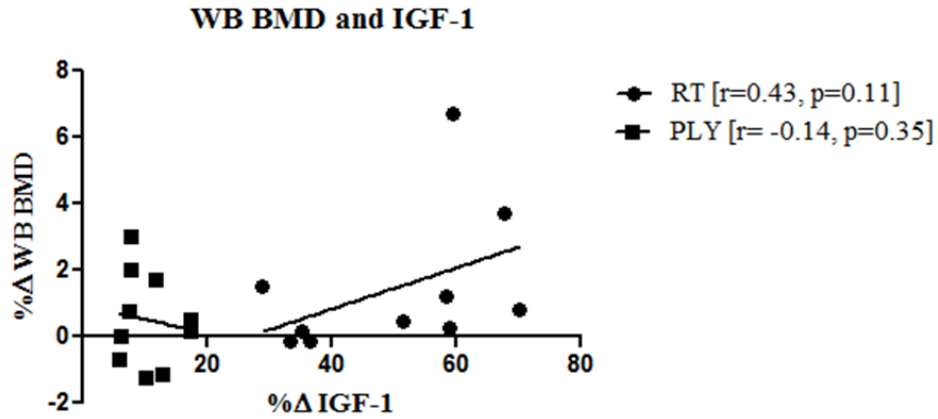


Fig 7 – Scatter plot of percent change in IGF-1 and percent change in whole body BMD.

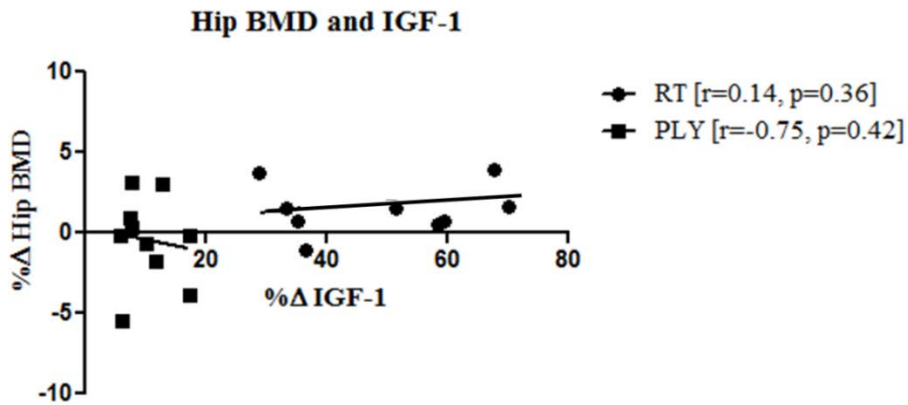


Fig 8 – Scatter plot of percent change in IGF-1 and percent change in Hip BMD.

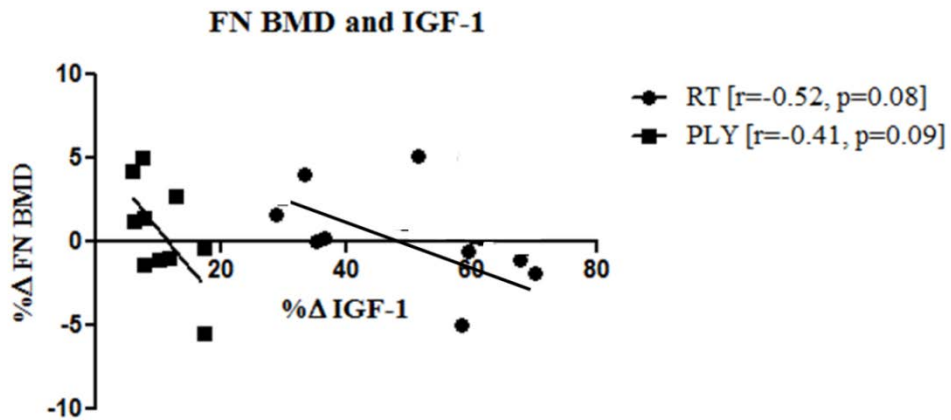
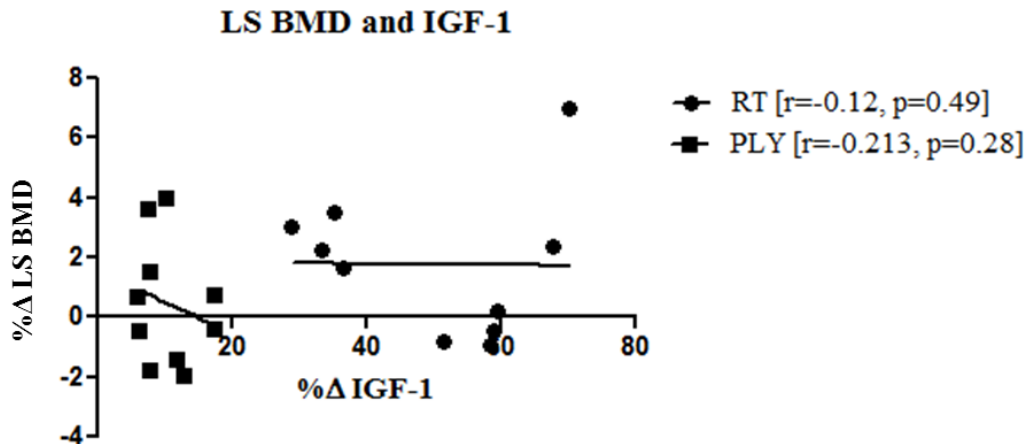


Fig 9 – Scatter plot of percent change in IGF-1 and percent change in femoral neck BMD.





**Fig 10 – Scatter plot of percent change in IGF-1 and percent change in lumbar spine BMD.**

## DISCUSSION

The present study investigated the relationship between changes in IGF-1 and changes in lumbar spine [LS BMD], hip [Hip BMD], whole body [WB BMD], and femoral neck [FN BMD] bone mineral density during a 12-mo exercise intervention in men with osteopenia. Our hypothesis was that changes in IGF-1 would be correlated with changes in BMD in the resistance training [RT] group, but not the plyometric training [PLY] group. This hypothesis was based on the influence of increased IGF-1 concentration on bone metabolism reported in both human and animal literature, the hormonal response to RT that has been demonstrated in the literature, and the dissimilarities between PLY and RT exercise that suggest the two modes should have dissimilar hormonal responses.

The first specific aim was to determine if RT and/or PLY would be effective at improving BMD of the whole body, lumbar spine, femoral neck, and hip of osteopenic men. We hypothesized that both RT and PLY would result in either increased or

maintained BMD at all of the aforementioned sites. Indeed, BMD was either increased or maintained at all sites in both groups [Fig 2-5]. Specifically, the RT group significantly increased BMD at the WB, LS, and Hip by 1.45%, 1.77%, and 1.46%, respectively while the PLY group significantly increased BMD at the WB and LS, by 0.50% and 0.46%, respectively, but not the Hip [Fig 2-5, Table 5]. Robling et al previously reported that a 5% increase in BMD resulted in nearly a 64% increase in bone strength and 94% increase in force absorbance prior to fracture. Thus, minimal changes in BMD can result in substantial improvements in bone strength. Other studies that have examined the effects of RT on BMD in men have reported mixed results (25, 62, 144, 237). Specifically, Menkes et al reported that 12 weeks of RT improved BMD of the LS and FN by 2.0% and 3.8%, respectively (144). Similarly, Braith et al reported that a 6-mo RT intervention in recent heart transplant patients restored BMD of the WB, FN, and LS to within 1.0%, 1.9%, and 3.6% of pre-operation values, respectively (25). Other studies have reported no effect of RT interventions up to 12 mos in length on BMD in either young or old men (62, 237). However, changes in BMD are typically not detectable for up to 12 mo due to the length of the bone remodeling process (35). Thus, some of the aforementioned studies may not have been long enough to observe valid changes in BMD. Additionally, Tsuzuku et al suggested that powerlifters, who regularly perform squats and deadlifts with near maximal loads, likely have increased BMD compared to matched controls due to the use of exercises that not only challenge the spine and hip with high-intensity muscle contractions, but directly compress these tissues with heavy loads (214). Thus, the aforementioned studies which reported no change in BMD with RT in men may have been flawed in program design and exercise selection. Data from the present study

support the existing literature that suggest that a properly designed RT program may be a valuable tool in the treatment and or/prevention of osteopenia in men.

Additionally, we found that subjects in the PLY group either maintained or increased BMD at all sites [Fig 2-5, Table 5]. The present study is the first to examine a PLY intervention in men, and the first to optimize loading parameters such as volume, intensity, and rest periods to induce osteogenesis in any population. Currently, the ACSM recommends RT as the main mode of exercise to improve and/or maintain BMD in adulthood (1). However, our data suggest that PLY may be an effective, time-efficient alternative to RT for bone health. This is the first study to show that exercise interventions specifically designed to be osteogenic can significantly improve BMD in men with osteopenia.

While the focus of this study was the use of an exercise intervention to treat osteopenia, pharmaceutical options are more common in practice. Bisphosphonates are an anti-resorptive drug often prescribed for treatment of osteoporosis. Studies have reported increases of hip, WB, and FN BMD by as much as 2.5%, 5.9%, and 2.6%, respectively over the course of 6-12mo (129, 171). However, these drugs are commonly associated with an increased occurrence of spiral fractures (194). These atypical fractures can be especially debilitating and painful (194). Thus, exercise may be a cost-effective and safer alternative to pharmaceutical therapies.

In the present study, only participants in the RT group experienced gains in Hip BMD. This apparent disparity of exercise effects may be due to the nature in which the skeleton is loaded during each mode of exercise. The main mechanisms by which PLY is

thought to exert strain on bone is through the high ground reaction forces [GRFs] induced during the landing phase of a jump, and the powerful muscle contraction involved in the initiation of the jump (170, 209, 241). Similarly RT, specifically exercises that axially load the skeleton, is thought to exert two types of strain on the bone: compressive forces via external load, and high muscle forces during muscle contraction. The main difference between RT and PLY exercise is the timing of the administration of these forces. In PLY, the bone experiences powerful muscle contractions, followed by compressive forces via the GRFs incurred upon landing. In RT, the bone experiences the compressive and muscle contractile forces simultaneously. Thus, it may be that the hip is strained to a greater extent, or more effectively during RT than PLY due to the simultaneous administration of compressive forces and powerful muscle contractions via the muscles that act at the hip. Indeed, both retrospective and longitudinal studies of RT in men and women have suggested that the combination compressive forces and muscle contractions may explain the site-specific adaptations of bone to RT (25, 144, 174, 214). We did find that changes in vertical jump max were significantly correlated with changes in Hip BMD, while changes in 1-RM in either the squat or deadlift were not significantly correlated to changes in BMD at any site [**Appendix N**]. Thus, it is possible that PLY may only be adequate to produce significant changes in Hip BMD so long as the training results in an increase in vertical jump height, while the effects of RT on Hip BMD may not be dependent on increases in strength. However, the contributions of these different forces to changes in bone metabolism have not been established previously, and cannot be elucidated from the present study. Further investigation is warranted.

Additionally, a portion of the subjects increased or maintained BMD at certain sites while others did not. This phenomenon has been described in other exercise studies, where researchers have defined these groups as “responders” and “non-responders (155).” In RT, baseline satellite cell number has been shown to predict the extent of adaptations to RT in men (162). We found that those subjects who had higher BMD at either the LS or Hip in the PLY group tended to experience less of a change in BMD at the LS and FN [**Appendices L and M**], suggesting that baseline BMD may predict changes in BMD in response to osteogenic exercise. These data are the first to suggest that having a higher BMD may lessen the osteogenic response to exercise in osteopenic men.

The second specific aim was to determine if IGF-1 concentrations would increase with participation in PLY or RT. We hypothesized that IGF-1 concentration would increase only in the RT group. However, we reported that IGF-1 concentration increased in both the RT and PLY groups, although the change was significantly higher in the RT group [Fig 6]. Mean IGF-1 concentrations increased from 165 ng/mL to 245 ng/mL in the RT group, and from 176 ng/mL to 193 ng/mL in the PLY group, equating to an approximate 49% change in the RT group, and 10% increase in the PLY group. These changes in the RT group are similar to that reported by Borst et al, who reported 40% increases in IGF-1 in men following 25 wks of RT (24). This is the first study to investigate the response of IGF-1 to chronic PLY. Our hypothesis was based on the idea that given the relative dissimilarities between PLY and exercise that has been shown in the literature to increase IGF-1 concentration, we would not see significant increases in IGF-1 in the PLY group. However, there is very little evidence in the literature of the

hormonal response to PLY, and those studies that have included PLY have often done so as part of a combined treatment or with different loading parameters that could cause a difference in the hormonal response when compared to the PLY intervention used in the present study (18, 70). The RT group also experienced significant increases in IGF-1, and this change was significantly greater than that in the PLY group [Fig 6]. This data is in agreement with current literature in both men and women that suggests that basal concentration of IGF-1 are significantly increased with chronic training (24, 111, 137, 183). This study is the first longitudinal study to show that RT can increase basal IGF-1 concentration in osteopenic men during a 12-mo exercise intervention.

The third and final specific aim was to determine if changes in BMD of the whole body, lumbar spine, femoral neck, and hip would be related to changes in IGF-1. We hypothesized that changes in IGF-1 concentration would be positively correlated with changes in BMD in the RT group, but not in the PLY group. However, we found no significant relationship between changes in IGF-1 and changes in BMD at any site in either group [Table 6, Fig 7-10]. This suggests that IGF-1 alone may not be a primary mediator of the effects of RT or PLY on BMD in osteopenic men. Indeed, RT typically results in a milieu of hormonal changes, including increases in other anabolic hormones such as testosterone, which may co-mediate the effects of RT on bone (114). In the present study, only concentrations of total IGF-1 were measured. Only IGF-1 that is not bound to binding proteins, also known as free IGF-1, can have a physiologic effect on target tissues (100). As previously mentioned, the hormonal response to PLY exercise in men is not well documented. Studies that have included combinations of high-power exercise and RT have reported an additional benefit of the high-power exercise on

testosterone responses to an acute exercise bout (18). However, the effects of exclusive PLY exercise training on hormone concentrations has not been investigated.

Several IGF-binding proteins have been identified in humans, with the most prominent being IGFBP3 (100). Studies of the IGFBP3 response to training are variable (24, 111). Koziris et al reported that elevations in IGF-1 concentration following four months of training in college swimmers were accompanied by equivalent increases in IGFBP3, and that the ratio of total IGF-1/IGFBP3, and indicator of free IGF-1 concentration, was unchanged (111). In contrast, Borst et al reported that IGFBP3 concentration decreased by 20% in men following 25 wks of RT involving a 3-set-per-exercise protocol, and no change in IGFBP3 in men following the same duration of a 1-set-per-exercise protocol (24). These changes in IGFBP3 were accompanied by increases in IGF-1 in both groups of men, resulting in an increase in the total IGF-1/IGFBP3 ratio (24). Data from the animal literature suggest that increased IGFBP3 concentration can disrupt the normal association between IGF-1 concentrations and bone (167, 195). Thus, it is possible that increases in IGF-1 concentration were accompanied by increases in concentration of IGFBP3 in the present study that resulted in very little actual physiologic effect of the increased circulating IGF-1 on bone.

Additionally, while the majority of circulating IGF-1 and IGFBPs are produced in the liver, they are also produced in muscle and bone and serve as an autocrine and paracrine messenger in these tissues (98). Two isoforms of IGF-I that are expressed and released from skeletal muscle cells have recently been identified (76, 140, 244). One of them, IGF-IEa, is very similar to the isoform produced by the liver, and thus, may have an endocrine function. This isoform is expressed both in working [exercised] and

nonworking [non-exercised] muscle (140). The other isoform, mechano-growth factor, or IGF-IEc, is expressed primarily in working muscle. Studies suggest that this isoform has an autocrine and paracrine function, stimulating myofibrillar protein synthesis and satellite cell activation in response to an acute bout of mechanical loading (4, 12, 66, 76). Additionally, it is known that bone cells produce IGF-1 and various IGFBPs, including IGFBP-5 (98). In-vitro data suggest that IGFBP-5 may associate with the cell surface of bones, thereby localizing bound IGF-1 to the bone, and potentiating the effects of IGF-1 on bone rather than inhibiting it (147). Thus, it is possible that exercise stimulates increases in local production of IGF-1 and beneficial IGFBPs such as IGFBP-5 that help mediate exercise induced changes in bone metabolism. However, tissue-born IGF-1 measurements cannot be obtained from blood analysis, and require tissue samples (98). Due to the invasiveness and ethical concerns of a bone biopsy in an osteopenic population, tissue-specific measures of IGF-1 were not obtained in the present study. Indeed, no studies to date have investigated the effects of exercise on local IGF-1 production in bone in humans. However, both in vitro and in vivo data suggest that mechanical loading can increase IGF-1 production in bone cells (172, 213, 242). Thus, it is possible that IGF-1 acts mainly through paracrine and autocrine, rather than endocrine pathways to influence bone metabolism in response to exercise stimulus, and that changes in these tissue-born IGF-1 concentration are simply not measurable via blood analysis.

Furthermore, exercise may act to induce osteogenesis through the mechanical strain which it places on the bone via both muscle contraction and compressive forces (214). As previously mentioned, both RT and PLY are thought to impose two separate



modes of mechanical strain on bone: shear force via muscle contraction, and compressive force via the external load supported by the lifter (59, 61, 214). Thus, other factors, both hormonal and non-hormonal, may be more important mediators of the effects of RT and PLY on BMD in osteopenic men. Further research should elucidate the precise mechanisms by which these modes of exercise induce an osteogenic effect.

The present study had several strengths. This study was the first to investigate the effects of a 12-mo osteogenic exercise intervention on changes in BMD in osteopenic men, filling an important void in the literature surrounding the under-appreciated problem of bone health in men. At the time of publication, this is also the first study to compare the effects of RT and PLY on BMD in any population, and the first to relate these changes to changes in IGF-1, a potentially important mediator of the anabolic effects of exercise. The 12-mo duration of the study allowed adequate time for changes in BMD to be observed via DXA. Throughout the 12-mo duration, subjects were provided daily calcium/Vitamin D supplements in order to control for low dietary intake of both of these critical bone-health nutrients. Additionally, both exercise interventions were specifically designed for the purpose of loading the skeleton and inducing osteogenesis, using data from *in vivo* and *ex vivo* animal studies in order to determine parameters such as rest intervals, volume, and intensity. Furthermore, all exercise sessions were supervised and recorded by trained study personnel to assure consistency and completion. Compliance to the aforementioned protocols was excellent [Table 4].

The present study also had several limitations. A lack of a control group made it impossible to compare changes in BMD in exercise groups to non-exercise groups. A control group was not included because the authors did not find it ethical to randomize

subjects into a group that would be required to do nothing about a very serious health issue for a year. However, our lab previously reported that BMD tends to decrease at weight-bearing sites over 3 years in adult, osteopenic men who are not performing bone-loading exercise (238). Additionally, at the time of data analysis, only 20 subjects had completed the 12-mo protocol. However, the specificity of the population, and 12-mo duration of the study made recruiting difficult. Also, this study is merely a preliminary assessment of a larger study in which 40 subjects [20 RT, 20 PLY] will eventually complete the 12-mo protocol. Finally, measurement of only total IGF-1, not free IGF-1 or IGFBP3, makes it difficult to draw conclusions on the importance of changes in IGF-1 on the osteogenic effect of exercise.

In summary, the present study is the first to suggest that a 12-mo intervention of either PLY or RT may be effective at increasing or maintaining BMD in osteopenic men. Additionally, IGF-1 may not be an important mediator of the effects of either RT or PLY on BMD in osteopenic men. This may have important relevance to the clinician or exercise professional who works with osteopenic men. Specifically, this study suggests that the inclusion of high-load, weight bearing exercise in a fitness program may help alleviate the economic and social burden of osteopenia and osteoporosis by improving or maintaining BMD in high risk individuals. However, the results of the present study suggest that changes in circulating IGF-1 concentrations are not related to changes in BMD during a 12-mo exercise intervention in men with osteopenia.

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## **OSTEOPOROSIS: A MEN'S HEALTH ISSUE**

Bone health is a critical aspect of men's health that is often overlooked. Osteopenia and osteoporosis are often thought to be health issues that only occur in women. However, recent literature suggests that the prevalence of osteoporosis in men is significant (104, 154). In the United States alone, more than 2 million men are osteoporotic and 12 million more have osteopenia (154). Additionally, research suggests that low bone mass is underdiagnosed in men, suggesting that these figures are underreported (2). Perhaps the most alarming figure is that the 12-month mortality rate in men following a hip fracture is 15-32% greater than in women (104). Furthermore, it is estimated that number of men and women with low bone mass will exceed 61 million by 2020 if nothing is done to attenuate this health issue (104).

### **Economic and Social Burden**

The increased prevalence of low bone mass in men has led to a heightened concern about the incidence of osteoporotic fracture in men. Research suggests that the number of men who suffer an osteoporotic hip fracture worldwide may reach 1.8 million by 2050 (104). Research also suggests that around 75% of all hip and 80% of all spine fractures in men can be attributed to low BMD (143). The health care cost of treating these factors is alarming: around \$18 billion, including the loss of productivity. In addition to the economic burden, those who suffer fractures also experience significant reductions in emotional and physical health, social well-being, and life expectancy. Furthermore, men who suffer an osteoporotic fracture are at an increased risk for future fractures (135, 142). Given these facts, there is little debate to whether or not this health issue demands significantly more attention in men than has previously been given.

## **Pathology**

Men develop two types of osteoporosis: idiopathic and secondary osteoporosis.

Idiopathic osteoporosis has no known causes, although it is hypothesized that genetic predisposition may play a major role (157). Secondary osteoporosis is that which is attributable to other behaviors and lifestyle choices, such as smoking, weight loss/low body mass, excessive drinking, sedentary lifestyle, hypogonadism, and glucocorticoid excess/treatment (80, 93, 157, 190).

## **Treatment in Men**

Although pharmaceutical treatment options are readily available for osteoporosis, the rate of use of them in men who have suffered an osteoporotic fracture is less than that in women, despite the approval of bisphosphonate alendronate treatment in men for over a decade (54, 109, 158). Indeed, Feldstein et al reported that only approximately 7% of men aged 65 years and older were prescribed a medication for osteoporosis during the 18-month period following an osteoporosis-related fracture (53). Instead, men are more likely to be prescribed a supplementation of vitamin D and calcium (109). However, bisphosphonate treatment has been shown to have both short-term and long-term adverse effects. Documented short-term effects of bisphosphonate treatment include nausea, abdominal pain, gastritis, fever, myalgia, and arthralgia lasting up 48hrs following administration (103). The most widely-reported long-term effect of bisphosphonate treatment is osteonecrosis of the jaw (141, 185). This effect is purported to take place due to the interference of normal bone turnover through the osteoclast inhibition caused by bisphosphonate treatment (5). While research has only reported osteonecrosis of the jaw, it is possible that long term bisphosphonate treatment could impact the whole

skeleton in similar fashion. Thus, physicians should use caution when prescribing bisphosphonates to patients who are not in immediate need of therapy, and alternative approaches such as weight bearing exercise should be considered.

## **BONE BIOLOGY**

### **Structure**

Two types of bone can be distinguished in the human skeleton: cortical bone and trabecular bone. Cortical bone is very dense and protective, providing structural integrity to the skeleton. Trabecular, or “spongy”, bone is much more porous and cancellous in nature. Both types of bone are integral to the function of the skeleton in the human body, which is comprised of approximately 80% cortical and 20% trabecular bone. Most bones in the body are made up of both types of bone, with cortical bone comprising mainly the outer surface of the bone, and trabecular bone comprising the center of the bone.

However, individual bones differ in their composition. For example, the vertebrae exhibit much higher proportions of trabecular bone in comparison to the long bones, such as the femur. The skeleton is covered by a thin layer of tissue that is very descriptively known as the periosteum, which houses blood vessels, nerve endings, and the main components of the bone remodeling cycle: osteoblasts and osteoclasts. Also, a tissue known as endosteum lines the inside of the long bones of the skeleton. This tissue lines the cavity in which bone marrow is stored (44).

### **Remodeling Cycle**

The Process of bone modeling during childhood and young adulthood allows for the accrual of bone mass and strength, with peak bone mass accrual occurring at roughly 30 years of age. This process involves alterations in the shape and size of bone, as opposed

to the composition of the bone. In adulthood, this process occurs mainly in response to injury or severe trauma (191). The remodeling cycle continues through the remainder of life, and allows for the repair and maintenance of bone through adulthood (44). The process of remodeling occurs via the coordinated actions of osteoclasts and osteoblasts, which break down and build bone, respectively. This process serves to alter the density of existing bone structure so as to strengthen areas that are subject to the most strain (191). The remodeling cycle is a lengthy process, often taking as long as 6 months to observe detectable changes via Dual-Energy X-ray Absorptiometry [DXA] (157).

The remodeling cycle is a coupled system, such that bones constantly being removed via resorption and replaced via formation. This coupling is referred to as bone turnover. The bone turnover process is responsive to various physiological stimuli, including electrical, mechanical, and endocrine signals (131). When these processes are in equilibrium such that formation equals resorption, bone turnover is considered balanced. However, when the system is pushed in either direction by any of the aforementioned stimuli, bone loss or bone gain can occur (44).

At the center of the remodeling cycle is the basic-multicellular unit, or the BMU. A BMU is made up of osteoclasts and osteoblasts, vascular supply, innervation, and connective tissue. These parts work in coordination to break down and/or build up bone as is determined by the physiological and mechanical signals that influence bone (160). A healthy adult has anywhere from 3 to 4 million BMUs initiated per year, and each BMU lasts about 6 to 9 months (160). BMUs exhibit different functionality in either cortical or trabecular bone. In cortical bone, BMUs travel through the center of the bone mass as if through a tunnel. In trabecular bone, BMUs travel along the surface of the

bone mass as if through a trench (160). In either case, the BMUs act to break down and build up bone through the remodeling cycle as they travel throughout the tissue. Due to the manner in which BMUs travel through bone, high concentrations of BMUs in the bone can actually reduce the structural integrity of the bone. Thus, high levels of bone turnover, no matter the balance as described previously, can be detrimental to bone health.

As previously mentioned, the remodeling cycle is a lengthy process. This process occurs in four distinct phases: activation, resorption, reversal, and formation (44). The activation phase is the beginning of the cycle, during which pre-osteoclasts attach themselves to the area of bone that is to be remodeled. These cells eventually become mature osteoclasts, whereupon they begin breaking down the bone matrix via enzyme and acid secretions (160). This begins the resorption phase, which lasts approximately 4 to 8 weeks in any one BMU (44). The resorption phase concludes as the reversal phase begins, during which various growth factors secreted by the osteoclasts attract pre-osteoblasts to the remodeling site. These cells become mature osteoblasts, and begin the formation phase, whereupon the mature osteoblasts synthesize new bone matrix to replace that which was removed during the resorption phase (44). At the conclusion of the formation phase of the remodeling cycle, some osteoblasts may still remain, which become embedded in the bone matrix as osteocytes (44).

### **Characteristics of Osteogenic Exercise**

The idea that bone adapts to mechanical stress was first postulated by Wolff, who simply stated that bone remodels in proportion to the load placed upon it (59). This was refined by Frost in his mechanostat theory (59). The mechanostat involves

mechanosensory cells within the bone that detect and respond to mechanical strain (61). This theory states that bone responds to strain within a normal physiological range by remodeling so as to strengthen the area of bone that has been strained (59, 61). At the cellular level, stress is experienced via a shift in extracellular fluid around bone cells. This fluid shift leads causes a rapid influx of calcium through gap junctions, which is thought to communicate information regarding modeling and remodeling between osteocytes and osteoblasts (34, 121). Additionally, animal research suggests that this load must be dynamic in nature and of sufficient but not excess volume, intensity, and frequency (122, 182, 216, 218, 220).

### *Volume*

Volume refers the number of loading cycles incurred in a single bout of mechanical loading [i.e. number of repetitions per training session]. A loading cycle is the process in which a mechanical stress causes a fluid shift through the bone, increasing intracellular calcium flux, after which the fluid resets (121). Lanyon et al provided evidence that as few as 36 loading cycles per day was as effective as 1,800 loading cycles at inducing bone formation (122). This is in agreement with research from Umemura et al who reported that rats who jumped 5 times per day experienced similar increases in bone density and strength to rats who jumped 100 times per day (220). Turner and colleagues hypothesized that bone may become desensitized to the mechanical stimulus above a certain threshold (218).

### *Intensity*

As it relates to bone, intensity refers to both the magnitude and rate of strain imposed on the skeleton. In his mechanostat theory, Frost states that in order for a mechanical stress

to induce changes in bone metabolism, the strain must exceed a certain minimum threshold (61). He suggests that strain magnitude at or above 1500-3000 microstrain are required to cause an increase in osteogenesis (61). Indeed, Mosely et al reported that in vivo loading of male Sprague-Dawley rats at strain magnitudes higher than those experienced in everyday living resulted in increased ulnar BMD compared to rats who did not experience the additional loading (150). The in-vivo nature of this study allowed the animals to experience normal loading in between cycles of the additional loading, suggesting that the increases in BMD were the result of the bouts of increased magnitude loading. In addition to magnitude, rate of strain is an important determinant of the effect of mechanical loading on bone. Turner et al reported that rat ulnas exposed to external loading at frequencies of 0.5-Hz and 2.0-Hz experienced significant increases in bone formation rates, but that frequencies above this did not result in an increased effect (218). Furthermore, the authors reported that bone formation rates were directly related to strain rates, such that the highest strain rates were associated with the greatest changes in bone formation, as measured via histomorphologic analysis of the tibia (217).

### *Frequency*

Frequency refers to how many bouts of loading the bone experiences over a given period of time [i.e. number of training sessions per week]. Robling et al studied the effects of 360 loading cycles, divided evenly over 1, 2, 4, or 6 bouts per day (175). The authors reported that the animals receiving the 360 loading cycles divided over 4 or 6 bouts per day experienced greater bone formation rates than when the loading was either divided over 2 bouts or given in a single bout (175). The authors concluded that the same volume of mechanical stress divided into discrete loading bouts may be more effective at

inducing osteogenesis (175). Using a similar model, the same authors studied the effects of different recovery periods between loading bouts on the osteogenic response to mechanical loading (176). They concluded that full mechanosensitivity is recovered following 8hrs, but not 1hr after a loading bout (176). These data suggest that dividing exercise into smaller bouts separated by approximately 8 hrs may be more effective than the same volume of exercise performed in one continuous bout.

### *Rest Periods*

The inclusion of rest periods in between individual loading stimuli appears to also enhance the osteogenic response to mechanical loading. Srinivasan et al discovered that inserting a ten-second rest interval between individual loading cycles resulted in a greater rate of bone formation than loading cycles in which there was no rest period (202). This suggests that within a bout of exercise, small rest periods between repetitions may enhance the benefit of the exercise on osteogenesis.

### **Markers of Bone Formation and Resorption**

While measurement of BMD allows for a static assessment of bone health, markers of bone formation and resorption provide a method by which to assess acute changes in bone metabolism in response to various stimuli. Currently, these markers of bone turnover monitor the effectiveness of therapies in the short term, monitor pharmacological interventions, predict future bone loss, select patients for therapy, and possibly predict of fracture risk (181). However, concentrations of these markers in blood and/or urine typically differ depending on bone diseases, bed rest, malignancy, menstrual status, diurnal variations, and season (181).



**Formation Markers.** Serum markers of bone formation include bone-specific alkaline phosphatase [BAP], osteocalcin [OC], carboxyterminal propeptide of type I collagen [PICP], and aminoterminal propeptide of type I collagen [PINP]. While alkaline phosphatase is produced by many tissues in the body, bone-specific alkaline phosphatase, or BAP, has been shown to be a good marker of bone formation in humans (233). This is because BAP is produced by active osteoblasts and serves as a good indicator of osteoblast function and subsequent bone formation (13, 192). Studies have shown that deficiency in alkaline phosphatase results in osteomalacia, suggesting that BAP may be an integral part of the development of sufficient bone mass and structural integrity (212). OC is a small protein, rich in glutamic acid, and is referred to as a Gla-protein. OC is widely accepted as a serum marker of bone formation as it is released into the blood by osteoblasts during differentiation and bone formation, although its fragments are also released from the bone matrix during resorption (13, 181, 192). Additionally, procollagen extension peptides are byproducts of the synthesis of type I collagen and are useful serum measures of bone formation. Type I collagen is the major constituent of bone matrix; thus, increased concentrations of these peptides in blood or urine indicate increased bone formation. This includes both the amino- [PINP] and carboxy-[PICP] terminal procollagen 1 extension peptides (181).

**Resorption Markers.** Serum markers of bone resorption include total and free pyridinolines [Pyd], total and free deoxypyridinolines [Dpd], N-telopeptide of collagen crosslinks [NTx], C-telopeptide of collagen crosslinks [CTx], cross-linked C-telopeptide of type I collagen [ICTP], and tartrate-resistant acid phosphatase isoform 5b [TRAP5b] (181). Tartrate-resistant acid phosphatase has been used as a serum marker of bone

resorption. The 5-b isoform is released by osteoclasts during resorption, and is a good indicator of osteoclast number and activity (233). Pyr and Dpd are cross-links between type I collagen fibers and are released into the blood during breakdown of type I collagen in bone (233). NTx and CTx are fragments of type I collagen released during the breakdown of type I collagen in bone (233). Although collagen is a constituent of many tissues in the human body, the breakdown of type I collagen in bone appears to be the main contributor to blood concentrations of CTx (206).

***Effects of Exercise.*** There have been several studies examining the effects of short-term physical activity on bone turnover markers in men. However, there are far fewer data on the effects of chronic physical activity on these bone markers. The available literature has reported either an increase, or no change in bone formation markers in adult [age  $\geq$  18 yrs] men (62, 144, 186, 189, 230). Additionally, some studies have reported a reduction in biomarkers of bone resorption as a result of a long-term weight-bearing exercise intervention (236).

***Clinical Relevance.*** While higher rates of bone turnover are associated with greater and more rapid bone loss, screening individuals for osteoporosis or osteopenia by biochemical markers of bone turnover alone has been fairly unsuccessful, with research reporting conflicting results. The Rotterdam Study reported that women with increased urinary concentrations of Dpd, a bone resorption marker, had an increased risk of suffering a hip fracture (223). Similarly, Dresner-Pollak et al. reported that in elderly women, bone loss of the hip was significantly negatively correlated with markers of bone turnover including urinary NTx, free Pyr, total Pyr, total Dpd, hydroxyproline, serum OC

and BAP (47). However, Keen et al. reported no correlation between bone turnover markers and future bone loss (101).

An additional use of the aforementioned bone markers is to monitor treatment efficacy during interventions designed to improve bone health. Measuring markers of bone formation and resorption throughout an osteogenic intervention may provide more immediate feedback as to the effectiveness of the intervention than a DXA scan, which usually does not detect changes in BMD for approximately 6mo from beginning the treatment (134). A shift in these bone markers towards elevated formation and reduced resorption may indicate that the treatment is effective long before changes can be seen in BMD via DXA (134). Collectively, these data suggest that while biochemical markers of bone turnover provide a relatively easy, non-invasive means to track treatment of osteoporosis in the short term, the ability to predict bone loss and fracture risk is controversial.

### **BASIC ENDOCRINOLOGY OF BONE**

Bone turnover and metabolism is affected by a myriad of hormones.

Additionally, exercise is known to influence the concentrations and actions of many of the same hormones. Thus, to better understand the potential effects of exercise on bone, it is helpful to know the effects and mechanisms of these hormones. The hormones reviewed in the following section were chosen based on their direct effects on the remodeling cycle, and/or their responsiveness to exercise.

#### **Parathyroid Hormone [PTH]**

PTH plays a major role in calcium homeostasis in adult humans (26). In the presence of low calcium, PTH is secreted from the chief cells of the parathyroid gland in order to re-

establish normal levels of calcium. It does this by increasing calcium absorption from the gut, reducing calcium excretion from the kidneys, and releasing calcium from bone (26). The effects of PTH on the bone are mediated by increases in osteoclast activity so as to cause a shift in the balance of bone metabolism towards resorption.

The primary mechanism by which PTH causes a shift in bone metabolism is through its inhibition of osteoprotegerin [OPG] (26). OPG is secreted by osteoblasts, and exhibits an inhibitory effect on osteoclasts by competitively binding RANKL, a ligand that interacts with the RANK receptor on osteoclasts. PTH serves to inhibit OPG expression in osteoblasts, thus disrupting the ability of osteoblasts to inhibit osteoclastogenesis (26). This may suggest that PTH is catabolic to bone. And indeed, individuals with chronically elevated levels of PTH exhibit below average levels of bone mass and increased fracture risk (71, 107, 240). However, researchers have found that the duration of exposure to PTH determines whether the hormone elicits an anabolic or catabolic effect on bone (115). Miki et al administered weekly injections of a PTH synthetic to osteoporotic women, and found increases in lumbar spine BMD following 6 and 12 month treatments (146). Furthermore, Drake et al administered either PTH or no treatment daily to a group of postmenopausal women (46). After only 14 days, the women who had been treated with PTH saw robust increases in markers of bone formation, including OC (46). Additionally, both continuous and intermittent PTH exposure are associated with increases in markers of bone turnover (71, 83, 222). Thus, in humans, PTH appears to have deleterious effects on bone with continuous exposure, and beneficial effects with intermittent exposure, and these effects appear to be dependent on a shift in the bone remodeling cycle towards resorption or formation.

Researchers further investigated these discrepant findings in humans with in vitro studies to determine how either intermittent or prolonged exposure to PTH influences the bone remodeling cycle. Ishizuya et al exposed rat osteoblasts to either intermittent or continuous PTH treatment for 21 days (87). The cells exposed to intermittent treatment exhibited increases in BAP activity and expression, as well as osteocalcin mRNA compared with vehicle treated controls. The cells exposed to continuous treatment exhibited the opposite effects, which included suppression of BAP activity and expression, and reduced osteocalcin mRNA (87). The authors concluded that intermittent PTH exposure increases the differentiation and metabolic activity of osteoblasts, while continuous exposure decreases this differentiation and metabolic activity (87). Similarly, Locklin et al compared intermittent and continuous PTH treatment in cell cultures of mouse bone marrow cells (130). They reported that four days of intermittent treatment increased osteoblast proliferation. Conversely, continuous treatment had no effect on osteoblast activity, increased mRNA levels of RANKL, which promotes osteoclast proliferation, and decreased mature osteoclast apoptosis (130). Additionally, Jilka et al reported that intermittent treatment with PTH decreased osteoblast apoptosis compared to a vehicle treated control in mice (89). These studies collectively suggest that PTH has varying effects on bone metabolism, based largely on the dose and timecourse of treatment. The mechanism by which the effects of PTH vary is still not clearly understood, and further research is necessary in order to elucidate such mechanisms.

***Effects of exercise.*** PTH has been reported to increase acutely following either endurance training or RT (179, 180). This increase in PTH in response to an exercise stimulus is due to the reduction in serum calcium concentrations following repeated

muscle contractions, where it acts to increase calcium resorption from the skeleton and maintain calcium homeostasis (15). Rogers et al reported that a single bout of RT or PLY resulted in decreases in PTH that coincided with increases in markers of bone formation and decreases in markers of bone resorption, suggesting that changes in PTH following exercise may mediate changes in bone metabolism (179). No long term training studies have investigate this relationship. However, given the aforementioned evidence suggesting that increases in endogenous PTH production are beneficial to bone, it is possible that PTH fluctuations following exercise are a mediator of the beneficial effect of exercise on bone.

### **Thyroid Hormone [T3]**

The importance of T3 in bone health stems from research on hypo- and hyperthyroidism. Hypothyroidism in adults results in reduced bone turnover with impaired osteoclastic bone resorption and osteoblastic bone formation (52, 149). This increased duration of the bone remodeling cycle results in a prolonged period of secondary mineralization, reducing the structural quality of the bone (52). Indeed, population studies indicate that men with hypothyroidism are at increased risk for fracture (225, 227). Additionally, hyperthyroidism also appears to have deleterious effects on bone. In contrast to hypothyroidism, hyperthyroidism results in increased bone turnover. This results in a loss of BMD due to increased cortical bone porosity and accelerated bone loss (51, 118, 149, 226). Large cohort studies have indicated similar increased fracture risk in men with hyperthyroidism as those with hypothyroidism (149, 225, 227). However, the effects of T3 on bone are not limited to pathophysiological conditions. Roef et al reported that in men aged 25-45yrs, T3 levels in the highest quartile of normal were

associated with reduced BMD at the whole body, lumbar spine, and hip compared to men in the lower quartiles (178). These data suggest that even small fluctuations in T3 status in men may have significant consequences on bone health.

*Effects of Exercise.* The response of T3 to exercise remains a topic of debate, with studies reporting inconclusive and conflicting results (72, 73, 82, 90, 193).

Variations in responses are likely due to a lack of control for other factors that affect T3 status, such as emotional and environmental stress, energy balance, and training status (73). Thus, it is unclear as to the importance of T3 in regards to mediation of exercise effects on bone.

### **Glucocorticoids**

Cortisol is an endogenous glucocorticoid released by the adrenal glands in response to stress and/or low blood glucocorticoid levels. The main function of cortisol is to increase the breakdown of proteins to provide the necessary components for gluconeogenesis, primarily through the breakdown of collagen, one of the main constituents of bone matrix (159). Cortisol directly affects bone metabolism by inhibiting the differentiation and formation of osteoblasts and apoptosis of mature osteoblasts, resulting in a shift towards bone resorption (33, 161). Additionally, cortisol acts to reduce calcium absorption in the intestines (127). Thus, chronically elevated cortisol in response to chronic stress may be detrimental to bone health (159). This is evidenced in the well-documented effects of exogenous glucocorticoid treatment on bone mass. Corticosteroids are used to treat a variety of common ailments, such as rheumatoid arthritis, inflammatory bowel diseases, and chronic obstructive pulmonary disease. Additionally, endogenous corticosteroids are an important part of the natural immune response to physiologic and metabolic stress.

However, glucocorticoid treatment has deleterious effects on bone (27). Additionally, most of these underlying diseases already have deleterious effects on bone separate from the effects of the treatments (27).

The mechanism by which glucocorticoids affect bone formation is three-fold: reduced osteoblast activity, apoptosis of mature osteoblasts and osteocytes, and reduced secretion of type-I collagen (27, 29, 45, 235). Glucocorticoid treatment results in differentiation of pre-osteoblasts into adipocytes instead of osteoblasts, resulting in reduced osteoblastic activity and impaired structural integrity (45, 235). Glucocorticoids also act to promote osteoclastogenesis via stimulation of the RANK receptor on osteoclasts. This increase in osteoclasts and decrease in osteoblasts leads to a pronounced shift in bone metabolism in favor of resorption (235). Indeed, a number of researchers have reported deleterious effects of glucocorticoid treatment on bone (63, 94, 116). Fujita et al reported an increase in markers of resorption, decrease in markers of formation, and a decrease in BMD in kidney patients who were treated with prednisone for three months (63). Kanis et al performed a meta-analysis of several prospective studies involving glucocorticoid use and found that prior or current use resulted in a nearly 50% higher fracture risk than those who had never used glucocorticoids. Research from Laan et al supports these findings with reports of nearly complete recovery of BMD after within 20 weeks following glucocorticoid use (116).

Collectively, glucocorticoid treatment appears to negatively affect bone by simultaneously inhibiting bone formation and promoting bone resorption (27). These effects on bone are so deleterious that they have led to the simultaneous use of pharmacological agents that prevent bone loss with glucocorticoid treatment.



## **Sex Steroid Hormones**

Both estrogens and androgens play a major role in bone health. Specifically, in both men and women, androgens and estrogens have been shown to inhibit bone resorption and stimulate bone formation by inhibiting osteoclast activity and stimulating osteoclast apoptosis (224). In women, the loss of bone mass associated with menopause is thought to occur due to loss of endogenous estrogen production (181). Bone cells exhibit nearly equal expression of both testosterone and estrogen receptors in the plasma membrane and express the aromatase enzyme and 5  $\alpha$  reductase (181). These enzymes are responsible for the conversion of testosterone to either estradiol or dihydroxytestosterone, respectively.

**Androgens.** Both dihydrotestosterone and testosterone appear to stimulate proliferation of osteoblast precursors, as well as increase the differentiation of mature osteoblasts (84, 97). Additionally, dihydrotestosterone appears to inhibit bone resorption through osteoclast activity by binding directly to the androgen receptor on osteoclasts and inhibiting RANK-L mediated osteoclastogenesis (3, 86). The idea that androgens play an important role in the maintenance of skeletal health in humans is rooted in the observation that hypogonadal men have reduced BMD compared to men with normal gonadal function (57). Additionally, men with androgen resistance also display reduced BMD compared to age-matched, normal men, suggesting that this discrepancy in bone mass is not entirely due to a simultaneous reduction in estrogens associated with hypogonadism (20). However, it is still unclear as to whether the primary in vivo effects of androgens on bone health are through direct mechanisms, or through the conversion to estradiol via the aromatase enzyme (36).

*Estrogens.* In women, the loss of estrogen production during menopause is thought to be the major contributor to the subsequent increased prevalence of osteoporosis in post-menopausal women (163). However, defining the exact role of estrogen in bone health has proven problematic (163). While estrogen is not commonly thought of as an important metabolic mediator in men, its importance cannot be overlooked. Recent evidence suggests that one of the main roles of estrogen in the maintenance of bone metabolism is through the alpha-estrogen receptor [ER-alpha]. Variations in the ER-alpha gene in men have been associated with lower BMD, increased risk of fracture, increased bone loss, and decreased responsiveness to exercise (69, 108, 126, 139, 173, 205). These data suggest that estrogen and ER-alpha may play an important role in the maintenance of bone health and the mediation of the effects of exercise on osteogenesis in men.

Additionally, epidemiological data have consistently suggested a relationship between low concentrations of estradiol and low BMD in men, while a similar relationship between androgens and BMD in men has not been consistently shown (7, 106, 169, 198, 208). Case studies of men with genetic defects that result in low concentrations of estrogen receptors report abnormally tall stature and low BMD, despite normal circulating levels of androgens and estrogens (199). Similarly, defects in expression of the aromatase enzyme also result in low BMD in men. Treatment of these men with testosterone had no effect on BMD (30). However, when these men were treated with estradiol, epiphyseal growth plate closure occurred and BMD improved (22, 30, 246). Thus, despite the fact that anabolism in most other tissues is mediated by androgens,

estrogens play a very important role in the building and maintenance of bone mass in men.

*Effects of Exercise.* It is well documented that exercise, specifically RT, can have a significant effect on testosterone levels in men (41, 74, 113). Kraemer et al studied the effects of two different RT protocols in younger [18-22yrs] and older [40+] men (113). One protocol involved 3 min rest periods and 5RM loads, while the other involved 1min rest periods and 10RM loads. The authors reported increases in testosterone following both protocols (113). Some studies suggest that differences in program variables such as volume, intensity, and rest periods can affect the changes in testosterone following RT. Hakkinen et al reported that 10 sets of 10 repetitions at 70% of 1RM, but not 20 sets of 1 at 97% 1RM, increased testosterone in elderly men (75). Similarly, Crewther et al reported that testosterone concentrations increased in response to an RT bout involving moderate loads [75%], but not heavy loads [88%] in young male weight lifters (41). However, no research to date has examined the relationship between changes in testosterone and changes in bone density following an exercise program.

### **Leptin**

Leptin is a cytokine primarily produced in adipose tissue. The primary function of leptin is as a short-term signal of energy status. Serum concentrations of leptin increase as food intake increases and signal the hypothalamus to reduce appetite and increase energy expenditure in order to maintain homeostasis, and vice-versa (77). Additionally, leptin appears to have both direct and indirect effects on bone metabolism (77). Studies of the direct effects of leptin on bone metabolism have reported conflicting results. Holloway et al reported that in vitro administration of leptin to mesenchymal stem cells stimulated

production of osteoprotegerin and inhibited RANK ligand secretion, and thus, osteoclastogenesis (85). However, Martin et al reported that higher doses of leptin led to increased bone resorption and decreased bone formation in rats (136). It is important to note that adipose tissue located within the bone also produces and secretes leptin (117). Thus, it has been postulated that high levels of skeletal adiposity may lead to extremely high concentrations of leptin within the bone, and that this may have deleterious effects on bone, while increased circulating leptin from other adipose tissue may have beneficial effects (77). Further research is necessary to elucidate the mechanisms by which leptin seems to have conflicting effects on bone metabolism.

As previously mentioned, leptin may also exert an effect on bone metabolism via an indirect mechanism. Ducy et al suggested that leptin inhibits osteogenesis by binding to the leptin receptor in the hypothalamus, which stimulates the release of noradrenaline from nerve fibers projecting into bone (48). This noradrenaline then inhibits bone formation by binding to the beta-2 adrenergic receptors on osteoblasts (48). However, the effects of central leptin appear to be specific to the type of bone. Research in Ob/Ob mice suggests that leptin has deleterious effects on trabecular bone and beneficial effects on cortical bone, which may be a useful adaptation to increase bone strength in response to increases in body mass (78). Thus, Hamrick et al postulated that sympathetic signaling through beta-1, beta-2, and perhaps other receptor sub-types, could have different, possibly opposite, effects on the various bone compartments (77). Indeed, research in beta-1 and beta-2 double KO mice suggests that while stimulation of beta2-adrenergic receptors on osteoblasts triggers RANKL-mediated osteoclastogenesis, prompting trabecular bone loss, systemic beta1-adrenergic receptor-mediated activity could enhance

levels and/or activity of the GH-IGF1 axis, thereby contributing to the maintenance of cortical bone mass (16).

the aforementioned mechanistic research, studies of the relationship between serum leptin levels and BMD have reported mixed results. Obesity is associated with elevated serum leptin concentrations as well as increased bone size and enhanced cortical bone mineral density (125), suggesting a potential regulation of bone mass by leptin. Indeed, studies in women have reported a positive relationship between serum leptin and BMD (23, 211). However, in young men, there appears to be a negative association between serum leptin levels and BMD that is not seen in older men (132, 148). The exact mechanism by which leptin induces its effects on bone metabolism is yet to be determined. However, research in mice suggests that leptin may be a negative regulator of the effects of exercise on osteogenesis (95). These results collectively suggest that in humans, the effects of leptin may be specific to gender, age, and weight status.

***Effects of Exercise.*** The effect of exercise on leptin levels in men is not well documented. Of the available studies, most have suggested that both acute and chronic exercise has little effect on serum leptin levels in men (9, 70, 92). Thus, while leptin levels may be related to bone health, there is not enough evidence to suggest that leptin is an important mediator of the effects of exercise on bone.

APPENDIX A—Study Flyer



**WEIGHT BEARING EXERCISE  
AND BONE HEALTH**



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**The Department of Nutrition and Exercise Physiology  
is seeking individuals for a study on how different  
types of weight-bearing exercise affect bone health.**

**We are looking for:**

- **Healthy men, 25-60 years old**
- **Currently participating in 4hr/wk of physical activity for the last 2 years**
- **Not currently participating in strength training (weight- lifting) or plyometrics (high-intensity jumping)**

**You will receive:**

- **free and supervised weight-lifting or plyometric training**
- **body composition testing**
- **bone mineral density assessment**
- **\$1000 compensation after completing the study**

**Contact: N & EP Bone Study  
Dept. of Nutrition and Exercise Physiology  
106 McKee Gym, 882-9917  
Email: [mubonestudy@gmail.com](mailto:mubonestudy@gmail.com)**

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**APPENDIX B—Consent Form**

**CONSENT FORM TO PARTICIPATE IN A RESEARCH STUDY**

**INVESTIGATOR’S NAME: PAMELA S. HINTON PH.D.**

**PROJECT # 1095877**

**DATE OF PROJECT APPROVAL: SEPTEMBER 12, 2007**

<b>FOR HS IRB USE ONLY</b>
<b>APPROVED</b>
    <hr/>
_____ HS IRB Authorized Representative Date
<b>EXPIRATION DATE:</b>  <hr/>

**STUDY TITLE: EFFICACY OF PLYOMETRICS TO INCREASE BONE MASS IN MALES WITH LOW BONE MINERAL DENSITY**

**INTRODUCTION**

**This consent may contain words that you do not understand. Please ask the investigator or the study staff to explain any words or information that you do not clearly understand.**

This is a research study. Research studies include only people who choose to participate. As a study participant you have the right to know about the procedures that will be used in this research study so that you can make the decision whether or not to participate. The information presented here is simply an effort to make you better informed so that you may give or withhold your consent to participate in this research study.

Please take your time to make your decision and discuss it with your family and friends.

You are being asked to take part in this study because you are a healthy male who participates in leisure time physical activity.

This study is being sponsored by the Department of Nutrition and Exercise Physiology, University of Missouri-Columbia.

In order to participate in this study, it will be necessary to give your written consent.

#### **WHY IS THIS STUDY BEING DONE?**

The purpose of this research is to determine how effective long term (12 months) jump training (plyometrics) is at improving bone density and increasing hormones that promote bone formation, as compared to long-term resistance training. This research is being done because the long-term benefits of regular plyometric exercise or resistance training on bone health in males with below normal bone density are unclear.

#### **HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?**

About 250 people will take part in this study at the University of Missouri-Columbia.

#### **WHAT IS INVOLVED IN THE STUDY?**

**Visit 1:** Begin the informed consent process and describe the study purpose and the requirements.



All participants must: 1) be males between 25 and 60 years of age who participate exclusively in leisure time physical activity at least 4 hours per week for the past 24 months; 2) be apparently healthy; 3) be physically able to perform plyometrics or resistance training; 4) be willing to keep daily records of physical activity and food intake; 5) be willing and able to provide accurate information about your medical history; 6) follow a normal sleep/wake cycle; and 7) be willing to take a calcium and vitamin D supplement daily.

All participants must not: 1) smoke, or have quit smoking within the last 6 months; 2) drink excessive amounts of alcohol (more than 3 drinks per day); 3) take medication that affects bone; 4) have a disease that affects bone; or 5) participate regularly in plyometrics or resistance training.

**Visit 2:** If you decide to participate in the study you will come back for Visit 2 and sign the consent form. Then you will undergo a dual X-ray absorptiometry (DXA) bone density test. You will be required to lie still for approximately 10 minutes during this procedure. You will be exposed to a small amount of radiation during the scan, equivalent to 1/10<sup>th</sup> the radiation of a chest X-ray and about 1/1000 of a similar Computed Tomography scan. All study participants will undergo additional bone density tests at 6 and 12 months. **It is important to note that in order to be eligible to participate in this study, the DXA scan must indicate that you have below normal bone mineral density.** You will be provided with the results of your bone mineral density test. If you have any questions about the results you will need to contact your family practitioner. Interpretation of the results of your bone mineral density test must be performed by a physician.

You will also fill out a medical and physical activity history questionnaire. You must provide information about your medical history, including history of illness, injuries, and drug treatment that may affect your ability to safely and effectively participate in the study. You also must provide accurate information about your physical activity history.

If you meet the eligibility requirements of the study (i.e., age, activity level, no diseases or medications that affect bone, below normal bone mineral density), you will be provided a 7-Day diet record form to record your dietary intake and return at the next visit. You will also be given a form to record your physical training for 7 days.

**Visit 3:** You will have your blood drawn on five occasions during the study (0, 3, 6, 9, 12 months). Following an 8-12 hour fast, your height and weight will be measured and a blood sample will be taken from a vein in your forearm using the same procedure as would be followed at a health clinic. On three of these occasions (0, 6, and 12 months) additional blood samples (3) will be collected during the 24 hours after your normally scheduled training. The amount of the blood sample is very small and will not affect your health (15 mL, 1 tablespoon). The blood will be used to measure markers of bone formation and breakdown and hormone levels. Your blood will be analyzed for factors that may affect your bone mass.

Your blood will be kept frozen for 5 years after the study is completed and the results are published in a research journal. No additional tests will be performed on your blood sample.

The study will require regular visits to the Exercise Physiology Laboratory, each visit lasting 30-90 minutes during the course of the exercise intervention. On several occasions (0, 6, and 12 months) during your normally scheduled training we will determine your feelings of pain, fatigue and exertion using surveys to help determine your experience with the training program and monitor your risk for pain and/or injury.

You will continue your normal exercise program throughout the study and you will maintain your normal life at home, work or school. You are allowed to quit at any time without penalty or loss of any benefits. You will be asked to discontinue the study if the research and medical staff determine it is in your best interest to do so.

You will be “randomized” into one of the study groups described below. Randomization means that you are put into a group by chance. It is like flipping a coin. Neither you nor the researcher will choose what group you will be in. You will have an equal chance of being placed in either group.

**Interventions:** All exercise training sessions will be conducted at the McKee Gym Fitness Center, under the supervision of trained exercise personnel.

**Group 1: If you are participating in the plyometric intervention you will attend 3 training sessions per week until you complete the 12-month exercise intervention.**

Participants will complete 10 repetitions of 10 different exercises to accumulate 40-120 loading cycles (jumps). The plyometric exercise sets will include: squat jumps, forward hops, split squat jumps, lateral box push offs, bounding, bounding with rings (lateral), box drill with rings, lateral hurdle jumps, zigzag hops, single leg lateral hops, and progressive depth jumps (10-100cm). The intensity of plyometric training will progress, with low intensity jumps weeks 1-2, low and moderate jumps weeks 3-4, and high intensity jumps weeks 5-6, followed by a rest week. You will steadily increase the intensity and number of jumps over each training cycle.

**Group 2: If you are participating in the resistance training intervention you will attend 2 training sessions per week until you complete the 12-month exercise intervention.**

Each exercise session will be made up from the following resistance exercises: squats, bent over row, dead lift, military press, lunges, and calf raises. Prior to and every 6 weeks during the RET intervention, maximal strength testing will be performed. This will involve a warm-up set of 5-10 repetitions, equal to 40-60% of your perceived maximum for each exercise. After a brief rest period, a second set of 3-5 repetitions at an intensity between 60-80% of perceived maximum will be performed. Subsequent attempts will be conducted using incremental increases in weight until a failed attempt, typically within 3 to 5 maximal attempts. One repetition maximums (1RM) will be conducted for squat, dead lift, and military press exercises, and modified maximums (10 repetitions) will be calculated for exercises in which 1RM are not commonly performed.

To account for strength adaptations as a result of strength training improvements, a progressive exercise program will be used. Weeks 1-2 will include one warm-up set (10 repetitions at 20% 1RM) and 3 moderate intensity sets (10 repetitions at 50% 1RM) for each exercise performed. Weeks 3-4 will be comprised of one warm-up set (10 repetitions at 20% 1RM), two sets at a moderate intensity (10 repetitions at 60% 1RM), and one set at high intensity (6-8 repetitions at 70-75% 1RM). Weeks 5-6 will be comprised of one warm-up set (10 repetitions at 20% 1RM), two sets at moderate

intensity (10 repetitions at 60% 1RM), and one set at high intensity (3-5 repetitions at 80-90% 1RM). Week 7 will be a rest week.

### **HOW LONG WILL I BE IN THE STUDY?**

Completion of all exercise training and testing procedures will take approximately 12 months.

You can stop participating at any time. Your decision to withdraw from the study will not affect in any way your medical care and/or benefits.

### **WHAT ARE THE RISKS OF THE STUDY?**

While on the study, you are at risk for the side effects described below. You should discuss these with the investigator and/or your doctor. There may also be other side effects that we cannot predict.

Risks and side effects related to the study tests and procedures include:

There is a possibility of bruising and soreness at the site of the blood draw. Sterile procedures will be used so the chance of getting an infection is very remote.

There is a possibility of muscle and joint injury as a result of participating in the weight lifting exercises of the resistance training and the jumping of the plyometric training. Participants will be instructed in the safe and proper procedures for all exercise activities by qualified exercise physiologists and supervised by exercise personnel at all times. All exercise sessions will include warm-up and cool-down procedures to further minimize the risk of injury.

**Reproductive risks: The effects of the DXA scan on the male reproductive system are unknown but could cause harm. If you have any questions about the reproductive issues, please discuss them with the investigator or your doctor.**

You will be exposed to a small amount of radiation. Radiation effects are cumulative. You should always inform future doctors of your participation in this study.

For the reasons stated above the investigator will observe you closely during the study described above and, if you have any worrisome symptoms, notify the investigator immediately. Dr. Pam Hinton's telephone number is (573) 882-4137. For more information about risks and side effects, ask the investigator or contact Dr. Hinton at (573) 882-4137.

### **ARE THERE BENEFITS TO TAKING PART IN THE STUDY?**

If you agree to take part in this study, there may or may not be direct medical benefit to you. You may expect to benefit from taking part in this research to the extent that you are contributing to medical knowledge. We hope the information learned from this study will allow for more specific exercise prescriptions for men with low bone mineral density.

In addition, you will: 1) participate in a supervised exercise program; 2) potentially improve your bone mass, strength, and balance; 3) receive free bone mineral density screening and results; 4) receive free diet and physical activity analyses; 5) receive free calcium and vitamin D supplements; and 5) have free parking and access to the McKee Gym locker room and showers during exercise sessions.

### **WHAT OTHER OPTIONS ARE THERE?**

You have the option to not participate in this study.

### **WHAT ABOUT CONFIDENTIALITY?**

Information will be stored in the investigator's file and identified by a code number only. The code key connecting your name to specific information about you will be kept in a separate, secure location. Information contained in your records may not be given to anyone unaffiliated with the study personnel at the University of Missouri-Columbia in a form that could identify you without your written consent, except as required by law. If the investigator conducting this study is not your primary, or regular doctor, she must obtain your permission before contacting your regular doctor for information about your past medical history or to inform them that you are in this study.

It is possible that your medical and/or research record, including sensitive information and/or identifying information, may be inspected and/or copied by the study sponsor (and/or its agent), the Food and Drug Administration (FDA), federal or state government

agencies, University of Missouri Health Sciences Institutional Review Board or hospital accrediting agencies, in the course of carrying out their duties. If your record is inspected or copied by the study sponsor (and/or its agents), or by any of these agencies, the University of Missouri-Columbia will use reasonable efforts to protect your privacy and the confidentiality of your medical information.

The results of this study may be published in a medical book or journal or used for teaching purposes. However, your name or other identifying information will not be used in any publication or teaching materials without your specific permission.

### **WHAT ARE THE COSTS?**

There is no cost to you for the study procedures. You will not be charged for blood tests that are part of this research study.

### **WILL I BE PAID FOR PARTICIPATING IN THE STUDY?**

You will be compensated \$1000 for completion of the study. You will be paid \$300 for completion of the first six months of the study and an additional \$700 upon completion of the entire study.

### **WHAT IF I AM INJURED?**

It is not the policy of the University of Missouri to compensate human subjects in the event the research results in injury. The University of Missouri, in fulfilling its public responsibility, has provided medical, professional and general liability insurance coverage for any injury in the event such injury is caused by the negligence of the University of Missouri, its faculty and staff. The University of Missouri also will provide, within the limitations of the laws of the State of Missouri, facilities and medical attention to subjects who suffer injuries while participating in the research projects of the University of Missouri. In the event you have suffered injury as the result of participation in this research program, you are to contact the Risk Management Officer, telephone number (573) 882-1181, at the Health Sciences Center, who can review the matter and provide further information. This statement is not to be construed as an admission of liability.

### **WHAT ARE MY RIGHTS AS A PARTICIPANT?**

**Participation in this study is voluntary. You do not have to participate in this study. Your present or future care will not be affected should you choose not to participate.** If you decide to participate, you can change your mind and drop out of the study at any time without affecting your present or future care in the University of

Missouri-Columbia. Leaving the study will not result in any penalty or loss of benefits to which you are entitled. In addition, the investigator of this study may decide to end your participation in this study at any time after she has explained the reasons for doing so and has helped arrange for your continued care by your own doctor, if needed.

You will be informed of any significant new findings discovered during the course of this study that might influence your health, welfare, or willingness to continue participation in this study.

**WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?**

If you have any questions regarding your rights as a participant in this research and/or concerns about the study, or if you feel under any pressure to enroll or to continue to participate in this study, you may contact the University of Missouri Health Sciences Institutional Review Board (which is a group of people who review the research studies to protect participants' rights) at (573) 882-3181.

You may ask more questions about the study at any time. For questions about the study or a research-related injury, contact Dr. Pam Hinton at (573) 882-4137 or Dr. John Thyfault at (573) 882-9818.

A copy of this consent form will be given to you to keep.

**SIGNATURE**

I confirm that the purpose of the research, the study procedures, the possible risks and discomforts as well as potential benefits that I may experience have been explained to me. Alternatives to my participation in the study also have been discussed. I have read this consent form and my questions have been answered. My signature below indicates my willingness to participate in this study.

\_\_\_\_\_

Subject/Patient\*

\_\_\_\_\_

Date

\_\_\_\_\_

Legal Guardian/Advocate/Witness (if required)\*\*

\_\_\_\_\_

Date

\_\_\_\_\_

Additional Signature (if required) (identify relationship to subject)\*\*\*

\_\_\_\_\_

Date

\*A minor's signature on this line indicates his/her assent to participate in this study. A minor's signature is not required if he/she is under 7 years old. Use the "Legal Guardian/Advocate/Witness" line for the parent's signature, and you may use the "Additional Signature" line for the second parent's signature, if required.



\*\*The presence and signature of an impartial witness is required during the entire informed consent discussion if the patient or patient's legally authorized representative is unable to read.

\*\*\*The "Additional Signature" line may be used for the second parent's signature, if required. This line may also be used for any other signature which is required as per federal, state, local, sponsor and/or any other entity requirements.

"If required" means that the signature line is signed only if it is required as per federal, state, local, sponsor and/or any other entity requirements.

**SIGNATURE OF STUDY REPRESENTATIVE**

I have explained the purpose of the research, the study procedures, identifying those that are investigational, the possible risks and discomforts as well as potential benefits and have answered questions regarding the study to the best of my ability.

\_\_\_\_\_

Study Representative\*\*\*\*

\_\_\_\_\_

Date

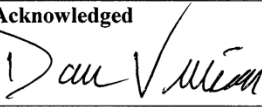

\*\*\*\*Study Representative is a person authorized to obtain consent. Per the policies of the University of Missouri Health Care, for any 'significant risk/treatment' study, the Study Representative must be a physician who is either the Principal or Co-Investigator. If the study is deemed either 'significant risk/non-treatment' or 'minimal risk,' the Study Representative may be a non-physician study investigator.

## APPENDIX C- HIPAA

UNIVERSITY OF MISSOURI-COLUMBIA  
Institutional Review Board

### HIPAA AUTHORIZATION FORM

**Authorization for the Use and Disclosure of Personal Health Information  
Resulting from Participation in a Research Study**

<b>FOR IRB USE ONLY</b>	
<b>Acknowledged</b>	
	
IRB Authorized Representative	Date

**Principal Investigator's Name:** Dr Hinton

**Project #** 1095877

**Project Title:** Efficacy of Plyometrics to Increase Bone Mass in Male Cyclists with Osteopenia

You have agreed to participate in the study mentioned above. This authorization form gives more detailed information about how your health information will be protected.

**1. Description of the information**

My authorization applies to the information described below. Only this information may be used and/or disclosed in accordance with this authorization:

Demographic and anthropomorphic information, results of bone density scan, blood tests, and medical history

**2. Who may use and/or disclose the information**

I authorize the following persons (or class of persons) to make the authorized use and disclosure of my PHI:

Principal investigator and graduate students research assistants.

**3. Who may receive the information**

I authorize the following persons (or class of persons) to receive my personal health information

University Missouri IRB, publications, research collaborators, and current and potential funding agencies

**4. Purpose of the use or disclosure**

My PHI will be used and/or disclosed upon request for the following purposes:

Publications and presentation that will not identify me, auditing, administrative and billing reviews, study outcomes including safety and efficacy

**5. Expiration date or event**

This authorization expires upon:

The following date: \_\_\_\_\_

End of research study

No expiration date

Other: \_\_\_\_\_

**6. Right to revoke authorization**

I understand that I have a right to revoke this authorization at any time. My revocation must be in writing in a letter sent to the Principal Investigator at 106 McKee Gym, University of Missouri, Columbia, MO 65211. I am

- aware that my revocation is not effective to the extent that the persons I have authorized to use and/or disclose my PHI have already acted in reliance upon this authorization.

**7. Statement that re-disclosures are no longer protected by the HIPAA Privacy Rule**

I understand that my personal health information will only be used as described in this authorization in relation to the research study. I am also aware that if I choose to share the information defined in this authorization to anyone not directly related to this research project, the law would no longer protect this information. In addition, I understand that if my personal health information is disclosed to someone who is not required to comply with privacy protections under the law, then such information may be re-disclosed and would no longer be protected.

**8. Right to refuse to sign authorization and ability to condition treatment, payment, enrollment or eligibility for benefits for research related treatment**

I understand that I have a right not to authorize the use and/or disclosure of my personal health information. In such a case I would choose not to sign this authorization document I understand I will not be able to participate in a research study if I do not do so. I also understand that treatment that is part of the research project will be conditioned upon my authorization for the use and/or disclosure of my personal health information to and for use by the research team.

**9. Suspension of right to access personal health information**

I agree that I will not have a right to access my personal health information obtained or created in the course of the research project until the end of the study.

**10.** If I have not already received a copy of the University of Missouri Healthcare Privacy Notice, I may request one. If I have any questions or concerns about my privacy rights I should contact, the HS Privacy Officer at 573-882-9054 or the Campus Privacy Officer at 573-882-7254.

**11. Individuals' signature and date**

I certify that I have received a copy of the authorization.

\_\_\_\_\_  
Signature of Research Participant \_\_\_\_\_  
Date

\_\_\_\_\_  
Research Participant's Legally Authorized Representative \_\_\_\_\_  
Date

\_\_\_\_\_  
Describe Representative Authority to Act for the Participant

**APPENDIX D - Physical Activity Readiness Questionnaire (PAR-Q)**

**Physical Activity Readiness Questionnaire**

Subject number \_\_\_\_\_

These questions ask about your readiness to participate in this research study's physical activity component. Please read each question carefully and answer each one honestly. Check **YES** or **NO**

YES

NO

1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?

2. Do you feel pain in your chest when you do physical activity?

3. In the past month, have you had chest pain when you were not doing physical activity?

4. Do you lose your balance because of dizziness or do you ever lose consciousness?

5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?

6. Do you have high blood pressure (systolic  $\geq 140$  mm Hg or diastolic  $\geq 90$  mm Hg)?

7. Is your doctor currently prescribing drugs (for example, water pills) for blood pressure or heart condition?

8. Do you know of any other reason why you should not do physical activity?

9. Do you have a family history of heart disease (for example, heart attack or sudden death) in first degree relative (male <55 years or female <65 years old)?

10. Currently a smoker or quit within previous 6 months?

11. Do you have high cholesterol? (Total cholesterol > 200 mg/dl, high-density lipoprotein cholesterol < 35 mg/dl, low-density lipoprotein > 130 mg/dl)

12. Do you have impaired fasting glucose? (for example  $\geq 110$  mg/dl)

\_\_\_\_\_ 13. During a typical week how many alcoholic beverages do you consume?

\_\_\_\_\_ 14. What is the greatest number of alcoholic beverages you may consume in a single day?

**APPENDIX E – Physical Activity History Questionnaire**

**Efficacy of Plyometrics to Increase Bone Mass in Men**

Medical and Physical Activity History Questionnaire

Subject number \_\_\_\_\_

Date \_\_\_\_\_

These questions ask about your medical and physical activity history. Please fill in the blank or circle the appropriate response.

1. Date of Birth: \_\_\_ / \_\_\_ / \_\_\_\_\_

2. Ethnicity:    Hispanic or Latino    Not Hispanic or Latino

3. Race:        African-American/Black

Alaskan Native

American Indian

Asian

Caucasian/White

Hawaiian or other Pacific Islander

Other: \_\_\_\_\_ (specify)

4. Do you regularly consume soy foods?    Yes    No

5. Do you currently take a calcium supplement?    Yes    No

What dose? \_\_\_\_\_mg

6. Are you currently taking any medications? Yes No

If so, which ones \_\_\_\_\_ (specify)

7. Are you currently taking any anti-inflammatory steroids? Yes No

If so, which ones? \_\_\_\_\_ (specify)

How long have you been taking them? \_\_\_\_\_ (specify)

8. Have you previously taken anti-inflammatory steroids? Yes No

If so, which ones? \_\_\_\_\_ (specify)

When and for how long? \_\_\_\_\_ (specify)

9. Do you have a family history of osteoporosis? Yes No

If so, please list affected family members, e.g., maternal grandmother.

\_\_\_\_\_  
—

10. Have you ever been diagnosed with a disease that affects bone (Cushing's disease, hyperthyroidism, leukemia, Crohn's disease, chronic liver disease, rheumatoid arthritis, etc)? Yes No

What was the diagnosis? \_\_\_\_\_

When was the diagnosis? \_\_\_\_\_

What is your current treatment? \_\_\_\_\_

11. Have you had any sports related fractures or stress fractures in the past 5 years?

Fracture: Yes No

Number \_\_\_\_\_

Location on body \_\_\_\_\_

\_\_\_\_\_

Year \_\_\_\_\_

Stress Fracture: Yes No

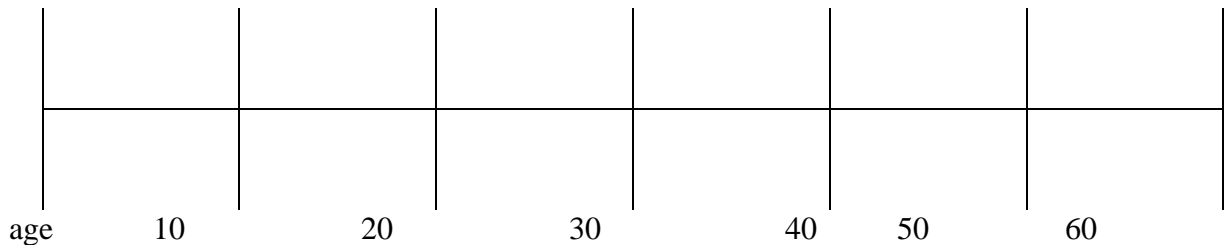
Number \_\_\_\_\_

Location on body \_\_\_\_\_

\_\_\_\_\_

Year \_\_\_\_\_

12. Please use the timeline below to indicate what leisure time physical activity and/or sports (include strength training) you participated in or played during your lifetime.





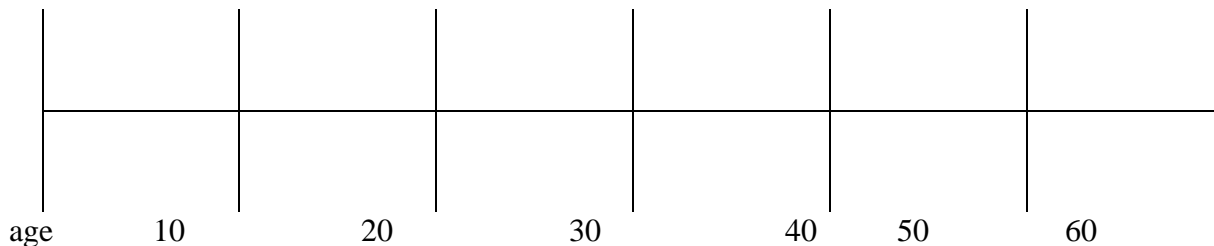
For each leisure time physical activity and/or sport listed, please describe approximately how many hours per week you participated or competed in this sport. If you competed, please indicate the level of competition.

Physical Activity or Sport	Ages	Hours per week	Weeks per year	Level of Competition

13. What leisure time physical activities and/or sports do you participate in now (include strength training)? How many hours per week do you train for or compete in this leisure time physical activity and/or sport? If you compete, please indicate the level of competition.

Physical Activity or Sport	Hours per week	Weeks per year	Level of Competition

14. Please use the timeline below to indicate job titles and physical activity you have had during your lifetime.



15. During the past 7 days, did you work for pay or as a volunteer (if yes, continue to questions 15-16)?      Yes      No

16. During the past 7 days, how many hours did you work for pay and/or as a volunteer?

\_\_\_\_\_ hours.

17. Which of the following categories best describes the amount of physical activity required on your job and/or volunteer work?

- a. Mainly sitting with slight arm movements. [Examples: office worker, watchmaker, seated assembly line worker, bus driver, etc.]
- b. Sitting or standing with some walking. [Examples: cashier, general office worker, light tool and machinery worker, etc.]
- c. Walking with some handling of materials generally weighing less than 50 pounds. [Examples: postal worker, waiter/waitress, construction worker, heavy tool and machinery worker, etc.]

- d. Walking and heavy manual work often requiring handling of materials weighing over 50 pounds. [Examples: lumberjack, stone mason, general laborer, etc]

18. Do you have a “normal” sleep pattern i.e., awake during the day, and asleep at night?      Yes      No

**APPENDIX F—Calcium-Focused Food Frequency Questionnaire**

**Food Frequency Questionnaire**

**INSTRUCTIONS:**

Think about **what you typically eat in a normal day**, including breakfast, lunch, dinner, and snacks.

For each item that you eat in the list of foods below, please enter the number of servings you usually eat each day.

Please enter servings in decimals, e.g., 1 serving or 2.5 servings.

<b>HC Foods</b>	<b>Serving Size</b>	<b>Number of Servings</b>
Nonfat or low-fat yogurt	1 cup (8 oz)	<input type="text"/>
Milk (whole, low-fat or nonfat)	1 cup (8 oz)	<input type="text"/>
Milkshake (any flavor)	1 cup (8 oz)	<input type="text"/>
Chocolate milk or hot chocolate (made with whole, low-fat or nonfat)	1 cup (8 oz)	<input type="text"/>
Cheese (Cheddar/Monterey Jack types)	1-½ oz.	<input type="text"/>
Processed cheeses (sliced American, string cheese)	1 item	<input type="text"/>
Soft cheeses (feta, camembert, brie)	1-½ oz.	<input type="text"/>
Ricotta cheese	½ cup	<input type="text"/>
Blended coffee drinks (e.g. lattes, mochas, made with milk)	1 - ½ cup	<input type="text"/>
Lasagna	1 large piece	<input type="text"/>
Enchilada, cheese	1 large	<input type="text"/>
Tofu processed with calcium	½ cup (4 oz)	<input type="text"/>

<b>MLC Foods</b>	<b>Serving Size</b>	<b>Number of Servings</b>
Custard or flan	½ cup (4 oz)	<input type="text"/>
Pudding	½ cup (4 oz)	<input type="text"/>
Frozen yogurt	½ cup (4 oz)	<input type="text"/>
Cottage cheese	1 cup (8 oz)	<input type="text"/>
Mustard greens, cooked	1 cup (8 oz)	<input type="text"/>
Bok choy, cooked	1 cup (8 oz)	<input type="text"/>

	oz)	
Canned fish with bones (salmon, mackerel)	2 oz.	<input type="checkbox"/>
Parmesan cheese	2 Tbsp.	<input type="checkbox"/>
Turnip greens, cooked	1 cup (8 oz)	<input type="checkbox"/>
Kale	1 cup (8 oz)	<input type="checkbox"/>
Ice milk (full fat, low-fat)	½ cup (4 oz)	<input type="checkbox"/>
Ice cream	½ cup (4 oz)	<input type="checkbox"/>
Almonds	¼ cup	<input type="checkbox"/>
Hot chocolate (made with packet)	1 cup (8 oz)	<input type="checkbox"/>
Broccoli	1 cup (8 oz)	<input type="checkbox"/>
Beans, refried beans or peas	1 cup (8 oz)	<input type="checkbox"/>
Corn tortillas	1 tortilla	<input type="checkbox"/>
Cream soup	1 cup (8 oz)	<input type="checkbox"/>
Sardines	1 3-inch sardine	<input type="checkbox"/>
Cream cheese	1 tablespoon	<input type="checkbox"/>
Spinach	1 cup (8 oz) fresh	<input type="checkbox"/>
Macaroni & Cheese	1 cup (8 oz)	<input type="checkbox"/>

CF Foods	Serving Size	Number of Servings
Calcium-fortified soy beverage	1 cup (8 oz)	<input type="checkbox"/>
Calcium-fortified orange juice	1 cup (8 oz)	<input type="checkbox"/>
Calcium-fortified frozen waffles	2 waffles	<input type="checkbox"/>
Calcium-fortified cereal (100 mg calcium/serving)	1 cup (8 oz)	<input type="checkbox"/>
Calcium-fortified energy bars	1 bar	<input type="checkbox"/>

## APPENDIX G - Diet Log

University of Missouri-Columbia Exercise Physiology Lab

Efficacy of Plyometrics to Increase Bone Mass in Men with Osteopenia

### 7-Day Dietary Record Instructions

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In order for us to assess your dietary habits, we need you to log your diet for seven consecutive days. Please be as accurate as possible. **Write down everything you eat, including snacks between meals, and drinks such as coffee, soft drinks, beer, wine and spirits.**

Below are some tips that will help you complete your diet record.

- 1) **Carry the log** with you as much as possible.
  
- 2) Write down **when** you ate.
  
- 3) Be **as specific as possible** about what you ate (e.g. include brand, restaurant), not just the type of food.
  
- 4) Write down the **exact amount** you ate using standard household measures (e.g. teaspoon, tablespoon, ounces, cups, number of slices, pieces).
  
- 5 ) Include **food preparation** techniques (e.g. baked, boiled, fried, sautéed, steamed).
  
- 6) Include any **condiments** you add (e.g. catsup, croutons, mayonnaise, mustard, onions, pickles).

- 7) Include any **side items** (e.g. French fries, vegetables, fruits, or salad).
  
- 8) Indicate if an item is low-fat, fat-free, etc.
  
- 9 ) Write down **everything** you eat, including snacks, nibbles, gum, and mints.
  
- 10) Record all **drinks/beverages**, other than water, **including alcohol**.
  
- 11) Record any nutritional and/or vitamin **supplements** (e.g. Powerbar, protein shakes, etc.).
  
- 12) Please keep your diet record up to date. Record immediately after each meal or snack.  
It is  
difficult to accurately remember what you ate if you wait until the end of the day to write it down.

Thank you, in advance, for your assistance in making this research study a success!

Please return the records to the lab at your earliest convenience.

ID #:		Date:	Day of week:		
Time of Day	Food/Drink	Brand	Amount (tsp, cup, oz)	Condiments	Location /Place
<b>BREAKFAST</b>					
<b>MORNING SNACK</b>					
<b>LUNCH</b>					
<b>AFTERNOON SNACK</b>					
<b>DINNER</b>					
<b>EVENING SNACK</b>					
<b>SUPPLEMENTS</b>					



**APPENDIX H—Physical Activity Log**



University of Missouri-Columbia

Exercise Physiology Lab



**Efficacy of plyometrics in male cyclists with osteopenia:**

*Physical Activity Log*

Subject # \_\_\_\_\_

Week \_\_\_\_\_

Dates \_\_\_/\_\_\_/\_\_\_ - \_\_\_/\_\_\_/\_\_\_

Weight (lbs) \_\_\_\_\_

Day	Date	Exercise Mode	Total Time (hrs:min)	Distance (miles/yards)	Average Pace (min/mile, mph, yds/min)	Max HR	Avg HR	Intensity (L, M, H)
Mon								
Tues								
Wed								
Thur								
Fri								
Sat								
Sun								

**APPENDIX I—Energy Intake and Energy Expenditure RMANOVA**

**Table 7.** Changes in energy intake and energy expenditure from 0-mo to 12-mo time points.

	PLY		RT		Time	Sig (p-value)	
	0-mo	12-mo	0-mo	12-mo		Group	Group x Time
Kilocalories (kcal)	2350 ± 201	2518 ± 234	2681 ± 295	2469 ± 215	0.99	0.22	0.64
Carbohydrates (g)	281 ± 30	302 ± 29	339 ± 28	311 ± 43	0.83	0.14	0.95
Protein (g)	99 ± 9	94 ± 7	107 ± 11	100 ± 8	0.47	0.16	0.96
Fat (g)	92 ± 8	98 ± 11	97 ± 16	88 ± 8	0.63	0.87	0.18
Calcium (mg)	1024 ± 142	969 ± 103	1168 ± 99	770 ± 91	0.06	0.71	0.32
Vitamin D (IU)	193 ± 50	134 ± 49	206 ± 66	222 ± 78	0.55	0.45	0.55

No significant differences from 0 to 12 months were observed in either group.

Data are means ± S.E.

**APPENDIX J – Covariate Matrix**

**Table 8.** Correlation matrix for covariates in RMANOVA analyses.

		<b>Δ IGF-1</b>	<b>Δ WB BMD</b>	<b>Δ LS BMD</b>	<b>Δ FN BMD</b>	<b>Δ Hip BMD</b>
Height (m)	RT	r = 0.493 p = 0.148 (n = 10)	r = 0.554 p = 0.097 (n = 10)	r = 0.297 p = 0.943 (n = 10)	r = -0.272 p = 0.479 (n = 9)	r = 0.028 p = 0.404 (n = 9)
	PLY	r = 0.014 p = 0.969 (n = 10)	r = -0.253 p = 0.481 (n = 10)	r = 0.245 p = 0.496 (n = 10)	r = -0.154 p = 0.671 (n = 10)	r = 0.017 p = 0.964 (n = 10)
Weight (kg)	RT	r = -0.23 p = 0.523 (n = 10)	r = -0.09 p = 0.805 (n = 10)	r = 0.303 p = 0.394 (n = 10)	r = -0.113 p = 0.771 (n = 9)	r = 0.117 p = 0.765 (n = 9)
	PLY	r = 0.423 p = 0.092 (n = 10)	r = -0.114 p = 0.753 (n = 10)	r = -0.016 p = 0.965 (n = 10)	r = -0.583 p = 0.077 (n = 10)	r = 0.122 p = 0.736 (n = 10)
Age (yrs)	RT	r = -0.104 p = 0.775 (n = 10)	r = 0.289 p = 0.418 (n = 10)	r = 0.351 p = 0.32 (n = 10)	r = -0.36 p = 0.341 (n = 9)	r = 0.51 p = 0.161 (n = 9)
	PLY	r = 0.409 p = 0.24 (n = 10)	r = 0.177 p = 0.624 (n = 10)	r = -0.13 p = 0.721 (n = 10)	r = -0.207 p = 0.566 (n = 10)	r = 0.287 p = 0.422 (n = 10)

No significant correlations were found [ $p \leq 0.05$ ].

**APPENDIX K – Bone Area RMANOVA**

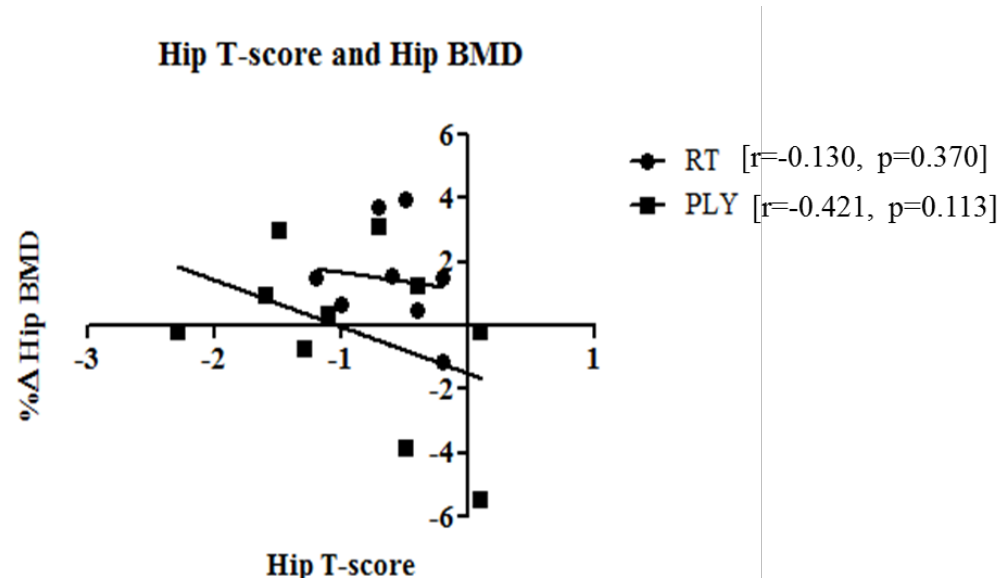
**Table 9.** Changes in bone area from 0 to 12-mo time points.

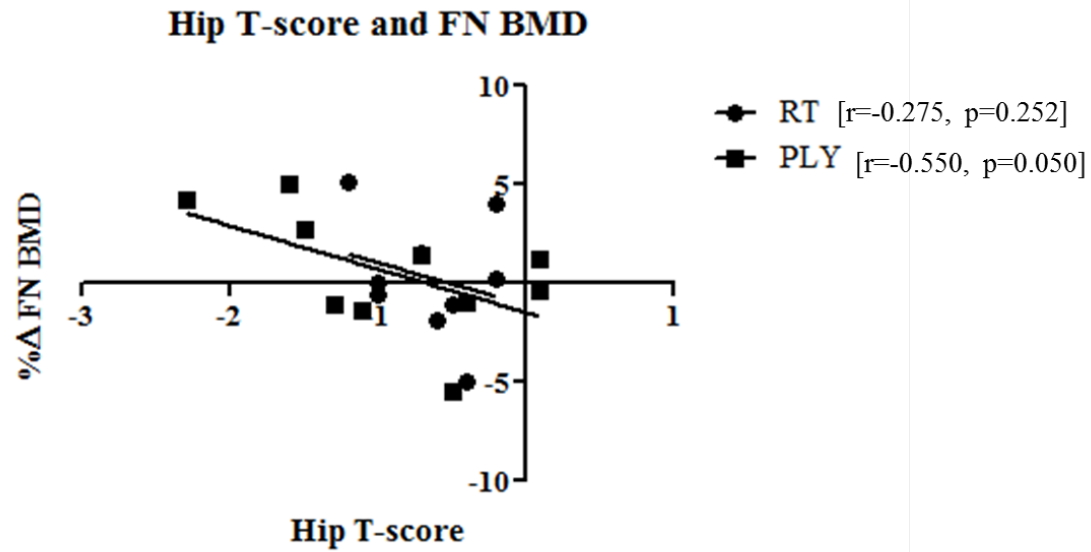
	<b>RT</b>		<b>PLY</b>		<b>Time</b>	<b>Sig (p-value)</b>	
	<b>0 Month</b>	<b>12 Month</b>	<b>0 Month</b>	<b>12 Month</b>		<b>Group</b>	<b>Group x Time</b>
<b>Whole Body (cm<sup>2</sup>)</b>	2359.71 ± 10.21	2368.44 ± 13.31	2182.85 ± 10.05	2176.91 ± 12.20	0.87	0.26	0.38
<b>Lumbar Spine (cm<sup>2</sup>)</b>	71.03 ± 0.63	71.09 ± 0.23	65.25 ± 0.18	65.63 ± 0.16	0.89	0.48	0.83
<b>Hip (cm<sup>2</sup>)</b>	40.51 ± 0.36	40.49 ± 0.32	39.1 ± 0.26	39.16 ± 0.21	0.34	0.56	0.11
<b>Femoral Neck (cm<sup>2</sup>)</b>	5.37 ± 0.26	5.44 ± 0.22	5.34 ± 0.16	5.13 ± 0.20	0.32	0.09	0.48

No significant changes were observed in either group.

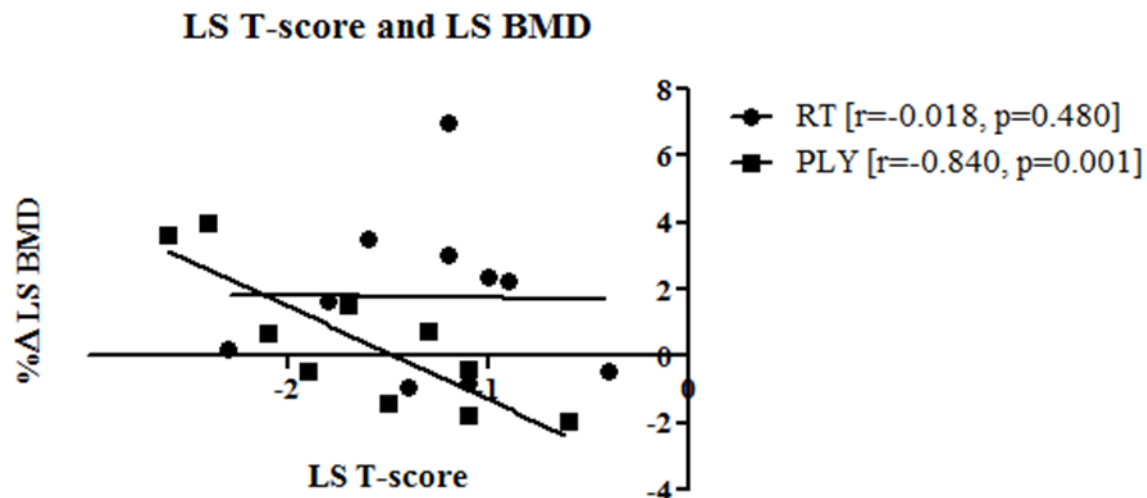
Data are means ± S.E.

**APPENDIX L – Correlation Between Baseline Hip T-score and Percent Change in Hip BMD and FN BMD**





**APPENDIX M – Correlation Between Baseline Lumbar Spine T-score and Percent Change in Lumbar Spine BMD**



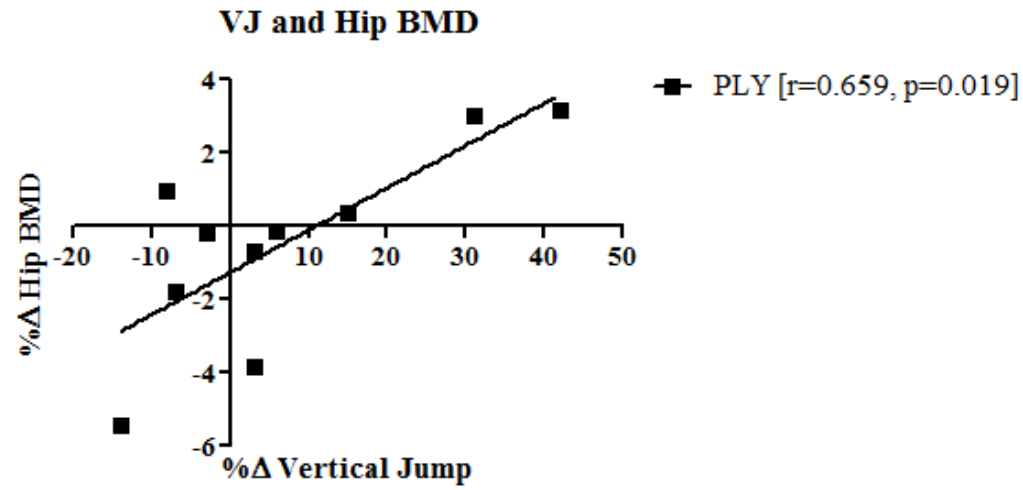
**Fig 13 – Scatterplot of lumbar spine T-scores and percent changes in lumbar spine BMD**  
**119APPENDIX N – Correlation Between Percent change in Vertical Jump or 1-RM and Percent change in BMD**

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**Table 10.** Relationships between changes in vertical jump or 1-RM and BMD at all sites

		<b>%Δ WB BMD</b>	<b>%Δ Hip BMD</b>	<b>%Δ FN BMD</b>	<b>%Δ LS BMD</b>
<b>RT</b>	<b>%Δ Squat 1-RM</b>	$r = 0.318$	$r = -0.248$	$r = -0.389$	$r = -0.515$
		$p = 0.202$	$p = 0.277$	$p = 0.164$	$p = 0.078$
		(n = 10)	(n = 9)	(n = 9)	(n = 10)
	<b>%Δ Deadlift 1-RM</b>	$r = 0.429$	$r = 0.031$	$r = 0.114$	$r = -0.220$
$p = 0.125$		$p = 0.471$	$p = 0.394$	$p = 0.285$	
	(n = 10)	(n = 9)	(n = 9)	(n = 10)	
<b>PLY</b>	<b>%Δ Vertical Jump</b>	$r = 0.143$	$r = 0.659$	$r = 0.092$	$r = -0.148$
		$p = 0.347$	$p = 0.019^*$	$p = 0.401$	$p = 0.341$
		(n = 10)	(n = 10)	(n = 10)	(n = 10)

\*Significant change from baseline,  $p \leq 0.05$ .



**Fig 14 – Scatterplot of Percent change in vertical jump and percent change in Hip BMD**