OSTEOGENIC EFFECT OF ENDURANCE CYCLING

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DEDICATION

This dissertation is dedicated to Perley G. Parr, Jr. and the late Kathreen E. Parr.

My success is now your success.

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ABSTRACT

The purpose of this dissertation was to determine a potential cause of low bone mineral density (BMD) in male cyclists and to test a novel treatment. Two studies were performed. The aim of the first study was to determine whether cortisol levels at the start or finish of a cycling competition were related to lumbar spine and hip BMD in road cyclists. A secondary aim of the first study was to determine factors associated with BMD in male cyclists. The sample included 35 male cyclists with a mean age of 43 years. Salivary cortisol was not correlated with BMD of the lumbar spine or hip. Cyclists who reported a higher number of minutes of weekly participation in weight lifting had higher BMD of the lumbar spine, total hip, femoral neck, and femoral trochanter. Cyclists with higher daily calcium intake had higher BMD of the lumbar spine and femoral neck. Cyclists with a greater number of years of experience had lower femoral neck BMD.

The aim of the second study was to administer calcium with vitamin D supplementation during training and racing in male cyclists in order to maintain and/or improve BMD across a 5-month competitive season. The sample concluded with 17 participating male cyclists with a mean age of 43 years. There were indicated no significant differences between the calcium supplement group and the control group. There was a significant decrease in femoral trochanter BMD across the competitive season (May to October) in the full sample.

Osteopenia was prevalent in these samples. It was found that half of the cyclists were considered at risk for fracture in the lumbar spine or hip in study one of this

dissertation. Further, in the second study, 71% (n = 12) of the cyclists were at risk for fracture in the lumbar spine or hip at the 5-month follow-up. Taken together, these findings suggest the importance of encouraging endurance cyclists to maintain a calcium-rich diet to help improve or maintain BMD. It is also important that cyclists include a weight training regimen to strengthen bones.

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CHAPTER I

PROJECT INTRODUCTION

Physical activity is generally considered a method to improve bone health and decrease the risk of osteoporosis. However, bicycling, a non-weight bearing sport, is associated with low bone mineral density (BMD). Osteopenia is prevalent in professional and amateur competitive male road cyclists. Thus, interest in bone health in this population is growing.

Bone mass is determined by two counteracting metabolic processes, formation and resorption. A balance in the ratio of bone deposition to bone resorption leads to maintenance of BMD and disruptions to the balance between formation and resorption may lead to low BMD. Untreated, low BMD leads to osteopenia and eventually osteoporosis. Osteoporosis weakens the microarchitecture of the bone which increases the risk of fracture. Bone remodeling is mediated by hormonal means to maintain calcium homeostasis in the blood. Calcium homeostasis is necessary for nerve impulse transmission and muscle contraction and is controlled by the parathyroid gland which releases parathyroid hormone (PTH) when blood serum calcium levels fall. Parathyroid hormone is a biochemical marker of bone metabolism that responds to the duration and intensity of the exercise. Other markers of bone metabolism are type I collagen C (CTX), which indicates bone resorption, and bone-specific alkaline phosphatase, which indicates bone formation (Maïmoun & Sultan, 2011). These biochemical markers of bone

metabolism respond to exercise more quickly than measures of BMD. However, BMD is the measurement used to classify one's bone health.

Studies showing low BMD in cyclists are numerous (Nichols, Palmer, & Levy, 2003; Rector, Rogers, Ruebel, & Hinton, 2008; Warner, Shaw, & Dalsky, 2002). Although athletes typically have higher BMD values than sedentary individuals (Bennell et al., 1997), BMD of cyclists is lower than that of sedentary controls in the lumbar spine and total hip (Nichols et al., 2003; Smathers, Bemen, & Bemben, 2009). Reports of lower BMD in competitive cyclists compared to age-matched controls are found in adult male cyclists (Smathers et al., 2009), male master cyclists with years of experience exclusively in cycling training (Nichols et al., 2003), and in postpubertal boy cyclists (Rico, Revilla, Villa, Gomez-Castresana, & Alvarez, 1993). In fact, researchers found that osteopenia is a health risk in professional and amateur male cyclists (Medelli, Lounana, Menuet, Shabani, & Cordero-MacIntyre, 2008; Rector et al., 2008). In an extreme example of bone mineral loss, during the Tour de France, researchers found a 25% decrease in BMD in 4 cyclists over the 3 week event. Other researchers have confirmed the negative trend of bone loss across a competitive season in male, road cyclists (Barry & Kohrt, 2008).

The cause of variation in markers of bone resorption during and post exercise is most likely multifactorial; thus far, factors that elicit bone mineral loss in response to endurance exercise are speculative. Although low BMD in cyclists has been attributed to low gravitational force of the non-weight bearing exercise, researchers are now exploring extended periods of hypersecretion of cortisol (Chiodini & Scillitani, 2008) or dermal

calcium loss through sweat (Barry & Kohrt, 2008) as major contributors to low BMD in cyclists.

Bone cell formation is disrupted during periods of elevated cortisol secretion due to a disruption in calcium homeostasis (Heshmati et al., 1998). Cortisol increases bone resorption and interferes with calcium absorption (Heshmati et al., 1998) which leads to a reduction in BMD (Hardy & Cooper, 2010). Abnormal overproduction of cortisol is accompanied by significant reduction in BMD in patients with Cushing's syndrome (Hardy & Cooper, 2010). Additionally, a negative correlation between cortisol and lumbar spine BMD has been documented in elderly (70 years of age) men and women (Raff et al., 1999). Cortisol is released in response to physical and psychological stress (Guyton, 1991), such as during athletic training and competition. Cortisol has been shown to elevate during cycling when intensity reaches 75% maximal oxygen consumption (Scott et al., 2011). However, the association between cortisol and BMD in competitive cyclists has not been reported.

Another cause of disruption to calcium homeostasis is dermal loss of calcium due to an increased sweat rate during long duration, intense exercise. This induces an increase in PTH. This has been confirmed by Guillemant, Accarie, Peres, and Guillemant (2004) and more recently by Barry et al. (2011). In both studies, PTH and CTX levels were compared during and after two intense cycling trials, one with a calcium supplementation and one with a placebo. The rise in PTH (Barry et al., 2011; Guillemant et al., 2004) and CTX (Guillemant et al., 2004) was attenuated by ingestion of calcium before and during exercise.

The attenuation of the increases in PTH and CTX with an oral calcium load during exercise indicates calcium supplementation may help in preventing low BMD in cyclists. Klesges et al. (1996) studied this by administering calcium to basketball athletes during practice over the course of a season. They were able to halt the decrease in BMD that was previously observed during the course of a season. This form of supplementation may also be beneficial in halting or reversing the documented decrease in BMD also observed across the competitive road cycling season.

Thus, the risk of low BMD in male cyclists may be caused by extended periods of elevated cortisol or dermal calcium loss due to excessive sweating during training and racing. Cortisol interferes with calcium absorption and dermal calcium loss disrupts serum calcium homeostasis. Although a single contributing factor has yet to be determined, it is certain that cycling lacks an osteogenic effect. Research is needed to determine possible causes and to find methods to improve BMD in male road cyclists.

Purpose

These studies were designed to investigate underlying causes for low BMD of the lumbar spine and the hip and to determine a strategy to attenuate decreases in BMD that occur throughout the competitive road cycling season.

The purpose of the first study was to determine whether or not elevated cortisol immediately pre- and post competition is part of the multifactorial cause of low BMD in male cyclists. Information garnered in this investigation determined whether or not cortisol levels at the start or finish of a competition were related to lumbar spine and hip BMD in road cyclists. **The purpose of the second study** was to improve bone

remodeling in favor of formation and reduce resorption in male, road cyclists. The aim was to administer calcium with vitamin D supplementation during bicycle training and racing in order to maintain and/or improve BMD across a competitive season. This examination documented preseason and postseason BMD of the lumbar spine and hip in competitive male road cyclists.

Significance of Studies

Recent reports of a high prevalence of osteopenia among competitive male road cyclists have gained the interest of the research community. Given the nature of the sport and risk of falls during training and competition, cyclists with osteopenia are at greater risk for fracture. Research is needed to determine the cause of the high prevalence of low BMD in male, road cyclists. Research is also needed to determine methods for maintaining and increasing BMD throughout the competitive season in this population. The investigations included in this dissertation present the first-known, season-long calcium and vitamin D supplementation intervention for male, road cyclists.

CHAPTER II

PROJECT LITERATURE REVIEW

This review includes justifications for conducting research on the relationship between exercise and bone mineral density (BMD). The chapter begins with a description of bone remodeling. This is followed by information pertaining to BMD and physical activity, specifically among road cyclists. Current theories of factors contributing to low BMD in road cyclists are examined. Next, the effect of exercise on biomarkers of bone metabolism is discussed. Current methods to attenuate loss of BMD in cyclists, such as calcium supplementations are outlined in detail. The chapter concludes with an overall summary.

Bone Remodeling

Bone is a dynamic tissue such that 5-7% of bone mass is recycled weekly (Marieb & Hoehn, 2008). Bone is the storage site for calcium and is under hormonal control by the parathyroid gland and vitamin D. Bone remodeling is the processes of bone mineral deposition and bone mineral resorption (removal) activated by osteoblasts and osteoclasts, respectively. Osteoblasts continually form new bone, whereas osteoclasts continually absorb minerals and break down protein in bone. In healthy young adults, the rate of bone deposit and resorption lead to net increases in bone density until the third decade of life (Marieb & Hoehn, 2008).

Bone remodeling is regulated by a negative feedback mechanism that serves to maintain serum calcium homeostasis in the blood. Calcium homeostasis is essential for

many physiologic processes such as nerve impulse transmission and muscle contraction. Endocrine mechanisms determine if and when remodeling occurs. The primary hormonal mechanism involves the parathyroid gland which releases parathyroid hormone (PTH) when blood serum levels of calcium fall. Parathyroid hormone stimulates osteoclasts to absorb bone mineral, releasing calcium into the blood to increase serum calcium levels. Parathyroid hormone causes vitamin D_3 (calcitriol) formation to increase renal calcium absorption, decrease renal phosphate absorption, which ultimately increases calcium excretion in urine (Guyton, 1991). In contrast, the thyroid gland releases the hormone calcitonin which promotes calcium deposition in bone, decreasing serum calcium concentration.

Parathyroid hormone also governs the effects of calcitriol on intestinal calcium absorption. Calcitriol plays an important role in intestinal calcium absorption as well as bone resorption and deposition. When calcium concentration is low, PTH secretion promotes stimulation of the active form of calcitriol. This increases absorption of calcium in the intestines and by the kidneys, causing calcium concentration to rise (Guyton, 1991). Calcitriol activation also leads to retention of phosphate, an ion which is needed to build bone, which is the source of 85% of the body's phosphate. When serum calcium level falls, phosphate is released from bone. Parathyroid hormone decreases renal reabsorption of phosphate and phosphate is then lost in urine. However, phosphate is retained with the presence of calcitriol in the kidneys. Thus, PTH enhances intestinal calcium and phosphate absorption by enhancing the activation of calcitriol. Excessive

dietary calcium and calcitriol intake, which increase serum calcium concentration, causes decreased parathyroid activity, and promote increases in BMD.

Measurement of bone density. A BMD test provides a measurement of bone strength and density. The most widely validated technique to measure BMD (g/m^2) is dual energy X-ray absorptiometry (DXA). A BMD measurement by DXA allows characterization of fracture risk which is classified by *t*-scores, a measurement representing the number of standard deviations above or below the average peak bone density for a young adult (age 25 – 29 years) of the same sex. The World Health Organization (WHO) classified osteopenia as a *t*-score that lies between -2.5 and -1.0 and osteoporosis is classified as a *t*-score less than -2.5 (Nordin, 1987). However, BMD is a static representation of bone metabolism which changes slowly. Bone metabolism is not reflected by the measurement of BMD. Slight and acute changes in bone structure are indirectly detected by bone biochemical markers in serum or urine, which are discussed later in this chapter.

Summary. Bone remodeling is the processes of bone mineral deposition and bone mineral resorption. Bone remodeling maintains serum calcium homeostasis and is regulated, primarily, by the release of PTH from the parathyroid gland. Parathyroid hormone stimulates the release of calcium from bone to blood, causes calcitriol formation, which stimulates intestinal and renal absorption of calcium. Calcitriol and phosphate assist PTH in the maintenance of serum calcium.

A balance in bone remodeling, or ratio of bone resorption to bone deposition, lends to maintenance of BMD. Bone remodeling is influenced by dietary calcium and

calcitriol intake, age, and physical activity. Immediate biochemical markers of bone metabolism are measured during exercise to determine the osteogenic effect induced by a specific activity. Long term measurements of BMD by DXA show that physical activity has an osteogenic effect on bone that is related to the mechanical force placed on the bone.

Effect of Exercise on BMD

Evidence has been established that physical activity reduces the risk of osteoporosis. Exercise is a generally accepted as a form of prevention and treatment of low BMD, which can lead to osteoporosis. Mechanical force placed on the skeleton is generated either by ground impact due to gravity or by muscular contractions. However, evidence is inconclusive as to which form of exercise induces greater osteogenic effects on bone health (Kohrt, Barry, & Schwartz, 2009). This is due partly to the difficulty of measuring isolated gravitational loading because muscle force is generated in both impact and non-impact exercises. Conversely, the lack of gravitational force, such as occurs in microgravity, causes an accelerated decline of BMD (Kohrt et al., 2009).

Exercise can be classified by the potential for an osteogenic effect through bone-loading such as exercises that induce high impact force vs. non-weight bearing exercises. Weight bearing activity is described as exercise that provides a high magnitude of mechanical force. When strain is unevenly distributed across bone, such as in participation in volleyball or soccer, the osteogenic effect of exercise is enhanced. This lends to further increases in BMD (Nikander, Sievänen, Heinonen, & Kannus, 2005).

There is clear evidence that individuals who participate in weight bearing exercises such as tennis and running have increased BMD compared to individuals who participate in less physical activity (Etherington et al., 1996). The long term effects of exercise on BMD are difficult to quantify, because this relationship is dependent on the type, duration, and intensity of exercise. Likewise, the time-frame necessary to increase BMD is relative to factors such as age, sex, nutritional status, and hormonal status.

Impact exercise during the growth period before puberty has been shown to significantly increase peak bone mass throughout the lifespan (Bass et al., 1998). For example, if high impact exercises (e. g., jumping, gymnastics, and plyometrics) are performed during childhood, peak BMD will be higher in adulthood (Kohrt, Bloomfield, Little, Nelson, & Yingling, 2004). Additionally, a 15-year longitudinal study showed that individuals who performed weight bearing exercise throughout childhood and young adulthood had the greatest peak bone mass which occurs at approximately 27 years of age (Welten et al., 1994).

Athletes have higher BMD than non-athletes, and power athletes have the highest BMD of athletic groups (Bennell et al., 1997; Suominen, 1993). Participation in high impact sports such as volleyball is associated with higher BMD compared to nonimpact sports such as swimming and cycling (Nikander et al., 2005). Although physical activity enhances bone health in most cases (Brahm, Piehl-Aulin, & Ljunghall, 1997), data are mixed in the case of endurance trained athletes such as runners, swimmers, and cyclists.

Since the 1980's, it was documented that females engaging in prolonged, endurance exercise may have difficulty maintaining optimal BMD (Drinkwater et al.,

1984; Fanning et al., 2007; Hawkins et al., 1999). A study was performed in order to determine the effect of chronic running on BMD in women. It was shown that 5 years of running more than 10 miles per week did not protect against age-related bone loss in master females (above 30 years of age). However, the same measurement performed 5 years later on most of the women from the previous study showed that (statistically) BMD was not further reduced. A regression analysis indicated age as the only predictor of change in BMD (Hawkins, Schroeder, Dreyer, Underwood, & Wiswell, 2003). Hawkins et al. (2003) concluded that chronic (10 years) running did not enhance nor did it reduce BMD.

Another study involving all female participants showed the mode of exercise is more important to bone health than the volume of physical activity. Nikander et al. (2005) measured femoral neck BMD of swimmers and cyclists (non-impact, repetitive exercise) and compared them to track athletes (high-impact) with a similar volume of training and to non-active referents. Both groups had approximately 10 years of experience. The femoral neck BMD of the track athletes, specifically hurdlers, was significantly greater than the swimmers. Femoral neck BMD of the cyclists and swimmers did not differ from that of the non-active referents (Nikander et al., 2005). Thus, non-impact sports participation does not provide a clear benefit to BMD, specifically of the femoral neck.

Hind, Truscott, and Evans (2006) measured lumbar spine BMD in 65 female perimenopausal runners and 44 male runners. The female, competitive endurance runners had lumbar spine BMD equal to that of age-matched sedentary controls. Lumbar

spine BMD of the males was similar to that of the female runners. There was a negative correlation between miles run per week and lumbar spine BMD in both males and females. Therefore, as weekly running mileage increased, BMD of the lumbar spine decreased which implies acceleration in bone loss. Male and female endurance runners may be at risk for stress fracture or osteoporotic fracture due to decreases in BMD (Bilanin, Blanchard, & Russek-Cohen, 1989; Hetland, Haarbo, & Christiansen, 1993).

In contrast, Stewart and Hannan (2000) found that runners had lumbar BMD greater than sedentary controls and cyclists, where the cyclists had the lowest values of the 3 groups. The authors attributed this disparity to the lack of loading on the skeleton during cycling. For example, jogging produces forces that are three times one's body weight (Marcus, 1996). In contrast, seated pedaling at 250 watts and 90 RPM produces pedal forces that are half that of the rider's body weight (Rowe, Hull, & Wang, 1998). This is because a cyclist's body weight is supported by five contact points: two hands on the handlebar, two feet on the pedals, and the pelvis on the saddle. Pedal loads also relate strongly to the riders weight and body position during cycling, i.e., seated or standing (Caldwell, Li, McCole, & Hagberg, 1998; Stone & Hull, 1995).

In summary, although exercise has been shown to benefit bone health, endurance athletes exhibit BMD of the lumbar spine (Hind et al., 2006) and femoral neck (Nikander et al., 2005) similar to age-matched sedentary controls. Individuals involved in low-impact endurance sports have BMD lower than that of individuals participating in high-impact sports (Stewart & Hannan, 2000). Researchers have determined that mode of exercise may be more important to BMD maintenance than duration (Nikander et al.,

2005). Male endurance, road cyclists, specifically, may be at risk for osteopenia (Medelli et al., 2008; Rector et al., 2008).

BMD in cyclists. Although cycling is beneficial to cardiovascular health, it does not provide a positive influence on bone health. Osteopenia may be a health risk in professional and amateur cycling (Medelli et al., 2008; Rector et al., 2008). Bone mineral density was measured in 23 professional male cyclists where 65% of the cyclists had measurements falling into the classification of osteopenia based on the definition by WHO (Medelli et al., 2008). Likewise, Rector et al. measured BMD in amateur cyclists and runners and found that 60% of the cyclists presented with osteopenia of the lower spine. Comparatively, 19% of the participating runners were osteopenic. The weight bearing athletes (runners) had significantly greater lumbar spine and whole body BMD as compared to the non-weight bearing participants (cyclists). When controlling for bone-loading exercise participation history, cyclists were still more likely to have osteopenia of the lower spine compared to runners. As mentioned, bone loading activity during youth is an important factor for peak bone mass (Welten et al., 1994). Thus, it is important to control for exercise history when examining BMD differences across groups.

A comparison was made of BMD between highly trained master cyclists and younger cyclists, aged 40-60 years and 25-35 years, respectively (Nichols et al., 2003). The master cyclists had 10 years experience and the young cyclists had 5 years of experience, both groups participated in and trained for cycling events exclusively. Similar to Medelli et al. (2008), Nichols et al. found that 66% of the master cyclists were classified as osteopenic in the lumbar spine and 63% in the total hip. Further, 4 of the 27

master cyclists were osteoporotic in the lumbar spine and/or total hip. In comparison, among the age-matched sedentary controls, 42% had osteopenia of the lumbar spine, but none were classified as osteoporotic. It is worthy to note that there were no statistically significant differences among groups in hours spent in weight bearing activity during youth. Overall, bone mass was 10% lower in the master cyclists than the referents (Nichols et al., 2003). Therefore, participation in road cycling alone may be harmful to bone health later in life. Additionally, participation in competitive cycling, where falling is prevalent, coupled with low BMD places cyclists at a high risk for fracture (Smathers et al., 2009). Smathers et al. recommended cyclists have regular BMD screening, perform supplemental high-impact or weight training physical activity, and consume adequate calcium and calcitriol.

Anecdotally, cyclists often do not engage in weight bearing activity, such as running or weight lifting, as it has been shown to have little influence on increasing aerobic power output (Bishop, Jenkins, MacKinnon, McEniery & Carey, 1999). Some cyclists train on the bicycle up to 30 hours per week. Recovery time is typically spent in a seated or reclining position further compounding time spent without bone loading. Cyclists who engage in some weight bearing activity throughout the year (2-6 months) still have lumbar spine BMD lower than controls matched by age and weight-lifting activity (Smathers et al., 2009). However, engaging in mountain biking has been shown to have a positive influence on BMD due to the osteogenic stimuli from different loading forces on varied ground surfaces such as rocks, logs, and drop-offs (Warner et al., 2002). A comparison among male mountain cyclists, road cyclists, and recreationally active

controls showed that road cyclists and controls had similar weight-adjusted proximal femur, lumbar spine, and total body BMD. Bone mineral density was greatest in the mountain cyclists. Therefore, a greater osteogenic stimulus may occur from mountain cycling compared to road cycling.

The previously summarized studies show that there is evidence that road cycling is a risk factor for low BMD in the lumbar spine and hip which leads to increased risk of osteopenia or osteoporosis. It is certain that road cycling lacks an osteogenic effect (Beatty, Webner, & Collina, 2010). Although a single contributing factor has yet to be determined, researchers postulate that long-term training causes microdamage of bone and muscle, contributing to the problem (Ehrnborg et al., 2003).

The observation of low BMD in the femoral neck, hip, and lumbar spine in road cyclists is surprising. Sport participation is typically beneficial to skeletal health, yet participation in cycling is detrimental to bone health despite the large, positive influence on cardiovascular fitness (Smathers et al., 2009). Although fracture rate is not well documented, falls occasionally occur. Cyclists are capable of reaching speeds of 45 mph on level ground to 65 mph on descents without wearing protective equipment. A fall at a high speed could cause any bone to fracture; but, the above research suggests that cyclists with low BMD may have much more serious injuries. The nature of the sport of competitive road cycling coupled with low BMD in the participants, places these athletes at risk for fracture (Smathers et al., 2009).

Summary. Endurance athletes exhibit BMD of the lumbar spine and femoral neck that is lower or similar to sedentary control groups. The mode of exercise plays an

important role in maintaining BMD. Cycling, specifically, is a non-weight bearing exercise. In a sport with participants displaying high levels of cardiovascular fitness, it is surprising that osteopenia is a risk factor in professional and amateur cyclists.

Laboratory cycling and running trials provide more insight into the physiology of bone metabolism during exercise. Biomarkers of bone metabolism, measured in blood serum, are elevated during exercise in laboratory trials. Direct insight into the effect of exercise on bone remodeling is indicated by measurement of hormonal and biochemical changes.

Effect of Exercise on Markers of Bone Metabolism

Biochemical markers of bone metabolism respond to exercise more quickly than DXA measurements of BMD. Thus, the measurement of blood serum indices of bone metabolism provides insight into an acute response of bone metabolism to exercise. The biochemical markers that measure bone metabolism activity are classified as indicators of bone formation and indicators of bone resorption. For example, osteocalcin and bone-specific alkaline phosphatase (BALP) indicate bone formation. Carboxyterminal cross-linked telopeptide of type I collagen (ICTP) and Type I collagen C (CTX) indicate bone resorption (Maïmoun & Sultan, 2011). With these tests, researchers are able to relate the intensity of exercise, duration of exercise, and training status of the athlete to bone remodeling.

Effect of intensity on markers of bone metabolism. Many researchers have documented the effect of intensity on markers of bone metabolism in endurance athletes. For example, a study performed by Salvesen et al. (1994) showed that PTH increases

with increases in intensity. The laboratory trial consisted of blood serum measurements of PTH from well-trained men as they performed a 50 minute run with a step-wise increase in velocity to reach 86% maximum heart rate. Blood samples were taken after the warm-up and every 10^{th} minute of the incremental work. Parathyroid hormone rose moderately in conjunction with intensity, and reached a plateau that was 50% greater than baseline. Serum calcium also rose with increases in workload. There was a correlation between increases in PTH and increases in lactate. However, the association between changes in PTH and calcium was not statistically correlated. The authors concluded that there is an exercise-induced elevation in PTH, relative to intensity, that may not be fully mediated by serum calcium concentration (Salvesen et al., 1994).

A study of competitive road cyclists who performed 50 minutes of cycling at moderate and high intensity defined as 15% below ventilatory threshold (VT) and 15% above VT, respectively, provides another example of the effect of intensity on biomarkers of bone metabolism (Maïmoun et al., 2006). Venous blood samples were drawn at the start of exercise, 30 and 50 minutes into exercise, and 15 minutes into recovery. Markers of bone remodeling were not correlated with anthropometric data or maximal oxygen consumption (VO_{2max}). During the high intensity trial, PTH increased 41% above baseline and peaked at 80% above baseline during recovery. On the other hand, PTH did not change during the low intensity trial. Serum calcium also increased during the high intensity trial, but did not reach statistical significance. A marker of bone resorption, CTX, increased 16% above baseline during the intense trial, but returned to baseline during the 15 minute recovery period. A significant rise in markers of bone formation

(osteocalcin and BALP) was found during exercise, but immediately returned to baseline following work. Thus, the rise in markers of bone formation and resorption during the high intensity trial, but not during the low intensity trial, suggest the existence of a minimal level of exercise intensity that will induce changes in biomarkers of bone metabolism (Maïmoun et al., 2006).

Insight into the relationship between exercise intensity and PTH was also provided by Brahm et al. (1997). Participants performed a treadmill exercise protocol consisting of 10 minute stages corresponding to workloads of 47% and 76% VO_{2max} followed by a maximal effort to exhaustion lasting 4 minutes. Serum PTH concentrations rose in proportion to exercise intensity and remained elevated throughout 24 hours of recovery. As illustrated by this study, it appears that the intensity level of exercise is a determinant of exercise-induced increases in PTH and calcium homeostasis (Maïmoun et al., 2006).

In summary, biochemical markers of bone resorption are related to the intensity of exercise. Parathyroid hormone increases linearly with increases in intensity until a plateau is reached. This leads to the idea that there is a threshold, determined by level of intensity, at which a significant elevation in PTH occurs. Zanglis, Andreopoulos, and Baziotis (2008) and Maïmoun et al., (2006) suggested that since intensity level affects markers of bone remodeling, one may assume there is a minimal intensity at which a modification of bone metabolism is induced.

Effect of duration on markers of bone metabolism. Short term exercise bouts, typical practice in untrained individuals, may increase bone formation hence optimizing

long term measurements of BMD. For example, Bouassida et al. (2003) measured the PTH response to exercise with and without a recovery period between bouts. On one day, participants performed running exercise with a recovery period of 40 minutes between two 20 minute bouts (70% and 85% VO_{2max}, respectively). On the second day, the participants performed the same bouts of running continuously, without a recovery period between sessions. The level of PTH was measured prior to, during, immediately following, and during recovery from exercise on both days. Parathyroid hormone was significantly lower in the trial including a 40 minute recovery period compared to the continuous run trial. The authors concluded that compared to a trial without recovery, running for two 20 minute bouts with a 40 minute recovery period in between, had an anabolic effect on bone (Bouassida et al., 2003).

Continuous administration of PTH causes bone resorption and intermittent administration of PTH causes bone deposition (Tam, Heersche, Murray, & Parsons, 1982). This may have implications in the positive or negative effect of exercise on the skeleton. Brahm et al. (1997) suggested low resting levels of PTH with a large increase during short term exercise produced favorable effects on bone health. In regards to the aforementioned work by Bouassida et al. (2003), it follows that since intermittent PTH release has an anabolic effect on bone, shorter bouts of exercise may ultimately increase BMD compared to extended bouts of exercise. Overall, the mechanisms by which PTH is stimulated by exercise are not well understood (Guillemant et al., 2004).

Most endurance athletes routinely include training bouts longer than 1 hour. Therefore, the 1 hour or less exercise bout most researchers assign to measure markers of

bone remodeling may not provide a clear picture of bone remodeling biomarkers for endurance athletes. Moderate intensity exercise performed for 2 hours increased PTH significantly over baseline measurement in runners (Zanglis, Andreopoulos, & Baziotis, 2008) and cyclists (Barry & Kohrt, 2007). Both sets of researchers corrected PTH for changes in plasma volume. Following the run, in the study by Zanglis et al., concentrations of PTH more than doubled, but returned to baseline 3 hours post run. For the cyclists, in the Barry and Kohrt sample, serum PTH rose from a mean of 40.6 pg/mL (SD = 15.6) to 69.5 pg/mL (SD = 25.5) immediately after the 2 hour trial. Barry and Kohrt also found an increase in unadjusted serum calcium from 9.3 pg/mL (SD = .03) to 9.6 mg/mL (SD = .03), p = .001. However, after adjusting for changes in hematocrit, the serum calcium concentration decreased from 9.3 mg/mL (SD = .03) at the start of exercise to 8.8 mg/mL (SD = .05) post-exercise ($p < .01$). Barry and Kohrt concluded with consideration of the similarities between endurance cyclists and patients with moderate hyperparathyroidism. These patients experience bone loss (Chappard, Houillier, & Paillard, 2001). Cyclists experience a similar state of elevated PTH during prolonged exercise. Thus, endurance cycling mimics hyperparathyroidism which may explain low BMD in athletes participating in extended bouts of cycling training.

The last example of the effect of duration on markers of bone metabolism is provided in a study by Ljunghall et al. (1986). A 5 hour cycling trial at 50% VO_{2max} was performed by 17 healthy males (Ljunghall et al., 1986). Plasma ionized calcium decreased and PTH increased during prolonged, low intensity exercise. There was a significant increase in PTH after the first hour which stayed elevated throughout the trial.

Thus providing further evidence of the exercise induced increase in PTH which is dependent on duration.

In conclusion, similar to the effect of intensity on changes in PTH during exercise, there appears to be a relationship in the effect of duration on changes in PTH during exercise. Competitive cyclists routinely train for long periods (2-5 hours) with bouts of intensity interspersed in the training session. Therefore, as Barry and Kohrt (2007) hypothesized, a prolonged state of elevated PTH may have the same effect on cyclists as hyperparathyroidism has on patients, low BMD. Conversely, an anabolic effect on bone was reported when participants performed repeated short bouts of intense exercise. Endurance training also affects the endocrine system, and in turn, may alter PTH secretion.

Effect of athlete training status on markers of bone metabolism. When the effect of endurance training is considered, it has been shown that consistent training lowers resting or baseline measures of PTH (Brahm et al., 1997). During a 50 minute, constant velocity treadmill run in long-distance runners and healthy men, PTH was measured. After 10 minutes, there was an increase in PTH and calcium that occurred in the well-trained men. In contrast, PTH in the healthy men did not increase, but calcium concentrations rose significantly.

Osteocalcin, a marker of bone formation, also responds to training. Nishiyama, Tomoeda, Ohta, Higuchi, and Matsuda (1988) found moderate intensity running for 30 minutes induced elevations in osteocalcin. The response time was different depending on training status of the participants. The authors concluded that because pre-exercise

osteocalcin and the maximal level of osteocalcin were higher in the trained participants, there is a higher rate of bone metabolism, in favor of deposition, in the athletic population. Similarly, osteocalcin was elevated immediately following a competition half-marathon run, but returned to baseline 3 hours post exercise (Zanglis et al., 2008). Without an accompanying longitudinal measurement of BMD, whether or not the experiment resulted in anabolic synthesis of bone is inconclusive.

Rector et al. (2008) measured markers of bone turnover at rest, following 24 hours without exercise. The authors found no difference in resting values of serum markers between runners and cyclists. The authors suggested the magnitude of bone turnover may be large at the start of an exercise program, but returns to baseline over time. This theory is supported by measurements of serum markers of bone turnover during extreme bone unloading, such as in extended periods of bed rest. There is a high rate of bone turnover at the start of the experiment. However, there is a decrease in serum markers of bone turnover over time (Zerwekh, Ruml, Gottschalk, & Pak, 1998).

Resting and low intensity exercise PTH concentration is lower in the endurance athlete compared to the sedentary individual. Data suggest bone remodeling occurs at a higher rate at the start of an exercise program, but returns to baseline over time. It appears that this baseline value may decrease depending on the magnitude of the exercise program.

Summary. Indicators of bone remodeling are elevated during exercise. This is mediated by the intensity of the exercise, the duration of the exercise bout, and the training status of the athlete. There is evidence of an intensity related bone metabolism

threshold, or a specific intensity level at which markers of bone metabolism rise (Maïmoun et al., 2006; Zanglis et al., 2008). Long-duration exercise induces an increase in markers of bone metabolism during exercise (Barry & Kohrt, 2007; Zanglis et al., 2008). Long term training decreases baseline markers of bone metabolism (Brahm et al., 1997).

As mentioned previously, PTH activates osteoclast activity. Extended periods of elevated PTH stimulation will lead to osteoclastic resorption of bone mineral which decreases BMD. This disruption in bone absorption and bone deposition balance causes weakened bones (Guyton, 1991). When intensity level of cycling is high enough, 15% above ventilatory threshold, PTH level will reach twice baseline level (Maïmoun et al., 2006). Although there is not a known, single contributing factor to the effect of exercise on biomarkers of bone remodeling, many theories have previously been reported.

Current Theories on Low BMD in Road Cyclists

Current theories for contributing factors affecting the relationship between endurance road cycling and low BMD include: dietary calcium intake, hormonal balance, dermal calcium loss, and metabolic acidosis (Barry & Kohrt, 2008; Ehrnborg et al., 2003; Ihle & Loucks, 2004; Maïmoun & Sultan, 2011; Marcus et al., 1985; Nichols et al., 2003; Rector et al., 2008; Stewart & Hannan, 2000).

Dietary calcium intake. Researchers have recommended adequate intake of calcium and calcitriol to attenuate the loss of BMD in cyclists (Nichols et al., 2003). However, other researchers have shown that dietary calcium does not influence BMD in road cyclists (Barry & Kohrt, 2008; Smathers et al., 2009). Calcium intake was measured

in competitive, road cyclists. The mean calcium intake (1,557 mg/d) exceeded the Institute of Medicine's recommended daily allowance. The BMD in these competitive, road cyclists was still lower than recreationally active controls (Smathers et al., 2009). In a similar study where the BMD of road cyclists was similar to that of controls (Warner et al., 2002), there was no difference between the groups in calcium and calcitriol intake. Barry and Kohrt (2008) measured BMD over the course of a year in cyclists finding a decrease throughout the competitive season. Although half of the participants received 1,500 mg calcium supplementation with meals, the decrease in BMD was the same in those with and without supplementation. Therefore, road cycling is not beneficial to bone health, regardless of dietary calcium intake.

Hormone balance. Endurance exercise may cause bone loss due to hormonally mediated suppression of bone deposition with accelerated bone resorption. Exercise stimulates PTH release (Barry & Kohrt, 2007) and increases cortisol secretion (Scott et al., 2011). Extended periods of hyperparathyroidism (Barry & Kohrt, 2007) and hypersecretion of cortisol (Chiodini & Scillitani, 2008) may lead to low BMD.

Cortisol is an adrenocortical steroid hormone secreted by the adrenal glands. Cortisol is secreted with physical or psychological stress. Types of stress that elevate cortisol are infection, intense heat or cold, trauma, blood volume loss, and exercise.

Elevated cortisol secretion has a negative effect on bone cell growth (Chiodini & Scillitani, 2008) due to effects on calcium homeostasis (Heshmati et al., 1998). Cortisol has been shown to increase bone resorption, interfere with calcium absorption (Heshmati et al., 1998), and reduce BMD (Hardy & Cooper, 2010). Patients with Cushing's

syndrome, characterized by marked overproduction of cortisol, have a significant reduction in BMD accompanied by an increased risk for fracture (Hardy & Cooper, 2010). Salivary cortisol and BMD are negatively related in elderly men and women (70 years of age). The negative correlation between cortisol and lumbar spine BMD suggests elevated cortisol may contribute to lower BMD in the elderly population (Raff et al., 1999).

Resting levels of cortisol increase in response to endurance training. Endurance training for 3 months, beginning with 15 hours per week extending to 26 hours per week, increased the resting serum cortisol concentration in professional road cyclists by 39%. There was no correlation between cortisol and performance (Hoogeveen & Zonderland, 1996). In a similar study, resting cortisol concentration was found to increase across 4 days of endurance cycling (120% above normal training loads) in female competitive cyclists (Bouget, Rouveix, Michaux, Pequignot, & Filaire, 2006). The purpose of these two studies was to determine the usefulness of assessing stress hormone elevation in relation to performance and overtraining, not in relation to bone metabolism. However, Rector et al. (2008) found a positive association between resting values of cortisol and CTX in cyclists and runners. This is meaningful because CTX is a marker of bone resorption.

Salivary cortisol is a simple and sensitive marker of changes in cortisol concentration. An examination of resting levels of cortisol in serum, saliva, and urine showed a high correlation among the three measurements (Neary, Malbon, & McKenzie, 2002). The use of salivary cortisol concentration provides a non-invasive and convenient

method to collect samples during exercise. It is important to consider circadian rhythms on cortisol secretion because cortisol will peak in the morning hours, 8:00 a.m., and will decline in the evening, 8:00 p.m. (Teo, McGuigan, & Newton, 2011). Cortisol should be measured at the same time of the day in order eliminate variations in circadian rhythm.

During exercise, changes in cortisol levels depend on intensity of the exercise. Serum cortisol was shown to increase during a laboratory endurance cycling trial when the intensity level reached approximately 75% of maximal oxygen consumption (Scott et al., 2011) and during a laboratory running trial to exhaustion (Hackney & Dobridge, 2009). Maïmoun et al. (2006) found that serum cortisol concentration decreased during cycling at 15% above and 15% below VT. However, individual variation was large enough to eliminate statistical significance. Serum cortisol concentration decreased further after 15 min of recovery. Cortisol was shown to return to resting levels within 2 hours of exercise (Fry, Morton, Garcia-Webb, & Keast, 1991).

Along with physiological stress, cortisol responds to psychological arousal during competition. Therefore, resting values of cortisol on competition day may be higher than on a rest day (Aubets & Segura, 1995; Crewther, Heke, & Keogh, 2011; Filaire, Alix, Ferrand, & Verger, 2009). Pre-event elevation in cortisol due to anticipation of mental and physical performance was determined in a recent study by Gatti and de Palo (2011). In this study, a quantitative measurement of pre-competition anxiety and salivary cortisol were related. Cortisol further responded to psychological stress as was indicated from pre- to post-competition increases.

Judo competitors completed the Competitive State Anxiety Inventory prior to competition as a measure of psychological stress. Salivary cortisol was collected as a measure of physiological stress. Salivary cortisol was significantly greater after the judo competition compared to before with a positive association with level of anxiety (Filaire, Sagnol, Ferrand, Maso, & Lac, 2001). Similarly, salivary cortisol was greater after a collegiate soccer competition than the pre-competition measurement (Edwards, Wetzel, & Wyner, 2006). Salivary cortisol was 130% higher after an Olympic weightlifting competition than before. The increase in cortisol was accompanied by an increase in performance compared to a competition simulated laboratory trial (Crewther et al., 2011).

The results of these studies indicate that salivary cortisol increases with high intensity exercise and is a measurement of physiological stress. Unfortunately, there are no known data relating salivary cortisol concentration to the physiological or psychological stress of road cycling competition in amateur cyclists.

Data relating cortisol to markers of bone resorption are mixed. Rector et al. (2008) found a positive association between cortisol and CTX, a marker of bone resorption. Cortisol was once considered a regulator of the PTH response to exercise. However, after a 60 minute cycling trial at 60% VO_{2max}, PTH increased 50% above baseline, but was not altered by the presence of serum cortisol (Tsai, Lin, Chen, Cheng, & Yang, 1997). There was no effect of cortisol, adrenocorticotropic hormone, calcium, magnesium, nor phosphorous on intact PTH secretion. Although cortisol also increases during high intensity exercise, there has been no correlation found between the rise in PTH and cortisol (Maïmoun et al., 2006; Tsai et al., 1997).

Testosterone is another hormone that may influence bone resorption and calcium homeostasis which ultimately alters BMD. Smathers et al. (2009) hypothesized that lumbar spine BMD would positively correlate with testosterone levels. However, bone turnover and sex hormone status, such as testosterone, was not altered by engaging in non-impact physical activity such as in road cycling (Smathers et al., 2009). Rector et al. (2008) found no difference between cyclists and controls in testosterone level, but lumbar spine BMD was lower in the cyclists. In contrast, Warner et al. (2002) found a positive correlation between testosterone and hip BMD in road cyclists. Thus, although one hypothesized contributing factor to low BMD in cyclists is sex hormone imbalance, results in the literature are mixed.

In summary, PTH is known as the main hormone regulating bone mineral turnover. Other hormones that may disrupt calcium homeostasis and bone deposition are cortisol and testosterone. Cortisol secretion has been shown to elevate during intense road cycling. Cortisol has also been shown to elevate prior to competition due to performance anxiety in soccer players and judo competitors. Amateur cyclists train up to 1,000 hours per year and most ride 6 days per week (Friel, 2003). During the competitive season, a high percentage of training and racing is at a high intensity. Although cortisol concentration during cycling competition and typical training has not been measured, cortisol is regularly elevated in these athletes and may potentially be linked to the lower BMD in road cyclists.

Dermal calcium loss. Dermal calcium loss has been estimated by Barry and Kohrt (2008) as approximately 70 mg/h^{-1} during moderate to vigorous cycling. It has

been postulated that dermal calcium loss decreases serum calcium which triggers the release of PTH and increases osteoclast activity, thus contributing to a deficit of serum calcium which is essential to bone deposition (Barry & Kohrt, 2007; Barry & Kohrt, 2008; Klesges et al., 1996). No correlation has been found between PTH and dermal calcium loss (Barry & Kohrt, 2007); however, interpretation of the relationship between PTH and serum calcium is difficult. A single point in time measurement of PTH and calcium may be misleading because an increase in PTH quickly normalizes a decrease in calcium. The notion that dermal calcium loss contributes to low BMD in cyclists was supported by documenting increases in PTH during a single bout of intense cycling, coupled with the amount of calcium lost through sweat (Barry et al., 2011; Barry & Kohrt, 2007; Guillemant et al., 2004).

Changes in serum calcium elicit an immediate endocrine response. It is possible that calcium loss due to excessive sweating during long duration, intense cycling triggers bone resorption that counteracts the beneficial effects of muscle loading on BMD during road cycling. The amount of calcium lost in sweat is dependent on intensity, temperature, hydration status, and acclimation to warm temperature. Thus, the failure to find similarities between exercise-induced changes in PTH and dermal calcium loss may be due to difficulty controlling measurements.

Metabolic acidosis. During a cycling competition and training, cyclists may experience metabolic acidosis (Ehrnborg et al., 2003; Maïmoun & Sultan, 2011). Bicycle competitions require athletes to sustain relatively high power outputs interspersed with bursts of anaerobic power throughout the duration of the race (Lepers, Sultana, Bernard,

Hausswirth, & Brisswalter, 2010). The competitive cyclist's ability to compete in races where power output oscillates greatly from low wattage to a high wattage close to one's maximum anaerobic power is important to a cyclist's success in the event. Often, race strategy rules over individual ability. The ability of the cyclist to maintain anaerobic power is mandatory during competition other than the individual time trial.

Metabolic acidosis may be a contributing factor to the increase in bone resorption activity during prolonged exercise at a high level of intensity which, in turn, may lead to low BMD (Ehrnborg et al., 2003; Maïmoun & Sultan, 2011). Chronic metabolic acidosis increases osteoclastic bone resorption which increases calcium release from bone and decreases osteoblast activity (Bushinsky, Parker, Alexander, & Krieger, 2001). The decrease in pH during exercise has traditionally been explained as lactic acidosis from the production of lactic acid. On the contrary, protons (H^+) are released from glycolysis and ATP hydrolysis. This does not cause an immediate decrease in pH because of the multiple components which bind to or consume H^+ to protect the cell against acidosis and regulate acid-base balance. Lactate removes protons by transporting H+ across the sarcolemma, thus leading to protection against metabolic acidosis. When the rate of H^+ production exceeds the biochemical capacity to remove protons, metabolic acidosis occurs in the skeletal muscle. Thus, metabolic acidosis is caused by reliance on nonmitochondrial ATP production. Blood lactate accumulation may be viewed as an indirect indicator for the potential of decreased cellular pH, but there is not a direct cause and effect relationship (Robergs, Ghiasvand, & Parker, 2004).

Applicable to the current topic, another potential mediator of the acid-base balance is the H^+ buffering by phosphate released from bone. Chronic metabolic acidosis does not alter intestinal calcium absorption, but increases urine calcium excretion. This suggests that the source of the additional urinary calcium is from bone mineral. The efflux of calcium is stimulated by a reduction in pH. Phosphate and proteins released from bone are used to buffer the additional hydrogen ions present in the plasma and body fluids (Bushinsky et al., 2003). This occurs during acute metabolic acidosis, but not respiratory acidosis (Bushinsky, 2001; Bushinsky et al., 2001). Osteoblasts synthesize collagen which is then mineralized to form bone. Metabolic acidosis inhibits genetic expression for osteoblastic collagen synthesis which is necessary for mineralization leading to bone formation (Bushinsky, 2001). Small changes in pH have a significant effect on osteoblastic function along with stimulation of osteoclastic resorption (Bushinsky, 2001; Bushinsky et al., 2003; Frick, Jiang, & Bushinsky, 1997).

During high-intensity exercise, it is common for blood pH to drop from the normal range of approximately 7.4 down to 7.08 at the end of a 30 second cycling sprint, which occurs often during bicycle racing and remained low into recovery (Bogdanis, Nevill, Boobis, Lakomy, & Nevill, 1995). Similarly, exceeding aerobic capacity causes a decrease in blood pH. Resistance exercise causes hypercalciuria, or excess calcium excreted in the urine (Ashizawa, Fujimura, Tokuyama, & Suzuki, 1997). Ashizawa et al. surmised that the most likely source of excess calcium is the alkaline calcium salts mobilized from bone for the maintenance of blood pH. In vitro studies showed an efflux

of calcium from bone during acute and chronic metabolic acidosis (Bushinsky et al., 2003).

In summary, during road cycling competition, a cyclist may experience metabolic acidosis which has been shown to cause damage to bone structure. Metabolic acidosis also leads to increased bone resorption. Blood pH is maintained by mobilization of phosphate from bone causing disruption to calcium homeostasis. Thus, metabolic acidosis is considered a part of the many physiological instigators leading to low BMD in road cyclists.

Summary. There are many theories of contributing factors leading to low BMD in road cyclists. Among these factors, low dietary calcium intake may exacerbate the problem by contributing to the disruption in serum calcium homeostasis. However, research shows that increased dietary calcium does not increase BMD in cyclists. During sports competition, elevated cortisol is common. Elevated cortisol has been connected to low BMD. Similarly, dermal calcium loss during intense cycling competition and training triggers the release of PTH to mobilize calcium from bone. Parathyroid hormone elicits an immediate response to a decrease in serum calcium, and is used as an indicator of bone remodeling during exercise. Barry and Kohrt (2007) suggested the prolonged elevation in PTH during endurance cycling may have the same effect on bone remodeling as in patients with hyperparathyroidism. These patients exhibit decreased BMD over time. Intense training and competition also may induce metabolic acidosis, another contributor to bone resorption. These may be underlying causes of long term changes in BMD in endurance cyclists.

Longitudinal Changes in BMD in Athletes

Longitudinal changes in BMD of the total hip, neck, shaft, and trochanter regions in competitive cyclists have been reported. Bone mineral density decreases throughout the course of a competitive season (Barry & Kohrt, 2008). It is unclear whether changes in BMD are unique to cycling or whether other athletes engaging in a high volume of endurance training are also at risk. For example, the same occurrence was not evident in male triathletes, an event consisting of swimming, cycling, and running (Maïmoun et al., 2004). In the triathletes, lumbar spine BMD increased after an 8 month racing season. However, the 7 triathletes were aged 18-20 years and may not yet have achieved peak bone mass.

Barry and Kohrt (2008) measured BMD of 20 cyclists at the start of the season, month 4.5, month 9, and month 12. Small, negative changes in BMD occurred by month 4.5, and BMD was statistically lower than baseline at month 9, the end of the competitive season, in most skeletal sites: total hip, femoral neck, femoral trochanter, and femoral shaft. Lumbar spine was the only exception. It is worthy to note that three of the participants had BMD of the lumbar spine classified as osteopenic by WHO.

Klesges et al. (1996) found a similar phenomenon among collegiate basketball players; thus, the notion that BMD is optimal in athletes performing vigorous weight-bearing exercise does not always hold true. The authors found that bone mineral content (BMC), a measurement similar to BMD, decreased by 3.8% from preseason to midseason. The authors speculated the loss of BMC occurred as a result of calcium imbalance during intense training due to dermal calcium loss. However, no association

was found between BMC and dermal calcium loss. There was a significant negative association with the estimated rate of dermal calcium loss and BMC at the hip, femoral neck, and femoral shaft. The authors concluded that athletes who train intensely for long periods may be at risk for decreases in BMC. Second, there was a relatively large amount of dermal calcium loss and the concentration of calcium lost through sweat decreased throughout the season.

Bone density was shown to decrease across a single competitive season in collegiate basketball players and in competitive cyclists. The hypothesized cause of the loss in BMC (basketball) and BMD (cyclists) was dermal calcium loss due to excessive sweating during training. The loss of calcium causes serum calcium to decrease, thus increasing the secretion of PTH. Secondary hyperparathyroidism is a medical condition that causes increased secretion of PTH in response to low serum calcium due to low calcitriol or inability of the intestine to absorb calcium. This condition is treated by correcting the calcium and calcitriol levels (Hyperparathyroidism, 2010). Exercise physiologists have investigated this method as a solution to compensate for the dermal calcium loss during exercise. The use of calcium supplementation with meals and during exercise may assist in the maintenance of calcium homeostasis.

Effect of Oral Calcium Supplementation on PTH

In an attempt to alter the change in BMC across the season in basketball players, Klesges et al. (1996) administered an oral calcium load during exercise. A calcium fortified sports beverage containing 600 mg calcium and 160 iu calcitriol was administered to the aforementioned basketball players during all practice sessions along

with daily calcium supplementation with meals throughout a competitive season. There was an increase of 6.1% in total body BMC. The increase in BMC occurred in an approximate 4 month period. The researchers suggest athletes engaging in long duration, strenuous exercise use a calcium supplementation during practice to preserve bone health (Klesges et al., 1996).

More recently, researchers have made attempts to potentially offset the dermal loss of calcium during exercise by administration of calcium supplementation with meals (Barry & Kohrt, 2008) or during exercise (Guillemant et al., 2004; Barry et al., 2011). Half of the cyclists from the year-long measurement of BMD in cyclists were prescribed 1,500 mg calcium to be taken in three divided doses with meals (Barry & Kohrt, 2008). The calcium dosage was based on the estimation that 124 mg/h of calcium is lost through sweat. Given that only one-third of a calcium supplement is absorbed, a 1,500 mg dose was expected to compensate for the dermal calcium loss over a 4 hour training period. The calcium supplementation group, however, showed no difference in BMD throughout the season compared to the control group.

Conversely, calcium supplementation taken during exercise has been shown to have a significant effect on biomarkers of bone metabolism (Barry et al., 2011; Guillemant et al., 2004). Cyclists performed a 1 hour trial at 80% VO_{2max} with and without calcium fortified mineral water (Guillemant et al., 2004). Prior to and during the trial, 486 mg/L was administered in 250 mL portions every 15 minutes for a total of 972 mg calcium and 2 L of water. Calcium, serum CTX, BALP, and PTH were measured every 30 minutes, starting 1 hour prior to exercise and ending 2 hours post exercise. An

estimation of changes in plasma volume corrected for concentrations of biochemical substances was used according to the formula by van Beaumont, Strand, Petrofsky, Hipskind, and Greenleaf (1973) which decreased by 9.9% at the end of the trial.

When participants drank plain water, calcium levels did not change, serum PTH increased significantly (3 times above baseline), BALP did not change, and CTX increased 48% above baseline. Also, without ingestion of calcium, CTX began increasing 30 minutes after the onset of exercise, reached statistical significance at the end of exercise, and peaked 1 hour after exercise. Guillemant et al. (2004) noted that due to this quick and significant increase in CTX, the response time of biochemical bone markers is rapid. There was a decrease in calcium 30 minutes after exercise. During the high-calcium water trial, calcium concentration peaked at the end of exercise, with a starting value of 2.35 mmol/L which increased to 2.51 mmol/L. There was a suppression of CTX, partial suppression of PTH, and no change in BALP. Thus, intake of calcium during exercise attenuated increases in markers of bone resorption.

Although the volume of sweat was not measured, it was estimated by Guillemant et al. (2004) that because calcium remained constant during the placebo trial, the loss of calcium was of the same degree as the decrease in plasma volume. Guillemant et al. surmised that, after exercise, although plasma volume had returned to baseline coupled with a decrease in serum calcium, the loss of calcium in sweat had not been replaced during the low calcium trial. In conclusion, the PTH response to exercise was likely due to calcium lost in sweat. Long term bone resorption following exercise may explain low BMD in endurance athletes. Ingestion of calcium before and during exercise was

suggested as a preventative measure to counteract the risk of osteoporosis in male road cyclists.

As an extension of the work by Guillemant et al. (2004), Barry et al. (2011) measured calcium citrate loading prior to and during exercise to determine the effect of markers of bone resorption in comparison to a placebo trial during a 35-km cycling time trial. Blood samples were drawn at the beginning of the lab trial, every 15 minutes during the approximate 1 hour trial, and at the end of the trial.

The beverages consisted of a sports drink with and without 1,000 mg calcium per liter. For the first test condition, the cyclists were instructed to consume 1,000 mL of the fortified beverage 20 minutes before exercise and the placebo every 15 minutes during exercise. In the second test condition, cyclists drank 1,000 mL of placebo before exercise, but consumed the fortified beverage every 15 minutes during the work bout. For the third condition, the cyclists consumed only the placebo in corresponding dosages. The researchers corrected for changes in plasma volume in the same manner as Guillemant et al. (2004) according to van Beaumont et al. (1973).

The rise in PTH during the placebo trial was attenuated by ingestion of calcium both before and during exercise. Small increases in CTX were recorded when calcium was administered prior to exercise; otherwise, bone resorption measured by CTX was not affected by calcium intake. In contrast, Guillemant et al. (2004) found that the rise in CTX reached statistical significance 30 minutes following exercise in the high calcium trial, but this measurement was not recorded in the study by Barry et al. (2011).

Similar to Guillemant et al. (2004), BALP and ionized calcium were not different during any trial in the Barry et al. (2011) study. Seemingly, dermal calcium loss was not affected by calcium ingestion. The major finding by Barry et al. was that calcium supplementation during exercise attenuated the exercise induced increase in PTH. Also, ingestion of calcium before exercise had the same effect on PTH as ingestion of calcium during exercise. Barry et al. postulated that the trigger for PTH increase was due to calcium loss through sweating. Ingestion of calcium provides a non-skeletal source of calcium to minimize the extent to which skeletal stores of calcium are released.

In an earlier work, calciotropic hormone response to submaximal running was measured in female long distance athletes (Grimston, Tanguay, Gundberg, & Hanley, 1993). A comparison was made between 45 minutes of running with and without ingestion of a calcium load via dairy products (plain yogurt with skim milk). Serum calcium increased during the 1,000 mg calcium load trial along with no significant change in PTH. An interesting feature of this study was the comparison between participants with normal lumbar spine BMD and those with low lumbar spine BMD. The authors found that despite an oral calcium load, participants with low lumbar spine BMD had a significant increase in PTH immediately post-exercise. In contrast, PTH of participants with normal BMD did not change post-exercise. Thus, percent change in PTH levels during exercise was negatively correlated with bone density. The reason for a greater PTH response to exercise in female runners with low BMD compared to runners with normal BMD is unclear. For runners with low BMD, participation in endurance

running may aggravate the existing condition. Assessment of BMD in female runners is important to maintain bone health.

Guillemant, Le, Guillemant, Delabroise, and Arnaud (1997) determined the time course of change in ionized calcium and PTH after ingestion of calcium rich mineral water. At rest, participants consumed 250 mg calcium in 0.5 L of mineral water. There was an inhibition of PTH which then decreased 43% below baseline after 1 hour. Ionized calcium increased significantly after 1 hour. However, the relationship between the calcium dose and the inhibited response of PTH was unclear. This shows that a calcium supplementation may help minimize in bone loss. Ingestion of calcium rich mineral water provides a low-calorie dietary supplement.

A note of interest pertaining to calcium intake during exercise is the finding of Messonier, Kristensen, Juel, and Denis (2007). Administration of sodium citrate, similar to calcium citrate, was used to examine mechanisms of pH regulation during supramaximal cycling to test maintenance of metabolic alkalosis. The authors found significantly greater pH when calcium was administered compared to the placebo trial at the end of exercise coupled with greater efficiency in performance. There was a negative correlation between supramaximal work capacity and pH. Thus, consumption of calcium citrate during exercise not only decreased biomarkers of bone resorption, and risk of metabolic acidosis, but may also lead to an increased work capacity during high-intensity exercise.

Summary. Ingestion of an oral calcium load prior to or during exercise such as cycling, running, and basketball playing attenuates the exercise induced rise in PTH.

Other biomarkers of bone resorption, such as CTX, were lower when participants ingested calcium during exercise. For athletes with low BMD, such as cyclists, taking calcium supplements during training may attenuate the decreases in BMD. Though calcitriol plays an important role in intestinal calcium absorption, current research experiments do not include simultaneous calcium with calcitriol supplementation in cyclists.

Overall Summary

In conclusion, although a single contributing factor has yet to be determined, it is certain that cycling lacks an osteogenic effect. The purpose of bone remodeling, the process of bone mineral deposition and bone mineral resorption, is to maintain serum calcium homeostasis. This process is regulated by the release of PTH which stimulates the release of calcium from bone to blood. Although exercise typically has the potential for a positive osteogenic effect on bone, it has recently been found that competitive, road cyclists are at risk for osteopenia, the precursor to osteoporosis.

Measurement of biochemical indicators of bone remodeling such as PTH and CTX are elevated during high intensity, long duration exercise. The cause of the increase is likely multifactorial and probable instigators include elevated cortisol during intense exercise, dermal calcium loss during excessive sweating, and metabolic acidosis accompanying high-intensity work. Stabilization in biochemical markers identified as having properties of bone resorption has been documented with an oral calcium load during cycling, but not when ingested at mealtime.

A decrease in BMC throughout the competitive season has been discovered in a team of collegiate basketball players. The exercise-related decreases in BMD over the course of a season were attenuated via oral calcium load during exercise in the basketball players. Decreases in site specific BMD over the course of a competitive season have also been reported in male, road cyclists. However, the longitudinal response of an oral calcium load during exercise has not been documented in male, road cyclists.

The association between cortisol level during a road cycling competition and BMD in cyclists is unknown. Thus, the purpose of study one was to examine the association between cortisol level during competition and BMD in road cyclists. The long term effect of an oral calcium load during exercise on changes in BMD throughout a competitive road cycling season is also unknown. More research is needed to find methods to improve bone remodeling in favor of formation and reduced resorption in competitive, male road cyclists. Thus, purpose of study two was to examine the effects of an oral calcium and calcitriol load during training and racing on changes in BMD across a 5 month period in male, road cyclists.

CHAPTER III

CORTISOL AND BONE MINERAL DENSITY IN COMPETITVE MALE CYCLISTS

Introduction

Interest in bone health among endurance athletes is growing. Studies showing low bone mineral density (BMD) in male cyclists are numerous (Nichols, Palmer, & Levy, 2003; Rector, Rogers, Ruebel, & Hinton, 2008; Warner, Shaw, & Dalsky, 2002) and osteopenia is prevalent in this population (Medelli, Lounana, Menuet, Shabani, & Cordero-MacIntyre, 2008; Rector et al., 2008). Reports of lower BMD in competitive cyclists compared to age-matched controls are found in adult male cyclists (Smathers, Bemben, & Bemben 2009), male master (over 30 years of age) cyclists (Nichols et al., 2003), and in male postpubertal cyclists (Olmedillas, González-Agüero, Moreno, Casajús, & Vicente-Rodríguez, 2011; Rico, Revilla, Villa, Gomez-Castresana, & Alvarez, 1993). Although not well documented, orthopedic injuries occur due to falls during cycling training and competition (Martinez, 2006) making the occurrence of low BMD even more concerning. The etiology of low BMD in cyclists is multifactorial and may be partially due to an imbalance in stress hormones, such as cortisol.

Cortisol triggers bone mineral resorption (removal) to free amino acids for use as an energy source through gluconeogenesis. Cortisol indirectly acts on bone by blocking calcium absorption which decreases bone cell growth (Chiodini & Scillitani, 2008). The disruption to serum calcium homeostasis (Heshmati, Riggs, Buritt, McAlister, Wollan, & Khosla, 1998) increases bone resorption (Heshmati et al., 1998) and ultimately reduces

BMD (Hardy & Cooper, 2010). Even a short bout of elevated cortisol secretion may cause a decrease in BMD. Excessive elevation of cortisol, such as in hypercortisolism or Cushing's syndrome, is linked to a high prevalence of osteoporosis and may be associated with the age-related decrease in BMD in the elderly (Raff et al., 1999).

Serum cortisol concentration elevates due to physiological and psychological factors. A cyclist's resting levels of cortisol will increase in response to endurance training (Bouget, Rouveix, Michaux, Pequignot, & Filaire, 2006; Hoogeveen & Zonderland, 1996), in response to intense cycling (Scott et al., 2011), and possibly in response to psychological arousal during competition (Aubets & Segura, 1995; Crewther, Heke, & Keogh, 2011; Filaire, Alix, Ferrand, & Verger, 2009; Maïmoun & Sultan, 2011). Although the cortisol response specific to cycling competition has not been reported, researchers have reported elevations in cortisol on competition day due to anticipation of mental and physical performance (Gatti & de Palo, 2011). Cortisol further responded to the physiological stress of competition as was indicated from pre- to post-competition concentration.

The purpose of this study was to determine whether or not elevated cortisol immediately pre- and/or post-competition contributes to low BMD in male cyclists. Information garnered in this investigation determined whether cortisol levels at the start or finish of a competition were related to lumbar spine, total hip, femoral neck, or femoral trochanter BMD in competitive male cyclists. It was hypothesized that salivary cortisol would be correlated with BMD within the male cyclist study group. A secondary

goal of the analysis was to determine the factors that are significantly associated with lumbar spine and hip BMD in competitive male cyclists.

Methodology

Participants

Amateur cyclists were recruited for participation in this study by contacting male competitors in a state championship time trial cycling competition. Among the participating cyclists ($N = 35$) were state, regional, and national competitors, with one former Olympian. Inclusion criteria consisted of cyclists over the age of 30 years, who trained regularly, and had at least 2 years of cycle-specific training experience. Exclusion criteria included use of medication known to affect the endocrine system and bone metabolism, or a history of endocrine disorders.

Instrumentation

BMD measurements. Lumbar spine (L1 – L4), total hip, femoral neck, and femoral trochanter BMD were measured using a Hologic Discovery QDR Series dual energy x-ray (DXA) absorptiometer (Bedford, MA) within 2 weeks of the cycling competition. The standard manufacturer protocols were followed and scans were conducted and analyzed by a licensed technician. The DXA was calibrated using a spine phantom prior to data collection, assuring that all calibrations fell within appropriate ranges. Data were recorded as *t*-scores and g/cm^2. Femoral neck and trochanter scans were performed on the cyclists' non-dominant leg, defined as the opposite leg used to hypothetically kick a ball. The hip scan included the femoral neck and the trochanteric region. To avoid false images on the scan, all cyclists wore hospital scrubs and removed

all metal objects from their bodies. Adequate distributions were found to categorize cyclists as having either normal BMD (t > -1.0 SD) or low BMD ($t \leq$ -1.0 SD) according to the definitions by the World Health Organization (WHO) for use in statistical analyses (Nordin, 1987; World Health Organization, 2004).

Body mass and height. Height was measured to the nearest 0.5 cm using a stadiometer (SECA model 222, seca Gmbh & Co. Kg, Hamburg, Germany) while cyclists stood barefoot on level ground with heels together and weight evenly distributed. Weight was measured to the nearest 0.1 kg without shoes after voiding using a digital scale (SECA model 770, Vogel & Halke, Hamburg, Germany). Height and weight were used to calculate body mass index (BMI) using weight in kilograms divided by height in meters squared.

Calcium intake. Calcium intake was measured using a 1 day dietary recall (see Appendix A) performed during the week prior to the race on a day representative of the typical diet. Food intake, including vitamin supplements, was analyzed for daily calcium (mg/d) by the principle investigator using the US Department of Agriculture website (http://www.mypyramid.gov).

Cortisol. Cortisol was measured within 10 minutes prior to the start of the race and within 5 minutes of race finish. Cyclists were informed of the method of saliva collection prior to racing. The cyclists provided a saliva sample in a Salivette (Sarstedt, Newton, NC), by placing a small cylindrical, non-toxic polymer oral swab under the tongue for 2 minutes. Saliva samples were stored on dry ice and were analyzed within 2 days of collection. Cortisol concentration within saliva was assayed in duplicate by

radioimmunoassay (Hormone Assay and Analytic Services Core, Vanderbilt University, Nashville, TN) with an intra-assay coefficient of variation of 3%.

Demographics. Participants reported age, number of years of bike-specific training, average number of minutes spent riding a bike (min/week), approximate number of races per season, number of minutes spent lifting weights (min/week), and number of minutes spent running (min/week) on a survey (see Appendix B).

Pre-race nervousness. Competitive state anxiety was used to measure pre-race nervousness. Due to time sensitivity of gathering questionnaire data immediately prior to a competitive event, three items were chosen from the Competitive State Anxiety Inventory 2 (Martens, Vealay, & Burton, 1990). A 4-point Likert-type scale was used to rate each item where a score of one represented 'not at all' and a score of four represented 'very much so.' Items for the shortened inventory were chosen to measure participants' cognitive and somatic anxiety. The items chosen were as follows: I am concerned about this competition; I feel nervous; My body feels tense. An average of the score for the three items produced the final score for pre-race nervousness. The shortened inventory demonstrated good reliability (Cronbach's alpha = .70) in practice.

Procedures

All participants were informed of the procedures of the study and signed an informed consent document (see Appendix C). Eligible participants reported to the University Exercise Science Lab within 2 weeks of the state championship time trial competition. Upon arrival, cyclists completed the demographic questionnaire and completed the 1-day dietary recall. Cyclists were provided with hospital scrubs and

removed shoes for all laboratory measurements. Measures of height and body mass were performed. Lastly, bone density scans were completed in randomized order. University Institutional Review Board approval was gained prior to data collection (see Appendix D).

The day of the race, cyclists reported to the start line where all measurements were performed 10 minutes prior to race start time. To avoid contamination of the saliva specimen from food or drink intake, cyclists were instructed to rinse their mouth with plain water and were not allowed to consume food or drink 10 minutes prior to saliva collection. A research assistant provided each cyclist with the competitive state anxiety questions on paper and a Salivette. As cyclists answered the questionnaire, the cotton swab from the salivette was placed in the mouth for 2 minutes to allow full saturation. Immediately post-race, the cyclists repeated the same procedure with a new Salivette. Saliva samples were placed on dry ice at the race site and transported to the laboratory for analysis.

Statistical Analyses

Anthropometric measurements and cortisol concentrations for pre- and post-race are reported as mean ± standard deviation. Pearson Product Moment correlations were used to assess the relationship between cortisol (pre- and post-race) and site specific BMD for the study population, for each age category (30 – 39, ≥ 40 years), and for each calcium intake group (≤ 1,200 mg/d and > 1,200 mg/d). A two-way repeated measures analysis of variance (ANOVA) was used to determine whether cortisol levels (pre- and post-race) were different for site specific BMD groups (low BMD, high BMD).

Linear regression analyses with stepwise selection were used to determine whether age, BMI, daily calcium intake, number of years cycling experience, number of minutes weight lifting (min/week), number of minutes running (min/week), pre-race cortisol (nmol/L), post-race cortisol (nmol/L), and pre-race nervousness were significantly associated with site-specific BMD. Due to the small sample size, three separate analyses were performed; each analysis consisted of three variables. The three variables for the first subgroup described participant demographics of age, BMI, and daily calcium intake. The second subgroup contained variables describing training regimen: years of cycling experience, weekly minutes of weight training, and weekly minutes of run training. Variables pertaining to hormonal and emotional characteristics, prerace cortisol, post-race cortisol, and prerace nervousness, were entered into the third stepwise linear regression. Significant variables from each subgroup were then combined into one final model to determine variables that were significantly associated with site-specific BMD. IBM SPSS version 19 was used to analyze the data.

Results

Of the 35 participants, three cyclists were excluded due to a flat tire during the competition. Additionally, BMD measurements were not obtained in two cyclists, one pre-race cortisol sample was contaminated, and eight post-race cortisol samples were contaminated. This resulted in 21 complete data sets.

The cyclists' mean age was 42.1 years (*SD* = 9.0 years, *CI* = 38.8 years to 45.5 years). The cyclists average height was 180.4 cm (*SD* = 6.0 cm, *CI* = 178.2 cm to 182.6 cm), and weight was 80.9 kg (*SD* = 6.9 kg, *CI* = 78.3 kg to 83.5 kg). The cyclists

participated in a mean of 22 (*SD* = 11, *CI* = 17 to 26) races per season. Descriptive statistics for cyclists' BMD scores, training history, and other characteristics are shown in Table 1. Based on WHO classifications, 50% of the male cyclists had either lumbar spine or hip BMD classified as osteopenia and 40% of the cyclists had osteopenia of the lumbar spine. Although not statistically significant, mean BMD (g/cm^2) of the total hip and femoral neck was lower in cyclists 30-39 years (*M* = 0.951, *SD* = 0.08; *M* = 0.789, *SD* = 0.10, respectively) of age than in cyclists over 50 years of age (*M* = 0.963, *SD* = 0.20; *M* = 0.851, *SD* = 0.19, respectively). Estimated daily calcium intake was at or below 1,200 mg per day in 50% of the participants, and approximately half of the participants (53%) were classified in the 30 – 39 year age category.

Correlations between Cortisol and BMD

Correlations between pre- and post-race salivary cortisol and BMD of the lumbar spine, total hip, trochanter, and femoral neck are presented in Table 1. Table 2 presents correlations across age categories and calcium intake groups. There was a significant positive relationship between post-race salivary cortisol concentration and total hip BMD for cyclist's ≥ 40 years of age. The association between post-race salivary cortisol concentration and femoral trochanter BMD approached significance in cyclists' ≥ 40 years of age. Additionally, there was no significant correlation between cortisol concentration and low (≤ 1,200 mg/d) or normal (> 1,200 mg/d) calcium intake groups.

Table 1

Means, Standard Deviations, and Correlations for BMD and Predictor Variables ($N = 30$)

Variable	*M*	*SD*	1	2	3	4	5	6	7	8	9	10	11	12
Predictor variable														
1. Age	42.1	9.0	--											
2. BMI	24.8	1.5	.00	--										
3. Calcium intake	1324.8	547.0	.07	.07	--									
4. Weight training	35.3	54.3	.01	.31	.62†	--								
5. Years of cycling	11.7	9.7	.67†	-.05	.00	-.12	--							
6. Run training	13.2	40.2	-.14	.42*	-.16	.20	-.23	--						
7. Pre-race cortisol[a]	9.4	4.1	-.05	.31	.15	.25	.08	.29	--					
8. Post-race cortisol[b]	20.8	14.5	-.02	-.24	.00	-.23	-.11	.19	-.08	--				
9. Prerace nervous	2.6	0.7	-.16	-.27	.06	-.03	-.05	-.21	-.19	-.08	--			
Dependent variables														
10. Lumbar spine	1.033	0.12	.02	-.11	.40*	.61†	-.22	-.44	-.04	.03	.03	--		
11. Total hip	0.971	0.12	-.24	.16	.21	.66†	-.28	.13	.16	-.02	-.03	.76†	--	
12. Femoral neck	0.817	0.12	-.27	.08	.38*	.75†	-.37*	.12	.11	-.08	-.11	.86†	.91†	--
13. Femoral trochanter	0.738	0.10	-.01	.05	.29	.64†	-.17	.09	.14	-.01	.10	.82†	.90†	.86†

Note. [a]$n = 29$, [b]$n = 22$; † denotes correlation is significant at the .01 level; * denotes correlation is significant at the .05 level. BMI represents body mass index. Calcium intake was estimated as mg/day. Weight training and run training were measured in min/wk. Salivary cortisol was measured in nmol/L. BMD was measured as g/cm^2.

Table 2

Correlations between Cortisol and BMD across Age and Calcium Intake Categories

	Age 30 – 39 years		Age 40 +		≤ 1,200 mg/d		> 1,200 mg/d	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Pre-race cortisol[a] versus:								
Lumbar Spine (L1-L4)	.14	.62	-.83	.53	-.15	.59	-.01	.98
Total Hip	.21	.46	.03	.93	-.02	.93	.22	.46
Neck	.25	.38	-.12	.68	.04	.88	.13	.67
Trochanter	.28	.32	.13	.67	.20	.47	.17	.56
Post-race cortisol[b] versus:								
Lumbar Spine (L1-L4)	-.13	.67	.55	.12	.21	.55	-.05	.87
Total Hip	-.18	.56	.72	.03*	.11	.77	-.08	.80
Neck	-.29	.34	.51	.16	.31	.37	-.22	.49
Trochanter	-.04	.90	.62	.07	.37	.29	-.01	.99

Note: [a]*n* = 29, [b]n = 22; Significance at the .05 level denoted by *; Salivary cortisol was measured in nmol/L. BMD was measured as g/cm^2.

Cortisol and Low vs. Normal BMD

A two-way repeated measures ANOVA was conducted to determine whether cortisol levels were different for BMD categories defined as low (t-score $\leq$ -1.0) and normal (t-score > -1.0). Means (SD) for pre- and post-competition cortisol levels across BMD classifications of low BMD were 8.47 nmol/L (2.20) and 18.40 nmol/L (8.00), respectively, and normal BMD were 10.79 nmol/L (4.38) and 23.35 nmol/L (19.22), respectively. There was not a significant interaction between cortisol levels and BMD categories, $F(1, 19) = 0.14$, $MSE = 126.34$, $p = .71$, $\omega^2 = .00$. There were no differences across BMD categories, $F(1, 19) = 1.25$, $p = .27$, $\omega^2 = .01$. Cortisol level did increase significantly from pre- to post-competition, $F(1, 19) = 10.48$, $p = .004$, $\omega^2 = .31$.

Variables Associated with BMD

Associations with lumbar spine BMD. Daily calcium intake ($\beta = 0.40$, $t = 2.30$, $p = .029$) was the only participant characteristic related to lumbar spine BMD. Weight training ($\beta = 0.61$, $t = 4.03$, $p < .001$) was the only training regimen characteristic that was statistically associated with lumbar spine BMD. Daily calcium intake and weight training were entered in a single stepwise linear regression to determine associations with BMD, but only weight training was selected ($R^2 = .37$). There was no statistically significant association among hormonal and pre-race nervousness variables and BMD.

Associations with total hip BMD. None of the participant characteristic variables were significantly associated with total hip BMD. Weight training was the only training characteristic variable associated with total hip BMD ($\beta = 0.62$, $t = 4.19$, $p < .001$, $R^2 =$

.39). There was no statistically significant association among hormonal and pre-race nervousness variables and BMD.

Associations with femoral neck BMD. Of the participant characteristic variables, daily calcium intake was associated with femoral neck BMD ($\beta = 0.37$, $t = 2.10$, $p = .045$). Weight training ($\beta = 0.72$, $t = 6.39$, $p < .001$) and years of cycling experience ($\beta = -0.29$, $t = -2.60$, $p = .015$) were both related to femoral neck BMD. Daily calcium intake, weight training, and years of cycling experience were entered in a stepwise regression analysis to determine associations with femoral neck BMD. Both weight training and years of cycling experience were significantly associated with femoral neck BMD ($R^2 = .63$). Results of the two variable model for femoral neck BMD are presented in Table 3. There was no statistically significant association among hormonal and pre-race nervousness variables and BMD.

Associations with femoral trochanter BMD. None of the participant characteristics variables were significantly associated with total hip BMD. Weight training was the only training characteristic variable related to femoral trochanter BMD ($\beta = 0.68$, $t = 4.92$, $p < .001$, $R^2 = .46$). There was no statistically significant association among hormonal and pre-race nervousness variables and BMD.

Discussion

Findings from this study support existing literature showing that concentration of cortisol is correlated with BMD in cyclist's $\geq$ 40 years of age (Raff et al., 1999). The results of this study also add to a growing body of literature pertaining to factors

Table 3

Linear Regression Analysis Summary for Participant Characteristics and Femoral Neck BMD ($N = 30$)

Variable	*B*	*SE B*	*β*	*t*	*p*
Weight training (min/wk)	0.01	0.002	0.72	6.39	< .001
Years of cycling experience	-0.03	0.010	-0.29	-2.60	0.015

Note. Variable contribution to femoral neck BMD: weight training, $R^2 = .57$; years of cycling experience $R^2 = .14$.

related to BMD in male, competitive cyclists. As indicated by R^2, an increase in weight training (min/wk) was associated with higher BMD of the lumbar spine (37%), total hip (38%), femoral neck (57%), and femoral trochanter (46%). Both weekly weight training and years of cycling experience together were used to create a statistical model that explained 63% of the observed changes in femoral neck BMD. There was a significant direct correlation (r = .62) between weight training and calcium intake, however the reason for this correlation is unknown. As a result, post hoc analyses were performed to determine the contribution of calcium intake to each BMD site. Bivariate linear regression analyses showed that estimated daily calcium intake significantly explained 16% and 14% of the variance in lumbar spine and femoral neck BMD, respectively. Existing literature and present data have shown a prevalence of osteopenia in male cyclists (Medelli et al., 2008; Rector et al., 2008). The present findings suggest that cyclists should include weekly weight training and consume adequate daily calcium to improve or maintain BMD.

Raff et al. (1999) measured cortisol and BMD in 130 men which is approximately four times the sample size of the present study. Because the Spearman r-values between lumbar spine BMD and cortisol in the two studies are similar, the correlation values in the present study may be due to the small sample size (N = 21). Raff et al. documented a significant negative correlation in a morning measurement of salivary cortisol and lumbar spine BMD. However, findings from the present study do not support the relationship between cortisol and BMD in cyclists and the notion that the decline in BMD may be partly due to excess glucocorticoid hormone secretion during races. Cyclists typically

train long hours at a high intensity and participate in many competitive events eliciting repeated elevations of cortisol over a prolonged period of time. One may assume that an accumulative effect of the increase in cortisol throughout the competitive season may compound the negative impact of cortisol on BMD.

A surprising significant positive correlation (r = .72) in post-race cortisol and total hip BMD and a near significant positive correlation (r = .62) between post-race cortisol and femoral trochanter BMD in male cyclist's $\geq$ 40 years of age were found. Due to the small sub-sample size (n = 9), one should be cautious in interpreting this correlation and further analysis about this phenomenon is warranted. Even when controlling for weight training and/or daily calcium intake, the positive correlation remained. Correspondingly, this finding is in line with the results of Raff et al. (1999) who documented a significant positive correlation in cortisol (at night) and lumbar spine BMD in elderly men, approximately 70 years of age.

Differences in cortisol concentration between cyclists with low BMD ($t \leq -1.0$) and cyclists with normal BMD ($t > -1.0$) were not supported by these data. Thus, the hypothesis that cyclists with low BMD would respond to a stressful event with a greater concentration of cortisol compared to cyclists with normal BMD was not supported. However, a significant increase in salivary cortisol concentration from pre- to post-competition, where time to complete the 40 k event ranged from 53 min to 1 hour 7 min, was documented in the study. Under normal physiological conditions, salivary cortisol concentration would decline throughout the morning hours due to the circadian rhythm of the hypothalamic-pituitary-adrenal axis with a peak in the early morning hours and a

nadir at night (Teo, McGuigan, & Newton, 2011). This finding suggests that a competitive cycling event provides strong psychological and physiological stimulation for the secretion of cortisol. This study also demonstrated that measurement of salivary cortisol is an appropriate method for assessing the hormonal response to competition stress in male cyclists.

Correlations between pre-race nervousness and pre-race cortisol were not supported in the present study. However, several studies have found significant correlations between pre-event cortisol and measures of pre-race nervousness (Aubets & Segura, 1995; Bouget et al., 2006; Crewther et al., 2011; Edwards, Wetzel, & Wyner, 2006; Filaire et al., 2009; Gatti & de Palo 2011; Maïmoun & Sultan, 2011; Scott et al., 2011). Due to pre-race time constraints in the present study, an abbreviated version of the Competitive State Anxiety Inventory 2 (Martens et al., 1990) was used. Use of the full scale may provide greater insight into the cycling competitor's pre-race psychological state.

Data relating cortisol to markers of bone mineral resorption (removal) are mixed. A disruption in the balance between bone mineral deposition and bone mineral resorption due to endurance cycling are hormonally mediated. Cycling induces secretion of parathyroid hormone (PTH) and type I collagen C (CTX), immediate measures of bone metabolism (Barry et al., 2011; Barry & Kohrt, 2007; Guillemant, Accarie, Peres, & Guillemant, 2004). Researchers have suggested that bone loss in response to endurance cycling results from the mediation of cortisol with markers of bone resorption, possibly inducing an acceleration of bone resorption (Rector et al., 2008). For example, CTX was

positively associated with cortisol in cyclists during a laboratory cycling trial. Also, cortisol was once considered a regulator of PTH during exercise; however, data on this result are mixed (Tsai, Lin, Chen, Cheng, & Yang, 1997).

Consistent with existing literature, 40% of the cyclists had osteopenia of the lumbar spine and 50% of the cyclists had low BMD ($t < 1.0$) of the lumbar spine or hip (Medelli et al., 2008; Rector et al., 2008). Participation in competitive cycling, where falling is prevalent, coupled with low BMD places cyclists at a high risk for fracture (Nichols et al., 2003; Smathers et al., 2009). Nichols et al. recommended cyclists have regular BMD screening, perform supplemental high-impact or weight training physical activity, and consume adequate calcium and vitamin D. The present findings support the recommendations of Nichols et al. Of the cyclists studied, 40% reported weekly participation in weight training. Participation in weight lifting was positively associated with BMD of the lumbar spine, total hip, femoral neck, and femoral trochanter. It should be noted that 14 participants with low BMD reported participation in less than 20 min of weight lifting per week; whereas 10 participants with normal BMD reported participation in at least 30 minutes of weight lifting per week. In contrast to Nichols' et al. suggestions, participation in running, a high impact exercise, was not significantly associated with BMD in the present population.

Consistent with the recommendations by Nichols et al. (2003), daily calcium intake was correlated with BMD of the lumbar spine and femoral neck. In addition, post hoc linear regression analyses revealed that daily calcium intake was significantly associated with higher lumbar spine and femoral neck BMD. Of the participating

cyclists, 50% reported daily calcium intake greater than 1,200 mg/d. Evidence supports adequate intake of calcium and vitamin D to attenuate the loss of BMD in cyclists (Barry et al., 2011; Nichols et al., 2003).

Nichols and Rauh (2011) measured BMD of master male cyclists in a 7-year longitudinal study. The cyclists experienced a greater decline in BMD compared to non-athletes. Of the cyclists, 6 became osteoporotic during the 7-year period. However, participants that reported participation in weight training experienced less bone loss compared to those who did not. A meta-analysis by Speckler (1996) showed evidence from 17 trials that high-impact physical activity had a greater impact on BMD when high calcium intake was included. The author concluded that impact exercise and high calcium intake may not affect BMD independently, but the beneficial impact of high calcium intake is present when impact activity is also present.

Researchers have shown that dietary calcium intake does not influence BMD in road cyclists (Barry & Kohrt, 2008; Smathers et al., 2009; Warner et al., 2002). According to Barry and Kohrt, there was no difference in BMD between cyclists taking a calcium supplement with meals and those without over the course of a year. However, researchers suggest the timing of calcium supplementation is crucial to increasing BMD. Calcium intake before (Guillemant et al., 2004) or during (Barry et al., 2011) endurance cycling may provide a positive osteogenic effect.

An association was found between the number of years of cycling experience and femoral neck BMD. An increase in the number of years of cycling experience was statistically associated with decreased femoral neck BMD. This confirms previous

research on BMD of master cyclists. Nichols et al. (2003) suggested that long-term participation in cycling may negatively impact BMD later in life. This assumption is presumably due to the accumulation of a high number of hours spent with supported body weight; a cyclist's body weight is supported by five contact points: two hands on the handlebar, two feet on the pedals, and the pelvis on the saddle. Pedal loads also relate strongly to the riders weight and body position during cycling, i.e., seated or standing (Caldwell, Li, McCole, & Hagberg, 1998; Stone & Hull, 1995).

The primary limitation to this study was the small sample size. Raff et al. (1999) found significant correlations in elevated salivary cortisol in an elderly population of 130 men. The significant correlation between post-race cortisol and total hip BMD in male cyclists over 40 years of age in the present study suggests that increasing the sample size of older cyclists in the present study may have produced more definite results. The high number of contaminated post-race salivary cortisol samples further indicates a need for measurement of a larger sample population. Another limitation is the cross-sectional design of the present study. The cyclists had a minimum of two years cycling experience, but may have had low BMD before initiating bicycle training and competition. In addition, testosterone, a hormone positively associated with markers of bone metabolism, may mediate bone resorption in concert with cortisol. For that reason, measuring the exercise induced response of hormones such as pre-race and post-race testosterone, PTH, and CTX may provide further insight into the hormonal mediation of accelerated bone resorption during intense cycling. This study provides original data on the assessment of pre- and post-competition measurements of salivary cortisol with

comparison to site-specific BMD in competitive male cyclists. Although the etiology of low BMD in male cyclists has yet to be determined, the cyclists' characteristics that are associated with BMD in this study provide topics for future research investigations. Furthermore, future research is needed to determine a weight training protocol along with a calcium supplementation needed to increase BMD of the lumbar spine and hip in male, competitive cyclists.

In conclusion, salivary cortisol was not a significant predictor of BMD in the whole sample population. However, an unexplained significant positive correlation between cortisol and BMD of cyclists over the age of 40 was found. On the basis of this data, low BMD in male competitive cyclists cannot be attributed to increased cortisol before or after competition. However, findings support the relationship between weekly participation in weight training and higher BMD of the lumbar spine and hip. The findings also support a positive relationship between daily calcium intake and BMD of the lumbar spine and femoral neck. It is recommended that cyclists participate in weight training and increase daily calcium intake in order to increase or maintain BMD.

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Chapter III Appendices

Appendix A

One-Day Dietary Recall

Name _____ Date _____

Please list all food intake with amount (i.e. 1 cup or 4 oz) for one full day. Specify whether or not the food has been fortified with calcium. Include any food intake during cycling. Please use record on a day typical of your daily diet. Include use of vitamins and supplements, Tums or Rolaids, with amount of calcium consumed.

Breakfast Food	Amount

Supplementation _____

_____

Lunch	Food	Amount

Dinner	Food	Amount

Snack	Food	Amount

Appendix B

Demographics Questionnaire

Name _____ Race Number _____

Age _____

How many years have you been training on a bike? _____

Approximately how many minutes do you ride weekly? _____

Approximately how many minutes do you run weekly? _____

Approximately how many minutes do you lift weights weekly? _____

Approximately how many races do you plan to attend this season? _____

What do you plan to eat and drink prior to and during the race?

_____

_____

Appendix C

Informed Consent Document for Research: Study I

Principal Investigator: Shannon Mathis
Study Title: Relationship between Cortisol and Bone Mineral Density in Competitive Male Cyclists
Institution: Middle Tennessee State University

Name of participant: _____ Age:_____
The following information is provided to inform you about the research project and your participation in it. Please read this form carefully and feel free to ask any questions you may have about this study and the information given below. You will be given an opportunity to ask questions, and your questions will be answered. Also, you will be given a copy of this consent form.

Your participation in this research study is voluntary. You are also free to withdraw from this study at any time. In the event new information becomes available that may affect the risks or benefits associated with this research study or your willingness to participate in it, you will be notified so that you can make an informed decision whether or not to continue your participation in this study.
For additional information about giving consent or your rights as a participant in this study, please feel free to contact the MTSU Office of Compliance at (615) 494-8918.

1. Purpose of the study:
You are asked to participate in a research study because low bone mineral density (BMD) is prevalent among male cyclists. Participation in this study may assist researchers determine a cause of low BMD in male cyclists.
The purpose of the study is to determine whether or not cortisol levels at the start and finish of a competition are related to lumbar spine and femoral neck bone mineral density (BMD) in road cyclists.

This study will utilize Dual Energy X-ray Absorptiomerty (DXA) to measure bone mineral density of the lumbar spine and hip.

2. Description of procedures to be followed and approximate duration of the study:
You are asked to provide approximately 1 hour at the MTSU Exercise Science Lab prior to the TN State Championship Time-Trial, 5 minutes prior to the race start and 5 minutes after the race finish.

Upon arrival at the lab, you will complete a demographic questionnaire with questions pertaining to racing and training history. Next, you will be asked to complete a 1-day dietary recall in order to determine daily dietary calcium intake. You will also be asked to complete a history of leisure activity questionnaire containing questions about your physical activity habits throughout your life. You will be provided with hospital scrubs to wear and must remove shoes for all laboratory measurements. Measures of height and body mass will then be performed. For the DXA assessment you will lie motionless on the cushioned table while the machine scans the body part. A hip and spine scan takes 45 seconds.

On the day of the race, you are asked to report to the start/finish line where all measurements will be performed 15 minutes prior to your race start time. A research assistant will give you 3 questions on a survey about your pre-race nervousness. Next, you will be asked to rinse your mouth with plain water, and will receive a Salivette (small plastic tube for saliva collection) labeled with your race number. You will then be asked to provide a saliva sample by placing a small cylindrical, non-toxic swab under your tongue for about 2 minutes. You will place the swab inside the Salivette without touching the swab with your fingers.

Immediately after your race, you are asked to repeat the same procedure with a new Salivette labeled with your race number. You will be asked 3 questions pertaining to your race and events during your race.

3. Expected costs:
There are no costs associated with your participation in this investigation.

4. Description of the discomforts, inconveniences, and/or risks that can be reasonably expected as a result of participation in this study:
You will be exposed to a small radiation dose as a part of the DXA assessment. The radiation dose is lower than what you would experience if you spent an entire day at the beach. It is recommended that you NOT participate in multiple investigations that utilize DXA as exposure to ionizing radiation has a cumulative effect.
There are no anticipated risks associated with your participation in this study. Inconveniences may include traveling to MTSU for the assessment.

5. Compensation in case of study-related injury:
MTSU and the research team conducting this study will not provide compensation in the case of study related injury.

6. Anticipated benefits from this study:
a) The potential benefits to science and humankind that may result from this study are determination of a cause of low BMD that is prevalent in male cyclists.
b) The potential benefits to you from this study are determination of your BMD, which is low in many male cyclists.

7. Alternative treatments
No treatment is given in this study.

8. Compensation for participation:
Participants will not be compensated for participation.

9. Circumstances under which the Principal Investigator may withdraw you from study participation:
You will be withdrawn from participation if you experience mechanical problem or a crash during the event.

Individuals using medication known to affect bone metabolism or bone metabolism are not eligible to participate. Also individuals with a history of endocrine disorders will not be eligible to participate.

10. What happens if you choose to withdraw from study participation:
There is no penalty for withdrawing from the study.

11. Contact Information.
If you should have any questions about this research study or possibly injury, please feel free to contact Shannon Mathis at (615) 400-8740 or my faculty advisor, Dr. Caputo, at 615-898-5547.

12. Confidentiality.
All efforts, within reason, will be made to keep the personal information in your research record private but total privacy cannot be promised. Your information may be shared with MTSU or the government, such as the Middle Tennessee State University Institutional Review Board, Federal Government Office for Human Research Protections if you or someone else is in danger or if we are required to do so by law.

STATEMENT BY PERSON AGREEING TO PARTICIPATE IN THIS STUDY
I have read this informed consent document and the material contained in it has been explained to me verbally. I understand each part of the document, all my questions have been answered, and I freely and voluntarily choose to participate in this study.

Date	Signature of patient/volunteer

Consent obtained by:

Date	Signature

MTSU
IRB Approved
Date: 5-3-11

Appendix D

MTSU Institutional Review Board Approval Letter: Study I

May 3, 2011

Shannon Leigh Mathis, Dr. Jennifer Caputo, Dr. Richard Farley, Dr. Dana Fuller, Dr. Amy Jetton
Department of Health and Human Performance
smathis@mtsu.edu, jcaputo@mtsu.edu

Protocol Title: "Relationship between Cortisol and Bone Mineral Density in Competitive Male Cyclists"

Protocol Number: 11-311

Dear Investigator(s),

The MTSU Institutional Review Board, or a representative of the IRB, has reviewed the research proposal identified above. The MTSU IRB or its representative has determined that the study poses minimal risk to participants and qualifies for an expedited review under 45 CFR 46.110 Category 4 and 7.

Approval is granted for one (1) year from the date of this letter for 100 participants.

According to MTSU Policy, a researcher is defined as anyone who works with data or has contact with participants. Anyone meeting this definition needs to be listed on the protocol and needs to provide a certificate of training to the Office of Compliance. **If you add researchers to an approved project, please forward an updated list of researchers and their certificates of training to the Office of Compliance (c/o Emily Born, Box 134) before they begin to work on the project.** Any change to the protocol must be submitted to the IRB before implementing this change.

Please note that any unanticipated harms to participants or adverse events must be reported to the Office of Compliance at (615) 494-8918.

You will need to submit an end-of-project form to the Office of Compliance upon completion of your research located on the IRB website. Complete research means that you have finished collecting and analyzing data. **Should you not finish your research within the one (1) year period, you must submit a Progress Report and request a continuation prior to the expiration date.** Please allow time for review and requested revisions. Your study expires **May 3, 2012**.

Also, all research materials must be retained by the PI or faculty advisor (if the PI is a student) for at least three (3) years after study completion. Should you have any questions or need additional information, please do not hesitate to contact me.
Sincerely,

Emily Born
Compliance Officer
Middle Tennessee State University
eborn@mtsu.edu

CHAPTER IV

EFFECTS OF A CALCIUM SUPPLEMENT ACROSS A COMPETITIVE SEASON ON BONE MINERAL DENSITY IN MALE CYCLISTS

Introduction

Participation in physical activity is linked with positive bone health (Etherington et al., 1996). However, road cycling, a non-weight bearing sport, has been shown to lack an osteogenic effect. Osteopenia has been documented in professional (Campion et al., 2010; Medelli, Lounana, Menuet, Shabani, & Cordero-MacIntyre, 2008) and competitive amateur male cyclists (Rector, Rogers, Ruebel, & Hinton, 2008). Road cyclists have bone mineral density (BMD) of the lumbar spine and total hip that are similar to or lower than healthy controls (Medelli, Shabani, Lounana, Fardellone, & Campion, 2009; Nichols, Palmer, & Levy, 2003; Smathers, Bemben, & Bemben, 2009; Warner, Shaw, & Dalsky, 2002) and runners (Rector et al., 2008; Stewart & Hannan, 2000). Adolescent cyclists under 17 years of age had 10% lower bone mineral content compared to age-matched controls involved in recreational sports (Olmedillas, González-Agüero, Moreno, Casajús, & Vicente-Rodríguez, 2011). Further, BMD was found to decrease throughout the competitive season in male road cyclists (Barry & Kohrt, 2008).

The direct cause of low BMD in road cyclists is unclear, and multiple pathogenic mechanisms contribute to the loss of bone. A theoretical contributing factor to low BMD in road cyclists is dermal calcium loss which will decrease blood serum calcium levels (Barry & Kohrt, 2007; Ehrnborg et al., 2003; Maïmoun & Sultan, 2011; Rector et al.,

2008; Stewart & Hannan, 2000). The need to maintain serum calcium elicits an immediate endocrine response regulated by the release of parathyroid hormone (PTH). Parathyroid hormone triggers the release of calcium from the skeleton to stabilize serum calcium (Barry & Kohrt, 2007; Barry & Kohrt, 2008; Klesges et al., 1996). The rate of dermal calcium loss is negatively associated with hip, femoral neck, and femoral shaft bone mineral content (Klesges et al., 1996) and BMD (Barry & Kohrt, 2008) suggesting excessive dermal calcium loss during endurance cycling may contribute to the adverse effect of cycling on BMD.

Ingestion of an oral calcium load prior to or during endurance exercise such as cycling (Barry et al., 2011; Guillemant, Accarie, Peres, & Guillemant, 2004) or running (Grimston, Tanguay, Gundberg, & Hanley, 1993) attenuates the exercise-induced rise in PTH. Therefore, administration of calcium before or during exercise compensates for the calcium loss through sweat (Barry et al., 2011; Klesges et al., 1996). Although no effect of calcium supplementation administered during meals was found on long term measurements of BMD in male cyclists (Barry & Kohrt, 2008), regularly consuming calcium during training may attenuate the exercise-induced elevation in PTH and ultimately maintain BMD. In collegiate basketball players, bone mineral content decreased during a 4 month training period, but was preserved when calcium was taken during practice (Klesges et al., 1996).

Given the challenge of maintaining BMD during a season of endurance bicycle competition, long term investigations of an oral calcium supplementation during training and racing are needed. Providing calcium during exercise may maintain serum calcium

which will attenuate the exercise-induced rise in PTH. For endurance athletes with low BMD, such as cyclists, taking calcium supplements during training and racing may maintain or increase BMD. Therefore, the purpose of this study was to examine the effects of a calcium supplementation during training and racing on changes in BMD across a 5 month competitive racing season in amateur, male road cyclists. It was hypothesized that calcium plus vitamin D_3 supplementation prior to exercise would maintain or increase lumbar spine, total hip, femoral neck, and femoral trochanter BMD compared to no supplementation.

Methodology

Participants

Amateur, male competitive road cyclists ($N = 29$) were recruited for this study. The criteria for study inclusion were (1) age 30 years or greater; (2) estimated daily calcium intake less than 2,500 mg/d, the upper limit for calcium intake as recommended by the Institute of Medicine (Institute of Medicine, 1997); and (3) participation in weight lifting, running, or mountain cycling less than 10% of total weekly training time. Cyclists with a history of kidney or gall stones, kidney disease, and use of medication known to affect bone metabolism were excluded from the study.

Instrumentation

BMD measurements. Lumbar spine (L1 – L4) and total hip, femoral neck, and trochanteric region BMD were measured using a Hologic Discovery QDR Series dual energy x-ray (DXA) absorptiometer (Bedford, MA). The standard protocols of the manufacturer were followed for all measures and all scans were conducted and analyzed

by a licensed technician. The DXA was calibrated using a spine phantom prior to data collection, assuring that all calibrations fell within appropriate ranges. Data were recorded as *t*-scores and g/cm^2. The cyclists' non-dominant leg was measured for hip BMD, defined as the opposite leg of the preferred kicking leg. Cyclists removed all metal objects from their bodies prior to BMD measurement and wore hospital scrubs to avoid false images on the scan.

Body mass and height. Height was measured to the nearest 0.5 cm using a stadiometer (SECA model 222, seca Gmbh & Co. Kg, Hamburg, Germany) while cyclists stood barefoot on level ground with heals together. After voiding, body mass was measured to the nearest 0.1 kg using a digital scale (SECA model 770, Vogel & Halke, Hamburg, Germany). Height and body mass were used to calculate body mass index (BMI) using body mass in kilograms divided by height in meters squared.

Supplementation. Cyclists were stratified into one of two groups based on baseline lumbar spine *t*-scores. A control group (CON) received no supplementation. The calcium supplement group (CAL) received 1,600 mg of calcium carbonate (Tums Ultra, GlaxoSmithKline, Middlesex, United Kingdom) taken prior to daily bicycle training or racing. The supplement group also received 1,000 iu of 1,25 dihydroxy vitamin D_3 (calcitriol) to increase intestinal absorption of the calcium (Spring Valley, US Nutrition, Inc., Bohemia, NY).

Demographics. Participants reported age, number of years of bike-specific training, average number of minutes spent riding a bike (min/week), approximate number

of races per season, number of minutes spent lifting weights (min/week), and number of minutes spent running (min/week) on a survey (see Appendix A).

Dietary recall. Prior to enrollment in the study, current dietary calcium intake was estimated using a 1 day dietary recall (see Appendix B). Additionally, calcium intake was measured monthly with a 1 day dietary recall throughout the 5 month intervention to ensure estimated daily calcium intake, excluding the study supplement, did not exceed 2,500 mg/d throughout the season. Food intake, including vitamin supplements, was analyzed for daily calcium (mg/d) by the principle investigator using the US Department of Agriculture website (http://www.mypyramid.gov).

Training log. A daily training log was obtained from each cyclist monthly during the 5 month intervention period. Training logs were collected either monthly via email or delivered upon return to the lab during post-season BMD testing.

Procedures

University Institutional Review Board approval was gained prior to data collection (see Appendix C) and written informed consent was obtained from all participants (see Appendix D). Participants were recruited from races, bicycle shops, and online cycling forums. Interested participants reported to the University Exercise Science Laboratory, completed the 1 day dietary recall and the demographics questionnaire to determine if participants met inclusion criteria. All cyclists meeting the inclusion criteria were invited to participate in the study. Eligible cyclists were provided with hospital scrubs and removed shoes for all laboratory measurements. Cyclist's had their height and body mass measured. Bone density scans were then completed, in randomized order.

Cyclists with any site *t*-score classified as low by the World Health Organization (2004) were asked to read and sign a statement informing them of the current state of their bone health (see Appendix E).

All eligible cyclists were placed into the CAL or CON group using stratified randomization based on lumbar spine *t*-scores. Cyclists in the CAL group were provided with written instructions on the timing of calcium and vitamin D_3 use; the CAL group was instructed to consume four Tums Ultra with a vitamin D_3 tablet prior to each daily training ride or cycling competition. All participating cyclists were instructed to consume a fiber containing snack, such as a piece of fruit or granola bar, prior to all rides and to avoid calcium containing foods within 2 hours prior to the training ride or cycling competition. Cyclists in the CAL group received the supplementation needed for a 5 month period. Cyclists also received a training log to record total training time.

Dietary calcium intake was estimated using a single day dietary recall monthly throughout the 5 month intervention. Cyclists were reminded to record food intake either verbally via telephone or with an email message. With the same monthly contact, cyclists were asked to provide daily training logs. The training logs contained number of minutes of cycling per day, whether or not the cyclist was ill or injured, and number of minutes of other forms of physical activity per day.

The intervention period began at the start of the competitive racing season (May) and concluded at the end of the season (October). Cyclists returned to the lab for post-intervention measurements of site specific BMD and body mass within one week of the end of the intervention period.

Statistical Analyses

Data were analyzed using IBM SPSS version 19. Baseline anthropometric measurements, participant demographics, and BMD were reported as mean and standard deviation. Pre-intervention anthropometric measurements and BMD for all cyclists were compared using independent samples *t*-tests. A two-way repeated measures ANOVA with time (pre-season, post-season) as a within-subjects factor and group (CAL, CON) as a between-subjects factor was conducted to analyze changes in lumbar spine, total hip, femoral neck, and femoral trochanter BMD. Statistical significance was set at .05 for all analyses.

Results

Of 29 eligible cyclists, 5 did not complete the study due to time constraints, illness, or injury and 7 cyclists were no longer responsive to emails or phone calls at some point throughout the season. Therefore, 9 CAL and 8 CON participants completed the 5 month study. There were no statistical differences in baseline measurements of lumbar spine and hip BMD, height, weight, BMI, and daily calcium intake among the 17 who completed the study. There were no statistical differences in training (hr/wk) between CAL and CON over the 5 month intervention. Descriptive statistics for all baseline measurements are given in Table 1. Osteopenia ($t < -1.0$) of the lumbar spine or hip was found in 10 (59%) cyclists at baseline and 12 (71%) cyclists at the 5-month follow-up. Pre- and post-season descriptive statistics for each BMD site are presented in Table 2. The changes in lumbar spine, total hip, femoral neck, and femoral trochanter BMD from May to October are shown in Figure 1.

Table 1

Baseline Characteristics of Male Cyclists ($N = 17$)

Variable	*M*	*SD*
Age (yr)	42.7	9.4
Height (cm)	180.6	6.1
Weight (kg)	78.0	8.5
BMI (kg/m^2)	23.8	1.5
Daily calcium intake (mg)	1153.9	432.6
Years of cycling	11.5	9.4
Cycling training (hr/mo)	36.5	14.1
Lumbar spine (g/cm^2)	1.017	0.084
Total hip (g/cm^2)	0.937	0.087
Femoral neck (g/cm^2)	0.773	0.096
Femoral trochanter (g/cm^2)	0.710	0.067
Lumbar spine (*t*-score)	-0.65	0.78
Total hip (*t*-score)	-0.57	0.70
Femoral neck (*t*-score)	-1.12	0.76
Femoral trochanter (*t*-score)	-0.49	0.65

Note. BMI represents body mass index.

Table 2

Descriptive Statistics for BMD across the Cycling Season

Group	BMD Site	Pre-Season		Post-Season	
		$M \pm SD$ (g/cm^2)	$M \pm SD$ (t-score)	$M \pm SD$ (g/cm^2)	$M \pm SD$ (t-score)
CAL	Lumbar spine	1.011 ± 0.11	-0.72 ± 0.97	1.011 ± 0.09	-0.73 ± 0.81
	Total hip	0.921 ± 0.10	-0.72 ± 0.66	0.912 ± 0.09	-0.81 ± 0.62
	Femoral neck	0.766 ± 0.11	-1.21 ± 0.81	0.756 ± 0.11	-1.26 ± 0.77
	Trochanter	0.701 ± 0.07	-0.61 ± 0.60	0.690 ± 0.07	-0.70 ± 0.55
CON	Lumbar spine	1.024 ± 0.06	-0.56 ± 0.53	1.010 ± 0.06	-0.70 ± 0.64
	Total hip	0.956 ± 0.07	-0.40 ± 0.75	0.949 ± 0.08	-0.44 ± 0.78
	Femoral neck	0.781 ± 0.09	-1.01 ± 0.74	0.772 ± 0.09	-1.09 ± 0.74
	Trochanter	0.719 ± 0.06	-0.35 ± 0.72	0.714 ± 0.06	-0.40 ± 0.69

Note. CAL indicates calcium supplement group, $n = 9$; CON indicates control group, $n = 8$.

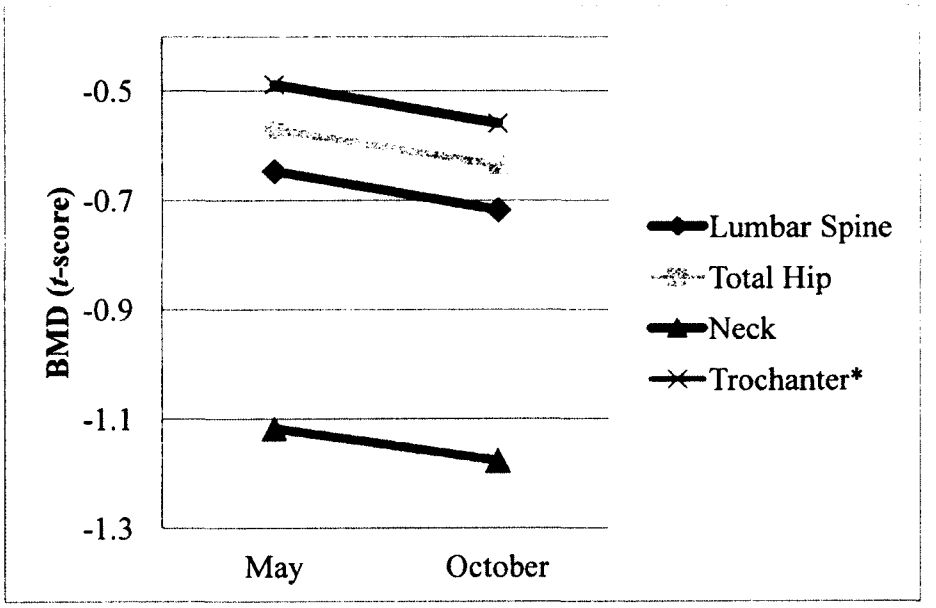

Figure 1. Changes in BMD *t*-Scores from May to October ($N = 17$); * denotes a significant decrease in trochanter BMD *t*-score from May to October, $p = .013$.

There were no significant interactions (group by time) for the lumbar spine ($F(1, 15) = 0.72$, $G\text{-}G\ p = .41$), total hip ($F(1, 15) = 0.02$, $G\text{-}G\ p = .88$), femoral neck ($F(1, 15) = 0.00$, $G\text{-}G\ p = .99$), and femoral trochanter ($F(1, 15) = 0.60$, $G\text{-}G\ p = .45$) BMD. The main effect of group (CAL, CON) was not significant for the lumbar spine ($F(1, 15) = 0.01$, $G\text{-}G\ p = .91$), total hip ($F(1, 15) = 0.76$, $G\text{-}G\ p = .40$), femoral neck ($F(1, 15) = 0.10$, $G\text{-}G\ p = .75$), or femoral trochanter ($F(1, 15) = 0.43$, $G\text{-}G\ p = .52$) BMD. The main effect of time (pre-season, post-season) was not significant for the lumbar spine ($F(1, 15) = 0.90$, $G\text{-}G\ p = .36$), total hip ($F(1, 15) = 1.47$, $G\text{-}G\ p = .25$), or femoral neck ($F(1, 15) = 2.24$, $G\text{-}G\ p = .16$) BMD. However, the main effect of time was significant for the femoral trocanter BMD ($F(1, 15) = 5.94$, $G\text{-}G\ p = .03$) which decreased from May to October.

Discussion

The primary purpose of this study was to determine whether or not an oral calcium supplement prior to training and racing would increase or maintain BMD throughout a competitive cycling season in male cyclists over 30 years of age. Although the CAL cyclists received 1,600 mg calcium carbonate and 1,000 iu vitamin D_3 prior to training, BMD of the lumbar spine, total hip, femoral neck, and femoral trochanter did not increase across the 5 month competitive season. On the contrary, femoral trochanter BMD significantly decreased during the 5 month competition season (May through October) in the male cyclists ($N = 17$).

Many theories are used to explain the cause of bone mineral loss in response to endurance exercise. Low BMD in cyclists has been attributed to a lack of gravitational

force due to the non-weight bearing nature of cycling (Kohrt, Barry, & Schwartz, 2009), dermal calcium loss (Barry et al., 2011; Klesges et al., 1996; Guillemont et al., 2004) and/or elevations in PTH during intense, prolonged cycling (Barry et al., 2011; Guillemont et al., 2004). In addition, researchers are now exploring the possibility that extended periods of hypersecretion of cortisol due to physiological stress of training and racing negatively impact bone (Chiodini & Scillitani, 2008). Compounding the aforementioned issues are common behaviors of competitive cyclists. Self-imposed diet restrictions can cause insufficient energy availability during training. Also, restriction of weight training in favor of recovery time or to increase cycling time may contribute to the effect of endurance cycling on BMD. A cyclist who trains with a high volume and intensity for a long competitive season over many years may experience serious bone loss (Nichols & Rauh, 2011).

Regardless of the underlying causes, cycling appears to be detrimental to bone health. As an illustration of this point, 59% (n = 10) of the cyclists' lumbar spine or hip BMD was classified as low according to WHO at baseline and 71% (n = 12) of the cyclists' lumbar spine or hip BMD was classified as low at the 5-month follow-up. The risk of fracture in the femoral neck of this relatively young (M = 42 years) sample of cyclists (see Table 1) highlights the importance of bone density scans for these athletes.

Decreased BMD of the femoral trochanter in the current full sample confirms the results of Barry and Kohrt (2008) who documented a decrease in BMD over the course of a competitive season in cyclists. Similar to the current findings, lumbar spine did not significantly decrease during this period. Although studies have defined the magnitude

of bone loss in cyclists, there is little information about the distribution of these changes within specific skeletal sites. The femoral trochanter is primarily made up of trabecular bone which is less dense than cortical bone. During a competitive cycling season, a period of rapid remodeling, it is possible that excessive bone resorption with a decrease in bone formation effects trabecular bone to a greater extent than cortical bone. The finding that femoral trochanter BMD significantly decreased in a short period of time illustrates the need to understand the effects of biomechanical actions of the muscle on hip bones during cycling. This is especially true because of the relationship between mechanical loading and BMD. A method for measuring muscle forces is not available; therefore, bone loading during cycling has to be determined from biomechanical model calculations (Brinckmann, Frobin, & Leivseth, 2002).

Contrary to the current results, Barry and Kohrt (2008) also found a significant decrease in total hip and femoral neck BMD from May to September, a 4.5 month period. Although half of the participants in Barry and Kohrt's study received a 1,500 mg calcium supplement with meals, the decrease in BMD was the same in those with and without supplementation (Barry & Kohrt, 2008). In contrast to the current findings and that of Barry and Kohrt, lumbar spine BMD increased after an 8 month racing season in triathletes (Maïmoun et al., 2004). However, the 7 triathletes were aged 18-20 years and may not yet have achieved peak bone mass.

The hypothesis that the timing of a calcium supplement, prior to training instead of with meals, would attenuate the decrease in BMD over a 5 month period was not supported. The basis for this hypothesis involves the actions of PTH which elevates in

response to a decrease in blood serum calcium levels to trigger the release of calcium from the skeleton. Parathyroid hormone stimulates osteoclasts to absorb bone mineral to elevate serum calcium, and increases renal calcium absorption. Exercise induced elevations in PTH have been documented with intense cycling (Maïmoun et al., 2006) and prolonged (2 to 5 hrs) moderate intensity cycling (Barry & Kohrt, 2007; Ljunghall, Joborn, Roxin, Rastad, Wide, & Åkerström, 1986).

As hypothesized by Barry and Kohrt (2007), cycling induced elevations of PTH mimic hyperparathyroidism and may lead to low BMD in athletes participating in extended bouts of cycling training. Thus, it was surmised that the supply of an oral calcium load during exercise, such as in the current study, would attenuate the exercise induced elevations in PTH. Previously, calcium supplement prior to (Barry et al., 2011; Guillemant et al., 2004) and during (Barry et al., 2011) laboratory cycling trials has been shown to significantly attenuate the rise in PTH compared to cycling trials with ingestion of plain water (placebo trials). Guillemant et al. and Barry et al. hypothesized that the increased PTH response to exercise in the placebo trials was likely due to calcium lost in sweat and the decreased PTH response to exercise in the calcium supplement trials was due to the provision of a non-skeletal source of calcium to be released as sweat. Changes in serum calcium elicit an immediate endocrine response. It is possible that calcium loss due to excessive sweating during long duration, intense cycling triggers bone resorption that counteracts the beneficial effects of muscle loading on BMD during cycling.

Contrary to the current findings, Klesges et al. (1996) administered 600 mg calcium with 160 iu vitamin D_3 to a team of basketball players during practice throughout

an approximate 4 month period. There was an increase of 6.1% in bone mineral content (BMC) when calcium was administered compared to a 3.8% decrease in BMC during the previous season without supplemental calcium. The authors speculated the loss of BMC occurred as a result of calcium imbalance during intense training in the heat due to dermal calcium loss. There are two notable differences between the study by Klesges et al. and the current data. Bone mass peaks during the third decade of life and the mean age of the basketball players was 20 years, whereas the mean age of the cyclists in the current study was 43 years. This implies that calcium intake during exercise may have a greater effect during the bone building phase of life. Additionally, Speckler (1996) documented evidence that impact activity has a greater effect on BMD when high calcium intake is present. Basketball, a weight bearing sport, has greater gravitational loading due to forces generated from impact with the ground. Cycling, a weight-supported exercise, generates a high level of muscle force, but lacks the force of impact (Kohrt et al., 2009).

The calcium dosage in the current study was determined from the work of Barry and Kohrt (2008). Dermal calcium loss was estimated at approximately 124 mg/h (Barry & Kohrt, 2008). Given that only 1/3 of a calcium supplement is absorbed, a 1,600 mg dose of calcium was expected to compensate for the dermal calcium loss over a 4 hour training period. A 1,000 IU dose of vitamin D_3 was included to increase calcium absorption. The amount of calcium lost in sweat is dependent on intensity, temperature, hydration status, and acclimation to warm temperature. However, the cyclists in the current study live in a hot, humid environment (Southeastern US); thus, the amount of

calcium prescribed in this study may not have been enough to compensate for the dermal calcium loss in this sample of cyclists.

The type of calcium administered during the cycling may have also have had a significant effect on the outcomes. In the current study, calcium carbonate was administered to the cyclists. Calcium carbonate is best absorbed when taken with meals, and cyclists were encouraged to consume a fiber containing snack with the supplement. Provision of calcium citrate may alleviate another speculated cause of lost BMD in cyclists, metabolic acidosis (Ehrnborg et al., 2003; Maïmoun & Sultan, 2011). Metabolic acidosis causes an increase in bone resorption activity during prolonged exercise at a high level of intensity which, in turn, may lead to low BMD (Ehrnborg et al., 2003; Maïmoun & Sultan, 2011). Chronic metabolic acidosis increases osteoclastic bone resorption by increasing calcium released from bone (Frick, Jiang, & Bushinsky, 1997) and decreases osteoblast activity by inhibiting the genetic expression for collagen synthesis (Bushinsky, Parker, Alexander, & Krieger, 2001). Similarly, an efflux of calcium is stimulated by a reduction in pH when phosphate is released from bone to buffer the additional hydrogen ions present in plasma (Bushinsky et al., 2003). During high-intensity exercise, such as a 30 second cycling sprint, it is common for blood pH to drop from the normal range of approximately 7.4 down to 7.08 (Bogdanis, Nevill, Boobis, Lakomy, & Nevill, 1995). These small changes in pH have a significant effect on osteoblastic function along with stimulation of osteoclastic resorption (Bushinsky et al., 2001; Bushinsky et al., 2003; Frick et al., 1997). Future research is encouraged examining the use of this alternate

form of calcium supplementation as well as the timing and dosage needed to positively affect BMD.

This is the first prospective study of a season long calcium supplementation during training and racing in male cyclists and several limitations to the current study exist. First, the high drop-out rate of the participants resulted in a small sample size (N = 17) effecting the statistical power of the between-participant analyses. Although training logs were collected, these data only account for training volume and not training intensity. Participant's average weekly training time over the 5 month period ranged from 7 hr/wk to 18 hr/wk. Average estimated daily calcium intake ranged from 400 mg/d to 1900 mg/d. This may be seen as a limitation; however, inclusion of cyclists that were representative of the population was purposeful in this study. Finally, although representative of a cyclist's competitive season, a 5 month period may not be long enough to see gains in BMD from calcium supplementation, as it has been noted that response to a calcium supplement may take years to detect improvement (Guillemont et al., 2004).

In conclusion, until researchers are able to identify which factors most prominently lead to this altered bone profile in road cyclists, it is important that awareness is raised concerning the state of bone health within the cycling community. Practitioners and coaches working with cyclists need to understand the deleterious effect cycling has on bone health and to understand precautionary measures that can be taken to improve bone health.

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Chapter IV Appendices

Appendix A

Demographics Questionnaire

Name _____ ID Number _____

Age _____

How many years have you been training on a bike? _____

Approximately how many minutes do you ride weekly? _____

Approximately how many minutes do you run weekly? _____

Approximately how many minutes do you lift weights weekly? _____

Approximately how many races do you plan to attend this season? _____

Appendix B

One-Day Dietary Recall

Name _____ Date _____

Please list all food intake with amount (i.e. 1 cup or 4 oz) for one full day. Specify whether or not the food has been fortified with calcium. Include any food intake during cycling. Please use record on a day typical of your daily diet. Include use of vitamins and supplements, Tums or Rolaids, with amount of calcium consumed.

Breakfast Food	Amount

Supplementation _____

_____

Lunch	Food	Amount

Dinner	Food	Amount

Snack	Food	Amount

Appendix C

MTSU Institutional Review Board Approval Letter: Study II

May 6, 2011

Shannon Leigh Mathis
Department of Health and Human Performance
smathis@mtsu.edu, jcaputo@mtsu.edu

Protocol Title: "Effect of Calcium Supplementation during Exercise throughout a Competitive Season on Bone Mineral Density in Male Cyclists"
Protocol Number: 11-316

Dear Investigator(s),

The MTSU Institutional Review Board, or a representative of the IRB, has reviewed the research proposal identified above. The MTSU IRB or its representative has determined that the study poses minimal risk to participants and qualifies for an expedited review under 45 CFR 46.110 Category 4 and 7.

Approval is granted for one (1) year from the date of this letter for 100 participants.

According to MTSU Policy, a researcher is defined as anyone who works with data or has contact with participants. Anyone meeting this definition needs to be listed on the protocol and needs to provide a certificate of training to the Office of Compliance. **If you add researchers to an approved project, please forward an updated list of researchers and their certificates of training to the Office of Compliance (c/o Emily Born, Box 134) before they begin to work on the project.** Any change to the protocol must be submitted to the IRB before implementing this change.

Please note that any unanticipated harms to participants or adverse events must be reported to the Office of Compliance at (615) 494-8918.

You will need to submit an end-of-project form to the Office of Compliance upon completion of your research located on the IRB website. Complete research means that you have finished collecting and analyzing data. **Should you not finish your research within the one (1) year period, you must submit a Progress Report and request a continuation prior to the expiration date.** Please allow time for review and requested revisions. Your study expires **May 6, 2012**.

Also, all research materials must be retained by the PI or faculty advisor (if the PI is a student) for at least three (3) years after study completion. Should you have any questions or need additional information, please do not hesitate to contact me.

Sincerely,

Emily Born
Compliance Officer
Middle Tennessee State University
eborn@mtsu.edu

Appendix D

Informed Consent Document for Research: Study II

INFORMED CONSENT FORM

Principal Investigator: Shannon Mathis
Study Title: Effects of Calcium Supplementation during Exercise throughout a Competitive Season on Bone Mineral Density in Male Cyclists

Institution: Middle Tennessee State University

Name of participant: _____ Age:_____

The following information is provided to inform you about the research project and your participation in it. Please read this form carefully and feel free to ask any questions you may have about this study and the information given below. You will be given an opportunity to ask questions, and your questions will be answered. Also, you will be given a copy of this consent form.

Your participation in this research study is voluntary. You are also free to withdraw from this study at any time. In the event new information becomes available that may affect the risks or benefits associated with this research study or your willingness to participate in it, you will be notified so that you can make an informed decision whether or not to continue your participation in this study.

For additional information about giving consent or your rights as a participant in this study, please feel free to contact the MTSU Office of Compliance at (615) 494-8918.

1. **Purpose of the study:**
   You are asked to participate in a research study because low bone mineral density (BMD) is prevalent among male cyclists. Participation in this study may assist researchers determine a treatment of low BMD in male cyclists.

   The purpose of this study is to examine the effects of a calcium supplementation during training and racing on changes in BMD across a 5 month competitive racing season in amateur, male road cyclists.

   This study will utilize Dual Energy X-ray Absorptiomerty (DXA) to measure bone mineral density of the lumbar spine and hip.

2. **Description of procedures to be followed and approximate duration of the study:**
   You are asked to provide approximately 1 hour at the MTSU Exercise Science Lab in May 2011, and again in the first week of November 2011.

Upon arrival to the lab, you will complete a demographics questionnaire with questions pertaining to racing and training history. Next, you will be asked to complete a one-day dietary recall in order to determine daily dietary calcium intake. You will be provided with hospital scrubs and must remove shoes for all laboratory measurements. Measures of height and body mass will then be performed. Lastly, bone density scans will be completed in which you will lie motionless on the cushioned table while the machine scans your lumbar spine and hip. A regional body part scan takes 45 seconds.

You are asked to provide a daily training log recording the number of minutes you ride a bike each day. You will be contacted via email or phone (based on your preference) every two weeks, to collect the training log.

In order to ensure your daily calcium intake does not exceed 1,200 mg, you are asked to provide a one-day dietary recall every two weeks. The research team will analyze the recall for total amount of calcium consumed. If your daily calcium intake is above 1,200 mg, then you will be counseled on the necessity to eliminate excess sources of calcium from your diet.

Before leaving the lab for your initial visit you will be randomly placed into one of two groups. Group 1 will consume calcium (1,600 mg) and vitamin D (800 IU) in the form of an over the counter supplements before every training ride or race. Group 2 will not receive any supplementation.

Group 1 will be provided with enough supplementation to last a 5 month period. Please contact Shannon Mathis at 615-400-8740 when supplies run low.

3. **Expected costs:**
   There are no costs associated with your participation in this investigation.

4. **Description of the discomforts, inconveniences, and/or risks that can be reasonably expected as a result of participation in this study:**
   You will be exposed to a small radiation dose as a part of the DXA assessment. The radiation dose is lower than what you would experience if you spent an entire day at the beach. It is recommended that you NOT participate in multiple investigations that utilize DXA as exposure to ionizing radiation has a cumulative effect.

   Inconveniences may include traveling to MTSU for the assessment.

5. **Compensation in case of study-related injury:**
   MTSU and the research team conducting this study will not provide compensation in the case of study related injury.

6. **Anticipated benefits from this study:**
   a) The potential benefits to science and humankind that may result from this study are determination of a method to increase or maintain BMD in male cyclists.

   b) The potential benefits to you from this study are determination of your BMD, which is low in many male cyclists.

7. **Alternative treatments**
   There are other pharmacological agents used to increase BMD. Contact your personal physician for more information.

8. **Compensation for participation:**
   Participants will not be compensated for participation.

9. **Circumstances under which the Principal Investigator may withdraw you from study participation:**

   Use of medication known to affect bone metabolism, history of endocrine disorders, history of kidney or gall stones, and a daily dietary intake of more than 1,200 mg/d of calcium are exclusionary criteria.

   Regular participation in weight lifting, running, or mountain cycling during the intervention period are also exclusion criteria, as these forms of exercise have been shown to increase bone density. Therefore, you are asked to refrain from regular participation in weight lifting, running, or mountain biking during the next 5 months.

10. **What happens if you choose to withdraw from study participation:**
    There is no penalty for withdrawing from the study.

11. **Contact Information.**
    If you should have any questions about this research study or possibly injury, please feel free to contact Shannon Mathis at (615) 400-8740 or my faculty advisor, Dr. Caputo, at 615-898-5547.

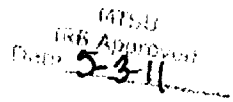
12. **Confidentiality.**
    All efforts, within reason, will be made to keep the personal information in your research record private but total privacy cannot be promised. Your information may be shared with MTSU or the government, such as the Middle Tennessee State University Institutional Review Board, Federal Government Office for Human Research Protections if you or someone else is in danger or if we are required to do so by law.

13. **STATEMENT BY PERSON AGREEING TO PARTICIPATE IN THIS STUDY**
I have read this informed consent document and the material contained in it has been explained to me verbally. I understand each part of the document, all my questions have been answered, and I freely and voluntarily choose to participate in this study.

Date	Signature of patient/volunteer

Consent obtained by:

Date	Signature
	Printed Name and Title



Appendix E

Letter to Participants Concerning Bone Health

Name _____ ID Number _____

Your bone mineral density score falls below the average for a person of comparable age and sex. If participation in this study does not increase your bone density, then it is recommended you visit your physician.

I have read the above statements and agree to participate in this study.

Name _____ Date_____

CHAPTER V

PROJECT CONCLUSIONS

The prevalence of osteopenia among amateur and professional male road cyclists has gained the interest of bone health researchers. Skeletal fragility in master cyclists may result from failure to reach optimal bone mass during the third decade of life and/or microdeterioration caused by excessive bone resorption with a decrease in bone formation. In a sport characterized by a high prevalence of falls, cyclists with low BMD are at risk for fracture. There are many studies showing low BMD in male cyclists of a wide range of ability and age. Low BMD or osteopenia has been found in professional cyclists (Campion et al., 2010; Medelli et al., 2008) and competitive amateur male cyclists (Rector et al., 2008). Competitive road cyclists have BMD of the lumbar spine and total hip that are similar to or lower than healthy controls (Medelli et al., 2009; Nichols et al., 2003; Smathers et al., 2009; Warner et al., 2002) and runners (Rector et al., 2008; Stewart & Hannan, 2000). A recent finding reported adolescent cyclists under 17 years of age had 10% lower bone mineral content compared to age-matched controls involved in recreational sports (Olmedillas, González-Agüero, Moreno, Casajús, & Vicente-Rodríguez, 2011). Cyclists' BMD was found to decrease throughout a competitive season, and the small increases during the off-season were not large enough to reach baseline measures (Barry & Kohrt, 2008).

An emerging area of research has been to determine specific causality of diminished bone health and to determine effective strategies to enhance bone health in

competitive male cyclists. Thus far, factors that elicit bone mineral loss in response to endurance cycling are speculative. Two key theories were examined in this dissertation.

First, extended periods of elevated cortisol disrupt bone cell formation, and interrupt blood serum calcium homeostasis. Abnormal increases in cortisol over an extended period of time lead to a significant reduction in BMD (Hardy & Cooper, 2010). A significant negative correlation between cortisol and lumbar spine BMD has been documented (Raff et al., 1999). As such, the physiological stress of training and racing may cause a significant increase in cortisol levels.

Second, calcium loss due to an increased sweat rate during training and racing causes a disruption to calcium homeostasis (Barry et al., 2011; Guillemant et al., 2004). A calcium supplement during exercise was shown to attenuate the rise in biochemical markers of bone resorption during laboratory cycling trials which leads to the belief that a calcium supplement during cycling training and racing would increase or maintain BMD in cyclists over time.

Hence, the main objectives of this dissertation project were to investigate the cause(s) of low BMD of the lumbar spine and hip and to determine a strategy to increase or maintain BMD. A total of 44 male cyclists were recruited to participate in either or both studies.

The purpose of the first study was to determine whether or not the psychological (pre-race) and physiological (post-race) stress of an individual cycling competition elevated cortisol. It was hypothesized that increased cortisol would be related to lumbar spine or hip BMD in road cyclists. A secondary goal of the study was to determine

factors significantly associated with lumbar spine and hip BMD. Salivary cortisol was measured immediately prior to and following a competitive cycling event in 35 cyclists. Other measurements included age, body mass, height, daily calcium intake, and training characteristics. There were no significant relationships between pre-or post-race cortisol and BMD of lumbar spine or hip. Weight training was statistically associated with increased BMD of the lumbar spine and hip. Also, increased number of years of cycling experience was associated with decreased BMD of the femoral neck. Although findings do not support the relationship between cortisol and BMD; findings do support recommendations that cyclists increase calcium intake and participate in weight training to increase or maintain BMD.

The purpose of the second study was to improve bone remodeling in favor of formation in male road cyclists. It has been hypothesized that excessive dermal calcium loss during intense, long endurance cycling may disrupt calcium homeostasis (Barry & Kohrt, 2008; Klesges et al., 1996). Thus, the provision of a non-skeletal source of calcium prior to training and racing may preserve bone over a period of time.

It was hypothesized that supplementation with calcium carbonate (1,600 mg) and vitamin D_3 (1,000 iu) prior to training and racing would maintain and/or improve BMD throughout a competitive season. A total of 29 cyclists were randomized into either a calcium supplementation group or a control group. Bone mineral density of the lumbar spine and hip were measured at the start of the competitive season (May) and again at the end of the season (October). Cyclists in the calcium group received calcium carbonate and vitamin D_3 to take prior to all cycling training or racing. A total of 17 cyclists

completed the 5 month study. There were no statistical group differences in anthropometric, BMD, or demographic measurements at baseline. There were no differences in BMD between the calcium and control group at the end of the competitive season. Lumbar spine, total hip, and femoral neck BMD was maintained throughout the season in the full sample. On the contrary, BMD of the femoral trochanter significantly decreased across all participants. Additionally, the WHO classification of femoral neck BMD changed from low to osteopenia. It is possible that the 5 month period was not long enough to observe significant changes to BMD.

In summary, current data confirm that competitive cycling is detrimental to bone health. Practitioners and coaches working with cyclists need to be aware of the importance of bone density scans for these athletes so that those riders at increased risk of fracture can be identified. In illustration of this point, half of the cyclists' lumbar spine or hip BMD was classified as low by WHO in study one of this dissertation. Further, in the second study, 59% (n = 10) of the cyclists' lumbar spine or hip BMD were classified as low at baseline and 71% (n = 12) of the cyclists' lumbar spine or hip BMD were classified as low at the 5-month follow-up. Taken together, these findings highlight the importance of encouraging endurance cyclists to maintain a calcium-rich diet to help improve or maintain BMD, especially in those with an increased number of years of cycling experience. It is also important that cyclists include a weight training regimen to increase BMD and strengthen bones. Future research on training protocols that may attenuate the decrease in BMD throughout the competitive cycling season is necessary. Additionally, it is important to continue inquiry into the timing, type, and dosage of

calcium intake needed to increase BMD in competitive male cyclists. Until researchers are able to identify what factors most prominently lead to this altered bone profile in road cyclists, it is important that the cycling community be educated with respect to the documented risk and precautionary measures that can be taken to improve bone health.

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