Effects of Calcium Collagen Chelate Consumption on Body Composition and Bone Biomarkers in Trained Male Cyclists

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EFFECTS OF CALCIUM COLLAGEN CHELATE CONSUMPTION ON BODY COMPOSITION AND BONE BIOMARKERS IN TRAINED MALE CYCLISTS

By

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A Dissertation submitted to the Department of Nutrition, Food & Exercise Sciences in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Degree Awarded:
Fall Semester, 2015
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I dedicated this to Suzi.
ACKNOWLEDGEMENTS

I would like to acknowledge the tireless efforts of some very important people involved in the completion of this project. The keystrokes of this document are ultimately mine, but the finished product belongs to my team to whom I am incredibly thankful. What will take up only a few lines of white space has more meaning to me than the entirety of this document.

First and foremost, to one of the most amazing women I have had the pleasure of knowing, I would like to thank Dr. Lynn Panton. Dr. P, you have made the life I wanted possible and fantastic over the past...years. Without your understanding, guidance and care I would not have made it to this point. I will be forever grateful and hope to make you proud.

Dr. Jeong-Su Kim, you have been a part of my education since the beginning and have been an inspiration. You have shown me that having an amazing family and an incredibly successful career as a prominent researcher can exist in the same person. Dr. Michael Ormsbee, although you arrived late to the party, you brought some incredible beer. I have learned a great deal about how to quickly make a big impact (in a good way) at my next position from you. Dr. Robert Contreras, thank you for your time and valuable feedback throughout this process. Your understanding and expertise have made working with you rewarding. And Dr. Bahram Arjmandi, our first encounter was a powerful one in which you challenged me to a test of grip strength while simultaneously inquiring about the impact factors of the journals I was reading. Thank you for showing me how to lead a team of colleagues with respect and focus.

Titch, Emily and Lyndsey, you have been like family and I plan to keep it that way. Mom and Dad, you continue to amaze me with your generosity and love. I hope to provide the same for my family and thank you for the example. Mom and Dad Moore, your support and love have helped more than you know. And finally to my ladies, Suzi, Dylan, Cameron and Evan, there are no words. So, I will spend the rest of my life showing you how much I appreciate each of you. This is the start of something big...
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ABSTRACT

PURPOSE: Objective 1 - To determine whether trained cyclists exhibit lower bone mineral density (BMD) than recreational cyclists. Objectives 2&3 - To determine the effects of 12-week supplementation with calcium collagen chelate (CCC) on body composition, bone and biomarkers of bone metabolism during habitual training in trained cyclists. A group of 29 male cyclists [9 recreational (<8 h/wk) and 20 trained (≥8 h/wk)] participated in the study. METHODS: Maximal exercise testing and 40-k time trials (TT) were performed on an electronically braked cycle ergometer. BMD of the whole body, lumbar spine (L1-L4) and both hips were measured using a Hologic Discovery-W DXA (Hologic, Waltham, MA, USA). For research Objective 1, a one-way analysis of variance (ANOVA) was used to evaluate the differences on dependent measures between the recreational and trained cyclists. Pearson product moment correlations and multiple regressions were used to examine relationships between the dependent variables. For research Objectives 2 and 3, trained cyclists were assigned to one of two groups (n = 9): 1) 6 g/d of CCC or 2) placebo control (CON) composed of maltodextrin with calcium and vitamin D equivalent to that found in the CCC. One-way ANOVA were used to compare baseline variables between the CON and CCC. Possible effects of the independent variable, CCC or CON supplementation, on the dependent variables, BMD (whole body, total hip, lumbar spine), bone alkaline phosphatase (BAP), tartrate resistant acid phosphatase 5b (TRAP5b) and sclerostin (SCL) were evaluated by two-way repeated measures ANOVA (group x time). The null hypothesis was that CCC supplementation would have no effect on the dependent variables. Tukey post hoc tests were used to find means that were significantly different from each other. Significance was accepted at p<0.05. RESULTS: Objective 1 - There were no differences in BMD at any site between recreational and trained cyclists. T-scores identified both recreational and trained cyclists as osteopenic (-1.16 and -1.49, respectively) at the lumbar spine. Twelve trained cyclists and two recreational cyclists were identified as osteopenic and three trained cyclists were identified as osteoporotic at the lumbar spine. Objectives 2 & 3 – No differences in BMD, body composition or biomarkers of bone metabolism were found between the CCC and CON groups. There were no group*time effects found for BMD, body composition or biomarkers of bone metabolism. Significant Pearson moment correlations were found between weekly training hours and TRAP5b (r = 0.53), BAP and VO2max (r = -0.56), and BAP/TRAP5b ratio and right/left hip BMD (r = -0.65 and r = -0.65, respectively). CONCLUSION: Our findings
demonstrate that male cyclists riding more than six hours per week have reduced BMD, particularly at the lumbar spine. While increased training volume leads to improved aerobic capacity, it may increase bone turnover and promote an environment that leads to significant bone loss over time. Additionally, 12 weeks supplementation of CCC did not affect body composition, BMD or biomarkers of bone metabolism. Further research is needed to determine whether low BMD compromises bone strength in male cyclists and to identify successful interventions for attenuating the loss of bone density in cyclists.

Supported in part by the Institute of Sports Sciences and Medicine.
Funded in part by The Graduate School and the College of Human Sciences at The Florida State University.
CHAPTER 1
INTRODUCTION

A major problem, physically and financially, facing our aging population is osteoporosis, more importantly the bone fragility and fractures that accompany this condition. It is currently estimated that 30-50% of women and 15-30% of men will suffer from osteoporotic fractures in their lifetime, significantly increasing morbidity rates (Nikander et al., 2010). Exercise, as prevention and treatment of osteoporosis, affects bone through the increased strain placed on the bone during physical activity. Sports with the highest strain intensities, e.g. gymnastics and weight lifting, have often been associated with significantly higher bone mineral density (BMD) at the loaded sites (Hind, Gannon, Whatley, Cooke, & Truscott, 2011; Morel, Combe, Francisco, & Bernard, 2001; Nikander, Sievanen, Uusi-Rasi, Heinonen, & Pekka, 2006; Nichols, Ruah, Barrack, & Barkai, 2007; Taffe, Robinson, Snow, & Marcus, 1997). In contrast, when the ground-reaction forces are reduced, the osteogenic stimulus appears to be attenuated. This effect has been shown in the lower body of swimmers and has the potential to be even more detrimental in cyclists, who may spend more time than swimmers training with no or low impact (Hind et al., 2011; Morel et al., 2001; Nikander et al., 2006).

Strain, the deformation of bone from mechanical loading, signals the osteogenic processes in bone and is influenced by ground-reaction forces (impact) or joint-reaction forces (muscle contraction). Increases in the magnitude, rate, duration and frequency of the applied strain must be sufficiently higher than those imposed by normal daily activities for any osteogenic response to result in significant, positive adaption of the bone (Barry & Kohrt, 2008a). When a group of cyclists were compared to age and weight matched recreationally active controls, spinal BMD was significantly reduced. Anterior-Posterior (AP) scans of L1-L4 were 7.1% lower (1.133 ± 0.022 vs. 1.220 ± 0.028 g/cm²), AP scans of L2-L4 were 6.5% lower (1.165 ± 0.023 vs. 1.246 ± 0.028 g/cm²) and lateral spine scans of B2-B3 were 14.3% lower (0.781 ± 0.025 vs. 0.911 ± 0.027 g/cm²) in the cyclist group (Smathers, Bembem, & Bembem, 2009).

Similarly, when BMD was compared in a group of age, weight and training volume matched runners (high ground-reaction forces) to that of cyclists (low ground-reaction forces), significant differences were observed (Rector, Rogers, Ruebel, & Hinton, 2008). Investigators found significantly lower whole body (1.26 ± .03 vs. 1.20 ± .01 g/cm³) and lumbar spine (1.10 ± .04 vs.
0.99 ± 0.02 g/cm³) BMD in the cyclists. No differences were found in serum hormone concentrations of testosterone, estradiol, cortisol, insulin-like growth factor (IGF-1) or parathyroid hormone (PTH). Additionally, comparison of serum bone markers bone alkaline phosphatase (BAP), osteocalcin (OC) and carboxy-terminal cross-linking telopeptide of type I collagen (CTX) were similar between groups.

Few longitudinal studies have looked at the effects of training on bone (Barry & Kohrt, 2008b; Nichols & Ruah, 2011), the most notable in cyclists investigated the effects of a competitive season on bone status in trained cyclists supplemented with either high (1500 mg/d) or low (250 mg/d) calcium citrate (Barry & Kohrt, 2008b). Over a 12-month period, a group of age, weight and performance matched amateur cyclists experienced losses in BMD whether supplementing with high or low calcium. Significant losses were recorded at the total hip (1.014 ± 0.038 vs. 0.999 ± 0.039 g/cm³ and 1.063 ± 0.024 vs. 1.048 ± 0.028 g/cm³) and femoral shaft (1.216 ± 0.046 vs. 1.198 ± 0.046 g/cm² and 1.280 ± 0.024 vs. 1.254 ± 0.027 g/cm³) in both the low and high dose groups, respectively. Although calcium supplementation of 1500 mg/d was not able to limit BMD loss in the cyclists, timing of calcium ingestion and vitamin D status can influence the effectiveness of supplementation. In the study, 25-hydroxyvitamin D concentrations were 24.0 ± 7.3 ng/mL in the low dose and 30.9 ± 6.4 ng/mL in the high dose group. Biochemical vitamin D insufficiency is classified as concentrations < 30 ng/mL (Barry et al., 2011); when combined with the lack of control over supplement timing, this may have significantly limited the potential benefits of calcium supplementation.

Dietary calcium and hydrolyzed collagen supplementation have been proposed as intervention strategies aimed at maintaining bone health due to their predominance in bone tissue (Barry et al., 2011; Guillemant, Accarie, Peres, & Guillemant, 2004; Cunco, Costa-Paiva, Pinto-Neto, Morais, & Amaya-Farfan, 2010). In a study of trained, adult male road cyclists and triathletes, investigators found that supplementation of a 1000mg/L calcium citrate malate drink consumed 20 minutes before exercise was able to significantly attenuate the increase in PTH following a 35-km time trial (TT) on a cycle ergometer (Barry et al., 2011). Although, BAP and CTX levels immediately post-exercise were not affected by calcium supplementation, it should be noted that 25-hydroxyvitamin D concentrations were low (32.7 ± 9.2 ng/mL), in fact nine of the participants were classified as insufficient (< 30 ng/mL). The poor vitamin D status may have contributed to reduced absorption of the calcium, limiting the beneficial effects of supplementation. The timing of the post-exercise blood draw may have also contributed to the lack of measured response to the
supplementation. A different group of investigators found significant increases in CTX levels were not evident until 30 minutes to two hours post-exercise following ingestion of high calcium (486 mg/h) mineral water during a 60-minute exercise bout at 80% of maximal oxygen consumption (VO₂max) on a cycle ergometer (Guillemant et al., 2004).

Supplementation of collagen has been shown to increase BMD, particularly in trabecular bone, as well as positively influencing biomarkers of bone turnover (Guilleminet et al., 2010; Wu, Fujioka, Sugimoto, Mu, & Ishimi, 2004). Ovariectomized rats, a post-menopausal osteoporosis model, had significant increases in whole body BMD, cortical area and increases in femur strength following 12 weeks of 2.5 g/kg/d of porcine derived collagen supplementation (Guilleminet et al., 2010). While this model was designed to simulate the effects of post-menopausal reductions in estrogen, the effects of this hormonal change resulted in increased osteoclast and attenuated osteoblast activity promoting unbalanced bone turnover favoring resorption. Uncoupled bone turnover is similar to the predicted mechanisms for the non-weight bearing loss of bone in cyclists (Campion et al., 2010; Nichols, Palmer, & Levy, 2003; Rector et al., 2008). In the study by Guilleminet et al. (2010), BAP levels were increased at four weeks but not at 12 weeks, while CTX was significantly reduced at 12 weeks. In a similar study, investigators found that a dose of 2 g/kg/d was more effective than 4 g/kg/d at increasing epiphyseal BMD, type I collagen and glycosaminoglycan content of the femur (Nomura, Oohashi, Watanabe, & Kasugai, 2005). It was suggested that an overdose of collagen might cause dyspepsia and defective absorption. Currently, the highest recommended dose for human trials is 10 g/d, roughly equivalent to 0.166 g/kg in a rat model; therefore, the risk of defective absorption due to overdose in human participants is minimal (Wu et al., 2004).

A human trial in a group of postmenopausal osteoporotic women found calcitonin supplementation combined with collagen hydrolysate (10 g/d) to be more effective than calcitonin alone. Over a six-month supplementation period, the hydrolyzed collagen group had greater reductions in markers of bone resorption than the control group, pyridinoline (PYD) (49.04% vs. 37.85% decrease) and deoxypyridinoline (DPD) (50.85% vs. 24.77% decrease), respectively (Adam, Spacek, Hulejova, Galianova, & Blabos, 1996). However, human trials have been limited and show mixed results (Adam et al., 1996; Cunco et al., 2010; Moskowitz, 2000). A human trial of hydrolyzed collagen supplementation, 10 g/d (bovine gelatin), showed no beneficial effect on CTX, BAP or OC levels following 24 weeks of supplementation. However, investigators noted that daily calcium
intake was low (621 – 685 mg/d) in all participants and there was no mention of vitamin D status (Cunco et al., 2010).

Trained cyclists spend a significant amount of time exercising in an unloaded position, particularly in the lumbar vertebrae, and this can lead to increased bone resorption. The USA Cycling Association states that almost 66% of their registered members are 35 years and over and almost 32% are 45 years and older, therefore it is important to understand the impact high levels of training will have on the bone health of this population. Intervention strategies utilizing calcium and hydrolyzed collagen have mixed results on BMD and biomarkers of bone turnover. This project was innovative in that calcium collagen chelate is a relatively new dietary formulation that has never been tested in an athletic population. Additionally, the current research project addressed limitations in previous supplementation studies by ensuring adequate vitamin D status, calcium and collagen supplementation by combining a chelated calcium collagen intervention with vitamin D supplementation.

**Purpose**

The purpose of the present investigation was to identify if recreational cyclists differed in body composition and BMD compared to trained cyclists and whether supplementation with calcium collagen chelate (CCC) during training would influence body composition (BMD, lean mass and fat mass) and biomarkers of bone turnover in trained cyclists.

**Research Objectives**

The present study was designed to answer the following research questions:

1. Is there a difference in body composition and bone status between recreational and trained cyclists?
2. To what extent will the ingestion of CCC (6 g/d) consumed for 12 weeks during regular training influence body composition and bone status compared to a CON supplement in trained cyclists?
3. To what extent will 12 weeks of calcium collagen chelate (CCC) supplementation affect resting levels of bone biomarkers compared to a control supplement (CON) in trained cyclists?

**Research Hypotheses**

The hypotheses of the present study included the following:
1. Trained cyclists will have lower fat mass and BMD, particularly at the lumbar spine, than recreational cyclists.

2. Trained cyclists participating in the CCC intervention will have greater total and regional (lumbar spine and hip) BMD after 12 weeks of CCC supplementation compared to CON.

3. Trained cyclists participating in the CCC intervention will demonstrate higher levels of biomarkers of bone formation and lower levels of biomarkers of bone resorption at rest after 12 weeks of CCC supplementation compared to CON.

**Assumptions**

Assumptions for the present study included the following:

1. All participants accurately reported their age, medical history, training history, current exercise status, and current dietary intake.

2. All participants followed the instructions given to them regarding the maintenance of their current dietary habits and current daily physical activity outside of the prescribed intervention.

3. All participants followed the instructions given to them regarding CCC and CON supplementation and honestly and accurately reported their adherence to the intervention when prompted to do so.

4. All laboratory equipment yielded accurate measurements over the course of repeated testing.

**Delimitations**

This study included the following delimitations:

1. Twenty-nine men, currently cycling at least three hours a week, between the ages of 18-54 years were recruited from the Tallahassee area to participate in a cross-sectional analysis (Objective 1).

2. Twenty trained men, determined by a VO$_2$max of $\geq$ 50 ml/kg/min currently cycling at least eight hours a week and with prior competition in cycling races, between the ages of 18-54 years were recruited from the Tallahassee area to participate in the 12-week intervention (Objectives 2 and 3).

3. Participants were healthy, without underlying disease or medical conditions and free of any contraindications to cycle ergometer endurance testing.

4. Participants were non-smokers and not taking any ergogenic aids.
Limitations

This study contained the following limitations:

1. Geographical bias was present since the participants were only recruited from the Tallahassee area.
2. Diet, sleep, training and other out-of-laboratory variables were not controlled throughout the study. However, participants were asked to maintain their normal diet and training volume throughout the study. Training was monitored daily using a self-reporting form.
3. Although participants had similar VO₂max values and training volumes, minimum and maximum performance standards based on race times were not established.

Definition of Terms

- **Biochemical Markers of Bone Turnover** - A blood or urine test to identify small changes in bone metabolism (medical-dictionary.thefreedictionary.com).
- **Body Composition** - the relative proportions of protein, fat, water, and mineral components in the body (medical-dictionary.thefreedictionary.com).
- **Bone Mineral Content** - A measurement of bone mass, expressed as the amount of total mineral in grams (americanbonehealth.com).
- **Bone Mineral Density** - A measurement of bone mass, expressed as the amount of mineral, in grams divided by the area scanned in cm² (americanbonehealth.com).
- **Chelate Compound** - A heterocyclic compound having a metal ion attached by coordinate bonds to at least two nonmetal ions (medical-dictionary.thefreedictionary.com).
- **Collagen** - Any class of extracellular proteins abundant in higher animals, especially in the skin, bone, cartilage, tendon, and teeth, forming strong insoluble fibers and serving as connective tissue between cells, yielding gelatin when denatured by boiling (dictionary.com).
- **Maximal Oxygen Consumption (VO₂ max)** - The maximum rate at which oxygen can be taken up and used by the body during exercise (Brooks, Fahey and Baldwin, 2005).
- **Osteoblasts** - A cell from which bone develops, a bone forming cell (medical-dictionary.thefreedictionary.com).
• **Osteocytes** - An osteoblast that has become embedded within the bone matrix, occupying a bone lacuna and sending, through the canaliculi, slender cytoplasmic processes that make contact with processes of other osteocytes (medical-dictionary.thefreedictionary.com).

• **Osteopenia** - Low bone mass that is 10-25% lower than a normal healthy adult. BMD that falls between one and two-and-one-half standard deviations below the average for a normal healthy adult (-2.5 < T-score < -1.0) (americanbonehealth.org).

• **Osteoporosis** - A disorder in which the bones become increasingly porous, brittle, and subject to fracture, owing to loss of calcium and other mineral components, sometimes resulting in pain, decreased height, and skeletal deformities. BMD that falls two and one half standard deviations or more below the average for a normal healthy adult (T-score ≤ -2.5) (americanbonehealth.org).

• **Resorption** - The destruction, disappearance, or dissolution of a tissue or part by biochemical activity, as the loss of bone or of tooth dentin (dictionary.com).

• **Time Trial** - A competitive event (as in bike racing) in which individuals are successively timed over a set course or distance (merriam-webster.com).
CHAPTER 2

REVIEW OF LITERATURE

A major problem, physically and financially, facing our aging population is osteoporosis, more importantly the bone fragility and fractures that accompany this condition. It is currently estimated that 30-50% of women and 15-30% of men will suffer from osteoporotic fractures in their lifetime, significantly increasing morbidity rates (Nikander et al., 2010). This review will focus on the effects of aerobic exercise, more specifically cycling, on bone status and metabolism. This review will also examine the potential for calcium, vitamin D and hydrolyzed collagen supplementation to promote improvements in bone health when combined with exercise.

The principal functions of bone are the mechanical support of the body, calcium dynamic deposition and hemopoiesis (Banfi, Lombardi, Colombini, & Lippi, 2010). Two counteracting metabolic processes are continuously occurring in bone, formation (osteoblasts) and resorption (osteoclasts), in order to support the above functions of the bone (Guadalupe-Grau, Fuentes, Guerra, & Calbet, 2009). Among the numerous pharmacologic and non-pharmacologic interventions that have been proposed to help maintain equilibrium, exercise is a widely accepted and recommended defense against declining bone health (Nikander et al., 2010). This is due to the fact that bone turnover, the rate of bone formation relative to bone resorption, relies on the balance of endogenous (e.g. hormones, growth factors and cytokines) and exogenous (e.g. mechanical loading) influences that increase in response to exercise (Banfi et al., 2010). As it pertains to exercise, the most important function of bone is to maintain its strength, allowing for the transmission of muscular force development without breaking. Bone strength is a multi-factorial concept that includes the amount of tissue (i.e. bone mass and size), the intrinsic material composition as well as the organization of materials within bone (Guadalupe-Grau et al., 2009; Nikander et al., 2010).

The amount of bone tissue within a given area, bone mineral density (BMD), is the most common measure of bone quality (Banfi et al., 2010; Guadalupe-Grau et al., 2009; Nikander et al., 2010). In fact, osteoporosis is defined as a reduction in BMD of 2.5 standard deviations below the mean for healthy persons at the age of attainment of peak bone mass (T-score), which is approximately 30 years of age, or matched to a reference population for age, sex and race (Z-score); the latter is the preferred measure for individuals under the age of 50 years (Nagle & Brooks, 2011).
Increased BMD augments the stiffness of bone, increasing its overall strength. Therefore, a significant loss in BMD could predispose an individual for increased risk of fracture, but it has been shown that the majority of low-trauma fractures occur in individuals who are normal or osteopenic (BMD from 1.1 - 2.4 standard deviations below normal) (Smathers et al., 2009). Although the amount of bone tissue is exceedingly important in maintaining bone health, studies measuring bone mass distribution, orientation of osteons and bone shape have shown maintenance of bone strength in the presence of decreased BMD (Banfi et al., 2010; Guadalupe-Grau et al., 2009; Nikander et al., 2010; Puustjarvi et al., 1999; Robling, Hinant, Burr, & Turner, 2002a).

The ability to directly measure whole bone strength, through loading the bone until failure, is not realistic in human studies. However, it has been shown that very small changes in BMD and even decreases in BMD following exercise are able to improve or maintain whole bone strength (Puustjarvi et al., 1999; Robling et al., 2002a). In a canine running study (Puustjarvi et al., 1999), investigators used a 1-year progressive training program to evaluate changes in bone structure and strength. Twenty young, female beagles were split into a control and exercise group made up of sister pairs. The exercise group ran from the age of 15 weeks to 70 weeks, 5 days per week with the distance per week gradually increased to 40 km/wk. The 40 km/wk training was maintained for the final 15 weeks of the study. There was no difference in the physical activity of the control or exercise groups while they were in their cages. Following one year of training, investigators found BMD in the running group to be significantly lower than controls as measured by quantitative computed tomography (QCT) in the thoracic (−6.7 ± 2.7%) and lumbar (−9.5 ± 2.8%) vertebrae. BMD was also measured by DXA and was significantly lower in the thoracic vertebrae (−8.6 ± 6.2%) and iliac bones (−9.7 ± 2.8%). However, even though BMD was significantly lower in the running group, there was no difference in whole bone strength as measured by the force needed to fracture cancellous bone of the vertebrae or iliac bone. Puustjarvi et al. (1999) proposed a reorganization of collagen fibers into a parallel arrangement, as a result of the imposed strain experienced during running, was responsible for the maintained strength in the face of reduced BMD.

**Bone Metabolism**

In addition to providing the mechanical framework required for movement during exercise, bone is a complex and dynamic tissue with significant metabolic responsibilities. Bone is classified based on its structure as either trabecular or cortical tissue. Trabecular bone is found in cuboidal, flat and the epiphysis of long bones; the composition of trabecular bone is a highly porous network
of struts and plates. Cortical bone, on the other hand, is a much denser tissue with low porosity found in the diaphysis of long bones. The microarchitecture of cortical bone is a system of open spaces that helps pattern the remodeling process; the framework is maintained by cellular open spaces called lacunae, the small interconnecting canaliculi and the larger osteonal canals that allow vascularization of bone (Zernicke et al., 2006).

The adaptation of bone to mechanical stimuli, like that experienced during exercise, is the responsibility of three distinctly different cell types: osteocytes, osteoblasts (formation) and osteoclasts (resorption). Osteocytes are considered the primary mechanosensory cells due to their highly interconnected communication network within bone. When compared to osteoblasts and osteoclasts, they are the most abundant and permanent cells in bone. Osteocytes are formed by quiescent osteoblasts that become trapped in the mineralized bone matrix. The newly formed osteocytes remain in contact with the osteoblasts on the surface, from which they were derived, and influence bone formation (Burger et al., 1999; Zernicke, MacKay, & Lorincz, 2006).

Bone modeling, the shaping of bone prior to maturation, and remodeling, the dominant shaping process after skeletal maturation, are the responsibility of two closely linked cell types. The activity of osteoblasts, originating from mesenchymal or stromal stem cells, and osteoclasts, originating from hematopoietic stem cells, are closely coupled during bone remodeling (Horwood, Elliot, Martin, & Gillespie, 1998). Following mechanical loading, bone experiences micro-damage to its structure and preosteoclasts are activated. Preosteoclasts bind to the damaged bone matrix and form annular sealing zones around bone-resorbing compartments beneath the multinucleated osteoclasts, which then release proteolytic enzymes and hydrogen ions to degrade and remove the tissue. Osteoblasts respond by synthesizing unmineralized collagenous organic matrix into the void created by the osteoclasts then either undergo apoptosis (50-75%) or await their progression into bone-lining cells and osteocytes as mineralization buries them in the matrix (Clark, 2008; Zernicke et al., 2006). Mechanotransduction, the conversion of mechanical stimuli into chemical activity, is the result of perturbations in cell shape; the autocrine and paracrine factors most likely responsible for the resultant osteogenesis involve integrins, cytoskeletal elements, ion channels and a number of secondary signaling molecules (Burger & Klein-Nulend, 1999; Zernicke et al., 2006). Osteocytes appear to be the major player in the response of bone metabolism to mechanical loading and unloading (Lin et al., 2009).

Human osteocytes and osteoblastic cells express a number of factors that interact with bone and govern cell migration, adhesion, proliferation and differentiation. Two major pathways involved
in the regulation of bone metabolism include the β-catenin dependent wingless integration factor (Wnt) and extracellular signal-related kinase (ERK) mitogen activated protein kinase (MAPK) signaling pathways. The Wnt signaling pathway promotes the commitment of mesenchymal/stromal stem cells to the osteoblast lineage as well as the proliferation and differentiation of osteoblast precursor cells into mature osteoblasts (Kramer et al., 2010).

The ERK-MAPK pathway is one of the primary signaling pathways resulting from integrin-matrix interactions and may also play a key role in osteoblast growth and differentiation (Lai et al., 2001; Ge, Xiao, Jiang, & Franceschi, 2007). The potentially osteogenic effect of the integrin-matrix interactions, like those involving type I collagen, are ultimately transmitted via cytoskeletal components like the interconnected network of microfilaments, microtubules and intermediate filaments. In addition to these signaling pathways, intracellular inositol 1,4,5-triphosphate (IP3)-dependent Ca\(^{2+}\) signaling via L-type voltage-dependent and stretch-activated channels is a critical component in the regulation of bone remodeling (Chen et al., 2000).

Finally, secondary signaling molecules such as nitric oxide (NO) and prostaglandins are produced by bone following mechanical loading and promote an osteogenic environment. NO exerts its effects mainly by inhibiting resorption and does so by directly inhibiting osteoclast activity and indirectly by decreasing the expression of receptor activator of nuclear factor-κB ligand (RANKL) and increasing osteoprotegerin (OPG) production by osteoblasts. The activity of RANKL and OPG regulate the entry of osteoclastic precursors, reducing the recruitment of osteoclasts to the surface and limiting resorption (Fan et al., 2004). Implicated in both bone resorption and formation, the actions of cyclooxygenase (COX)-2 and prostaglandin (PG)E2 g-protein signaling can provide significant stimulus for osteogenic activity in bone following mechanical loading (Blackwell, Raisz, & Pilbeam, 2010).

One of the primary components of bone is the calcium-phosphate complex, hydroxyapatite. Calcium is only available to the body through dietary sources and is a major determinant of calcium balance. The calcium requirement of an individual varies and is regulated by parathyroid hormone (PTH), 1,25-dihydroxyvitamin D (1,25(OH)\(_2\)D) and the amount of ionized calcium present in the blood (Peacock, 2010). More than 99% of the total body calcium (approximately 1000g) is located in bone where it serves two primary functions: provides structural support and serves as a dynamic store of calcium required to maintain intra- and extra-cellular equilibrium. The relatively small remaining calcium in adults (approximately 10g) is tightly controlled. Serum ionized calcium, the most abundant form of calcium in this small pool (51%), is maintained within a physiologic range of

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4.4 to 5.4 mg/dL (1.10-1.35 mM). Forty percent of the remaining calcium is found in protein-bound complexes, such as the serum proteins albumin and globulin and inside the cell bound to calmodulin. The remaining nine percent forms ionic complexes such as calcium phosphate and calcium carbonate (Peacock, 2010).

Calcium balance is controlled by intestinal absorption, renal reabsorption and bone turnover. When there are inadequate levels of serum calcium, an environment that promotes negative bone balance, the calcium-sensing receptors (CaR) in the body are activated and respond by increasing circulating PTH levels (secondary hyperparathyroidism). Increased PTH causes an increase in calcium reabsorption in the kidneys as well as increased bone resorption and subsequent bone loss. PTH also increases the level of vitamin D released from the kidneys. The higher levels of 1,25(OH)₂D activate receptors in the intestine, which increase the calcium absorbed during digestion (Lips et al., 2010). When serum calcium levels are restored, the CaRs are inactivated and the negative feedback loop starts to reduce serum calcium. This rapidly accessible pool of calcium is necessary to support the many responsibilities of calcium within the body, including nerve impulse transmission, muscular contraction and cellular signaling (Peacock, 2010).

A number of urinary and serum bone metabolism markers have been identified in an attempt to measure changes in bone turnover following exercise. Bone turnover and the consequent alterations in bone mass are the result of two opposing processes, bone formation and resorption. Bone formation markers, which appear to be more sensitive than resorptive markers, are typically measured in serum or plasma and are the products of osteoblast activity. Often measured bone formation markers include bone alkaline phosphatase (BAP), osteocalcin (OC), carboxyterminal propeptide of type I procollagen (PICP) and aminoterminal propeptide of type I procollagen (PINP) (Banfi et al., 2010).

Bone mineralization is associated with the presence of BAP and OC. The bone specific isoenzyme (BAP) is involved in all phases of mineralization and therefore serves as a specific indicator of osteoblast activity (Magnusson, Larsson, Magnusson, Davie, & Sharp, 1999; van Straalen, Sanders, Prummel, & Sanders, 1991). OC is a protein exclusively synthesized by osteoblasts, odontoblasts and hypertrophic chondrocytes that is also involved in mineralization, but its role is unclear (Hauschka, Lian, Cole, & Gundberg, 1989). In addition to binding calcium, OC has an active role in organizing the extracellular matrix and in energy metabolism. However, the use of OC to assess bone formation is limited for two reasons: it is difficult to differentiate the influence of increased energy requirement from exercise on increasing OC and there is an absence of
methodological standardization when measuring the various types of OC-derived fragments circulating in the bloodstream (Confavreux, Levine, & Karsenty, 2009; Bell, 1997).

The final bone formation markers are PICP and PINP and represent the activity of type I collagen. Ninety percent of the bone matrix is composed of type I collagen (Bjarnason & Christiansen, 2000). The two different procollagen markers result from the cleaving of peptides from the carboxy and amino terminals prior to the collagen molecule’s secretion into the extracellular space, this direct relationship allows PICP and PINP to be used as quantitative markers of type I collagen formation (Hassager et al., 1991; Eriksen et al., 1993).

Due to the predominance of type I collagen in bone, bone resorption markers such as the cross-links hydroxyllysylpyridinoline (PYD) and deoxypyridinoline (DPD) are simply degradation products of type I collagen (Seibel, 2005). Two other markers result from the degradation of cross-linked telopeptides, amino-terminal (NTX) and carboxy-terminal (CTX) are cleared by the kidneys and measureable in the urine (Bonde, Qvist, Fledelius, Riis, & Christiansen, 1994). Unlike bone formation markers, PYD and DPD are also best measured in the urine (Garnero, Gineyts, Arbault, Christiansen, & Delmas, 1995). PYD is found in a number of tissues, including cartilage, bone, tendon and connective tissue of vessels, whereas DPD is found mainly in bone and dentin (Eyre, Dickson, & Van Ness, 1988).

Another indication of bone resorption is found by measuring osteoclast activity. The osteoclast specific enzyme tartrate-resistant acid phosphatase subform 5b (TRAP5b), a sialic acid-free form of the TRAP5 isoform, can be measured in serum. The TRAP5 isoform is the only L(+)-tartrate resistant member of a family of acid phosphatases found in the prostate, bone, spleen, platelets, erythrocytes and macrophages. TRAP5 has shown two subforms, the osteoclast specific 5b and subform 5a that is expressed by macrophages and other non-identified sources (Halleen et al., 2000).

Increased understanding of bone metabolism regulation has identified the osteocyte, once thought to have limited involvement, as highly influential in regulating bone turnover. Osteocytes promote mineralization of osteoid laid down by osteoblasts by releasing protein phosphate-regulating gene with homologues to endopeptidases on the x-chromosome (PHEX), which blocks the binding of matrix extracellular phosphoglycoprotein (MEPE) to acidic serine aspartate-rich MEPE-associated motif (ASARM) (Guo et al., 2002). On the other hand, osteocytes increase bone resorption, by stimulating osteoclast activity through the release of RANKL in response to bone matrix damage, bone disuse, unloading and high levels of cortisol (Atkins & Findlay, 2012).
It has also been shown that osteoclast activity is increased in areas where osteocytes have undergone apoptosis as a result of microdamage that disrupts the extensive network of canaliculi (Clark et al., 2005). Osteocytes use this branched network to contact other osteocytes, stay in contact with surface osteoblasts and influence mesenchymal and haemopoietic cells in the marrow (Kamioka, Honjo, & Takano-Yamamoto, 2001). Not only a major producer of RANKL, osteocytes have been found to release sclerostin (SCL), another marker for measuring increased bone resorption (Atkins et al., 2012). SCL is involved in the negative regulation of bone formation pathways resulting from bone morphogenic protein (BMP) and canonical wingless integration (Wnt) signaling, negative regulation of these pathways is necessary to produce the appropriate amount of bone. SCL, released almost exclusively from osteocytes, has been shown to inhibit Wnt and BMP pathways and osteoblast to osteocyte transition; these effects reduce formation and mineralization, respectively (Winkler et al., 2003). Dysfunction of the SOST gene, responsible for SCL production, has been associated with increased bone mass and has become a target for anti-resorptive therapy (Atkins et al., 2012; ten Dijke, Krause, de Gorter, Lowik, & van Bezooijen, 2008).

**Bone Health: Bone Density vs. Bone Quality**

Increased BMD reduces the risk of fractures from falls, low-trauma and no-trauma injuries. However, according to The International Society for Clinical Densitometry, the diagnosis of osteoporosis using BMD may only be used in postmenopausal women and men older than 50 years of age (Clin Soc Den, 2007). Therefore, there must be more to optimal bone health than simply maintaining BMD. In an effort to maintain the health of our bones, the only intervention that has been found to increase bone mass and bone strength, while at the same time reducing the risk of falling is exercise (Barry & Kohrt, 2008a).

Exercise, as prevention and treatment of osteoporosis, affects bone through the increased strain placed on the bone during physical activity. Strain, the deformation of bone from mechanical loading, signals the osteogenic processes in bone and is influenced by ground-reaction forces (impact) or joint-reaction forces (muscle contraction). Increases in the magnitude, rate, duration and frequency of the applied strain must be sufficiently higher than those imposed by normal daily activities for any osteogenic response to result in significant, positive adaption of the bone. Intensity, magnitude and rate of strain, appears to be the most important variable determining bone adaptation to exercise (Barry & Kohrt, 2008a).
The magnitude of strain is in reference to the actual amount of bone deformation experienced during physical activity, not the relative intensity of the exercise. However, since increased ambulation and increased muscular resistance have been shown to increase the magnitude of strain, aerobic exercise intensity measured by percent of maximal heart rate and resistance exercise intensity measured by percent of one-repetition maximum (1RM) are often used to represent the magnitude of strain (Barry & Kohrt, 2008a). Strain rate refers to how quickly a given magnitude is loaded onto the bone during mechanical deformation; a positive relationship exists between strain rate and an osteogenic response (Burr, Robling, & Turner, 2002). However, there is a desensitization of bone to continuously repeated loading cycles, which reduces the osteogenic effect of increasing the time spent loading bone during exercise (Umemura, Ishiko, Yamauchi, Kurono, & Mashiko, 1997).

One in vivo animal study found significant improvements in bone mineral content (BMC) of the surgically isolated ulnae of a bird, following 36 repetitions of high-intensity loading. A pneumatic piston designed to impose a 0.5-hertz intermittent compressive load consisting of .95s on/off intervals increased BMC 134 ± 8% over 5 weeks. No additional benefit was found when the number of load cycles was increased to 360 or 1800 repetitions (Rubin & Lanyan, 1984). Due to the desensitization of bone to prolonged, repeated cycles of loading performed during a continuous exercise session, studies investigating frequency have utilized varying rest intervals to assess the impact of frequency on positive bone adaptation.

In a study by Robling et al. (2002b), investigators found when they separated 360 load cycles per day into 4 exercise sessions separated by 3 hours of recovery, the loaded ulnas of rats were able to absorb 165% more energy and 87% more total force before failing than the unloaded control ulnas. This increase represented a 75% and 35% improvement, respectively, over a group that received the 360 load cycles in a single session. In an earlier study by Robling et al. (2002a), using the same protocol, investigators found that these large increases in bone strength were associated with very modest increases in BMD (5.4 – 8.6% increase) and BMC (6.9 – 11.7% increase). The improvements in strength were attributed to site-specific increases in bone formation.

Bone requires a recovery period in order for the strain response to have an osteogenic effect. Due to this recovery effect, increasing the number of exercise bouts performed within the same day (e.g. four short sessions instead of one long session) has a greater effect on improving bone health (Turner & Robling, 2003; Robling, Hinant, Burr, & Turner, 2002a). Taking the concept of recovery even further, within the same exercise bout, augmented responses have been found when the rest
time between individual repetitions increase (e.g. 10-14s between each repetition) (Srivanasan et al., 2007; Lamothe & Zernicke, 2004; Robling, Burr, & Turner, 2001). Although increasing the rest time between individual contractions during aerobic exercise would be counterproductive to performance, it emphasizes the importance of a recovery period on osteogenesis.

Often times, studies have shown athletes to have higher BMD than non-athletic populations, but even within the athletic population there are variations depending on the nature of the sport (Bennell et al., 1997; Hind, Gannon, Whatley, Cooke, & Truscott, 2011; Morel, Combe, Francisco, & Bernard, 2001). Those sports with the highest ground-reaction (e.g. gymnastics and running) and joint-reaction (e.g. weight-lifting and racket sports) forces are often associated with significantly increased BMD at the loaded sites (Nikander et al., 2006; Morel et al., 2001; Nichols et al., 2007; Taffe, Robinson, Snow, & Marcus, 1997). Increased BMD resulting from exercise strengthens bone and should reduce the risk of fracture.

The goal of exercise training is to produce bone that is mechanically adequate to resist fracture, but does not cause excess metabolic cost for locomotion. Since fractures occur when loads exceed the capacity of bone to withstand them and we know both geometry [cross-sectional area (CSA), cross-sectional area moment of inertia (CSMI) and hip axis length (HAL)] and BMD are components of skeletal integrity, it is not enough to simply look at BMD (LaCroix et al., 2010; Leslie, Pahlavan, Tsang, & Lix, 2009). In a study by Hind et al. (2011), they used hip structural analysis (HSA) techniques to measure bone quality in the narrow neck region of the proximal femur in male runners, gymnasts, swimmers and non-athletic controls. The investigators found that BMD (g/cm³) of the gymnasts and runners, 1.223 (SE 0.4) and 1.114 (SE 0.3) respectively, were significantly greater than controls, 1.044 (SE 0.0). They also found increased CSA (mm²) in the gymnasts and runners, 214.8 (SE 8.2) and 204.8 (SE 5.1) respectively, versus controls, 179.9 (SE 5.6). There was an increased resistance against bending forces (CSMI) in the runners, 193.1 (SE 7.5) mm⁴, when compared to the controls, 169.7 (SE 8.2) mm⁴. And finally, the investigators looked at a variable called femoral strength index (FSI). FSI combines BMD, femoral geometry, age, height and body mass to indicate fracture risk associated with forces generated during a fall on the greater trochanter. The greater the FSI, the lower the hip fracture risk from a fall on the greater trochanter. FSI is calculated as strength divided by stress (Faulkner et al., 2006; Yoshikawa et al., 1994). FSI was significantly higher in gymnasts and runners, 1.8 (SD 0.1) and 2.06 (SD 0.3) respectively, than in the control group, 1.4 (SD 0.3). There were no differences found between the swimmers and controls in BMD, CSMI or FSI when adjusted for age, weight and body mass. Although a trend toward
reduced BMD, CSA and CSMI was present when comparing swimmers to the runners and gymnasts, the only significant difference was found when comparing FSI of the runners to the swimmers, 2.06 (SD 0.3) and 1.5 (SD 0.3), respectively. Considering the swimmers had more lean body mass, similar body fat percentages and were overall heavier than the runners and gymnasts, it would appear that the increased ground-reaction forces experienced in the weight-bearing exercises loaded the bones more effectively during training.

In another cross-sectional study by Nikander et al. (2006), they used peripheral quantitative computed tomography (pQCT) to assess differences in loading-related bone characteristics in female athletes. The advantage of this technique is that it does more than evaluate the bone mass of an individual; it is able to measure the structural characteristics of bone, the primary determinant of whole bone strength (Jarvinen et al., 2005). More specifically, the loading of bone in an effort to promote osteogenic activity that lays the foundation for optimal bone strength seems to be most influential in the cortical bone (Adami, Gatti, Braga, Bianchini, & Rossini, 1999; Frost, 2003). In the study, investigators compared 113 premenopausal competitive national level female athletes with 30 nonathletic referents. Of particular interest were the racket sport (n=23), soccer (n=18) and swimming (n=27) athletes. Within these groups, the BMC of the tibia, both distal and shaft, were found to be 15.4% and 11.8% higher in the racket sport group and 22.1% and 21.3% higher in the soccer group than the controls as well as significantly higher than that of the swimming group. However, when measuring the radius, both distal and shaft, and humeral shaft, there were no differences between the racket sport, soccer or swimming groups. Also, all three groups had significantly higher BMC at the distal radius than the control group: 15.2% (racket sport), 16.8% (soccer) and 12.9% (swimming). The racket sport and swimming groups were also found to have 13.5% and 9.7% higher BMC at the humeral shaft than the controls, respectively. Structurally speaking in the lower body, total CSA and cortical wall thickness of the tibial shaft were 9.9% and 8.1% greater in the racket sport group and 16.3% and 15.6% greater in the soccer group than controls. No differences were found between the swimming and control groups. In the upper body, total CSA was increased 12.3% in the racket sport, 11.6% in the soccer and 12.5% in the swimming groups when compared to controls. It is important to note that swimming was almost equally beneficial to the non-weight bearing upper body as the impact-loaded racket sport group when comparing BMC and total CSA of the humeral shaft, this is most likely due to the vigorous muscle activity of swimming increasing the joint-reaction forces at this site. However, when comparing the lower body bone quality of the distal tibia and tibial shaft in swimmers to every other
sport, they were significantly lower in BMC, cortical wall thickness, trabecular density and section modulus (an index of bone strength against torsion and bending forces). In addition to being lower than the other sports, the tibial bone quality of the swimmers was no different than the controls (Nikander et al., 2006).

While DXA-derived measures of BMD and BMC are useful in determining total bone formation within a given area, the use of HSA-capable DXA and QCT scans are necessary to evaluate overall improvements in bone quality (Hind et al., 2011; Faulkner et al., 2006; Nikander et al., 2006). In a study by Ramamurthi et al. (2011), they compared the results of 2D DXA technology to the measurements obtained by 3D 64-slice CT scans at the narrow neck (NN) and intertrochanteric (IT) regions of the femur in 41 elderly women. High linear correlations were found at the NN and IT for CSA (r = 0.95 and r = 0.93), CSMI (r = 0.94 and r = 0.93), section modulus (r = 0.93 and r = 0.89) and width (r = 0.95 and r = 0.95), respectively. Therefore, using HSA to calculate these variables from DXA images is a safer (lower dose of radiation) and more convenient option than QCT when measuring overall bone quality.

Instead of merely attempting to measure the result of abnormal metabolism in bone through the use of static measures, DXA and QCT, bone metabolism biomarkers provide dynamic analyses of the rates of bone formation and resorption responsible for the eventual changes in BMD (Banfi et al., 2010). The use of bone turnover markers, however, is subject to significant biological variability. Most of the markers are present in other tissue and may be altered due to non-skeletal activity and some may reflect both bone resorption and formation (Banfi et al., 2010).

The deformation of bone, via ground-reaction (impact) or joint-reaction (muscle contraction) forces, signals the osteogenic processes responsible for improving bone quality. The increased deformation, or strain, during exercise must be sufficiently higher than normal activity in order to result in a significant, positive adaptation (Barry & Kohrt, 2008a; Burr et al., 2002). Sports with the highest strain intensities, e.g. gymnastics and weight lifting, have often been associated with significantly increased BMD at the loaded sites (Hind et al., 2011; Morel et al., 2001; Nikander et al., 2006; Nichols et al., 2007; Taffe et al., 1997). In contrast, when the ground-reaction forces are reduced, the osteogenic stimulus appears to be attenuated. This effect has been shown in the lower body of swimmers, and has the potential to be even more detrimental in cyclists who may spend more time than swimmers training with no or low impact (Hind et al., 2011; Morel et al., 2001; Nikander et al., 2006).
Effects of Cycling on Bone Health

Although aerobic exercise has long been prescribed as a viable method of prevention for multiple diseases, its role in the prevention of osteoporosis is not as well defined. A few reviews have been published regarding the effects of regular exercise in postmenopausal women, a population at increased risk for developing osteoporosis and suffering fractures associated with the condition (Barry & Kohrt, 2008a; Guadalupe-Grau et al., 2009; Howe et al., 2011), but considerably less work has been performed in young, healthy female and male populations. More specifically, investigations into the effect of cycling on bone health are quite rare. Cycling is a very popular mode of aerobic exercise, particularly with athletes looking to remain competitive into later years. The USA Cycling Association states that almost 66% of their registered members are 35 years and over and almost 32% are 45 years and older, it is imperative to understand the impact high levels of training will have on the bone health of this population.

The number of studies focused on the effects of cycling in athletic populations are even scarcer, the majority of which are cross-sectional in design. Most studies have recruited young to middle-aged, trained participants who are actively participating in organized competition. In a study by Smathers et al. (2009), they compared 32 competitively trained male cyclists with 30 recreationally active, age and weight matched controls. The cyclists had a mean age of 31.9 ± 1.2 yr, had 9.4 ± 1.1 yr of racing experience and trained 13.0 ± 0.7 hours per week, while the control group performed less than 3 hours per week of exercise. No athletic performance data were published (maximal oxygen uptake (VO₂max), anaerobic threshold, maximal power, etc.), however, the population was said to contain professional and highly ranked amateur racers. Using DXA scans to measure BMD, investigators found significantly lower BMD in the spine of the cyclists, but no difference between groups in the hip region (total hip, femoral neck and trochanter). The cyclists’ BMD of the anterior-posterior lumbar scan of L1-L4 was 7.1% lower (1.133 ± 0.022 vs. 1.220 ± 0.028 g/cm²), L2-L4 was 6.5% lower (1.165 ± 0.023 vs. 1.246 ± 0.028 g/cm²) and a lateral spinal scan recorded 14.3% lower BMD (0.781 ± 0.025 vs. 0.911 ± 0.027 g/cm²) than controls. There were no differences in total body BMD scores or BMD at the total hip, femoral neck or trochanter between the two groups. It is possible that the greater leg lean mass of the cyclists was able to provide enough joint reaction forces to maintain lower body BMD and offset any BMD loss at the spine. The total (24.6 ± 1.4 mmol/L) and free testosterone (42.7 ± 3.47 pmol/L) levels were within the normal ranges and mean calcium intake was estimated at 1557 ± 132 mg/day (based on food frequency questionnaire) for the cyclists, more than the daily recommended intake (1,000 mg/d). The proposed mechanisms
for the lower BMD in cyclists were the lack of sufficient loading on the spine due to the flat positioning of the upper body while on the bike, dermal calcium loss through sweating and negative energy balance. Investigators controlled for smoking and drugs that have been shown to influence BMD, but did not control for resistance training history.

Another cross-sectional study from Warner et al. (2002) compared competitively trained male road (n=14) and mountain (n=16) cyclists with recreationally active (n=15) controls. The controls were age and weight matched to the road cyclists; road cyclists were significantly heavier and older than the mountain cyclists. Although the road cyclists also had more years of racing experience, due to their older age (31.4 ± 5.5 yr), the mountain cyclists (26.2 ± 5.0 yr) had significantly higher VO₂max values of 67.4 ± 4.6 ml/kg/min vs. 60.8 ± 3.6 ml/kg/min than the road cyclists. Each group regularly performed 11 hours of training per week, compared to that of less than three hours per week in the control group. Although no differences were found among the three groups for absolute BMD (g/cm²), relative comparisons (BMD/body weight) of DXA scans among the three groups highlighted significant differences in total body, spine and hip regions. The mountain cyclists had significantly higher relative BMD at lumbar spine, femoral neck, femoral trochanter, Ward’s triangle and total body than the road cyclists and controls. There was no difference reported at any site between road cyclists and the control group. Investigators proposed that the difference between mountain and road cyclists could be attributed to the inherent differences in the loads induced from controlling the bike and the varied terrain of mountain biking. Road cycling tends to be more predictable, i.e. less force from steering and braking, and is performed on a smoother and considerably less resistive surface. For example the drops, jumps, and rocks, that are common to mountain biking may provide increased load during the activity. However, keep in mind there was no difference among the groups when comparing actual BMD scores. Also, the BMD measurements of the three groups are in line with what is generally found in a normal population of healthy adult males. Therefore, while this study suggests there are no detrimental effects from cycling, there also appears to be no increased benefit to BMD.

Nichols et al. (2003) performed a cross-sectional analysis of 16 young (31.7 ± 3.5 yr) and master (51.8 ± 5.1 yr) highly trained male cyclists and recreationally active, master age and weight matched (51.6 ± 4.7 yr) controls. Again, no athletic performance data were published, but the investigators stated that there were regional, national and internationally competitive participants. The younger cyclists had been actively racing for 11 years, currently training 16 hours per week, while the master cyclists had been racing for 20 years and were training 12 hours per week. The
recreationally active control group had no race experience and exercised on average 5 hours per week, performing an unspecified cross-training program. DXA scans found no significant differences between young cyclists and the control group. However, the master cyclists were found to have lower BMD than the younger cyclists at all sites, the lumbar spine (L2-L4) \((1.07 \pm 0.15 \text{ g/cm}^2 \text{ vs. } 1.20 \pm 0.13 \text{ g/cm}^2)\), total hip \((0.93 \pm 0.11 \text{ g/cm}^2 \text{ vs. } 1.10 \pm 0.16 \text{ g/cm}^2)\), femoral neck \((0.91 \pm 0.18 \text{ g/cm}^2 \text{ vs. } 1.05 \pm 0.18 \text{ g/cm}^2)\), femoral trochanter \((0.79 \pm 0.11 \text{ g/cm}^2 \text{ vs. } 0.91 \pm 0.15 \text{ g/cm}^2)\) and total body \((1.16 \pm 0.09 \text{ g/cm}^2 \text{ vs. } 1.26 \pm 0.10 \text{ g/cm}^2)\), respectively. The master cyclists also had lower BMD than the control group at the femoral neck \((0.91 \pm 0.18 \text{ g/cm}^2 \text{ vs. } 0.99 \pm 0.16 \text{ g/cm}^2)\), trochanter \((0.79 \pm 0.11 \text{ g/cm}^2 \text{ vs. } 0.89 \pm 0.18 \text{ g/cm}^2)\) and total body \((1.16 \pm 0.09 \text{ g/cm}^2 \text{ vs. } 1.22 \pm 0.11 \text{ g/cm}^2)\), respectively. Of serious concern, four of the master cyclists had T-scores lower than -2.5, classifying them as osteoporotic (Nichols et al., 2003).

Nichols et al. (2011), performed a 7-year follow up with most of the master \((n=19)\) and control \((n=18)\) participants and found that in most regions there was no significant decrease within the groups for BMD scores, the exception being a significant decrease in total body BMD in the master cyclists \((1.180 \pm 0.093 \text{ g/cm}^2 \text{ vs. } 1.153 \pm 0.084 \text{ g/cm}^2)\). After seven years, the control participants had a somewhat greater BMD than the cyclists at the lumbar spine \((1.169 \pm 0.192 \text{ g/cm}^2 \text{ vs. } 1.052 \pm 0.180 \text{ g/cm}^2)\), but this difference was not significant \((p < 0.10)\). However, the control participants had significantly greater BMD than the cyclists at the total hip \((1.067 \pm 0.196 \text{ g/cm}^2 \text{ vs. } 0.924 \pm 0.112 \text{ g/cm}^2)\), femoral neck \((0.976 \pm 0.180 \text{ g/cm}^2 \text{ vs. } 0.866 \pm 0.107 \text{ g/cm}^2)\) and total body \((1.241 \pm 0.115 \text{ g/cm}^2 \text{ vs. } 1.153 \pm 0.084 \text{ g/cm}^2)\). Through exercise history questionnaires, the researchers noted that those participants who added resistance training programs over the 7 year period, both cyclists \((n=7)\) and controls \((n=4)\), had less BMD loss than those that did not (Nichols et al., 2011).

In a study by Barry and Kohrt (2008b), they compared the changes in BMD over a 12-month period that included a 9-month competitive racing season in 14 male cyclists (mean age of 34 yrs) with either high \((1500 \text{ mg/day})\) or low \((250 \text{ mg/day})\) supplemental calcium intake. The cyclists had 5-8 years of racing experience, currently trained 9 hours per week and had a mean \(\text{VO}_2\max\) of 51 ml/kg/min. DXA scans were performed at baseline, 4 months (mid-season), 9 months (end of season) and 12 months (prior to start of next season). Investigators found that after 12 months, participants in both high and low groups had significantly lower BMD at the total hip \((1.048 \pm 0.028 \text{ g/cm}^2 \text{ vs. } 1.063 \pm 0.024 \text{ g/cm}^2 \text{ and } 0.999 \pm 0.039 \text{ g/cm}^2 \text{ vs. } 1.014 \pm 0.038 \text{ g/cm}^2)\) and femoral shaft \((1.254 \pm 0.027 \text{ g/cm}^2 \text{ vs. } 1.280 \pm 0.024 \text{ g/cm}^2 \text{ and } 1.198 \pm 0.046 \text{ g/cm}^2 \text{ vs. } 1.216 \pm 0.046 \text{ g/cm}^2)\).
sites, respectively. Due to slight increases in BMD measured at the midpoint of the season, they also found decreases in BMD from the midpoint to the end of the season at the femoral neck and femoral trochanter, but the values were no different than baseline measurements after the 12-month period. At most sites, there was a slight decrease over the season with only partial recovery during the three-month off-season. However, the investigators also noted non-significant patterns toward continued loss at the lumbar spine and femoral trochanter. In an attempt to attenuate bone loss over the course of the competitive season, investigators supplemented the cyclists’ training with calcium citrate. The high dose group was assigned to take 1500 mg/day while the low dose group was assigned to consume 250 mg/day, the doses were to be taken three times each day for the course of the study. There were no differences in BMD at any site between groups; increased calcium supplementation did not appear to have any effect on the rate of BMD loss over the competitive season.

However, a study published out of the same laboratory found the timing of calcium supplementation to be a key component of successfully maintaining calcium homeostasis following exercise (Barry et al., 2011). Only when 1000 mg of calcium was supplemented 20 minutes before exercise did the investigators find PTH levels significantly reduced. Additionally, two other studies that found calcium supplementation to be effective against bone loss administered calcium at regular intervals during exercise (Guillemant et al., 2004; Klesges et al., 1996). If dermal calcium loss through sweating plays a role in reduced BMD and since calcium is absorbed in the small intestine, calcium availability at the time of exercise may have been inadequate in the previous study by Barry and Kohrt (2008b). If that had been the case, the body may have responded by increasing bone resorption during exercise to maintain the tightly controlled calcium homeostasis.

**Mechanisms of Cycling on Bone Turnover**

Several mechanisms have been proposed to explain the increased bone turnover with increased resorption experienced by cyclists, including increased levels of PTH, cortisol and pro-inflammatory cytokines in combination with decreased energy availability, sex hormones and weight bearing cross-training. Serum calcium level is the main factor that influences PTH secretion, a powerful promoter of bone resorption (Guillemant et al., 2004). Since training has been shown to increase the response of PTH to exercise, it is not a surprise that many studies have shown PTH to increase following acute bouts of endurance exercise (Bouassida et al., 2003; Guillemant et al., 2004; Maimoun et al., 2006; Salvesen et al., 1994; Thorsen et al., 1997; Zerath, Holy, Douce, Guzeennec,
& Chatard, 1997). The increase in PTH may be partially explained by increased dermal calcium loss through sweat (Barry & Kohrt, 2008b; Kleges et al., 1996). In studies that have measured sweat loss and BMD, the average amount of calcium lost through sweat was found to be 68-126 mg/h during indoor cycling (Barry & Kohrt, 2007, 2008b; Barry et al., 2011) and 124 mg/h (Kleges et al., 1996) during basketball practice over a single training session. It should be noted that Barry and Kohrt (2008b) did not find any relationship between the amount of dermal calcium loss and changes in BMD during indoor cycling. However, they did find a negative relationship between total sweat loss during exercise and baseline BMD. It is possible that chronically high calcium loss through sweat is associated with lower BMD. Since cycling is performed outdoors, for prolonged periods of time and often in hot environments, excessive sweating that results in unbalanced dermal calcium loss may lead to reduced BMD over time. However, PTH was not measured in this study and therefore we cannot identify this as a potential mechanism for reduced BMD. This study emphasizes the importance of timing the calcium ingestion and the potentially higher daily calcium requirement of trained cyclists.

In addition to increasing PTH, exercise has also been shown to increase cortisol and pro-inflammatory cytokines, both of which are known to promote bone resorption (Chennaoui et al., 2004; Pederson, Ostrowski, Rohde, & Bruunsgaard, 1998; Yun & Lee, 2004). There is a direct effect of cortisol on osteoblast and osteocyte function. The presence of glucocorticoids, such as cortisol, induces apoptosis of both cell types. Damaging the replication, differentiation and function of osteoblasts and osteocytes significantly decreases new bone formation. In addition to suppressing osteogenesis, cortisol also prolongs the lifespan of osteoclasts, which leads to augmented bone resorption (Saag et al., 2007; Sambrook, Hughes, Nelson, Robinson, & Mason, 2003; Weinstein et al., 2002). Cortisol directly increases the survival of osteoclasts by attenuating the pro-apoptotic effects of bisphosphonates; Cortisol has also been shown to enhance RANKL expression and inhibit OPG expression, further contributing to enhanced bone resorption (Jia, O'Brien, Stewart, Manolagas, & Weinstein, 2006). Another action of cortisol is to limit the ability of the small intestine to absorb calcium as well as the kidneys to reabsorb calcium, which may contribute to further increasing PTH secretion and subsequent bone resorption (Kalpakcioglu, Engelke, & Genant, 2011). Additionally, the inflammatory response to exercise leads to increases in cytokines such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-6, IL-1β and C-reactive protein (CRP), which may influence osteoclast number and function (Mundy, 2007).
The U-shaped relationship between exercise and disease suggests that while moderate doses of activity are healthy, excess activity may lead to tissue destruction from an excessive inflammatory reaction (Walsh et al., 2011). During repeated muscle contraction, cytokines and other peptides are produced that have various paracrine and autocrine effects. It has been shown that increased levels of CRP, IL-6 and TNF-α are associated with decreased BMD and are strong predictors of non-traumatic fractures (Lencel & Magne, 2011). In addition to directly stimulating osteoclasts, TNF-α and IL-1β have been shown to inhibit osteoblast differentiation, collagen expression and secretion of osteocalcin (OC) that effectively reduces bone formation (Lencel & Magne, 2011). In a study by Serrano et al. (2010), investigators followed six professional cyclists (age 24.8 ± 1.2 yr) over 4 days during the Andalusian Tour. Blood samples were taken at rest one day before the race and within 20 minutes following the end of the final day of racing. During the tour, participants covered 647.6 km, characterized by submaximal endurance series interspersed with sprint bouts, distributed as follows: day 1-123.4 km, day 2-176.2 km, day 3-174.5 km and day 4-173.5 km. There was a significant increase in IL-6 (0.92 ± 0.28 ng/L vs. 2.92 ± 0.57 ng/L) and TNF-α (5.14 ± 1.98 ng/L vs. 11.87 ± 2.43 ng/L) following the tour.

However, while isolated instances of prolonged, exhaustive exercise may lead to increased levels of pro-inflammatory cytokines, chronic aerobic exercise training has been shown to reduce plasma concentrations of CRP (Mattusch et al., 2000). Fourteen healthy male runners between the ages of 25 and 40 years were followed over nine-months while training for a marathon. Investigators tested running economy prior to training by measuring the running velocity when blood lactate concentration reached 4mmol/L (anaerobic threshold - AT). Absolute training intensity increased from a speed of 3.82 ± 0.29 m/s to 4.17 ± 0.17 m/s following training. Training volume increased from 31± 9 km/week at the beginning of training to 53 ± 15 km in the month before the marathon. Training sessions consisted of 3-4 sessions per week, each lasting 50 minutes at 75% of the AT. After the 9-month training program, even though exercise volume and intensity were increased, investigators found circulating CRP had decreased from a pre-training level of 1.19 mg/L to 0.82 mg/L (Mattusch, Dufaux, Heine, Mertens, & Rost, 2000). So, although acute bouts of increased aerobic activity may lead to an augmented inflammatory response, this study shows that there is a strong protective adaptation accompanying aerobic exercise training.

Although they exert pro-inflammatory responses as stated above, certain cytokines produced from skeletal muscle, “myokines” as coined by Pedersen et al. (2009), may also double as anti-inflammatory factors. These myokines have been show to exert a direct anti-inflammatory effect as
well as an indirect anti-inflammatory effect by increasing fat metabolism and stimulating the release of anti-inflammatory components (Walsh et al., 2011). The first and most pronounced anti-inflammatory myokine released during exercise, from both type I and II fibers, is IL-6 (Walsh et al., 2011). In addition to inducing well-known anti-inflammatory cytokines, IL-1 receptor antagonist (IL-1ra) and IL-10, it suppresses increases in TNF-α. IL-6 also acts locally by activating 5’ adenosine monophosphate activated protein kinase (AMPK) and phosphatidylinositol-3-kinase (PI3K), which increase fat oxidation and glucose uptake. Therefore, the protective effect of exercise may be in part a result of the reduction in fat mass, which is a significant source of pro-inflammatory cytokines (Lencel & Magne, 2011; Walsh et al., 2011). As a population, cyclists tend to have lower body mass and fat mass. While the lower body mass reduces the overall load on bone, potentially reducing bone formation, lower fat mass may reduce the release of pro-inflammatory cytokines and the subsequent bone resorption. Therefore, the bone resorptive potential of inflammatory cytokines may be offset by the chronic anti-inflammatory adaptations and reduced body fat that results from aerobic exercise training.

In addition to improving the environment for bone resorptive stimulants like PTH, cortisol and pro-inflammatory cytokines, cycling also has the potential to decrease the effects of bone formation agonists by decreasing testosterone, dehydroepiandrosterone (DHEA) sulfate, energy availability and the inclusion of weight-bearing, cross-training activities. Testosterone levels have shown varied responses following intense bouts of exercise in cyclists (Lucia et al., 2001), runners (Hackney, Szczepanowska, & Viru, 2003; Kenefick et al., 1998; Tremblay et al., 2005) and cross-country skiers (Ronsen et al., 2004). The trend appears to be that testosterone levels may increase during and immediately following shorter bouts of exercise (≤ 2hr) (Kenefick et al., 1998; Tremblay, Copeland, & Van Helder, 2005), but eventually may decrease below pre-exercise levels following longer efforts (≥ 2hr) (Lucia et al., 2003; Ronsen et al., 2004; Tremblay et al., 2005). However, in a longer duration training study involving a taper-like protocol, investigators found a potential adaptation in the testosterone response to aerobic training (Chennaoui et al., 2004). The investigators measured basal testosterone levels after one day of recovery from a 16-day high-volume training period (T1 = 48-54 hrs at 70-80% maximal heart rate (HRmax)) and then again after a 20-day reduced-volume training period (T2 = 30-32 hrs at 80-90% HRmax). Following T1, investigators found a significant decrease in testosterone from pre-training levels (14.63 vs. 9.85 nmol/L), however, testosterone had significantly increased back to pre-training levels following T2 (9.85 vs. 14.10 nmol/L). It is possible that the prolonged efforts common to aerobic endurance
training may lead to lower basal levels than those found in untrained men (Hackney et al., 2003; Lucia et al., 2001). Low levels of testosterone have been shown to increase osteoclast lifespan and decrease osteoblast activity resulting in increased bone turnover and subsequent bone loss. Testosterone acts directly, via androgen receptors, promoting osteoclast apoptosis and stimulating osteoblast activity. Testosterone has also been shown to upregulate its own receptors in osteoblasts, further promoting bone formation (Vanderschueren et al., 2004). However, due to the varied response of testosterone, it has been proposed that total and bioavailable estradiol may be a better predictor of BMD. It has been found in studies of older males, that estradiol deficiency is a stronger predictor of osteoporosis than testosterone deficiency. Therefore, if estradiol is a stronger predictor of reduced BMD and it is more stable in exercising males, it may be a better indicator of an environment that promotes bone loss (Fink et al., 2006; Khosla, Melton, & Riggs, 2002).

In addition to altering testosterone levels, studies involving trained cyclists (Chennaoui et al., 2004) and trained runners (Tremblay et al., 2005) have shown increased DHEA sulfate following exercise. Although not androgenic itself, DHEA sulfate is a metabolic intermediate in the biosynthesis pathway of androgens such as testosterone and 5α-dihydrotestosterone as well as 17β-estradiol. Decreased DHEA sulfate has been associated with increased risk of osteoporotic fractures; this effect is due to the subsequent reduction in estradiol and testosterone (Garnero, Gineyts, Arbault, Christiansen, & Delmas, 2000). Therefore, altered DHEA may be representative of changes in androgens and estrogens; its concentration is considered to be reflective of adrenal activity (Chennaoui et al., 2004; Garnero et al., 2000). Since longer duration aerobic exercise (> 2 hr) has been associated with reduced anabolic:catabolic hormone status, it is possible that the exercise training common among highly-trained cyclists could lead to an environment that promotes bone resorption.

Growth hormone (GH) and insulin-like growth factor-I (IGF-I) are also involved in the maintenance of bone by augmenting bone turnover (Ueland, 2005). Although osteoclastogenesis and the activation of mature osteoclasts increase as a result of GH and IGF-I, the anabolic effect of osteoblast activation predominates. Bone expresses receptors for both GH and IGF-I; GH exerts its influence on bone directly via GH receptors and indirectly through stimulation of IGF-I production in the osteoblasts (Tritos et al., 2009). IGF-I is primarily produced by the liver and released into systemic circulation, but is also produced locally in bone where it acts in an autocrine/paracrine fashion. Circulating IGF-I is often bound to one of the IGF-I binding proteins
(e.g. IGFBP-3) and is the primary mediator of GH actions (Wahl, Zinner, Achtzehn, Bloch, & Mester, 2010).

In support of the bone formation effects of GH, investigators have often found that deficient levels of GH are associated with below-normal to normal BMD (de Boer et al., 1994; Holmes et al., 1994; O'Halloran et al., 1993; Murray et al., 2004; Toogood et al., 1997). The effects of GH deficiency (GHD) on BMD has found that child-onset GHD leads to significantly decreased BMD (de Boer et al., 1994; O'Halloran et al., 1993), while the effects of GHD in adults shows varied impact on BMD (Holmes, Economou, Whitehouse, Adams, & Shalet, 1994; Murray, Columb, Adams, & Shalet, 2004; Toogood, Adams, O’Neill, & Shalet, 1997). In a study by Murray et al. (2004) of GHD, they separated groups by age (< 30, 30-45, 45-60 and > 60yr) and found that the most significant decrease in BMD, z scores of less than -1.0, was in younger adults (< 30 yr). In the youngest group, they found Z-scores of -1.25 ± 1.03 (lumbar spine), -1.55 ± 1.20 (femoral neck), -1.34 ± 1.09 (total hip), -1.14 ± 1.10 (ultra distal radius) and -1.74 ± 1.19 (distal radius). The only other group with below normal z-scores was the 30-45 yr group and that was only found at the distal radius (-1.12 ± 0.99). In line with previous work, when the groups were sub-divided by the timing of onset (childhood vs. adulthood), the child-onset group had significantly lower BMD at all sites. The investigators proposed that when the reduction in GH was experienced during peak bone formation years (< 30yrs), it has a greater impact than deficiency that arises later in life. Due to this relationship, the age at which the cyclist begins intensive training could impact how the bone responds during training studies and may be responsible for some variations in BMD.

Endurance exercise training has been shown to increase levels of GH, IGF-I and IGFBPs (Gilbert, Stokes, Hall, & Thompson, 2008; Manetta, Brun, Maïmoun, Fédou, Préfaut, & Mercier, 2003; Wahl et al., 2010). In a study of 11 young, healthy endurance trained men (age 26.5 ± 5.6 yr; VO$_2$max 64.6 ± 7.1 ml/kg/min), investigators performed three separate exercise trials following either a high-intensity interval training (HIT) or high volume endurance training (HVT) protocol (Wahl et al., 2010). Following a 10-minute warm-up at 50% of VO$_2$max, the HIT session consisted of four 30s maximal effort isokinetic (120rpm) bouts on a cycle ergometer separated by 5 minutes passive rest; the HVT was performed at 50% of VO$_2$max for one hour. Measurement of GH, IGF-I and IGFBP were performed prior to exercise then again at 10, 60 and 240 minutes post exercise bout. Investigators found an Increase in hGH levels at 10 minutes post exercise following HIT training of 72 ± 21 pg/mL, but no significant increase following HVT (17 ± 15 pg/mL). However, in a study by Gilbert et al. (2008), investigators found a significant increase in peak GH following a
30-minute exercise bout performed at 70% of VO₂max in both young (age 22 ± 1 yr; 0.11 ± 0.09 vs. 19.40 ± 11.58 μg/L) and middle-aged (44 ± 4 yr; 0.10± 0.14 vs. 4.76 ± 4.00 μg/L) participants. It should also be noted that peak GH levels were achieved prior to the cessation of exercise; there was a drop in GH levels after only 10 minutes of recovery (Gilbert et al., 2008). They also found IGFBP-3 to be significantly elevated (411 ± 184 pg/mL) from pre-exercise levels at 10 minutes post exercise only after HIT session. No differences were found in IGF-I at any time point. It was proposed that a drop in pH resulting from increased lactate concentration following HIT was partly responsible for the augmented response of GH and IGFBP-3. However, as stated above, it is possible that the HVT was successful in augmenting an anabolic response, but at the time of measurement the levels of GH had already returned to normal.

In a four-month training study following a group of eight competitive cyclists and 8 age-matched controls, investigators found significant differences in IGF-I, IGFBP-1 and IGFBP-3 (Manetta et al., 2003). The training program consisted of 500 km per week (~17h) with the following schedule: Monday 45 km recovery, Wednesday 100-140 km endurance, Thursday 30 km recovery, Friday 50 km intervals, Saturday 100-140 km endurance/racing and Sunday 80-100 km endurance/racing. The first month was performed at a lower intensity (120-160 bpm HR) compared to the final three months (>170bpm during intervals and following hill climbs). In addition to the cyclists experiencing a significant improvement in VO₂max following training (56.5 ± 1.4 vs. 70.3 ± 1.5 ml/kg/min), they also experienced a 38% increase in IGFBP-1 and a 20% increase in IGFBP-3. This increase is believed to represent an improvement in the GH-IGF axis, since IGFBP-3 is considered to be an index of GH action (Ueland et al., 2005). It would appear that increased aerobic exercise training does not impair the anabolic potential of GH-IGF and is most likely not a concern for competitive cyclists, particularly as the cyclist get older, since it appears that changes in GH-IGF have less impact on bone turnover in older adults.

The impact of prolonged endurance exercise on energy balance allows endurance athletes to maintain low body mass. While this is beneficial in preserving sports performance, it does not help maintain BMD. Furthermore, it has been shown that acute periods of decreased energy availability, like those experienced during cycling training programs, impair bone formation and increase resorption. When energy availability dropped below 30 kcal/kg of fat-free mass/day in a group of young, healthy women, bone formation was impaired and bone resorption was increased (Ihle et al., 2004). Energy availability is defined as dietary energy intake minus exercise energy expenditure normalized to fat-free mass (Lambrinoudaki & Papadimitriou, 2010; Nattiv, Loucks, Manroe,
Sanborn, Sundgot-Borgen, & Warren, 2007). Although the referenced study focused on women, it is possible that the calorie restriction and/or incredibly high exercising energy expenditure common in competitive cycling could lead to the same type of uncoupled bone turnover in male athletes. Also, it is common for cyclists to limit their exposure to resistance training and ground-impact cross training, two modes of exercise known to increase BMD, in an effort to maximize the specificity of their training. Some cyclists even ignore recommendations from health/wellness professionals, as evidenced by the low percentage (37%) of athletes who incorporated resistance training and/or impact exercise into their programs even after being told they had low or declining BMD (Nichols et al., 2011).

**Calcium/Vitamin D and Hydrolyzed Collagen Supplementation and Bone Health**

Calcium and vitamin D metabolism are some of the main determinants of bone turnover. Vitamin D is required for the intestinal absorption of calcium; deficiencies in either can disrupt the tightly controlled serum calcium concentration and lead to secondary hyperparathyroidism. The bone turnover associated with elevated PTH results in reduced mineralization, increased resorption and ultimately bone loss (Lips et al., 2010). To maintain bone health, current recommendations for calcium intake have been set at 1000 mg/day for adults under the age of 50 and 1200 mg/day for older adults (Yates, Schlicker, & Sutor, 1998). Vitamin D status is based on a deficiency threshold of 50-75 nmol/L of serum 25(OH)D and should be maintained with 800IU/day of vitamin D (Lips et al., 2010). Calcium and vitamin D supplementation beyond these levels does not seem to provide any additional benefit, and in the case of calcium, may have harmful side effects such as cardiovascular irregularity (Bolland et al., 2008) and the development of kidney stones (Jackson et al., 2006).

Studies have shown that short, intense aerobic exercise lasting less than 1 hour (Bouassida et al., 2003; Guillemant et al., 2004; Salvesen et al., 1994) as well as long, moderate-intensity endurance exercise (Barry & Kohrt, 2007) can increase PTH levels. In a study of 20 competitive male road cyclists, investigators found that 2 hours of cycling significantly increased PTH by 40.6 ± 15.6 pg/mL to 72.8 ± 24.6 pg/mL and serum calcium levels from 9.3 ± 0.3 mg/dL to 9.6 ± 0.3 mg/dL (Barry & Kohrt, 2007). The exercise bout consisted of 120 minutes of moderate intensity exercise based on the participant’s power output at ventilatory threshold (VT), the athletes performed a repeated pattern of 10 minutes at 60% and 20 minutes at 75% on a cycle ergometer. The increase in
serum calcium concentration was a result of decreased plasma volume. Although dermal sweat loss averaged 69 ± 36 mg/hour, no significant associations could be made between dermal sweat loss nor changes in serum calcium and the increase in PTH. However, as investigators pointed out, this does not mean calcium loss during exercise is not a moderator of increased PTH. The response of PTH to changes in serum calcium concentrations occur within minutes, quickly restoring calcium homeostasis, attempting to derive a relationship for this highly dynamic process by evaluating a single measurement point after exercise most likely does not represent the relationship that occurs during the exercise bout.

Studies have attempted to limit the increase in PTH, and more importantly the resultant increase in bone resorption and loss of BMD, by supplementing with calcium at various time points before, during and after exercise (Barry et al., 2011; Barry & Kohrt, 2008b; Guillemant et al., 2004; Klesges et al., 1996). In a study of 20 adult male road cyclists and triathletes (age 37.0 ± 7.6 yr, VO₂max 53.0 ± 7.0 ml/kg/min), investigators used a 35-km time trial to assess the effects of acute calcium ingestion on calcium homeostasis (Barry et al., 2011). After establishing VO₂max during an incremental exercise test on an electronically braked cycle ergometer, participants returned to the laboratory on three separate days separated by at least 2 days but no more than 7 days and performed a 35-km time trial. In order to minimize the effects of diurnal and nutritional variation on the measured variables, the exercise bouts were performed at the same time of day and participants were instructed to consume the same foods and drinks before each session. The participants were supplied with either a calcium-free sports beverage or a calcium-fortified sports beverage containing 1000 mg/L of calcium citrate malate. Under the first condition, 1000 mL calcium drink was consumed in its entirety 20 minutes before exercise and then 250 mL of the placebo drink at 0, 15, 30 and 45 minutes of the exercise. The second condition had the athletes consume 1000 mL of the placebo drink 20 minutes before exercise then 250 mL of the calcium drink at 0, 15, 30 and 45 minutes of exercise. And finally, the third condition had the placebo consumed at all time points. During the exercise, sweat was collected using patches attached at the forearms, upper chest and below the scapula for two 20-minute intervals: the first 20 minutes and from minutes 30-50 minutes. Pre- to post-exercise changes in PTH, C-terminal telopeptide of Type I collagen (CTX; a marker of bone resorption), bone-specific alkaline phosphatase (BAP; a marker of bone formation) and ionized calcium were measured. The major finding of the study was that only calcium supplementation before exercise resulted in a significant attenuation of the increase in PTH following exercise, PTH increased 52.06 ± 15.01 pg/mL instead of the 70.10 ± 14.18 pg/mL.
increase in the placebo trial. Calcium supplementation during exercise may attenuate the increase in PTH, but the decrease was not significant in the present study. Although PTH was lower following calcium supplementation, CTX (bone resorption marker) levels remained unchanged. However, it has been shown previously that CTX levels peak 1 hr after exercise and remain elevated beyond 2 hours of recovery (Guillemant 2004). In the current study, CTX was measured immediately post-exercise and the differences may not have been detectable yet. Also, it is important to note that the mean 25(OH)D status of the athletes, 32.7 ± 9.2 ng/mL, is considerably lower than the deficiency threshold of 50-75 ng/mL previously discussed; nine of the participants had 25(OH)D levels below 30 ng/mL (Lipps et al., 2010; Willis, Peterson, & Larson-Meyer, 2008). Impaired intestinal absorption of calcium may have contributed to limiting the effectiveness of the calcium supplementation in the current study.

In another study, 12 male triathletes (age 30.7 ± 4.2 yr, VO₂max 61.7 ± 6.3 ml/kg/min) performed two 60-minute exercise sessions at 80% of VO₂max on a cycle ergometer separated by at least one week (Guillemant et al., 2004). Participants consumed either high-calcium mineral water (486 mg/L) or low-calcium mineral water (9 mg/L) in 250 mL fractions every 15 minutes starting one-hour prior to exercise; no calcium water was consumed during the 2-hour recovery. Investigators found that the 46% increase in total CTX adjusted for plasma volume following the low calcium water was significantly reduced following increased calcium supplementation during exercise. Similar to the findings of the previously discussed study, Barry et al. (2011), there was no difference in the CTX levels immediately after exercise. However, at 30 minutes, one-hour post- and two hours post-exercise the total CTX levels were considerably elevated in the low-calcium water trial.

Collagen, specifically type I collagen, is the primary organic component of trabecular bone (Guillerminet et al., 2010). In addition to providing the structural foundation for bone tissue, type I collagen is responsible for the elastic properties of bone, reducing the risk of fracture when bone deforms under load (Wu et al., 2004). Hydrolyzed collagen supplementation has been shown to promote the synthesis of extracellular matrix proteins and has been used for treatment of osteoarthritis and other joint disorders (Bello et al., 2006). Considering the role of type I collagen in extracellular matrix protein calcification in bone, hydrolyzed collagen has been proposed as potential intervention for individuals suffering from osteoporosis (Cunco et al., 2010; Wu et al., 2004).

In a study by Guillerminet et al. (2010), investigators found 12 weeks of porcine-derived hydrolyzed collagen supplementation to improve overall bone status in ovariectomized (OVX) mice.
Following 12 weeks of 2.5 g/kg/day of hydrolyzed collagen supplementation, investigators found increased whole body BMD (0.02 ± 0.0023 g/cm² vs. 0.017 ± 0.0019 g/cm²), larger cortical area (1.25 ± 0.11 vs. 1.14 ± 0.07 mm³) of the femur and lower CTX (9.45 ± 1.33 vs. 12.42 ± 1.70 ug/mL) when compared to the OVX mice without hydrolyzed collagen. Ultimate strength of the femur, as measured by a three-point bending test, was higher in the hydrolyzed collagen group (30.80 ± 2.88 vs. 28.67 ± 2.07 N). Further analysis of the geometrical properties of the bone found that the larger cortical area in the hydrolyzed collagen group was due to increased external diameter of the femur, suggesting periosteal apposition. Although BAP was not increased after 12 weeks of supplementation, it was significantly elevated at four weeks and may have had a rapid but transient influence on formation.

A human trial in a group of postmenopausal osteoporotic women found calcitonin supplementation combined with collagen hydrolysate (10 g/d) to be more effective than calcitonin alone in attenuating loss of bone. Over a six-month supplementation period, the hydrolyzed collagen group had greater reductions in markers of bone resorption than the control group, PYD (49.04% vs. 37.85% decrease) and DPD (50.85% vs. 24.77% decrease), respectively (Adam 1996). In a later human trial of hydrolyzed collagen supplementation, 10 g/d (bovine gelatin), no beneficial effect was shown on CTX, BAP or OC levels following 24 weeks of supplementation. However, investigators noted that daily calcium intake was low (621-685 mg/d) in all participants and there was no mention of vitamin D status (Cuneo et al., 2010). Very little work has been done in human trials investigating the effects of hydrolyzed collagen supplementation on bone metabolism and nothing has been published on the potential effects in an athletic population (Adam et al., 1996; Cuneo et al., 2010; Moskowitz et al., 2000).

Closing Remarks

As the population of older adults continues to increase, so does the prevalence and incidence of osteoporotic fractures. Among the many responsibilities bone serves in the body, maintaining its strength to provide adequate mechanical support is of extreme importance when attempting to reduce the risk of osteoporotic fractures through regular exercise. The mechanical loading, production of hormones, growth factors and cytokines experienced during exercise help balance the counterproductive efforts of the bone building osteoblasts and bone resorbing osteoclasts. Despite the osteogenic effects generally associated with exercise, athletes participating in predominantly non-
weight bearing types of endurance sports have shown declines in bone density similar to, and in some cases worse than, sedentary individuals.

In particular, cyclists appear to be at an increased risk of falling below the normal range for BMD. BMD is positively influenced by increased mechanical loading of the bones, but since competitive cyclists are lighter than the average person and the majority of their training is non-weight bearing, they reduce the potential osteogenic effect of ground-impact forces. In addition to potentially lower bone formation activity, the long duration moderate to high intensity efforts common in their training may promote increased bone resorption via hormonal alterations, increased inflammatory response and the disruption of calcium homeostasis. The uncoupling of these processes may reduce the healthy bone turnover increases usually associated with exercise.

Although it is unclear whether cycling detrimentally reduces BMD, it does not appear to have the beneficial effect of increasing BMD. The literature reviewed has been inconclusive as to the effect of cycling on BMD, markers of bone turnover and the importance of BMD in determining overall bone strength. The goal of an aerobic athlete’s exercise program is to produce bone capable of resisting fracture, but at the same time limits excess metabolic cost for movement. Therefore, it is possible that the best bone structure is not the densest for a competitive cyclist. Further investigation is required to identify the long-term BMD adaptations experienced by highly trained cyclists, whether those changes actually compromise bone strength and if the maintenance of calcium homeostasis through supplementation is able to promote an osteogenic environment.
CHAPTER 3

RESEARCH DESIGN AND METHODS

Study Overview

We recruited 29 (see Data Analyses for sample size determination) healthy male cyclists to participate in a study designed to achieve the following objectives:

Objective 1: In a cross-sectional design, measurements of body composition (dual-energy x-ray absorptiometry DXA), BMC, BMD, maximal oxygen uptake (VO_{2}\text{max}; TrueOne Parvomedics Metabolic Cart, Sandy, Utah) and training history were assessed for twenty-nine trained (n=20) and recreational cyclists (n=9). HR, BP and blood samples were taken at rest. All tests and measurements were performed on the same visit (Visit 1). Those individuals who qualified as trained (cycling ≥ 8 h/wk; VO_{2}\text{max} ≥ 50 ml/kg/min), continued on to Objectives 2 and 3 and participated in a 40-km time trial (TT) during this first visit (Visit 1). Those that did not qualify were classified as recreational cyclists, did not perform the TT and did not return to the lab for visit 2.

Objectives 2 and 3: In a stratified, placebo-controlled, double blind protocol, twenty qualifying participants from Objective 1, performed a 40-km TT exercise bout following a 15-minute rest from the VO_{2}\text{max} test (TrueOne Parvomedics Metabolic Cart, Sandy, Utah) and then were assigned to one of two groups (matched for BMD, body weight and training status): 1) 6 g/d of CCC (n = 10) or 2) placebo control (CON) (n = 10) composed of maltodextrin with calcium and vitamin D equivalent to that found in the CCC. Supplementation began the day after Visit 1. Participants in both groups consumed their respective supplements twice daily (four pills in the morning and four pills in the evening) for the next 12 weeks. Participants returned empty supplement containers and compliance logs every two weeks, at which time they were given supplements for the next two weeks.

Measurements of body composition, exercise performance and bone biomarkers were assessed at baseline (Visit 1) and after 12 weeks of supplementation (Visit 2). All participants were instructed to consume their normal diet and follow their normal in-season training programs throughout the entirety of the experiment. Physical activity was monitored with training logs. Any participant that dropped below six hours per week of training for two consecutive weeks or a total of four weeks during the intervention period was removed from the study. Any participant that dropped below 80% compliance with their respective supplement was removed from the study.
Inclusion Criteria

Normal-weight (BMI 18.5 – 24.9 kg/m²), healthy males (18 – 54 yrs) engaged in more than three hours per week of cycling training were recruited for the study. Men training no more than two days per week in an activity other than cycling were allowed to participate.

Exclusion Criteria

Men who engaged in any exercises, other than cycling, for more than two days per week for more than 40 minutes per session (within the past six months), had uncontrolled hypertension (blood pressure (BP) >160/100 mmHg), currently taking BP medications, had been diagnosed with cardiovascular disease, stroke, diabetes, thyroid or kidney dysfunction, and/or had any musculoskeletal complications (i.e. osteoarthritis or injury) that would impede exercise testing and training were not able to participate in the study. In addition, those who smoked, were prescribed cholesterol medication or used any medications known to affect bone metabolism were excluded.

Data Collection

Recruitment of participants was done through flyers and contact through cycling clubs in the Tallahassee and regional areas. The participants were screened via the telephone (Telephone Screening – Appendix A) to ensure their eligibility. Once eligibility was determined, qualifying participants were given through email or mailed a three-day dietary log (Three-Day Dietary Log – Appendix B) and an appointment was made to meet with the investigators to obtain consent and begin baseline measurements. Laboratory testing was completed on two occasions, each visit lasted approximately three hours.

Baseline (Visit 1)

On the first visit, participants reported to the laboratory following a three-hour fast at least 24 hours after their last strenuous exercise bout and were given an informed consent document (Informed Consent – Appendix C) that had been approved by the University Institutional Review Board (Appendix I), a medical history form (Medical History Form – Appendix D) and physical activity readiness questionnaire (PAR-Q – Appendix E) to complete before they participated in the study. Once these forms were completed, participants completed questionnaires regarding nutritional habits, training and racing history (Medical and Training History Form – Appendix F).
Participants then had their resting BP, resting HR, anthropometric and isometric handgrip strength measurements performed.

Handgrip strength was assessed using an isometric handgrip dynamometer (Lafayette Instrument Corp., Lafayette, IN). Participants performed three maximal effort attempts on each hand, alternating hands between efforts; the score was calculated as the sum of the highest score from the left and right hands. Blood was sampled via venipuncture from the antecubital space of the forearm, gently mixed and allowed to clot, centrifuged at 3500 rpm for 15 minutes at 4 °C, divided into aliquot samples and stored at -80 °C for later analysis. Height and body weight were measured using a stadiometer and digital scale (SECA, Birmingham, United Kingdom), respectively. The same technician throughout the study measured resting HR and BP; the average of two consecutive measurements was used for data analysis.

Body composition was evaluated by dual energy X-ray absorptiometry (DXA; Hologic Discovery W, Bedford, MA). Total body, hip and lumbar BMD scans were performed. Before testing, participants were asked to change into clothing that was free of metal and/or hard plastic (buttons, zippers, snaps, etc.) and asked to remove all metal from the body (jewelry, eyeglasses, hair accessories, etc.). A total of four scans were performed on each participant: 1) anteroposterior (AP) view of the total body with the participant lying supine; 2) AP view of the lumbar (L1-L4) spine with the participant lying supine with hips and knees supported at a 90° angle; and 3) AP view of the right and left femoral neck with the participant lying supine with thigh internally rotated. Testing was completed according to the manufacturer’s instructions and specifications by a certified X-ray technician.

Maximal Performance Testing (Visit 1 continued)

Participants then performed an incremental exercise test on an electronically braked cycle ergometer (RacerMate; Velotron Dynafit Pro, Seattle, WA). Participants began exercising at 50 W; resistance increased by 50 W every two minutes up to 150 W. Thereafter, resistance increased by 25 W every minute until volitional fatigue. The criterion for achievement of VO2max was fulfilled by reaching at least three of the following: 1) a plateau in oxygen consumption for an increase in exercise intensity (< 2.0 ml/kg/min increase), 2) respiratory exchange ratio = 1.05), 3) heart rate = 85% of an age predicted maximum (as determined by 220-participant’s age), 4) voluntary cessation of the test by the participant and 5) a rating of perceived exertion (RPE) > 18 on the Borg Scale (Chen 2002). Expired gasses were measured continuously using a TrueMax 2400 metabolic cart.
(ParvoMedics, Sandy, UT). All mouthpieces, breathing hoses and nose clips were sterilized and cleaned with disinfecting solutions.

**40-km Time Trial (TT) Testing (Visit 1 continued)**

Following 15 minutes of recovery from the maximal performance testing, those participants identified as trained cyclists (training ≥ 8 hrs/wk and VO₂ max ≥ 50 ml/kg/min), performed a 40-km TT. A computer display provided participants with gearing, current and average speed, cadence, grade and distance. Participants were instructed to complete the TT as quickly as possible. For purposes of standardization, no outside encouragement was offered during the TT and participants were not allowed to listen to music.

**Supplementation**

In an effort to maintain homogeneous groups, participants were stratified based on age, weight and lumbar BMD measurements. The first two participants were randomly assigned to a group and each new participant was placed in the group most likely to maintain homogeneity. Starting on the morning following the first visit, participants in both groups consumed their respective supplements twice daily (four pills in the morning and four pills in the evening) for the next 12 weeks. They returned empty supplement containers and training/compliance logs (Training and Supplement log – Appendix G) every two weeks, at which time they were given supplements for the next two weeks. Any participant that dropped below six hours per week of training for two consecutive weeks or a total of four weeks during the intervention period were removed from the study. Any participant that dropped below 80% compliance with their respective supplement was removed from the study. Compliance was recorded throughout the 12 weeks (Supplement and Training Compliance – Appendix H). Visit 1 was approximately three hours to complete.

**Post Intervention (Visit 2)**

Following 12 weeks of supplementation, participants reported to the laboratory following a three-hour fast and at least 24 hours after the last strenuous exercise bout. Participants were asked to replicate their three-day dietary log during the final three days of the intervention, return it at Visit 2 and repeat all measurements and exercise testing performed during Visit 1 (BP, HR, blood collection, DXA [body composition, bone status], VO₂ max and 40-km TT). Visit 2 was approximately three hours to complete.
Biochemical Analyses

Blood samples were collected on two occasions (resting baseline and resting post-intervention) under sterile conditions (single-use collection instruments and appropriate handling/disposal of biohazard waste and sharps) and the total amount of 20 ml (two blood draws per each visit) from a forearm vein (the space between the upper and lower arm at the inside of the elbow) were stored for later analysis. Serum samples were centrifuged at 3500 revolutions per minute for 15 minutes at 4 °C; aliquot samples were prepared and stored at -80 °C for later batch analysis of BAP, TRAP5b and SCL. All assays were performed in duplicate using enzyme linked immunosorbent assays (ELISA) according to the manufacturer’s instructions (BioTek Instruments, USA).

Participant Recruitment, Retention and Compliance

Normal weight, healthy male cyclists who lived in the Tallahassee metropolitan and rural areas within reasonable commuting distances were recruited to participate in this study. Participants were recruited via flyers posted on The Florida State University campus, in health facilities, grocery stores, gas stations, and bike shops. E-mail distribution lists from campus organizations and local cycling clubs were also utilized to recruit participants. During the study, supplementation and physical activity were recorded daily and collected every two weeks to enhance compliance and adherence to the supplement intervention. Dietary analysis was conducted at baseline and 12 weeks of supplementation.

Anticipated Risks and Solutions

The risks associated with cycle ergometer exercise testing are minimal and the selected protocols have been previously used in other studies. However, there is a possibility that muscle soreness could occur from the exercise testing. The risk was reduced by using proper warm-up and rest intervals and by using qualified supervisors for the testing. The trained personnel had current cardiopulmonary resuscitation (CPR) and automated external defibrillator (AED) certifications. These precautions minimized the risk for participants. The risks from the blood draw were small and include local discomfort with possible bruising or swelling. The risk of infection was also small. The risk was minimized by the use of skilled technicians using sterile techniques and equipment. There were no risks of adverse events from the calcium collagen chelate or placebo supplements.
Statistical Analyses

Statistical analyses were performed using SPSS version 20. Sample size estimation was determined a priori as a function of the significance criterion (α), the statistical power and effect size (ES). For this experiment, an effect size of 0.72 was calculated based on Barry and Kohrt (2008a) who examined changes in total hip BMD over a four and a half month period during a competitive season in trained cyclists.

\[
ES = \frac{\bar{X}_1 - \bar{X}_2}{s} = \frac{1.061 - 1.040}{0.029} = 0.724
\]

\(\bar{X}_1\) = mean total hip BMD (g/cm\(^3\)) at 4.5 months

\(\bar{X}_2\) = mean total hip BMD (g/cm\(^3\)) at 9 months

\(s\) = standard deviation total hip BMD at 4.5 months

Statistical significance was set at an α = 0.05, ES = 0.72 and a statistical power of 0.80, which yielded a minimum of 10 participants per group. We were able to recruit 29 participants (9 recreational, 10 trained CCC and 10 trained CON). Descriptive statistics (means ± SD) were performed on demographic, anthropometric, and serum biomarker variables.

For research Objective 1, a one-way analysis of variance (ANOVA) was used to evaluate the differences on dependent measures between the recreational and trained cyclists. Pearson product moment correlations and multiple regressions were used to examine relationships between the dependent variables.

For research Objectives 2 and 3, one-way ANOVA were used to compare baseline variables between the CON and CCC. Possible effects of the independent variable, CCC or CON supplementation, on the dependent variables, BMD (whole body, total hip, lumbar spine), BAP, TRAP5b and SCL were evaluated statistically by two-way repeated measures ANOVA (group x time). The null hypothesis was that CCC supplementation would have no effect on the dependent variables. Tukey post hoc tests were used to find means that were significantly different from each other. All significance was accepted at p ≤ 0.05.
CHAPTER 4
RESULTS

Participant Data and Performance Measures: Research Objective 1

Thirty-nine healthy, non-smoking men, currently riding a bicycle at least three hours per week, were contacted to participate in this study. Email and telephone screening were used to identify twenty-nine volunteers eligible for participation. Six of the men were screened out of the study due to performing running exercise more than two days per week. The other four men were removed from participation due to their extensive and recent (within the last six months) resistance training history. Following initial screening and VO₂max testing, the participants were separated into two groups: those who had previous race experience, trained ≥ 8 hr/wk and had VO₂max ≥ 50 ml/kg/min, qualified as trained (n = 20) and the remainder were classified as recreational cyclists (n = 9). Table 1 presents descriptive characteristics of the trained and recreational cyclists. It should be noted that one of the trained cyclists fell below 50 ml/kg/min for his VO₂max but was include in the analysis based on his racing history (20 yr) and weekly training hours (9 h). Also, two recreational riders had VO₂max scores higher than the mean for the trained group but were not included in the trained group because they did not meet the minimum number of weekly training hours, had only recently started riding (2-3 yr) and had no racing experience. One of these individuals competed at the collegiate level as a swimmer and the other in football. The only significant differences between the groups were found in the training related variables, trained cyclists had higher handgrip strength (109.6 ± 15.2 vs. 90.7 ± 7.4 kg), VO₂max (57.3 ± 4.3 vs. 53.1 ± 6.2 ml/kg/min), years of training (15.2 ± 9.1 vs. 7.0 ± 4.8 yr) and weekly training volume (10.4 ± 2.2 vs. 6.2 ± 0.9 h/wk) than recreational cyclists.

Table 1. Descriptive characteristics of the participants (N=29).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Trained Cyclists (n=20)</th>
<th>Range</th>
<th>Recreational Cyclists (n=9)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>38.7 (8.7)</td>
<td>21 – 54</td>
<td>39.4 (8.9)</td>
<td>22 – 53</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.80 (0.07)</td>
<td>1.68 – 1.95</td>
<td>1.75 (0.08)</td>
<td>1.61 – 1.84</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.7 (9.0)</td>
<td>65.0 – 99.4</td>
<td>74.3 (8.3)</td>
<td>64.2 – 88.7</td>
</tr>
<tr>
<td>Body Fat %</td>
<td>19.5 (3.6)</td>
<td>14.2 – 27.9</td>
<td>21.8 (3.3)</td>
<td>17.9 – 28.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.7 (1.5)</td>
<td>20.8 – 26.7</td>
<td>24.4 (1.9)</td>
<td>21.8 – 27.0</td>
</tr>
</tbody>
</table>
Table 1 - continued

<table>
<thead>
<tr>
<th>Variables</th>
<th>Trained Cyclists (n=20)</th>
<th>Range</th>
<th>Recreational Cyclists (n=9)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBM (kg)</td>
<td>58.0 (6.1)</td>
<td>48.3 – 74.9</td>
<td>54.6 (6.4)</td>
<td>45.1 – 66.4</td>
</tr>
<tr>
<td>Handgrip Strength (kg)</td>
<td>109.6 (15.2)*</td>
<td>87.0 – 138.0</td>
<td>90.7 (7.4)</td>
<td>83.5 – 103.5</td>
</tr>
<tr>
<td>VO2max (ml/kg/min)</td>
<td>57.3 (4.3)*</td>
<td>48.2 – 65.4</td>
<td>53.1 (6.2)</td>
<td>38.8 – 59.8</td>
</tr>
<tr>
<td>Training History (yr)</td>
<td>15.2 (9.1)*</td>
<td>4.0 – 30.0</td>
<td>7.0 (4.8)</td>
<td>2.0 – 15.0</td>
</tr>
<tr>
<td>Training Volume (h/wk)</td>
<td>10.4 (2.2)**</td>
<td>7.0 – 16.0</td>
<td>6.2 (0.9)</td>
<td>5.0 – 7.5</td>
</tr>
</tbody>
</table>

Values are means (SD)

BMI = Body Mass Index; LBM = Lean Body Mass; VO2max = Maximal Oxygen Uptake

*Trained Cyclists significantly greater than Recreational Cyclists, p < 0.05

**Trained Cyclists significantly greater than Recreational Cyclists, p < 0.001

BMD measurements are presented in Table 2. There were no significant differences in BMD between the trained and recreational cyclists at any of the measurement sites. Evaluation of T-scores identified lumbar BMD as osteopenic (-2.5 < T-score < -1.0) in both trained and recreational cyclists (-1.49 ± 1.09 and -1.16 ± 0.84, respectively). When lumbar scans were evaluated individually, 12 trained cyclists and two recreational cyclists were identified as osteopenic and three trained cyclists were identified as osteoporotic (T-score ≤ 2.5). None of the recreational cyclists were classified as osteoporotic. When Z-scores were used, considered more appropriate for populations under the age of 50, the same individuals were identified as osteopenic and osteoporotic. Significant Pearson moment correlations were found when VO2max Power (max power achieved during the maximal graded exercise test) and whole body BMD (r = 0.396) were compared, no other significant correlations were found.

Table 2. Bone mineral density (g/cm²) of the participants (N=29).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Trained Cyclists (n=20)</th>
<th>T-score / Z-score</th>
<th>Recreational Cyclists (n=9)</th>
<th>T-score / Z-score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Body</td>
<td>1.115 (0.083)</td>
<td>-0.77 (0.81) / -0.72 (0.79)</td>
<td>1.134 (0.085)</td>
<td>-0.39 (0.57) / -0.36 (0.55)</td>
</tr>
<tr>
<td>Lumbar Spine (L1-L4)</td>
<td>0.925 (0.119)</td>
<td>-1.49 (1.09) / -1.38 (1.06)</td>
<td>0.989 (0.071)</td>
<td>-1.16 (0.84) / -1.01 (0.89)</td>
</tr>
<tr>
<td>Right Hip</td>
<td>0.900 (0.088)</td>
<td>-0.88 (0.58) / -0.69 (0.55)</td>
<td>0.954 (0.136)</td>
<td>-0.66 (0.90) / -0.48 (0.88)</td>
</tr>
<tr>
<td>Left Hip</td>
<td>0.896 (0.080)</td>
<td>-0.91 (0.52) / -0.72 (0.50)</td>
<td>0.944 (0.132)</td>
<td>-0.73 (0.85) / -0.54 (0.81)</td>
</tr>
</tbody>
</table>

Values are means (SD)

**Participant Data and Performance Measures: Research Objectives 2 & 3**

Following initial screening and performance of the VO2max testing, 20 participants were identified as eligible for participation in the trained cyclists group. Two participants were later
removed from the study after falling below six hours per week of training and failing to take the supplement at least six out of seven days for two consecutive weeks. The remaining 18 participants completed the entire protocol and were 100% compliant on supplementation. See Table 3 for the descriptive characteristics of the trained cyclists. There were no significant differences between the supplement (CCC) and control (CON) groups for any of the variables presented in Table 3.

Table 3. Descriptive characteristics of the trained cyclists (N=18).

<table>
<thead>
<tr>
<th>Variables</th>
<th>CCC (n=9)</th>
<th>Ranges</th>
<th>CON (n=9)</th>
<th>Ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>38.6 (11.2)</td>
<td>21 – 54</td>
<td>38.6 (7.0)</td>
<td>30 – 52</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.77 (0.08)</td>
<td>1.68 – 1.95</td>
<td>1.82 (0.06)</td>
<td>1.71 – 1.89</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.2 (10.5)</td>
<td>65.0 – 99.4</td>
<td>78.4 (8.7)</td>
<td>66.4 – 95.5</td>
</tr>
<tr>
<td>Body Fat %</td>
<td>18.2 (4.3)</td>
<td>14.2 – 27.9</td>
<td>20.4 (2.9)</td>
<td>15.6 – 23.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.9 (1.5)</td>
<td>22.5 – 26.1</td>
<td>23.6 (1.8)</td>
<td>20.8 – 26.7</td>
</tr>
<tr>
<td>LBM (kg)</td>
<td>57.6 (6.1)</td>
<td>48.3 – 65.7</td>
<td>58.8 (7.0)</td>
<td>50.9 – 74.9</td>
</tr>
<tr>
<td>Handgrip Strength (kg)</td>
<td>104.4 (13.6)</td>
<td>87 – 126</td>
<td>114.8 (15.7)</td>
<td>87 – 138</td>
</tr>
<tr>
<td>Training History (yrs)</td>
<td>16.3 (11.2)</td>
<td>4.0 – 30.0</td>
<td>14.6 (7.9)</td>
<td>5.0 – 26.0</td>
</tr>
<tr>
<td>Training Volume (hrs/wk)</td>
<td>10.4 (2.3)</td>
<td>7.5 – 16.0</td>
<td>10.3 (2.5)</td>
<td>7.0 – 16.0</td>
</tr>
</tbody>
</table>

Values are means (SD)
BMI = Body Mass Index; LBM = Lean Body Mass

Trained participants performed VO₂max exercise testing and 40-km TT bouts pre- and post-intervention. The tests were used to qualify the participants as trained cyclists and to ensure sustained training volume during the intervention. The hourly training volumes by week are found in Figure 1. There was no group*time effect on hourly training volume. The results of these performance tests are found in Table 4.

Repeated measures ANOVA did not identify any significant group*time effects on the performance measures related to maximal exercise testing or the time trial exercise bout. Repeated measures ANOVA did identify significant time effects on TT performance. TT average power increased from 248.9 ± 35.6 W to 261.6 ± 42.5 W in the CCC group and increased from 264.0 ± 58.6 W to 267.6 ± 65.3 W in the CON group over the 12-wk period. TT power to weight increased from 3.3 ± 0.4 W/kg to 3.5 ± 0.4 W/kg in the CCC group and increased from 3.3 ± 0.5 W/kg to 3.4 W/kg in the CON group over the 12-week period. Time to completion improved from 66.9 ± 3.6 min to 65.5 ± 3.9 min in the CCC group and improved from 65.6 ± 4.8 min to 65.3 ± 5.3 min in the CON group over the 12-week period. However, there were no differences in improvements over time between the two groups for any variable.
Figure 1. Hourly training volumes by week of the trained cyclists (N=18).

Table 4. Performance measures of the trained cyclists pre- and post-intervention (12 weeks) (N=18).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group</th>
<th>Pre</th>
<th>Post</th>
<th>P values</th>
<th>Group</th>
<th>Time</th>
<th>Group x Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO$_{2\max}$ (ml/kg/min)</td>
<td>CCC</td>
<td>58.7 (3.5)</td>
<td>59.1 (4.2)</td>
<td>0.464</td>
<td>0.382</td>
<td>0.739</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>57.0 (4.8)</td>
<td>57.8 (5.1)</td>
<td>0.673</td>
<td>0.119</td>
<td>0.590</td>
<td></td>
</tr>
<tr>
<td>VO$_{2\max}$ Power (W)</td>
<td>CCC</td>
<td>402.8 (42.3)</td>
<td>397.2 (50.7)</td>
<td>0.673</td>
<td>0.119</td>
<td>0.590</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>416.7 (61.2)</td>
<td>405.6 (65.9)</td>
<td>0.676</td>
<td>0.282</td>
<td>0.716</td>
<td></td>
</tr>
<tr>
<td>VO$_{2\max}$ Power/Wt (W/kg)</td>
<td>CCC</td>
<td>5.4 (0.4)</td>
<td>5.3 (0.4)</td>
<td>0.676</td>
<td>0.282</td>
<td>0.716</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>5.3 (0.5)</td>
<td>5.2 (0.6)</td>
<td>0.676</td>
<td>0.282</td>
<td>0.716</td>
<td></td>
</tr>
<tr>
<td>Time Trial Avg Power (W)</td>
<td>CCC</td>
<td>248.9 (35.6)</td>
<td>261.6 (42.5)*</td>
<td>0.569</td>
<td>0.017</td>
<td>0.853</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>264.0 (58.6)</td>
<td>275.0 (65.7)*</td>
<td>0.569</td>
<td>0.017</td>
<td>0.853</td>
<td></td>
</tr>
<tr>
<td>Time Trial Power/Wt (W/kg)</td>
<td>CCC</td>
<td>3.3 (0.4)</td>
<td>3.5 (0.4)*</td>
<td>0.826</td>
<td>0.035</td>
<td>0.823</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>3.3 (0.5)</td>
<td>3.4 (0.5)*</td>
<td>0.826</td>
<td>0.035</td>
<td>0.823</td>
<td></td>
</tr>
<tr>
<td>Time To Completion (min)</td>
<td>CCC</td>
<td>66.9 (3.6)</td>
<td>65.5 (3.9)*</td>
<td>0.585</td>
<td>0.019</td>
<td>0.642</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>65.6 (4.8)</td>
<td>64.6 (5.1)*</td>
<td>0.585</td>
<td>0.019</td>
<td>0.642</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD  
VO$_{2\max}$ = Maximal Oxygen Consumption; Wt = Weight; Avg = Average  
* Post significantly different than Pre (p < 0.05)

Bone Mineral Density and Biomarkers of Bone Metabolism

Bone mineral density was assessed pre- and post-intervention and are presented in Table 5. No differences in BMD were found at any of the measurement sites at baseline (pre) or following 12-weeks of the intervention (post) in the CCC or CON groups. Repeated measures ANOVA did not identify any significant time or group*time effects on BMD in either group. Strong positive
Pearson moment correlations were found between weekly training hours and lumbar BMD (r = 0.473).

Similar to the relationship found in Objective 1 significant positive Pearson moment correlations were found when VO2 max power (max power achieved during the maximal graded exercise test) and whole body BMD were compared (r = 0.549). Left hip BMD was also positively correlated to VO2 max power (r = 0.558). When comparing VO2 max, strong positive correlations were found with right and left hip BMD (r = 0.495 and r = 0.522, respectively). VO2 max power to weight ratio was strongly correlated to lumbar, right and left hip BMD (r = 0.47, r = 0.528 and r = 0.521, respectively). Better performance during the 40-km TT, as determined by shorter time to completion, TT average power output and TT power to weight ratio, was strongly correlated with left hip BMD (r = -0.591, r = 0.509 and r = 0.547, respectively).

Table 5. Bone mineral density (g/cm^2) pre- and post-intervention (12 weeks) during habitual training (N=18).

<table>
<thead>
<tr>
<th>Variables (g/cm^2)</th>
<th>Group</th>
<th>Pre</th>
<th>Post</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Group</td>
</tr>
<tr>
<td>Whole Body</td>
<td>CCC</td>
<td>1.120 (0.082)</td>
<td>1.088 (0.066)</td>
<td>0.801</td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>1.113 (0.095)</td>
<td>1.114 (0.098)</td>
<td></td>
</tr>
<tr>
<td>Lumbar Spine (1.1 – 1.4)</td>
<td>CCC</td>
<td>0.929 (0.118)</td>
<td>0.923 (0.119)</td>
<td>0.907</td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>0.916 (0.139)</td>
<td>0.922 (0.136)</td>
<td></td>
</tr>
<tr>
<td>Right Hip</td>
<td>CCC</td>
<td>0.933 (0.062)</td>
<td>0.940 (0.064)</td>
<td>0.111</td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>0.871 (0.104)</td>
<td>0.866 (0.101)</td>
<td></td>
</tr>
<tr>
<td>Left Hip</td>
<td>CCC</td>
<td>0.924 (0.054)</td>
<td>0.932 (0.054)</td>
<td>0.121</td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>0.868 (0.100)</td>
<td>0.865 (0.101)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD

Biomarkers of bone metabolism were analyzed using ELISA techniques, the results are presented in Table 6. Repeated measures ANOVA did not identify any significant time or group*time effects on BAP, TRAP5b, SCL or BAP/TRAP5b ratio in either group. Strong Pearson moment correlations were found between age and TRAP5b as well as BAP/TRAP5b ratio (r = -0.664 and r = 0.589, respectively). Training years and training hours were also strongly correlated with TRAP5b (r = -0.546 and r = 0.531, respectively). Fat mass percentage was the only other descriptive characteristic correlated to the resting biomarkers, negatively related to TRAP5b (r = -0.556) and positively to BAP/TRAP5b ratio (r = 0.522). Comparison of VO2 max to the biomarkers revealed a negative correlation between BAP and BAP/TRAP5b ratio (r = -0.561 and -0.597,
respectively). No other relationships were found between performance measurements and biomarkers of bone metabolism.

In regards to the relationships between measures of BMD and the biomarkers of bone metabolism, BAP and BAP/TRAP5b ratio were found to have significant correlations. BAP was negatively correlated to right hip BMD ($r = -0.660$) and was approaching a significant ($p = 0.059$) negative correlation to left hip BMD ($r = -0.467$). BAP/TRAP5b ratio was found to be negatively correlated to both right and left hip BMD ($r = -0.649$ and $r = -0.646$, respectively). No other biomarkers were correlated with BMD at any site.

**Table 6.** Resting biomarkers of bone metabolism pre- and post-intervention (12 weeks) during habitual training (N=18).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group</th>
<th>Visit</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>BAP (U/L)</td>
<td>CCC</td>
<td>31.73 (7.63)</td>
<td>32.87 (5.37)</td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>36.16 (7.72)</td>
<td>35.61 (7.39)</td>
</tr>
<tr>
<td>TRAP5b</td>
<td>CCC</td>
<td>3.29 (0.95)</td>
<td>3.45 (0.88)</td>
</tr>
<tr>
<td>(U/L)</td>
<td>CON</td>
<td>2.97 (0.72)</td>
<td>2.99 (0.67)</td>
</tr>
<tr>
<td>SCL (ng/ml)</td>
<td>CCC</td>
<td>0.45 (0.13)</td>
<td>0.45 (0.08)</td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>0.44 (0.07)</td>
<td>0.44 (0.06)</td>
</tr>
<tr>
<td>BAP/TRAP</td>
<td>CCC</td>
<td>9.93 (2.42)</td>
<td>9.91 (2.31)</td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>12.64 (3.35)</td>
<td>12.51 (3.83)</td>
</tr>
</tbody>
</table>

Values are means ± SD
BAP = Bone Alkaline Phosphatase; TRAP = Tartrate Resistant Acid Phosphatase; SCL = Sclerostin
CHAPTER 5

DISCUSSION

The purpose of this study was three-fold, 1.) Identify whether there were differences in measures of body composition and BMD between healthy, non-smoking age and weight matched trained (≥ 8 h/wk of cycling) and recreational (3 < h/wk of cycling < 8) cyclists, 2.) determine effects of 12-week calcium collagen chelate (CCC) supplementation on body composition/BMD during normal training and 3.) Determine whether 12 weeks of CCC supplementation would affect resting biomarkers of bone metabolism during habitual training. The main findings of this study were 1.) no differences in body composition or BMD were measured between our recreationally active group and our high-volume, competitively training group of cyclists, 2.) no change in BMD over the course of 12 weeks of training (> 10 h/d) and no effect from CCC supplementation and 3.) no change in resting biomarkers of bone metabolism over the course of 12 weeks of training and no effect from CCC supplementation. Therefore, we were not able to reject the null hypothesis for Objective 1, there was no difference between age and size matched male recreational cyclists and competitively trained cyclists in regard to their body composition and BMD. We were unable to reject the null hypotheses for Objectives 2 & 3; there were no differences in BMD or biomarkers of bone metabolism between the CCC and CON groups. There were no differences in BMD or resting biomarkers of bone metabolism over the course of 12 weeks in competitively trained male cyclists.

Recreational vs. Trained Cyclists

Objective 1 involved a cross-sectional analysis of two groups of healthy, active adult males who maintain their fitness almost exclusively through bicycling. The fundamental difference between these two groups of men was one group was “competitive racers” and the other group was recreational cyclists. This distinction was supported by significant differences in training hours per week (10.4 ± 2.2 vs. 6.2 ± 0.9 h/wk) and VO₂ max values (57.3 ± 4.3 vs. 53.1 ± 6.2 ml/kg/min), with the competitively trained group having higher values. The competitively trained group also had considerably more years of exposure to bicycle exercise (15.2 ± 9.1 vs. 7.0 ± 4.8 yr) than the recreational group. In addition to differences in these bike specific training variables, the trained group also had higher handgrip strength than the recreational riders (109.6 ± 15.2 vs. 90.7 ± 7.4 kg).
When analyzing the range of performances on the handgrip test, it should be noted that the trained group had a very large range of values (87.0 – 138.0 kg) when compared to the recreational group (83.5 – 103.3 kg). Anecdotally, the trained group seemed to have a more “athletic and competitive” background. Therefore, this score may have been due to inherently better motor control and coordination affording this group better performance on a test of upper body strength, an area in which neither group trains.

Comparison of BMD between the trained and recreational groups at the whole body (1.115 ± 0.083 vs. 1.134 ± 0.085 g/cm²), lumbar (0.925 ± 0.119 vs. 0.989 ± 0.071 g/cm²), right (0.900 ± 0.088 vs. 0.954 ± 0.136 g/cm²) and left hip (0.896 ± 0.080 vs. 0.944 ± 0.132 g/cm²) regions showed no differences at any site, respectively. Although there were no differences in BMD between the groups of cyclists, analysis of the T-score and more appropriately Z-score for this younger (< 50 yr) adult population identified both trained (-1.49 ± 1.09 and -1.38 ± 1.06, respectively) and recreational cyclists (-1.16 ± 0.84 and -1.01 ± 0.89, respectively) as osteopenic at the lumbar spine, similar to previous findings (Smathers et al., 2009). Though statistical significance was not achieved at any site, when the lumbar scans were evaluated individually, 15 of the trained cyclists were identified as osteopenic (n = 12) or osteoporotic (n = 3) while only two of the recreational group were identified as osteopenic with none of the recreational cyclists being classified as osteoporotic. Seventy-five percent prevalence of below average BMD in the trained group versus 22% prevalence in the recreational group is a noteworthy difference.

Similar to the study by Smathers et al. (2009) where they found 7.1% lower L1-L4 lumbar BMD in competitive road cyclists when compared to age and body-mass matched controls, our lumbar scans show almost a 7% difference in BMD and were approaching significance (p = 0.146); it is possible that a small sample size or significant training effect in the recreational group affected this analysis. Additionally, one of the cyclists from the recreational group competed at the collegiate level in distance swimming, which has been shown to impact BMD in ways similar to cycling (Hind et al., 2011). When this individual was removed from the recreational group, differences approached significance at the lumbar (p = 0.078), right hip (p = 0.052) and left hip (p = 0.060) sites and all of the measurement sites fell within the normal range for BMD based off of T- and Z-scores. These findings suggest that trained male cyclists performing high-volume training have lower BMD than age and weight matched male cyclists performing lower volume training.

In response to the sample size issue, recruitment for the recreational group proved difficult; many individuals that expressed interest in participating were involved in too many hours of load-
bearing exercise to be eligible for testing. In regards to the training effect on the recreational group, in the study by Smathers et al. (2009), the recreationally active group was not cyclists and exercised no more than three days per week. When the aerobic fitness and/or training volume of our recreational group was compared to other studies investigating BMD they were similar to trained cyclists (Barry and Kohrt, 2008b) and higher than recreationally active groups (Nichols et al., 2003; Nichols et al., 2011; Smathers et al., 2009). It is possible, that the recreational group from our study was too highly trained on the bicycle to show any significant difference from our trained group. Observation of the weekly training volume over 6 hours in the recreational group may be enough to contribute to the non-weight bearing effects from cycling proposed by previous research (Nichols et al., 2003; Rector et al., 2008).

Investigating potential differences in the biomarkers of bone metabolism may have shed more light on the differences between these two groups, but was beyond the scope of the current project. Previous work with cyclists has focused on those individuals involved in racing and who participate in excessively large volumes of training (Barry and Kohrt, 2008b; Guillaume et al., 2012; Nichols et al., 2003; Nichols et al., 2011; Rector et al., 2008; Smathers et al., 2009). Our work shows the need to investigate the effects of cycling in more recreational populations, particularly considering the charge in our society to maintain activity level with aging and the increasing popularity of the sport of cycling. Future work in this area should be performed with larger and more distinctly separate groups of competitively trained and recreationally active cyclist.

It should be noted that even with the “swimmer” removed from the group, our recreational cyclists fell into the low-normal range with lumbar BMD almost osteopenic at a Z-score of -0.9. Although data on the prevalence of osteoporotic fractures specific to aging populations of cyclists are not available, low BMD has been shown to be a strong predictor of fracture risk in the general adult population (Nikander et al., 2010). The necessity of overall bone health is key due to the role of bone in maintaining calcium balance, hemopoiesis and providing a structural foundation for the maintenance of daily physical activity (Banfi et al., 2010). Even though the majority of low-trauma fractures occur in people who are normal to osteopenic, suggesting there is more to bone strength than density, achieving normal levels of bone density in cyclists should be attainable (Smathers et al., 2009). Attempts at promoting bone growth in adult populations have utilized calcium, collagen and vitamin D supplementation but never combined in an athletic population (Barry et al., 2011; Guillemant et al., 2004; Cuneo et al., 2010).
Maintenance of Low Bone Density in Trained Cyclists

The purpose of Objectives 2 and 3 was to investigate the potential effects of 12 weeks of supplementation with the dietary supplement calcium collagen chelate (CCC) had on body composition, bone density and biomarkers of bone metabolism when consumed during habitual training in the competitive season of a trained male cyclist. The competitively trained groups were selected from the group of cyclists that volunteered to participate in the cross-sectional analysis component of Objective 1 who met the following guidelines: history of racing, VO_{2} max \geq 50 ml/kg/min and training \geq 8 h/wk on the bike with less than two weight-bearing exercise sessions per week. From the original group, 20 men were selected to participate. Once qualified, the men were stratified into groups based on age, weight and lumbar BMD. Two of the men were later removed from the study due to failure to comply with training and supplementation adherence, the remaining 18 men completed the entire study.

Participants were asked to consume, a maltodextrin placebo plus a 600 mg calcium carbonate and 400 I.U. vitamin D supplement (CON) or the six g/d calcium collagen chelate plus 600 mg calcium carbonate and 400 I.U. supplement (CCC) daily for 12 weeks while maintaining their normal training volume. The compliance rate for the 18 men completing the study was 100%; collecting used containers every two weeks prior to distributing the next two weeks of supplements monitored compliance. Baseline analysis of the descriptive characteristics of the CCC and CON groups revealed no differences in age, height, weight, lean body mass, handgrip strength, training history or training volume.

No significant correlations were found between weekly training hours, VO_{2} max, max power, average power during the TT, lean body mass or fat mass. However, measures of power to weight ratio in both the max test (r = 0.599) and the TT (r = 0.507) were positively associated with weekly training hours. Therefore, the number of hours spent training on the bike must be focused on optimizing performance not simply increasing mileage. To determine whether training volume had been maintained, VO_{2} max and 40-km TT tests were performed at the end of the 12-week period. There were no performance differences in either group for the maximal exercise test or 40-km TT pre- or post-intervention, suggesting 12 weeks supplementation of the CCC did not have a measureable effect on performance. While no improvements were seen in VO_{2} max, both groups experienced significant improvements in 40-km TT performance indicating maintenance of training volume. TT average power increased (5.1% and 4.2%), TT power to weight ratio increased (5.1% and 4.3%) and time to completion improved (2.1% and 1.5%) in the CCC and CON groups,
respectively. The improvements in TT performance were most likely due to a learning effect between the first and second trials considering there was no increase in training volume during the intervention. Most of the men did not have experience working with a stationary cycle ergometer and seemed to have more successful pacing during the second trial.

Baseline BMD was measured at four sites, whole body, lumbar (L1-L4) spine, right and left hips; no differences were present between groups at any site. Following 12 weeks of training and supplementation, no differences were measured in either group at any site. These results are noteworthy when compared to previous research showing a decline in BMD over an 18-week interval, specifically at the hip and femur that persisted at a 12-month follow-up in trained male cyclists over the course of a competitive season (Barry and Kohrt, 2008b). Comparison of BMD measurements at the total hip from Barry and Kohrt (2008b), showed a significant decline in BMD from 1.061 g/cm² to 1.040 g/cm² in the high-dose calcium group and from 1.013 g/cm² to 0.999 g/cm² in the low-dose group. In contrast, our men saw no change over the course of 12 weeks at the right hip, 0.904 g/cm² to 0.905 g/cm², or left hip 0.897 g/cm² to 0.900 g/cm².

The values obtained by Barry and Kohrt (2008b) are considerably higher than the values for our trained cyclists, but are similar to those measured in our group of recreational cyclists at the right (0.983 g/cm²) and left (0.971 g/cm²) hip. This relationship is likely due to the fact that our recreational cyclists were similar to their trained cyclists. Comparison of our trained and recreational cyclists to their trained cyclists in terms of years of training (15.2 ± 9.1 vs. 7.0 ± 4.8 vs. 6.6 ± 4.8 yr, respectively) and VO₂max (57.3 vs. 53.1 vs. 51.7 ml/kg/min, respectively) show the extent of similarity between the two groups.

It is possible our group of trained cyclists had reached a critical level of BMD, no longer losing bone due to training. In a study by Nichols et al. (2011), investigators measured BMD in master cyclists (50.7 ± 4.0 yr) who had trained for 20.4 ± 6.7 yr at over 10 hr/wk. They found BMD similar to our group of trained cyclists at the lumbar (0.937 vs. 0.925 g/cm², respectively) and hip regions (0.871 g/cm² total hip vs. 0.900 right and 0.896 left hip). In fact, in our trained group, Pearson moment correlations revealed a strong positive relationship between weekly training hours and lumbar BMD (r = 0.473), suggesting more cycling activity was beneficial to BMD in our group of men. This is in contrast to the theory that significant time in a non-weight bearing position on the bike seat contributes to declines in BMD, particularly at the lumbar spine (Rector et al., 2008; Samthers et al., 2009). It is possible that an adaptation to high-level training on the bicycle is a
reduction in BMD that reduces the energy cost of locomotion without sacrificing strength to resist fracture.

Continuing along the same trend, VO₂max was positively associated with right (r = 0.495) and left (r = 0.522) hip BMD and VO₂max power with whole body (r = 0.549) and left hip (r = 0.558) BMD. Power to weight ratio during the VO₂max test also revealed strong positive correlations with lumbar (r = 0.47), right (r = 0.509) and left (r = 0.547) hip BMD. This is in contrast to previous work showing a negative association with VO₂max in collegiate male athletes (Ackerman et al., 2011). However, even though higher performance values were associated with higher BMD they are still lower than the general population. Our findings were the result of measuring a fairly homogenous population of individuals with low BMD.

The relationship between higher power output and higher BMD should not come as a surprise due to the increased contractile muscle-induced joint strain that would be experienced in athletes with greater power output (Barry and Kohrt, 2008a). In order to explain the relationship between training hours and lumbar BMD, energy availability and body composition may provide some support for our findings. Diminished energy availability, the difference in energy consumed and energy used during exercise, has been shown to directly and indirectly affect bone metabolism by suppressing triiodothyronine (T3), insulin-like growth factor 1 (IGF-1), insulin and testosterone (Ducher et al. 2011; Ihle and Loucks, 2004). In our study, the men in both groups maintained bodyweight and LBM during the 12-week intervention, suggesting adequate energy availability. Additionally, in a study of collegiate male athletes, investigators found that total and free estradiol levels were positively associated with BMD, noting that increased body fat is associated with higher levels of estradiol (Ackerman et al. 2011). Maintenance of LBM (57.6 ± 6.1 and 58.8 ± 7.0 kg, respectively) combined with the relatively high body fat (18.2 ± 4.3 and 20.4 ± 2.9 %, respectively) in the CCC and CON groups of our study may have acted as a protective mechanism in maintaining BMD during high levels of training. However, Barry and Kohrt (2008b) had similar values for body fat at 18.7% and fat free mass of 59.1 kg and did not experience the same protective mechanism. This provides further support for a potential critical level of BMD that is maintained in the body of trained cyclists. In an effort to identify underlying mechanisms possibly responsible for the reduction in BMD often found in cyclists, biomarkers of bone metabolism were measured.
Biomarkers of Bone Metabolism

Due to the highly dynamic nature of bone tissue, the measurement of biomarkers associated with bone metabolism has the potential to offer insight into the physiological changes responsible for reducing BMD. In order to get a clearer picture of the balancing act of maintaining healthy bone turnover, we measured markers associated with the three cell types found in bone responsible for resorption and formation activity. BAP and TRAP5b were analyzed to measure the activity of osteoblasts responsible for bone formation and the osteoclasts responsible for bone resorption, respectively. SCL was measured to determine the influence of osteocytes, the most prominent cell type found in bone, since they have been shown to be the most receptive to mechanical stimulation and to mediate bone turnover (Burger et al., 1999; Lin et al., 2009; Zernicke et al., 2006).

Few studies have investigated the effects of cycling on biomarkers of bone metabolism and only one of these studies looked at longitudinal changes under regular training conditions (Barry et al., 2011; Guillaume et al. 2012; Guillemant et al., 2004; Lombardi et al., 2012; Rector et al., 2008). Baseline measurements of BAP revealed that our cyclists had no change in BAP over the 12-week period in either group. These results were similar to other studies, both acute and long-term, that showed no change in BAP in cyclists (Barry et al., 2011; Guillemant et al., 2004; Lombardi et al., 2012). Comparison of baseline BAP revealed that our values were higher than those reported by Rector et al. (2008) at 7.0 ± 1.0 U/L, Lombardi et al. (2012) at less than 20 U/L and Barry et al. (2011) at 21.98 ± 1.53 – 24.95 ± 1.71 U/L. Although reference ranges have not been established in healthy men, our values of 31.73 ± 7.63 – 36.16 ± 7.72 U/L fell within the normal range established for healthy pre-menopausal women of 14.8 to 38.8 U/L (Eastell et al., 2012). In our group of cyclists, BAP was negatively associated with VO\text{max} (r = -0.561) but none of the men were below the normal range. When BAP was compared to measures of BMD, negative relationships were found at the right hip (r = -0.660) and left hip T-score (r = -0.491). Although BAP is often considered a measurement of bone formation activity, it can also represent a marker of whole bone turnover. Therefore, higher levels of BAP could represent an environment of increased bone turnover that does not allow for the maturation of bone and leads to reductions in BMD as seen in our hip scans (Avbersek-Luznik et al. 2007).

A number of biomarkers for bone resorption exist. However, many of them are related to the measurement of degraded byproducts of bone resorption like CTX and NTX, whereas TRAP5b is the only measure of osteoclast number and activity (Civitelli et al., 2009; Vasikaran et al., 2011). In a study by Lombardi et al. (2012), investigators followed professional cyclists during an intense
three-week cycling race. Over 22 days, they found TRAP5b levels to significantly increase from approximately 2.5 to over 3.5 U/L. Although we did not see the same increase in TRAP5b, our values were similar at 2.97 – 3.45 U/L. These values appear to be slightly higher than those measured in physically active, healthy men of similar age (Rogers et al., 2011). Since reference values for TRAP5b do not exist, it is difficult to determine the clinical significance of these values.

When TRAP5b was compared to age and training years, negative correlations were found suggesting that older athletes with more years of training had lower levels of TRAP5b. When TRAP5b was compared to lumbar BMD, higher levels of TRAP5b were associated with higher values of BMD. Our men were intentionally recruited beyond the age of peak bone mass attainment and prior to the age of any potential accelerated bone loss, these correlational relationships may be less important than the fact that TRAP5b did not increase over the course of regular training, suggesting that bone resorption did not increase as a result of training. However, it is unclear whether these values were high at baseline and therefore less likely to increase any farther over the course of the intervention. For instance, when baseline TRAP5b was compared to training hours per week, those athletes with greater hours of training had higher levels of TRAP5b. Whether these levels would be considered unusually high has yet to be determined.

Due to the lack of established reference values for the biomarkers of bone metabolism, we also look at the relationship between bone formation and bone resorption in the form of BAP/TRAP5b ratio. Higher values for this ratio may represent an environment that favors formation or may simply represent higher levels of bone turnover due to elevated BAP (Avbersek-Luznik et al., 2007). When the BAP/TRAP5b ratio was compared to measures of BMD, the only relationships found were negative at the right ($r = -0.649$) and left ($r = -0.646$) hips. In our population, higher BAP/TRAP5b ratio may simply represent increased bone turnover instead of formation. This increased turnover could limit the amount of mature bone that can be maintained and over time could result in lower BMD.

The final marker measured in our study was SCL, released almost exclusively by osteoclasts, and may be the most sensitive to changes in mechanical loading (Atkins et al., 2012; Winkler et al., 2003). Increased levels of SCL have been associated with reductions in osteoblast activity and could result in reduced BMD. Previous research has linked high levels of SCL to increased risk of vertebral fracture (Yamamoto et al., 2013). This association is likely due to the Wnt pathway inhibition resulting in reduced osteoblast and bone formation activity (Winkler et al., 2003). Although baseline SCL levels of 0.44 – 0.45 ng/mL in our trained cyclists were low when compared

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to a study in healthy young men (0.81 ng/mL) and older adult men (1.79 ng/mL), SCL has been shown to be elevated in the short-term and potentially reduced in the long term during exposure to non-weight bearing activities (Spatz et al., 2012; Yamamoto et al., 2013). In a short-term bed rest study, 90 days, investigators found SCL increased from 0.81 ng/mL to 1.12 ng/mL (Spatz et al. 2012). A cross-sectional study by Yamamoto et al. (2013) involving spinal cord injury patients found those individuals with less than three years of injury duration to have higher SCL levels than those with three to five years. It was proposed that over time increased SCL resulted in lower BMD, less mature bone and consequently less SCL. Due to the lengthy training history and low BMD of our cyclists, it is possible that they are producing less SCL due to less mature bone.

**Conclusion**

The results of our study suggest that while cycling is beneficial to cardiovascular, pulmonary and metabolic health outcomes, bone does not respond in the same favorable manner. Although there was no difference in BMD between the trained and recreational cyclists, both groups had lower than normal BMD. Of most concern was the high prevalence of osteopenic and osteoporotic BMD at the lumbar spine. As noted previously, when the “swimmer” was removed from the recreational group, the difference between trained and recreational cyclists became more distinct. Since most people who decide to use cycling as their primary form of exercise will fall into this recreational category, future research should be done to determine what effects moderate levels of training on the bike will have on bone health.

When the bone health of the trained group of cyclists was investigated, BMD was significantly reduced leading to 12 men identified as osteopenic and three osteoporotic. However, although BMD was low across the entire group, the fitter and higher performing the cyclist, the better their BMD. When the BMD was compared from our study to others looking at highly trained cyclists, BMD was very similar. It is possible that reduced BMD may be an adaptation to cycling that makes it easier to perform the activity due to reduced body mass but maintains strength due to structural changes in bone.

When determining the effectiveness of the calcium collagen chelate supplement to increase BMD, affect body composition or biomarkers of bone metabolism, we found no effect from supplementation. Higher weekly training hours were associated with higher baseline levels of TRAP5b. However, BAP, TRAP5b and SCL remained at the same level throughout the study. This is most likely the result of high bone turnover that remained high during the ≥ 10 h/wk of bicycle
training performed by the participants over the 12 weeks. Chronically high levels of bone turnover would result in less mature bone being maintained in the skeleton. Future work investigating structural changes in bone, as well as long-term fracture incidence and prevalence rates in trained cyclists should be performed to determine whether or not the reduced BMD experienced by cyclists places these athletes at greater risk for fractures.
APPENDIX A

TELEPHONE SCREENING
The Florida State University
Nutrition, Food, and Exercise Sciences

Name ___________________  Date _________  ID ______________

Phone screenings will be completed in order for the research team to understand if interested participants are eligible and to avoid any unnecessary time commitment for potential participants. Because this study requires participants to be healthy male cyclists and to be free from the use of performance and bone enhancing supplements for at least 4 weeks, the researchers directly involved with this study (Chris Mojock, Dr. Ormsbee, Dr. Kim and Dr. Panton) will conduct a phone or email screening with the following questions.

Nutritional Supplements and Medications
Please list all vitamins, minerals and herbs and other nutritional (performance) supplements as well as medications you are currently taking. (examples: creatine monohydrate, nitric oxide, hydroxy-beta-methylbutyrate (HMB), androsterone derivatives, vitamin E, pharmacological agents, etc.)

How long have you been taking them?

How frequently?

If you are currently taking any of these supplements are you willing to stop taking them for a period of one month and through the duration of the 12-week study and through pre and post testing?

EXERCISE
Do you perform at least three hours of training per week on the bike?  Y  N
Do you perform at least eight hours of training per week on the bike?  Y  N
Do you perform any other forms of exercise regularly?  Y  N
What kinds of exercise?

How often? Please be detailed in a description of your average week of training.

Please list the last three cycling events (races, centuries, time trials, etc.) that you have participated in and how you finished/placed (e.g. Century – 4:20 or 32 mile mtb race – 3rd place Sport class):
Do you have any current conditions that might prevent you from completing maximal exercise testing and a 35-km time trial (examples: tendonitis, pulled muscle, torn ligament, knee or back problem)?

**MEDICAL**
Do you have uncontrolled hypertension (BP>160/100 mmHg)?
Do you have diagnosed cardiovascular disease, stroke, or diabetes?
Do you have thyroid or kidney dysfunction?
Do you currently smoke or chew tobacco? How many/much? Frequency?
Do you take cholesterol medication or blood pressure medication? If so, what do you take?
Do you have any allergies?
APPENDIX B

THREE-DAY DIETARY LOG

1. Keep your 3-day food record on three consecutive days.

2. Please record each food you eat immediately after you eat it.

3. Record only one food item per line.

3. Be as specific as possible when describing a food eaten: how it was cooked and the amount you ate. Don’t forget to include all beverages you drink. For example: Coffee with 1 tsp. Cream, 12 oz. Regular Coke, or 8 oz. Sweetened Tea.

4. Include brand names or labels from food items whenever possible.

5. Record amounts eaten in household measures. For example: one cup nonfat milk, 3 ounces grilled chicken, 2 tablespoons ranch dressing, 1 medium fruit, 2 slices cheese.

6. Include the method used to prepare the food item. For example: fresh, frozen, stewed, fried, baked, canned, broiled, raw, braised.

7. For canned foods, include the liquid in which it was canned. For example: Sliced peaches in heavy syrup or Fruit cocktail in light syrup.

8. If you eat at a restaurant, do your best to estimate portion size and list the restaurant you ate at. List any visible fat, oil, or sauces added to your food.

9. List amount and type of oil or butter you use in the preparation of your food.

10. Do not alter your diet while you are keeping a food record.

11. Please indicate the activities you participated in during each of the days that you record your diet along with the duration of activity.
Date _______________  Participant ID # _______________

Day of the Week _______________

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<thead>
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Did you consume your supplement? (Y/N) ______________

Was this a typical day's intake? (Y/N. If no, please explain).

______________________________________________________________________________

______________________________________________________________________________

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APPENDIX C

INFORMED CONSENT FORM

The Effects of Calcium Collagen Chelate on Bone Status in Trained Cyclists

Informed Consent Form

1. I voluntarily and without element of force or coercion, consent to be a participant in the research project entitled "The effects of calcium collagen chelate on bone status in trained cyclists." This study is being conducted by Chris Mojock, Dr. Lynn Panton, Dr. Jeong-Su Kim, Dr. Mike Ormsbee and Dr. Bahram Arjmandi of the Department of Nutrition, Food & Exercise Sciences at The Florida State University.

2. The purpose of the proposed study is to examine how calcium collagen chelate (CCC) supplementation during daily training affects body composition, bone mineral content (BMC), bone mineral density (BMD), biomarkers of bone metabolism and blood hormone levels. Sixty normal-weight, healthy male (18 to 54 years of age) cyclists (cycling > 3 hrs/wk) will be recruited for this study.

3. My participation in this study will require coming to the Human Performance Laboratory at The Florida State University for testing on one or two different occasions over 12 weeks to complete the measurements and assessments as described below. Total time commitment will be approximately three hours per visit.

I will come to the Human Performance Laboratory in a fasted state (no food or drink, except water for at least three hours) at least 24 hours after my last strenuous exercise bout and will turn in my three-day food log that was sent to me by email after my phone interview. On my first visit, I will be given an informed consent document to sign and a medical history form to complete before I can participate in the study. I cannot participate in this study if I have uncontrolled hypertension (blood pressure (BP) >160/100 mmHg), currently taking BP medications, have been diagnosed with cardiovascular disease, stroke, diabetes, thyroid or kidney dysfunction, and or any musculoskeletal complications (i.e. osteoarthritis or injury) that would impede exercise testing and training. In addition, I will be excluded if I currently smoke, take cholesterol medication or use any medications known to affect bone metabolism or regularly partake in planned exercise, other than cycling, for more than two days per week for more than 40-minutes per session (within the past six months).

During Visit 1, I will answer questionnaires regarding nutritional habits, training and racing history. I will have my resting BP, resting heart rate (HR), anthropometric measurements, blood collection and maximal exercise testing performed. Height and weight will be assessed using a standardized scale. My body composition, bone mineral content (BMC) and bone mineral density (BMD) will be measured using dual energy X-ray absorptiometry (DXA). Very low doses of radiation are used; however, this test is non-invasive. I will lie on a padded table for approximately 10 minutes while the scan is being completed. Testing will be completed according to the manufacturer's instructions and specifications by a certified X-ray technician.

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The Effects of Calcium Collagen Chelate on Bone Status in Trained Cyclists

I will repeat my three-day food log again during the sixth week of the 12-week training period and turn it into the research staff. Following 12 weeks of supplementation, I will report to the lab following a three-hour fast and at least 24 hours after my last strenuous bout of exercise to complete Visit 2. I will replicate my three-day food log during the final three days of the intervention, return it to the investigators and repeat all measurements and exercise testing performed during Visit 1 (BP, HR, blood collection, DXA [body composition, bone status], maximal performance testing and 35-km TT). This visit will take approximately three hours.

4. I understand there is a minimal level of risk involved if I agree to participate in this study. I may experience some muscle soreness from the exercise testing sessions. The risks associated with exercise testing are minimal and the selected protocols have been previously used in other studies. There is the possibility of muscle fatigue or soreness related with exercise training or testing. The risk will be minimized by using qualified investigators to supervise testing and ensure proper procedures. The risk of a cardiovascular event during testing will be minimized by careful review of my medical history and monitoring of my exercise sessions. All mouth pieces, breathing hoses and nose clips will be sterilized and cleaned with disinfecting solutions.

The risk of blood drawing is small and there may be some local discomfort at the site of needle placement with possible bruising or swelling. The risk of local infection is also small. These risks will be minimized by the use of skilled technicians using sterile techniques and equipment (single-use collection instruments and appropriate handling/disposal of biohazard waste and sharps).

Body composition will be evaluated by Dual-Energy X-ray Absorptiometry (DXA). This involves low exposure to radiation less than 5 mREMs per DXA scan. Doses received from DXA examinations are small in comparison to other common radiation sources and are believed to represent no significant health risk. No risk of adverse health conditions have been established for lower exposures of 5000 mREMs less. By comparison, natural background radiation is about 300 mrem/year, an x-ray of the spine is 70 mREM, a mammogram is 45 mREM, and a round trip transcontinental plane flight is 6 mREM. The measurement of body composition using DXA is non-invasive.

The dietary supplement is a calcium ion and collagen peptide chelated product. It describes an association of a metal ion having a valence of two or more to form a structure wherein the positive electrical charges of the metal ion are neutralized by the electrons available through collagen/hydrolyzed collagen. The binding represents physical associations of calcium with collagen peptide, mostly through ionic interactions. It mimics the status of natural presence of calcium in food. Collagen in the dosage of 10g per day has been used in other trials with no

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I will also have my blood drawn under sterile conditions (2 blood draws per each visit), the total amount of 20 milliliters from a forearm vein (between the upper and lower arm) will be stored for later analysis. The blood samples will be analyzed for calcium level, 25-hydroxyvitamin D, and parathyroid hormone (PTH). In addition, biomarkers of bone metabolism will be measured including, alkaline phosphatase (B-ALP), tartrate resistant acid phosphatase (TRAP), osteocalcin (OC), sclerostin (SCL), carboxy- and amino-terminal propeptide of type I procollagen (PINP or PICP), and cross-linked C- and N-telopeptide of type I collagen (CTX or NTX). I will then perform an incremental exercise test on an electronically braked cycle ergometer (RacerMate; Velotron Dynafit Pro, Seattle, WA). I will begin exercising at 50 W; resistance will increase by 50 W every two minutes up to 200 W. Thereafter, resistance will increase by 25 W every two minutes until I can no longer maintain performance. During the test I will wear headgear with a mouthpiece attached, a nose clip, and a heart rate monitor around my chest. This will be done to measure my aerobic fitness.

If I meet the requirements to be classified as a trained cyclist (cycling ≥ 8 hrs/wk, maximal oxygen consumption (VO2max) ≥ 50 ml/kg/min), I will rest for 15 minutes and then perform a 35-km time trial (TT). If I do not meet the requirements, I will not be able to participate in the training portion of the study, but my data will be used for a cross-sectional analysis comparison between recreational and trained cyclists. For the TT, a computer display will provide me with gearing, current and average speed, cadence, grade, and distance while I am cycling on the Velotron cycle. I will be instructed to complete the TT as quickly as possible. For purposes of standardization, no outside encouragement will be offered during the TT and I will not be allowed to listen to music. Blood will be collected immediately and 30 minutes post-exercise for later analysis. I will be given three-day food logs (to list all foods and beverages consumed over three days) to return filled out at six weeks and again on the final visit and will receive instructions on how to complete these forms. I will also be given compliance logs (to track daily supplementation and training) to return filled out every two weeks. This visit will take approximately three hours.

After finishing Visit 1, I will be randomly assigned to one of two intervention groups for the duration of the twelve-week intervention: 1) 6 g/d of CCC or 2) placebo control (CON) composed of an inert compound with calcium and vitamin D equivalent to that found in the CCC. Participants in both groups will consume their respective supplements twice daily (four pills in the morning and four pills in the evening) for the next 12 weeks. I will return my empty supplement containers and compliance logs every two weeks, at which time I will be given my supplements for the next two weeks. I will maintain my normal diet and weekly training volume throughout the duration of the study. If I drop below six hours per week of training for two consecutive or a total of four weeks during the intervention period, I will be removed from the study. If I drop below 80% compliance with my respective supplement, I will be removed from the study.

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reported side effects although participants may experience gastrointestinal discomfort such as bloating and/or constipation.

5. The possible benefits of this research for both groups include knowledge of body composition, bone mineral content (BMC), bone mineral density (BMD), resting vital measures, maximal oxygen uptake, biomarkers of bone metabolism and hormone status. I will have the potential to improve body composition and bone status.

6. The results of this study may be published but my name or identity will not be revealed. Information obtained during the course of the study will remain confidential, to the extent allowed by law. My name will not appear on any of the results. No individual responses will be reported. Only group responses will be reported in the publications. Confidentiality will be maintained by assigning each subject a code number and recording all data by code number. The only record with the participant’s name and code number will be kept by the principal investigator, Chris Mojock, in a locked drawer in his office. Data will be kept for 10 years and then destroyed.

7. In case of an injury, first aid (free of charge) will be provided to me by the laboratory personnel working on the research project. However, any other treatment or care will be provided at my expense.

8. Any questions I have concerning the research study or my participation in it, before or after my consent, will be answered by the investigators or they will refer me to a knowledgeable source. I understand that I may contact Chris Mojock at cmojock@fsu.edu, Dr. Mike Ormsbee at (850) 644-4793 (mormsbee@fsu.edu) or Dr. Lynn Panton at (850) 644-4685 (lpanton@fsu.edu) for answers to questions about this research study or my rights. Group results will be sent to me upon my request.

9. In case of an injury, or if I have questions about my rights as a subject/participant in this research, or if I feel I have been placed at risk, I can contact the chair of the Human Subjects Committee, Institutional Review Board, through the office of the Vice President of Research at (850) 644-8633 (humansubjects@magnet.fsu.edu).

10. The nature, demands, benefits and risks of the study have been explained to me. I knowingly assume any risk involved.

11. I have read the above informed consent form. I understand that I may withdraw my consent and discontinue participation at any time without penalty or loss of the benefits to which I may otherwise be entitled. In signing this consent form, I am not waiving my legal claims, rights or remedies. A copy of this consent form will be given to me.

Approved

FSU Human Subjects Committee Approved on 2/14/2013. Void after 2/12/2014. HSC # 2013.9887
The Effects of Calcium Collagen Chelate on Bone Status in Trained Cyclists

Print name

Signature       Date

Approved

FSU Human Subjects Committee Approved on 2/14/2013. Void after 2/12/2014. HSC # 2013.9887
# APPENDIX D

## MEDICAL HISTORY

Florida State University  
Dept. of Nutrition, Food & Exercise Sciences  
Tallahassee, FL 32306

ID# __________  
Initials __________  
DATE __________

Answer the following questions, indicating the month and year of the event or diagnosis where appropriate.

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
<th>Month/Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Has a doctor ever told you that you have heart disease?</td>
<td>___</td>
<td>___</td>
<td><em><strong>/</strong></em></td>
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<tr>
<td>2. Have you ever had a heart attack?</td>
<td>___</td>
<td>___</td>
<td><em><strong>/</strong></em></td>
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<tr>
<td>3. Have you ever had chest pain?</td>
<td>___</td>
<td>___</td>
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<td>4. Have you ever had cardiac catheterization?</td>
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<td>5. Have you ever had balloon angioplasty?</td>
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<td><em><strong>/</strong></em></td>
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<tr>
<td>6. Have you had coronary artery bypass graft surgery?</td>
<td>___</td>
<td>___</td>
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<tr>
<td>If yes, list date and number of grafts:</td>
<td>___</td>
<td>___</td>
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<td><strong><strong>/</strong></strong> # grafts: ___ 1 ___ 2 ___ 3 ___ 4+</td>
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<td>7. Have you ever had a stroke?</td>
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<td>8. Do you have hypertension (high blood pressure)?</td>
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<td>If yes, how long have you had hypertension?</td>
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<td>____ less than 1 year</td>
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<td>____ 1-5 years</td>
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<tr>
<td>____ 6-10 years</td>
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<td>____ more than 10 years</td>
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</tbody>
</table>
9. Do you have diabetes mellitus?  
   Yes No Month/Year

10. Do you take insulin for diabetes?  
   If yes, how long have you taken insulin?
   
   _____ less than 1 year
   _____ 1-5 years
   _____ 6-10 years
   _____ more than 10 years

11. Do you take oral hypoglycemics for diabetes?  
   Yes No

12. Do you have a cardiac pacemaker?  
   If yes, how long have you had a cardiac pacemaker?
   
   _____ less than 1 year
   _____ 1-5 years
   _____ 6-10 years
   _____ more than 10 years

13. Have you had a carotid endarterectomy?  
   Yes No Month/Year

14. Has your doctor ever told you that you have a heart valve problem?  
   Yes No Month/Year

15. Have you had heart valve replacement surgery?  
   Yes No Month/Year
   
   If yes, what heart valves were replaced?  
   _____ mitral  _____ aortic

16. Have you had cardiomyopathy?  
   Yes No Month/Year

17. Have you had a heart aneurysm?  
   Yes No Month/Year

18. Have you had heart failure?  
   Yes No Month/Year

19. Have you ever suffered cardiac arrest?  
   Yes No Month/Year
20. OTHER MEDICAL PROBLEMS: Indicate if you have had any of the following medical problems:

<table>
<thead>
<tr>
<th>Past</th>
<th>Now</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>___</td>
<td>Alcoholism</td>
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<td></td>
<td>___</td>
<td>Allergies</td>
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<td></td>
<td>___</td>
<td>Anemia</td>
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<td></td>
<td>___</td>
<td>Arthritis</td>
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<td></td>
<td>___</td>
<td>Asthma</td>
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<td>___</td>
<td>Back injury or problem</td>
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<td>___</td>
<td>Blood clots</td>
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<td>___</td>
<td>Bronchitis</td>
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<td>Claudication</td>
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<td>Eye problems</td>
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<td>Headaches</td>
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<td>Hernia</td>
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<td>___</td>
<td>Hip, knee, or ankle problems</td>
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<td>Kidney disease</td>
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<td>Liver disease</td>
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<td>Lung disease</td>
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<td>Mental illness</td>
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<td>Neck injury or problem</td>
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<td>___</td>
<td>Obesity/overweight</td>
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<td>___</td>
<td>Osteoporosis</td>
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<td></td>
<td>___</td>
<td>Parkinson's disease</td>
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<td></td>
<td>___</td>
<td>Seizure disorder</td>
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<td></td>
<td>___</td>
<td>Thyroid disease</td>
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<td></td>
<td>___</td>
<td>Tumors or cancer - List type:</td>
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<tr>
<td></td>
<td>___</td>
<td>Ulcers</td>
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<td></td>
<td>___</td>
<td>Other - specify:</td>
</tr>
</tbody>
</table>

List medications you are taking below:

<table>
<thead>
<tr>
<th>Name of Drug</th>
<th>Dosage</th>
<th>Times/day</th>
<th>Duration of drug use</th>
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<tbody>
<tr>
<td></td>
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APPENDIX E

PAR-Q

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?</td>
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<tr>
<td>2. Do you feel pain in your chest when you do physical activity?</td>
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<tr>
<td>3. In the past month, have you had chest pain when you were not doing physical activity?</td>
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<tr>
<td>4. Do you lose your balance because of dizziness or do you ever lose consciousness?</td>
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<td>☐</td>
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<tr>
<td>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?</td>
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<tr>
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<td>☐</td>
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<tr>
<td>6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?</td>
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<td>☐</td>
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<tr>
<td>7. Do you know of any other reason why you should not do physical activity?</td>
<td></td>
</tr>
</tbody>
</table>

If you answered YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

Delay becoming much more active:

- If you are not feeling well because of a temporary illness such as a cold or a fever — wait until you feel better; or
- If you are or may be pregnant — talk to your doctor before you start becoming more active.

Please note: If you have health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME ___________________________ DATE ____________

SIGNATURE ___________________________ WITNESS ___________________________

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority)

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.
APPENDIX F

MEDICAL AND TRAINING HISTORY

Human Performance Laboratory
Florida State University
Nutrition, Food, and Exercise Sciences

The following questions are designed to obtain a thorough preliminary medical history. The information you provide will help us to make the best determination about your eligibility for a particular study or other studies. Please answer all questions and provide as much information as you possibly can. This questionnaire, as well as any other medical information you provide will be kept confidential and will not be shared with any unauthorized person or organization unless you specifically request us to do so.

Name: ____________________________________________________________
Street Address: ____________________________________________________
City, State, Zip code: ______________________________________________
Telephone Number: H ( ) __________ W ( ) __________
Email address: _____________________________________________________
Date of Birth: ___________ Age: ___________
    (mm/dd/yy)
Sex:  M  F
Personal Physician’s Name: ______________________ Phone: ( ) __________
    Address: ______________________________________________________
    ______________________________________________________________
Height ________ in. _________ cm
Weight ________ lb. ________ kg

Participant ID Number: __________________________

Signature: ________________________________
Occupation
Current occupation:

Race

Personal Health History
Have you ever been hospitalized or had surgery? Yes____ No____
Please list all hospitalizations and surgeries to the best of your recollection.

<table>
<thead>
<tr>
<th>Hospitalized for</th>
<th>Age when hospitalized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease/Operation</td>
<td>Duration</td>
</tr>
</tbody>
</table>

List any disease or illness you have had not listed above (e.g., mumps, measles, broken bones, etc.)

Are you allergic, sensitive or intolerant of any foods or medications? Yes____ No____
If yes, please describe:
- Food
- Medication
- Other

Are you currently seeing a doctor or other health care provider for any reason?
Yes_____ No_____
If yes, please explain:
1. Have you ever been diagnosed as having any of the following and if yes, how are you currently treating the condition?

   Y   N   High Blood Pressure
   Please indicate last known reading:
   Blood pressure: ______/_____

   Y   N   High Cholesterol or High Triglycerides
   Please indicate last known reading:
   Cholesterol: ______
   Triglycerides: ______

   Y   N   Diabetes (Circle: Type 1 or Type 2)
   Note: Type 1 diabetes is insulin-dependent diabetes mellitus. It is typically diagnosed at an early age and requires insulin shots or an insulin pump immediately upon diagnosis. Type 2 diabetes is often diagnosed at an older age (past age 20) and is usually initially treated with changes in diet and/or medication (pills).

   Y   N   Hypoglycemia (low blood sugar)

   Y   N   Asthma

2. Have you ever had a glucose tolerance test?   Y   N
   If yes, what were the results?

3. Have you ever had a fasting blood sugar test?   Y   N
   If yes, what were the results?

4. Does anyone in your family (immediate family including your grandparents) have a history of cardiovascular disease (heart attacks, stroke, etc.)? Please explain:

5. Do you have any neurological problems including fainting, dizziness, headaches or seizures?

6. Do you have any orthopedic or other health problems that may affect your ability to perform exercise? If yes, please explain:

7. Do you smoke or use smokeless tobacco?   Y   N
   If yes, how many cigarettes per day? ______

8. Do you drink coffee or other caffeinated beverages?   Y   N
   What kind, how much and how often?
9. Please list all vitamins, minerals and herbs and other nutritional (performance) supplements as well as medications you are currently taking. How long have you been taking them and how frequently?

Are you willing to stop taking all nutritional supplements you are currently on for the duration of this research study? (Y/N) ___________________

10. Do you have any food allergies or intolerances (e.g., allergic to dairy or lactose intolerance)? Please describe:

11. How would you describe the type of diet you currently eat? Have you recently been on any special diets, e.g. used to lose weight or lower cholesterol? Please list and describe:

12. What changes have you made in your diet in the last six months?

13. Please describe your current cycling training volume. Please be detailed in your description of an average week of training and specify whether the training sessions are road or trail.

   Days per week:

   Miles and/or hours per week:

   Miles and/or hours per training session:

   Intensity level per training session:

If possible, please provide an average weekly training schedule

   Ex. Sunday - 45 miles steady, moderate intensity (Road)
       Monday - Rest

   Sunday:
   Monday:
   Tuesday:
   Wednesday:
   Thursday:
   Friday:
   Saturday:
14. Please list the last three cycling events (races, centuries, time trials, etc.) that you have participated in and how you finished/placed (e.g. Century – 4:20 or 32 mile mtb race – 3rd place Sport class):

15. What is your current USAC racing category? How long have you been in that category?

16. Do you perform any other forms of exercise regularly? Y N
   What kinds of exercise?
   How often? Please be detailed in a description of your average week of non-cycling training.

17. How does your current exercise and physical activity compare to 6 months ago? 1 year ago?

18. Have you had a physical exam in the past 2 years? Y N
   Please describe your assessment of your overall health:
# APPENDIX G

## TRAINING AND SUPPLEMENT LOG

### Training / Supplement Log - Weeks: ___ & ___

<table>
<thead>
<tr>
<th>Name:</th>
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<td>Date: <em><strong>/</strong></em></td>
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<td>Date: <em><strong>/</strong></em></td>
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<tr>
<td>Training:</td>
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<td>Training:</td>
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<td>Training:</td>
<td>Training:</td>
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<td>Training:</td>
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<td>Training:</td>
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<tr>
<td>Supplementation</td>
<td>Training</td>
<td>Calendar</td>
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<td>-----------------</td>
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<tr>
<td>Please circle Y if you consumed your supplement or N if you did not. Please try to consume pills at the same time of day throughout the study.</td>
<td>Please give a detailed description of your daily training. Include distance, duration, intensity and indicate road or trail. Be sure to include any non-cycling exercise as well.</td>
<td>Please fill-in the following: Weeks, e.g. 3 &amp; 4 Day (of the week, e.g. Monday) Date (mm/dd/yy)</td>
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</tbody>
</table>
APPENDIX H

SUPPLEMENT AND TRAINING COMPLIANCE

Calcium Collagen Chelate Supplement and Cycling Training Study

DATE:__-__-__

<table>
<thead>
<tr>
<th>Subject No:</th>
<th>Subject Initials:</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Supplement (given - initial/returned - initial)</th>
<th>Training (≥ 6 hrs – initial)</th>
<th>Reminder (called - date and initial)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>____ <em><strong>/</strong></em>_ ____ ____ ____</td>
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<td>Week 2</td>
<td>____ <em><strong>/</strong></em>_ ____ ____ ____</td>
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<td>Week 3</td>
<td>____ <em><strong>/</strong></em>_ ____ ____ ____</td>
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<td>Week 4</td>
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<td>Week 5</td>
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<td>Week 6</td>
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<td>Week 7</td>
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<td>Week 8</td>
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<td>Week 9</td>
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<td>Week 10</td>
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<td>Week 11</td>
<td>____ <em><strong>/</strong></em>_ ____ ____ ____</td>
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<tr>
<td>Week 12</td>
<td>____ <em><strong>/</strong></em>_ ____ ____ ____</td>
<td>_________________</td>
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</tbody>
</table>
APPENDIX I

HUMAN SUBJECTS COMMITTEE APPROVAL LETTER

Office of the Vice President For Research
Human Subjects Committee
Tallahassee, Florida 32306-2742
(850) 644-8573 - FAX (850) 644-4392

APPROVAL MEMORANDUM

Date: 01/10/2013

To: CHRISTOPHER JOINK

Address:  

Dept.: NUTRITION FOOD AND EXERCISE SCIENCES

From: Thomas L. Jacobson, Chair

Re: Use of Human Subjects in Research
   The Effects of Calcium Collagen Chelate on Bone Status in Trained Cyclists

The application that you submitted to this office in regard to the use of human subjects in the research proposal referenced above has been reviewed by the Human Subjects Committee at its meeting on 04/11/2012. Your project was approved by the Committee.

The Human Subjects Committee has not evaluated your proposal for scientific merit, except to weigh the risk to the human participants and the aspects of the proposal related to potential risk and benefit. This approval does not replace any departmental or other approvals which may be required.

If you submitted a proposed consent form with your application, the approved stamped consent form is attached to this approval notice. Only the stamped version of the consent form may be used in recruiting research subjects.

If the project has not been completed by 04/10/2013, you must request a renewal of approval for continuation of the project. As a courtesy, a renewal notice will be sent to you prior to your expiration date; however, it is your responsibility as the Principal Investigator to timely request renewal of your approval from the Committee.

You are advised that any change in protocol for this project must be reviewed and approved by the Committee prior to implementation of the proposed change in the protocol. A protocol change/amendment form is required to be submitted for approval by the Committee. In addition, federal regulations require that the Principal Investigator promptly report, in writing, any unanticipated problems or adverse events involving risks to research subjects or others.

By copy of this memorandum, the chairman of your department and/or your major professor is reminded that he/she is responsible for being informed concerning research projects involving human subjects in the department, and should review protocols as often as needed to ensure that the project is being conducted in compliance with our institution and with DHHS regulations.

This institution has an Assurance on file with the Office for Human Research Protection. The Assurance Number is IRB0000446.

Cc: LYNN PANTON, Advisor
    HSC No. 2012.8566
APPENDIX J

HUMAN SUBJECTS COMMITTEE RE-APPROVAL LETTER

Office of the Vice President For Research
Human Subjects Committee
P. O. Box 3062742
Tallahassee, Florida 32306-2742
(850) 644-8673 · FAX (850) 644-4392

RE-APPROVAL MEMORANDUM

Date: 02/18/2013

To: CHRISTOPHER MOJOCK

Address: xxxxxxxxxxxxxxxxxxxx

Dept.: NUTRITION FOOD AND EXERCISE SCIENCES

From: Thomas L. Jacobson, Chair

Re: Re-approval of Use of Human subjects in Research:

The Effects of Calcium Collagen Chelate on Bone Status in Trained Cyclists

Your request to continue the research project listed above involving human subjects has been approved by the Human Subjects Committee. If your project has not been completed by 02/12/2014, you are required to request renewed approval by the Committee.

If you submitted a proposed consent form with your renewal request, the approved stamped consent form is attached to this re-approval notice. Only the stamped version of the consent form may be used in recruiting of research subjects. You are reminded that any change in protocol for this project must be reviewed and approved by the Committee prior to implementation of the proposed change in the protocol. A protocol change/amendment form is required to be submitted for approval by the Committee. In addition, federal regulations require that the Principal Investigator promptly report in writing, any unanticipated problems or adverse events involving risks to research subjects or others.

By copy of this memorandum, the Chairman of your department and/or your major professor are reminded of their responsibility for being informed concerning research projects involving human subjects in their department. They are advised to review the protocols as often as necessary to insure that the project is being conducted in compliance with our institution and with DHHS regulations.

CC:

HSC No. 2013.9887
REFERENCES


29. Clark, WD, Smith, EL, Linn, KA, Paul-Murphy, JR, Muir, P, and Cook, ME. Osteocyte apoptosis and osteoclast presence in chicken radii 0–4 days following osteotomy. Calcif Tissue Int 77: 327-336, 2005.


BIOGRAPHICAL SKETCH

Christopher Dylan Mojock
Florida State University
Department of Nutrition, Food & Exercise Sciences
436 Sandels Building
Tallahassee, FL 32306

EDUCATION

Ph.D. Florida State University, Tallahassee, FL. December 2013.
Major: Exercise Science with Specialization in Exercise Physiology
Advisor: Lynn B. Panton, Ph.D.
Dissertation: Effects of calcium collagen chelate consumption on body composition and
bone biomarkers in trained male cyclists.

M.S. Florida State University, Tallahassee, FL. May 2009.
Major: Exercise Science with Specialization in Exercise Physiology
Advisor: Lynn B. Panton, Ph.D.
Thesis: Effects of static stretching on running economy and endurance performance in
female distance runners.

B.S. Florida State University, Tallahassee, FL. April 2002.
Major: Marketing

PROFESSIONAL EXPERIENCE

Teaching Assistant in Exercise Science, Department of Nutrition, Food and Exercise Sciences,
Florida State University, Tallahassee, FL. May, 2008 to present.

Graduate Assistant Strength Coach, Strength and Conditioning, Florida State University,

PUBLICATIONS - REFEREED

   on running economy and endurance performance in female distance runners during treadmill

**PUBLISHED/REFEREED ABSTRACTS - PRESENTED AT NATIONAL AND INTERNATIONAL CONFERENCES**


**UNPUBLISHED/REFEREED ABSTRACTS - PRESENTED AT NATIONAL, INTERNATIONAL, AND REGIONAL CONFERENCES**


**INVITED SPEAKER FOR COMMUNITY AND UNIVERSITY ORGANIZATIONS**


**GRANTS**

Florida State University Office of Graduate Studies Dissertation Research Grant. Effects of calcium collagen chelate consumption on body composition and bone biomarkers in trained male cyclists.
Total Award: $750.00  
Funded Date: 05/13 – 08/13  
Role: Principal Investigator

Florida State University College of Human Sciences Dissertation Award Program. Effects of calcium collagen chelate consumption on body composition and bone biomarkers in trained male cyclists.  
Total Award: $1000  
Funded Date: 08/13 – 12/13  
Role: Principal Investigator

National Strength and Conditioning Association Graduate Research Grant – Doctoral. The effects of calcium collagen chelate on bone status in trained cyclists.  
Not Funded  
Date: 03/12  
Role: Principal Investigator

COURSES TAUGHT

ACADEMIC

PET3361 Nutrition and Sports  
PET3102 Introduction to Exercise Sciences  
PET3322L Functional Anatomy & Physiology – Laboratory Sections  
PET3380L Applied Exercise Physiology – Laboratory Sections  
PET4551L Exercise Testing & Prescription – Laboratory Sections

ACTIVITY

RealRyder Indoor Cycling Group Exercise

PROFESSIONAL CERTIFICATIONS

Certified Strength and Conditioning Specialist (CSCS) - National Strength and Conditioning Association  
Basic X-Ray Machine Operator (BMO) - Florida Department of Health  
Cardio Pulmonary Resuscitation and Automated Defibrillator (CPR/AED) - American Red Cross  
Bloodborne Pathogens, Hazardous Waste, Biosafety Level 2 and Above Laboratory Safety - Florida State University Environmental Health and Safety
HONORS AND PROFESSIONAL AFFILIATIONS

Dean’s Scholar
Hortense Glenn Honor Society
KON (Human Sciences Honor Society)
Jean A. Reutlinger and Lillian H. Munn Scholarship
Florence Smith McAllister Endowed Fellowship
Cora & Ross Evans Scholarship
Pao-sen Chi Scholarship
American College of Sports Medicine
Southeast Chapter of the American College of Sports Medicine
National Strength and Conditioning Association
The Center for Advancing Exercise and Nutrition Research on Aging