Calcium and Vitamin-D supplementation on bone structural properties in young male Jockeys: a randomised controlled trial

Submitted by

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A thesis submitted in total fulfilment of the requirements for the degree of

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23 March 2016

Statement of Sources

This thesis contains no material published elsewhere or extracted in whole or part from a thesis by which I have qualified for or been awarded another degree or diploma.

No other person's work has been used without due acknowledgement in the main text of the thesis.

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All research procedures reported in the thesis received the approval of the Australian Catholic University Human Research Ethics Committee (2012 114N).

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Abstract

Introduction: Young male jockeys compromise bone health by engaging in caloric restriction and high volumes of physical activity during periods of musculoskeletal growth and development. Failure to attain peak bone mass (PBM) during growth can have adverse short and long term musculoskeletal effects, with numerous studies demonstrating inferior bone health in jockey populations. However, no study to date has addressed counteracting the deleterious effects that participation in this sport has on bone health. The purpose of this six-month double-blind randomised placebo controlled trial was to examine the efficacy of 800mg calcium and 400 IU vitamin D daily supplementation on improving bone mineral properties at the tibia (weight-bearing) and radius (non-weight-bearing) using peripheral Quantitative Computed Tomography (pQCT) and blood-borne markers of bone turnover.

Three inter-related studies were designed following the findings of a systematic review and meta-analysis examining the effects of calcium and vitamin D supplementation on bone mineral density in healthy males. **Study one** examined the effect that the protocol had on markers of bone metabolism and bone properties of the radius. **Study two** was designed to investigate the impact of six months supplementation on weight-bearing bone while **study three** further explored alterations to radial and polar cortical bone properties at the tibial mid-shaft following the clinical trial.

Methods: Twenty-nine male jockeys (age=20.18 ± 3.23yrs) were originally recruited to the study with 17 completing the intervention. Bone properties at the ultra-distal (4%) and proximal (66%) radius and tibia using pQCT and serum vitamin D, Procollagen type 1 N propeptide (P1NP) and C-terminal telopeptide of type I collagen (CTx) were

assessed at baseline and six months. Bone properties at the 66% tibial site were further analysed using BoneJ pQCT distribution plug-in. Polar and radial volumetric bone mineral density (vBMD) was measured in 36, ten degree cortical sectors (polar) and three concentric cortical divisions (radial). Polar distribution was further consolidated into four, 90 degree quadrants aligned to anatomical planes. Cortical mineral mass, endocortical and pericortical radii were also analysed.

Results: After co-varying for height, body mass and baseline bone measurements, the analysis of co-variance (ANCOVA) results of these combined studies demonstrate that six months calcium and vitamin D supplementation stimulated a reduction in bone resorption together with significant improvements to bone material properties at the proximal tibia in the supplemented group. ANCOVA serum analysis indicated significantly higher vitamin D levels (18.1%, p=0.014) and lower CTx (ng/L) (-24.8%, p=0.011) in the supplemented group with P1NP unchanged. The supplemented group displayed greater post-intervention bone properties at the 66% proximal site with cortical content (mg·mm) 6.6% greater (p<0.001), cortical area (mm^2) 5.9% larger (p<0.001), cortical density ($mg \cdot cm^3$) 1.3% greater (p=0.001), and total area (mm^2) 4% larger (p=0.003). No alterations were observed to bone material properties at the radius, nor the ultra-distal tibia or bone strength indices.

When cortical bone of the proximal tibia was examined in greater detail the supplemented group demonstrated greater endocortical vBMD in the posterior region of bone (1140.5 ± 6.3 vs 1116.2 ± 5.9; p=0.018) with a trend suggesting supplementation improved mineral mass and stimulated bone apposition in the posterior and lateral regions of the tibia.

Conclusion: This is the first randomised controlled trial to examine the efficacy of calcium and vitamin D supplementation in improving bone properties in a highly vulnerable, young athletic, weight-restricted population. Results indicate beneficial effects of supplementation on bone properties in as little as six months. Although the study size is small, this intervention appears promising as a strategy for improving bone health in young athletes in weight-restricted sports.

Acknowledgements

While undertaking a PhD is a very personal endeavour, it is very much a team effort, and I know that I would not have managed to complete this thesis without all of the support and encouragement that I have received.

I would like to specially thank Associate Professor David Greene, my principal supervisor, who took me on as an unknown quantity. I could not have been luckier to stumble across such an encouraging and supportive supervisor! Dave - you provided me all of the support that I required to complete this project, always gave me timely feedback and were available for me whenever needed. Thank you also, to my co-supervisor Dr Michael Baker for his assistance whenever I asked for it, especially in relation to undertaking systematic reviews.

I would like to acknowledge the NSW and VIC Jockeys Associations for assisting in recruiting the jockeys for the study and providing facilities for us to undertake all of the testing. A special thank you goes to all of the young men who volunteered their bodies to science in order for me to complete this study.

Malik, good friends are hard to find. Thank you for being around to distract me from my studies when I needed a break and encouraging me whenever self-doubt crept in.

And last but not at all least, my family – what can I say, except you are all wonderful! You provide me with a safe haven which allows me to securely venture out into the unknown. Everyone said it wouldn't be easy to raise a family and write a thesis, but you have all made it seem effortless. It is always a good feeling to know that people are proud of you, no matter how old you are. I love you all.

Completing this thesis might mark the end of my PhD studies, but it is the beginning of a new phase of my life, which I hope will continue to be filled with discovery and learning.

"If we knew what it was we were doing, it would not be called research, would it?"

- Albert Einstein

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Journal Publications:

Silk, L. N., Greene, D. A., & Baker, M. K. (2015). The Effect of Calcium or Calcium and Vitamin D Supplementation on Bone Mineral Density in Healthy Males: A Systematic Review and Meta-analysis. *International Journal of Sport Nutrition and Exercise Metabolism, 25*(5), 510-524.

Silk, L. N., Greene, D. A., Baker, M. K., & Jander, C. B. (2015). Tibial bone responses to 6-month calcium and vitamin D supplementation in young male Jockeys: A randomised controlled trial. *Bone, 81*, 554-561.

Conference Abstracts:

Greene, D. A., Silk, L. N., & Baker, M. K. (2014). The effect of calcium and vitamin D supplementation on bone mineral density in healthy males: A meta-analysis. *J Bone Miner Res, 29* (Suppl 1).

Silk, L., Greene, D., & Baker, M. (2015). Racing to better bone health! A 6-month calcium and vitamin D randomised controlled trial in young male jockeys. *Bone Abstracts, 4*(P22).

Awards:

- New Investigator Award, International Conference on Children's Bone Health, 2015 Salzburg Austria.
- Best research poster, International Conference on Children's Bone Health, 2015 Salzburg Austria.
- Australian Post-Graduate Award, Commonwealth Government of Australia, 2013.

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List of Abbreviations

Areal bone mineral density (aBMD): The amount of bone mineral content per projected bone scanned area expressed in $g \cdot cm^2$, as measured by DXA.

Bone mineral content (BMC): The amount of bone mineral per anatomical region expressed in grams (g).

Bone strength index (BSI): An estimate of compressional bone strength at 4% ultra-distal scan sites measured in $mg^2 \cdot mm^4$ calculated using the formula: total area x (total density x $0.001)^2$ at the 4% site (Kontulainen et al., 2008), as measured by pQCT.

Calcidiol (25(OH)D): also known as 25-hydroxycholecalciferol, or 25-hydroxyvitamin D. This is a pre-hormone form of vitamin produced in the liver and commonly used to determine vitamin D status.

Calcitriol (1,25(OH)$_2$D): also known as 1,25-dihydroxyvitamin D, or vitamin D_3. This is the hormonally active metabolite of vitamin D.

Calcium (Ca^{2+}): calcium ions found in human body.

Calcium carbonate ($CaCO_3$*):* most common and least expensive calcium supplement with high absorptive properties (Zhao, Martin, & Weaver, 2005).

Calcium citrate ($Ca_3(C_6H_5O_7)_2$): used as a calcium supplement and contains around 21% calcium.

Carbohydrate (CHO): dietary sugars.

Cortical area: the cortical portion of total bone area (mm^2).

Cortical density: the volumetric bone mineral density of the cortical region of the bone ($mg \cdot cm^3$).

Cortical thickness: the average thickness of the cortical region of bone (mm).

C-terminal telopeptide of type I collagen (CTx): bone resorption marker comprised of collagen molecules which are released when collagen within the bone is broken down.

Distal: anatomical term describing locations further away from the trunk of the body.

Dual energy x-ray absorptiometry (DXA): a type of scanner that provides two-dimensional images of regional areas or the whole body, using two x-ray beams of differing energy levels to measure the absorption of each beam in order to calculate bone mineral (An & Draughn, 1999).

Endocortical: compact bone located on the inner surface of bone adjacent to the medullary cavity.

Grams (g): metric unit of measure equal to 1/1000 kilogram.

Hydroxyapatite: mineral substance found in bone comprised of calcium, phosphate, hydroxyl and carbonate with trace elements of other minerals such as iron, zinc, magnesium, sodium and potassium.

Kilocalorie (kcal): imperial measurement of one unit of nutritional energy. One kcal equals 4.19 kJ.

Kilogram (kg): base unit of mass in metric system.

Kilojoules (kJ): metric measurement of amount of nutritional energy. One kJ equals 0.24 kcal.

Mid-cortical: ring of cortical bone located between the pericortical and endocortical surfaces.

Peak bone mass (PBM): the amount of bony tissue present at the end of the skeletal maturation.

Pericortical: compact bone located on the outer surface of the bone.

Peripheral quantitative computed tomography (pQCT): a type of bone scanner that provides high-resolution three-dimensional images of the peripheral skeleton and uses absorptiometry techniques to measure the attenuation of radiation passing through the scanned site in order to provide measures of volumetric bone density (An & Draughn, 1999).

Procollagen type 1 N propeptide (P1NP): bone formation marker cleaved from type 1 collagen molecules during the process of incorporating collagen into the bone matrix.

Proximal: anatomical description of location closer to the trunk of the body.

Stress strain index (SSI): a surrogate measures of cortical bone strength expressed in mm^3 combining bone geometry with cortical vBMD. This measure has been validated in both animal and human studies (Rauch & Schoenau, 2008).

Total area: cross-sectional area of the bone in mm^2.

Total density: total volumetric bone mineral density in $mg \cdot cm^3$.

Trabecular area: cross-sectional area of trabecular bone in mm^2.

Trabecular density: volumetric bone mineral density of trabecular bone in $mg \cdot cm^3$.

Ultra-distal: anatomical description of location furthest away from trunk of body.

Urine specific gravity (Usg): a measurement of the concentration of all chemical particles in the urine.

Volumetric bone mineral density (vBMD): The amount of bone mineral content as a function of bone volume expressed in $g \cdot cm^3$ as assessed by pQCT.

1 Introduction

1.1 Introduction

Horse racing requires jockeys to be slight in body mass in order to meet the handicap weight restrictions imposed on horses that they ride. Typically, jockeys commence their riding career with small, immature stature. Male apprentice jockeys in Australia require a body mass of between 45kg and 48kg (Racing-NSW, 2014) which places apprentice riders in the lowest 5th percentile for international weight-for-age scales (Kuczmarski et al., 2000). Despite a restricted body mass, jockeys must demonstrate strength, endurance, and balance in order to control animals ten times their body mass over distances ranging from 800m to 3,600m (flat riding) or up to 5,500m in jump races (Douglas, Price, & Peters, 2012; Hitchens, Blizzard, Jones, Day, & Fell, 2011; Trowbridge, Cotterill, & Crofts, 1995; Waldron-Lynch et al., 2010).

Jockeys represent a unique group of weight-category athlete. Unlike other weight category sports which have competitive seasons, jockeys are required to maintain their restricted weight throughout a full calendar year (Hitchens et al., 2011). Jockeys can ride in multiple events each day and must weigh in both before and after each race. As such, they are not afforded the ability to rehydrate or increase energy levels prior to competing, especially when subsequent races require lower handicap weights (Dolan et al., 2011; Wilson, Drust, Morton, & Close, 2014). To remain within specific weight limits, jockeys often engage in unhealthy weight-loss behaviours that rely on rapid, short-term weight loss and include an increased propensity to engage in disordered eating (Leydon & Wall, 2002; Moore, Timperio, Crawford, Burns, & Cameron-Smith, 2002; Wilson et al., 2015). Thus, there is a high risk of inadequate nutrition in an effort to maintain low body

weight. Increasing evidence indicates that maintaining a restricted weight can negatively impact on physiological and cognitive health. More specifically, participating in weight restricted activity can limit the attainment of peak bone mass (PBM) during growth and have damaging short and long term musculoskeletal effects (De Souza & Williams, 2005).

Previous research has found jockeys to have low calcium intakes and subsequent indicators of compromised musculoskeletal health (Caulfield & Karageorghis, 2008; Dolan, McGoldrick, et al., 2012; Dolan et al., 2011; Greene, Naughton, Jander, & Cullen, 2013; Leydon & Wall, 2002; Moore et al., 2002; Waldron-Lynch et al., 2010; Warrington et al., 2009). Evidence of vitamin D levels indicate young jockeys are vitamin D deficient (Guillemant et al., 2001; Wilson, Sparks, Drust, Morton, & Close, 2013). Approximately 50% of jockeys demonstrate osteopenia as young as 20 years of age (Leydon & Wall, 2002; Warrington et al., 2009) and apprentice riders display reduced bone strength (Greene et al., 2013).

Biomechanical analysis of horse riding is very limited, however faster gaits adopted during racing force riders to utilise a riding positon which causes loading through a rider's legs rather than hips (Douglas et al., 2012). Generally, upper extremities are non-weight bearing highlighting the action of muscle strain in improving bone strength (Nikander, Sievänen, Uusi-Rasi, Heinonen, & Kannus, 2006). Previous research has found jockeys to have positive bone adaptations at the forearm (Greene et al., 2013; Leydon & Wall, 2002) suggesting muscular forces incurred at the radius during riding may be in excess of common habitual loads. The additional strains incurred by jockeys through repetitive daily activities may result in excessive bone strain and increase fracture risk.

Consequently, lifestyle factors potentially place jockeys, particularly young apprentice riders, in a high risk group for poor bone health.

The age of PBM for males is unclear, with some studies indicating 18-20 years of age for spine and hip PBM whilst others have found PBM to be 25-29 years (Boot et al., 2010; Henry et al., 2010; Lorentzon, Mellström, & Ohlsson, 2005; Szulc, Marchand, Duboeuf, & Delmas, 2000). However, there is evidence supported by both dual energy x-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT) indicating the long bones of limbs do not reach PBM until 40-50 years of age in males (Henry et al., 2010; Lorentzon et al., 2005; Szulc et al., 2000). This suggests bone-optimising strategies should extend well beyond early adulthood in order to maximise bone mineral properties in males.

One available strategy to potentially optimise the attainment of PBM in young jockeys involves calcium and vitamin D supplementation. Currently there is conflicting evidence about the beneficial effects of calcium and vitamin D supplementation on bone mineral density during growth and insufficient evidence relating to male populations (Abrahamsen et al., 2010; Chung et al., 2009; Cranney et al., 2007; Lips, Gielen, & van Schoor, 2014; Shea et al., 2002; Tang, Eslick, Nowson, Smith, & Bensoussan, 2007; Winzenberg, Shaw, Fryer, & Jones, 2010). However it appears populations who incur the greatest compromises to musculoskeletal health may also benefit the most from calcium and vitamin D supplementation (Winzenberg et al., 2010). Apprentice jockeys therefore represent an at-risk population who may potentially achieve a positive musculoskeletal response to a simple and effective intervention strategy.

To date DXA has predominantly been used to assess jockey bone health (Caulfield & Karageorghis, 2008; Dolan, McGoldrick, et al., 2012; Dolan et al., 2011; Leydon & Wall, 2002; Moore et al., 2002; Waldron-Lynch et al., 2010; Warrington et al., 2009). This technology has a number of limitations, such as an inability to differentiate between cortical and trabecular bone or the assessment of bone size and shape with acceptable accuracy (Khan et al., 2001). Conversely, pQCT is able to distinguish between trabecular and cortical bone, provide measures of volumetric bone mineral density (vBMD) and assess bone size, strength and geometry (Khan et al., 2001). Minor alterations to the distribution of bone mass or bone structure may have considerable impact on bone strength without altering overall bone mineral density (BMD) (Nikander et al., 2010). Accordingly, pQCT should allow for more accurate assessments of potential changes in the structural properties of bone arising from supplementation.

While BMD may take months or years to respond to stimuli, bone turnover markers (BTM) may begin to detect changes in bone metabolism within days or weeks (Vasikaran, Eastell, Bruyere, et al., 2011). A wide variety of BTMs has been used to assess bone turnover in jockeys with no consistency (Dolan, McGoldrick, et al., 2012; Waldron-Lynch et al., 2010; Wilson, Fraser, et al., 2013). The International Osteoporosis Foundation (IOF) recommends the use of serum procollagen type I N propeptide (s-PINP) and serum C-terminal telopeptide of type I collagen (s-CTx) be adopted as markers of bone formation and bone resorption. Therefore, to standardise the assessment of BTMs in clinical trials, s-P1NP and s-CTx should be utilised.

1.2 Study rationale

Lifestyle factors place jockeys in a high risk group for poor bone health. In addition to compromised nutrition, young jockeys are exposed to the highest risks of musculoskeletal conditions by repetitive loading on bones. Injury-related data from around the world shows that majority of injuries sustained by jockeys are fractures (Foote, McIntosh, V'Landys, & Bulloch, 2011). Upper limb fractures are the most commonly reported fracture in both the USA and the UK/Ireland, and while Australian injury data is not well documented, recent reports indicated that 78% of all jockeys surveyed incurred a fracture due to riding (Foote et al., 2011).

Recommended strategies to improve bone health are not feasible for jockeys as the weight restricted nature of horse racing prohibits the additional development of lean tissue mass that arises from resistance training. Furthermore, the introduction of a modified diet to increase calcium-rich food choices may be challenging given the daily demands of racing and propensity for disordered eating habits of jockeys.

As such, the purpose of this thesis was to examine the efficacy of calcium and vitamin D supplementation on improving bone mineral properties at the tibia (weight-bearing) and radius (non-weight-bearing) using pQCT and blood-borne markers of bone turnover. A combination of weight-bearing and non-weight-bearing bones were selected to control, in part, the influence of habitual daily loading.

1.3 Thesis Overview

The primary aim of this thesis was to examine the effectiveness of 800 mg calcium (citrate and carbonate) and 400 IU vitamin D supplementation daily for six months in

improving bone material properties and blood borne markers of bone turnover in young male jockeys.

Secondary aims were to:

(i) compare the effects of supplementation on weight-bearing (tibia) and non-weight bearing (radius) bones

(ii) elucidate the way in which physical loading affects alterations to bone material properties in conjunction with calcium and vitamin D supplementation.

1.4 Intervention studies and hypotheses

The intervention studies undertaken were as follows:

1.4.1 Study One

Effect of calcium and vitamin D supplementation on bone turnover markers and radial bone properties in young male jockeys: A Randomised Controlled Trial.

1.4.1.1 *Aim*

This study aimed to examine the effectiveness of calcium and vitamin D supplementation to positively alter bone metabolism, in order to improve the bone material properties of non-weight bearing bone (radius).

1.4.1.2 *Hypotheses*

i. Calcium and vitamin D supplementation will be an effective and feasible strategy for improving bone material properties of the non-weight bearing radius in young male jockeys;

ii. Calcium and vitamin D supplementation will result in improved blood-borne markers of bone metabolism.

1.4.2 Study Two

Tibial bone responses to 6-month calcium and vitamin D supplementation in young male jockeys: A randomised controlled trial.

1.4.2.1 *Aim*

This study aimed to examine the effectiveness of calcium and vitamin D supplementation to positively improve the bone material properties of weight bearing bone (tibia).

1.4.2.2 *Hypothesis*

Calcium and vitamin D supplementation will be an effective and feasible strategy for improving bone material properties of the tibia in young male jockeys.

1.4.3 Study Three

Cortical bone distribution at the tibial shaft in young male jockeys after 6-months calcium and vitamin D supplementation: A randomized controlled trial.

1.4.3.1 *Aim*

To compare the polar and radial cortical vBMD distribution at the tibial mid-shaft in young male jockeys exposed to 6-months calcium and vitamin D supplementation with age- and gender-matched jockeys receiving a placebo.

1.4.3.2 *Hypothesis*

Calcium and vitamin D supplementation is associated with reduced cortical vBMD, particularly at the mid- and pericortical bone divisions.

1.5 Limitations

The following limitations to the study are acknowledged:

1. The number of participants initially recruited and completing the study were lower than originally anticipated.

2. The length of the intervention was short in comparison to a number of other calcium and vitamin D intervention studies. A longer study length may have resulted in more clarity in relation to alterations in bone strength. However, to maximise compliance and participant retention, a longer intervention period may have proven impractical.

3. Regulating dietary intake was beyond the control of the study. Estimates of total energy intake as well as calcium and vitamin D intake were monitored through the completion of dietary intake questionnaires; however, control over actual intakes each day were beyond the scope of the study.

4. Measuring energy expenditure was not feasible. For safety and competition reasons, jockeys are not permitted to wear equipment that would estimate energy expenditure during racing or training.

5. The influence of muscular strain on bone properties due to the actions of horse racing have not been elucidated. Equipment required to measure the biomechanical strain would be unsafe during racing or training.

6. Genetics are known to influence bone mineral density. Controlling for genetics fell outside of the scope of the study; however, the randomised controlled nature of the study assisted in reducing selection bias and minimise genetic influences.

7. The study did not measure parathyroid hormone (PTH) levels pre- or post-intervention. PTH levels influence both osteoclastic and osteoblastic activity and work in concert with vitamin D in order for the body to

maintain calcium homeostasis. Levels of PTH are usually adequate when vitamin D levels are also adequate. We have monitored serum vitamin D levels.

8. Efforts were taken to monitor compliance, however, despite a number of attempts to obtain the remaining tablet containers, we were unable to do so. Compliance was verbally provided at the time of data collection and evidenced by alterations to serum vitamin D levels in the supplemented group.

1.6 Delimitations

The following delimitations were implemented:

1. Only male Apprentice jockeys undertaking a Certificate IV in Racing in New South Wales (NSW) or Victoria (VIC) were recruited to the study.

2. All participants were required to be in good health with no known history of fracture in the scanned limbs, no known history of metabolic bone or muscle disease and not taking medications or other supplements that may influence bone metabolism.

3. Data collection was delimited to two testing sessions, six months apart with data collection times scheduled for the same time period at each testing session.

4. Musculoskeletal assessment was restricted to the use of bone imaging techniques of pQCT (XCT 2000, Stratec Medizintechnik, Pforzheim, Germany).

5. Further analysis of the bone scans obtained from the pQCT was restricted to the pQCT distribution plug-in available in BoneJ (Doube et al., 2010; Rantalainen, Nikander, Heinonen, Daly, & Sievänen, 2011).

6. Blood borne markers of bone turnover were delimited to s-P1NP and s-CTx as recommended by the IOF.

7. Anthropometric data was collected in accordance with procedures approved and described by the International Society for the Advancement of Kinanthropometry (ISAK) (Stewart, Marfell-Jones, Olds, & de Ridder, 2011)

1.7 Assumptions

The following assumptions have been made:

1. All participants completed the questionnaires accurately and in full detail.

2. Any previous injuries or medical conditions were declared and did not affect the results of the study.

3. Participants consumed the active or placebo tablets as instructed for the duration of the study period.

4. Participants did not take any other supplements or medications during the study period that would conflict with the results.

1.8 Thesis Presentation

This thesis is presented in eight chapters. Chapter Two presents a narrative review of both generalised and jockey-specific literature pertaining to bone and influences on

bone health. Chapter Three presents a published systematic review and meta-analysis of the effect of calcium or calcium and vitamin D supplementation on bone mineral density in healthy males, the results of which informed the three clinical trial studies. Methods applicable to all three intervention studies are described in Chapter Four. The three intervention studies follow in Chapters Five, Six and Seven and the final chapter presents discussion.

2 Narrative Review of the Literature

2.1 Bone anatomy

Human skeletons are living dynamic tissue structures containing 206 bones once adulthood is reached (Marieb, 2000). Bone undergoes constant change throughout the lifespan and plays an important metabolic role in calcium homeostasis. Modelling and remodelling occurs in order to help the skeleton adapt to biomechanical stresses and remove damaged bone, replacing it with new bone in order to preserve bone strength (Clarke, 2008). Four broad categories of bone type exist:

Type of bone	Examples
Short	Carpal, Tarsal, Patellae
Long	Radius, Tibia, Femur, Humerus
Irregular	Vertebrae, Coccyx
Flat	Ilium, Skull, Mandible

While each type of bone serves differing structural functions, they all contribute to mineral homeostasis, acid-base balance and provide growth factors directly associated with red blood-cell formation (Taichman, 2005). Long bones, such as the tibia and radius, primarily act as levers and allow for locomotion through their ability to transfer loads.

2.1.1 Macroscopic structure of long bones

Long bone structures have a distinct macroscopic shape with clearly defined regions comprised of differing bone tissue and having specific functions (refer Figure 2-1). The end region of the bone is referred to as the epiphysis and the middle region is called the diaphysis (bone shaft). Located in the middle of the diaphysis is the medullary cavity which is filled with both red marrow (responsible for production of red blood cells) and yellow marrow (fat storage) (White & Folkens, 2005). The flared region between the

epiphysis and diaphysis is known as the metaphysis. Located between the metaphysis and the epiphysis is the epiphyseal disk (or growth plate), the region responsible for longitudinal bone growth. The epiphysis is the location where bones meet to form joints, allowing for movement. Articular cartilage coats the epiphysis, allowing for shock absorption and reducing friction between the two bones. This cartilage contains osteogenic cells (refer 2.2), blood and nerve fibres (Clarke, 2008).

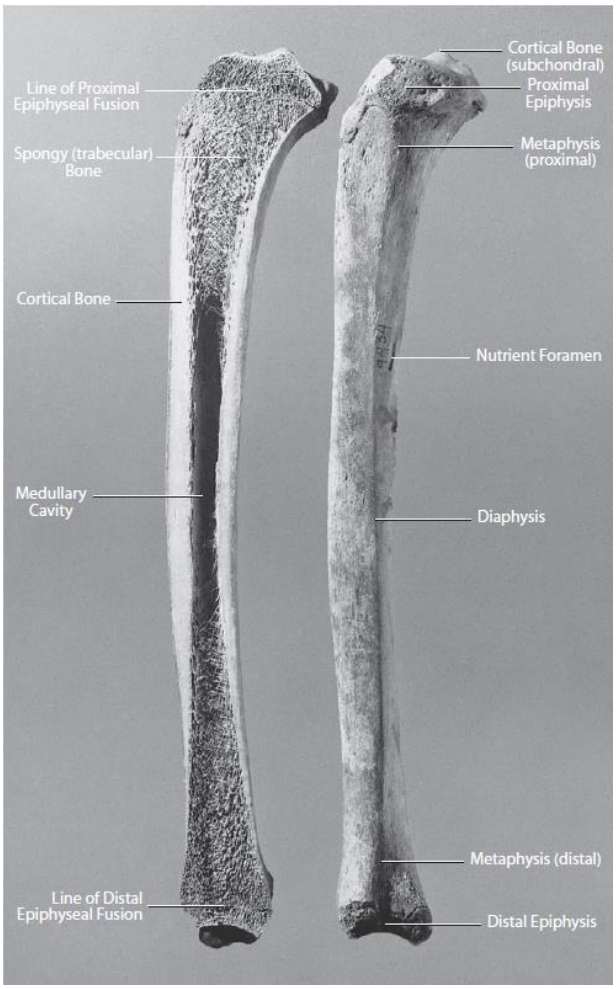

Figure 2-1: Labelled diagram of a human tibia showing gross anatomy of long bone. Reprinted from *The Human Bone Manual* (p. 41), by T White and P Folkens, 2005, Burlington, MA: Elsevier Academic Press. Copyright 2005 by Elsevier Inc. Reprinted with permission.

Long bones are covered by two layers of dense connective tissue known as the periosteum. The outer layer is comprised of fibroblasts and collagen fibres whilst the

inner layer contains osteogenic cells (Standring, 2008). The internal surface of the bone is lined with a cellular structure known as the endosteum and contains blood vessels, osteoclasts and osteoblasts (refer 2.2) (White & Folkens, 2005).

2.1.2 Bone composition

Subject to site specificity, two types of tissue may exist within the bone: woven tissue and lamellar tissue (Khan et al., 2001). Woven tissue is immature bone with collagen arranged in random formation within the tissue. It is the predominant type of bone at birth, at fracture sites or sites of extreme mechanical loading. Woven tissue is formed by active osteoblasts and stimulated by fracture or growth factors (Standring, 2008). Lamellar tissue has collagen arranged along lines of force in alternating directions, giving bone its strength (Clarke, 2008). Both tissue types are organised into compartments of either cortical or trabecular bone. Lamellar tissue is predominately arranged in cylindrical structures known as Haversian canals which form the osteons, or basic units of bone (Standring, 2008).

Bones consist of two different structural components: trabecular (or spongy) bone and cortical (or compact) bone (refer Figure 2-1). Cortical bone is typically 80% - 90% calcium salts whilst calcium only accounts for approximately 15-25% of the bone volume of trabecular bone (Khan et al., 2001). The cellular and molecular structure of both types of bone is similar however they differ in their degree of porosity (White & Folkens, 2005). Most bone tissue of epiphyses and metaphyses are comprised of trabecular bone. It consists of lamellae arranged in an irregular latticework of thin plates of bone called trabeculae, giving it a spongy, porous structure. The spaces between trabeculae are filled with red bone marrow. Cortical bone has a high mineral content, making it very dense,

and is also less metabolically active than trabecular bone (Clarke, 2008). The structure of cortical bone is based on Haversian systems (cortical osteons) which are located in the diaphysis (see Figure 2-2). Cortical bone also covers trabecular bone in the epiphyses. The functions of Haversian systems are to protect, support, and resist stress. Human adults have more trabecular area than cortical area. There are approximately 14 x 10^6 trabecular osteons with a total area of 7m^2, whilst there are estimated to be 21 x 10^6 cortical osteons with a total Haversian modelling area of 3.5m^2 (Clarke, 2008).

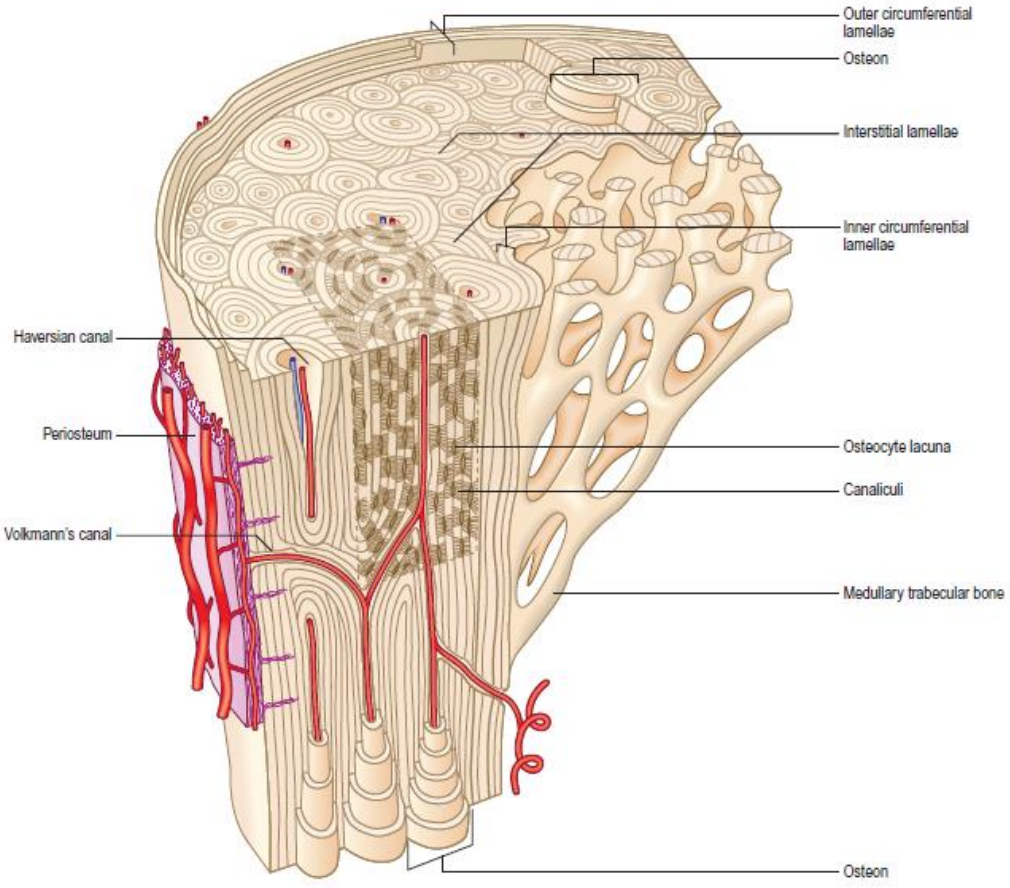

Figure 2-2: Main features of the microstructure of mature lamellar bone. Areas of compact and trabecular (cancellous) bone are included.
Reprinted from *Gray's Anatomy (40th Ed)* (p. 89), by S Standring (Editor-in-Chief), 2008: Churchill Livingstone Elsevier. Copyright 2008 by Elsevier Inc. Reprinted with permission.

2.2 Microstructure of bone

2.2.1 Bone cells

Three main cell types are responsible for the formation and maintenance and remodelling of bone tissue: osteoblasts, osteocytes and osteoclasts (Khan et al., 2001; Standring, 2008; White & Folkens, 2005).

2.2.1.1 *Osteoblasts*

Osteoblasts are created from osteoprogenitor cells located in the bone marrow and other bone connective tissue such as periosteum and endosteum (Standring, 2008). Osteoblasts are seen on the surfaces of growing or (re)modelling bone and function as the bone formation cells, producing and laying down bone material. Osteoblastic function is hormonally driven, primarily by parathyroid hormone (PTH), vitamin D_3 ($1,25(OH)_2D_3$) and growth hormone (Khan et al., 2001). Activity of osteoblasts, particularly during growth, influences bone shape and size in response to mechanical loading, otherwise known as bone modelling. In adults, osteoblasts are primarily located on endosteal rather than periosteal surfaces, and are also found deep within cortical bone where osteons are remodelled (Standring, 2008). Osteoblasts produce large amounts of pre-bone tissue (osteoid), an un-calcified collagen matrix. Osteoids contain type-I collagen as well as glycoproteins such as osteocalcin (Standring, 2008). Calcification occurs when hydroxyapatite (refer 2.2.2.1) is deposited into the osteoid matrix through the actions of osteocalcin which binds calcium molecules (White & Folkens, 2005). Once the osteoblasts are surrounded by the bony matrix and embedded they become osteocytes.

Osteoblasts also play an important role in bone resorption, expressing receptors for $1,25(OH)_2D_3$, parathyroid hormone (PTH) and other bone resorption proteins. The

osteoblasts directly stimulate osteoclast differentiation through the PTH receptors, and conversely down-regulate osteoclast activity when conditions favour bone deposition (Standring, 2008).

2.2.1.2 *Osteoclasts*

Osteoclasts are specialised cells derived from the macrophage family and are responsible for bone resorption (removal) (Teitelbaum, 2000). These cells are directly involved in modelling and remodelling of bone and hence, maintenance of bone mass. They are large, multi-nucleated cells which function by destroying bone matrix to allow for the repair and geometric optimisation, and therefore strength, of the bone (Clarke, 2008; Khan et al., 2001). Osteoclasts remove local bone during growth and remodelling of osteons and surface bone. Osteoclasts attach to bone, releasing enzymes to create an acidic environment which demineralises the bone. Following this, enzymes are also released to destroy the organic collagen matrix (Clarke, 2008). Upon completion, the osteoclasts then move to the next bone degradation site (Teitelbaum, 2000). These cells are stimulated by various influences including osteoblastic signalling and hormones such as PTH and $1,25(OH)_2D_3$, while calcitonin produced by the thyroid gland reduces osteoclastic activity (Standring, 2008).

2.2.1.3 *Osteocytes*

Osteocytes are mature bone cells located within osteons (see Figure 2-2). They are derived from osteoblasts and are essential for bone maintenance. Mature bone cells do not divide, nor do they secrete new bone matrix. Each osteocyte maintains contact with adjacent osteocytes allowing for inter-cellular communication within each osteon; however, they do not communicate with neighbouring osteon systems. Osteocytes

release biochemical signals in response to mechanical strain or a lack of strain, thereby recruiting osteoblasts or osteoclasts to regulate and repair bone mass (Hughes & Petit, 2010). Osteocytes are responsive to $1,25(OH)_2D_3$ and PTH, and may be involved in mineral exchange at adjacent bone surfaces (Standring, 2008). Osteocytes have a long lifespan which is measured in years and influenced by the metabolic activity of the individual bone (Standring, 2008). Their function has been shown to be affected by limitations to calcium availability, which may suppress bone formation in response to loading and therefore affect remodelling when osteocytes die (Hughes & Petit, 2010).

2.2.2 Bone matrix

Bone is a strong organic matrix comprised of around 70% calcium hydroxyapatite and 30% collagen fibres and non-collagenous proteins (Khan et al., 2001; Standring, 2008). The combination of mineral and collagen fibres enables bones to be both stiff and flexible in order to accommodate constant loading in a variety of planes (White & Folkens, 2005). Calcium in bone provides compressional strength; collagen fibres contribute to tensile strength; and proteins play an important role in bone remodelling (Khan et al., 2001; Seeman & Delmas, 2006).

2.2.2.1 *Bone minerals*

The mineral substance found in bone is commonly referred to as hydroxyapatite and provides rigidity and strength to bone. (White & Folkens, 2005). Bone mineral is predominantly calcium, phosphate, hydroxyl and carbonate with trace elements of other minerals such as iron, zinc, magnesium, sodium and potassium also being present (Clarke, 2008; Standring, 2008). The hydroxyapatite is deposited with the gaps in the collage matrix with the help of a number of phosphate- and calcium-binding proteins (Clarke,

2008). In immature osteons, the mineral content is low but this increases with age and is highest in older more peripheral regions (Standring, 2008).

2.2.2.2 *Collagen*

Around 85% to 90% of bone proteins are collagens which form slightly elastic, flexible fibres enabling bones to bend without breaking under loading and providing compressive and tensile strength (White & Folkens, 2005). Of the differing types of collagen found in bone, the predominant form is type 1 collagen, although it's structure in bone differs from other parts of the body (Standring, 2008). Osteoblasts synthesise collagen fibres, which have a cross-linkage structure that provides strength and space for mineral deposits within the spaces formed by this structure. Around 2/3 of all mineral deposits in bone are found within collagen fibrils (Standring, 2008).

2.2.2.3 *Other organic compounds*

Other non-collagenous proteins comprise around 10-15% of the extracellular bone proteins, such as serum proteins and growth factors which are thought to assist in mineralisation of the bony matrix and regulation of bone cell production (Clarke, 2008). Osteocalcin is released by both osteoblastic and osteoclastic activity and is thought to inhibit bone formation, whist osteonectin (the most prevalent protein) affects osteoblast proliferation and mineralisation of the bone matrix (Clarke, 2008).

2.3 Bone growth, modelling and remodelling

Growth refers to lengthening and thickening of the entire skeleton through a process of continual bone tissue deposition while modelling refers to the process which changes the shape of bones in response to mechanical loading or physiological influences.

Remodelling is the continual process of bone renewal in order to maintain bone strength and mineral homeostasis (Clarke, 2008).

2.3.1 Growth

Bone growth involves two simultaneous processes: appositional growth, which allows for the enlargement of shaft diameters; and longitudinal growth, which refers to the lengthening of the skeleton. Appositional growth occurs through a process whereby osteoblasts located in the periosteum deposit new bone, while osteoclasts situated in the endosteum remove older bone tissue, allowing the bone diameter bone to increase while maintaining a central cavity (White & Folkens, 2005). Lengthening of the skeleton occurs by replacing cartilage cells produced on the epiphyseal side of the growth plate with bone cells on the diaphyseal side in a process known as endochondral ossification (White & Folkens, 2005). This replacement process allows bone to increase in length while maintaining epiphyseal plate thickness. Eventually cartilage cells stop dividing and cartilage is replaced by bone, producing a new structure called the epiphyseal line (Khan et al., 2001).

2.3.1.1 *Attainment of peak bone mass*

During bone growth, bone mass increases through the mineralisation of the bone matrix. Approximately 25% of PBM is gained in the two year period surrounding peak height velocity during adolescence with bone mineral accrual lagging behind linear growth by approximately 6-12 months (Bailey, Faulkner, & McKay, 1996). At peak height velocity, adolescents have reached approximately 90% of adult height but only 57% of their adult bone mineral content (BMC) (Bailey, 1997; Bailey, McKay, Mirwald, Crocker, & Faulkner, 1999). Following linear growth, bone modelling continues, so that

approximately 90% of PBM is acquired by 18 years of age (Baxter-Jones, Mirwald, McKay, & Bailey, 2003).

Substantial increases in total body, radial, femoral neck and lumbar spine aBMD have been reported with advancing sexual maturity in male and females during growth (Blimkie et al., 1996). In males, radial and lumbar spine aBMD continues to increase from late puberty into early adulthood (Blimkie et al., 1996). Males generally have slightly higher radial, femoral neck and lumbar spine aBMD than females by full sexual maturity during the later years of adolescence due to males generally having larger bone size (Wren, Liu, Pitukcheewanont, & Gilsanz, 2005).

Similar to results found using dual x-ray absorptiometry (DXA), Schoenau, Neu, Rauch, and Manz (2001), used pQCT to demonstrate that for any given cortical bone mass, males have stronger bones than females during growth. Males deposit bone on the periosteal surface while females deposit bone on the endocortical surface. It is suggested that female bone deposits have a small effect on bone stability in order to provide a source of calcium during child-bearing (Schoenau et al., 2001). However, it is plausible that male bones require more strength as a result of higher muscle mass generating greater mechanical strain on bone.

Bone area has been shown to peak around two years before BMC (Baxter-Jones, Faulkner, Forwood, Mirwald, & Bailey, 2011). Literature regarding age of attained PBM for males is equivocal, with some studies indicating that PBM of lumbar spine and hip is not achieved until 25-29 years of age (Szulc et al., 2000), whilst others indicate PBM of lumbar spine, and femoral neck in males is reached by 18-20 years (Boot et al., 2010; Henry et al., 2010; Lorentzon et al., 2005). There is evidence to suggest, however, that

PBM of the long bones of limbs and whole body does not occur until 40-50 years of age in males, supported by both DXA and pQCT (Henry et al., 2010; Lorentzon et al., 2005; Szulc et al., 2000). Consolidation of long bone PBM is achieved through increased cortical thickness and further mineralisation of cortical bone (Lorentzon et al., 2005).

2.3.2 Modelling

Modelling refers to the actions of osteoblasts and osteoclasts in order to optimise bone shape and size in response to growth and mechanical loading. The Mechanostat theory suggests the existence of a homeostatic regulatory mechanism which is responsible for creating or resorbing bone in response to mechanical loading or unloading (Frost, 1987). The theory proposes that modelling and remodelling are two separate mechanisms within bone (Frost, 1998, 2004; Jee, 2000). When strain exceeds a threshold, bone formation occurs on the existing structure to increase bone strength and repair micro-damage within the bone (Frost, 2004). Modelling occurs through “drifts” involving independent actions of osteoblasts and osteoclasts. Formation drifts control osteoblastic activity, adding bone to periosteal surfaces while resorption drifts remove bone from endocortical surfaces (Frost, 2004). These drifts are also crucial to reshaping bones as they lengthen during growth (Hughes & Petit, 2010), refer Figure 2-3.

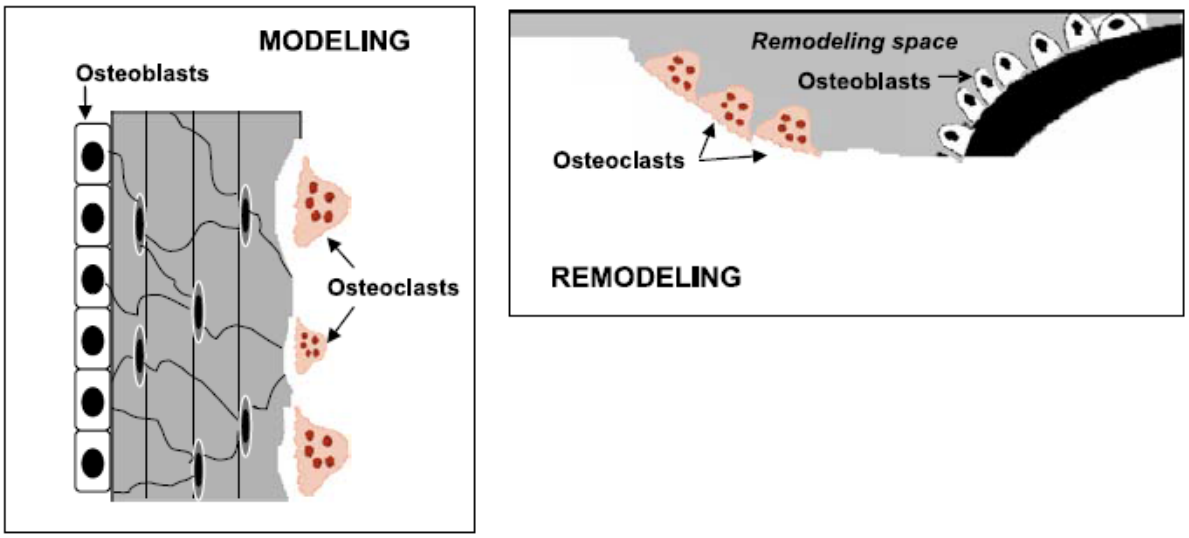

Figure 2-3: Modelling and remodelling of bone. Modelling can be seen to occur on both the periosteal and endosteal surfaces of the bone allowing for growth. Remodelling occurs on one surface, with old bone removed and replaced with new bone.
Reprinted from "Methods for measurement of paediatric bone," by Binkley, Berry, & Specker, 2008, *Reviews in Endocrine and Metabolic Disorders, 9*(2), 95-106. Copyright 2008 by Springer. Reprinted with permission.

Bone modelling occurs in response to muscular contraction as well as external physical loading in order to optimise bone strain (Frost, 1998; Schoenau & Frost, 2002). Bone undergoes modelling via a negative feedback loop in response to strain and bone strength (Frost, 1998). The effects of activity on bone are site specific, so that only bone exposed to physical loading will undergo modelling. Persistent strain above the stress thresholds can accumulate, causing stress fractures in athletes. Studies in athletic populations have demonstrated that physical loading can increase site-specific bone strength (Greene, Naughton, Bradshaw, Moresi, & Ducher, 2012; Jürimäe, Purge, Jürimäe, & Duvillard, 2006) and that non-weight bearing physical activity can have a negative impact on bone density (Smathers, Bemben, & Bemben, 2009). Macdonald, Cooper, and McKay (2009) found that physical activity improved bone strength at the tibial shaft in young boys. Further, their results suggest that observed alterations occur in different

quadrants of the bone shaft, reflecting bone adaptation directly in response to site-specific physical loading.

2.3.3 Remodelling

Bone remodelling is a localised surface-based phenomenon that first involves the removal of 'old' bone via the actions of osteoclasts, followed by the deposition of 'new' bone via osteoblasts at the same site (Hughes & Petit, 2010) (Figure 2-3). Remodelling continues throughout the lifespan to maintain skeletal mechanical integrity by repairing micro-damage within the bone (Clarke, 2008; Dalsky, 1990). In healthy populations, remodelling is directly related to both calcium homeostasis and mechanical strain (Rizzoli, Bianchi, Garabédian, McKay, & Moreno, 2010). Calcium loss from bone is influenced by site-specific mechanical loading with bone exposed to the highest mechanical loads experiencing less bone mineral loss than non-loaded sites (Harada & Rodan, 2003).

2.4 Other influences on bone

While the Mechanostat theory has been adopted as the most significant influence on bone strength and development, there are a number of other important influences on bone, without which, bone strength cannot be achieved or maintained. The most important of these is interlinked actions of calcium, vitamin D and PTH which affects calcium absorption, and bone mineralisation.

2.4.1 Influence of Calcium on bone health

Calcium is the most abundant mineral in the human body, comprising the main structural element of teeth and bones and playing a vital role in physiological functioning. Calcium is involved in: muscle contraction, blood vessel regulation, nerve impulse transmission, hormone secretion, and is required as a coenzyme for a number proteins

and enzymes (Higdon & Drake, 2011). In order to maintain normal physiological functioning, serum and fluid calcium levels are maintained within strict range through the actions of the parathyroid gland and vitamin D (refer Figure 2-4).

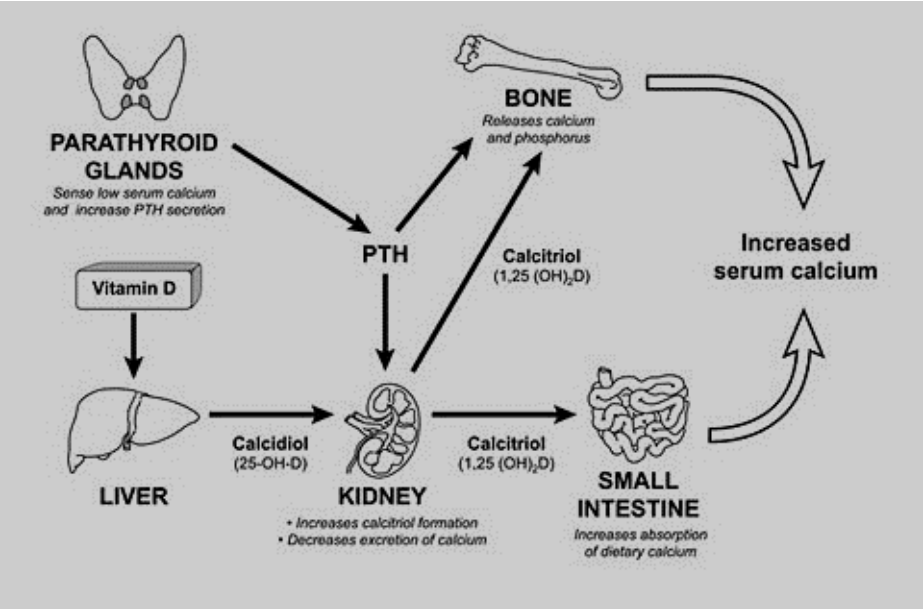

Figure 2-4: The regulation of serum calcium via the actions of 1-25 $(OH)_2D_3$ vitamin D (Calcitriol) and parathyroid hormone (PTH). Both PTH and Calcitriol act directly on bone to release calcium in order to regulate serum and fluid calcium levels.
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Maintenance of calcium homeostasis overrides all other functions of the skeleton (Harada & Rodan, 2003) and is regulated primarily by PTH and vitamin D (Raisz, 1999). When blood calcium concentrations fall below normal levels, PTH is released by the parathyroid gland which in turn stimulates production of $1,25(OH)_2D_3$ by the kidneys (Khan et al., 2001). Together these hormones activate osteoclasts which stimulate bone resorption and the release of calcium into the bloodstream. Similarly, when serum levels of $1,25(OH)_2D_3$ fall below $30ng{\cdot}mL^{-1}$, PTH levels increase, stimulating osteoclastic bone activity (Angeline, Gee, Shindle, Warren, & Rodeo, 2013). Conversely, as serum calcium

levels rise the thyroid gland releases calcitonin which inhibits osteoclastic activity, although this is thought to have a minor effect on calcium homeostasis (Khan et al., 2001; Raisz, 1999).

2.4.1.1 *Calcium requirement*

Adequate calcium intake is considered one of the most important preventative interventions contributing to optimal bone health in young adults (Nattiv & Armsey, 1997). Recommended dietary intakes for calcium ensure gains in bone mass during adolescence and minimise bone loss that occurs with ageing (Bachrach, 2001). The skeleton of a new-born contains approximately 25 grams of calcium increasing to over 1000 grams in a healthy adult. Increasing calcium demands of the growing skeleton must be provided from dietary sources for essential increases in calcium between birth and adulthood (Heaney et al., 2000). Calcium is stored in bone tissue, however excess intake cannot be maintained, as bone is regulated by mechanical stimulus. Greater calcium intake results in a higher level of PBM acquisition; however, calcium behaves as a threshold nutrient (Heaney et al., 2000) with a regulatory system in place limiting bone growth and resulting in higher levels of urinary calcium output if exceeded (Vicente-Rodríguez et al., 2008). It appears that stimulation of bone acquisition may only occur in those with inadequate calcium intakes (Lee et al., 1994).

2.4.1.2 *Calcium intake*

Collectively, calcium and vitamin D contribute between 3% and 10% of bone strength (Schoenau & Frost, 2002). During adolescence, when peak bone mineral accrual occurs, males have calcium accretion rates of approximately 359mg·day^{-1} (Bailey, Martin, McKay, Whiting, & Mirwald, 2000). Based on DXA scans taken over a period of six years,

Bailey (1997) demonstrated that around 36% of total body BMC is accrued in the 4 year period surrounding peak height velocity.

To provide adequate supplies of calcium in bone and maintain bone integrity throughout adulthood, nutrition guidelines recommend a daily calcium intake of 1,300mg·day^{-1} for males 12-18 years of age and 1,000mg·day^{-1} for 19-30 year old males (National Health and Medical Research Council, 2006a). The higher adolescent requirement reflects the large accrual of calcium in bones during the two-year period surrounding PBM. Young adults (18-30 years) do not experience longitudinal growth of the skeleton; however, consolidation of PBM occurs (refer section 2.3.1.1). It is estimated that approximately 10-15% of adult total calcium accretion occurs during this period (Heaney et al., 2000), suggesting that there is an opportunity to maximise skeletal consolidation provided calcium intakes are also optimised during young adulthood. It should be noted, however, that without adequate levels of vitamin D (refer section 2.4.2) calcium will not be optimally absorbed (Heaney, 2008).

Previous meta-analyses show that calcium supplementation with or without vitamin D has a small but positive effect on bone strength and a reduction in fracture risk (Abrahamsen et al., 2010; Chung et al., 2009; Cranney et al., 2007; Lips et al., 2014; Shea et al., 2002; Tang et al., 2007; Winzenberg et al., 2010). However, the majority of studies have used children or exclusively female populations and few studies have examined the effects such supplementation may have on BMD in men.

2.4.2 Influence of vitamin D on bone health

Vitamin D is a complex fat soluble vitamin which has physiological actions similar to hormones. Through a complex process, sunlight converts cholesterols into vitamin D

precursors which are converted via the liver to 25(OH) vitamin D (calcidiol). Parathyroid hormone then stimulates the conversion of calcidiol by the kidney, into the biologically active form of $1,25(OH)_2D_3$ or calcitriol (Higdon & Drake, 2011). Vitamin D facilitates the absorption of calcium through digestion and is essential for optimal bone health in children, ensuring adequate mineralisation of developing bones (Bouillon, Bischoff-Ferrari, & Willett, 2008; McCann & Ames, 2008; Rovner & O'Brien, 2008; Stewart & Rittweger, 2006). In adult populations, deficiency can lead to increased bone turnover and osteoporosis, with severe vitamin D inadequacy resulting in osteomalacia, a condition whereby the ratio of collagenous bone matrix increases as a result of bone demineralisation (Higdon & Drake, 2011).

Vitamin D in both calcidiol and calcitriol forms are required for optimal absorptive functioning (Heaney, 2008). The $1,25(OH)_2D$ form of vitamin D appears to act specifically to regulate serum calcium homeostasis by acting on calcium absorption and also stimulating bone resorption in order to release calcium when required. Conversely, 25(OH)D appears to act solely on calcium absorption, correlating to absorptive efficiency whilst serum $1,25(OH)_2D$ levels do not (Heaney, Barger-Lux, Dowell, Chen, & Holick, 1997). Highlighting the tight interaction between vitamin D, calcium and PTH, it has been demonstrated that (i) PTH levels will be within ideal ranges if vitamin D levels are adequate even with calcium intakes are lower than $800mg \cdot day^{-1}$ and (ii) intakes of calcium over $1,200mg \cdot day^{-1}$ permits a lower serum 25(OH)D in order to maintain PTH levels (Steingrimsdottir, Gunnarsson, Indridason, Franzson, & Sigurdsson, 2005).

Adequate intake levels for vitamin D in Australia is set at 5 $\mu g \cdot day^{-1}$ (200IU) for adult men aged 18-30 years, equating to the amount required to maintain serum

25(OH)D at a level of at least 27.5 nmol·L^{-1} with minimal exposure to sunlight (National Health and Medical Research Council, 2006b). Others recommend serum levels should be maintained at a minimum of 50 nmol·L^{-1} (Osteoporosis Australia Medical & Scientific Advisory Committee, 2014). However, these levels may not be adequate to optimise calcium absorption. In post-menopausal women, it has been demonstrated that calcium absorption effectiveness was between 45% to 65% higher when 25(OH)D were at 86.5 nmol·L^{-1} compared to 50 nmol·L^{-1}; however, levels above 86.5 nmol·L^{-1} did not elicit a greater response (Heaney, Dowell, Hale, & Bendich, 2003). A recent review of vitamin D intakes to optimise all health outcomes recommends serum concentrations of 25(OH)D begin at 75 nmol·L^{-1} and suggests optimal bone health outcomes are achieved between 90 and 100 nmol·L^{-1} (Bischoff-Ferrari, Giovannucci, Willett, Dietrich, & Dawson-Hughes, 2006) and others suggest a minimum of 70 nmol·L^{-1} (Vieth, 2004). This has prompted a revision of the recommended daily intake of vitamin D for all adults to be 1000 IU·day^{-1} (25µg) vitamin D (cholecalciferol) in order to bring 25(OH)D concentrations up to 75 nmol·L^{-1} (Bischoff-Ferrari et al., 2006).

Concerns for vitamin D status in the general population have recently been extended to young athletes (Willis, Peterson, & Larson-Meyer, 2008). Some young athletes are at high risk of vitamin D insufficiency due to lack of sun exposure and/or low energy intake (and therefore low vitamin D intake) in disciplines emphasizing a lean physique. Examples include young people who undertake horse riding, ballet dancing, running or gymnastics. Recent studies conducted in Australia and New Zealand showed that 30 to 70% of male adolescents present with vitamin D insufficiency (25(OH)D < 50 nmol·L^{-1}) (Jones, Dwyer, Hynes, Parameswaran, & Greenaway, 2005; Rockell et al., 2005).

2.4.3 Influence of physical activity on bone health

Mechanical stressors act on bone to illicit a response however, loading must be beyond habitual levels for an osteogenic response to occur (Frost, 1998). For the lower extremities, such as the tibia, a combination of muscle contractions, ground reaction forces, and body mass contribute to bone adaptations (Nikander et al., 2006). Physical activity under varying loading conditions has been shown to have differing impact on the tibia. In athletic pursuits which involve weight-bearing, such as gymnastics, soccer or volleyball, greater distal (5% site) and tibia shaft (50% site) adaptations have been found, whereas non-weight bearing pursuits such as swimming and water polo do not impart greater adaptations compared to controls (Greene, Naughton, Bradshaw, et al., 2012; Nikander et al., 2006). As athletic populations regularly engage in loading it is accepted that additional stimuli such as additional calcium or muscular force is required for (re)modelling to occur.

The radius is considered a load-bearing bone; however, it is not habitually weight-bearing for most individuals. General populations who undertake high levels of physical activity show little difference in bone strength at the radius than those undertaking low levels of habitual physical activity (Duckham et al., 2014). Conversely, elite athletes such as tennis players and young gymnasts who undergo regular impact loads and strain at the radius demonstrate greater bone properties at the radius (Dowthwaite, Kanaley, Spadaro, Hickman, & Scerpella, 2009; Haapasalo et al., 2000; Ireland et al., 2013). In tennis players, the adaptations at the radius are observed to result in a larger bone size, without a concurrent increase in volumetric bone density except at the distal radius where there was a significant positive difference in the dominant arm (Haapasalo et al., 2000).

Similarly, jockeys have been found to demonstrate positive bone effects in the forearm (Greene et al., 2013; Leydon & Wall, 2002). Greater trabecular density at the distal radius and greater bone strength (SSI) at the proximal radius is evident in jockeys using pQCT (Greene et al., 2013). Differences in distal radius aBMD as compared to whole body, spine and hip measures have been observed using DXA (Leydon & Wall, 2002).

2.4.4 Influence of energy restriction on bone health

Bone turnover and bone mass are directly influenced by nutritional habits (Heaney et al., 2000). The interaction of loading and nutrition may ultimately enhance skeletal integrity of active individuals. Osteogenic responses to mechanical loading are typically site specific whereas the influence of diet is more diffuse, acting on the whole skeleton. Physically active individuals experience greater energy expenditure and if this exceeds energy intake, an energy deficit will result. It has been suggested that energy intakes below 125.6 $kJ \cdot kgbw \cdot day^{-1}$ (30 kcal) is insufficient to maintain normal physiological functioning in athletes (Loucks, 2007). Prolonged periods of energy deficit culminate in reduced body weight, altered body composition, a reduction in bone mass and disturbances in endocrine function (Hotta et al., 2000; Hotta, Shibasaki, Sato, & Demura, 1998; Warren et al., 2002).

Bone loss due to increased bone turnover can result from endocrine changes that mobilize stored fuels. Prolonged energy expenditure resulting from physical activity places as a greater demand muscle and liver glycogen (Brooks & Mercier, 1994; Hagerman, 1992). Depletion of glycogen results in an attenuation of insulin release during prolonged physical activity. Coupled with inadequate energy intake, this results in increasing quantities of tissue protein being used as a substrate for gluconeogenesis

(Wagenmakers et al., 1991). A subsequent decrease in plasma levels of insulin and insulin-like growth factor 1 (IGF-1) with a concomitant increase in plasma concentrations of cortisol and growth hormone (GH), exerts a direct effect on the function of bone cells. Osteoblast function is retarded while osteoclast activity is accelerated (De Souza et al., 2008; Ihle & Loucks, 2004).

Female athlete triad is a recognised condition, resulting in reproductive disorders and demineralisation of the skeleton. These athletes demonstrate a number of metabolic and substrate abnormalities including mobilisation of fat stores, declining glucose utilisation, and a reduced metabolic rate (Loucks, 2007). Similar abnormalities have been observed in male athletes in weight-restricted and endurance sports (Loucks, 2004). Disturbances to GH, IGF-1 and testosterone have been observed in male wrestlers preparing for a season of wrestling (Loucks, 2004).

Poor bone health through excessive energy expenditure coupled with restricted energy intake, is typical of a number of athletic populations (Ebeling, 2008; Fredericson et al., 2007; Greene et al., 2013; Nichols & Rauh, 2011; Rector, Rogers, Ruebel, & Hinton, 2008; Smathers et al., 2009). Studies specifically examining males relating to energy deficit and bone health are limited, however male long distance runners and cyclists have displayed compromised bone health (Fredericson et al., 2007; Rector et al., 2008; Smathers et al., 2009). It is suggested this may be due to energy imbalance, as endurance athletes undertake activity that consistently and cumulatively loads their bones. Male endurance runners have similarly low aBMD to female long distance runners (Hind, Truscott, & Evans, 2006). Further, Zanker and Swaine (2000) found markers of bone

formation were suppressed when male distance runners were placed on an energy restricted diet.

2.5 Assessment of bone health

Various methods of assessment are used to examine bone health. Whole bone testing provides the most accurate measure of bone strength, however this method is impractical *in vivo* (Donnelly, 2011). As a result, imaging techniques must be employed to examine bone strength and structural properties. Most frequently, DXA has been considered the 'gold standard' (Lee & Gallagher, 2008), mainly due to low cost and ease of use however, DXA has a number of inherent weaknesses limiting its ability to fully explore bone health (Binkley, Berry, & Specker, 2008). Peripheral QCT provides a three-dimensional assessment of bone properties, allowing for a more detailed analysis of bone health (Sievänen et al., 1998).

2.5.1 Variables examined when determining bone health

Ultimately, bone health is determined by the structural integrity of the bone. This comprises total bone mass, bone geometry, and properties of bone tissue (Donnelly, 2011). Although a number of different factors contribute to bone strength, alterations to bone mineral properties are commonly reported as changes to bone mineral density measured by DXA (aBMD). However, this overall measure has reduced capacity to identify structural changes occurring within the bone (Donnelly, 2011). Changes in bone geometry caused through modelling and remodelling have transient or permanent effects on bone health. For example, alterations to cortical thickness and total cross-sectional area result from modelling whilst vBMD is a function of both modelling and remodelling (Binkley et al., 2008). In the short term, it may be that changes due to remodelling resulting in

modifications to bone geometry play a greater role than alterations to bone mass (Heaney & Weaver, 2005). Bone mineral density is determined by both mass and volume and these differ in trabecular and cortical compartments of the bone (Rauch & Schoenau, 2001).

2.5.2 Dual energy x-ray absorptiometry (DXA)

Studies examining bone density have predominantly used DXA to assess bone health. In clinical populations, DXA is widely used for diagnosing osteoporosis and osteopenia; however, concerns regarding technical limitations of DXA-based measurements, particularly the assessment of aBMD, have been highlighted in recent years (Faulkner, 2000; Lee & Gallagher, 2008; Schoenau, Neu, Beck, Manz, & Rauch, 2002; Seeman, 2002). Dual energy x-ray absorptiometry measures BMC in grams and bone area (cm^2) which are then combined to approximate 'areal' BMD in grams·cm^2. The planar two-dimensional assessment capabilities of DXA present difficulties in accurately scanning a three dimensional bone structure. Attempts to adjust aBMD in order to reflect volumetric BMD have been made, including bone mineral apparent density (BMAD) for the femoral neck and spine (Binkley et al., 2008). Although bone length and width can be measured, depth can only be estimated by DXA technology. An increase in "density" may be due to greater bone size and not necessarily an increase in mass per unit volume of bone (Seeman, 2002). Concerns about bone size are most relevant when research involves participants of varying size or where bone size may change rapidly during a study, such as studies of growing children and adolescents (Haapasalo et al., 2000). Areal BMD is therefore size dependent, particularly in children. In addition, DXA is unable to differentiate between cortical and trabecular bone and due to its two dimensional nature, bone size and shape is difficult to quantify with acceptable accuracy (Khan et al., 2001).

Small alterations in the distribution of bone mass or bone structure may have considerable impact on bone strength without altering overall BMD (Nikander et al., 2010)

2.5.3 Peripheral quantitative computed tomography (pQCT)

Unlike DXA, pQCT is a three dimensional imaging device capable of assessing bone size, strength and geometry (Khan et al., 2001). Specifically, pQCT is able to differentiate between trabecular and cortical bone and determine endosteal and periosteal circumferences. The technology provides a measure of vBMD in the peripheral skeleton, and quantifies cross sectional area (CSA) of bone. Furthermore, pQCT provides surrogate measures of bone strength: strength-strain index (SSI) and bone strength index (BSI) (Binkley et al., 2008). The SSI combines bone geometry with properties of cortical bone (cortical vBMD) and has been validated in both animal and human studies (Rauch & Schoenau, 2008). Research shows that up to 80% of low-trauma fractures occur in normal or osteopenic, not osteoporotic, individuals indicating it may be structure, not necessarily BMD, that is the cause for weakness (Nikander et al., 2010). Thus, pQCT allows for a more detailed assessment of bone properties and is an important assessment tool for estimating fracture risk.

Despite pQCT's superior ability to differentiate between trabecular and cortical bone compartments and its ability to precisely examine bone geometry, limitations derived from *in vitro* and *in vivo* precision studies exist (Augat, Gordon, Lang, Iida, & Genant, 1998; Grampp et al., 1995; Sievänen et al., 1998). A lack of spatial resolution prevents the precise identification of areas where a thin cortical rim of bone exists, such as at the ultra-distal radius. Standard geometric analysis assigns the outer 55% of the

radial cross-section as “cortical and subcortical” bone and the remaining area is considered trabecular bone. As actual cortical thickness in adults is considerably smaller, the percentage of CSA consisting of cortical bone is much lower than 55%. Determining cortical vBMD at the ultra-distal region appears subject to analysis imprecision (Neu, Manz, Rauch, Merkel, & Schoenau, 2001).

2.6 Other indicators of bone health

Calcium supplementation appears to have a transient effect on bone remodelling producing reductions in bone fragility well before augmentation of bone mass can be measured (Heaney & Weaver, 2005). Biochemical markers of bone turnover provide a means of detecting and monitoring osteoblastic and osteoclastic activity in the absence of, and complimentary to, BMD data (Vasikaran, Eastell, Bruyere, et al., 2011). These include blood-borne markers of bone turnover and vitamin D status.

2.6.1 Blood borne markers of bone turnover

To date, multiple biochemical markers have been used for clinical and research purposes with limited consistency. Further, rather than identifying bone formation and bone resorption separately, some markers may reflect both, and most are present in other tissue besides bone (Delmas, Eastell, Garnero, Seibel, & Stepan, 2000). Each marker has its benefits and inherent weaknesses. Consequently, the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) have recommended that one standard marker be used for each bone formation and resorption for clinical studies: serum procollagen type 1 N propeptide (s-P1NP) for bone formation and serum C-terminal telopeptide of type I collagen, (s-CTx) for bone resorption (Vasikaran, Eastell, Bruyere, et al., 2011).

Procollagen type 1 N propeptide is cleaved from type 1 collagen molecules during the process of incorporating collagen into the bone matrix while CTx is released when collagen within the bone is broken down (Szulc, Kaufman, & Delmas, 2007). Gender differences are apparent in P1NP and CTx levels, with males having higher levels of bone metabolism markers than females between 20-40 years due to persistent bone activity in the long bones (Delmas et al., 2000). Circadian rhythms are also known to affect bone turnover markers, peaking between 2am and 6am and reaching their lowest points between 1pm and 11pm. CTx levels may be twice as high at the peak compared to the lowest levels, whereas P1NP may vary by around 20%, highlighting the need to regulate blood collection times (Delmas et al., 2000). Serum CTx is also influenced by renal and kidney function and diet (Vasikaran, Eastell, Bruyere, et al., 2011). Exercise may also effect bone turnover markers through both acute and chronic effects although this is highly dependent on the age of participants and type of activity (Delmas et al., 2000; Rantalainen et al., 2009; Vasikaran, Eastell, Bruyere, et al., 2011).

Serum levels of both CTx and P1NP for male cohorts aged between 20-29 have been reported, averaging 528 (453–676) ng/mL for CTx and 61 (45–78) μg/L for P1NP respectively (Jenkins et al., 2013). These levels compare to levels reported in French men aged 19-30 of 600 ng/mL for CTx and 74 ng/ml for P1NP (Szulc, Garnero, Munoz, Marchand, & Delmas, 2001).

2.6.2 Serum vitamin D

It is recommended that vitamin D status be assessed using serum 25(OH)D as this measure accounts for both subcutaneous and dietary sources of the vitamin (National Health and Medical Research Council, 2006b). Current classifications of deficiency for

Australian adults are defined as: mild (25 and 50 nmol·L^{-1}); moderate (12.5 and 25 nmol·L^{-1}) and, severe (<12.5 nmol·L^{-1}) (Diamond et al., 2005). As discussed in section 2.4.2, recommendations regarding the optimal range of serum 25(OH)D are equivocal. Levels of 25(OH)D are currently recommended to be around 50 nmol·L^{-1} (Osteoporosis Australia Medical & Scientific Advisory Committee, 2014) with others calling for minimum recommendations to be as high as 90 and 100 nmol·L^{-1} to achieve optimal bone health outcomes (Bischoff-Ferrari et al., 2006; Dawson-Hughes et al., 2005). When concentrations of 25(OH)D concentrations are in the deficient range, serum PTH levels are inversely proportional to 25(OH)D levels, and can therefore also be a valuable indication of inadequate vitamin D status.

2.7 Jockeys: current literature

2.7.1 Bone health assessment of jockeys

A number of studies have examined the bone health of jockeys (see Table 2-1). With the exception of the study by Greene et al. (2013), all studies have utilised DXA to assess bone health. Previous studies have demonstrated that jockeys have compromised bone health, with smaller, flat jockeys having inferior bone health compared to larger jockeys. Evidence of osteopenia in whole body, spine and hip measures were found in apprentices, flat and national hunt jockeys (Dolan, McGoldrick, et al., 2012; Leydon & Wall, 2002; Warrington et al., 2009). One criticism of DXA technology is the underestimation of aBMD in smaller individuals. This is predominantly of concern where height is not controlled for, or in longitudinal studies where growth may be a factor. While height may be a limitation of the DXA outcomes reported in previous jockey research (see Table 2-1), all of the studies either used subjects of similar stature or controlled for differences in body size. Using pQCT, Greene et al. (2013) found evidence

of compromised bone health in jockeys compared to age- and gender-matched controls. Specifically, apprentice jockeys displayed reduced BMC, cortical area and bone strength at the distal tibia and radius compared to controls. While DXA and pQCT outcomes are not directly comparable, jockeys assessed by both Leydon and Wall (2002) and Greene et al. (2013) demonstrated positive bone effects at the forearm. Leydon and Wall (2002) reported a significantly positive difference in distal wrist aBMD as compared to whole body, spine and hip measures while Greene et al. (2013) found greater trabecular density at the distal radius and greater bone strength (SSI) at the proximal radius in jockeys compared to controls. It is postulated that muscular forces incurred at the radius during riding may be in excess of common habitual loads and therefore provide a positive osteogenic benefit.

2.7.2 Assessment of markers of bone turnover in jockeys and vitamin D status

Typically, jockeys have high bone turnover (Dolan, McGoldrick, et al., 2012; Waldron-Lynch et al., 2010; Wilson, Fraser, et al., 2013) linked to low energy and calcium intakes. Examination of CTx levels in jockeys have found them to be low but within normal reference ranges (bottom 5^{th} percentile for age) (Jenkins et al., 2013; Wilson, Fraser, et al., 2013). This contrasts with other findings showing elevated levels of bone resorption urinary cross linked N-telopeptides of type 1 collagen (NTx) and free deoxypyridinoline crosslinks (fDPD) (Dolan, McGoldrick, et al., 2012; Waldron-Lynch et al., 2010). High levels of serum P1NP have been found by previous research examining jockey bone turnover (Dolan, McGoldrick, et al., 2012; Waldron-Lynch et al., 2010).

Previous investigations of serum vitamin D levels in young male jockeys highlight 70% of riders being vitamin D deficient (serum (25(OH)D < 25 $nmol \cdot L^{-1}$) in winter

(Guillemant et al., 2001; Wilson, Fraser, et al., 2013). Jump and flat jockeys from the United Kingdom recorded serum levels of 25(OH)D averaging 35 $nmol \cdot L^{-1}$ and 38 $nmol \cdot L^{-1}$ during winter (Wilson, Sparks, et al., 2013). In another study, jockeys sampled during winter had serum 25(OH)D levels below 50 $nmol \cdot L^{-1}$ whilst those measured in the summer exceeded 90 $mmol \cdot L^{-1}$ (Waldron-Lynch et al., 2010).

Table 2-1: Existing literature examining bone mineral density in jockeys

Study	Subjects	Gender	No of subjects	Age (mean)	Weight (mean)	Height (mean)	Scan equipment	Sites scanned	Outcomes (mean value)	Comments
Leydon and Wall (2002) (New Zealand)	Apprentices (a) Seniors (s) All flat racing	Male & Female	2 (a); 4 (s) 9 (a); 5 (s)	20.5 (a) 28.7 (s)	49.6 (a) 51.3 (s)	157.5 (a) 158.7 (s)	DXA	Whole body Lumbar spine Hip Distal wrist	Reported for all jockeys combined: T-scores Whole body: -1.80 ± 0.76 Lumbar spine: -0.36 ± 1.0 Hip: -0.54 ± 1.1 Distal wrist: 0.26 ± 1.0	Overall 44% of jockeys classified as osteopenic, 60% of apprentices and 25% of seniors. 2 males osteopenic (33%) Actual values not reported
Warrington et al. (2009) (Ireland)	Flat (f) National Hunt (h)	Male	17 (f) 10 (h)	26.7 (f) 28.3 (h)	53.1 (f) 66.2 (h)	1.60 (f) 1.73 (h)	DXA	Whole body Lumbar spine Hip	aBMD($g \cdot cm^2$): Whole body: 1.05 ± 0.07 (f) 1.21 ± 0.06 (h) Lumbar spine: 1.12 ± 0.11 (f) 1.22 ± 0.15 (h) Hip: 0.99 ± 0.1 (f) 1.08 ± 0.13 (h)	Whole body: flat jockeys 53% osteopenia, 12% osteoporotic; hunt jockeys 10% osteopenia Hip: flat jockeys 41% osteopenia, hunt jockeys 20% osteopenia Spine: flat jockeys 35% osteopenia; hunt jockeys 40% osteopenia
Hitchens et al. (2011) (Australia)	Jockeys (j) Track-work riders (t)	Male & Female	5 (j) 6 (t)	27 (j) 36 (t)	51.7(j) 65.9 (t)	163.4 (j) 168.2 (t)	DXA	Whole body	aBMD($g \cdot cm^2$): 1.157 ± 0.07 (j) 1.312 ± 0.10 (t)	T-scores not reported
Dolan, Crabtree, et al. (2012) (Ireland)	Flat (f) National Hunt (h) Boxers (b) Control (c)	Male	14 (f) 16 (h) 14 (b) 14 (c)	25 (f) 25 (h) 21 (b) 23 (c)	54.6 (f) 64.3 (h) 65.3 (b) 69.2 (c)	1.65 (f) 1.72 (h) 1.74 (b) 1.79 (c)	DXA	Whole body Lumbar spine Hip	aBMD($g \cdot cm^2$): Whole body: 1.09 ± 0.6 (f) 1.17 ± 0.05 (h) 1.29 ± 0.1 (b) 1.26 ± 0.06 (c) Lumbar spine: 1.10 ± 0.09 (f) 1.15 ± 0.1 (h) 1.48 ± 0.16 (b) 1.26 ± 0.14 (c) Hip: 1.05 ± 0.07 (f) 1.07 ± 0.11 (h) 1.25 ± 0.11 (b) 1.19 ± 0.5 (c)	Authors utilised equations in order to calculate an apparent volumetric bone density that DXA does not provide. This was done to determine whether jockeys physical size had an effect on bone density as compared to other populations. The findings indicated that irrespective of measure used, jockeys displayed lower bone mass than boxers or control group.

Study	Subjects	Gender	No of subjects	Age (mean)	Weight (mean)	Height (mean)	Scan equipment	Sites scanned	Outcomes (mean value)	Comments
Dolan, McGoldrick, et al. (2012) (Ireland)	Mixed group of jockeys (j) Control (c)	Male	20 (j) 20 (c)	25.9 (j) 23.9 (c)	61.1 (j) 69.5 (c)	1.7 (j) 1.78 (c)	DXA	Whole body Lumbar spine Hip	aBMD($g \cdot cm^2$): Whole body: 1.134 ± 0.05 (j) 1.27 ± 0.06 (c) Lumbar spine: 1.11 ± 0.08 (j) 1.28 ± 0.12 (c) Hip: 1.06 ± 0.09 (j) 1.15 ± 0.13 (c)	Blood and urine markers of bone turnover were also included in the study. Findings indicated: (i) jockeys had lower aBMD than controls but when adjusted for height, jockeys have wider bones (ii) blood and urine markers indicate an elevated rate of bone loss in jockeys.
Greene et al. (2013) (Australia)	Apprentice jockeys (j) Controls (c)	Male & Female	25 (J) 25 (c)	20.2 (j) 20.1 (c)	48.9 (j) 72.7 (c)	155.7 (j) 168.6 (c)	pQCT	Tibia Radius	Tibia: Cortical area (mm^2) 66 % proximal 311.4 (40.5) (j) 351.6 (66.9) (c) SSI (mm^3) 4 % distal 1 683.3 (123.7) (j) 1 998.1 (117.5) (c) 66 % proximal 1 953.9 (326.8) (j) 2 501.8 (481.6) (c) Radius: Cortical area (mm^2) 66 % proximal 81.4 (16.7) (j) 82.4 (17.2) (c) SSI (mm^3) 4 % distal 391.9 (69.4) (j) 423.4 (75.3) (c) 66 % proximal 297.3 (88.3) (j) 261.2 (81.9) (c)	Overall results indicate that jockeys have reduced bone density compared to controls, with the exception of trabecular density at the distal radius and a higher stress strain index at proximal radius.

2.7.3 Energy intake of jockeys

Jockeys show evidence of disordered eating characteristics and unhealthy weight loss practices in order to achieve race weight (Cotugna, Snider, & Windish, 2011; Dolan et al., 2011; Greene et al., 2013; Leydon & Wall, 2002; Moore et al., 2002). Table 2-2 shows dietary analyses of daily energy intake and bone related micronutrients.

Table 2-2: Dietary intake for flat and jump jockeys

Author	Jockey	Weight (kg)	Total Energy ($kg \cdot day^{-1}$)	Est. energy ($kj \cdot kgbw \cdot day^{-1}$)	Calcium ($mg \cdot day^{-1}$)	Dietary Vitamin D ($\mu g \cdot day^{-1}$)
Dolan et al. (2011)	Flat (m) Jump (m)	53.1 ± 4.1 66.2 ± 2.9	7012 ± 1824 8462 ± 2979	132.1 127.8	619 ± 295 (total)	1.5 ± 0.8 (total)
Greene et al. (2013)	Flat (m & f)	48.9 ± 2.8	7516 ± 2272	153.7	775 ± 68.4	NR
Leydon and Wall (2002)	Flat (m & f)	52.8 ± 2.4 (m) 49.3 ± 3.4 (f)	6359 ± 1671	124.6	449 ± 158	NR
Waldron-Lynch et al. (2010)	Flat and Jump (m)	NR	7369 ± 1184	n/a	541 ± 106	1.4 ± 0.8
Wilson, Fraser, et al. (2013)	Flat (m) Jump (m)	56.1 ± 2.9 65.3 ± 2.5	6111 ± 1,250 7470 ± 830	108.9 114.4	557 ± 240 758 ± 193	1.6 ± 1.6 2.3 ± 1.6

* Values shown as mean ± SD; kgbw: kilograms of body weight; f: female; m: male; NR: not reported.

Total energy intake consistently reveals estimates well below estimated daily requirements with reported intakes ranging between 6,111 ± 1,671 kJ and 7,516 ± 2,272 kJ per day. Recommended daily minimum energy intakes of approximately 188-210 $kj \cdot kgbw \cdot day^{-1}$ are prescribed for athletes (Sundgot-Borgen & Garthe, 2011), whilst jockeys average between 108 $kj \cdot kgbw \cdot day^{-1}$ and 153 $kj \cdot kgbw \cdot day^{-1}$. Jockeys spend approximately 4 hours per day (24.6 hrs per week with an assumed day off) training (Greene et al., 2013) in activities involving riding horses for exercise and fast paced work, cleaning stables, washing and feeding horses (Wilson, Sparks, et al., 2013). There is some inference that a habitually low energy intake may result in a suppressed metabolic rate (Dolan et al.,

2011), however the physical and energy demands of horse racing and training remain under investigated.

In addition to severe energy intake restrictions, jockeys habitually undertake a variety of dehydration practices in order to make weight such as exercising to sweat, saunas and vomiting (Cotugna et al., 2011; Dolan et al., 2011; Moore et al., 2002). Researchers have reported moderate levels of dehydration on non-race days (1.022+0.005 Usg), increasing on race days to 1.028+0.005 Usg, with over 50% of jockeys competing in a severely dehydrated state (Usg>1.030) (Warrington et al., 2009).

2.7.3.1 *Calcium and vitamin D intake*

Average calcium intake for jockeys have been found to range between 450 $mg \cdot day^{-1}$ and 775 $mg \cdot day^{-1}$ (refer Table 2-2) well below nutritional guidelines (National Health and Medical Research Council, 2006a). Dietary intake of vitamin D ranged between 1.4 $\mu g \cdot day^{-1}$ to 2.3 $\mu g \cdot day^{-1}$ being reported in some UK based jump jockeys. Again, this is well below minimums of 5 $\mu g \cdot day^{-1}$ which may also be insufficient in the absence of adequate sunlight (refer section 2.4.2).

2.7.4 Energy expenditure

Assessment of energy balance in jockeys has been difficult to achieve to due numerous safety and regulatory restrictions with the sport of horse racing. Standard equipment used to assess energy expenditure has not been permitted in research during race conditions (Wilson et al., 2014).

Early studies examining energy expenditure of experienced equestrian riders has focussed on oxygen uptake, which has been shown to vary with horse gaits (Devienne &

Guezennec, 2000; Westerling, 1983). Rider VO_2 is seen to vary between 0.5 $L \cdot min^{-1}$ when the horse is walking to 1.9 $L \cdot min^{-1}$ when cantering, representing a maximum of approximately 75% VO_{2max} (Devienne & Guezennec, 2000). Work by Trowbridge et al. (1995) examined heart rate and blood lactate levels of National Hunt jockeys who typically race up to six races per day. Heart rates were seen to range between 136 and 188 $beats \cdot min^{-1}$ in the first race of the day and remained elevated above resting levels at the commencement of each subsequent race. Recent work by Hitchens et al. (2011) compared VO_{2max} in a group of Australian jockeys and track-work riders. Results showed jockeys had a mean VO_2 of 48.55$ml \cdot kg^{-1} \cdot min^{-1}$ compared to 43.18$ml \cdot kg^{-1} \cdot min^{-1}$ for track-work riders. The study, however, was conducted in laboratory conditions and does not give an accurate indication of oxygen consumption during races or track work. Results are comparable to Westerling (1983) who found mean VO_{2max} of approximately 43.8$ml \cdot kg^{-1} \cdot min^{-1}$ using an ergometer.

Wilson, Sparks, et al. (2013) simulated race conditions for a group of National Hunt jockeys using a mechanical "push" horse to estimate energy expenditure during race time using respiratory gas analysis and a commercial heart rate monitor (HRM). Energy expenditure for a typical day (24hr) was measured using a HRM, while energy intake was recorded using 7-day self-reported food record diaries. Findings suggest that energy expenditure is approximately 1,800kJ per race and typical non-race energy expenditure is approximately 11,260 $kj \cdot day^{-1}$. Energy intake was found to be approximately 7,240$kj \cdot day^{-1}$ which is in agreement with other studies that examined energy intake (Dolan et al., 2011; Leydon & Wall, 2002). National Hunt jockeys, however, are typically around 10kg heavier than apprentice and flat jockeys and the race style differs from flat racing.

2.8 Conclusion

The review of literature indicates bone health is influenced through a combination of factors: mechanical stressors which influence bone modelling and remodelling; adequate energy intake; and adequate vitamin and mineral intakes. It is apparent that sufficient calcium is required in order to maintain bone integrity and that this is facilitated by vitamin D. However, at the present time there are equivocal recommendations in relation to vitamin D status and bone health.

The literature to date indicates jockeys have compromised health status in a number of areas related to prolonged inadequate energy intake as a result of the weekly cycles of making weight in order to compete. Jockeys have consistently been shown to have inadequate energy intakes with calcium intakes well below adequate levels. Whilst findings to date pertaining to the bone health status of jockeys shows compromised bone health, research has heavily relied on DXA technology which has inherent weaknesses, particularly in relation to body size and aBMD. Further, numerous methods of assessing bone turnover markers have been utilised, leaving inconsistencies in interpretation.

3 The Effect of Calcium or Calcium and Vitamin D Supplementation on Bone Mineral Density in Healthy Males: A Systematic Review and Meta-analysis

As published in: *International Journal of Sport Nutrition and Exercise Metabolism*, (2015) vol. 25 (5) pp. 510-524. Authors: Silk, L.N, Greene, D.G and Baker, M.K.

3.1 Abstract

Research examining the preventative effects of calcium and vitamin D supplementation has focused on children and females, leaving the effects on male bone mineral density (BMD) largely unexplored. Thus, the aim of this systematic review and meta-analysis is to examine the efficacy of calcium supplementation, with or without vitamin D for improving BMD in healthy males. Medline, EMBASE, SPORTDiscus, Academic Search Complete, CINHAHL Plus and PubMed databases were searched for studies including healthy males which provided participants calcium supplementation with or without vitamin D and used changes to BMD as the primary outcome measure. Between trial standardised mean differences of percentage change from baseline in BMD of femoral neck, lumbar spine, total body and total hip sites were calculated. Nine studies were included in the systematic review with six references totalling 867 participants contributing to the meta-analysis. Significant pooled effects size (ES) for comparison between supplementation and control groups were found at all sites included in the meta-analysis. The largest effect was found in total body (ES=0.644; 95% CI=0.406 to 0.883; p=0.000), followed by total hip (ES=0.483, 95% CI= 0.255 to 0.711, p=0.000), femoral neck (ES=0.402, 95% CI=0.233 to 0.570, p=0.000) and lumbar spine (ES=0.306, 95% CI=0.173 to 0.440, p=0.000). Limited evidence appears to support the use of calcium and vitamin D supplementation for improving BMD in older males. There is a need for high quality randomised controlled trials, especially in younger and middle-aged male

cohorts and athletic populations to determine whether supplementation provides a preventative benefit.

KEYWORDS: Musculoskeletal, Randomised controlled trials, prevention

3.2 Introduction

Osteoporosis is commonly associated with females, remaining an under-diagnosed and under-treated disorder men (Briot et al., 2009). The incidence of osteoporosis varies by world-wide geographical regions with higher rates in developed nations, such as the USA, Europe and Australia (Dhanwal, Dennison, Harvey, & Cooper, 2011). The primary outcome of osteoporosis is fragility fractures. Globally, the residual lifetime risk of osteoporotic fracture for males is currently estimated to be 27% (Cooley & Jones, 2001). In 2000, 39% of total fragility fractures and approximately 33% of all hip fractures occurred in men (Ebeling, 2014; Johnell & Kanis, 2006). Mortality rates for male fragility fractures are two to three times higher than seen in females (Briot et al., 2009). Whilst ageing is the primary cause of bone weakness in males, common secondary causes include inadequate vitamin D and calcium intakes (Ebeling, 2008). It is therefore worthwhile exploring the potential benefits of calcium and vitamin D supplementation on bone density in healthy adult males as a primary prevention strategy, prior to the onset of osteoporosis.

Male fracture incidences have two peaks, one occurs during the ages of 15-45 years and the other after 70 (Briot et al., 2009). Low body mass index (BMI) and excessive exercise, as often found in athletes, also contribute to osteoporosis (Ebeling, 2008). Studies on male athletic populations have demonstrated that non-weight bearing physical activity can have a negative impact on bone density (Smathers et al., 2009). Low BMD has

been observed in both weight and non-weight bearing male athletic populations such jockeys, cyclists and endurance runners (Fredericson et al., 2007; Greene et al., 2013; Nichols & Rauh, 2011; Rector et al., 2008; Smathers et al., 2009)

The recommended dietary intakes for calcium focus on maximizing bone mass accretion during adolescence and minimising bone loss during ageing (Bachrach, 2001). Vitamin D is essential for optimal bone health, ensuring adequate mineralisation of bones (Higdon & Drake, 2011). Without adequate levels of vitamin D, calcium absorption is limited to around 12.5% of dietary intake (Aloia et al., 2010). The combination of weight-bearing exercise, adequate dietary calcium and vitamin D intakes, as well as appropriate sun exposure to ensure vitamin D levels maintain or improve BMD are not always practical or possible, especially for athletic populations. These individuals already undertake high amounts of physical activity and may have dietary restrictions related to competition. Supplementation is recommended for improving bone health for populations unable to meet daily recommended intakes of calcium and vitamin D through diet alone (Rizzoli et al., 2008).

Previous meta-analyses show that calcium and vitamin D supplementation has a small but positive effect on bone strength and a reduction in fracture risk (Abrahamsen et al., 2010; Chung et al., 2009; Cranney et al., 2007; Lips et al., 2014; Shea et al., 2002; Tang et al., 2007; Winzenberg et al., 2010). However, the majority of studies have used children or exclusively female populations and few studies have examined the effects of calcium supplementation, with or without vitamin D on BMD in men. The aim of this systematic review and meta-analysis is to examine the efficacy of calcium and vitamin D

supplementation on improving bone mineral density in otherwise healthy males across the adolescent and adult lifespan.

3.3 Methods

3.3.1 Eligibility criteria for study inclusion/exclusion

Studies that were trials of calcium or calcium and vitamin D supplementation without an exercise intervention conducted with healthy males aged 16 years and over as participants were eligible for inclusion. Athletes were included provided the study did not require additional exercise beyond habitual training or exercise. Participants were required to be healthy, showing no signs of osteoporotic fractures and not taking any medications that would influence bone metabolism.

All studies required a minimum of baseline and post-intervention bone density measurements in the form of areal or volumetric BMD or BMC as measured by DXA, ultrasound, QCT or peripheral quantitative computed tomography (pQCT). Studies must have provided participants with calcium supplementation, or a combination of calcium and vitamin D supplementation in the form of supplement, dietary interventions or both for a minimum of six months. Trials which included an exercise intervention were included in the analysis provided they also included a non-exercise supplementation group in addition to a placebo or control group. In these studies, the exercise intervention groups were excluded from analysis.

3.3.2 Data Sources

A comprehensive, systematic search was conducted on 16 April 2013 for manuscripts, using Ovid MEDLINE 1946 to April week 2 2013, Ovid EMBASE 1974 to 2013 April 15, EBSCO SPORTDiscus, Academic Search Complete, CINHAHL Plus and PubMed

databases. Searches were conducted, without language restrictions, using the following four groups of keyword terms for Ovid: (i) Calcium, calcium carbonate, calcium citrate, caltrate, Ca2+, calcium adj 3 diet, calcium supplement, (ii) vitamin D, vitamin D2, vitamin D3, 25-OH, 1, 25-OH, calcitriol, cholecalciferol, ergocalciferol, (iii) bone adj 5 density, bone mineral density, BMC, BMC, BMD, aBMD, vBMD , (iv) men , male, adolescent adj 4 male, male adj 4 athlete. Adjustments made to the Boolean phrases for the EBSCO searches. Terms in each group of keywords were combined with OR and the final four groups were combined with AND. All titles were then manually searched and review articles were examined for further references for possible inclusion in the review.

3.3.3 Design

3.3.3.1 *Data extraction and synthesis*

Studies selected for inclusion into the review were assessed for quality based upon a modified Delphi list (Verhagen et al., 1998). Quality criteria extracted included reporting of eligibility criteria, randomisation, allocation concealment, blinding of treatment, compliance and drop-out rates, power calculations, type of analysis undertaken and statistical analysis.

Two reviewers (LS, DG) developed the review protocol and determined inclusion and exclusion criteria. The articles extracted were independently assessed by two reviewers (LS, DG) for potential inclusion into the review. A third reviewer (MB) was available if consensus on inclusion could not be reached. Data relating to: age, baseline calcium and vitamin D intakes and /or serum levels, type and dosages of calcium and/or vitamin D supplements given, compliance rates, baseline BMC and/or BMD and

percentage change from baseline were extracted for analysis. Where data was incomplete, authors were contacted for further information if possible.

3.3.3.2 *Outcome measures*

Changes to BMD were used as the outcome measure for this review. Data was extracted on BMC, aBMD and vBMD yielding data on 8 different measurement sites. Sufficient pre to post change bone mineral data was available to conduct meta-analysis on the following sites: lumbar spine, femoral neck, total hip, and total body.

3.3.4 Statistical analysis

Between-trial standardised mean differences, or effect size (ES) were calculated using Hedges *g* and 95% confidence interval at each site where there was sufficient data. Variability between studies was examined using the I^2 measure of inconsistency to provide an indication of how much variability between studies was due to heterogeneity rather than chance. Funnel plot asymmetry was not assessed as there were fewer than 10 studies in the meta-analysis (Sterne et al., 2011). No meta-regression was performed, due to the limited number of studies available.

3.3.5 Meta-analyses

Pooled estimates of the effect of supplementation on bone mineral density at the femoral neck, lumbar spine, total body and total hip, using the percentage change from baseline as the outcome measure, were obtained using a random-effects model. Analysis of these site was also conducted excluding the young age cohort (Prentice et al., 2005). Sub-group analysis examining: duration of study less than or greater than one year; low baseline calcium intake (defined as below 1000mg·day^{-1}); Participants' age, and whether the study included vitamin D were conducted to determine whether any of the factors

have had an effect on supplementation. This was limited to the lumbar spine site due to sufficiency of data. One study had more than one supplement group (Reid, Ames, Mason, & et al., 2008). In this instance, only control and the higher supplement groups were included in the meta-analysis. All analyses were conducted using Comprehensive Meta-Analysis v2 (Biostat Inc, Englewood, NJ, USA).

3.4 Results

3.4.1 Study inclusion/exclusion

The process for study inclusion is shown in Figure 3-1. The initial search strategy yielded a total of 3930 references after duplicates were removed of which 3898 were excluded during the initial screening of the titles and abstracts. Thirty-two articles were included for a full text review, of which 11 references to 9 studies were included in the systematic review. Two studies presented results for QCT and DXA in separate papers although participants were either a sub-set of the initial study (Daly, Bass, & Nowson, 2006) or the same participants after an extension of the study period (Kukuljan et al., 2011). These were not included in the meta-analysis. The main reason studies were excluded was that the participants were too young (n=6). Other reasons for exclusion were: no bone measures/ no post-study measures (n=2), participants had osteoporotic fractures (n=2), conference abstract only (n=3), or study included exercise intervention with no control group (n=1). Two studies (Dawson-Hughes, Harris, Krall, & Dallal, 1997; Peacock et al., 2000) included both males and females; however, the results were reported separately allowing inclusion of the male data in this analysis. One study (Kukuljan, Nowson, et al., 2009; Kukuljan et al., 2011) included exercising groups as well as supplementation and control groups. Only the supplementation and control groups

have been included in this analysis. Additional data was provided from one author (Reid et al., 2008). Three studies were analysed for study quality; however, they were excluded from the subsequent meta-analysis due to inadequate reporting of final results and/or the absence of a control group (Barry & Kohrt, 2008; Klesges et al., 1996; Peacock et al., 2000). This resulted in 6 studies including 867 participants being included in the meta-analysis.

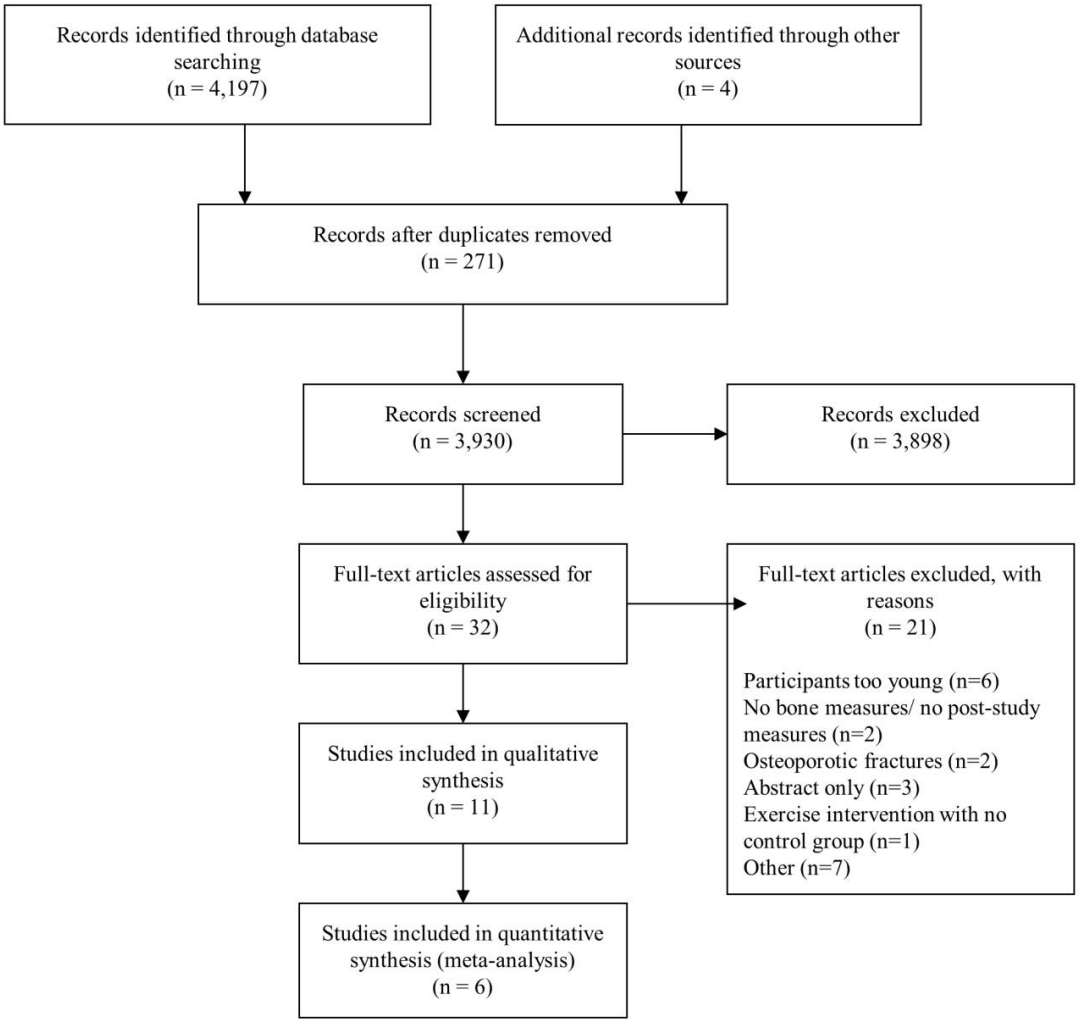


Figure 3-1: Study inclusion flow diagram.
(Moher, Liberati, Tetzlaff, Altman, & Group, 2009)

3.4.2 Study quality/Bias risk

Studies were assessed for quality on a range of criteria which is presented in Table 3-1. With the exception of one study (Klesges et al., 1996), treatment allocation was randomised with the majority of studies using computer generated randomisation sequence for allocation into groups. Five studies were conducted as double-blind, placebo controlled studies although the blinding process was not fully described. Two studies used a non-placebo control group (Daly, Brown, Bass, Kukuljan, & Nowson, 2006; Kukuljan, Nowson, et al., 2009) and two studies used varying amounts of supplementation without a control group (Barry & Kohrt, 2008; Klesges et al., 1996). Results were complete in all but two studies (Klesges et al., 1996; Peacock et al., 2000); however, not all studies reported bone measurements at all data points. Analysis was evenly divided between intention to treat and per protocol. With the exception of two studies (Barry & Kohrt, 2008; Klesges et al., 1996) compliance rates, drop-out rates and missing data were reported. Based on study quality and/or duplication of cohorts, 5 trials were eliminated from the subsequent meta-analysis (as indicated in Table 3-1).

3.4.3 Participant characteristics

Extracted participant characteristics are shown in Table 2. Participants included in all studies were considered to be healthy male adults without evidence of osteoporotic incidence. Exclusions were related to known osteoporotic fractures; bone related diseases or treatments which affect bone metabolism; previous calcium/vitamin D supplementation usage; smoking and alcohol intake. Subjects ranged in age from adolescent males (16 to 18 years) through to elderly men (67 to 84 years). The majority of subjects fell within an age range of 42 to 70 years of age. One study specifically recruited males over 65 years of age (Dawson-Hughes et al., 1997). In the majority of studies, the

participants' average ages were between 55 and 63 years. Baseline calcium intakes ranged from a low of 629mg/d to 1159mg/d. Serum vitamin D concentrations were not reported for two studies (Dawson-Hughes et al., 1997; Prentice et al., 2005). Reporting of baseline vitamin D levels varied between serum concentrations and intake, with studies reporting serum vitamin D concentrations ranging from 52 to 85.7nmol/L.

Table 3-1: Study Quality

Author	Orwoll, Oviatt, McClung, Deftos, and Sexton (1990)	Klesges et al. (1996)	Dawson-Hughes et al. (1997)	Peacock et al. (2000)	Prentice et al. (2005)	Daly, Brown, et al. (2006)	Daly, Bass, et al. (2006)	Barry and Kohrt (2008)	Reid et al. (2008)	Kukuljan, Nowson, et al. (2009)	Kukuljan et al. (2011)
Eligibility criteria specified	●		●	●	●	●	●	●	●	●	●
Baseline characteristics reported	●	●	●	●	●	●	●	●	●	●	●
Treatment allocation randomised	●		●	●	●	●	●	●	●	●	●
Double blinded?	●		●	●	●				●		
Placebo/ control group	●		●	●	●	●	●		●	●	●
Power calculations	●				●				●		
Full results reported	●		●	●	●	●	●	●		●	●
Type of Analysis	PP	PP	ITT	ITT	ITT	PP	PP	PP	ITT	ITT	ITT
Missing data accounted for	●		●	●	●	●	●		●	●	●
Compliance reported			●	●	●	●	●		●	●	●
Drop outs reported	●		●	●	●	●	●	●	●	●	●
Statistical analysis	ANCOVA, linear regression	ANOVA	Two sample t-tests, ANCOVA	ANOVA	ANOVA ANCOVA	Pooled time series regression with random effects models	t-tests, ANCOVA	t-tests, ANOVA	Mixed model repeated measures	ANOVA	Pooled time series regression

Author	Orwoll, Oviatt, McClung, Deftos, and Sexton (1990)	Klesges et al. (1996)	Dawson-Hughes et al. (1997)	Peacock et al. (2000)	Prentice et al. (2005)	Daly, Brown, et al. (2006)	Daly, Bass, et al. (2006)	Barry and Kohrt (2008)	Reid et al. (2008)	Kukuljan, Nowson, et al. (2009)	Kukuljan et al. (2011)
SD or CI	CI	CI	SD	SD	SD, SE	SD and 95% CI	SD and 95% CI	SD and 95% CI	SE and 95% CI	SD and 95% CI	SD and 95% CI
Bone measures at all data points					●	●	●	●	●	●	
Included in meta-analysis	●		●		●	●			●	●	

Key: ● = Yes; ANOVA= analysis of variance; ANCOVA=analysis of covariance; CI=confidence intervals; ITT= intention to treat; PP = per protocol; SD=standard deviation; SE=standard error

3.4.4 Study interventions

Study interventions are reported in Table 3-2. Intervention length ranged from 1 year to 4 years, with the largest study (by number of participants) being 2 years in length(Reid et al., 2008). Four studies utilised both calcium and vitamin D supplementation, the others using calcium only. Calcium dosages ranged from 500mg/d to 1200mg/d and were in the form of calcium carbonate, calcium citrate malate, and milk fortified with calcium salts derived from fresh whey. All studies supplementing with vitamin D used vitamin D3 (cholecalciferol) in dosages ranging from 400IU/d to 1000IU/d and supplements were generally provided twice daily. One study (Reid et al., 2008) included two different supplement groups. The high supplement group was analysed in the meta-analysis as the results could not be pooled.

Table 3-2: Participant characteristics and study interventions

Author	Orwoll et al. (1990)	Dawson-Hughes et al. (1997)	Prentice et al. (2005)	Daly, Brown, et al. (2006)	Reid et al. (2008)	Kukuljan, Nowson, et al. (2009)
Participants	Healthy adult males	Healthy, ambulatory, over 65 years of age.	Male sixth form students aged between 16 and 18 years of age.	Males > 50 years Community living	Men over 40 years in good general health	Males aged 50-79 Community dwelling Normal to below average BMD
Number of Male participants	41 (s) 36 (p)	86 (s) 90 (p)	73 (s) 70 (p)	85 (s) 82 (c)	107 (p) 108 (600) 108 (1200)	45 (s) 44 (C)
Age	60 ± 12 (s) 55 ± 13 (p)	70 ± 4 (s) 71 ± 5 (p)	16.8 ±0.5 (p) 16.8 ± 0.4(s)	62.1 ± 7.7 (s) 61.7 ± 7.7 (c)	57 ± 10 (p) 55± 10 (600) 57 ± 10 (1200)	61.7 ± 7.7 (s) 59.9 ± 7.4(c)
Baseline calcium intake(mg/d) or serum (mmol/L)	Intake for group: 1159 ± 576 Serum: 2.35 ± 0.1 (s); 2.37 ± 0.1 (p)	Intake: 748 ± 391 (s) 673 ± 349 (p)	Intake: 1197 ± 463 (s) 1199 ± 437 (p)	Serum: 2.35 ± 0.12 (s) 2.37 ± 0.13 (c)	Intake: 800 ± 360 (p) 870 ±470 (600) 930 ± 510 (1200)	Intake: 1039 ± 455 (s) 996 ± 293 (c) Serum: 2.39 ± 0.16 (s) 2.41 ± 0.18 (c)
Baseline vitamin D intake or serum	Serum 25(OH)D (nmol/L): 60 ± 17 (s); 52 ± 15 (p) Serum 1,25 (OH) D (pg/mL): 33 ± 18 (s); 33 ± 17 (p)	Intake (IU): 202 ± 104 (s) 197 ± 117 (p)	NR	Serum 25(OH)D (nM): 77.2 ± 22.6 (s) 76.1 ± 23.5 (c)	Intake (µg/d): 3.2 ± 4.8 (p) 2.7 ± 3.3 (600) 2.9 ± 3.2 (1200) Serum 25(OH)D (ng/mL): 38 ± 13 (p) 38 ± 14 (600) 35 ± 12 (1200)	Serum 25(OH)D (nmol/L): 83.6 ± 32.7 (s) 85.7 ± 40.3 (c)
Length of intervention	3 years	3 years	13 months	2 years	2 years	12 months
Total calcium supplement amount	1000mg/d	500mg/d	1000mg/d (500mg/tablet)	1000mg/d (500mg/ 200ml)	1200mg/d (h) or 600mg/d (l)	1000mg/d (500mg/ 200ml)
Total vitamin D supplement amount	25µg/d (1000IU/d)	700IU/d	N/A	800 IU/d (400IU/200 ml)	N/A	800 IU/d (400IU/200ml)

Key: 25(OH)D = 25-hydroxyvitamin D; BMD: bone mineral density; c= control group; ca= calcium; NR=not reported; p=placebo; s=supplement; t=total; vD=vitamin D

International Reference values (Ross et al., 2011):

Calcium- males aged 19-50: 1,000 mg·day^{-1}, 51+: 1,200 mg·day^{-1}

Vitamin D- males up to 70yrs: 15 µg·day^{-1} or 600 IU·day^{-1}, 71+: 20 µg·day^{-1} or 800 IU·day^{-1}, Serum 25(OH)D- 20ng/mL or 50nmol/L

3.4.5 Study Outcomes

Study outcomes are detailed in Table 3. Studies utilized DXA for bone density measures, with a wide variation in the sites measured. However, the majority of studies took measurements at lumbar spine and hip. Bone density was most commonly measured every six months although only half the studies reported measurements at each data point. Results relating to bone density were, in all cases, reported as a percentage change from baseline. Confidence intervals (CI) were reported in four studies, whereas standard deviation and effect size with CI were reported in two. In all but one of the studies included in the meta-analysis (Orwoll et al., 1990), supplement compliance rates were reported, ranging between 65.2% and 92%.

The supplemented group in the young age cohort (Prentice et al., 2005) demonstrated significant higher gains in bone density over the control group at the total body, total hip and femoral neck sites, with non-significant improvement at the lumbar spine. Among the older age cohorts there were mixed results. Two studies reported significant positive improvements in bone density at the lumbar spine (Dawson-Hughes et al., 1997; Kukuljan, Nowson, et al., 2009) one reported a lesser decrease in bone density for total body (Dawson-Hughes et al., 1997), one reported a significant positive improvement at the total hip (Kukuljan, Nowson, et al., 2009) and two showed significant improvements in bone density at the femoral neck (Daly, Brown, et al., 2006; Dawson-Hughes et al., 1997). Other studies showed non-significant changes at the lumbar spine (Daly, Brown, et al., 2006; Orwoll et al., 1990), total hip (Orwoll et al., 1990) and femoral neck (Kukuljan, Nowson, et al., 2009), with one study not reporting significance (Reid et al., 2008).

Table 3-3: Outcomes reported by studies included in the meta-analysis

Study Name	Bone density equipment	Outcome reported	Supplement compliance (mean rate)	Pre-intervention	Post-intervention	P value
Orwoll et al. (1990)	Radial: single photon absorptiometry; Lumbar: CT	BMC (g/cm - radial) (mg/cm^3 – vertebrae)	NR	Vertebral (mg/cm^3): 112 ± 32 (s) 121 ± 27 (p) Proximal radius (g/cm): 1.24 ± 0.18 (s) 1.29 ± 0.14 (p) Distal radius (g/cm): 1.32 ± 0.14 (s) 1.28 ± 0.25 (p)	Vertebral: -2.5 (-3.3 to - 1.7) (s) -2.1 (-2.7 to -1.5) (p) Proximal Radial: -0.9 (-1.2 to -0.55) (s) -1.3 (-1.8 to -0.55) (p) Distal Radial: -1.1 (-1.7 to -0.53) (s) -0.8 (-1.3 to -0.29) (p) Reported as % change	0.41 0.29 0.46
Dawson-Hughes et al. (1997)	DXA	BMD (g/cm^2)	92% ± 10 calcium tablet 93% ± 10 vitamin D tablet	Total body: 1.22 ± 0.09 (s) 1.19 ± 0.09 (p) Spine: 1.32 ± 0.21 (s) 1.27 ± 0.20 (p) Femoral neck: 0.99 ± 0.14 (s) 0.95 ± 0.12 (p)	Total body: 0.34 ±1.40 (s) -0.85 ± 1.53 (p) Spine: 2.93 ±3.42 (s) 1.74 ±3.85 (p) Femoral neck: 0.95 ± 4.07 (s) -1.35 ±4.70 (p) Reported as % change	<0.001 0.03 <0.001

Study Name	Bone density equipment	Outcome reported	Supplement compliance (mean rate)	Pre-intervention	Post-intervention	P value
Prentice et al. (2005)	DXA	BMD (g/cm^2)	65.2% ± 27.9 calcium tablet 52.2% ± 32 placebo	Total body: 1.17 ± 0.10 (s) 1.18 ± 0.10 (p) Lumbar spine: 1.00± 0.11 (s) 0.99 ± 0.11 (p) Total hip: 1.10 ± 0.12 (s) 1.12 ± 0.13 (p) Femoral neck: 0.91 ± 0.13 (s) 0.99 ± 0.12 (p) Trochanter: 0.86 ± 0.11 (s) 0.87 ± 0.12 (p) Intertrochanter: 1.246 ± 0.14 (s) 1.27 ± 0.16 (p) UD radius: 0.44 ± 0.06 (s) 0.44 ± 0.05 (p) 33% radius: 0.67 ± 0.05 (s) 0.69 ± 0.06 (p)	Total body: 1.27 ± 0.55 Lumbar spine: 2.51 ± 0.86 Total hip: 2.32 ± 0.92 Femoral neck: 2.36± 1.03 Trochanter: 1.10 ± 1.24 Intertrochanter: 2.66 ± 1.05 UD radius: 0.39 ± 0.91 33% radius: 0.76± 0.51 Intervention effect (%) reported to account for skeletal growth S vs P ± SE	<0.05 NS <0.01 <0.05 NS <0.01 NS NS
Daly, Brown, et al. (2006)	DXA	BMD (g/cm^2)	85.1%	Lumbar spine: 1.22 ± 0.16 (s) 1.21 ± 0.16 (c) Total hip: 1.02 ± 0.12 (s) 1.04 ± 1.12 (c) Femoral neck: 0.95 ± 0.11 (s) 0.95 ± 0.1 (c) UD radius: 0.42 ± 0.05 (s) 0.42 ± 0.05 (c) 33% radius: 0.79 ± 0.07 (s) 0.78 ± 0.07 (c)	Lumbar spine: 0.69 (-0.33, 1.72) Total hip: 0.90 (-0.04, 1.84) Femoral neck: 1.51 (0.55, 2.48) UD radius: 1.57 (0.79, 2.35) 33% radius: 0.40 (-0.24, 1.05) Mean difference between groups, 95% CI	0.08 0.05 0.001 0.001 NS
Reid et al. (2008)	DXA	BMD (g/cm^2)	85% (p) 86% (l) 83% (h)	Total body: 1.26 ± 0.10 (p) 1.25 ± 0.08) (l) 1.26 ± 0.10 (h) Lumbar spine: 1.24 ± 0.16 (p) 1.25 ±0.15 (l) 1.26 ± 0.18 (h) Total hip: 1.08 ± 0.13 (p) 1.07 ± 0.13 (l) 1.09 ± (h)	Total body: -0.13 ± 1.26 (p) -0.11 ± 1.19 (l) 0.74 ± 1.17 (h) Lumbar spine: 0.79± 3.04 (p) 0.43 ±2.70 (l) 1.48 ± 2.44 (h) Total hip: -0.42 ± 1.79 (p) -0.19 ± 1.43 (l) 0.89± 1.61 (h) % change. 24mth results (SD)	NR

Study Name	Bone density equipment	Outcome reported	Supplement compliance (mean rate)	Pre-intervention	Post-intervention	P value
Kukuljan, Nowson, et al. (2009)	DXA	aBMD (g/cm^2)	87%	Lumbar spine: 1.21 ± 0.15 (s) 1.24 ± 0.17 (c) Total hip: 1.00 ± 0.08 (s) 1.01 ± 1.12 (c) Femoral neck: 0.92 ± 0.08 (s) 0.93 ± 0.08 (c) Trochanter: 0.88 ± 0.09 (s) 0.89 ± 0.12 (c)	Lumbar: 2.1 (1.1, 3.0) (s) 0.6 (-0.1, 1.3) (c) Total hip: 1.2 (0.7, 1.8) (s) 0.5 (0.0, 1.0) (c) Femoral neck: -0.4 (-1.1, 0.3) (s) -0.2 (-0.9, 0.6) (c) Trochanter: 1.6 (0.7, 2.5) (s) 0.8 (0.0, 1.5) (c) Mean % change (95% CI)	 0.001 NS 0.001 NS NS NS 0.001 NS within group

Key: BMC= bone mineral content; BMD = bone mineral density; c= control group; ca= calcium only group; DXA=dual energy x-ray absorptiometry; h= high calcium supplement; I_{polar} = density-weighted polar moment of inertia; l= low calcium supplement; NR=not reported; NS= not significant; QCT = quantitative computer tomography; p=placebo group; s=supplement group; t=total; UD= ultra-distal; vBMD= volumetric bone mineral density; vD=vitamin D only group.

3.4.6 Effects of Supplementation on bone mineral density (meta-analyses)

The effect of calcium or calcium and vitamin D supplementation on bone mineral density is shown in Figure 3-2. Moderate (non-significant) heterogeneity was observed among studies for total body (I^2 =46.233%, p=0.156) and total hip (I^2=48.842%, p=0.118). Given the low number of studies, a random effects model was used to account for variability which may have arisen from differences in: age, baseline calcium intakes, baseline bone mineral density, supplementation dosages and duration. Overall, significant pooled ES for comparison between supplementation and placebo/control groups at all sites included in the meta-analyses was found. The largest effect was found in total body (ES=0.644; 95% CI=0.406 to 0.883; Z=5.302; p=0.000), followed by weight bearing sites of total hip (ES=0.483; 95% CI=0.255 to 0.711; Z=4.156; p=0.000) and femoral neck (ES=0.402; 95% CI=0.233 to 0.570; Z= 4.667; p=0.000). Supplementation had the least effect on the lumbar spine (ES=0.306; 95% CI=0.173 to 0.440; Z= 4.499; p=0.000).

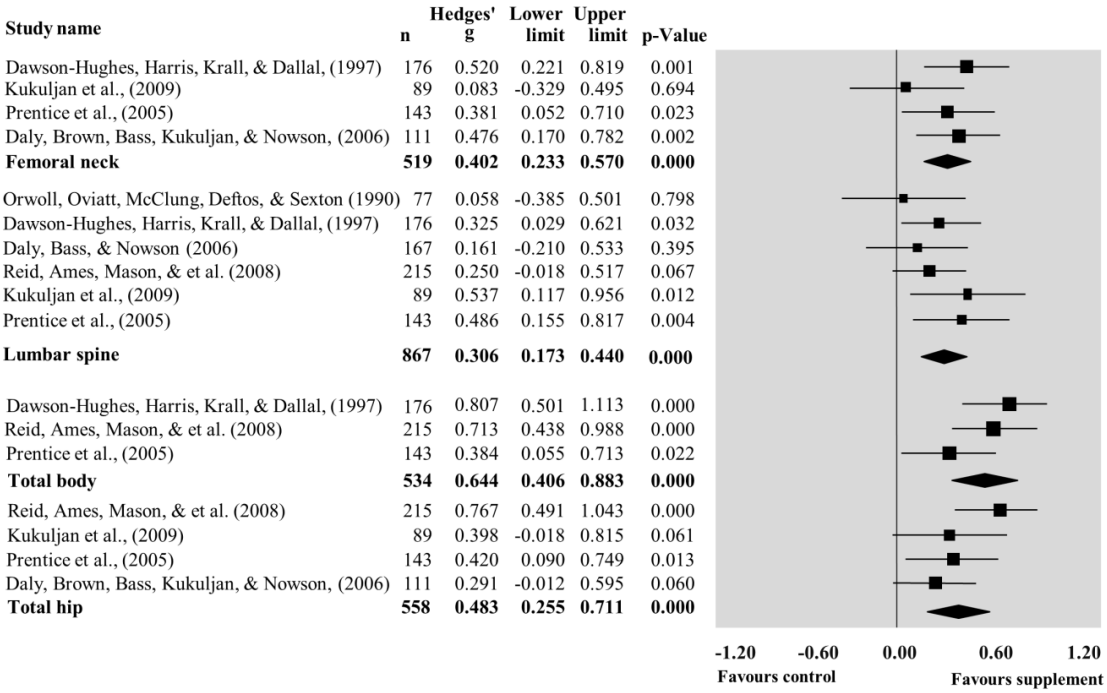


Figure 3-2: Forest plots of main effects of calcium or calcium and vitamin D supplementation on bone mineral density of lumbar spine, femoral neck, hip and total body.

The results for the lumbar spine, femoral neck, hip and total body excluding the young age cohort (Prentice et al., 2005) showed significant pooled ES for comparison between supplementation and placebo/control groups at all sites. Total body was based on two studies (Dawson-Hughes et al., 1997; Reid et al., 2008), demonstrating a larger effect size than when the younger group was included (ES=0.755; 95% CI=0.551 to 0.960; Z=7.237.302; p=0.000). Total hip was also larger (ES=0.493; 95% CI=0.183 to 0.814; Z=3.099; p=0.002). The femoral neck (ES=0.395; 95% CI=0.154 to 0.636; Z= 3.213; p=0.001) and lumbar spine (ES=0.271; 95% CI=0.126 to 0.417; Z= 3.649; p=0.000) sites were lower without the younger cohort, but demonstrated similar effect sizes.

3.4.7 Sub-group analyses

Sub-group analyses of study duration, age of participants, baseline calcium intake and inclusion of vitamin D in supplementation regime is presented for the lumbar spine region (Figure 3-3). The effect size for participants with low baseline calcium was found to be smaller than the group with adequate baseline calcium levels. As can be seen in Table 3-2, this also included the group with the lowest supplementation level (Dawson-Hughes et al., 1997). A small difference was seen between the participants who received vitamin D in conjunction with calcium (ES=0.281; 95% CI=0.107 to 0.455; p=0.002) and those receiving calcium only (ES=0.347; 95% CI=0.119 to 0.574; p=0.003). Despite a paucity of research, it appears the age of participants had a greater influence on the bone mineral density outcome at the lumbar spine with younger participants demonstrating a greater ES. Furthermore, results also suggest that additional years of supplementation have no additional benefit although this may be affected by age. Sub group analyses at other sites showed similar trends however there were fewer studies to compare, weakening the analysis.

Group	Participants n	Studies n	Hedges' g	Lower limit	Upper limit	p-Value
Baseline calcium < 1000mg [29,32,34]	309	3	0.260	0.094	0.426	0.002
Baseline calcium ≥ 1000mg [24,31,35]	558	3	0.486	0.155	0.817	0.004
Calcium Only [24,32]	358	2	0.347	0.119	0.574	0.003
Calcium plus vitamin D [29,31,34,35]	509	4	0.281	0.107	0.455	0.002
Intervention ≤1 yr [24,31)]	232	2	0.505	0.245	0.765	0.000
Intervention >1 yr [29,32,34,35]	635	4	0.235	0.079	0.390	0.003
Subjects ≤ 55 yrs age [24]	143	1	0.486	0.155	0.817	0.004
Subjects >55 yrs age [29,31,32,34,35]	724	5	0.271	0.126	0.417	0.000

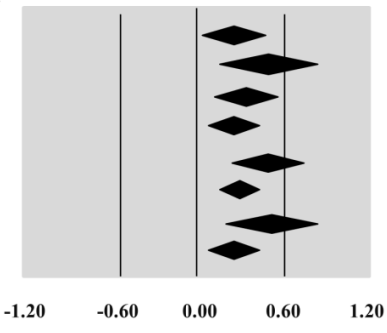


Figure 3-3: Forest plots of subgroup analysis of the effect of inclusion of vitamin D, study duration and age of participants on bone mineral density of lumbar spine**.**

3.5 Discussion

Calcium and vitamin D supplementation has previously shown a positive effect on bone strength in females and children (Abrahamsen et al., 2010; Chung et al., 2009; Cranney et al., 2007; Shea et al., 2002; Tang et al., 2007; Winzenberg et al., 2010), however the preventative effects of supplementation on healthy male bone remaining largely unexplored. This is the first systematic review and meta-analysis to examine the effects of calcium supplementation with or without vitamin D on BMD in healthy adult male populations. The data appears to demonstrate that, when compared to a control intervention, calcium supplementation with or without vitamin D has a small to moderate effect on bone mineral density at the femoral neck, lumbar spine, total body and total hip. Total body bone mineral density demonstrated the greatest impact of supplementation followed by the weight bearing sites of total hip and the femoral neck, with the smallest impact observed at the lumbar spine region. For the younger cohort, this resulted in positive acquisition of bone, while in older cohorts this resulted in an attenuation of the rate of bone loss.

The meta-analysis included six studies involving a total of 867 participants (435 in control groups and 432 in supplemented groups) ranging in age from 16 years to 84 years of age with the average age of participants was over 55 years. Baseline calcium intakes reported in the majority of studies were below recommended daily intake levels being 1,000-1,200 $mg{\cdot}day^{-1}$ (National Health and Medical Research Council, 2006a; Ross et al., 2011). Intake levels ranged from a low of $629mg{\cdot}day^{-1}$ to $1159mg{\cdot}day^{-1}$. Sub group analysis indicated that lower baseline calcium intake resulted in a lower effect size at the lumbar spine. Others have found that treatment effect is higher in groups whose daily calcium

intake is low (Tang et al., 2007). These results must be interpreted with caution, however, given the low number of studies available for inclusion in the analysis. There was a wide variation in individual calcium intakes amongst participants in all studies as well as variation in the amount of supplement provided. One study included in the low baseline group also provided the lowest daily calcium supplement of 500mg·day^{-1} (Dawson-Hughes et al., 1997). A lack of sufficient data makes it difficult to determine whether the results may have been different at other sites.

Similarly, sub group analysis indicates that the addition of vitamin D did not enhance bone outcomes at the lumbar spine which may be due to the initial adequacy of vitamin D levels in the participants included in the meta-analysis in all but one study (Dawson-Hughes et al., 1997), being 50nmol/L or above (National Health and Medical Research Council, 2006b; Ross et al., 2011). Again, the power to detect such differences may be low given the small number of studies available for inclusion in the analysis. Meta-analysis examining vitamin D supplementation in children has shown vitamin D to be effective in improving bone density when serum levels are low, but not when they are at normal levels (Winzenberg, Powell, Shaw, & Jones, 2011). It is suggested that vitamin D supplementation for healthy adults, defined as 20-65 years of age, needs to be in the range of 2000IU per day in order to achieve improvements to BMD (Ebeling et al., 2013). Aloia et al (2010) examined the effect of 1,200 mg·day^{-1} calcium supplementation with or without 100µg·day^{-1} vitamin D (4000IU) on bone turnover markers in healthy adults aged 20-80 years of age over a three - month period. The research found that the increased calcium intake lowered markers of bone turnover whilst the vitamin D alone or together with the calcium, had no effect on bone turnover markers. While some report modest reductions in fracture risk in older adults with a combination of calcium and vitamin D

supplementation (Ebeling et al., 2013), most report vitamin D alone does not appear to significantly influence fracture risk (Lips et al., 2014), nor bone mineral density, with a recent meta-analysis finding a small positive effect at the femoral neck only, in older, predominantly female adults (Reid, Bolland, & Grey, 2014) Further, a recent meta-analysis assessing the reduction of fracture risk associated with vitamin D and/or calcium supplementation showed similar results to this meta-analysis with no difference found between calcium only and calcium plus vitamin D groups (Tang et al., 2007). As such, current evidence suggests that calcium supplementation may be more important than vitamin D in improving BMD, however, some caution should be applied due to the paucity of results.

Amongst the studies there was inconsistency in intervention length. Not all measurements were reported at each data point, making it difficult to determine at which stage supplementation began to have an effect on bone density. Furthermore, sub-group analysis showed little benefit in supplementation extending beyond one year at the lumbar spine site, however this is limited by the low effect size at the lumbar spine site. Other sub-group analyses indicated a higher benefit but are limited by the numbers of studies included. While supplementation beyond one year does not appear to provide an additional improvement, follow-up research suggests that continued supplementation maintains the initial benefit of the supplementation (Dawson-Hughes, Harris, Krall, & Dallal, 2000).

3.5.1 Limitations

Consideration of several limitations should be made when interpreting these results. First and foremost is the limited data available to examine the effectiveness of

supplementation. This has an impact in both the overall effects of supplementation for all age groups as well as being able to fully assess the impact that differing dosages, baseline intakes, age, supplementation length, vitamin D all may have on the effectiveness of the supplementation.

Only one study included adolescent males, demonstrating improvements in bone density in the supplementation group beyond the normal growth related acquisition. While included in the systematic review, the two studies examining athletic male populations (young adult males) were unable to be incorporated into the meta-analysis due to the absence of control groups. This makes it unclear of the effects of calcium supplementation on growing bone in male populations. While approximately 90% of PBM is acquired by 18 years of age (Baxter-Jones et al., 2003), peak BMD appears to be achieved by the end of the 20's or early 30's (Baxter-Jones et al., 2011). Further, in athletic male populations with low BMD, it is unclear whether supplementation would be an effective and feasible strategy for improving BMD. In addition, there was variation in baseline BMD of the participants, baseline calcium intakes and vitamin D status, as well the dosages of supplementation provided.

It is worth noting that the studies in this meta-analysis used DXA to measure changes in BMD at a variety of sites. The inherent limitations of two-dimensional DXA technology are well known. Peripheral quantitative computed tomography (pQCT) provides measures of volumetric bone mineral density and bone geometry in the arms and legs, and therefore represents a viable alternative to address the question of skeletal adaptations to calcium and vitamin D supplementation, particularly in healthy populations.

Despite these limitations, our results indicate that supplementation provides small to moderate benefits in slowing the rate of bone loss in older male populations. Research in healthy young male populations is required to assess the impact of supplementation on BMD in cohorts that have not fully reached PBM. Further, research with athletic cohorts known to have low BMD is also necessary to establish whether supplementation would be an effective intervention. Follow-up studies would be beneficial to establish whether supplementation provides lasting or transitory benefits.

3.6 Conclusion

With the growing incidence of male osteoporosis, the results of this paper may be relevant when considering the primary prevention strategies to curtail future osteoporotic fracture. The available evidence shows that when compared to a control intervention, supplementation with calcium, in combination with vitamin D, has a small to moderate effect on bone mineral density at the femoral neck, lumbar spine, total body and total hip in healthy males. For the younger cohort, this resulted in positive acquisition of bone over normal growth related bone acquisition, while in older cohorts this resulted in an attenuation of the rate of bone loss. However, caution should be applied when interpreting these results. There is a very limited number of studies on male participants to adequately determine the efficacy of vitamin D and/or calcium supplementation, and there are no studies of any sufficient quality examining supplementation in athletic populations although evidence of compromised bone health exists.

Novelty statement

Provides the first meta-analysis examining the effects of calcium and vitamin D supplementation exclusively on healthy male BMD.

Practical application

The very limited evidence appears to support calcium and vitamin D supplementation for improving BMD in older males. Available research examining male cohorts do not provide an estimate of fracture risk reduction, and the paucity of research highlights the need for high quality research in all male age cohorts and male athletic populations.

Contributors: Study design: LS, DG, MB. Data collection: LS. Data analysis: LS, DG. Data interpretation: LS, DG, MB. Drafting manuscript: LS. Revising manuscript content: DG, MB. Approving final version of manuscript: LS, DG, MB. LS takes responsibility for the integrity of the data analysis.

Competing interests: All authors declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Note: Refer to Appendix 9: PRISMA Checklist for Systematic Review and Meta-Analysis for detailed PRISMA Checklist (Moher et al., 2009).

4 Methodology

This chapter describes the protocols and procedures generic to all results chapters within this thesis.

4.1 Study Design

The study was developed as a prospective double-blind placebo controlled trial of calcium and vitamin D supplementation with repeated measures taken at baseline and six months in order to examine the efficacy of supplementation to illicit positive skeletal adaptations at the tibia and radius in young male jockeys. A six-month intervention period was selected based upon previous research undertaken by ACU Researchers (Greene & Naughton, 2011).

4.2 Ethics Approval

Approval was obtained from the Human Research Ethics Committee at the Australian Catholic University (ACU) (Approval number 2012-114N) prior to commencement of the study (Appendix 2: Ethics Approval).

4.3 ANZ Clinical Trials Registration

The study was registered with the Australian New Zealand Clinical Trials Registry (registration no: ACTRN126000374864) (Appendix 1: ANZ Clinical Trials Registration).

4.4 Participants

Participants were male Apprentice Jockeys aged 16 to 34 years who were currently completing a Certificate IV in Racing in New South Wales (NSW) and Victoria (VIC). Of the available pool of Apprentices, approximately one-third was female; however, dietary supplementation in young males and females would be unlikely to have a

homogeneous response and therefore females were excluded from recruitment. Results would be difficult to interpret and the number of female jockeys was insufficient to provide power for a sub-group analysis. Apprentice jockeys were selected over licenced jockeys due to the difficulty in recruiting large numbers of licenced jockeys. Whilst the jockeys do have an industry Association, they are individually employed, making recruitment difficult. Further, they work and race in a variety of locations and no convenient central location to test large numbers of jockeys was available.

To control for selection bias, specific inclusion and exclusion criteria was used:

1. In good health with no systemic illness lasting more than 2 weeks in last 6 months.
2. No known history of fracture or recurrent fracture complications in last 6 months.
3. No known history of metabolic bone or muscle disease.
4. No medication, hormones, calcium or vitamin D preparations in preceding 6 months and willing to remain free of such medications for the 6 months of data collection.

4.5 Power Analysis

The sample size of 15 per group will allow detection of a significant difference if the mean key outcome variable (bone strength index (BSI) at 4% sites and stress strain index (SSI) at 66% sites) for the supplemented group is at least 1.5 SD higher than the mean value in the control group. This sample size allows for a 35% drop out (power = 80%, $p>0.05$) (Peat & Barton, 2005).

4.6 Recruitment of Participants

The Australian Jockey Association, with support from the Australian Racing Board assisted with participant recruitment. The jockeys were available for testing on "Apprentice Day" held once per month at TAFE NSW and Racing Victoria. Unforeseen relocations and requirements to race at short notice reduced the original pool of 40 jockeys to 30 available for initial participation in the study (refer Figure 4-1). A total of 29 participants were recruited for the study, after excluding one jockey for health reasons, and were randomised into either the intervention or placebo groups. All participants were provided with an information statement and informed consent was obtained from all participants prior to participation in the study (Appendix 4: Information statement and Appendix 5: Informed consent). Where participants were under 18 years of age, permission was obtained from a parent or guardian.

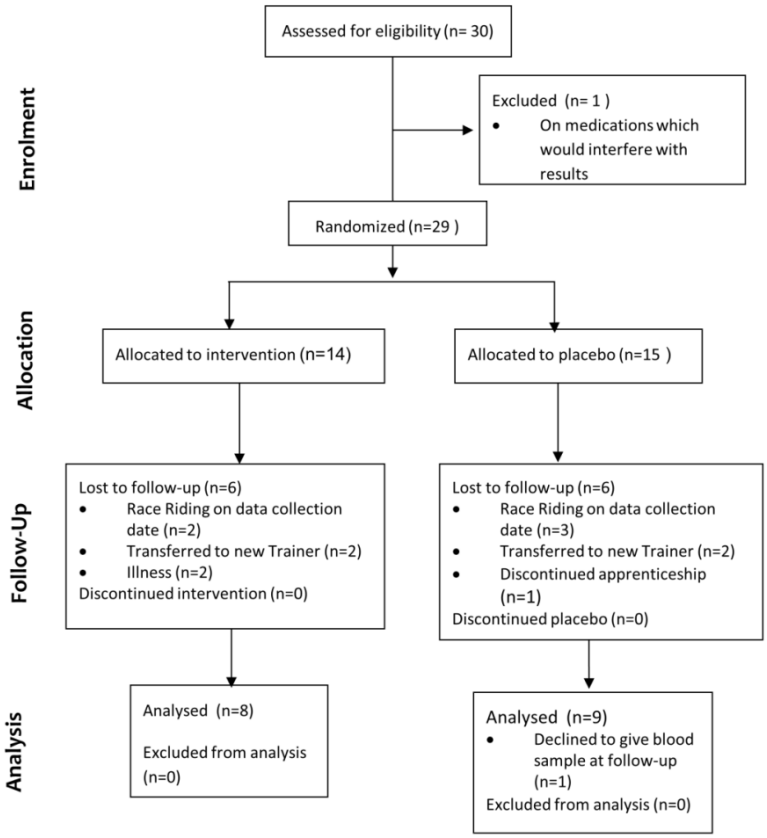


Figure 4-1: Number of participants available at each stage of the intervention

4.7 Randomisation and blinding

Participants were randomly allocated into the active or placebo group using a computerised four block randomisation process (www.sealedenvelope.com). Active and placebo supplements were in tablet form and were identical in colour, taste, texture and appearance (Figure 4-2). The tablets were provided in opaque plastic containers which were placed in envelopes labelled A or B and group allocation was recorded for later analysis. Participants were provided with enough tablets to last the duration of the study. Both researchers and participants were blinded to group allocation and remained blinded until after the data analysis had been completed.

Figure 4-2: Tablets and containers used for active and placebo groups.

4.8 Calcium and Vitamin D supplement

The active group received 800 mg calcium and 400 IU vitamin D in tablet form per day for a period of six months (USANA Pty Ltd, Sydney, Australia) divided into two tablets taken twice per day (morning and evening). The placebo comprised predominantly cellulose (Microcrystalline cellulose 814.42mg·g^{-1}). The full list of ingredients appears in Table 4-1.

Table 4-1: List of ingredients contained in both active and placebo tablets.

Active tablet	Placebo tablet
Calcium (equiv 200 mg) as:	Microcrystalline cellulose 814.42mg
Calcium citrate hydrate 371 mg	Pre-gelatinised starch 56.82mg
Calcium carbonate 304 mg	Croscarmellose sodium 37.88mg
Magnesium (equiv 100 mg) as:	Ascorbyl palmitate 18.94mg
Magnesium citrate 370 mg	Colloidal silicon dioxide 18.94mg
Magnesium amino acid chelate 83 mg	Dextrin 6.90mg
Magnesium oxide 82 mg	Dextrose 0.96mg
Cholecalciferol (vitamin D3, 100 IU) 2.5 µg	Lecithin 0.95mg
Phytomenadine (Vitamin K) 15 µg	Sodium CMC 0.47 mg
	Sodium citrate 0.19 mg

Participants were sent identical reminders via text messaging, every second day for two weeks, then twice per week for one month, followed by weekly reminders until the end of the third month. From months three to six, fortnightly reminders were sent.

4.9 Data Collection Overview

Data collection was undertaken at baseline and six months using equipment from the Exercise Science laboratory of ACU Strathfield campus. NSW jockeys were tested at Canterbury Race Course during their monthly TAFE Apprentice course. Victorian jockeys were tested at Racing Victoria Apprentice School located at Flemington Race Course. At baseline, information such as date of birth and contact details were collected to allow reminder messages to be sent during the trial. At both baseline and six months, the following data was collected:

- Anthropometric data
- Bone geometry, density and strength at both radius and tibia
- Serum for analysis of bone turnover markers and vitamin D status
- Hydration status
- Dietary questionnaire
- Health and lifestyle questionnaire

At the six-month data collection date, participants were requested to return any unconsumed tablets in their containers. Jockeys were not compliant in returning the containers and were agreeable to returning them by mail. A letter and pre-paid, pre-addressed envelope was forwarded to each jockey which was then followed-up by text message.

4.9.1 Anthropometric characteristics

Body mass was measured using an electronic scale accurate to 500 g (Wedderburn UW150, Sydney, Australia) with participants dressed in light clothing and without shoes. Participants were instructed to have their feet evenly placed on the scales, with mass evenly distributed, ensuring they were looking straight ahead.

Standing and seated height were measured to 0.1 cm using a stadiometer (SECA height rod model 220, Hamburg, Germany). Participants were required to stand in anatomical position, with heals together and back against the rod of the stadiometer. The head was placed in the Frankfort plane and standing height was then recorded as the maximal distance from the floor to the vertex of the skull. To take seated height, participants were required to be seated in an erect position on a flat chair. Again the head was positioned in the Frankfort plane and the maximal distance from the floor to the vertex of the skull was recorded. To calculate seated height, the height of the chair (47cm) was deducted from the recorded height.

Anthropometric measures (Figure 4-3) included:

- Skinfolds: triceps, subscapular, supraspinale, abdominale, front thigh and calf
- Girths: head, arm relaxed, arm flexed, forearm, wrist, chest, waist, gluteal, upper thigh, mid-thigh, calf and ankle.
- Bone breadths: biacromial, biiliocristale, transverse chest, A-P chest depth, biepicondylar humerus, wrist, biepicondylar femur and ankle.

- Bone lengths: radial length (ulnar styloid and olecranon processes) and tibial length (tibilale mediale to malleolus mediale) were made.

These measures were taken using Harpenden skinfold callipers (Baty International, UK), flexible steel tape measure (Lufkin W606PM), segmometer (University of Western Australia), large and small sliding bone callipers (Rosscraft, Inc. Canada). All measures were taken twice and made in accordance with International Society for the Advancement of Kinanthropometry (ISAK) guidelines (Stewart et al., 2011) by an accredited Level 3 ISAK Anthropometrist (LS).

A **B**

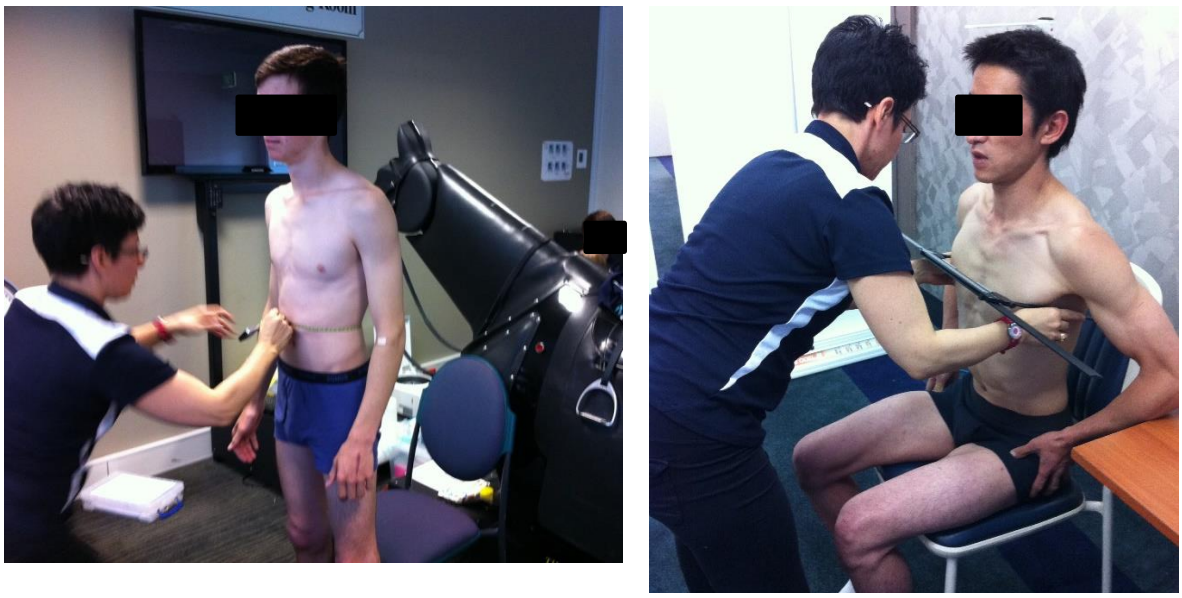


Figure 4-3: Anthropometric assessment.

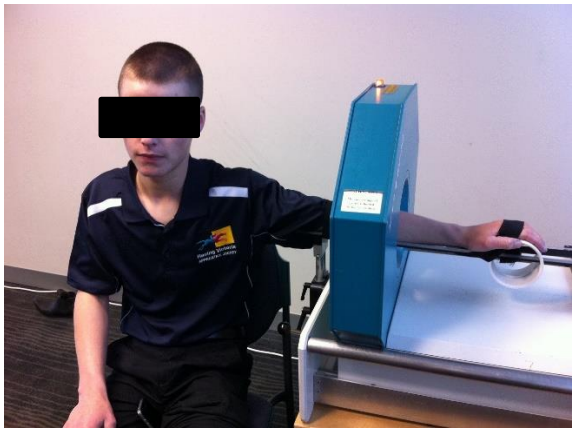
4.9.2 Bone material properties and fracture risk

The non-dominant radius and tibia were measured using a Stratec XCT-2000L pQCT bone scanner (Stratec Medizintechnic, Pforzheim, Germany) with software version 5.50d. The scanner was positioned at the anatomical reference line (cortical end plate) of the radius that corresponded to 4% (distal) and 66% (proximal) of radial length (Figure

4-4). Radial length was measured externally as the distance between the mid-point of the ulnar styloid and olecranon processes. For the tibia, the scanner was positioned at the anatomical reference line (cortical end plate) of the tibia that corresponded to 4% (distal) and 66% (proximal) of tibial length (Figure 4-4). Tibial length was measured externally as the distance between the mid-point of the distal medial malleolus and the proximal medial tibial plateau landmarks.

A planar scout scan was first conducted to determine the anatomical reference line for both the radius and tibia. Tomographic slices of 1 mm thickness were obtained at the 4% and 66% sites measured distally. Scan speed and voxel size were 30 mm/s and 0.5 mm respectively. A contour mode with a threshold of 180mg/cm^3 was used to separate soft tissue and bone in order to analyse trabecular bone. Cortical bone was identified and removed using a constant default threshold of 711mg/cm^3. Muscle cross-sectional area was assessed using contour mode 3, peel mode 2, and with a threshold of 40 mg/cm3. A region of interest (ROI) to identify the radius and tibia within each scan were automatically identified with manual adjustments made as necessary to ensure the entire radial ROI was enclosed. Volumetric BMD, bone geometry, bone strength, and muscle cross-sectional areas were assessed at the two scanned sites

A



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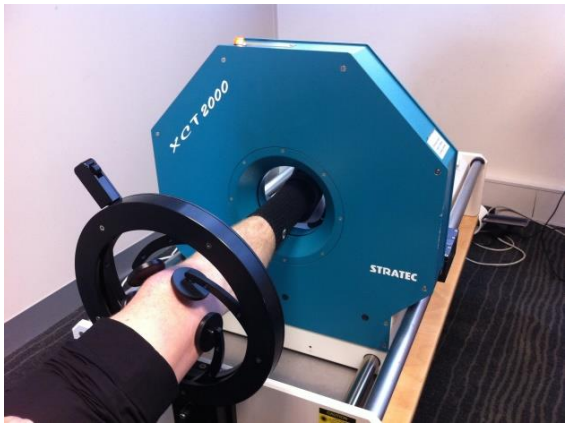

Figure 4-4: Positioning of participants for measurement of radius (A) and tibia (B) in pQCT.

Outcome measures for both the radius and tibia automatically calculated by the pQCT are shown in Table 4-2 below. Estimates of compressional bone strength (bone strength index in $mg^2 \cdot mm^4$) were subsequently calculated using the formula: total area x (total density x 0.001)^2 at the 4% site (Kontulainen et al., 2008).

Table 4-2: Outcome variables assessed at 4% and 66% sites for both Radius and Tibia

Radius	Tibia
4% distal site	**4% distal site**
Total Area (mm^2)	Total Area (mm^2)
Total density ($mg \cdot cm^3$)	Total density ($mg \cdot cm^3$)
Trabecular area (mm^2)	Trabecular area (mm^2)
Trabecular density ($mg \cdot cm^3$)	Trabecular density ($mg \cdot cm^3$)
Bone strength Index ($mg^2 \cdot mm^4$)	Bone strength Index ($mg^2 \cdot mm^4$)
66% proximal site	**66% proximal site**
Cortical area (mm^2)	Total area (mm^2)
Cortical density ($mg \cdot cm^3$)	Cortical area (mm^2)
SSI-Polar 66% (mm^3)	Cortical content (mg.mm)
Total Bone Area (mm^2)	Cortical density ($mg.cm^3$)
Muscle Area (mm^2)	Cortical thickness (mm)
Endocortical circumference (mm)	SSI-Polar 66% (mm^3)
Pericortical circumference (mm)	

Figure 4-5 provides a pictorial overview of scan sites, measurement variables and range of interest identified in the scans. The precision of repeat measurements on the pQCT in the ACU laboratory is 0.7% to 1.4% radius and 0.8% to 2.9% tibia after

repositioning in eight adults. (Greene et al., 2013). Scans were performed at baseline and 6-months by the same investigator.

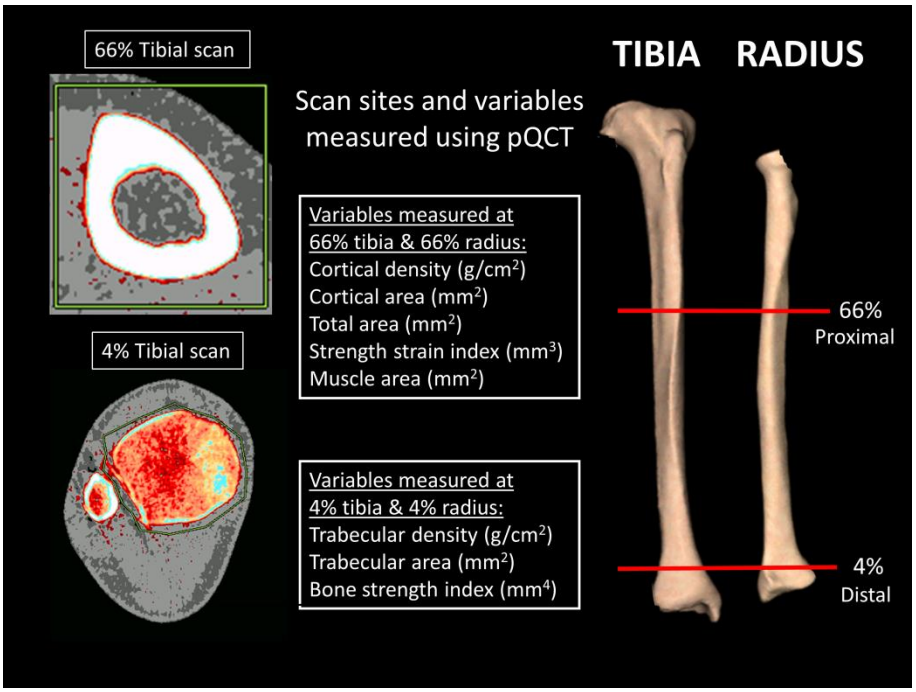

Figure 4-5: Location of bone scan sites at tibia and radius, together with variables measures at each scan site and range of interest examples provided.

4.9.3 Bone shape analysis

Bone shape analysis was performed using ImageJ 1.47v Medical Research Software (National Institutes of Health, USA) BoneJ plug-in, pQCT distribution analysis (Doube et al., 2010; Rantalainen, Nikander, Heinonen, et al., 2011). Images derived from pQCT scans were further analysed using standard settings in BoneJ (Figure 4-6). Endocortical and pericortical radii (mm) measured as the distance from the centroid to the endocortical and pericortical edge, together with mineral mass (mg) was assessed at the 66% tibial site for 36 x 10 ten degree sectors. Polar cortical vBMD ($mg \cdot cm^3$) was assessed using 36 x 10 degree sectors, whilst radial cortical vBMD ($mg \cdot cm^3$) was assessed using 3 concentric rings equally spaced from the centroid (Figure 4-6).

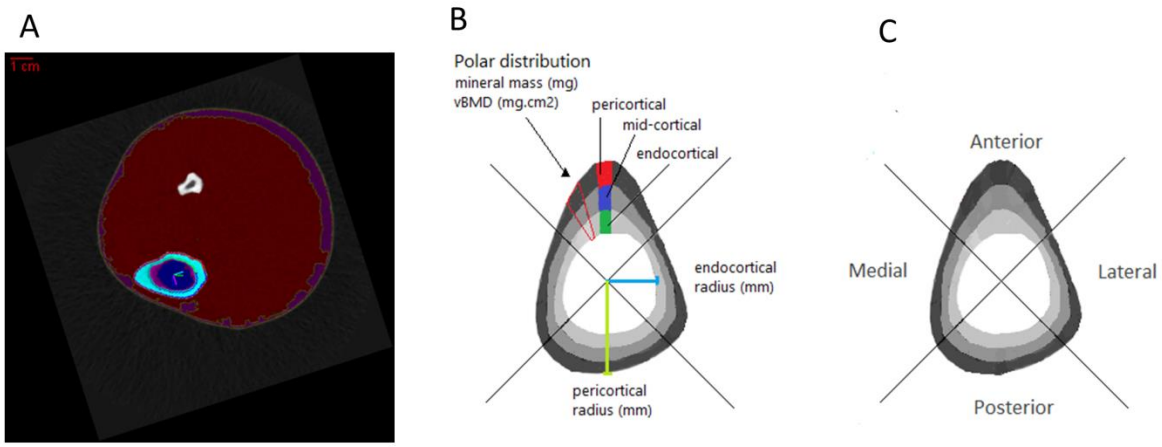

Figure 4-6: pQCT image as treated by BoneJ pQCT distribution plug-in (A), an illustration of the radial and polar distribution (B) and the location of the anterior, posterior, lateral and medial planes (C).
Source: Images B and C adapted from Rantalainen, Nikander, Heinonen, et al. (2011)

The cortical cross-section was further consolidated into four 90^{o} polar sectors and three cortical radial divisions representing the anterior, posterior, lateral and medial anatomical planes (Figure 4-6). Mean endocortical and pericortical radii (mm), mean mineral mass (mg) and mean polar cortical and radial vBMD (mg·cm^{3}) was calculated for each 90^{o} sector by averaging the sum of each 10^{o} sector within the defined planes.

4.9.4 Markers of bone turnover and Vitamin-D

Blood samples were collected by a qualified phlebotomist at the same time of day at each data collection period. Ten ml blood was drawn using a lithium heparin collection tube and each sample was centrifuged within 15 minutes at 4000 rpm using a Centurion centrifuge (Scanspeed406G, Labogene, Scandanavia). Immediately following separation, clear plasma was transferred into three eppendorf tubes with aliquot which were labelled with names and dates and then placed in an ice box. The samples were transferred to freezers at -80^{o} C within six hours of blood draw. Serum P1NP has been shown to be stable at both room (20^{o} C) and refrigerator temperatures (2^{o} to 8^{o} C) for up to 7 days while serum CTx separated in lithium heparin is stable for less than 24 hours at

refrigerator temperatures (Stokes, Ivanov, Bailey, & Fraser, 2011). A number of studies have found that vitamin D is stable at both room and refrigerator temperatures for up to 72 hours, as well as multiple freeze-thaw cycles and light exposure (Wielders & Wijnberg, 2009; Zerwekh, 2008). However, the most consistent results for vitamin D status were achieved when serum was stored in the dark and at refrigerator temperatures if freezing was unavailable.

Frozen blood samples were subsequently collected by an external NATA accredited laboratory (Melbourne Pathology, Victoria, Australia) and analysed for Procollagen type 1 N propeptide (P1NP) (ug/L), C-terminal telopeptide of type 1 collage (CTx) (ng/L), and serum 25-hydroxy vitamin D [25(OH)D] (nmol/L). A DIALAB 25-OH Vitamin D total ELISA kit, using a solid phase enzyme-linked immunosorbent assay was used to measure 25(OH)D (nmol/L). Using a denaturation buffer to extract 25(OH)D from Vitmin D binding protein (VDBP), samples were pretreated in separate vials. After mixing with enzyme conjugate and enzyme complex, the samples were transferred to microtiter plate wells. Incubation for 60 minutes at 37 degrees was followed by a washing step and a colour reaction was stopped after 15 minutes at room temperature. All wells were read within 10 minutes after the addition of the stop solution. Intra-assay coefficient of variation (CV) was 3.2% (n=20) and the inter-assay CV was 6.9% (n=30). Bone markers were analysed using enzyme-linked immunosorbent assay kits (Cloud-Clone Corp., USA) for cross linked c-telopetide type I collagen (CTx) and procollagen 1 N-terminal propeptide (P1NP). Intra-assay coefficient of variation (CV) for CTx and P1NP were <10% (3 samples tested 20 times on one plate) and the inter-assay CV was <12% (3 samples tested on 3 different plates, 8 replications in each plate).

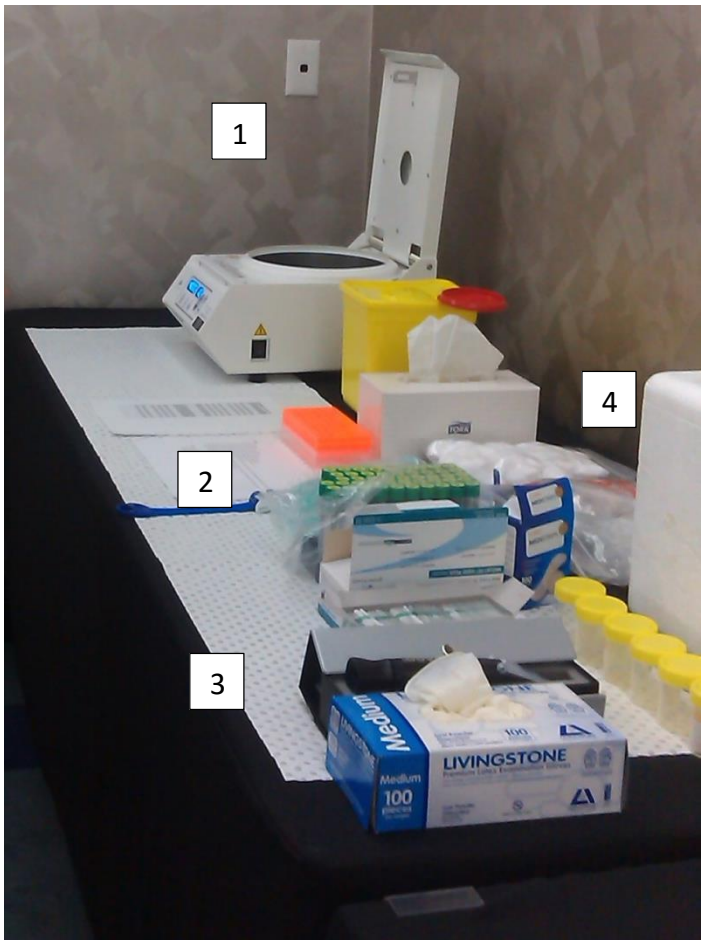

Figure 4-7: Blood collection and hydration equipment including centrifuge (1), collection tubes (2), refractometer (3) and ice-box (4).

4.9.5 Hydration

Urine specific gravity (Usg) was analysed using a handheld refractometer (MASTER URC/NM, Atago Co. Ltd, Japan. This method of field testing has been demonstrated to be a viable method of determining hydration status (Oppliger, Magnes, Popowski, & Gisolfi, 2005). Participants were provided with urine sample jars and instructions to provide a "first morning" urine sample at each data collection point. Usg was assessed at the time of sample collection using standard refractometer procedures to determine dehydration status. Samples stored at room temperature for up to four hours after collection have been shown to provide accurate results (Veljkovic et al., 2012).

The refractometer was calibrated prior to testing and after every ten samples by placing distilled water on the glass as the sample, and adjusting the scale to read 1.000 to ensure that the calibration remained accurate. A small drop of urine was placed on the glass plate after ensuring it is cleaned and dried. After closing the flap, the Usg is read off the scale located inside the eyepiece by holding the refractometer up to natural light. The specific gravity reading was located at the point where the light and dark areas intersect the scale.

The results were recorded on a collection sheet for later analysis. Hydration status was determined based upon the findings of Armstrong et al. (1994), defined as: well-hydrated: Usg < 1.013; euhydrated: Usg 1.013-1.029; hypohydrated: Usg > 1.029.

4.9.6 Health and Lifestyle Questionnaire

A modified version of a diet, health and lifestyle questionnaire designed using focus groups and pilot testing with approximately 100 Australian Professional jockeys by University of Sydney was administered (Dolan et al, 2011) (Appendix 6: Lifestyle questionnaire). The questionnaire contains 16 open- and closed-ended questions pertaining to training volume, physical activity outside of racing, current injuries and injury history, fluid intake, smoking, alcohol consumption and medications or supplements. Jockeys who required assistance completing the lifestyle questionnaire had the questions read out to them and their responses were recorded.

4.9.7 Dietary intake estimation

A Dietary Questionnaire for Epidemiological Studies (DQES) was administered to assess dietary calcium (mg), vitamin D and energy intake (kJ). Appendix 7 shows an uncompleted form. The DQES form is designed to be self-administered and has adequate

reliability (Hodge et al, 2000). The participants were required to complete each answer in pencil. Jockeys who required assistance completing the DQES had the questions read out to them and their responses were recorded. Upon completion, the DQES were forwarded to the Cancer Council of Victoria for processing and raw data (in excel spreadsheet format) was returned for analysis. Calcium and energy intake was calculated as absolute daily intake and expressed as a mean daily value.

4.10 Statistical Analysis

All statistical analyses were performed using SPSS version 22.0 (SPSS, Chicago, IL, USA). Independent samples t-tests were performed on all baseline descriptive characteristics. Homogeneity of variance was tested using Levene's Test for Equality of Variances and where this was significant, the 2-tailed significance of the t-tests for equality of means was determined using the assumption that variances were not equal. Baseline descriptive data are reported as mean ± SD as applicable.

Bone variables were compared using analysis of covariance (ANCOVA) after controlling for weight, body mass and baseline bone measurements to derive regression equations to measure the effect size and probability of between-group differences (Vickers & Altman, 2001). Blood variables were controlled for baseline blood variables. All data were first checked for normality using Shapiro-Wilk test, described as the most appropriate normality test for small sample sizes (Field, 2013). Where data were found to violate the assumption of normality, they were log10 transformed prior to parametric testing and completing the ANCOVA analysis. Following the normality tests, the data were tested for homogeneity of regression by comparing group*baseline variable interactions. Homogeneity of variance assumes that the variance of one variable is relatively similar to

all levels of another variable (Field, 2013). Where this result was significant, heterogeneity of regression is found, meaning the error rate of the test is overstated and the ability to test effects is not maximized (Field, 2013). In these instances, ANCOVA should not be performed. Post-hoc Bonferroni analysis were used in pair-wise comparisons to compute confidence intervals.

Normally distributed data are presented in mean ± standard deviations (SD) throughout the results presented and have been treated with parametric analysis. ANCOVA results are presented as adjusted group means (± SE) together with adjusted mean differences (± SE). Statistical significance was set at an alpha level of 0.05 for all tests with the exception of ANCOVA where both the Levene's test for equality of variances and the group differences were significant. As the sample size was small and the group sizes were uneven, significance was set at an alpha level of 0.02. Effect size for the treatment is presented as partial η^2 where define small, medium, and large effects are defined as being based upon values of F statistic that correspond to values of partial η^2 of 0.0099, 0.0588, and 0.1379 (Richardson, 2011).

5 Effect of calcium and vitamin D supplementation on bone turnover markers and radial bone properties in young male Jockeys: A Randomised Controlled Trial

As submitted to *Journal of Sports Sciences*, January 2016. Authors: Silk, L.N, Greene, D.A, Baker, M.K and Jander, C.B.

5.1 Abstract

Purpose: Engagement in high volumes of physical activity coupled with energy restriction during periods of musculoskeletal development may compromise bone health. Young male jockeys regularly limit caloric intakes from their teens. The aim of this trial was to establish whether calcium and vitamin D supplementation would improve bone turnover markers (BTM) and non-weight bearing bone properties of young male jockeys. **Methods:** Two groups of apprentice jockeys (age=20.18 ± 3.23yrs) were supplemented with 800mg of calcium and 400IU of vitamin D (n=8) or a placebo (n=9) daily. Bone properties at the ultra-distal (4%) and proximal (66%) radius using pQCT and serum vitamin D, P1NP and CTx were assessed at baseline and six months. **Results:** ANCOVA indicated higher vitamin D levels (18.1%, p=0.014) and lower CTx (ng/L) (-24.8%, p=0.011) in the supplemented group with P1NP unchanged. No differences were observed in bone properties post-intervention. **Conclusion:** This trial is the first examining the efficacy of calcium and vitamin D supplementation in improving non-weight bearing bone properties in a young male athletic population. Analysis indicated positive alterations to bone metabolism, however longer duration appears required to detect changes in bone properties at the radius. Further examination of such interventions in weight-restricted athletes is warranted.

5.2 Introduction

Jockeys represent a unique group of weight-restricted athletes who engage in repetitive energy restriction, often from their late teens. Failure to attain PBM during growth can have adverse short and long term musculoskeletal effects (De Souza & Williams, 2005). Opportunities to maximise loading during growth are therefore essential; however, when excessive loading is coupled with inadequate nutrition, compromised bone health can occur (Ebeling, 2008; Rantalainen, Nikander, Heinonen, Suominen, & Sievänen, 2010). As a consequence, there is increasing evidence of compromised musculoskeletal health in this at-risk population (Dolan, McGoldrick, et al., 2012; Greene et al., 2013; Warrington et al., 2009).

Unlike other weight-restricted sports with defined off-seasons, jockeys are required to maintain a specific riding weight throughout a full calendar year (Hitchens et al., 2011). Additionally, strategies to maximise PBM such as resistance training and/or the addition of calcium-rich foods may be incongruous with effective weight management. One strategy available to jockeys to improve bone health involves calcium and vitamin D supplementation which has been found to elicit a positive response at the weight-bearing tibia in young jockeys (Silk, Greene, Baker, & Jander, 2015). Whilst load-bearing,upper extremities are generally not weight-bearing, highlighting the action of muscle strain in improving bone strength (Nikander et al., 2006). Previous research has found jockeys to have overall compromised bone health with positive bone adaptations at the forearm (Greene et al., 2013; Leydon & Wall, 2002) suggesting muscular forces incurred at the radius during riding may be in excess of common habitual loads. Thus, the addition of

calcium through supplementation combined with the use of forearms during riding, may provide the stimulus to improve bone properties at the radius.

Minor alterations to the distribution of bone mass or bone structure may have considerable impact on bone strength without altering overall bone mineral density (BMD) (Nikander et al., 2010). Further, measures of BMD are less responsive to loading than cortical bone (Nikander et al., 2006). Assessment of cortical bone is possible via peripheral quantitative computed tomography (pQCT) together with measures of bone size, strength and geometry. Specifically, pQCT is able to distinguish between trabecular and cortical bone, provide measures of volumetric BMD (vBMD) and quantify cross sectional area (CSA) (Khan et al., 2001). To date, however, dual energy x-ray absorptiometry (DXA) has predominantly been used to assess jockey bone health (Dolan, McGoldrick, et al., 2012; Leydon & Wall, 2002; Warrington et al., 2009). The use of DXA has a number of limitations, such as an inability to differentiate between cortical and trabecular bone or the assessment of bone size and shape with acceptable accuracy (Khan et al., 2001). Thus pQCT should allow for a more accurate assessment of potential changes in the structural properties from supplementation.

While BMD may take months or years to respond to stimuli, bone turnover markers (BTM) may detect change within days or weeks of commencing treatment (Vasikaran, Eastell, Bruyère, et al., 2011). In order to detect possible stimulus in bone turnover through supplementation, changes in BTMs should be concurrently assessed. A wide variation in both markers and methods has been used to assess bone turnover in jockeys (Dolan, McGoldrick, et al., 2012; Waldron-Lynch et al., 2010; Wilson, Fraser, et al., 2013). To standardise the assessment of BTMs it is recommended by the International

Osteoporosis Foundation (IOF) that the use of serum procollagen type I N propeptide (s-PINP) and serum C-terminal telopeptide of type I collagen (s-CTx) be adopted as markers of bone formation and bone resorption (Vasikaran, Eastell, Bruyère, et al., 2011).

Apprentice jockeys represent a vulnerable population who may potentially achieve a positive musculoskeletal response to a simple and effective intervention strategy. However, little is known about the effectiveness of calcium and vitamin D supplementation on improving BTMs and bone properties in male athletes (Silk, Greene, & Baker, 2015). Therefore, the aim of this study was to assess the efficacy of six months calcium and vitamin D supplementation in young male jockeys in improving BTMs and bone properties at the radius.

5.3 Methods

5.3.1 Participants

The present study is a further exploration of data obtained from young male jockeys who participated in a six month randomised double-blind placebo controlled trial of calcium and vitamin D supplementation (Silk, Greene, Baker, et al., 2015). Twenty-nine apprentice male jockeys (mean age 20.2± 3.2) completing a Certificate IV in Racing in New South Wales or Victoria, Australia (3 first year, 5 second year and 21 third year apprentices) were originally recruited for the study. Participants were available for testing on Apprentice School days, held one day each month. To control for selection bias, specific inclusion and exclusion criteria was used: in good health in last six months with no systemic illness lasting more than 2 weeks; no known history of fracture or recurrent fracture complications in last six months; no known history of metabolic bone or muscle disease; and no medication, hormones or calcium and/or vitamin D preparations in

preceding six months and willing to remain free of such medications for the six months of data collection. All participants provided written informed consent with parental consent provided for those under 18 years of age. Ethical approval was granted by the Human Research Ethics Committee at the Australian Catholic University (2012 114N) and the study was registered with the Australian New Zealand Clinical Trials Registry (registration no: ACTRN126000374864).

5.3.2 Research design

A double-blind placebo controlled trial was used to assess the effect of six-months calcium and vitamin D supplementation on bone material properties. Data was collected at baseline (May) and at six months (November). Participants were randomly allocated into the active (S) or placebo (P) group using a computerised four block randomisation process (www.sealedenvelope.com). Active and placebo supplements were in tablet form and were identical in colour, taste, texture and appearance. Participants were each provided with enough tablets to last the duration of the study. The active group received 800 mg calcium (citrate and carbonate) and 400 IU vitamin D together in tablet form per day (USANA Pty Ltd, Sydney, Australia) divided into two doses (morning and evening). Participants were instructed to take a double dose if they missed the morning or evening dose. At completion of the study, all used and unused containers were required to be returned in order to assess compliance. Researchers and participants were blinded to group allocation and remained blinded until after the trial was completed.

5.3.3 Anthropometric and descriptive characteristics

Standing and seated height was measured to 0.1 cm using a stadiometer and body mass was measured using an electronic scale accurate to 500g (Wedderburn UW150,

Sydney, Australia) with participants dressed in light clothing and without shoes. Skinfolds (triceps, subscapular, supraspinale, abdominale, front thigh and calf), girths (forearm and wrist), bone breadths (biepicondular humerus and wrist), and radial length (ulnar styloid and olecranon processes). These measures were taken using Harpenden skinfold calipers (Baty International, UK), flexible steel tape measure (Lufkin W606PM), segmometer (University of Western Australia), large and small sliding bone calipers (Rosscraft, Inc. Canada). All measures were made in accordance with ISAK guidelines by an accredited Level 3 ISAK Anthropometrist (LS).

A Dietary Questionnaire for Epidemiological Studies (DQES) was administered to assess dietary calcium (mg), and energy intake (kJ). The DQES form is designed to be self-administered and has adequate reliability (Hodge, Patterson, Brown, Ireland, & Giles, 2000). In addition, a modified version of a diet, health and lifestyle questionnaire previously used with jockeys (Dolan et al., 2011) was completed by each participant. Each participant was given instructions on how to complete the questionnaires and where assistance was required, questions were read out to the participant for them to answer.

5.3.4 Musculoskeletal parameters

The non-dominant radius was measured using a Stratec XCT-2000L peripheral Quantitative Computed Tomography (pQCT) bone scanner (Stratec Medizintechnic, Pforzheim, Germany) using software version 5.50d. The scanner was positioned at the anatomical reference line (cortical end plate) of the radius that corresponded to 4% (distal) and 66% (proximal) of radial length. Radial length was measured externally as the distance between the mid-point of the ulnar styloid and olecranon processes. A planar scout scan was conducted to determine an anatomical reference line. Tomographic slices

of 1 mm thickness were obtained at the 4% and 66% tibia measured distally. Scan speed and voxel size were 30 mm/s and 0.5 mm respectively. Contour mode 1 with a threshold of 180mg/cm^3 was used to separate soft tissue and bone in order to analyse trabecular bone. Cortical bone was identified and removed using a constant default threshold of 711mg/cm^3. A region of interest (ROI) to identify the radius was automatically identified with manual adjustments made as necessary to ensure the entire radial ROI was enclosed. The precision of repeat measurements on the pQCT in our laboratory is 0.7% to 1.4% radius after repositioning in eight adults (Greene et al., 2013). Scans were performed at baseline and six months by the same investigator (DG).

5.3.5 Markers of bone turnover and vitamin-D

Blood samples were collected by a qualified phlebotomist (CJ) at the same time of day at each data collection period. Ten ml blood was drawn using a lithium heparin collection tube and each sample was centrifuged within 15 minutes at 4000 rpm using a Centurion centrifuge (Scanspeed406G, Labogene, Scandanavia). Clear plasma was transferred into three eppendorf tubes with aliquot, housed temporarily in an ice box and then stored at -80^{o}C within six hours of blood draw. Blood samples were analysed for Procollagen type 1 N propeptide (P1NP) (ug/L), C-terminal telopeptide of type 1 collage (CTx) (ng/L), and serum 25-hydroxy vitamin D [25(OH)D] (nmol/L).

All blood samples were tested by a NATA accredited external laboratory (Melbourne Pathology, Victoria, Australia). A DIALAB 25-OH Vitamin D total ELISA kit, using a solid phase enzyme-linked immunosorbent assay was used to measure 25(OH)D (nmol/L). Intra-assay coefficient of variation (CV) was 3.2% (n=20) and the inter-assay CV was 6.9% (n=30). Bone markers were analysed using enzyme-linked immunosorbent assay

kits (Cloud-Clone Corp., USA) for cross linked c-telopetide type I collagen (CTx) and procollagen 1 N-terminal propeptide (P1NP). Intra-assay coefficient of variation (CV) for CTx and P1NP were <10% and the inter-assay CV was <12%.

5.3.6 Statistical methods

Data were tested for normality using Shapiro-Wilk test and t-tests were performed on baseline characteristics. Normally distributed data are presented in mean ± standard deviations (SD) and treated with parametric analysis. Baseline descriptive data are reported as mean ± SD as applicable. Bone variables were compared using analysis of covariance (ANCOVA) to derive regression equations to measure the effect size and probability of between-group differences after controlling for body mass, height and baseline bone measurements. Data used in ANCOVA was tested for homogeneity of regression. Statistical analyses were performed using SPSS version 22.0 (SPSS, Chicago, IL, USA). A sample size of 9 per group enabled detection of a significant difference if the mean key outcome variable (BSI at 4% and SSI at 66% sites) for the supplemented group was at least 1.5 SD higher than the mean value in the control group. To allow for a 35% drop out, at least 15 jockeys were allocated to each group (power = 80%, $p>0.05$).

5.4 Results

5.4.1 Descriptive results

Twenty-nine jockeys were originally recruited to the study and subsequently randomised into either the placebo or intervention group. Of the original 29 participants, 17 were available for follow-up measurements. A number of jockeys had relocated outside of data collection areas or were required to race on the assigned data collection day. All other jockeys completed the six month intervention (refer Figure 4-1).

Baseline characteristics indicate the two groups were homogenous, with no significant differences in age, height or body mass (Table 5-1). Anthropometric characteristics reveal a high degree of similarity between forearm characteristics of the two groups with no differences found at baseline or six months. Similarly, no differences between groups were observed in either body mass index (BMI) or sum of six skinfolds. Dietary analysis revealed wide variations in total kilojoules in both groups; however, mean values were not statistically different. Mean calcium intake (excluding supplementation) in each group was well below standard dietary recommendations of 1,000 mg per day (National Health and Medical Research Council, 2006a) while baseline serum vitamin D was above minimum recommendations of 50 nmol/L (National Health and Medical Research Council, 2006b).

Table 5-1: Characteristics for participants completing the study

	Baseline			Six months		
	Supplement group (n=8) Mean (SD)	Placebo group (n=9) Mean (SD)	*p*	Supplement group (n=8) Mean (SD)	Placebo group (n=9) Mean (SD)	*p*
Age (yrs)	22.3 (5.0)	19.3 (1.8)	0.152	22.9 (5.0)	19.9 (1.8)	0.151
Height (cm)	165.6 (4.4)	167.3 (4.3)	0.517	166.3 (4.8)	167.7 (4.1)	0.521
Body mass (kg)	52.7 (3.6)	52.6 (3.3)	0.453	53.7 (3.6)	53.8 (3.9)	0.933
BMI ($kg \cdot m^2$)	19.3 (1.7)	18.8 (1.1)	0.507	17.3 (6.7)	19.1 (1.3)	0.429
Sum of six skinfolds (mm)	34.4 (5.9)	32.9 (5.1)	0.588	36.4 (7.0)	34.6 (7.2)	0.602
Forearm girth (cm)	25.0 (0.7)	24.7 (0.8)	0.490	25.1 (0.5)	24.9 (0.3)	0.596
Wrist girth (cm)	15.5 (0.4)	15.8 (0.5)	0.243	15.4 (0.4)	15.6 (0.4)	0.359
Biepicondular humerus (cm)	6.6 (0.3)	6.7 (0.3)	0.848	6.7 (0.4)	6.8 (0.3)	0.405
Wrist breadth (cm)	5.5 (0.2)	5.6 (0.2)	0.476	5.6 0.3)	5.6 (0.2)	0.720
Hydration levels (Usg)	1.022 (0.01)	1.020 (0.01)	0.348	1.020 (0.01)	1.019 (0.01)	0.705
Energy intake ($kJ \cdot day^{-1}$)	7,723 (2,974)	8700 (2,454)	0.469	9,035 (6,044)	9,626 (4,758)	0.825
Alcohol consumption ($g \cdot day^{-1}$)	16.1 (18.7)	3.2 (2.7)	0.092	17.3 (17.7)	12.1 (14.8)	0.518
Calcium intake ($mg \cdot day^{-1}$)	669.7 (274.3)	790.4 (423.9)	0.503	740.3 (481.5)	888.7 (363.6)	0.481
25OH Vit D ($nmol \cdot L^{-1}$)	64.6 (19.5)	81.2 (24.4)	0.146	75.6 (20.8)	73.4 (20.8)	0.832

Three-quarters (n=21) of the jockeys had been training for three years. Results from the health and lifestyle questionnaire revealed that 90% (87% S, 93% P) reported completing in excess of six or more hours training per week. One third reported engaging in additional physical activity outside of training. Sixty per cent of the jockeys reported having previous fractures, with half of the supplemented group and one third of placebo group experienced fractures due to a riding injury. Approximately 87% of supplemented group and 57% of placebo group reported drinking alcohol. Smoking habits showed 40% of the supplemented group and 14% of placebo group smoked on a daily basis

5.4.2 Bone variables

Radial bone variables were evaluated at the 4% distal and 66% proximal sites. Group means (± SD) for baseline and six months together with adjusted mean differences (± SE) and 95% confidence intervals for mean differences are presented for both sites (Table 5-2). After controlling for any variations in baseline height, body mass or bone variability, no post-intervention differences were observed in trabecular, cortical or total-area, content or density; total bone area; pericortical or endocortical circumferences. Similarly, no significant post-intervention differences were found in muscle area or in bone strength indices at either the 4% or 66% sites.

5.4.3 Bone turnover markers and vitamin D

Serum Vitamin D levels were higher in the placebo group at baseline (81.2 ±24.4 $nmol \cdot L^{-1}$ vs 64.6 ±19.5 $nmol \cdot L^{-1}$), however this was not significantly different (p=0.146). At six months, unadjusted values for 25(OH)D had increased to 75.6 (±20.8) $nmol \cdot L^{-1}$ for the supplemented group while unadjusted the placebo groups levels fell slightly to 73.4

(±20.8) nmol·L^{-1}. At six months, ANCOVA indicated 25OH(D) levels were 18% higher in the supplemented group than the placebo group (p= 0.014).

No difference in BTMs was evident between groups at baseline. Bone resorption marker levels (CTx) decreased, both in absolute terms and on an adjusted mean basis in the supplemented group. Baseline values were similar at 371.3± 201.0 (S) vs 380.0 ± 141.1 (P) while at six months they had fallen in the supplemented group and increased in the placebo group. Between group differences showed CTx to be almost 25% lower in the supplemented group (p=0.011) while no post-intervention differences were observed in the bone formation marker, P1NP between groups (refer Table 5-3).

Table 5-2: Baseline and adjusted six month bone variables at the 4% distal site and 66% proximal radius for supplemented (S) and placebo (P) groups after covarying for baseline height, weight and bone variables.

	Baseline values		Six mth values		ANCOVA results six months		
	S group (n=8) mean (SD)	P group (n=9) mean (SD)	S group (n=8) mean (SD)	P group (n=9) mean (SD)	Adj Mean diff (SE)	95% CI for diff	p-value
Distal radius 4% site							
Trabecular area (mm^2)	207.49 (27.69)	205.17 (17.50)	206.74 (32.29)	204.50 (17.03)	2.65 (5.17)	-8.61 to 13.91	0.618
Trabecular density ($mg \cdot cm^3$)	239.11 (43.57)	226.59 (46.80)	231.21 (34.70)	215.43 (40.82)	4.88 (11.27)	-19.66 to 29.44	0.672
Total content (mg.mm)	149.50 (19.30)	145.87 (19.35)	149.87 (22.27)	149.00 (18.86)	-3.94 (5.56)	-16.19 to 8.32	0.495
Total area (mm^2)	461.34 (61.52)	456.14 (38.88)	459.67 (71.78)	454.75(37.84	5.81 (11.50)	-19.25 to 30.88	0.623
Total density ($mg \cdot cm^3$)	338.05 (40.82)	317.95 (43.41)	336.71 (47.20)	315.85 (33.48)	1.37 (8.82)	-17.83 to 20.59	0.879
Bone strength index ($mg^2 \cdot mm^4$)	52.66 (11.45)	46.63 (12.31)	51.71 (11.40)	45.84 (10.66)	0.65 (2.86)	-5.58 to 6.88	0.825
Proximal radius 66% site							
Cortical area (mm^2)	85.55 (12.81)	80.68 (8.71)	85.75 (11.64)	80.57 (8.63)	0.57 (0.66)	-0.867 to 2.01	0.404
Cortical density ($mg \cdot cm^3$)	1119.87 (43.66)	1114.28 (16.00)	1132.62 (38.46)	1121.27 (23.87)	10.05 (11.80)	-15.66 to 35.78	0.411
Cortical content (mg.mm)	111.87 (17.77)	106.37 (12.18)	111.25 (18.1)	106.37 (11.55)	-0.52 (1.24)	-3.25 to 2.22	0.685
Total bone area (mm^2)	305.77 (28.40)	310.32 (45.61)	305.33 (22.60)	311.84 (46.40)	-5.79 (8.72)	-24.79 to 13.21	0.519
SSI-Polar (mm^3)	302.55 (64.43)	302.63 (84.23)	328.73 (155.65)	363.15 (126.33)	-31.09 (61.50)	-165.09 to 102.90	0.622
Muscle Area (mm^2)	4141.91 (281.41)	4127.49 (219.55)	4189.00 (256.25)	4138.82 (378.00)	-5.4 (109.24)	-243.38 to 232.58	0.961
Endocortical circumference (mm)	26.12 (2.24)	28.18 (4.04)	26.62 (3.62)	28.27 (3.99)	-1.54 (2.17)	-6.22 to 3.32	0.517
Pericortical circumference (mm)	41.95 (2.67)	42.70 (3.67)	42.25 (2.84)	42.87 (3.53)	-0.32 (1.78)	-4.24 to 3.59	0.859

Table 5-3: Baseline and six month mean values together with adjusted mean differences (95% CI) in blood variables: vitamin D levels, CTx and P1NP between supplemented (S) and placebo (P) groups after covarying for baseline blood variables.

	Unadjusted baseline values		ANCOVA six-months adjusted values					
	S group (n=8) Mean (SD)	P group (n=8) Mean (SD)	S group (n=8) Adj Mean (SE)	P group (n=8) Adj Mean (SE)	Adjusted Mean diff (SE)	95% CI diff	partial Eta^2	p-value
25OH Vit D (nmol/L)	64.6 (19.5)	81.2 (24.4)	81.9 (3.6)	67.1 (3.6)	14.8 (5.2)	3.6 to 26.1	0.38	***0.014***
CTx (ng/L)	371.3 (201.0)	380.0 (141.1)	357.5 (21.3)	446.3 (21.3)	-88.8 (30.2)	-154.0 to -23.6	0.40	***0.011***
P1NP (ug/L)	104.2 (46.4)	108.9 (31.6)	107.3 (5.7)	101.9 (5.7)	5.4 (8.0)	-11.9 to 22.7	0.03	0.511

5.5 Discussion

Young jockeys clearly have compromised bone health (Dolan, Crabtree, et al., 2012; Greene et al., 2013; Warrington et al., 2009), however no research to date has examined the efficacy of strategies for improvement in this at-risk population. This is the first randomised controlled trial (RCT) to assess markers of bone turnover and non-weight bearing bone responses to six months of calcium and vitamin D supplementation in young male jockeys or any other male athletic population known to have compromised bone health. Results demonstrate supplementation stimulated a response in bone turnover markers (BTM) through a reduction in bone resorption indicators, however this was not reflected in alterations to bone material properties.

Typically, jockeys have high bone turnover linked to low energy and calcium intakes (Dolan, McGoldrick, et al., 2012; Waldron-Lynch et al., 2010; Wilson, Fraser, et al., 2013). Despite being within reference ranges, CTx levels were in the bottom 5th percentile while P1NP levels were approximately 30% higher than averages for 19-30 year old males (Jenkins et al., 2013). Results for CTx were in line with some previous findings (Wilson, Fraser, et al., 2013) but contrasting with other research showing high levels of bone resorption (Dolan, McGoldrick, et al., 2012; Waldron-Lynch et al., 2010). Possible explanations for such differences are variations in the bone resorption markers analysed or the mean age of the participants. Jockeys in the current study averaged five years younger than those previously reported. Following the intervention period, bone resorption levels (CTx) were significantly lower in the supplemented group. Conversely, both groups continued to demonstrate elevated levels of bone formation (P1NP) over the intervention period. While this may be in part due to the age of the participants, high

levels of P1NP are supported by previous research examining jockey bone turnover (Waldron-Lynch et al., 2010).

Calcium kinetics indicate supplementation results in an increase in absorbed calcium and suppression of bone resorption (Wastney et al., 2000). Acute BTM marker responses in male athletes undertaking endurance cycling demonstrate a suppression of bone resorption (CTx) in the presence of calcium loading (Guillemant, Accarie, Peres, & Guillemant, 2004). Chronic BTM responses to calcium supplementation in athletic populations are unknown to date. However, supplementation with 1000mg calcium and 5µg vitamin D daily for a period of 16 weeks in pre-menopausal females resulted in a >30% decrease in CTx, despite baseline calcium intakes approaching adequate intakes (Kruger et al., 2006). Differing from this group; however, P1NP levels were 15% lower after 16 weeks, possibly due to age differences between the participants. In older males, calcium supplementation is also associated with reductions in CTx (-14%) and P1NP (-16%) without significant changes to BMD (Kukuljan, Ducher, Nowson, Ebeling, & Daly, 2009). In the current study, it is speculated that decreased resorption markers, combined with no alteration to bone formation markers potentially increased calcium availability resulting in a reduction in bone resorption.

Calcium and vitamin D are known to act synergistically in order to provide adequate mineralisation during growth and maintenance of the skeleton through adulthood (Bailey et al., 1996). Results of meta-analyses indicate greater bone gain in younger populations and an attenuation of bone loss in the elderly (Chung et al., 2009; Heaney & Weaver, 2005; Lips et al., 2014; Tang et al., 2007). However this has not been adequately determined in young male populations, and in particular male athletes (Silk,

Greene, & Baker, 2015). Previous calcium and vitamin D supplementation trials using male participants have predominantly used DXA to assess changes to bone properties with improvements to BMD found within six-months in healthy older males (Reid et al., 2008). Supplementation over the course of 12 months in competitive male road cyclists failed to ameliorate BMD (Barry & Kohrt, 2008). Examination of bone responses using pQCT to calcium and vitamin D supplementation at the weight-bearing tibia in young jockeys indicate a positive response (Silk, Greene, Baker, et al., 2015). However, the stimulus provided at the tibia differs from that of the radius which is typically non-weight bearing. Consequently, the current study provides new knowledge about bone responses to calcium supplementation in young athletic adult males.

Athletes in sports where use of the lower limbs are stressed and general populations who undertake high levels of physical activity show little difference in bone strength in the radius compared to those undertaking low levels of habitual physical activity (Duckham et al., 2014; Wilks et al., 2009). A previous analysis conducted with female participants examining the interaction between physical activity and calcium intake indicated exercise had no discernible effect on distal radius BMD at calcium intakes below 1000mg·day^{-1} (Specker, 1996). Conversely, elite athletes such as tennis players and young gymnasts who undergo regular impact loads and strain at the radius demonstrate greater bone properties at the radius (Dowthwaite et al., 2009; Haapasalo et al., 2000; Ireland et al., 2013). Similarly, jockeys have been found to demonstrate positive bone effects in the forearm (Greene et al., 2013; Leydon & Wall, 2002). It was postulated that additional calcium made available through supplementation, combined with additional exercise-induced strains in excess of habitual loads experienced at the radius, may have produced improvements in bone properties. However, this was not evident over the

duration of the intervention, suggesting that greater stimulus and / or intervention period may be required to produce osteogenic responses to non-weight bearing bone.

Poor bone health has been shown to arise through excessive energy expenditure coupled with restricted energy intake, typical of a number of athletic populations (Fredericson et al., 2007; Greene et al., 2013; Hind et al., 2006; Nichols & Rauh, 2011; Smathers et al., 2009). Jockeys in this study demonstrated insufficient energy intake and low calcium levels supporting previous findings (Greene et al., 2013; Leydon & Wall, 2002; Warrington et al., 2009). Daily energy consumption was below recommended minimum energy intakes of approximately 188-210kj·kgbw·day^{-1} for athletes (Sundgot-Borgen & Garthe, 2011), averaging between 144-152kj·kgbw·day^{-1}. Very low sum of six skinfolds observed in this group of jockeys places them in the bottom 5th percentile for skinfold measures in athletic populations (Garrido-Chamorro, Sirvent-Belando, González-Lorenzo, Blasco-Lafarga, & Roche, 2012), and body mass index for this group was also below levels reported by other groups of jockeys (Wilson et al., 2014), further substantiating the energy imbalance which may be negatively effecting bone properties. Apprentice jockeys undertake around 25 hours per week of rigorous physical activity (Greene et al., 2013), similar to observations made in cyclists and endurance runners (Barry & Kohrt, 2008; Fredericson et al., 2007; Hind et al., 2006) who also report low BMD.

A number of strengths and limitations exist within this study. Using pQCT to assess bone properties has enabled a more detailed analysis of the adaptations that occurred at the proximal and distal radius as a result of calcium and vitamin D supplementation. By using a randomised controlled study design, we have minimised selection bias and attempted to reduce possible genetic influence on bone properties. Additionally, we have

statistically controlled for baseline variations in body composition and bone variables. Serum analysis of bone formation and resorption markers were conducted in accordance with recommendations from the IOF (Vasikaran, Eastell, Bruyère, et al., 2011) with results indicating that supplementation positively affected bone metabolism. It is recognised that, given a longer intervention period, bone outcomes may have reflected further improvement. However, the number of drop-outs that may have occurred beyond six months would have further compromised outcomes. Testing opportunities were limited to one day per month, making follow-up difficult. Further, the participants were subject to unforeseen relocation and race riding requirements on the allocated testing days. The number of drop-outs during the study reduced the sample size which has impacted upon the power of the study and we acknowledge that outcomes would have been strengthened if participants lost to follow up were minimised. Despite instructions to return all used and unused supplement containers as the final data collection period, few jockeys returned the containers as instructed. Verbal assurances were received from the participants regarding compliance with the supplementation regime at the time of data collection, and attempts to follow-up with regard return of containers proved fruitless. Nonetheless, positive vitamin D blood results suggest that jockeys in the supplemented group were compliant throughout the six-month intervention period.

This is the first randomised controlled trial to examine the effects of calcium and vitamin D supplementation on BTMs and non-weight bearing bone properties in young male jockeys using pQCT. While BTMs suggest supplementation may be influencing bone metabolism, our findings indicate that 800mg of calcium and 400IU of vitamin D per day for a period of six months is not an adequate duration to produce positive bone responses at the distal and proximal radius. This is in contrast to weight-bearing limbs

which appear to respond more rapidly. Extended supplementation in this cohort and other at-risk athletic populations demonstrating compromised bone health would additionally improve our understanding of structural changes to bone properties arising from the synergistic benefits calcium and vitamin D supplementation.

6 Tibial bone responses to 6-month calcium and vitamin D supplementation in young male Jockeys: A randomised controlled trial

As published in *Bone*, 2015, vol 81, pp 554-561. Authors: Silk, L.N, Greene, D.A, Baker, M.K and Jander, C.B.

6.1 Abstract

Young male jockeys compromise bone health by engaging in caloric restriction and high volumes of physical activity during periods of musculoskeletal growth and development. The aim of this randomised, double-blinded, placebo-controlled trial was to establish whether calcium and vitamin D supplementation would improve bone properties of young male jockeys. We conducted a 6-month trial with two groups of weight-, height- and age-matched apprentice male jockeys (age=20.2 ±3.2yrs). Participants were supplemented with 800mg of calcium and 400IU of vitamin D (S, n=8) or a placebo (cellulose) (P, n=9) daily for 6-months. Baseline calcium intake was (669.7±274.3 (S) vs 790.4±423.9 (P) and vitamin D 64.6±19.5 (S) vs 81.2 ±24.4 (P) with no statistical differences. Peripheral quantitative computed tomography (pQCT) measured ultra-distal (4%) and proximal (66%) tibial bone properties at baseline and 6 months. Blood-borne markers of bone turnover, P1NP and CTx and vitamin D concentration were assessed. After co-varying for height, weight and baseline bone measurements, the supplemented group displayed greater post-intervention bone properties at the 66% proximal site with cortical content (mg·mm) 6.6% greater ($p<0.001$), cortical area (mm^2) 5.9% larger ($p<0.001$), cortical density ($mg \cdot cm^3$) 1.3% greater ($p=0.001$), and total area (mm^2) 4% larger ($p=0.003$). No other between group differences in bone variables were observed. Blood analysis indicated higher vitamin D levels (18.1%, $p=0.014$) and lower CTx (ng/L) (-24.8%, $p=0.011$) in the supplemented group with no differences observed in

P1NP. This is the first randomised controlled trial to examine the efficacy of calcium and vitamin D supplementation in improving bone properties in a highly vulnerable, young athletic, weight-restricted population. Results using pQCT indicate beneficial effects of supplementation on bone properties in as little as six months. Although the study size is small, this intervention appears promising as a strategy for improving bone health in young athletes in weight-restricted sports.

KEYWORDS: Cortical bone, Peripheral QCT, young males, calcium, supplementation

6.2 Introduction

Jockeys represent a unique group of weight-category athletes, required to maintain their restricted weight throughout a full calendar year instead of seasonal competitions (Hitchens et al., 2011). Participation in weight restricted activity may limit the attainment of PBM during growth which could have deleterious short and long term musculoskeletal effects (De Souza & Williams, 2005). Typically, jockeys enter their profession around 16 years of age when they are still to attain PBM. Approximately 90% of male PBM occurs by 18-20 years of age, however this appears to be site specific (Baxter-Jones et al., 2003; Henry et al., 2010; Lorentzon et al., 2005; Szulc et al., 2000). Whilst loading during growth predominantly determines bone size and shape during adulthood (Rantalainen et al., 2010) other environmental factors, including diet, are known to influence male bone (Ebeling, 2008). Recommended strategies to improve bone health are not always feasible and/or acceptable to jockeys. The weight restricted nature of riding precludes additional gains in muscle mass from strength exercise and there is a belief, albeit unsupported with scientific fact, that adding calcium-rich foods, such as dairy products, may also be incongruous with weight management demands. One

strategy to potentially maximise the attainment of PBM during growth in young jockeys involves calcium and vitamin D supplementation. However, there is scant evidence regarding the beneficial effects of calcium and vitamin D supplementation on bone mineral density in male athletic populations (Silk, Greene, & Baker, 2015). Accordingly, we have an imperfect understanding of the role of supplementation on bone development in this at-risk population.

Male apprentice jockeys in New South Wales (Australia) are recommended to have a body weight 45kg to 48kg (Racing-NSW, 2014), placing them in lowest 5th percentile for international weight-for-age scales (Kuczmarski et al., 2000). To remain within specific weight limits, jockeys often engage in unhealthy weight-loss behaviours relying on rapid, short-term weight loss and demonstrate an increased propensity to engage in disordered eating (Leydon & Wall, 2002; Moore et al., 2002). Thus there is a high risk of inadequate nutrition in an effort to maintain low body weight. Previous research has found jockeys to have low calcium intakes and subsequent indicators of compromised musculoskeletal health (Caulfield & Karageorghis, 2008; Dolan, McGoldrick, et al., 2012; Dolan et al., 2011; Greene et al., 2013; Leydon & Wall, 2002; Moore et al., 2002; Waldron-Lynch et al., 2010; Warrington et al., 2009) and there is evidence of inadequate serum vitamin D, particularly in the northern hemisphere (Close et al., 2012; Guillemant et al., 2001; Waldron-Lynch et al., 2010; Wilson, Fraser, et al., 2013; Wilson, Sparks, et al., 2013). Approximately 50% of jockeys demonstrating osteopenia as young as 20 years of age (Leydon & Wall, 2002; Warrington et al., 2009) and apprentice riders displaying reduced bone strength (Greene et al., 2013).

Current assessment of jockey bone health has predominately used dual energy x-ray absorptiometry (DXA) (Dolan, Crabtree, et al., 2012; Dolan, McGoldrick, et al., 2012; Hitchens et al., 2011; Leydon & Wall, 2002; Warrington et al., 2009) with only one study using pQCT (Greene et al., 2013). The two-dimensional nature of DXA has a number of inherent limitations, being unable to differentiate between cortical and trabecular bone, and difficulty in quantifying bone size and shape with acceptable accuracy (Khan et al., 2001). Small alterations in the distribution of bone mass or bone structure may have considerable impact on bone strength without altering overall BMD (Nikander et al., 2010). Peripheral quantitative computed tomography (pQCT) provides a more accurate assessment of bone size, strength and geometry (Khan et al., 2001). Specifically, pQCT differentiates between trabecular and cortical bone, provides a measure of volumetric BMD (vBMD), and quantifies cross sectional area (CSA) of bone. It is suggested that cortical bone appears to be more responsive to loading than BMD (Nikander et al., 2006), thus pQCT should allow for a more accurate assessment of potential changes in the structural properties through supplementation. While BMD can take months or years to respond to stimuli, bone turnover markers (BTM) may detect change within days or weeks of treatments beginning (Vasikaran, Eastell, Bruyère, et al., 2011). Changes in BTMs have been assessed in jockeys; however, a wide variation in markers and methods exists (Dolan, McGoldrick, et al., 2012; Waldron-Lynch et al., 2010; Wilson, Fraser, et al., 2013). The current recommended markers of bone formation and bone resorption are serum procollagen type I N propeptide (s-PINP) and serum C-terminal telopeptide of type I collagen (s-CTx) (Vasikaran, Eastell, Bruyère, et al., 2011).

The efficacy of calcium and vitamin D supplementation on improving bone properties, particularly in male athletes and younger male age cohorts remains unknown

(Silk, Greene, & Baker, 2015). It appears, however, that populations who incur the greatest compromises to musculoskeletal health may also benefit the most from calcium and vitamin-D supplementation (Winzenberg et al., 2010). Apprentice jockeys represent an at-risk population who may potentially achieve a positive musculoskeletal response to a simple and effective intervention strategy. Therefore, the aim of this study was to assess the efficacy of 6-months calcium and vitamin D supplementation in young male jockeys in improving bone properties at the tibia.

6.3 Methods

6.3.1 Participants

From a total pool of 40 apprentice male jockeys, 30 jockeys were available for recruitment to the study. After excluding one jockey for health reasons, twenty-nine apprentice male jockeys aged 16 to 32 years (mean age 20.2± 3.2) representing 72.5% of all male apprentices were recruited to participate. All participants were completing a Certificate IV in Racing in New South Wales or Victoria, Australia (3 first year, 5 second year and 21 third year apprentices). Participants were only available for testing on Apprentice School days which were held one day each month. All other days, they were apprenticed to a Trainer and required to train or ride and were therefore unavailable for testing. To control for selection bias, specific inclusion and exclusion criteria was used: in good health in last 6 months with no systemic illness lasting more than 2 weeks; no known history of fracture or recurrent fracture complications in last 6 months; no known history of metabolic bone or muscle disease; and no medication, hormones or calcium/vitamin D preparations in preceding 6 months and willing to remain free of such medications for the 6 months of data collection. All participants provided informed

consent. Ethical approval was granted by the Human Research Ethics Committee at the Australian Catholic University and the study was registered with the Australian New Zealand Clinical Trials Registry (registration no: ACTRN12612000374864).

6.3.2 Research design

A double-blind placebo controlled trial was used to assess the effect of 6-months calcium and vitamin D supplementation on bone material properties. Data were collected at baseline and at six months. Participants were randomly allocated into the active (S) or placebo (P) group using a computerised four block randomisation process (www.sealedenvelope.com). Active and placebo supplements were in tablet form and were identical in colour, taste, texture and appearance. Participants were each provided with enough tablets to last the duration of the study. The active group received 800 mg calcium (citrate and carbonate) and 400 IU vitamin D3 (Cholecalciferol) in tablet form per day (USANA Pty Ltd, Sydney, Australia) divided into two doses (morning and evening). The placebo comprised predominantly cellulose (Microcrystalline cellulose 814.42mg·g^{-1}). Researchers and participants were blinded to group allocation and remained blinded until after the trial was completed.

6.3.3 Anthropometric and descriptive characteristics

Standing and seated height was measured to 0.1 cm using a stadiometer and weight was measured using an electronic scale accurate to 500g (Wedderburn UW150, Sydney, Australia) with participants dressed in light clothing and without shoes. Tibial (tibiale mediale to malleolus mediale) length was also measured. Measures were made in accordance with ISAK guidelines by an accredited Level 3 ISAK Anthropometrist (LS).

A Dietary Questionnaire for Epidemiological Studies (DQES) was administered to assess dietary calcium (mg), and energy intake (kJ). The DQES form is designed to be self-administered and has adequate reliability (Hodge et al., 2000). In addition, a modified version of a diet, health and lifestyle questionnaire previously used with jockeys (Dolan et al., 2011) was completed by each participant. Years of training, frequency of training, other activities outside of riding, injury rates and types, and smoking and alcohol consumption were assessed. Each participant was given instructions on how to complete the questionnaires and where assistance was required, questions were read out to the participant for them to answer.

6.3.4 Bone material properties and fracture risk

The non-dominant tibia was measured using a Stratec XCT-2000L peripheral Quantitative Computed Tomography (pQCT) bone scanner (Stratec Medizintechnic, Pforzheim, Germany) using software version 5.50d. The scanner was positioned at the anatomical reference line (cortical end plate) of the tibia that corresponded to 4% (distal) and 66% (proximal) of tibial length. Tibial length was measured externally as the distance between the mid-point of the distal medial malleolus and the proximal medial tibial plateau landmarks. A planar scout scan was conducted to determine an anatomical reference line. Tomographic slices of 1 mm thickness were obtained at the 4% and 66% tibia measured distally. Scan speed and voxel size were 30 mm/s and 0.5 mm respectively. A contour mode with a threshold of 180mg/cm^3 was used to separate soft tissue and bone in order to analyse trabecular bone. Cortical bone was identified and removed using a constant default threshold of 711mg/cm^3. A region of interest (ROI) to identify the tibia was automatically identified with manual adjustments made as necessary to ensure the entire tibial ROI was enclosed. Volumetric bone mineral density

(vBMD), bone geometry, and bone strength, were assessed at the two scanned sites. Outcome measures included trabecular and cortical density (mg/cm^3) and content (mg·mm), cortical cross-sectional area (mm^2), total cross-sectional area (mm^2), cortical thickness (mm), and a surrogate marker of bone strength, namely stress strain index (SSI in mm^3). Estimates of bone strength (BSI in mg^2·mm^4) were also made using the formula: total area x (total density x 0.001)^2 at the 4% site. The precision of repeat measurements on the pQCT in our laboratory is 0.7% to 1.4% radius and 0.8% to 2.9% tibia after repositioning in eight adults (Greene et al., 2013). Scans were performed at baseline and 6-months by the same investigator (DG).

6.3.5 Markers of bone turnover and Vitamin-D

Blood samples were collected by a qualified phlebotomist (CJ) at the same time of day at each data collection period. Ten ml blood was drawn using a lithium heparin collection tube and each sample was centrifuged within 15 minutes at 4000 rpm using a Centurion centrifuge (Scanspeed406G, Labogene, Scandanavia). Clear plasma was transferred into three eppendorf tubes with aliquot, housed temporarily in an ice box and then stored at -80^{o}C within six hours of blood draw. Blood samples were analysed for Procollagen type 1 N propeptide (P1NP) (ug/L), C-terminal telopeptide of type 1 collage (CTx) (ng/L), and serum 25-hydroxy vitamin D [25(OH)D] (nmol/L). A DIALAB 25-OH Vitamin D total ELISA kit, using a solid phase enzyme-linked immunosorbent assay was used to measure 25(OH)D (nmol/L). Using a denaturation buffer to extract 25(OH)D from Vitamin D binding protein (VDBP), samples were pre-treated in separate vials. After mixing with enzyme conjugate and enzyme complex, the samples were transferred to microtiter plate wells. Incubation for 60 minutes at 37 degrees was followed by a washing step and a colour reaction was stopped after 15 minutes at room temperature. All wells

were read within 10 minutes after the addition of the stop solution. Intra-assay coefficient of variation (CV) was 3.2% (n=20) and the inter-assay CV was 6.9% (n=30). All blood samples were tested by a NATA accredited external laboratory (Melbourne Pathology, Victoria, Australia).

6.3.6 Hydration status

Urine specific gravity (Usg) was analysed using a handheld refractometer. Participants were provided with urine sample jars and instructed to provide a "first morning" urine sample at each data collection point. Calibration was repeated after every ten samples to ensure accuracy and consistency. Urine specific gravity was assessed at the time of sample collection with the reading taken at the point where the light and dark areas intersect the scale. Hydration status was defined as: well-hydrated Usg < 1.013; euhydrated Usg 1.013-1.029; hypo-hydrated Usg > 1.029 (Armstrong et al., 1994).

6.3.7 Statistical methods

All variables were tested for normality using Shapiro-Wilk test and t-tests were performed on baseline characteristics. Normally distributed data are presented in mean ± standard deviations (SD) and treated with parametric analysis. Baseline descriptive data are reported as mean ± SD as applicable. Bone variables were compared using analysis of covariance (ANCOVA) to derive regression equations to measure the effect size and probability of between-group differences (Vickers & Altman, 2001) after controlling for weight, height and baseline bone measurements. Data used in ANCOVA was tested for homogeneity of regression by examining the statistical significance of the interaction of covariates and the independent variables. Statistical analyses were performed using SPSS version 22.0 (SPSS, Chicago, IL, USA). A sample size of 9 per group will allow us to detect a

significant difference if the mean key outcome variable (BSI at 4% and SSI at 66% sites) for the supplemented group is at least 1.5 SD higher than the mean value in the control group. To allow for a 35% drop out, at least 15 jockeys will be allocated to each group (power = 80%, p>0.05) (Peat & Barton, 2005).

6.4 Results

Twenty-nine jockeys were originally randomised into either the placebo or intervention group after excluding one available jockey. Of the original 29 participants, 17 were available for follow-up measurements. A number had been apprenticed to new trainers located outside of data collection areas during the intervening period making them unavailable for follow-up, whilst others were required to race on the assigned data collection day. All other jockeys completed the six month intervention which took place between October 2013 and May 2014 (Figure 4-1).

6.4.1 Descriptive characteristics

Analysis of baseline characteristics revealed the two groups were homogenous with no significant differences in age, height or weight (Table 6-1). There were no significant differences between the groups at baseline or six months in hydration levels, nor in dietary variables. Dietary analysis revealed wide variations in total kilojoules in both groups; however, mean values were not statistically different. Average calcium intake in each group was well below standard dietary recommendations of 1,000 mg per day for men aged 18-30 years (National Health and Medical Research Council, 2006a). Excluding the effects of supplementation, no differences were seen between baseline and six month in hydration status, total energy intake, macronutrient or calcium intakes (see Table 6-1).

Table 6-1: Descriptive characteristics at baseline and six months for participants completing the trial

	Baseline values					Six months values				
	Supplement group (n=8)		Placebo group (n=9)			Supplement group (n=8)		Placebo group (n=9)		
	mean	SD	Mean	SD	p	mean	SD	Mean	SD	p
Age (yrs)	22.3	5.0	19.3	1.8	0152	22.9	5.0	19.9	1.8	0.151
Height (cm)	165.6	4.4	167.3	4.3	0.517	166.3	4.8	167.7	4.1	0.521
Weight (kg)	52.7	3.6	52.6	3.3	0.453	53.7	3.6	53.8	3.9	0.933
BMI ($kg \cdot m^2$)	19.3	1.7	18.8	1.1	0.507	19.4	1.7	19.1	1.3	0.682
Hydration levels (Usg)	1.022	0.01	1.020	0.01	0.348	1.020	0.01	1.019	0.01	0.705
Energy intake ($kJ \cdot day^{-1}$)	7,723	2,974	8700	2,454	0.469	9,035	6044	9,626	4758	0.825
Carbohydrate intake ($g \cdot day^{-1}$)	166.8	68.2	198.0	53.0	0.305	179.9	106.6	208.7	94.9	0.565
Fat intake ($g \cdot day^{-1}$)	73.4	30.8	87.1	32.6	0.388	88.8	653	103.7	62.3	0.638
Protein intake ($g \cdot day^{-1}$)	95.8	39.5	121.4	48.1	0.252	123.2	115.9	110.4	45.6	0.764
Alcohol ($g \cdot day^{-1}$)	16.1	18.7	3.2	2.7	0.092	17.3	17.7	12.1	14.8	0.518
Dietary calcium intake ($mg \cdot day^{-1}$)	669.7	274.3	790.4	423.9	0.503	740.3	481.5	888.7	363.6	0.481

Results from the health and lifestyle questionnaire both at baseline and six months revealed that 72% of jockeys had been training for over two years with 90% (87% S, 93% P) reporting in excess of six or more hours training per week. Additionally, one third reported engaging in other physical activity outside of training exceeding six hours per week in activities such as running, soccer and martial arts. Approximately 40% of the supplemented group and 14% of placebo group reported smoking while 87% of supplemented group and 57% of placebo group reported drinking alcohol. Of those, four jockeys reported drinking daily with the majority reporting consumption 1-2 times per week. Sixty per cent of the jockeys reported having previous fractures. Approximately half of the supplemented group and one third of placebo group experienced fractures due to a riding injury.

6.4.2 PQCT bone variables

Tibial bone variables were evaluated at the 4% distal and 66% proximal sites after co-varying for baseline weight, height and bone measurements. Adjusted group means (±

SE) at six months together with adjusted mean differences (± SE) are presented for both sites (Table 6-2). Normality tests showed all variables to be normally distributed with the exception of cortical density at the 66% proximal site. This variable was $\log_{10}$ transformed, however non-transformed values are presented in the table as the significance and partial η^2 were identical. There were no significant post-intervention differences observed in trabecular content, trabecular density or trabecular area between groups. Similarly, total density and total area at the 4% distal site showed no significant differences post-intervention (refer Table 6-2). At the 66% proximal site, results demonstrate the supplemented group displayed 4% larger total area ($p=0.003$), 6.6% greater cortical content ($p<0.001$), 1.3% greater cortical density ($p=0.001$) and 5.9% larger cortical area ($p<0.001$) post-intervention while there was no significant difference in cortical thickness between the groups. For the significant variables, partial η^2 indicate moderate to high treatment effect, ranging from 0.53 for total area to 0.7 for cortical content. No significant post-intervention differences were seen in the stress-strain index (SSI tibia) or bone strength index over the six month period.

Table 6-2: Baseline and six-month adjusted bone variables at the 4% distal site and 66% proximal tibia for supplemented (S) and placebo (P) groups after co-varying for baseline height, weight and bone variables.

	Unadjusted baseline values		ANCOVA six-months adjusted values						
	S group (n=8) baseline Mean (SD)	P group (n=9) baseline Mean (SD)	S group (n=8) Adj Mean (SE)	P group (n=9) Adj Mean (SE)	Adjusted Mean diff (SE)	% diff	Partial Eta^2 (η^2)	95% CI	p-value
4% distal Tibia									
Trabecular density (mg·cm^3)	241.0 (28.1)	246.8 (33.9)	259.3 (5.6)	244.3 (5.3)	14.9 (7.8)	5.7%	0.23	-2.1 to 31.9	0.080
Trabecular content (mg·mm)	227.0 (26.0)	211.4 (42.3)	220.8 (6.6)	220.8 (5.8)	-0.02 (9.1)	0.0%	0.00	-20.0 to 19.9	0.998
Trabecular area (mm^2)	861.1 (160.2)	854.7 (126.7)	892.0 (49.7)	870.0 (18.5)	22.0 (27.4)	2.5%	0.05	-37.7 to 81.7	0.437
Total area (mm^2)	1109.2 (81.7)	1044.0 (137.8)	1063.9 (15.9)	1090.0 (14.9)	-26.1 (22.8)	-2.5%	0.10	-75.8 to 23.6	0.275
Total density (mg·cm^3)	300.1 (29.0)	286.6 (40.9)	292.2 (8.0)	293.9 (7.6)	-1.7 (11.3)	-0.6%	0.00	-26.2 to 22.8	0.882
Bone strength index (mg^2·mm^4)	100.1 (17.5)	86.6 (22.4)	90.6 (4.5)	96.5 (4.2)	-5.8 (6.4)	-6.4%	0.07	-19.7 to 8.1	0.380
66% proximal Tibia									
Cortical content (mg·mm)	296.5 (35.7)	293.8 (52.4)	318.3 (2.9)	297.1 (2.7)	21.1 (4.0)	6.6%	0.70	12.4 to 29.8	*<0.001*
Cortical area (mm^2)	267.1 (29.3)	263.4 (43.8)	282.6 (2.3)	266.0 (2.2)	16.6 (3.2)	5.9%	0.69	9.6 to 23.5	*<0.001*
Cortical density (mg·cm^3)	1101.5 (24.7)	1113.4 (22.1)	1127.4 (2.5)	1112.9 (2.3)	14.5 (3.5)	1.3%	0.59	6.9 to 22.1	*0.001*
Total area (mm^2)	492.2 (59.6)	519.8 (75.6)	524.6 (4.2)	503.4 (3.9)	21.2 (5.8)	4.0%	0.53	8.5 to 33.9	*0.003*
Cortical thickness (mm)	4.2 (0.5)	3.8 (0.6)	4.1 (0.0)	4.0 (0.0)	0.08 (0.04)	1.9%	0.25	-0.01 to 0.2	0.066
SSI Tibia (mm^3)	2100.5 (329.2)	2140.0 (489.6)	2207.1 (80.3)	2127.1 (75.6)	80.0 (111.6)	3.6%	0.04	-163.1 to 323.1	0.487

6.4.3 Blood borne variables

No significant differences in BTM was evident between groups at baseline. Serum Vitamin D levels were higher in the placebo group at baseline (81.2 ±24.4 nmol·L^{-1} vs 64.6 ±19.5 nmol·L^{-1}), however this was not significantly different (p=0.146). At six months, unadjusted values for 25(OH)D had increased to 75.6 (±20.8) nmol·L^{-1} for the supplemented group while unadjusted the placebo groups levels fell slightly to 73.4 (±20.8) nmol·L^{-1}. ANCOVA revealed adjusted 25(OH)D levels were 18% higher in the supplemented group than the placebo group (p= 0.014) at six months. Bone resorption marker levels (CTx) were almost 25% lower in the supplemented group (p= 0.011) while no post-intervention differences were observed in the bone formation marker, P1NP between groups (refer Table 6-3).

Table 6-3: Baseline and six month adjusted mean values and adjusted mean differences (95% CI) in blood variables: vitamin D levels, CTx and P1NP between supplemented (S) and placebo (P) groups after covarying for baseline blood variables.

	Unadjusted baseline values		ANCOVA six-months adjusted values						
	S group (n=8) Mean (SD)	P group (n=8) Mean (SD)	S group (n=8) Adj Mean (SE)	P group (n=8) Adj Mean (SE)	Adjusted Mean diff (SE)	% diff	partial Eta^2	95% CI diff	p-value
25OH Vit D (nmol/L)	64.6 (19.5)	81.2 (24.4)	81.9 (3.6)	67.1 (3.6)	14.8 (5.2)	18.1%	0.38	3.6 to 26.1	***0.014***
CTx (ng/L)	371.3 (201.0)	380.0 (141.1)	357.5 (21.3)	446.3 (21.3)	-88.8 (30.2)	-24.8%	0.40	-154.0 to -23.6	***0.011***
P1NP (ug/L)	104.2 (46.4)	108.9 (31.6)	107.3 (5.7)	101.9 (5.7)	5.4 (8.0)	5.0%	0.03	-11.9 to 22.7	0.511

6.5 Discussion

There is clear evidence that jockeys have compromised bone health (Caulfield & Karageorghis, 2008; Dolan, McGoldrick, et al., 2012; Dolan et al., 2011; Greene et al., 2013; Leydon & Wall, 2002; Moore et al., 2002; Waldron-Lynch et al., 2010; Warrington et al., 2009); however, no research to date has examined the efficacy of strategies for improvement in this at-risk population. This is the first randomised controlled trial (RCT) to assess bone responses to six months of a combined calcium and vitamin D supplement in young male jockeys or any other young male athletic group. Furthermore, this is the first RCT to use pQCT to examine bone responses at the distal and proximal tibia in an at-risk athletic population known to have compromised bone health (Greene et al., 2013). Results show that the supplemented group displayed greater bone density and geometry at the proximal tibia post intervention suggesting supplementation may be a viable strategy to counteract the deleterious skeletal effects of engaging in a weight-restricted activity in the lower limb.

Unlike previous studies examining bone responses following calcium supplementation in male populations, the present study utilised pQCT rather than DXA (Daly, Brown, et al., 2006; Dawson-Hughes et al., 1997; Kukuljan, Nowson, et al., 2009; Orwoll et al., 1990; Prentice et al., 2005; Reid et al., 2008). While DXA-derived BMD may be a surrogate predictor of fracture risk (Kanis et al., 2008) the two-dimensional technology is limited by its inability to adequately assess bone geometry and consequently, bone strength. Minor changes in bone mass and bone geometry may lead to increases in bone strength independent of changes in BMD (Adami, Gatti, Braga, Bianchini, & Rossini, 1999; Järvinen, Sievänen, Jokihaara, & Einhorn, 2005). Furthermore,

the importance of cortical bone in relation to whole bone strength is well established (Järvinen et al., 2005). The results of this study demonstrate improvements in cortical content, density and area at the proximal tibia following six months of supplementation. While the primary outcome of the study (changes to strength strain index), indicated some improvement in the supplemented group, this was not significantly different. Despite no evidence of greater strength strain index in the supplemented group, it is plausible that an insufficient intervention period restricted improvements in bone strength (Nikander et al., 2010). Nonetheless, it is plausible that greater bone geometry and bone material at the proximal tibia may have resulted from calcium and vitamin D supplementation over a six month period.

Typically, jockeys have high bone turnover (Dolan, McGoldrick, et al., 2012; Waldron-Lynch et al., 2010; Wilson, Fraser, et al., 2013) linked to low energy and calcium intakes. Blood analysis of bone turnover markers (BTM) demonstrated a significant decrease in bone resorption markers (CTx) in the supplemented group over the intervention period. Despite being within reference ranges, CTx levels were in the bottom 5^{th} percentile (Jenkins et al., 2013). Bone resorption markers in the current study are similar to previous findings (Wilson, Fraser, et al., 2013) but contrast with other research which show high levels of bone resorption (Dolan, McGoldrick, et al., 2012; Waldron-Lynch et al., 2010). Differences may arise from a variety of bone resorption markers analysed or the average age of the participants (Jenkins et al., 2013; Michelsen et al., 2013). At 20 years of age, jockeys in the current study average five years younger than those previously reported. In contrast, P1NP remained unchanged over the intervention, revealing both groups to have elevated levels of bone formation markers (95^{th} percentile for age) (Jenkins et al., 2013; Michelsen et al., 2013). This may be in part due to the

average age of the participants; however, high levels of P1NP are supported by previous research examining jockey bone turnover (Waldron-Lynch et al., 2010). Calcium kinetics indicate that supplementation results in an increase in absorbed calcium and suppression of bone resorption (Wastney et al., 2000). While approximately 90% of PBM is acquired by 18 years of age (Baxter-Jones et al., 2003), the age of attainment of PBM for males is equivocal with some studies indicating 18-20 years of age for spine and hip PBM whilst others have found this is achieved around 25-29 years (Boot et al., 2010; Henry et al., 2010; Lorentzon et al., 2005; Szulc et al., 2000). Tibial bone appears to continue to undergo cortical bone mineral accrual up to 50 years of age (Lorentzon et al., 2005). As such, it is speculated that decreases in CTx observed in this study, combined with no change in P1NP, resulted from continued bone mineral accrual stimulated by an increase in available calcium.

The actions of calcium on bone are well known, being required for adequate mineralisation during growth (Bailey et al., 1996) and maintenance of the skeleton through adulthood (Bachrach, 2001). Multiple meta-analyses have demonstrated the effectiveness of supplementation in improving BMD in children and female populations(Abrahamsen et al., 2010; Chung et al., 2009; Cranney et al., 2007; Lips et al., 2014; Shea et al., 2002; Tang et al., 2007; Winzenberg et al., 2010), however, effectiveness has not been demonstrated adequately in young male populations, and in particular male athletes (Silk, Greene, & Baker, 2015). A previous controlled trial of calcium supplementation in young males (16-18 years) found calcium appeared to stimulate skeletal growth; however, once differences in height were accounted for, no difference in BMD was reported (Prentice et al., 2005). Study duration of calcium trials using male participants has varied from 12 months (Kukuljan, Nowson, et al., 2009) to 3

years. Where multiple time points have been examined, supplementation begins to improve BMD within a six-month period in healthy older males (Reid et al., 2008). It should be noted that DXA, not pQCT, has been used to measure changes in bone mineral properties. Positive alterations to bone properties using pQCT has been demonstrated in a six month period of calcium supplementation in a group of female peri-pubertal twins (Greene & Naughton, 2011).

Previous investigations of serum vitamin D levels in young male jockeys highlight 70% of riders being vitamin D deficient (serum (25(OH)D < 25 nmol·L^{-1}) in winter (Guillemant et al., 2001; Wilson, Fraser, et al., 2013). Jump and flat jockeys from the United Kingdom recorded serum levels of 25(OH)D averaging 35 nmol·L^{-1} and 38 nmol·L^{-1} during winter (Wilson, Sparks, et al., 2013). In another study, jockeys sampled during winter had serum 25(OH)D levels below 50 nmol·L^{-1} whilst those measured in the summer exceeded 90 mmol·L^{-1}(Waldron-Lynch et al., 2010). Recent studies conducted in Australia and New Zealand showed that 30 to 70% of male adolescents present with vitamin D insufficiency (25(OH)D < 50 nmol·L^{-1}) (Jones et al., 2005; Rockell et al., 2005). In the current study, vitamin D levels in both groups were above the current recommended serum levels of 50 nmol·L^{-1} (National Health and Medical Research Council, 2006b), with serum vitamin D increasing over the intervention period in the supplemented group from 64.6 ±19.5 nmol·L^{-1} to 75.6 (±20.8) nmol·L^{-1}. Without adequate levels of vitamin D bone mineralisation may be inadequate as calcium absorption becomes limited (Aloia et al., 2010; Higdon & Drake, 2011); however, there is divergence of opinion as to what level of serum vitamin D represents “adequate” in relation to bone outcomes. There is evidence, albeit in a different population, that calcium absorption effectiveness was found to be between 45% to 65% higher when 25(OH)D levels were at 86.5 nmol·L^{-1} compared to 50

nmol·L^{-1} in post-menopausal women (Heaney et al., 2003). Fracture risk in older males has been significantly reduced when serum vitamin D levels were between 71 to 99 nmol·L^{-1} compared with serum vitamin D levels between 54–62 nmol·L^{-1} (Dawson-Hughes et al., 2005). A recent review of vitamin D intakes to optimise all health outcomes recommends serum concentrations of 25(OH)D begin at 75 nmol·L^{-1}, and suggests optimal bone health outcomes and reductions in fracture risk are achieved between 90 and 100 nmol·L^{-1} (Bischoff-Ferrari et al., 2006). Consequently, it is possible that supplementation with vitamin D in this group of jockeys may have assisted in optimising the calcium absorption. However, given that the jockeys in this study had vitamin D levels deemed adequate under current recommendations, the additional vitamin D may not have had an effect.

Furthermore, energy deficits as a result of restrictions in energy intake and excessive exercise typical of a number of athletic populations can result in poor bone health outcomes (Ebeling, 2008; Fredericson et al., 2007; Greene et al., 2013; Nichols & Rauh, 2011; Rector et al., 2008; Smathers et al., 2009). Jockeys in this study demonstrated insufficient energy intakes and low calcium intakes supporting previous findings (Greene et al., 2013; Leydon & Wall, 2002; Moore et al., 2002; Warrington et al., 2009). Additionally, lifestyle behaviours such as smoking (Kanis, Johnell, et al., 2005) and excessive alcohol intakes (Kanis, Johansson, et al., 2005) have been shown to contribute to osteoporosis, particularly in males. A number of the jockeys in this study reported smoking (33%) and regularly drinking alcohol (72%). Although jockeys report training on a weekly basis in this study, others have shown jockeys spend approximately 25 hours per week training (Greene et al., 2013) in track-work activities involving riding horses for exercise and fast paced work, cleaning stables, washing and feeding horses (Dolan et al.,

2011; Wilson, Sparks, et al., 2013). The combination of low energy intake, high amounts of physical activity, smoking and alcohol consumption seen in this group could be expected to negate the benefits of the calcium supplementation, however, this does not appear to be the case. It is plausible that the synergistic nature of vitamin D and calcium, combined with physical activity undertaken by jockeys has produced positive bone outcomes in the supplemented group.

A number of strengths and limitations exist within this study. The use of pQCT to assess bone properties has allowed a more detailed analysis of musculoskeletal adaptations at the tibia as a result of calcium and vitamin D supplementation. The randomised controlled design of this study has minimised selection bias and attempted to reduce, where possible, genetic influence on bone properties. Additionally, we have statistically controlled for baseline variations in body composition and bone variables. Changes to bone properties have been further supported by serum analysis of bone formation and resorption markers, in accordance with recommendations from the International Osteoporosis Foundation (Vasikaran, Eastell, Bruyère, et al., 2011). The 6-month duration of this study was shorter than other research examining calcium and vitamin D supplementation. Bone outcomes, particularly measures of bone strength (SSI and BSI), may have reflected further improvement had the intervention period been longer. However, compliance beyond six months may have compromised outcomes. The population is not readily accessible, with testing opportunities limited to one day per month. Further, they are subject to unforeseen relocation, making follow-up difficult at times, as evidenced in this study. The number of drop-outs during the study reduced the sample size which has impacted upon the power of the study. It should be noted, however, that the differences in cortical bone were found to be highly significant

suggesting that an effect does exist, although we acknowledge that outcomes would have been strengthened if participants lost to follow up were minimised. Further, we were unable to report compliance with the supplementation regime. While verbal assurances regarding compliance were provided by participants at data collection, very few jockeys returned supplement containers as instructed. Despite the lack of reported compliance, changes to both blood and bone variables suggest that jockeys in the supplemented group were compliant throughout the 6-month intervention period.

This is the first randomised controlled trial to examine the effects of calcium and vitamin D supplementation on bone properties in young male jockeys using pQCT. Our findings indicate that supplementation with 800mg of calcium and 400IU of vitamin D per day for a period of 6-months improves bone properties at the proximal tibia and therefore supplementation may be a viable strategy for improving bone outcomes in at-risk male athletic populations. Longitudinal supplementation in this cohort and other weight-restricted athletic populations demonstrating compromised bone health would further improve our understanding of the synergistic benefits calcium and vitamin D supplementation.

6.6 Disclosures

Nil

6.7 Acknowledgements

Study design: DG. Study conduct: LS and DG. Data collection: LS, DG, CJ. Data analysis: LS. Data interpretation: LS and DG. Drafting manuscript: LS. Revising manuscript content: LS, DG and MB. Approving final version of manuscript: LS, DG, MB and CJ. LS takes responsibility for the integrity of the data analysis.

We would like to acknowledge all of the participants for assisting in this research project. Recruitment was only possible with the support and assistance of the Australian Jockeys Association, Racing NSW and Racing Victoria. Supplements were kindly supplied by USANA Health Sciences (Australia).

Note: Refer to Appendix 10: Consort Statement for checklist of information to include when reporting a randomised trial (Moher, Schulz, & Altman, 2001).

7 Cortical bone distribution at the tibial shaft in young male Jockeys after 6-months calcium and vitamin D supplementation: A randomized controlled trial

Silk, L.N, Greene, D.A, Baker, M.K (2016). Submitted to BONE journal 11 February 2016.

7.1 Abstract

Cortical bone distribution in long bones varies both along the axial length of long bones and in cross-sections. Regional-specific alterations to cortical bone distribution are apparent with minimal variations in volumetric cortical bone mineral density (vBMD). Using three-dimensional images acquired using peripheral quantitative computed tomography (pQCT), an open source analysis tool (BoneJ) measured the distribution of cortical bone within circumferential layers within the cortex (radial distribution) and in sectors around the neutral axis (polar distribution). The aim of this study was to compare the polar and radial distribution of cortical bone at the tibial shaft in young male jockeys exposed to 6-months calcium and vitamin D supplementation. Cortical distribution at the tibial shaft (66% of tibial length measured distally) was assessed using pQCT in two groups of male jockeys aged 17 to 32 years (mean 20.2± 3.2). Participants were supplemented with 800mg of calcium and 400IU of vitamin D (n=8) or a placebo (cellulose) (n=9) daily for 6-months. Polar and radial vBMD was measured in 36, ten degree cortical sectors (polar) and three concentric cortical divisions (radial). Polar distribution was further consolidated into four, 90 degree quadrants aligned to anatomical planes. Cortical mineral mass, endocortical and pericortical radii were also analysed. After covarying for height, weight, and baseline measurements, the supplemented group demonstrated greater endocortical vBMD in the posterior region of bone (1140.5 ± 6.3 vs 1116.2 ± 5.9; p=0.018). While the study was not quite long enough to produce significant results,

trends support previous suggestions that alterations in bone density and geometry are a combined result of site-specific loading and anti-resorptive responses to supplementation.

Keywords: Cortical bone; BoneJ; calcium

7.2 Introduction

Designed for optimal function, long bones are predominately comprised of cortical bone that is light, rigid and accommodating of loads experienced during movement. During growth the diaphysis of long bones alters its mineral mass and positions cortical bone away from the neutral axis resulting in increased bone size and bone strength (Kontulainen, Macdonald, & McKay, 2006). However, the dispersion of cortical bone in long bones is not uniformly distributed. Furthermore, the effect of loading on radial and polar cortical bone distribution highlights a region-specific response (Cooper, Ahamed, Macdonald, & McKay, 2008; Kontulainen et al., 2006; Lai, Qin, Hung, & Chan, 2005). In particular, the distribution of cortical volumetric bone mineral density (vBMD) appears responsive to region-specific effects of loading with little evidence of commensurate changes in whole bone cortical vBMD.

Strain enhances apposition of periosteal layer and/or reduces resorption in the endocortical region (Kukuljan et al., 2011). Examination of athletes reveals that expansion of bone cross-sectional area rather than changes to vBMD appear to influence bone strength (Rantalainen, Nikander, Daly, Heinonen, & Sievänen, 2011). Conversely, calcium and vitamin D supplementation appears to have anti-resorptive influences, preserving BMD through a reduction in endocortical resorption and maintenance of cortical thickness (Daly, Duckham, & Gianoudis, 2014). This suggests calcium and bone strain

work in concert to improve bone properties, particularly in the presence of initial calcium deficiency, but it is unclear if increases or decreases in vBMD occurs with supplementation.

To date, a variety of analysis tools to assess cortical vBMD distribution have been used with limited validity (Kontulainen et al., 2006; Lai et al., 2005). In recent years an open source image analysis tool, built as an ImageJ plug-in, was validated as a method of analysing cortical vBMD distribution around the neutral axis (polar distribution) and in circumferential layers within the cortex (radial distribution) using pQCT scans (Rantalainen, Nikander, Heinonen, et al., 2011). Whilst examinations of the distribution of cortical vBMD have been undertaken in athletic and non-athletic populations (Cooper et al., 2008; Greene, Naughton, Moresi, & Bradshaw, 2012; Macdonald et al., 2009; Rantalainen et al., 2014; Rantalainen, Weeks, Nogueira, & Beck, 2015; Weidauer, Binkley, Berry, & Specker, 2013), no study to date has examined changes to cortical bone following an intervention aimed at improving bone material properties via calcium and vitamin D supplementation.

Our aim was to compare the radial and polar cortical vBMD distribution at the tibial mid-shaft in young male jockeys exposed to 6-months calcium and vitamin D supplementation with age- and gender-matched jockeys receiving a placebo. We hypothesised that jockeys receiving calcium and vitamin D supplementation is associated with reduced cortical vBMD, particularly at the mid- and pericortical bone divisions.

7.3 Methods

7.3.1 Research design

The present study is a further exploration of data obtained from young male jockeys who participated in a six month randomised double-blind placebo controlled trial of calcium and vitamin D supplementation (Silk, Greene, Baker, et al., 2015). Participants in the study were young male jockeys, mean age 20.2± 3.2 years. Data was collected at baseline and six months with the supplemented group (n=8) having received 800mg of calcium and 400IU of vitamin D per day whilst the placebo group (n=9) were provided with cellulose tablets. Specific inclusion and exclusion criteria was used: in good health in last 6 months with no systemic illness lasting more than 2 weeks; no known history of fracture or recurrent fracture complications in last 6 months; no known history of metabolic bone or muscle disease; and no medication, hormones or calcium/vitamin D preparations in preceding 6 months and willing to remain free of such medications for the 6 months of data collection. All participants provided informed consent. Ethical approval was granted by the Human Research Ethics Committee at the Australian Catholic University and the study was registered with the Australian New Zealand Clinical Trials Registry (registration no: ACTRN126000374864).

7.3.2 Anthropometric characteristics

Standing and seated height was measured to 0.1 cm using a stadiometer and weight was measured using an electronic scale accurate to 500g (Wedderburn UW150, Sydney, Australia) with participants dressed in light clothing and without shoes. Tibial length was measured externally as the distance between the mid-point of the distal medial malleolus and the proximal medial tibial plateau landmarks.

7.3.3 Bone material properties

The non-dominant tibia was scanned using a peripheral quantitative computed tomography (pQCT) bone scanner (XCT-2000L, software version 5.50d, Stratec Medizintechnic, Pforzheim, Germany) and has been previously described (Silk, Greene, Baker, et al., 2015). The pQCT images were further analysed using BoneJ (v 1.47v) (Doube et al., 2010), pQCT density distribution plug-in (Rantalainen, Nikander, Heinonen, et al., 2011) using standard settings (see Figure 7-1 A). Radial and polar vBMD distribution**,** endocortical and pericortical radii (mm) together with polar cortical vBMD (mg·cm^3) and mineral mass (mg) were calculated by BoneJ for 36, ten degree sectors.

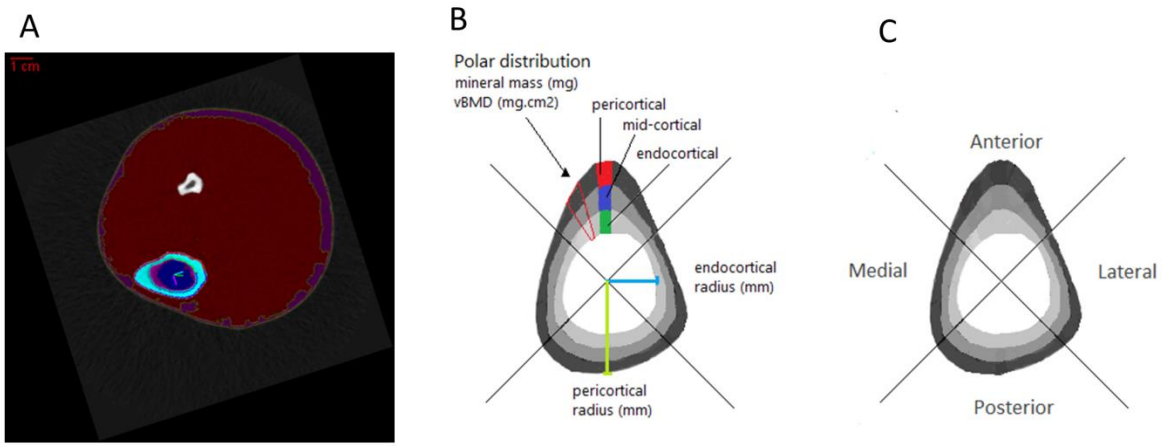

Figure 7-1: pQCT image as treated by BoneJ pQCT distribution plug-in (A), an illustration of the radial and polar distribution (B) and the location of the anterior, posterior, lateral and medial planes (C).
Images B and C adapted from Rantalainen, Nikander, Heinonen, et al. (2011)

The cortical cross-section was divided into four 90^o polar sectors and three cortical radial divisions representing anterior, posterior, lateral and medial planes (Figure 7-1 B and C). Mean endocortical and pericortical radii (mm), mean mineral mass (mg) and mean polar cortical vBMD (mg·cm^3) was calculated for each 90^o sector by averaging the sum of each 10^o sector within the defined planes (Macdonald et al., 2009).

7.3.4 Blood borne markers of bone turnover and vitamin D status

Blood samples were collected and analysed for Procollagen type 1 N propeptide (P1NP) (ug/L), C-terminal telopeptide of type 1 collage (CTx) (ng/L), and serum 25-hydroxy vitamin D [25(OH)D] (nmol/L). Methods have been previously described (Silk, Greene, Baker, et al., 2015).

7.3.5 Statistical methods

All variables were tested for normality using Shapiro-Wilk test and t-tests were performed on baseline characteristics. Normally distributed data are presented in mean ± standard deviations (SD) and treated with parametric analysis. Baseline descriptive data are reported as mean ± SD as applicable. Bone variables were compared using analysis of covariance (ANCOVA) to derive regression equations to measure the effect size and probability of between-group differences (Vickers & Altman, 2001) after controlling for weight, height and baseline bone measurements. Data used in ANCOVA was tested for homogeneity of regression by examining the statistical significance of the interaction of covariates and the independent variables. Statistical analyses were performed using SPSS version 22.0 (SPSS, Chicago, IL, USA).

7.4 Results

7.4.1 Descriptive characteristics

Following attrition during the study due predominantly to race-related commitments and apprenticeship transfers, 17 jockeys completed final data collection. Descriptive statistics for participants who completed the study are shown in Table 7-1. The two groups were homogenous, with no significant differences in age, height or weight. Average calcium intake in each group was well below standard dietary

recommendations of 1,000 mg per day (National Health and Medical Research Council, 2006a) while baseline serum vitamin D was above minimum recommendations of 50 nmol·L^{-1} (National Health and Medical Research Council, 2006b). Excluding the additional calcium intake in the supplemented group, six monthly values for all variables remained consistent.

Table 7-1: Baseline characteristics of study completers.

	Baseline values				
	Supplement group (n=8)		Placebo group (n=9)		
	mean	SD	mean	SD	p
Age (yrs)	22.3	5.0	19.3	1.8	0.152
Height (cm)	165.6	4.4	167.3	4.3	0.517
Body mass (kg)	52.7	3.6	52.6	3.3	0.453
BMI (kg·m^2)	19.3	1.7	18.8	1.1	0.507
Calcium intake (mg·day^{-1})	669.7	274.3	790.4	423.9	0.503
25OH Vit D (nmol·L^{-1})	75.93	21.20	79.00	22.00	0.705

7.4.2 BoneJ

7.4.2.1 *Mineral mass*

Mineral mass distribution for both groups was seen to be highest in the anterior region (Figure 7-2). ANCOVA results in the four anatomical planes indicated non-significant differences in mineral mass following supplementation. There was a trend towards higher mineral mass in the posterior region (12.03 mg ±0.11 vs 11.73 mg ±0.10) for the supplemented group.

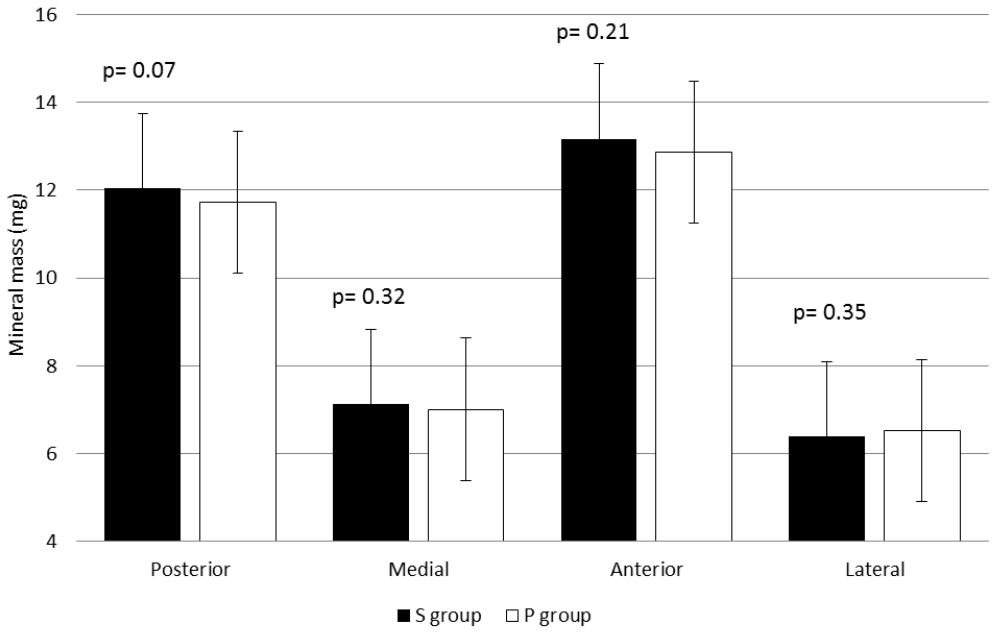

Figure 7-2: Six month adjusted mineral mass (SE) presented in the 4 anatomical planes; S = supplemented group, P = placebo group**.**

7.4.3 Cortical vBMD

Post-supplementation vBMD was examined in the endocortical, mid-cortical and pericortical radii in all 36 sectors as well as anatomical regions. Results by radial division demonstrated no significant between group differences. For both groups, the mid-cortical region displayed the greatest vBMD and the endocortical region demonstrated the lowest vBMD (Table 7-2).

Table 7-2 : **Unadjusted six months vBMD (mg·cm^3) by radial division**

	Endocortical			**Mid-cortical**			**Pericortical**		
	Mean (mg·cm^3)	SD	p-value	Mean (mg·cm^3)	SD	p-value	Mean (mg·cm^3)	SD	p-value
Supplemented	1103.72	175.81	0.28	1147.97	186.01	0.18	1108.31	172.19	0.17
Placebo	1158.77	28.02		1214.57	30.31		1192.35	51.60	

When examined in anatomical regions (Figure 7-3), ANCOVA results (adjusted mean ±SE) indicate endocortical posterior vBMD was significantly greater in the

supplemented group (1140.5 ± 6.3 vs 1116.2 ± 5.9; p=0.018) with vBMD in the anterior pericortical radial division also greater (1160.1 ± 8.8 vs 1137.4 ± 8.3; p=0.09).

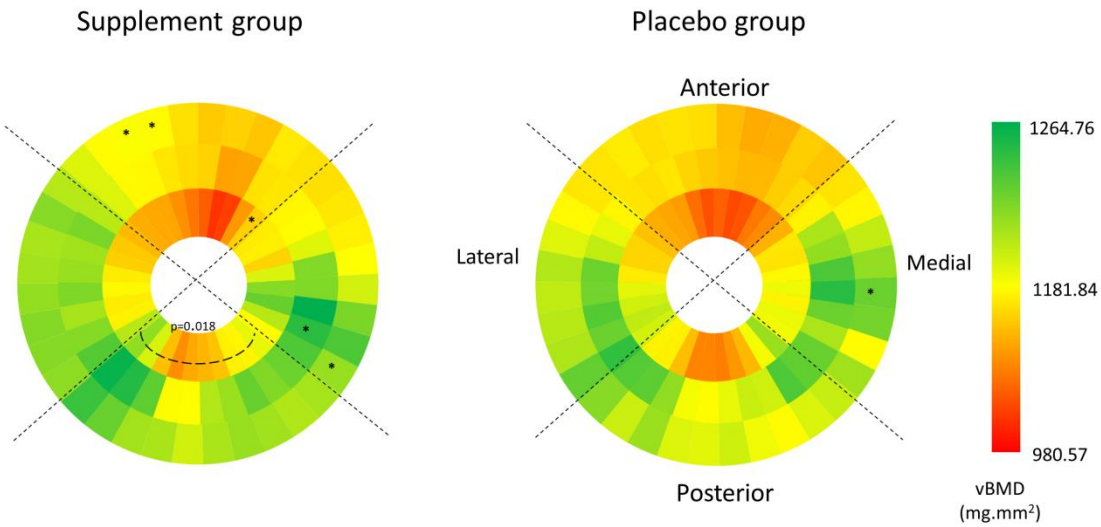

Figure 7-3: Plot of ANCOVA results for vBMD (mg·cm^3) by sector in endocortical, mid-cortical and pericortical radii. * indicates significant difference in 10^o sector (p<0.05).

7.4.3.1 *Radius*

Results of ANCOVA for the endocortical and pericortical radius are shown as adjusted mean (SE error bars) in Figure 7-4. Pericortical radius were highest in the anterior region whilst endocortical radius was similar in the anterior and posterior regions. While not significant, endocortical radius adjusted mean (SE) in the lateral region was greater in the supplemented group (p=0.067) and pericortical radius adjusted mean (SE) in the supplemented group was greater in the posterior region (p=0.076).

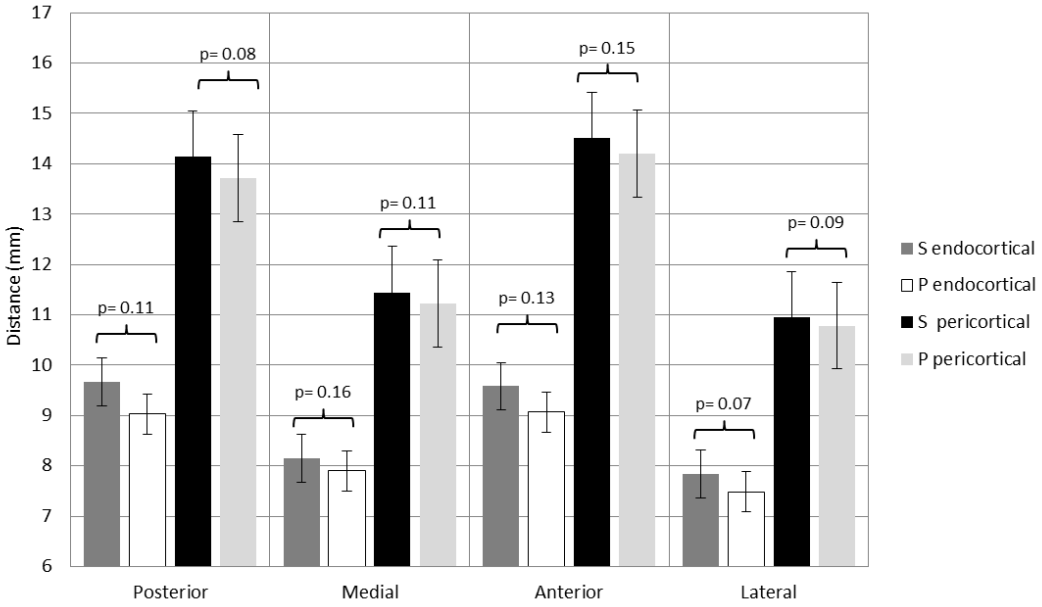

Figure 7-4: Six month adjusted mean (SE) for endocortical and pericortical radius (mm) presented in the 4 anatomical planes; S = supplemented group, P = placebo group.

7.5 Discussion

This is the first study to compare cortical bone distribution at the tibial mid-shaft in male jockeys after 6-months calcium and vitamin D supplementation or a placebo. Results of the BoneJ pQCT distribution plug-in analysis show greater vBMD at the endocortical posterior region of the tibia in the supplemented group compared to the placebo group. No other between-group differences were found. While the majority of results were non-significant, there was a general trend to suggest that calcium supplementation stimulated bone apposition in the posterior and lateral regions of the tibia with increasing mineral mass in the posterior region of the bone.

Exercise-induced changes to bone appear to be found in regions of loading, resulting in geometric alterations rather than changes to vBMD. Studies in athletic populations have demonstrated that loading can increase site-specific bone strength (Greene, Naughton, Bradshaw, et al., 2012; Jürimäe et al., 2006) and that non-weight bearing physical activity can have a negative impact on bone density (Smathers et al.,

2009). Weight-bearing loading has been shown to improve bone strength at the tibial shaft in young boys (Macdonald et al., 2009). Further, results suggest that observed alterations occur in different quadrants of the bone shaft, reflecting bone adaptation directly in response to site-specific loading in the anterior-posterior plane (Macdonald et al., 2009). In the current study, the trend towards expansion of the radii in the supplemented group in the anterior/posterior plane appears consistent with these findings.

As previously shown (Rantalainen, Nikander, Daly, et al., 2011; Weidauer et al., 2013) distribution of vBMD in both groups was highest in the mid-cortical layer of bone whereas endocortical vBMD was lowest, reflecting higher metabolic activity in this region. Following supplementation, vBMD in the posterior region of the endocortical radius was significantly greater in the supplemented group. Loading influences bone in a site-specific way, enhancing periosteal apposition and/or reducing resorption in the endocortical region (Kukuljan et al., 2011). In adults, supplementation with calcium and vitamin D appears to have an anti-resorptive influence, decreasing remodelling and essentially preserving BMD by reducing endocortical resorption and maintaining cortical thickness (Daly et al., 2014). It is suggested that calcium and loading work in concert to improve bone properties particularly where there is an initial calcium deficiency (Daly et al., 2014).

As professional athletes, jockeys are habitually physically active; however, the extent to which horse riding loads the tibia is unknown. Both groups presented with low calcium intake which persisted in the non-supplemented group, suggesting increases in vBMD in the endocortical posterior region may be in response to both supplementation and loading. Observed reductions to bone resorption markers (CTx) in this population

coupled with high levels of bone formation marker (P1NP) (Silk, Greene, Baker, et al., 2015) also appears to further support the premise that the calcium and vitamin D supplementation may have anti-resorptive effects.

Peripheral quantitative computed tomography (pQCT) data indicate that males deposit bone on the periosteal surface (Schoenau et al., 2001). Furthermore, consolidation of long bone PBM (PBM) is achieved through increased cortical thickness and mineralisation of cortical bone (Lorentzon et al., 2005). Little is currently known about the site specific effects of calcium supplementation on male bone (Silk, Greene, & Baker, 2015). With a few exceptions (Daly, Bass, et al., 2006; Kukuljan et al., 2011; Silk, Greene, Baker, et al., 2015), supplementation has focused on changes to BMD using DXA. Few male studies have examined changes in bone geometry subsequent to supplementation using pQCT. Supplementation with calcium and vitamin D fortified milk in a group of men aged 60.7 ± 7.1 over a period of 18 months had no effect on total or cortical area, or cortical vBMD at the mid-tibia measured by QCT (Kukuljan et al., 2011). Similarly, in a separate study, no differences were observed at the mid-femur after 2 years using a calcium-vitamin D fortified milk supplement (Daly, Bass, et al., 2006). However, when divided into under- and over-62 years age groups, the older group demonstrated a reduction in the rates of medullary cavity expansion and cortical vBMD loss at the mid-femur (Daly, Bass, et al., 2006). Results of the present study, albeit with a younger male cohort, show a similar trend in the supplemented group regarding vBMD at the endocortical posterior region of the tibia.

A number of strengths and limitations exist within this study. The randomised controlled design has minimised selection bias and assisted in reducing possible genetic

influence on bone properties. The pQCT has allowed a more detailed analysis of musculoskeletal adaptations at the tibia as a result of calcium and vitamin D supplementation as compared to DXA and the additional analysis through the BoneJ cortical distribution plugin (Doube et al., 2010; Rantalainen, Nikander, Heinonen, et al., 2011) has provided further insight. Additionally, baseline variations in body composition and bone variables have been statistically controlled throughout the analysis. It is acknowledged that the 6-month duration of this study is shorter than other research examining calcium and vitamin D supplementation. Whilst pQCT variables indicated significant changes in the supplemented group over the intervention period, this was not the case in the subsequent detailed analysis. Had the intervention period been longer and/or the sample size had remained higher, we expect that alterations to the different bone parameters may have become evident. However, compliance beyond six months may have compromised outcomes, as the group was not readily accessible and subject to unforeseen relocation, making follow-up difficult as evidenced in this study. It should be noted, however, that differences in cortical bone measured by pQCT previously reported (Silk, Greene, Baker, et al., 2015), were found to be highly significant suggesting that an effect does exist, although we acknowledge that outcomes would have been strengthened if participants lost to follow up were minimised.

This is the first randomised controlled trial to compare cortical bone distribution using a custom-designed analysis tool (BoneJ) at the tibial mid-shaft in male jockeys exposed to 6-months calcium and vitamin D supplementation. Longitudinal supplementation in this cohort and other weight-restricted athletic populations demonstrating compromised bone health would further improve our understanding of

the synergistic benefits calcium and vitamin D supplementation and the influence of loading on cortical bone distribution.

7.6 Disclosures

Nil

7.7 Acknowledgements

Study design: DG. Study conduct: LS and DG. Data collection: LS, DG. Data analysis: LS. Data interpretation: LS and DG. Drafting manuscript: LS. Revising manuscript content: LS, DG and MB. Approving final version of manuscript: LS, DG, MB. LS takes responsibility for the integrity of the data analysis.

We would like to acknowledge all of the participants for assisting in this research project. Recruitment was only possible with the support and assistance of the Australian Jockeys Association, Racing NSW and Racing Victoria. Supplements were kindly supplied by USANA Health Sciences (Australia).

7.8 Funding

This project was supported by Faculty of Health Sciences funding from the Australian Catholic University.

8 Thesis Summary

This thesis examined the efficacy of 800mg of calcium and 400IU of vitamin D daily on improving the bone material properties at the weight-bearing tibia and non-weight bearing radius in of a group of young male jockeys, through a double-blind, placebo controlled intervention for a period of six months.

A narrative review of the literature provided contextual information relating to bone growth, development and maintenance, and outlined influences on bone health including calcium, vitamin D and physical activity and energy restriction. The strengths and weaknesses of major imaging techniques used to assess the structural integrity of bones were outlined. Other indicators of bone health, ie vitamin D status and blood-borne markers of bone turnover were also discussed. Following this background information, a review of the literature directly pertaining to the bone health of jockeys was presented. Specifically finding in relation to bone health assessments, blood-borne markers of bone turnover, energy intake and calcium and vitamin D status were presented.

8.1 Effects of calcium and vitamin D supplementation on male bone material properties

A systematic review and meta-analysis was conducted examining the effectiveness of vitamin D and/or calcium supplementation on improving bone mineral properties in healthy males. The systematic review, which met all CONSORT statement guidelines (Moher et al., 2001), revealed a distinct lack of studies on male participants in order to adequately determine the efficacy of vitamin D and/or calcium supplementation. Specifically, it highlighted that no studies of sufficient quality examining supplementation

in male athletic populations exist, despite evidence of compromised bone health in athletic cohorts. Additionally, the results revealed a heavy reliance on DXA data in analysing the effects of supplementation on bone material properties. Further, it cannot be established from the studies, the stage at which supplementation begins to affect BMD. While many studies made multiple time point measures the majority only reported final outcomes.

The results of the subsequent meta-analysis suggest that supplementation with calcium, in combination with vitamin D, has a small to moderate effect on bone mineral density in healthy males, indicating differing responses between weight-bearing and non-weight bearing bone. In younger cohorts, supplementation results in positive acquisition of bone over normal growth related bone acquisition, whilst in older populations supplementation appeared to attenuate bone loss.

The combined results of the systematic review and meta-analysis informed the three clinical studies. Alterations to bone strength and geometry may translate into strength gains without overall alterations to BMD, suggesting that imaging techniques such as pQCT should be employed in studies to further elucidate the effects of supplementation on both weight bearing and non-weight bearing bone.

8.2 Influence of supplementation on blood-borne markers of bone turnover

Alterations to bone material properties may take a considerable length of time to respond to stimuli whilst blood-borne markers of turnover are more responsive. Chapter five incorporated an examination of the effects of calcium and vitamin D supplementation on markers of bone turnover, hypothesising that supplementation 'will be an effective

and feasible strategy for improving bone turnover markers'. Specifically, the bone formation marker P1NP and the bone resorption marker CTx were monitored at baseline and six months to evaluate whether the supplementation regime was having an effect on bone metabolism.

As outlined in section 2.7.2, results of previous studies examining levels of bone resorption markers in jockeys are equivocal, with serum markers of CTx being low but within normal ranges, whilst examination of urinary markers of bone resorption by other groups suggest elevated bone resorption. Bone formation markers in jockeys have been shown to be high. Compared to values for Australian males aged 20-29, the baseline results for this group of young jockeys revealed low levels of CTx and elevated levels of P1NP, in line with other jockey-related research. Following the intervention, CTx levels ($ng \cdot L^{-1}$) declined in the supplemented group being 350.00 ± 204.66 compared to 371.3 ± 201.00 at baseline while the placebo group demonstrated an increase in CTx levels from 380.0 ± 141.1 to 453.75 ± 164.92. The supplemented group's CTx levels were found to be approximately 24% lower than the placebo group on an adjusted mean basis (p=0.011). The reduction in CTx over the course of the trial was modest compared to those observed in pre-menopausal females (-30%) and older men (-14%) (Kruger et al., 2006; Kukuljan, Ducher, et al., 2009). Conversely, bone formation markers remained elevated with no changes in P1NP levels observed in either group. This result differs from other trials of calcium supplementation, where decreases in P1NP levels approximating 15% have been observed (Kruger et al., 2006; Kukuljan, Ducher, et al., 2009). The average age of jockeys in this study is 20 years, making it plausible that P1NP levels remained elevated due to sustained bone mass consolidation, which has been found to continue into the mid-20s in

males in the spine and hip (Szulc et al., 2000) and later for long bones (Henry et al., 2010; Lorentzon et al., 2005).

Overall, supplementation appears to have impacted bone metabolism which in turn, influenced bone resorption. This is evidenced by alterations to cortical bone material properties as measured by pQCT in the tibia (Chapter 6) and a reduction in bone resorption in the endocortical region of bone estimated by BoneJ analysis (Chapter 7).

8.3 Effect of supplementation on the non-weight bearing radius

Chapters 5 investigated whether non-weight bearing bone, i.e. the radius, would display improvements in bone material properties following 800mg calcium and 400IU vitamin D supplementation daily for a period of six months. Differences in pQCT derived structural properties of bone between the supplemented and control group following the intervention period were derived for the 4% distal and 66% proximal radius. It was hypothesised that the supplementation would be 'an effective and feasible strategy for improving the bone material properties at the radius' in this group of young male jockeys. Unlike the weight-bearing tibia, the supplementation regime did not have any measurable positive effects on the bone properties of the radius, thus rejecting the hypothesis.

Superior bone material properties at the radius have been observed in jockeys (Greene et al., 2013; Leydon & Wall, 2002) suggesting a positive influence of physical strain on bone adaptations. However, measurement of trabecular area, trabecular density, total content, total area and total density at the 4% distal site demonstrated no between-group differences following the intervention. Similarly, cortical area, cortical density, cortical content and total bone area at the proximal radius remained consistent

between the two groups. Further, no observed differences were seen in measures of bone strength (BSI or SSI-Polar), or endocortical circumference and pericortical circumference. When the results of the radius are considered in light of the alterations to bone turnover markers and the observed changes in the tibia, it is plausible that six months supplementation may have been an inadequate intervention period to observe changes to bone material properties in the non-weight bearing radius.

8.4 Effect of supplementation on weight-bearing bone

Chapters 6 investigated the influences of 800mg calcium and 400IU vitamin D supplementation daily at the weight-bearing tibia using pQCT. The study examined the differences in pQCT-derived structural properties of bone at the 4% distal and 66% proximal sites of the tibia between the supplemented and control group following six months supplementation. It was hypothesised that the supplementation would be 'an effective and feasible strategy for improving the bone material properties at the tibia'.

This hypothesis was supported at the 66% proximal site, with analysis of the pQCT data revealing adjusted post-intervention bone properties in the supplemented group being 6.6% greater for cortical content (mg·mm), 5.9% larger cortical area (mm^2), 1.3% greater cortical density ($mg·cm^3$) and total area (mm^2) being 4% larger after the six-month intervention. No changes were observed in cortical thickness. For the significant variables, partial η^2 indicate moderate to high treatment effect, ranging from 0.53 for total area to 0.7 for cortical content, suggesting an effect does exist for supplementation on cortical bone material properties.

The hypothesis was rejected at the 4% distal site, with no between-group differences being observed in trabecular content, trabecular density or trabecular area.

Further, no significant post-intervention differences were seen in total density or total area at the 4% distal site. The lack of response in the trabecular variables suggests that jockeys may incur lower levels of ground reaction forces compared to torsional stressors to the bone. Together with the relatively short duration of the intervention, this could possibly explain the lack of response in trabecular bone. While the SSI improved in the supplemented group over the period, the differences were not significant. When coupled with the higher than anticipated drop-out rate which may have impacted the power of the study, this suggests that six months may not have been long enough to produce a significant change in the key outcome variable.

8.5 Influence of physical strain in concert with calcium and vitamin D supplementation

In light of the positive responses observed within the cortical bone of the tibia, Chapter 7 further explored the changes to bone material properties within this cohort of jockeys. Regional responses to bone strain together with calcium and vitamin D supplementation were assessed using BoneJ pQCT distribution plug-in (Rantalainen, Nikander, Heinonen, et al., 2011) to further analyse the bone scans obtained through pQCT. It was hypothesised that 'supplementation in concert with mechanical loading from weight-bearing activity is associated with reduced cortical vBMD'.

As outlined in Chapter 2, bone (re)modelling maintains structural integrity and results from a combination of physical strain and calcium homeostasis. Physical activity appears to affect bone in a site specific manner, so that only bone exposed to loading will undergo modelling (see sections 2.3.2 and 2.3.3). Strain enhances periosteal apposition and/or reduces resorption in the endocortical region (Kukuljan et al., 2011). Examination

of athletes revealed that expansion of bone cross-sectional area, rather than changes to vBMD, appear to influence bone strength (Rantalainen, Nikander, Daly, et al., 2011). Conversely, calcium and vitamin D supplementation appears to have anti-resorptive influences, decreasing remodelling. Supplementation appears to preserve BMD through a reduction in endocortical resorption and maintenance of cortical thickness (Daly et al., 2014). Together, this suggests calcium and bone strain work in concert to improve bone properties, particularly where there is an initial calcium deficiency, but makes it unclear whether increases or decreases to vBMD will occur.

Results from the BoneJ analysis revealed a trend towards increasing mineral mass following the intervention, particularly in the posterior region of the tibia. Adjusted mean mineral mass (mg) was 12.03 ±0.11 for supplemented group vs 11.73 ±0.10 (p=0.072). Further there was a trend towards expansion of the radii in the supplemented group particularly in the posterior and lateral regions.

Examination of vBMD ($mg \cdot cm^3$) within the endocortical, mid-cortical and pericortical radii by anatomical sectors showed significantly greater vBMD in the posterior endocortical radius for the supplemented group (1140.5 ± 6.3 (S) vs 1116.2 ± 5.9 (P); p=0.018). Anterior pericortical radial vBMD for the supplemented group was also larger although not significant (1160.1 ± 8.8 vs 1137.4 ± 8.3; p=0.09). These results support the suggestion that supplementation reduces endocortical resorption (Daly et al., 2014).

8.6 Other influences on bone

8.6.1 Vitamin D

Supplementation resulted in the active group experiencing an increase in vitamin D levels ($nmol \cdot L^{-1}$) from 64.6 ± 19.5 to 75.6 ± 20.8 whilst the placebo group's serum levels

fell from 81.2 ± 24.4 to 73.4 ± 20.8. Without adequate levels of vitamin D, calcium absorption will be limited, thus making it essential that vitamin D be available. At the present time, consensus as to adequacy of vitamin D levels is not unanimous. A minimum of 50 nmol·L^{-1} serum vitamin D is suggested by the International Osteoporosis Foundation as adequate to prevent bone related disorders with general nutrition guidelines being as low as 25 nmol·L^{-1} (National Health and Medical Research Council, 2006b). Others suggest that vitamin D levels should be around 75 nmol·L^{-1}, particularly for optimisation of bone health outcomes (Bischoff-Ferrari et al., 2006; Dawson-Hughes et al., 2005; Vieth, 2004, 2006).

Findings suggest that vitamin D levels may be more essential to maintain calcium metabolism and PTH levels than high calcium intakes (Steingrimsdottir et al., 2005). Optimisation of calcium absorption appears to correlate to threshold levels of 80 nmol·L^{-1} serum vitamin D, below which calcium absorption may be impaired (Heaney et al., 2003), implying that higher levels of serum vitamin D should be maintained for bone health.

Given that the supplemented group ANCOVA results were 81.9nmol·L^{-1} following the intervention, it is plausible that the additional vitamin D provided to the jockeys assisted in enhancing calcium absorption in the supplemented group. This was reflected in significant improvements in cortical bone material properties as well as indications that mineral mass and bone radii were altered at the tibia.

8.6.2 Dietary intake and energy imbalance

The dietary analysis incorporated into Chapters 5 and 6 found both groups had insufficient daily energy intakes as well as low calcium levels. Daily calcium intake (mg) was estimated to be between 669.7 ± 274.3 and 790.4 ± 423.9 at baseline, well below the

recommended levels of 1,000 mg per day. While there was a wide variation in reported energy intakes (kJ) (7,723 ± 2,974 to 9,626 ± 4,758), the questionnaire used has adequate reliability and supported previous findings relating to jockeys (refer section 2.7.3). Recommended minimum daily energy intakes for athletes are approximately 188-210 kj·kgbw·day^{-1} (Sundgot-Borgen & Garthe, 2011), whilst the jockeys in this study averaged between 144-152 kj·kgbw·day^{-1}. Limited energy intake coupled with excessive energy expenditure typical of a number of athletic populations has been found to contribute to poor bone health (Ebeling, 2008; Fredericson et al., 2007; Greene et al., 2013; Nichols & Rauh, 2011; Rector et al., 2008; Smathers et al., 2009).

Anthropometric measures revealed this group of jockeys to be very lean compared to other weight category athletes (Garrido-Chamorro et al., 2012), with sum of six skinfolds (mm) of between 32.9 ± 5.1 and 36.4 ± 7.0 being observed over the six month period. Further, body mass index in this group ranged between 17.3 - 19.3 kg·m^2 which was below the 20 kg·m^2 reported by others (Wilson et al., 2014). These measures further support low levels of energy balance and reported energy intakes.

Bone cell function is directly affected by endocrine changes that mobilize stored fuels which must be accessed during times of prolonged energy expenditure and/or inadequate energy intake (De Souza et al., 2008; Ihle & Loucks, 2004). The continual habitual restriction of energy availability that prevails amongst this population may counter any benefits of increased availability of calcium and vitamin D. However, the supplementation regime appears to be adequate to elicit a positive bone response.

8.6.3 Lifestyle factors

Findings from the lifestyle questionnaire indicate that 17 of the apprentices in this study had incurred at least one fracture of which 12 reported riding related injuries with the others sporting related. Most reported multiple fractures. Numbers of injuries and locations were:

- Wrist – 5
- Hand or fingers – 7
- Forearm – 5
- Collarbone – 5
- Pelvis – 3
- Leg - 6

Injury related data from around the world shows that majority of injuries sustained by jockeys are fractures (Foote et al., 2011). Upper limb fractures are the most commonly reported fracture in both the USA and the UK/Ireland, and while Australian injury data is not well documented, recent reports indicated that 78% of all jockeys surveyed incurred a fracture due to riding (Foote et al., 2011). There is a distinct lack of follow-up data in relation to bone health in retired jockeys with recent work not reporting bone related data despite the use of DXA for body composition purposes (Cullen et al., 2016). The number of injuries highlights the need for improving bone material properties in this group.

8.7 Contributions to existing literature

The use of calcium and vitamin D supplementation as a means of improving bone material properties has been under-explored in male cohorts. The systematic review and meta-analysis has been rigorously conducted, highlighting a number of gaps within the

existing literature, namely: a distinct absence of data in younger male cohorts and athletic populations; a heavy reliance on DXA aBMD measures with no cortical or trabecular bone material property information available; no indication as to what time period is required for supplementation to have a significant effect on bone properties; and further, no data which examines the impact of calcium and vitamin D supplementation on reducing fracture risk in otherwise healthy male cohorts.

To date, research pertaining to jockeys has focussed on a number of key areas, namely: dietary practices and weight management (Caulfield & Karageorghis, 2008; Cotugna et al., 2011; Dolan et al., 2011; Leydon & Wall, 2002; Moore et al., 2002; Wilson et al., 2014; Wilson et al., 2015); hormonal and health status (Cullen et al., 2016; Dolan, McGoldrick, et al., 2012; Guillemant et al., 2001; Warrington et al., 2009; Wilson, Fraser, et al., 2013) and; bone health (Dolan, Crabtree, et al., 2012; Dolan, McGoldrick, et al., 2012; Greene et al., 2013; Leydon & Wall, 2002; Waldron-Lynch et al., 2010; Warrington et al., 2009). A select number of studies has attempted to quantify energy expenditure of jockeys (Trowbridge et al., 1995; Wilson, Sparks, et al., 2013); however, this has proven difficult given restrictions of using equipment out in the field due to safety and competition requirements. No randomised controlled trials have been conducted in this athletic cohort to date.

The overall consensus in relation to bone health is that jockeys prematurely display reduced bone material properties, however no study to date has addressed a means of rectifying this situation. The current research has tested whether a simple intervention, i.e. daily calcium and vitamin D supplementation would result in positive bone outcomes. New knowledge has been gained relating to vBMD, cortical and

trabecular bone properties, of both weight- and non-weight bearing bone in jockeys as existing literature predominantly used DXA to assess bone health. Additionally, by using IOF recommended BTMs, data obtained in this group has added to current information of BTM profiles of jockeys and will be comparable to other populations. Anthropometric data obtained has added physical characteristics to the existing knowledge base as anthropometric data has previously been limited to height, weight and estimates of body fat obtained via DXA.

The response to the supplementation in this group adds new knowledge, not only to jockeys, but also to the existing literature relating to both BTMs and bone material property responses to supplementation in: weight-restricted athletes; male athletes known to have compromised bone health; and healthy male populations. Further, the post-processing of the pQCT data through BoneJ has provided some new insight into site-specific ways in which weight-bearing bone reacts to supplementation. Previous examinations of bone properties using BoneJ have characterised bone in general and athletic populations; however, with the exception of one study (Macdonald et al., 2009) these analyses have not followed an intervention and none have examined responses following calcium and vitamin D supplementation.

It is acknowledged that the final study size was small, however it should be recognised that three-quarters of the male apprentice jockey population located in NSW and Victoria were recruited the study. Professional obligations on the part of the jockeys resulted in a large number of the participants being lost to follow-up. Coupled with the shorter study duration, this may have impacted on the power of the study, affecting our ability to detect significance in the strength strain index. Six months is recognised as being

a short duration in terms of manifestations to bone alterations. A year-long intervention may have borne out significant alterations to the radius bone properties and strength indices. Unfortunately, this was impractical with this group of participants as 21 of the original 29 jockeys were in their final apprenticeship year and would most likely have been lost to follow-up. Nevertheless, recognition should be given to the highly significant results obtained at the tibia and within the bone turnover markers despite the reduced final study size.

8.8 Directions and Future Research

To strengthen the findings found in this research, future studies should:

- Extend the intervention period beyond six months to further explore changes to bone material properties at both the tibia and radius. It is acknowledged that six months represent a relatively short time period to observe adaptations to bone properties. Had the timeframe been longer, changes to parameters such as cortical thickness and bone strength indices (SSI and BSI) may have been observed. Further alterations to trabecular properties may have been observed in the tibia as well as improvements to radial bone material properties.

- Replicate the study using female jockeys to establish whether the supplementation regime would result in positive modifications to bone material properties. The current study excluded female participants as the numbers of female apprentice jockeys available was insufficient to provide power for a sub-group analysis. Further, females deposit bone on the endocortical surface while males deposit bone on the periosteal surface,

suggesting there would not be a homogenous response (Schoenau et al., 2001).

- Extend the scope of the study to other athletic populations known to demonstrate low BMD, such as cyclists and endurance runners, who habitually undertake excessive amounts of exercise and limit body weight in order to maximise power to weight ratios.

- Include additional blood markers of bone homeostasis, specifically PTH, as this hormone works in concert with vitamin D to regulate osteoclastic activity. Increasing levels of PTH would indicate continued bone resorption, while decreasing levels of PTH would be indicative of an inhibition of osteoclastic activity. This would further assist in clarifying alterations to BTMs.

- Extend the blood parameters to include markers of energy metabolism to further establish the effects of prolonged weight regulation on bone material properties. Additionally, these markers of energy metabolism would assist in interpreting dietary information provided by the subjects, particularly in the absence of energy expenditure data. Specific measures could include: plasma levels of insulin and IGF-1, cortisol and growth hormone which are known to have an effect on the function of bone cells. (De Souza et al., 2008; Ihle & Loucks, 2004).

- Studies with older, retired jockeys specifically examining bone material properties would assist in establishing whether there are prolonged

effects of persistent weight regulation. Whilst the health characteristics of retired jockeys has recently been examined, this did not include data relating to bone health.

- Elucidate impact loads at the tibia and radius through the habitual physical activity of jockeys would assist in establishing the level of musculoskeletal strain incurred through horse riding.

- Quantification of energy expenditure encompassing training and routine physical activity would help establish levels of energy deficit.

8.9 Final Remarks

At a time when energy intake should be optimised to build muscle and bone, apprentice jockeys actively restrict energy consumption, compromising growth and impacting bone health, amongst other things. The results of this double-blind randomised placebo controlled trial of calcium and vitamin D supplementation are encouraging as a means of counteracting the deleterious effects of engaging in a prolonged weight-restricted sport. Reductions in bone resorption together with improvements to the tibial shaft suggest that 800mg of calcium combined with 400IU of vitamin D daily would assist in improving bone outcomes in this group of weight-restricted athletes, potentially reducing fracture risk and improving their longer-term bone health.

References

Abrahamsen, B., Masud, T., Avenell, A., Anderson, F., Meyer, H. E., Cooper, J. C., . . . Johansen, A. (2010). Patient level pooled analysis of 68 500 patients from seven major vitamin D fracture trials in US and Europe. The DIPART (vitamin D Individual Patient Analysis of Randomized Trials) Group. *British Medical Journal*, 340:b5463.

Adami, S., Gatti, D., Braga, V., Bianchini, D., & Rossini, M. (1999). Site-Specific Effects of Strength Training on Bone Structure and Geometry of Ultradistal Radius in Postmenopausal Women. *Journal of Bone and Mineral Research, 14*(1), 120-124.

Aloia, J., Bojadzievski, T., Yusupov, E., Shahzad, G., Pollack, S., Mikhail, M., & Yeh, J. (2010). The relative influence of calcium intake and vitamin D status on serum parathyroid hormone and bone turnover biomarkers in a double-blind, placebo-controlled parallel group, longitudinal factorial design. *Journal of Clinical Endocrinology & Metabolism, 95*(7), 3216-3224.

An, Y. H., & Draughn, R. A. (1999). *Mechanical testing of bone and the bone-implant interface*: CRC press.

Angeline, M. E., Gee, A. O., Shindle, M., Warren, R. F., & Rodeo, S. A. (2013). The effects of vitamin D deficiency in athletes. *The American Journal of Sports Medicine, 41*(2), 461-464.

Armstrong, L. E., Maresh, C. M., Castellani, J. W., Bergeron, M. F., Kenefick, R. W., LaGasse, K. E., & Riebe, D. (1994). Urinary indices of hydration status. *International journal of sport nutrition, 4*(3), 265.

Augat, P., Gordon, C. L., Lang, T. F., Iida, H., & Genant, H. K. (1998). Accuracy of cortical and trabecular bone measurements with peripheral quantitative computed tomography (pQCT). *Physics in Medicine and Biology, 43*(10), 2873.

Bachrach, L. K. (2001). Acquisition of optimal bone mass in childhood and adolescence. *Trends in Endocrinology & Metabolism, 12*(1), 22-28.

Bailey, D. A. (1997). The Saskatchewan Pediatric Bone Mineral Accrual Study: bone mineral acquisition during the growing years. *Int J Sports Med, 18*(S 3), S191-S194.

Bailey, D. A., Faulkner, R. A., & McKay, H. A. (1996). Growth, physical activity, and bone mineral acquisition. *Exercise & Sport Sciences Reviews, 24*(1), 233-266.

Bailey, D. A., Martin, A. D., McKay, H. A., Whiting, S., & Mirwald, R. L. (2000). Calcium accretion in girls and boys during puberty: a longitudinal analysis. *Journal of Bone and Mineral Research, 15*(11), 2245-2250.

Bailey, D. A., McKay, H. A., Mirwald, R. L., Crocker, P. R. E., & Faulkner, R. A. (1999). A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: The University of Saskatchewan Bone Mineral Accrual Study. *Journal of Bone and Mineral Research, 14*(10), 1672-1679.

Barry, D. W., & Kohrt, W. M. (2008). BMD decreases over the course of a year in competitive male cyclists. *Journal of Bone and Mineral Research, 23*(4), 484-491.

Baxter-Jones, A. D. G., Faulkner, R. A., Forwood, M. R., Mirwald, R. L., & Bailey, D. A. (2011). Bone mineral accrual from 8 to 30 years of age: An estimation of peak bone mass. *Journal of Bone and Mineral Research, 26*(8), 1729-1739.

Baxter-Jones, A. D. G., Mirwald, R. L., McKay, H. A., & Bailey, D. A. (2003). A longitudinal analysis of sex differences in bone mineral accrual in healthy 8-19-year-old boys and girls. *Annals of Human Biology, 30*(2), 160-175.

Binkley, T. L., Berry, R., & Specker, B. L. (2008). Methods for measurement of pediatric bone. *Reviews in Endocrine and Metabolic Disorders, 9*(2), 95-106.

Bischoff-Ferrari, H. A., Giovannucci, E., Willett, W. C., Dietrich, T., & Dawson-Hughes, B. (2006). Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *The American Journal of Clinical Nutrition, 84*(1), 18-28.

Blimkie, C. J. R., Rice, S., Webber, C. E., Martin, J., Levy, D., & Gordon, C. L. (1996). Effects of resistance training on bone mineral content and density in adolescent females. *Canadian journal of physiology and pharmacology, 74*(9), 1025-1033.

Boot, A. M., de Ridder, M. A. J., van der Sluis, I. M., van Slobbe, I., Krenning, E. P., & de Muinck Keizer-Schrama, S. M. P. F. (2010). Peak bone mineral density, lean body mass and fractures. *Bone, 46*(2), 336-341.

Bouillon, R., Bischoff-Ferrari, H., & Willett, W. (2008). Vitamin D and health: perspectives from mice and man. *Journal of Bone and Mineral Research, 23*(7), 974-979.

Briot, K., Cortet, B., Trémollières, F., Sutter, B., Thomas, T., Roux, C., & Audran, M. (2009). Male osteoporosis: Diagnosis and fracture risk evaluation. *Joint Bone Spine, 76*(2), 129-133.

Brooks, G. A., & Mercier, J. (1994). Balance of carbohydrate and lipid utilization during exercise: the "crossover" concept. *Journal of Applied Physiology, 76*(6), 2253-2261.

Caulfield, M. J., & Karageorghis, C. I. (2008). Psychological effects of rapid weight loss and attitudes towards eating among professional jockeys. *Journal of Sports Sciences, 26*(9), 877-883.

Chung, M., Balk, E. M., Brendel, M., Ip, S., Lau, J., Lee, J. E., . . . Trikalinos, T. A. (2009). Vitamin D and calcium: a systematic review of health outcomes. *Evidence Report Technology Assessment (Full Rep), Aug*(183), 1-420.

Clarke, B. (2008). Normal Bone Anatomy and Physiology. *Clinical Journal of the American Society of Nephrology, 3*(Supplement 3), S131-S139.

Close, G. L., Russell, J., Cobley, J. N., Owens, D. J., Wilson, G., Gregson, W., . . . Morton, J. P. (2012). Assessment of vitamin D concentration in non-supplemented professional athletes and healthy adults during the winter months in the UK: implications for skeletal muscle function. *Journal of Sports Sciences, 31*(4), 344-353.

Cooley, H., & Jones, G. (2001). A population-based study of fracture incidence in southern Tasmania: lifetime fracture risk and evidence for geographic variations within the same country. *Osteoporosis International, 12*(2), 124-130.

Cooper, D. M. L., Ahamed, Y., Macdonald, H. M., & McKay, H. A. (2008). Characterising cortical density in the mid-tibia: intra-individual variation in adolescent girls and boys. *British Journal of Sports Medicine, 42*(8), 690-695.

Cotugna, N., Snider, O. S., & Windish, J. (2011). Nutrition assessment of horse-racing athletes. *Journal of Community Health, 36*(2), 261-264.

Cranney, A., Horsley, T., O'Donnell, S., Weiler, H., Puil, L., Ooi, D., . . . Mamaladze, V. (2007). Effectiveness and safety of vitamin D in relation to bone health. . *Evidence Report/Technology Assessment, No. 158*, 1-235.

Cullen, S., Donohoe, A., McGoldrick, A., McCaffrey, N., Davenport, C., Byrne, B., . . . Warrington, G. (2016). Physiological and health characteristics of ex-jockeys. *Journal of Science and Medicine in Sport, 19*(4), 283-287.

Dalsky, G. P. (1990). Effect of excerise on bone: permissive influene of estrogen and calcium. *Medicine & Science in Sports & Exercise, 22*(3), 281-285.

Daly, R. M., Bass, S., & Nowson, C. (2006). Long-term effects of calcium-vitamin-D3-fortified milk on bone geometry and strength in older men. *Bone, 39*(4), 946-953.

Daly, R. M., Brown, M., Bass, S., Kukuljan, S., & Nowson, C. (2006). Calcium- and vitamin D3-fortified milk reduces bone loss at clinically relevant skeletal sites in older men: a 2-year randomized controlled trial. *Journal of Bone & Mineral Research, 21*(3), 397-405.

Daly, R. M., Duckham, R. L., & Gianoudis, J. (2014). Evidence for an interaction between exercise and nutrition for improving bone and muscle health. *Current osteoporosis reports, 12*(2), 219-226.

Dawson-Hughes, B., Harris, S. S., Krall, E. A., & Dallal, G. E. (1997). Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. *New England Journal of Medicine, 337*(10), 670-676.

Dawson-Hughes, B., Harris, S. S., Krall, E. A., & Dallal, G. E. (2000). Effect of withdrawal of calcium and vitamin D supplements on bone mass in elderly men and women. *American Journal of Clinical Nutrition, 72*(3), 745-750.

Dawson-Hughes, B., Heaney, R. P., Holick, M. F., Lips, P., Meunier, P. J., & Vieth, R. (2005). Estimates of optimal vitamin D status. *Osteoporos Int, 16*(7), 713-716.

De Souza, M. J., West, S. L., Jamal, S. A., Hawker, G. A., Gundberg, C. M., & Williams, N. I. (2008). The presence of both an energy deficiency and estrogen deficiency exacerbate alterations of bone metabolism in exercising women. *Bone, 43*(1), 140-148.

De Souza, M. J., & Williams, N. I. (2005). Beyond hypoestrogenism in amenorrheic athletes: energy deficiency as a contributing factor for bone loss. *Current Sports Medicine Reports, 4*(1), 38-44.

Delmas, P. D., Eastell, R., Garnero, P., Seibel, M. J., & Stepan, J. (2000). The Use of Biochemical Markers of Bone Turnover in Osteoporosis. *Osteoporosis International, 11*(6), S2-S17.

Devienne, M.-F., & Guezennec, C.-Y. (2000). Energy expenditure of horse riding. *European Journal of Applied Physiology, 82*(5-6), 499-503.

Dhanwal, D. K., Dennison, E. M., Harvey, N. C., & Cooper, C. (2011). Epidemiology of hip fracture: Worldwide geographic variation. *Indian journal of orthopaedics, 45*(1), 15.

Diamond, T. H., Eisman, J. A., Mason, R. S., Nowson, C. A., Pasco, J. A., Sambrook, P. N., & Wark, J. D. (2005). Vitamin D and adult bone health in Australia and New Zealand: a position statement. *Medical journal of Australia, 182*(6), 281-285.

Dolan, E., Crabtree, N., McGoldrick, A., Ashley, D. T., McCaffrey, N., & Warrington, G. (2012). Weight regulation and bone mass: a comparison between professional jockeys, elite amateur boxers, and age, gender and BMI matched controls. *Journal of Bone and Mineral Metabolism, 30*(2), 164-170.

Dolan, E., McGoldrick, A., Davenport, C., Kelleher, G., Byrne, B., Tormey, W., . . . Warrington, G. (2012). An altered hormonal profile and elevated rate of bone loss are associated with low bone mass in professional horse-racing jockeys. *Journal of Bone and Mineral Metabolism, 30*(5), 534-542.

Dolan, E., O'Connor, H., McGoldrick, A., O'Loughlin, G., Lyons, D., & Warrington, G. (2011). Nutritional, lifestyle, and weight control practices of professional jockeys. *Journal of Sports Sciences, 29*(8), 791-799.

Donnelly, E. (2011). Methods for Assessing Bone Quality: A Review. *Clinical Orthopaedics and Related Research®, 469*(8), 2128-2138.

Doube, M., Kłosowski, M. M., Arganda-Carreras, I., Cordelières, F. P., Dougherty, R. P., Jackson, J. S., . . . Shefelbine, S. J. (2010). BoneJ: Free and extensible bone image analysis in ImageJ. *Bone, 47*(6), 1076-1079.

Douglas, J. L., Price, M., & Peters, D. M. (2012). A systematic review of physical fitness, physiological demands and biomechanical performance in equestrian athletes. *Comparative Exercise Physiology, 8*(1), 53-62.

Dowthwaite, J., Kanaley, J., Spadaro, J., Hickman, R., & Scerpella, T. (2009). Muscle indices do not fully account for enhanced upper extremity bone mass and strength in gymnasts. *Journal of Musculoskeletal and Neuronal Interactions, 9*(1), 2-14.

Duckham, R. L., Baxter-Jones, A. D., Johnston, J. D., Vatanparast, H., Cooper, D., & Kontulainen, S. (2014). Does Physical Activity in Adolescence Have Site-Specific and Sex-Specific Benefits on Young Adult Bone Size, Content, and Estimated Strength? *Journal of Bone and Mineral Research, 29*(2), 479-486.

Ebeling, P. R. (2008). Osteoporosis in men. *New England Journal of Medicine, 358*(14), 1474-1482.

Ebeling, P. R. (2014). *Osteoporosis in Men: Why change needs to happen*. Retrieved from

Ebeling, P. R., Daly, R. M., Kerr, D. A., Kimlin, M. G., Bailey, C., Banks, E., . . . English, D. (2013). Building healthy bones throughout life: an evidence-informed strategy to prevent osteoporosis in Australia. *MJA Open, 2*(Supp 1:1).

Faulkner, K. G. (2000). Bone matters: Are density increases necessary to reduce fracture risk? *Journal of Bone and Mineral Research, 15*(2), 183-187.

Field, A. (2013). *Discovering statistics using IBM SPSS statistics* (3rd ed.). Los Angeles, London: Sage.

Foote, C., McIntosh, A., V'Landys, P., & Bulloch, K. (2011). *Health and saftey in Australian horse racing*. Rural Industries Research and Development Corporation.

Fredericson, M., Chew, K., Ngo, J., Cleek, T., Kiratli, J., & Cobb, K. (2007). Regional bone mineral density in male athletes: a comparison of soccer players, runners and controls. *British Journal of Sports Medicine, 41*(10), 664-668.

Frost, H. M. (1987). Bone "mass" and the "mechanostat": a proposal. *The anatomical record, 219*(1), 1-9.

Frost, H. M. (1998). Changing concepts in skeletal physiology: Wolff's Law, the Mechanostat, and the "Utah Paradigm". *American Journal of Human Biology, 10*(5), 599-605.

Frost, H. M. (2004). A 2003 update of bone physiology and Wolff's Law for clinicians. *The Angle orthodontist, 74*(1), 3-15.

Garrido-Chamorro, R., Sirvent-Belando, J. E., González-Lorenzo, M., Blasco-Lafarga, C., & Roche, E. (2012). Skinfold sum: reference values for top athletes. *International Journal of Morphology, 30*(3), 803-809.

Grampp, S., Lang, P., Jergas, M., Glüer, C. C., Mathur, A., Engelke, K., & Genant, H. K. (1995). Assessment of the skeletal status by peripheral quantitative computed tomography of the forearm: Short-term precision in vivo and comparison to dual X-ray absorptiometry. *Journal of Bone and Mineral Research, 10*(10), 1566-1576.

Greene, D. A., Naughton, G., Moresi, M., & Bradshaw, E. (2012). Cortical bone distribution at the tibial shaft in adolescent female athletes. *Journal of Science and Medicine in Sport, 15, Supplement 1*(0), S227.

Greene, D. A., & Naughton, G. A. (2011). Calcium and vitamin-D supplementation on bone structural properties in peripubertal female identical twins: a randomised controlled trial. *Osteoporosis International, 22*(2), 489-498.

Greene, D. A., Naughton, G. A., Bradshaw, E., Moresi, M., & Ducher, G. (2012). Mechanical loading with or without weight-bearing activity: influence on bone strength index in elite female adolescent athletes engaged in water polo, gymnastics, and track-and-field. *Journal of Bone and Mineral Metabolism, 30*(5), 580-587.

Greene, D. A., Naughton, G. A., Jander, C. B., & Cullen, S. J. (2013). Bone Health of Apprentice Jockeys Using Peripheral Quantitative Computed Tomography. *Int J Sports Med, 34*(EFirst), 688-694.

Guillemant, J., Accarie, C., Peres, G., & Guillemant, S. (2004). Acute Effects of an Oral Calcium Load on Markers of Bone Metabolism During Endurance Cycling Exercise in Male Athletes. *Calcified Tissue International, 74*(5), 407-414.

Guillemant, J., Le, H. T., Maria, A., Allemandou, A., Pérès, G., & Guillemant, S. (2001). Wintertime vitamin D deficiency in male adolescents: effect on Parathyroid Function and response to vitamin D3 supplements. *Osteoporosis International, 12*(10), 875-879.

Haapasalo, H., Kontulainen, S., Sievänen, H., Kannus, P., Järvinen, M., & Vuori, I. (2000). Exercise-induced bone gain is due to enlargement in bone size without a change in volumetric bone density: a peripheral quantitative computed tomography study of the upper arms of male tennis players. *Bone, 27*(3), 351-357.

Hagerman, F. C. (1992). Energy metabolism and fuel utilization. *Medicine & Science in Sports & Exercise, 24*(9), 309-314.

Harada, S.-I., & Rodan, G. A. (2003). Control of osteoblast function and regulation of bone mass. *Nature, 423*(6937), 349.

Heaney, R. P. (2008). Vitamin D and calcium interactions: functional outcomes. *The American Journal of Clinical Nutrition, 88*(2), 541S-544S.

Heaney, R. P., Abrams, S., Dawson-Hughes, B., Looker, A., Looker, A., Marcus, R., . . . Weaver, C. (2000). Peak bone mass. *Osteoporosis International, 11*(12), 985-1009.

Heaney, R. P., Barger-Lux, M. J., Dowell, M. S., Chen, T. C., & Holick, M. F. (1997). Calcium Absorptive Effects of Vitamin D and Its Major Metabolites. *The Journal of Clinical Endocrinology & Metabolism, 82*(12), 4111-4116.

Heaney, R. P., Dowell, M. S., Hale, C. A., & Bendich, A. (2003). Calcium Absorption Varies within the Reference Range for Serum 25-Hydroxyvitamin D. *Journal of the American College of Nutrition, 22*(2), 142-146.

Heaney, R. P., & Weaver, C. M. (2005). Newer Perspectives on Calcium Nutrition and Bone Quality. *Journal of the American College of Nutrition, 24*(sup6), 574S-581S.

Henry, M. J., Pasco, J. A., Korn, S., Gibson, J. E., Kotowicz, M. A., & Nicholson, G. C. (2010). Bone mineral density reference ranges for Australian men: Geelong Osteoporosis Study. *Osteoporosis International, 21*(6), 909-917.

Higdon, J., & Drake, V. (2011). *An evidence-based approach to vitamins and minerals: health benefits and intake recommendations*. New York, NY: Thieme.

Hind, K., Truscott, J. G., & Evans, J. A. (2006). Low lumbar spine bone mineral density in both male and female endurance runners. *Bone, 39*(4), 880-885.

Hitchens, P., Blizzard, L., Jones, G., Day, L., & Fell, J. (2011). Are physiological attributes of jockeys predictors of falls? A pilot study. *BMJ open, 1*(1).

Hodge, A., Patterson, A. J., Brown, W. J., Ireland, P., & Giles, G. (2000). The Anti Cancer Council of Victoria FFQ: relative validity of nutrient intakes compared with weighed food records in young to middle-aged women in a study of iron supplementation. *Australian and New Zealand Journal of Public Health, 24*(6), 576-583.

Hotta, M., Fukuda, I., Sato, K., Hizuka, N., Shibasaki, T., & Takano, K. (2000). The relationship between bone turnover and body weight, serum insulin-like growth factor (IGF) I, and serum IGF-binding protein levels in patients with anorexia nervosa. *Journal of Clinical Endocrinology & Metabolism, 85*(1), 200-206.

Hotta, M., Shibasaki, T., Sato, K., & Demura, H. (1998). The importance of body weight history in the occurrence and recovery of osteoporosis in patients with anorexia nervosa: evaluation by dual X-ray absorptiometry and bone metabolic markers. *European Journal of Endocrinology, 139*(3), 276-283.

Hughes, J., & Petit, M. (2010). Biological underpinnings of Frost's mechanostat thresholds: the important role of osteocytes. *Journal of Musculoskeletal and Neuronal Interactions, 10*(2), 128-135.

Ihle, R., & Loucks, A. B. (2004). Dose-response relationships between energy availability and bone turnover in young exercising women. *Journal of Bone and Mineral Research, 19*(8), 1231-1240.

Ireland, A., Maden-Wilkinson, T., McPhee, J., Cooke, K., Narici, M., Degens, H., & Rittweger, J. (2013). Upper limb muscle-bone asymmetries and bone adaptation in elite youth tennis players. *Medicine and science in sports and exercise, 45*(9), 1749-1758.

Järvinen, T. L. N., Sievänen, H., Jokihaara, J., & Einhorn, T. A. (2005). Revival of Bone Strength: The Bottom Line. *Journal of Bone and Mineral Research, 20*(5), 717-720.

Jee, W. (2000). Principles in bone physiology. *Journal of Musculoskeletal and Neuronal Interactions, 1*(1), 11-13.

Jenkins, N., Black, M., Paul, E., Pasco, J. A., Kotowicz, M. A., & Schneider, H. G. (2013). Age-related reference intervals for bone turnover markers from an Australian reference population. *Bone, 55*(2), 271-276.

Johnell, O., & Kanis, J. (2006). An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. *Osteoporosis International, 17*(12), 1726-1733.

Jones, G., Dwyer, T., Hynes, K. L., Parameswaran, V., & Greenaway, T. M. (2005). Vitamin D insufficiency in adolescent males in Southern Tasmania: prevalence, determinants, and relationship to bone turnover markers. *Osteoporosis International, 16*(6), 636-641.

Jürimäe, J., Purge, P., Jürimäe, T., & Duvillard, S. (2006). Bone metabolism in elite male rowers: adaptation to volume-extended training. *European Journal of Applied Physiology, 97*(1), 127-132.

Kanis, J. A., Johansson, H., Johnell, O., Oden, A., De Laet, C., Eisman, J. A., . . . Tenenhouse, A. (2005). Alcohol intake as a risk factor for fracture. *Osteoporosis International, 16*(7), 737-742.

Kanis, J. A., Johnell, O., Oden, A., Johansson, H., De Laet, C., Eisman, J. A., . . . Tenenhouse, A. (2005). Smoking and fracture risk: a meta-analysis. *Osteoporosis International, 16*(2), 155-162.

Kanis, J. A., McCloskey, E. V., Johansson, H., Oden, A., Melton, L. J., 3rd, & Khaltaev, N. (2008). A reference standard for the description of osteoporosis. *Bone, 42*(3), 467-475.

Khan, K., McKay, H. A., Kannus, P., Bailey, D. A., Wark, J. D., & Bennell, K. (2001). *Physical activity and bone health*. Champaign, Ill.: Human Kinetics.

Klesges, R. C., Ward, K. D., Shelton, M. L., Applegate, W. B., Cantler, E. D., Palmieri, G. M., . . . Davis, J. (1996). Changes in bone mineral content in male athletes. Mechanisms of action and intervention effects. *JAMA, 276*(3), 226-230.

Kontulainen, S. A., Johnston, J. D., Liu, D., Leung, C., Oxland, T. R., & McKay, H. A. (2008). Strength indices from pQCT imaging predict up to 85% of variance in bone failure properties at tibial epiphysis and diaphysis. *Journal of Musculoskeletal and Neuronal Interactions, 8*(4), 401-409.

Kontulainen, S. A., Macdonald, H. M., & McKay, H. A. (2006). Change in Cortical Bone Density and Its Distribution Differs between Boys and Girls during Puberty. *The Journal of Clinical Endocrinology & Metabolism, 91*(7), 2555-2561.

Kruger, M. C., Booth, C. L., Coad, J., Schollum, L. M., Kuhn-Sherlock, B., & Shearer, M. J. (2006). Effect of calcium fortified milk supplementation with or without vitamin K on biochemical markers of bone turnover in premenopausal women. *Nutrition, 22*(11–12), 1120-1128.

Kuczmarski, R. J., Ogden, C. L., Grummer-Strawn, L. M., Flegal, K. M., Guo, S. S., Wei, R., . . . Johnson, C. L. (2000). CDC growth charts: United States. *Adv Data*(314), 1-27.

Kukuljan, S., Ducher, G., Nowson, C. A., Ebeling, P. R., & Daly, R. M. (2009). Long-term effects of exercise and calcium-vitamin D3 supplementation on biochemical

markers of bone turnover and their association with changes in BMD in older men. *Bone, 44*, S82.

Kukuljan, S., Nowson, C. A., Bass, S. L., Sanders, K., Nicholson, G. C., Seibel, M. J., . . . Daly, R. M. (2009). Effects of a multi-component exercise program and calcium-vitamin-D3-fortified milk on bone mineral density in older men: a randomised controlled trial. *Osteoporosis International, 20*(7), 1241-1251.

Kukuljan, S., Nowson, C. A., Sanders, K. M., Nicholson, G. C., Seibel, M. J., Salmon, J., & Daly, R. M. (2011). Independent and combined effects of calcium-vitamin D3 and exercise on bone structure and strength in older men: an 18-month factorial design randomized controlled trial. *Journal of Clinical Endocrinology & Metabolism, 96*(4), 955-963.

Lai, Y. M., Qin, L., Hung, V. W. Y., & Chan, K. M. (2005). Regional differences in cortical bone mineral density in the weight-bearing long bone shaft—A pQCT study. *Bone, 36*(3), 465-471.

Lee, S. Y., & Gallagher, D. (2008). Assessment methods in human body composition. *Current Opinions in Clinical Nutrition and Metabolic Care, 11*(5), 566-572.

Lee, W. T., Leung, S. S., Wang, S. H., Xu, Y. C., Zeng, W. P., Lau, J., . . . Cheng, J. C. (1994). Double-blind, controlled calcium supplementation and bone mineral accretion in children accustomed to a low-calcium diet. *American Journal of Clinical Nutrition, 60*(5), 744-750.

Leydon, M., & Wall, C. (2002). New Zealand Jockeys' dietary habits and their potential impact on health. *Int J Sport Nutr Exerc Metab, 12*(2), 220-237.

Lips, P., Gielen, E., & van Schoor, N. M. (2014). Vitamin D supplements with or without calcium to prevent fractures. *Bonekey Rep, 3*, 512.

Lorentzon, M., Mellström, D., & Ohlsson, C. (2005). Age of Attainment of Peak Bone Mass Is Site Specific in Swedish Men—The GOOD Study. *Journal of Bone and Mineral Research, 20*(7), 1223-1227.

Loucks, A. B. (2004). Energy balance and body composition in sports and exercise. *Journal of Sports Sciences, 22*(1), 1-14.

Loucks, A. B. (2007). Low energy availability in the marathon and other endurance sports. *Sports Medicine, 37*(4-5), 348-352.

Macdonald, H. M., Cooper, D. M., & McKay, H. A. (2009). Anterior-posterior bending strength at the tibial shaft increases with physical activity in boys: evidence for non-uniform geometric adaptation. *Osteoporos Int, 20*(1), 61-70.

Marieb, E. N. (2000). *Essentials of human anatomy & physiology* (8th ed.). San Francisco CA: Pearson Benjamin Cummings.

McCann, J. C., & Ames, B. N. (2008). Is there convincing biological or behavioral evidence linking vitamin D deficiency to brain dysfunction? *The FASEB Journal, 22*(4), 982-1001.

Michelsen, J., Wallaschofski, H., Friedrich, N., Spielhagen, C., Rettig, R., Ittermann, T., . . . Hannemann, A. (2013). Reference intervals for serum concentrations of three bone turnover markers for men and women. *Bone, 57*(2), 399-404.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., & Group, P. (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ, 339*, b2535.

Moher, D., Schulz, K. F., & Altman, D. G. (2001). The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomised trials. *The Lancet, 357*(9263), 1191-1194.

Moore, J. M., Timperio, A. F., Crawford, D. A., Burns, C. M., & Cameron-Smith, D. (2002). Weight management and weight loss strategies of professional jockeys. *International Journal of Sport Nutrition and Exercise Metabolism, 12*(1), 1-13.

National Health and Medical Research Council. (2006a). Calcium. *Nutrient reference values for Australia and New Zealand including recomended dietary intakes. Commonwealth of Australia.* Retrieved from http://www.nrv.gov.au/nutrients/calcium.htm

National Health and Medical Research Council. (2006b). Vitamin D. *Nutrient reference values for Australia and New Zealand including recomended dietary intakes. Commonwealth of Australia.* Retrieved from http://www.nrv.gov.au/nutrients/calcium.htm

Nattiv, A., & Armsey, T. D. (1997). Stress injury to bone in the female athlete. *Clinics in sports medicine, 16*(2), 197-224.

Neu, C. M., Manz, F., Rauch, F., Merkel, A., & Schoenau, E. (2001). Bone densities and bone size at the distal radius in healthy children and adolescents: a study using peripheral quantitative computed tomography. *Bone, 28*(2), 227-232.

Nichols, J. F., & Rauh, M. J. (2011). Longitudinal changes in bone mineral density in male master cyclists and nonathletes. *Journal of Strength & Conditioning Research, 25*(3), 727-734.

Nikander, R., Sievanen, H., Heinonen, A., Daly, R. M., Uusi-Rasi, K., & Kannus, P. (2010). Targeted exercise against osteoporosis: A systematic review and meta-analysis for optimising bone strength throughout life. *BioMed Central Medicine, 8*, 47.

Nikander, R., Sievänen, H., Uusi-Rasi, K., Heinonen, A., & Kannus, P. (2006). Loading modalities and bone structures at nonweight-bearing upper extremity and weight-

bearing lower extremity: a pQCT study of adult female athletes. *Bone, 39*(4), 886-894.

Oppliger, R. A., Magnes, S. A., Popowski, L. A., & Gisolfi, C. V. (2005). Accuracy of urine specific gravity and osmolality as indicators of hydration status. *International Journal of Sport Nutrition and Exercise Metabolism, 15*(3), 236-251.

Orwoll, E. S., Oviatt, S. K., McClung, M. R., Deftos, L. J., & Sexton, G. (1990). The rate of bone mineral loss in normal men and the effects of calcium and cholecalciferol supplementation. *Annals of Internal Medicine, 112*(1), 29-34.

Osteoporosis Australia Medical & Scientific Advisory Committee. (2014). Vitamin D Consumer guide. Retrieved from http://www.osteoporosis.org.au/vitamin-d?cta=Autumn%20is%20here.%20Check%20your%20vitamin%20D.

Peacock, M., Liu, G., Carey, M., McClintock, R., Ambrosius, W., Hui, S., & Johnston, C. C. (2000). Effect of calcium or 25OH vitamin D3 dietary supplementation on bone loss at the hip in men and women over the age of 60. *Journal of Clinical Endocrinology & Metabolism, 85*(9), 3011-3019.

Peat, J. K., & Barton, B. (2005). *Medical statistics: A guide to data analysis and critical appraisal.* Massachusetts, USA: Blackwell Publishing BMJ Books.

Prentice, A., Ginty, F., Stear, S. J., Jones, S. C., Laskey, M. A., & Cole, T. J. (2005). Calcium supplementation increases stature and bone mineral mass of 16- to 18-year-old boys. *Journal of Clinical Endocrinology & Metabolism, 90*(6), 3153-3161.

Racing-NSW. (2014). Apprentice Jockey. Retrieved from http://www.racingnsw.com.au/default.aspx?s=jockey

Raisz, L. G. (1999). Physiology and pathophysiology of bone remodeling. *Clinical Chemistry, 45*(8), 1353+.

Rantalainen, T., Duckham, R. L., Suominen, H., Heinonen, A., Alén, M., & Korhonen, M. T. (2014). Tibial and Fibular Mid-Shaft Bone Traits in Young and Older Sprinters and Non-Athletic Men. *Calcified Tissue International, 95*(2), 132-140.

Rantalainen, T., Heinonen, A., Linnamo, V., Komi, P. V., Takala, T. E., & Kainulainen, H. (2009). Short-term bone biochemical response to a single bout of high-impact exercise. *Journal of sports science & medicine, 8*(4), 553.

Rantalainen, T., Nikander, R., Daly, R. M., Heinonen, A., & Sievänen, H. (2011). Exercise loading and cortical bone distribution at the tibial shaft. *Bone, 48*(4), 786-791.

Rantalainen, T., Nikander, R., Heinonen, A., Daly, R., & Sievänen, H. (2011). An open source approach for regional cortical bone mineral density analysis. *Journal of musculoskeletal & neuronal interactions, 11*(3), 243-248.

Rantalainen, T., Nikander, R., Heinonen, A., Suominen, H., & Sievänen, H. (2010). Direction-specific diaphyseal geometry and mineral mass distribution of tibia and fibula: a pQCT study of female athletes representing different exercise loading types. *Calcified Tissue International, 86*(6), 447-454.

Rantalainen, T., Weeks, B. K., Nogueira, R. C., & Beck, B. R. (2015). Effects of bone-specific physical activity, gender and maturity on tibial cross-sectional bone material distribution: a cross-sectional pQCT comparison of children and young adults aged 5–29 years. *Bone, 72*, 101-108.

Rauch, F., & Schoenau, E. (2001). Changes in Bone Density During Childhood and Adolescence: An Approach Based on Bone's Biological Organization. *Journal of Bone and Mineral Research, 16*(4), 597-604.

Rauch, F., & Schoenau, E. (2008). Peripheral quantitative computed tomography of the proximal radius in young subjects–new reference data and interpretation of results. *Journal of Musculoskeletal Neuronal Interactions, 8*(3), 217-226.

Rector, R. S., Rogers, R., Ruebel, M., & Hinton, P. S. (2008). Participation in road cycling vs running is associated with lower bone mineral density in men. *Metabolism, 57*(2), 226-232.

Reid, I. R., Ames, R., Mason, B., & et al. (2008). Randomized controlled trial of calcium supplementation in healthy, nonosteoporotic, older men. *Archives of Internal Medicine, 168*(20), 2276-2282.

Reid, I. R., Bolland, M. J., & Grey, A. (2014). Effects of vitamin D supplements on bone mineral density: a systematic review and meta-analysis. *The Lancet, 383*(9912), 146-155.

Richardson, J. T. E. (2011). Eta squared and partial eta squared as measures of effect size in educational research. *Educational Research Review, 6*(2), 135-147.

Rizzoli, R., Bianchi, M. L., Garabédian, M., McKay, H. A., & Moreno, L. A. (2010). Maximizing bone mineral mass gain during growth for the prevention of fractures in the adolescents and the elderly. *Bone, 46*(2), 294-305.

Rizzoli, R., Boonen, S., Brandi, M. L., Burlet, N., Delmas, P., & Reginster, J. Y. (2008). The role of calcium and vitamin D in the management of osteoporosis. *Bone, 42*(2), 246-249.

Rockell, J. E., Green, T. J., Skeaff, C. M., Whiting, S. J., Taylor, R. W., Williams, S. M., . . . Wohlers, M. W. (2005). Season and ethnicity are determinants of serum 25-Hydroxyvitamin D concentrations in New Zealand children aged 5–14 y. *Journal of Nutrition, 135*, 2602–2608.

Ross, A. C., Manson, J. E., Abrams, S. A., Aloia, J. F., Brannon, P. M., Clinton, S. K., . . . Shapses, S. A. (2011). The 2011 report on dietary reference intakes for calcium and vitamin D from the institute of medicine: what clinicians need to know. *Journal of Clinical Endocrinology & Metabolism, 96*(1), 53-58.

Rovner, A. J., & O'Brien, K. O. (2008). Hypovitaminosis D among healthy children in the United States - A review of the current evidence. *Archives of Pediatractic and Adolescent Medicine, 162*(6), 513-519.

Schoenau, E., & Frost, H. M. (2002). The "Muscle-Bone Unit" in children and adolescents. *Calcified Tissue International, 70*(5), 405-407.

Schoenau, E., Neu, C. M., Beck, B., Manz, F., & Rauch, F. (2002). Bone mineral content per muscle cross-sectional area as an index of the functional muscle-bone unit. *Journal of Bone and Mineral Research, 17*(6), 1095-1101.

Schoenau, E., Neu, C. M., Rauch, F., & Manz, F. (2001). The development of bone strength at the proximal radius during childhood and adolescence. *Journal of Clinical Endocrinology & Metabolism, 86*(2), 613-618.

Seeman, E. (2002). An exercise in geometry. *Journal of Bone and Mineral Research, 17*(3), 373-380.

Seeman, E., & Delmas, P. D. (2006). Bone quality—the material and structural basis of bone strength and fragility. *New England Journal of Medicine, 354*(21), 2250-2261.

Shea, B., Wells, G., Cranney, A., Zytaruk, N., Robinson, V., Griffith, L., . . . Guyatt, G. (2002). VII. Meta-analysis of calcium supplementation for the prevention of postmenopausal osteoporosis. *Endocrine Reviews, 23*(4), 552-559.

Sievänen, H., Koskue, V., Rauhio, A., Kannus, P., Heinonen, A., & Vuori, I. (1998). Peripheral quantitative computed tomography in human long bones: Evaluation of

in vitro and in vivo precision. *Journal of Bone and Mineral Research, 13*(5), 871-882.

Silk, L. N., Greene, D. A., & Baker, M. K. (2015). The Effect of Calcium or Calcium and Vitamin D Supplementation on Bone Mineral Density in Healthy Males: A Systematic Review and Meta-analysis. *International Journal of Sport Nutrition and Exercise Metabolism, 25*(5), 510-524.

Silk, L. N., Greene, D. A., Baker, M. K., & Jander, C. B. (2015). Tibial bone responses to 6-month calcium and vitamin D supplementation in young male jockeys: A randomised controlled trial. *Bone, 81*, 554-561.

Smathers, A. M., Bemben, M. G., & Bemben, D. A. (2009). Bone density comparisons in male competitive road cyclists and untrained controls. *Medicine & Science in Sports & Exercise, 41*(2), 290-296.

Specker, B. L. (1996). Evidence for an interaction between calcium intake and physical activity on changes in bone mineral density. *Journal of Bone and Mineral Research, 11*(10), 1539-1544.

Standring, S. (2008). *Gray's Anatomy: The anatomical basis of clinical practice, expert consult* (40th ed.). Spain: Churchill Livingstone Elselvier.

Steingrimsdottir, L., Gunnarsson, O., Indridason, O. S., Franzson, L., & Sigurdsson, G. (2005). Relationship between serum parathyroid hormone levels, vitamin d sufficiency, and calcium intake. *JAMA, 294*(18), 2336-2341.

Sterne, J. A. C., Sutton, A. J., Ioannidis, J. P. A., Terrin, N., Jones, D. R., Lau, J., . . . Higgins, J. P. T. (2011). Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. *British Medical Journal, 342:d4002*.

Stewart, A., Marfell-Jones, M., Olds, T., & de Ridder, H. (2011). *International Standards for Anthropometric Assessment*: International Society for the Advancement of Kinanthropometry.

Stewart, C. E. H., & Rittweger, J. (2006). Adaptive processes in skeletal muscle: Molecular regulators and genetic influences. *Journal of Musculoskeletal and Neuronal Interactions, 6*(1), 73-86.

Stokes, F. J., Ivanov, P., Bailey, L. M., & Fraser, W. D. (2011). The effects of sampling procedures and storage conditions on short-term stability of blood-based biochemical markers of bone metabolism. *Clinical Chemistry, 57*(1), 138-140.

Sundgot-Borgen, J., & Garthe, I. (2011). Elite athletes in aesthetic and Olympic weight-class sports and the challenge of body weight and body compositions. *Journal of Sports Sciences, 29*(sup1), S101-S114.

Szulc, P., Garnero, P., Munoz, F., Marchand, F., & Delmas, P. D. (2001). Cross-Sectional Evaluation of Bone Metabolism in Men*. *Journal of Bone and Mineral Research, 16*(9), 1642-1650.

Szulc, P., Kaufman, J., & Delmas, P. (2007). Biochemical assessment of bone turnover and bone fragility in men. *Osteoporosis International, 18*(11), 1451-1461.

Szulc, P., Marchand, F., Duboeuf, F., & Delmas, P. D. (2000). Cross-sectional assessment of age-related bone loss in men: the MINOS study. *Bone, 26*(2), 123-129.

Taichman, R. S. (2005). Blood and bone: two tissues whose fates are intertwined to create the hematopoietic stem-cell niche. *Blood, 105*(7), 2631-2639.

Tang, B. M. P., Eslick, G. D., Nowson, C., Smith, C., & Bensoussan, A. (2007). Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures

and bone loss in people aged 50 years and older: a meta-analysis. *The Lancet, 370*(9588), 657-666.

Teitelbaum, S. L. (2000). Bone Resorption by Osteoclasts. *Science, 289*(5484), 1504-1508.

Trowbridge, E. A., Cotterill, J. V., & Crofts, C. E. (1995). The physical demands of riding in National Hunt races. *European Journal of Applied Physiology and Occupational Physiology, 70*(1), 66-69.

Vasikaran, S., Eastell, R., Bruyere, O., Foldes, A. J., Garnero, P., Griesmacher, A., . . . Kanis, J. A. (2011). Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. *Osteoporos Int, 22*(2), 391-420.

Vasikaran, S., Eastell, R., Bruyère, O., Foldes, A. J., Garnero, P., Griesmacher, A., . . . Kanis, J. A. (2011). Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. *Osteoporosis International, 22*(2), 391-420.

Veljkovic, K., Rodríguez-Capote, K., Bhayana, V., Pickersgill, R., Beattie, J., Clark, L., & Kavsak, P. A. (2012). Assessment of a four hour delay for urine samples stored without preservatives at room temperature for urinalysis. *Clinical Biochemistry, 45*(10–11), 856-858.

Verhagen, A. P., de Vet, H. C., de Bie, R. A., Kessels, A. G., Boers, M., Bouter, L. M., & Knipschild, P. G. (1998). The Delphi list: a criteria list for quality assessment of randomized clinical trials for conducting systematic reviews developed by Delphi consensus. *Journal of clinical epidemiology, 51*(12), 1235-1241.

Vicente-Rodríguez, G., Ezquerra, J., Mesana, M. I., Fernández-Alvira, J. M., Rey-López, J. P., Casajus, J. A., & Moreno, L. A. (2008). Independent and combined effect of

nutrition and exercise on bone mass development. *Journal of Bone and Mineral Metabolism, 26*(5), 416-424.

Vickers, A. J., & Altman, D. G. (2001). Analysing controlled trials with baseline and follow up measurements. *British Medical Journal, 323*, 1123-1124.

Vieth, R. (2004). Why the optimal requirement for Vitamin D3 is probably much higher than what is officially recommended for adults. *The Journal of Steroid Biochemistry and Molecular Biology, 89–90*, 575-579.

Vieth, R. (2006). What is the optimal vitamin D status for health? *Progress in Biophysics and Molecular Biology, 92*(1), 26-32.

Wagenmakers, A. J., Beckers, E. J., Brouns, F., Kuipers, H., Soeters, P. B., van der Vusse, G. J., & Saris, W. H. (1991). Carbohydrate supplementation, glycogen depletion, and amino acid metabolism during exercise. *American Journal of Physiology - Endocrinology and Metabolism, 260*(6), E883-E890.

Waldron-Lynch, F., Murray, B. F., Brady, J. J., McKenna, M. J., McGoldrick, A., Warrington, G., . . . Barragry, J. M. (2010). High bone turnover in Irish professional jockeys. *Osteoporosis International, 21*(3), 521-525.

Warren, M. P., Brooks-Gunn, J., Fox, R. P., Holderness, C. C., Hyle, E. P., & Hamilton, W. G. (2002). Osteopenia in exercise-associated amenorrhea using ballet dancers as a model: A longitudinal study. *Journal of Clinical Endocrinology & Metabolism, 87*(7), 3162-3168.

Warrington, G., Dolan, E., McGoldrick, A., McEvoy, J., MacManus, C., Griffin, M., & Lyons, D. (2009). Chronic weight control impacts on physiological function and bone health in elite jockeys. *Journal of Sports Sciences, 27*(6), 543-550.

Wastney, M. E., Martin, B. R., Peacock, M., Smith, D., Jiang, X.-Y., Jackman, L. A., & Weaver, C. M. (2000). Changes in Calcium Kinetics in Adolescent Girls Induced by High Calcium Intake. *The Journal of Clinical Endocrinology & Metabolism, 85*(12), 4470-4475.

Weidauer, L., Binkley, T., Berry, R., & Specker, B. (2013). Variation in cortical density within the cortical shell of individuals across a range in densities and ages. *Journal of Musculoskeletal and Neuronal Interactions, 13*(1), 89-96.

Westerling, D. (1983). A Study of the physical demands in riding. *European Journal Applied Physiology, 50*, 373-382.

White, T. D., & Folkens, P. A. (2005). *The human bone manual*: Academic Press.

Wielders, J. P., & Wijnberg, F. A. (2009). Preanalytical stability of 25 (OH)–vitamin D3 in human blood or serum at room temperature: Solid as a rock. *Clinical Chemistry, 55*(8), 1584-1585.

Wilks, D. C., Winwood, K., Gilliver, S., Kwiet, A., Chatfield, M., Michaelis, I., . . . Felsenberg, D. (2009). Bone mass and geometry of the tibia and the radius of master sprinters, middle and long distance runners, race-walkers and sedentary control participants: a pQCT study. *Bone, 45*(1), 91-97.

Willis, K. S., Peterson, N. J., & Larson-Meyer, D. E. (2008). Should we be concerned about the vitamin D status of athletes? *Int J Sport Nutr Exerc Metab, 18*(2), 204-224.

Wilson, G., Drust, B., Morton, J. P., & Close, G. L. (2014). Weight-making strategies in professional jockeys: Implications for physical and mental health and well-being. *Sports Medicine, 44*(6), 785-796.

Wilson, G., Fraser, W., Sharma, A., Eubank, M., Drust, B., Morton, J., & Close, G. (2013). Markers of bone health, renal function, liver function, anthropometry and

perception of mood: a comparison between Flat & National Hunt jockeys. *International journal of sports medicine, 34*(5), 453-459.

Wilson, G., Pritchard, P., Papageorgiou, C., Phillips, S., Kumar, P., Langan-Evans, C., . . . Close, G. (2015). Fasted exercise and increased dietary protein reduces body fat and improves strength in jockeys. *International journal of sports medicine*.

Wilson, G., Sparks, S. A., Drust, B., Morton, J. P., & Close, G. L. (2013). Assessment of energy expenditure in elite jockeys during simulated race-riding and a working day: Implications for making-weight. *Applied Physiology, Nutrition, and Metabolism*(38), 415-420.

Winzenberg, T., M., Powell, S., Shaw, K. A., & Jones, G. (2011). Effects of vitamin D supplementation on bone density in healthy children: systematic review and meta-analysis. *BMJ Online, 342:c7254*.

Winzenberg, T., M., Shaw, K., A., Fryer, J., & Jones, G. (2010). Calcium supplementation for improving bone mineral density in children. *Cochrane Database of Systematic Reviews,* (4).

Wren, T. A. L., Liu, X., Pitukcheewanont, P., & Gilsanz, V. (2005). Bone Acquisition in Healthy Children and Adolescents: Comparisons of Dual-Energy X-Ray Absorptiometry and Computed Tomography Measures. *The Journal of Clinical Endocrinology & Metabolism, 90*(4), 1925-1928.

Zanker, C. L., & Swaine, I. L. (2000). Responses of bone turnover markers to repeated endurance running in humans under conditions of energy balance or energy restriction. *European Journal of Applied Physiology, 83*(4-5), 434-440.

Zerwekh, J. E. (2008). Blood biomarkers of vitamin D status. *The American Journal of Clinical Nutrition, 87*(4), 1087S-1091S.

Zhao, Y., Martin, B. R., & Weaver, C. M. (2005). Calcium bioavailability of calcium carbonate fortified soymilk is equivalent to cow's milk in young women. *Journal of Nutrition, 135*(10), 2379-2382.

Appendix 1: ANZ Clinical Trials Registration

From: info@actr.org.au [mailto:info@actr.org.au]
Sent: Monday, 2 April 2012 11:13 AM
To: David Greene
Subject: Your ACTRN (registration number): ACTRN12612000374864

Dear David,

Re: For young male jockeys, will 6-month calcium and vitamin D supplementation compared to a placebo increase tibial and radial bone strength and density

Thank you for submitting the above trial for inclusion in the Australian New Zealand Clinical Trials Registry (ANZCTR).

Your trial has now been successfully registered and allocated the ACTRN: ACTRN12612000374864

Web address of your trial: http://www.ANZCTR.org.au/ACTRN12612000374864.aspx
Date submitted: 30/03/2012 10:18:44 AM
Date registered: 2/04/2012 11:13:04 AM
Registered by: David Greene

If you have already obtained Ethics approval for your trial, could you please send the ANZCTR a copy of at least one Ethics Committee approval letter? A copy of the letter can be sent to

info@actr.org.au (by email) OR (61 2) 9565 1863, attention to ANZCTR (by fax).

Please be reminded that the quality and accuracy of the trial information submitted for registration is the responsibility of the trial's Primary Sponsor or their representative (the Registrant). The ANZCTR allows you to update trial data, but please note that the original data lodged at the time of trial registration and the tracked history of any changes made will remain publicly available.

The ANZCTR is recognised as an ICMJE acceptable registry (http://www.icmje.org/faq.pdf) and a Primary Registry in the WHO registry network (http://www.who.int/ictrp/network/primary/en/index.html).

If you have any enquiries please send a message to info@actr.org.au or telephone +61 2 9562 5333.

Kind regards,
ANZCTR Staff

T: +61 2 9562 5333
F: +61 2 9565 1863
E: info@actr.org.au
W: www.ANZCTR.org.au

Appendix 2: Ethics Approval

Human Research Ethics Committee
Committee Approval Form

Principal Investigator/Supervisor: David Green Sydney Campus
Co-Investigators: Melbourne Campus
Student Researcher: Melbourne Campus

Ethics approval has been granted for the following project:
Calcium and vitamin D supplementation on bone structural properties in young male jockeys: A randomised controlled trial.
for the period: 13/06/2012-27/02/2013
Human Research Ethics Committee (HREC) Register Number: 2012 114N

Special Condition/s of Approval

Prior to commencement of your research, the following permissions are required to be submitted to the ACU HREC:
N/A

The following standard conditions as stipulated in the *National Statement on Ethical Conduct in Research Involving Humans* (2007) apply:

(i) that Principal Investigators / Supervisors provide, on the form supplied by the Human Research Ethics Committee, annual reports on matters such as:

- security of records
- compliance with approved consent procedures and documentation
- compliance with special conditions, and

(ii) that researchers report to the HREC immediately any matter that might affect the ethical acceptability of the protocol, such as:

- proposed changes to the protocol
- unforeseen circumstances or events
- adverse effects on participant. The HREC will conduct an audit each year of all projects deemed to be of more than low risk. There will also be random audits of a sample of projects considered to be of negligible risk and low risk on all campuses each year.

Within one month of the conclusion of the project, researchers are required to complete a *Final Report Form* and submit it to the local Research Services Officer.

If the project continues for more than one year, researchers are required to complete an *Annual Progress Report Form* and submit it to the local Research Services Officer within one month of the anniversary date of the ethics approval.

Signed: ..... Date: .....15/06/2012.....
(Research Services Officer, Melbourne Campus)

Appendix 3: Study results

Appendix 3 Table 1: Descriptive Characteristics results baseline and six months

		BASELINE					SIX MONTHS				
ID	Group	Age (yrs)	Weight (kg)	Height (cm)	BMI	Hydration (Usg)	Age (yrs)	Weight (kg)	Height (cm)	BMI	Hydration (Usg)
JCAL01	1	32.5	55.9	168.1	1.020	19.78	33.1	55.6	168.0	19.70	1.025
JCAL02	1	19.5	50.4	170.6	1.023	17.32	20.1	50.9	171.0	17.41	1.023
JCAL03	2	18.7	55.6	167.0	1.017	19.94	19.3	55.4	167.1	19.84	1.025
JCAL04	2	17.9	51.5	171.5	1.014	17.49					
JCAL05	1	22.4	56.3	169.6	1.025	19.57					
JCAL06	2	18.1	53.9	171.0	1.020	18.43					
JCAL07	1	22.7	52.8	161.6	1.022	20.22	23.3	54.0	161.5	20.70	1.017
JCAL08	2	19.8	54.5	167.3	1.010	19.47	20.4	55.0	168.2	19.44	1.015
JCAL09	1	21.3	54.4	160.8	1.019	21.04					
JCAL10	2	23.6	53.1	166.8	1.025	19.09	24.2	53.7	167.6	19.12	1.003
JCAL11	2	18.9	48.7	159.0	1.018	19.26	19.5	49.3	159.4	19.40	1.023
JCAL12	1	22.3	58.8	178.8	1.030	18.39					
JCAL13	2	20.7	47.1	160.8	1.005	18.22					
JCAL14	1	27.2	58.0	161.8	1.028	22.15	27.8	60.2	163.4	22.53	1.005
JCAL15	2	19.5	50.6	162.3	1.032	19.21	20.0	54.1	163.5	20.22	1.021
JCAL16	1	18.7	59.2	173.3	1.028	19.71					
JCAL17	2	17.0	54.6	170.8	1.018	18.72	17.6	54.9	171.1	18.75	1.018
JCAL18	1	18.6	50.4	159.2	1.021	19.89	19.1	50.5	159.0	19.96	1.024
JCAL19	1	20.0	51.3	167.6	1.022	18.26					
JCAL20	2	18.2	51.2	169.7	1.018	17.78	18.7	53.4	169.6	18.55	1.017
JCAL21	2	18.9	47.6	171.1	1.010	16.26	19.4	47.6	171.5	16.17	1.017
JCAL22	2	18.2	52.4	170.0	1.021	18.13					
JCAL23	1	17.7	54.6	174.6	1.026	17.91					
JCAL24	1	19.1	48.3	165.0	1.021	17.74	19.6	52.1	165.5	19.02	1.020
JCAL25	1	16.7	49.7	174.0	1.028	16.42					
JCAL26	1	19.9	56.5	168.8	1.024	19.81	20.4	56.1	170.8	19.23	1.026
JCAL27	2	19.2	57.7	172.1	1.030	19.46	19.7	61.5	172.0	20.79	1.029
JCAL28	2	18.9	60.7	169.6	1.029	21.10					
JCAL29	1	19.0	49.9	170.1	1.021	17.25	19.4	50.4	171.6	17.12	1.020

Appendix 3 Table 2: Baseline pQCT results – Radius

ID	Group	Total Area (mm^2)	Total content ($mg \cdot mm$)	Total density ($mg.cm^3$)	Trabecular area (mm^2)	Trabecular density ($mg.cm^3$)	Bone strength Index ($mg^2 \cdot mm^4$)	Endo circ (mm)	Peri circ (mm)
JCAL01	1	366.0	125	355.6	164.5	235.4	46.3	40.50	24.50
JCAL02	1	440.3	148	320.3	198.0	205.0	45.2	41.61	25.38
JCAL03	2	427.8	124	269.8	192.3	183.0	31.1	43.31	29.66
JCAL04	2	419.3		401.1	188.5	387.6	67.5		
JCAL05	1	554.5		290.8	229.5	155.5	46.9		
JCAL06	2	462.8		310.3	208.0	242.7	44.6		
JCAL07	1	534.5	163	303.9	240.5	210.5	49.4	41.15	25.07
JCAL08	2	498.8	153	364.7	224.3	327.5	66.3	42.24	26.76
JCAL09	1	448.5		373.0	187.3	240.7	62.4		
JCAL10	2	472.3	171	352.7	212.5	239.7	58.8	42.47	28.96
JCAL11	2	477.0	136	279.2	214.5	201.1	37.2	35.93	20.82
JCAL12	1	433.0		395.2	156.6	223.6	67.6		
JCAL13	2	430.8		335.8	193.8	264.9	48.6		
JCAL14	1	447.0	177	400.8	201.0	281.8	71.8	48.31	30.80
JCAL15	2	465.3	158	335.3	209.3	247.9	52.3	49.47	34.91
JCAL16	1	574.3		283.5	240.0	198.5	46.2		
JCAL17	2	486.3	139	272.3	218.8	196.6	36.1	42.09	27.69
JCAL18	1	445.3	162	393.8	200.3	318.8	69.1	41.49	23.65
JCAL19	1	542.5		228.1	204.0	143.5	28.2		
JCAL20	2	444.8	167	345.6	200.0	255.9	53.1	42.21	26.11
JCAL21	2	464.0	133	272.5	208.8	182.6	34.5	41.76	27.69
JCAL22	2	550.0		291.3	247.3	230.5	46.7		
JCAL23	1	476.8		275.5	192.5	164.0	36.2		
JCAL24	1	421.8	127	309.0	189.8	244.7	40.3	39.51	25.44
JCAL25	1	519.5		286.4	233.8	228.9	42.6		
JCAL26	1	478.0		325.8	215.0	183.9	50.7		
JCAL27	2	369.0	119	369.4	166.0	205.0	50.4	43.88	30.55
JCAL28	2	410.0		319.3	184.6	181.6	41.8		
JCAL29	1	557.8	161	295.2	250.8	232.8	48.6	41.30	26.47

KEY: Endo circ = endocortical circumference; Peri circ = Pericortical circumference

Appendix 2 Table 2: Baseline pQCT results – Radius cont'd

ID	Group	Cortical content (mg.mm)	Cortical area 66% (mm^2)	Cortical density 66% ($mg.cm^3$)	SSI-Polar 66% (mm^3)	Total Bone Area (mm^2)	Total Muscle + Bone Area (mm^2)	Total Bone Area (mm^2)	Muscle Area (mm^2)
JCAL01	1	108	83.3	1171.3	314.9	198.5	4698.8	291.3	4407.5
JCAL02	1	117	85.3	1075.6	223.8	206.0	3884.3	366.0	3518.3
JCAL03	2	103	74.8	1119.5	292.2	173.3	4449.8	282.3	4167.5
JCAL04	2		86.3	1123.1	281.6	189.0	3677.8	271.8	3406.0
JCAL05	1		72.3	1082.6	354.4	218.5	4601.5	344.8	4256.7
JCAL06	2		79.8	1034.6	314.7	181.8	4127.0	353.3	3773.7
JCAL07	1	110	86.0	1150.9	324.2	209.5	4570.0	307.5	4262.5
JCAL08	2	110	85.8	1099.7	295.4	213.5	4495.3	335.3	4160.0
JCAL09	1		76.3	1101.1	256.7	202.0	4917.0	307.3	4609.7
JCAL10	2	101	77.8	1132.1	315.5	187.5	4608.3	301.8	4306.5
JCAL11	2	87	69.5	1127.5	188.8	166.0	4442.8	244.3	4198.5
JCAL12	1		75.7	1132.3	268.3	208.0	4364.3	321.0	4043.3
JCAL13	2		75.3	1174.7	246.5	174.5	4115.3	254.5	3860.8
JCAL14	1	149	112.5	1142.3	427.3	183.3	4573.8	311.5	4262.3
JCAL15	2	129	96.3	1104.0	492.5	226.3	4952.0	411.3	4540.7
JCAL16	1		72.2	1061.2	343.4	199.3	4620.0	343.8	4276.2
JCAL17	2	103	79.5	1097.8	295.0	185.5	4292.5	297.8	3994.7
JCAL18	1	118	91.5	1146.0	312.8	214.0	4533.8	293.3	4240.5
JCAL19	1		61.3	1023.4	287.8	178.0	4490.3	316.3	4174.0
JCAL20	2	115	88.3	1140.8	344.2	196.3	4279.0	294.8	3984.2
JCAL21	2	98	74.8	1105.2	242.7	183.8	4075.3	302.8	3772.5
JCAL22	2		91.0	1094.4	336.0	208.8	4547.3	340.5	4206.8
JCAL23	1		72.5	1067.5	289.1	187.3	4441.5	322.5	4119.0
JCAL24	1	89	69.0	1039.2	221.9	167.3	4338.5	273.0	4065.5
JCAL25	1		68.8	1039.4	232.8	172.8	4562.5	320.3	4242.2
JCAL26	1		76.5	1122.5	301.0	186.0	4658.5	317.8	4340.7
JCAL27	2	103	79.3	1101.9	257.4	194.3	4345.3	322.5	4022.8
JCAL28	2		80.5	1120.7	289.8	197.3	4788.5	342.3	4446.2
JCAL29	1	106	80.3	1111.2	294.5	185.8	4323.8	285.8	4038.0

Appendix 3 Table 3: Six months pQCT results – Radius

ID	Group	Total Area (mm^2)	Total content ($mg \cdot mm$)	Total density ($mg.cm^3$)	Trabecular area (mm^2)	Trabecular density ($mg.cm^3$)	Bone strength Index ($mg^2 \cdot mm^4$)	Endo circ (mm)	Peri circ (mm)
JCAL01	1	346.8	125	361.3	156.0	227.3	45.3	40.92	25.07
JCAL02	1	497.0	140	297.5	223.5	192.6	44.0	47.33	34.19
JCAL03	2	416.8	132	281.7	187.5	194.8	33.1	44.20	30.10
JCAL04	2								
JCAL05	1								
JCAL06	2								
JCAL07	1	522.0	162	311.3	234.8	218.6	50.6	41.34	25.07
JCAL08	2	442.5	182	345.9	199.0	255.1	52.9	43.20	28.08
JCAL09	1								
JCAL10	2	470.8	167	362.6	211.8	253.5	61.9	42.35	28.58
JCAL11	2	469.8	133	288.7	211.3	197.6	39.2	36.50	21.42
JCAL12	1								
JCAL13	2								
JCAL14	1	425.0	178	416.5	191.0	282.9	73.7	46.02	26.53
JCAL15	2	471.3	156	336.1	212.0	245.6	53.2	49.12	34.69
JCAL16	1								
JCAL17	2	506.0	132	274.6	227.5	204.1	38.2	41.45	26.82
JCAL18	1	421.0	174	384.0	189.3	282.3	62.1	40.11	21.42
JCAL19	1								
JCAL20	2	477.8	154	349.2	214.8	262.2	58.3	41.79	25.25
JCAL21	2	460.0	126	288.4	206.8	183.5	38.3	41.61	28.14
JCAL22	2								
JCAL23	1								
JCAL24	1	416.3	129	305.6	187.3	231.0	38.9	39.44	26.23
JCAL25	1								
JCAL26	1	474.5		337.7	213.5	194.1	54.1		
JCAL27	2	377.8	136	315.5	169.8	142.5	37.6	44.38	31.21
JCAL28	2								
JCAL29	1	574.8	165	279.8	258.5	220.9	45.0	41.23	26.29

KEY: Endo circ = endocortical circumference; Peri circ = Pericortical circumference

Appendix 3 Table 3: Six months pQCT results – Radius cont'd

ID	Group	Cortical content (mg.mm)	Cortical area 66% (mm^2)	Cortical density 66% ($mg.cm^3$)	SSI-Polar 66% (mm^3)	Total Bone Area (mm^2)	Total Muscle + Bone Area (mm^2)	Total Bone Area (mm^2)	Muscle Area (mm^2)
JCAL01	1	109	82.8	1171.9	307.7	198.0	4671.5	290.3	4381.2
JCAL02	1	121	86.5	1164.2	262.6	204.3	4184.0	308.3	3875.7
JCAL03	2	104	73.0	1120.1	289.9	170.3	4211.8	279.8	3932.0
JCAL04	2								
JCAL05	1								
JCAL06	2								
JCAL07	1	110	84.8	1160.8	327.1	207.5	4861.0	305.8	4555.2
JCAL08	2	112	85.0	1127.4	582.4	215.8	4232.0	327.8	3904.2
JCAL09	1								
JCAL10	2	100	76.8	1143.1	325.6	189.0	4731.3	303.3	4428.0
JCAL11	2	89	68.3	1141.4	206.9	165.8	4725.0	238.3	4486.7
JCAL12	1								
JCAL13	2								
JCAL14	1	147	110.3	1125.8	698.4	175.5	4798.3	347.5	4450.8
JCAL15	2	128	97.8	1111.5	500.2	229.0	5199.8	412.5	4787.3
JCAL16	1								
JCAL17	2	100	80.0	1099.5	283.2	188.5	4304.3	318.3	3986.0
JCAL18	1	116	92.5	1124.9	196.4	217.3	4576.8	312.5	4264.3
JCAL19	1								
JCAL20	2	113	87.5	1160.5	346.1	196.0	4594.3	298.8	4295.5
JCAL21	2	97	77.8	1098.3	474.1	189.3	4101.0	306.5	3794.5
JCAL22	2								
JCAL23	1								
JCAL24	1	86	72.8	1049.5	233.0	171.8	4225.8	275.3	3950.5
JCAL25	1								
JCAL26	1		76.3	1130.6	298.0	186.5	4323.0	318.5	4004.5
JCAL27	2	105	79.0	1089.6	260.0	186.5	3956.5	321.3	3635.2
JCAL28	2								
JCAL29	1	104	80.0	1133.3	306.6	182.8	4314.3	284.5	4029.8

Appendix 3 Table 4: Baseline pQCT results – Tibia

ID	Group	Trabecular content (mg·mm)	Trabecular density ($mg.cm^3$)	Trabecular area (mm^2)	Total density ($mg.cm^3$)	Total Area (mm^2)	Bone strength Index ($mg^2 \cdot mm^4$)
JCAL01	1	215.02	208.90	952.00	286.60	1152.25	94.65
JCAL02	1	202.99	216.30	913.75	283.30	1108.00	88.93
JCAL03	2	184.47	246.50	748.50	286.90	918.00	75.56
JCAL04	2	186.94	206.70	904.50	244.70	1100.75	65.91
JCAL05	1	189.97	200.40	922.75	253.30	1117.50	71.70
JCAL06	2	237.86	232.00	1025.25	252.80	1231.50	78.70
JCAL07	1	216.76	242.70	811.50	314.40	988.00	97.66
JCAL08	2	192.69	272.00	708.50	352.30	910.25	112.98
JCAL09	1	236.67	308.80	806.50	353.00	989.20	123.26
JCAL10	2	238.05	240.60	989.25	266.20	1187.75	84.17
JCAL11	2	134.92	173.30	778.75	214.00	949.75	43.49
JCAL12	1	198.20	204.70	913.75	285.10	1131.00	91.93
JCAL13	2	244.05	305.00	800.25	348.60	983.00	119.46
JCAL14	1	278.80	256.50	989.25	331.90	1189.75	131.06
JCAL15	2	215.22	269.20	799.50	295.30	973.75	84.91
JCAL16	1	205.02	207.40	959.25	251.90	1163.50	73.83
JCAL17	2	228.51	240.30	951.00	264.80	1144.50	80.25
JCAL18	1	241.85	300.60	804.50	350.20	989.30	121.33
JCAL19	1		213.60	616.50	244.20	1370.50	81.73
JCAL20	2	283.35	259.70	1091.25	285.90	1308.50	106.96
JCAL21	2	186.69	227.90	819.25	275.60	1006.00	76.41
JCAL22	2	263.31	266.00	989.75	284.80	1192.00	96.68
JCAL23	1	210.12	201.50	1009.00	257.50	1210.75	80.28
JCAL24	1		246.80	505.80	280.10	1124.00	88.18
JCAL25	1	178.51	210.90	842.25	248.70	1021.50	63.18
JCAL26	1	225.41	230.60	989.20	287.60	1204.00	99.59
JCAL27	2	235.48	292.10	806.25	338.60	997.75	114.39
JCAL28	2	225.45	235.50	957.50	272.20	1158.00	85.80
JCAL29	1	208.45	225.80	923.00	266.40	1118.50	79.38

Appendix 3 Table 4: Baseline pQCT results – Tibia cont'd

ID	Group	Cortical content (mg.mm)	Cortical density 66% (mg.cm^3)	Cortical area 66% (mm^2)	Cortical thickness (mm)	Total Bone Area (mm^2)	SSI-Polar 66% (mm^3)
JCAL01	1	343.71	1124.30	303.75	4.61	521.00	2462.91
JCAL02	1	314.36	1126.00	289.50	4.56	454.25	2085.24
JCAL03	2	280.90	1116.90	251.50	3.80	484.75	1920.14
JCAL04	2	288.79	1103.30	261.75	3.67	542.75	2067.81
JCAL05	1	269.93	1101.00	249.00	3.40	566.25	2237.49
JCAL06	2	272.13	1102.80	246.75	3.88	454.50	1840.00
JCAL07	1	232.63	1047.90	228.25	4.16	392.50	1482.23
JCAL08	2	260.05	1097.30	237.00	3.73	450.50	1777.96
JCAL09	1	293.22	1110.00	239.50	4.04	462.50	2008.11
JCAL10	2	313.37	1121.20	279.50	3.53	647.75	2717.28
JCAL11	2	186.07	1092.90	170.25	2.77	391.50	1108.08
JCAL12	1	321.84	1103.80	292.30	3.78	604.00	2694.26
JCAL13	2	359.66	1144.50	314.25	4.97	493.75	2338.09
JCAL14	1	302.57	1091.90	263.00	3.79	587.75	2567.12
JCAL15	2	296.29	1136.30	260.75	3.83	509.25	2206.84
JCAL16	1	309.12	1111.60	242.00	3.84	540.00	2290.19
JCAL17	2	276.82	1072.90	258.00	3.49	569.75	2223.47
JCAL18	1	318.41	1106.20	289.30	4.64	449.75	2095.45
JCAL19	1	259.09	1052.40	230.25	3.17	614.75	2400.54
JCAL20	2	359.77	1140.30	315.50	4.86	509.50	2319.66
JCAL21	2	318.82	1112.80	286.50	4.16	531.50	2340.65
JCAL22	2	325.71	1086.60	299.75	4.33	545.25	2295.44
JCAL23	1	245.88	1087.20	230.75	3.34	531.00	2007.95
JCAL24	1	317.60	1100.50	287.75	4.58	489.75	2080.18
JCAL25	1	243.94	1004.00	237.75	3.49	505.25	1809.64
JCAL26	1	278.20	1114.20	235.25	3.73	515.00	2093.83
JCAL27	2	352.34	1130.20	311.75	4.32	583.50	2645.82
JCAL28	2	335.68	1132.10	296.50	4.03	591.25	2649.41
JCAL29	1	264.53	1101.00	240.25	3.38	527.50	1937.64

Appendix 3 Table 5: Six months pQCT results – Tibia

ID	Group	Trabecular content (mg·mm)	Trabecular density ($mg.cm^3$)	Trabecular area (mm^2)	Total density ($mg.cm^3$)	Total Area (mm^2)	Bone strength Index ($mg^2 \cdot mm^4$)
JCAL01	1	227.94	239.20	953.00	282.50	1162.75	92.79
JCAL02	1	225.96	239.30	944.25	286.30	1153.75	94.57
JCAL03	2	187.22	241.00	776.75	280.00	949.25	74.42
JCAL04	2						
JCAL05	1						
JCAL06	2						
JCAL07	1	188.70	224.40	841.00	249.90	1017.25	63.53
JCAL08	2	195.41	275.20	710.00	361.10	935.25	121.95
JCAL09	1						
JCAL10	2	246.24	243.70	1010.25	274.40	1218.75	91.77
JCAL11	2	150.98	184.50	818.50	224.60	997.75	50.33
JCAL12	1						
JCAL13	2						
JCAL14	1	279.64	299.20	934.75	345.60	1155.25	137.98
JCAL15	2	221.49	264.50	837.25	292.80	1016.75	87.17
JCAL16	1						
JCAL17	2	220.15	240.10	916.75	265.50	1105.00	77.89
JCAL18	1	271.39	325.10	834.75	359.70	1024.25	132.52
JCAL19	1						
JCAL20	2	264.27	251.20	1052.00	281.50	1266.25	100.34
JCAL21	2	196.78	231.20	851.00	283.70	1049.25	84.45
JCAL22	2						
JCAL23	1						
JCAL24	1	226.06	269.80	837.75	314.20	1029.50	101.63
JCAL25	1						
JCAL26	1	218.09	242.00	901.25	294.30	1108.25	95.99
JCAL27	2	245.50	283.80	865.00	328.40	1065.00	114.86
JCAL28	2						
JCAL29	1	192.52	218.50	881.25	258.10	1066.50	71.05

Appendix 3 Table 5: Six months pQCT results – Tibia cont'd

ID	Group	Cortical content (mg.mm)	Cortical density 66% ($mg.cm^3$)	Cortical area 66% (mm^2)	Cortical thickness (mm)	Total Bone Area (mm^2)	SSI-Polar 66% (mm^3)
JCAL01	1	376.31	1152.60	326.50	4.87	538.50	2613.16
JCAL02	1	346.16	1146.20	302.00	4.76	489.00	2183.52
JCAL03	2	279.92	1115.20	251.00	3.82	480.50	1868.30
JCAL04	2						
JCAL05	1						
JCAL06	2						
JCAL07	1	267.46	1072.00	249.50	4.23	413.25	2168.44
JCAL08	2	261.77	1102.20	237.50	3.75	448.25	1775.26
JCAL09	1						
JCAL10	2	315.22	1128.80	279.25	3.52	648.50	2796.06
JCAL11	2	196.41	1112.80	176.50	2.92	382.75	1178.77
JCAL12	1						
JCAL13	2						
JCAL14	1	329.54	1120.90	294.00	3.87	608.00	2676.75
JCAL15	2	299.47	1140.80	262.50	3.87	508.75	2179.04
JCAL16	1						
JCAL17	2	267.55	1070.20	250.00	3.46	545.00	2086.33
JCAL18	1	344.90	1133.60	304.25	4.87	481.25	2208.00
JCAL19	1						
JCAL20	2	360.97	1143.20	315.75	4.83	515.00	2331.15
JCAL21	2	321.40	1118.90	287.25	4.17	533.50	2397.80
JCAL22	2						
JCAL23	1						
JCAL24	1	338.20	1125.50	300.50	4.68	493.25	1609.80
JCAL25	1						
JCAL26	1	298.34	1123.70	265.50	3.79	534.75	2170.17
JCAL27	2	351.38	1126.20	312.00	4.33	580.75	2604.03
JCAL28	2						
JCAL29	1	265.45	1102.60	240.75	3.39	526.75	1954.18

Appendix 3 Table 6: Blood borne variables data baseline and six months

		BASELINE			SIX MONTHS		
ID	Group	250H Vit D (nmol·L^{-1})	CTx (ng·L^{-1})	Total P1NP (ug·L^{-1})	250H Vit D (nmol·L^{-1})	CTx (ng·L^{-1})	Total P1NP (ug·L^{-1})
JCAL01	1	58	190	69	63	160	51.3
JCAL02	1	47	250	100.7	50	230	113.9
JCAL03	2	103	200	102.1	86	190	91.5
JCAL04	2	86	400	159.8			
JCAL05	1	106	480	76.6			
JCAL06	2	60	550	187.4			
JCAL07	1	58	260	53	65	220	65.8
JCAL08	2	53	440	153.1	50	540	160.1
JCAL09	1	101	210	75.4			
JCAL10	2	93	230	92	92	240	90.6
JCAL11	2	62	610	115.8	54	620	110.7
JCAL12	1	81	180	56			
JCAL13	2	79	450	64.5			
JCAL14	1	64	180	53.2	88	160	64.6
JCAL15	2	75	270	63.4	52	400	52.2
JCAL16	1	90	610	191.7			
JCAL17	2	90	330	101.5			
JCAL18	1	98	280	108.4	108	250	98.9
JCAL19	1	98	570	113.3			
JCAL20	2	86	440	125.7	88	470	121.9
JCAL21	2	47	350	153.5	63	550	146.1
JCAL22	2	98	470	93.4			
JCAL23	1	86	430	133.1			
JCAL24	1	41	510	115.2	58	580	105.8
JCAL25	1	60	540	127.9			
JCAL26	1	89	610	184.4	100	570	152.9
JCAL27	2	122	550	73.4	102	620	64.1
JCAL28	2	52	440	125.9			
JCAL29	1	62	690	149.5	73	630	183.6

Appendix 3 Table 7: Baseline Anthropometric data

ID	Group	Stretch stature (cm)	Sitting Height (cm)	Triceps s/f (mm)	Subscap s/f (mm)	Supraspinale s/f (mm)	Abdominal s/f (mm)	Front Thigh s/f (mm)	Medial Calf s/f (mm)	sum of six s/f (mm)
JCAL01	1	168.1	86.8	4.95	6.45	3.2	4.9	5.4	3.3	28.2
JCAL02	1	170.6	85.3	5.2	5.35	3.6	5.15	5.1	4.45	28.85
JCAL03	2	167	84.5	6.65	6.35	5.55	7.95	6.65	4.85	38
JCAL04	2	171.5	87	5.5	5.8	5	5.75	6.7	4.85	33.6
JCAL05	1	169.6	83.5	5.3	5.35	3.75	5.1	6.2	4.2	29.9
JCAL06	2	171	85	6.45	6	4.1	6	7.05	5.75	35.35
JCAL07	1	161.6	85	5.8	6.2	5.2	8.95	7.35	4.5	38
JCAL08	2	167.3	84.8	6.5	5.8	4.25	6.25	7.65	5.25	35.7
JCAL09	1	160.8	82	7.6	7.45	6.15	8.8	7.8	4.35	42.15
JCAL10	2	166.8	85.2	4.35	6.2	4	5.3	6.95	3.45	30.25
JCAL11	2	159	84.6	5.15	5.95	4.5	5.5	6.1	3.3	30.5
JCAL12	1	178.8	89.6	5.5	4.9	3.4	5.1	6.25	3.15	28.3
JCAL13	2	160.8	84	5.5	5.45	3.6	5.05	6.15	3.55	29.3
JCAL14	1	161.8	83	7	5.95	5.85	10.45	9.8	3.55	42.6
JCAL15	2	162.3	81	5.25	6.4	3.4	5.15	6.55	4.55	31.3
JCAL16	1	173.3	83.4	10.1	5.75	4.2	8.05	12.3	5.35	45.75
JCAL17	2	170.8	83.4	9	6.25	4.65	7	8.6	6.4	41.9
JCAL18	1	159.2	78.8	6.55	7.1	4	5.7	10.25	4.7	38.3
JCAL19	1	167.6	83	4.45	4.8	2.9	4.5	5.9	3	25.55
JCAL20	2	169.7	83.2	5.8	5.65	3.6	5.8	7.9	4.75	33.5
JCAL21	2	171.1	84.5	3.45	4.9	3.5	5.05	4	3.55	24.45
JCAL22	2	170	82.9	4.35	5.15	3.85	4.4	6.3	4.15	28.2
JCAL23	1	174.6	84	4.55	5.9	6.05	7.8	4.75	5.2	34.25
JCAL24	1	165	79.2	5.3	4.4	3.75	5.5	6.1	3.8	28.85
JCAL25	1	174	84.5	6.2	5.7	5.6	11.3	9.8	5.75	44.35
JCAL26	1	168.8	84.9	6.3	5.65	5.1	8.95	8.9	5.1	40
JCAL27	2	172.1	84.9	4.85	5.05	3.2	5.05	8.4	4.65	31.2
JCAL28	2	169.6	83.4	7.4	5.9	4.75	6.35	10.6	4.25	39.25
JCAL29	1	170.1	82	4.65	5.3	4.25	5.95	5.7	4.9	30.75

Appendix 3 Table 7: Baseline Anthropometric data cont'd

ID	Group	Head girth (cm)	Arm girth relaxed (cm)	Arm girth flexed (cm)	Forearm girth (cm)	Wrist girth (cm)	Chest girth (cm)	Waist girth (cm)	Gluteal girth (cm)	Thigh girth (1 cm dist. glut. line) (cm)	Thigh girth (mid (cm)	Calf girth (max.) (cm)	Ankle girth (min.) (cm)
JCAL01	1	55.7	28.3	30.3	26.1	15.5	88.4	68.5	84.7	48	45.2	34.3	19.5
JCAL02	1	55.2	24.8	27.3	24.3	15.3	82	66	81.5	45.2	40.7	29.5	20.2
JCAL03	2	53	27	29.6	25.8	16.1	90	69.5	86.1	48	45.6	32	20.4
JCAL04	2	52	24.7	27	23.7	15.4	83	64.5	82.5	46.8	42	31.5	20.5
JCAL05	1	53.4	27.5	30.9	25.7	15.8	90.5	68.3	83.3	49	47	32.5	21.3
JCAL06	2	56.3	25.8	28.8	24	16.3	85.3	69.5	82.5	47.7	43	29.7	20.7
JCAL07	1	52.1	27.5	31.3	25	15.6	87.8	69.4	82	49.4	45	32.2	19.8
JCAL08	2	55.7	25.4	28.5	24.7	16.5	87.2	68	85.5	46.3	43.5	31.3	20.3
JCAL09	1	56	29	31.8	25.7	15.7	86.6	70.3	84	48.7	45.2	30.3	20
JCAL10	2	56.2	27	28.1	24.7	15.5	85.7	65.2	82.3	45	40.8	33.3	20.7
JCAL11	2	53.2	27.2	29.9	24.5	15.1	86.8	67.8	78.5	44.7	41.8	31.3	19.5
JCAL12	1	55.3	27.5	29.3	24.9	15.2	88.5	67	84.8	46.2	43	30.8	19.8
JCAL13	2	52.5	25.8	28.1	23.3	14.8	83.2	63	81	45	42.3	29.8	20.3
JCAL14	1	58.2	29.4	31	26	15.8	87.6	71.2	87.5	49	45.6	35.6	20.5
JCAL15	2	54.5	27.4	30.2	25.7	16	87.8	68	82.3	44.3	40	29.5	20
JCAL16	1	54.5	27.3	30.3	24.6	15.7	87.4	70.8	86.7	48.5	44.7	31.4	20.7
JCAL17	2	57.2	26	28.5	24.5	15.3	85.6	69.2	84.4	47.1	44	30.3	21
JCAL18	1	58	26.8	29.8	24.6	14.8	84.6	68.8	80.6	46.5	43.5	31.1	19.5
JCAL19	1	56.2	25.7	28.3	23.8	16.1	80.2	65	82	46.1	43.9	31.1	21.9
JCAL20	2	56.5	27	29.5	24.2	16.4	83.7	67.5	83	44.5	43.2	32	20.9
JCAL21	2	53.9	26	28.2	23.2	15.6	86.1	60.8	79.8	42.3	39.3	30.5	19.3
JCAL22	2	53.2	26.8	29	25.4	16.8	87	65.3	82	44.5	41.3	31.8	21.4
JCAL23	1	57	25.5	27.8	25.3	15.8	87.5	67.2	84.4	45.1	40.8	31.5	20.1
JCAL24	1	52.2	26.2	28.3	24	15	81.8	66.1	81.8	44.4	40.8	29.8	19.7
JCAL25	1	54.6	26.3	29.4	24.8	15.3	85.7	68.8	85.3	47.3	42.4	32.3	19.9
JCAL26	1	55.3	26	28.8	25.1	16	86.3	65.8	87.6	50.4	45.5	32.1	19.7
JCAL27	2	55.6	27.3	29.3	25.2	15.5	89	68.5	85.2	48.5	46	33	20.2
JCAL28	2	57.4	28.7	30.8	26.5	16.1	86.2	69.5	90.3	53	48	33	20.7
JCAL29	1	53.2	25.5	28.1	24.8	16	82.8	60.7	81.4	45.5	40.8	29.4	19.1

Appendix 3 Table 7: Baseline Anthropometric data cont'd

ID	Group	Biacromial breadth (cm)	Biiliocristal breadth (cm)	Transverse chest breadth (cm)	A-P Chest depth (cm)	Humerus breadth (biepicondylar) (cm)	Wrist breadth (cm)	Femur breadth (biepicondylar) (cm)	Ankle breadth (cm)
JCAL01	1	38.2	26.4	28.4	16.7	6.7	5.3	9.4	6.7
JCAL02	1	38.2	24.8	28	16.3	6.6	5.7	9.1	6.6
JCAL03	2	36.1	24.7	28.6	17.9	6.4	5.5	8.7	6.8
JCAL04	2	37.1	26.15	25.6	18.7	7	5.4	9.2	6.7
JCAL05	1	38.95	24.7	28.3	18.1	6.8	6	9.5	6.9
JCAL06	2	38	25	27.3	17.8	6.7	6.2	9.6	7.5
JCAL07	1	36.7	23.7	30.5	18.3	6.4	5.5	9.5	7
JCAL08	2	37.2	26.7	28.8	17.3	7	5.8	9.5	6.8
JCAL09	1	36.5	24.4	26.5	18.8	6.3	5.6	8.7	6.6
JCAL10	2	39.2	23.8	27.6	17.2	6.6	5.7	9.9	7.4
JCAL11	2	37.3	23.4	27.6	17.8	7	5.2	8.8	6.8
JCAL12	1	40.5	25.2	28.3	18.7	6.9	5.6	9.2	6.9
JCAL13	2	35.5	23.5	26.5	16.2	6.5	5.4	9.1	6.8
JCAL14	1	36.5	26.7	26.8	18.9	6.2	5.8	9.7	7.5
JCAL15	2	38	25.7	28.3	19	6.9	5.8	9.1	6.9
JCAL16	1	41.4	27	29.7	17.6	7	5.7	10.8	6.8
JCAL17	2	37.7	24.5	27.7	19.1	6.7	5.8	9.4	7.4
JCAL18	1	36.9	24.9	27.6	18	6.3	5.2	9.3	6.6
JCAL19	1	37.4	25.7	26.4	16.7	6.7	6	9.3	7.3
JCAL20	2	35	25.7	25.8	17.2	6.7	5.8	9.4	6.9
JCAL21	2	37.8	24	26.7	18.1	6.5	5.7	9	6.6
JCAL22	2	39.1	25.5	28.8	17.7	7.3	5.6	9.4	7.2
JCAL23	1	39.3	25.5	28	17.6	6.8	6.1	9.6	7.1
JCAL24	1	36.7	25.6	26.9	16.6	6.9	5.3	9.2	6.5
JCAL25	1	36.3	27	28.7	17.3	7	5.3	9.4	7.2
JCAL26	1	38	25.8	26.4	19.7	7	5.8	9.4	7
JCAL27	2	37.2	25.2	29.8	19.5	6.3	5.3	9.2	7.1
JCAL28	2	37.7	25.6	29.4	16.6	6.8	5.9	9.8	7.5
JCAL29	1	35.5	24.2	25.8	18.3	7.1	5.7	9.1	6.9

Appendix 3 Table 8: Six month Anthropometric data

ID	Group	Stretch stature (cm)	Sitting Height (cm)	Triceps s/f (mm)	Subscapular s/f (mm)	Supraspinale s/f (mm)	Abdominal s/f (mm)	Front Thigh s/f (mm)	Medial Calf s/f (mm)	sum of six s/f (mm)
JCAL01	1	168	78.1	5.6	7.35	3.7	6.05	6.25	3.3	32.25
JCAL02	1	171	75.4	5.5	5.65	3.7	5.6	6.8	5.6	32.85
JCAL03	2	167.1	79.3	6.7	6.7	6.15	8.95	6.55	4.6	39.65
JCAL04	2									
JCAL05	1									
JCAL06	2									
JCAL07	1	161.5	76.7	6.9	7.25	6.15	12.5	8.5	4.4	45.7
JCAL08	2	168.2	78.4	6.3	6.35	4.3	6.7	7.95	5.8	37.4
JCAL09	1									
JCAL10	2	167.6	77	3.8	5.55	3.4	5.15	6.15	3.2	27.25
JCAL11	2	159.4	81.8	5.05	6.2	4.55	5.8	5.2	2.9	29.7
JCAL12	1									
JCAL13	2									
JCAL14	1	163.4	80.6	6.8	6.9	6.3	12.6	10.9	4	47.5
JCAL15	2	163.5	79.7	5.3	7.3	3.9	6.6	6.75	4.25	34.1
JCAL16	1									
JCAL17	2	171.1	80	9.3	6.6	5.2	7.95	9.05	5.8	43.9
JCAL18	1	159	73.7	5.8	6.85	4	6.1	8.5	4.1	35.35
JCAL19	1									
JCAL20	2	169.6	78.2	6.5	5.45	4.05	6.35	8.3	4.25	34.9
JCAL21	2	171.5	78	3.2	4.5	3.15	4.5	3.65	3.2	22.2
JCAL22	2									
JCAL23	1									
JCAL24	1	165.2	72.9	5.15	4.45	4.6	6.6	7.25	4.3	32.35
JCAL25	1									
JCAL26	1	170.8	78.2	6.3	5.8	4.8	8.15	8.25	5.1	38.4
JCAL27	2	172	80	6.65	6.65	5.2	9.05	10.35	4.55	42.45
JCAL28	2									
JCAL29	1	171.6	79.8	3.75	5.2	3.75	5.45	4.95	4.05	27.15

Appendix 3 Table 8: Six month Anthropometric data cont'd

ID	Group	Head girth (cm)	Arm girth relaxed (cm)	Arm girth flexed (cm)	Forearm girth (cm)	Wrist girth (cm)	Chest girth (cm)	Waist girth (min.) (cm)	Gluteal girth (max.) (cm)	Thigh girth (1 cm dist.) (cm)	Thigh girth (mid) (cm)	Calf girth (max.) (cm)	Ankle girth (min.) (cm)
JCAL01	1	55.8	28.5	30.8	26.1	15.6	87	70	86.4	49.4	45.6	33.8	19.2
JCAL02	1	54.8	25.3	27.8	24.3	15	82.5	68.5	82	45	41.2	29.5	19.4
JCAL03	2	52.8	27.5	29.1	25.7	15.5	90.5	66.8	85.2	48	45.7	32	20.5
JCAL04	2												
JCAL05	1												
JCAL06	2												
JCAL07	1	52.5	28.8	32.3	25.5	15.4	88.2	71.5	84.6	49.8	46.2	32.2	19.5
JCAL08	2	55.5	25.8	28.8	24.7	16	88	66	86.5	46.8	44.6	31.5	19.4
JCAL09	1												
JCAL10	2	55.7	26.8	27.4	24.3	15.5	82.4	65.2	81.5	44.5	40.4	32.5	20.4
JCAL11	2	53.3	27.3	30.3	25.8	14.8	86.2	67.3	78.7	45.5	42.7	30.8	19
JCAL12	1												
JCAL13	2												
JCAL14	1	57.8	30.3	31.5	26.5	15.7	86.3	72.3	88.4	51	46	35.9	20.5
JCAL15	2	54.7	28.8	32	26.1	16	94.8	71	84.2	45.4	41.2	29.8	19.9
JCAL16	1												
JCAL17	2	56.8	26.8	29.8	24.4	15.4	86.5	70.5	84.8	47.8	45.5	32.7	20.7
JCAL18	1	57.5	26.5	29.5	24.5	14.8	85.1	68.6	81.3	46.6	43.6	30.4	19.5
JCAL19	1												
JCAL20	2	56.4	26.8	29.4	24.5	16.4	85	65.5	82.5	46.3	44.5	31.5	21.2
JCAL21	2	53.6	25.7	28.6	23.3	15.6	86.5	62.3	79.5	41.7	39	30	18.7
JCAL22	2												
JCAL23	1												
JCAL24	1	51.8	26.4	30.2	25.1	15	85	70.5	85.6	47	42.4	30.8	19.7
JCAL25	1												
JCAL26	1	55.2	26.1	28.9	24.5	15.9	85.6	64.8	88.2	50.5	44.2	32	19.7
JCAL27	2	56	28.5	29.7	25.5	15.5	91.5	74.2	90	54	48	33.9	19.8
JCAL28	2												
JCAL29	1	52.8	25.8	27.9	24.7	16	84.5	61.5	80.8	44.4	40.5	29.2	19.3

Appendix 3 Table 8: Six months Anthropometric data cont'd

ID	Group	Biacromial breadth (cm)	Biiliocristal breadth (cm)	Transverse chest breadth (cm)	A-P Chest depth (cm)	Humerus breadth (biepicondylar) (cm)	Wrist breadth (cm)	Femur breadth (biepicondylar) (cm)	Ankle breadth (cm)
JCAL01	1	40.7	26.6	28.8	17.7	6.5	5.3	9.5	6.7
JCAL02	1	39.1	25.2	26.7	17.7	6.6	5.5	8.7	6.8
JCAL03	2	36	25.3	29.1	18.1	6.5	5.6	8.5	7
JCAL04	2								
JCAL05	1								
JCAL06	2								
JCAL07	1	36.5	23.7	29	18.5	6	5.6	9.5	7
JCAL08	2	37.2	26.7	28.7	36.8	7	5.7	9.3	6.8
JCAL09	1								
JCAL10	2	39	24.7	26.7	17	6.8	5.6	9.6	7.4
JCAL11	2	37.3	23.5	27.5	17.4	7	5.2	9	7
JCAL12	1								
JCAL13	2								
JCAL14	1	36.1	26.2	27.6	19.7	6.8	5.9	9.6	7.2
JCAL15	2	38.7	25.6	28.2	19.3	7	6	9.1	7.1
JCAL16	1								
JCAL17	2	38.1	24.5	27.5	18.4	7.1	5.6	9.1	7.3
JCAL18	1	37.3	25.4	26.3	18.6	6.3	5.1	9.1	6.5
JCAL19	1								
JCAL20	2	37.2	25.7	28.7	16.9	6.9	5.8	9.6	7.1
JCAL21	2	36.8	24.1	26.1	18.4	6.6	5.6	9	6.5
JCAL22	2								
JCAL23	1								
JCAL24	1	37.3	24.8	28.4	16.3	7	5.3	9.3	6.9
JCAL25	1								
JCAL26	1	38.1	24.3	26.6	19.6	7	5.8	9.2	7.5
JCAL27	2	36.8	25.6	29.7	19.6	6.3	5.4	8.9	6.7
JCAL28	2								
JCAL29	1	35.9	24.4	26.3	18.6	7.1	6	9.3	7.1

Appendix 3 Table 9: Baseline Dietary intake (DQES)

ID	Group	Portion (std factor)	Energy (kJ·day^{-1})	Alcohol (kJ·day^{-1})	Total kJ (kJ·day^{-1})	Fat (g·day^{-1})	Protein (g·day^{-1})	Carbohydrate (g·day^{-1})	Alcohol (g·day^{-1})	Calcium (mg·day^{-1})
JCAL01	1	1.1	6998.3	2064.2	9062.5	64.1	81.5	194.0	57.4	852.8
JCAL02	1	1.0	8857.9	621.2	9479.1	93.4	115.9	208.5	15.7	745.2
JCAL03	2	1.1	5946.7	60.8	6007.6	50.8	74.7	167.4	1.8	213.6
JCAL04	2	1.1	5475.5	0.0	5475.5	55.2	67.2	137.1	0.0	`
JCAL05	1	1.8	18326.3	697.1	19023.5	198.1	321.3	330.4	21.5	1360.2
JCAL06	2	0.8	3528.9	113.4	3642.3	41.0	34.8	85.4	3.2	201.3
JCAL07	1	1.9	9510.1	1056.9	10566.9	100.6	106.9	238.7	27.3	598.3
JCAL08	2	0.9	4475.9	0.0	4475.9	35.2	65.9	123.8	0.0	799.9
JCAL09	1	1.1	6092.6	245.8	6338.5	62.6	98.2	127.5	7.8	561.6
JCAL10	2	0.6	7357.2	71.6	7428.7	58.9	66.6	241.9	2.5	602.4
JCAL11	2	1.1	8738.2	226.8	8965.0	107.8	90.9	192.0	6.4	640.3
JCAL12	1	0.9	4296.4	371.8	4668.2	35.3	72.8	104.4	11.9	651.6
JCAL13	2	1.0	2913.9	0.0	2913.9	24.2	51.5	68.4	0.0	188.0
JCAL14	1	0.5	4240.5	318.0	4558.6	39.9	68.5	96.1	8.8	510.5
JCAL15	2	1.8	12378.6	250.1	12628.7	129.2	149.7	302.3	7.3	790.4
JCAL16	1	0.8	5401.7	281.4	5683.1	55.9	74.3	123.9	8.2	542.4
JCAL17	2	1.1	10880.2	0.0	10880.2	113.2	173.2	224.6	0.0	1141.4
JCAL18	1	1.2	9635.0	178.9	9813.9	83.9	145.3	238.9	6.2	1214.0
JCAL19	1	0.9	8324.1	291.4	8615.5	85.5	90.8	218.8	10.0	1233.6
JCAL20	2	0.8	8842.8	204.4	9047.2	83.6	139.3	203.8	5.6	1712.3
JCAL21	2	1.9	9277.4	107.4	9384.8	115.0	138.9	159.6	3.7	665.4
JCAL22	2	0.8	7917.7	354.8	8272.5	72.3	67.7	248.3	10.9	794.8
JCAL23	1	0.9	6710.7	0.0	6710.7	70.1	81.4	163.2	0.0	636.1
JCAL24	1	1.6	10119.3	61.3	10180.7	120.3	145.1	190.6	1.6	661.3
JCAL25	1	0.9	9602.1	0.0	9602.1	105.6	153.9	183.3	0.0	902.7
JCAL26	1	1.1	4611.8	375.1	4986.9	49.1	69.8	96.3	10.3	418.5
JCAL27	2	1.0	9433.6	51.1	9484.7	90.6	193.9	166.8	1.8	548.3
JCAL28	2	0.6	5759.0	2037.5	7796.5	51.8	69.2	160.8	59.1	529.4
JCAL29	1	0.6	3081.5	54.5	3136.0	36.0	33.2	71.3	1.5	357.1

Appendix 3 Table 9: Six months Dietary intake (DQES)

ID	Group	Portion (std factor)	Energy (kJ·day^{-1})	Alcohol (kJ·day^{-1})	Total kJ (kJ·day^{-1})	Fat (g·day^{-1})	Protein (g·day^{-1})	Carbohydrate (g·day^{-1})	Alcohol (g·day^{-1})	Calcium (mg·day^{-1})
JCAL01	1	1.2	8872.7	1776.9	10649.6	80.0	106.4	243.3	48.8	1070.2
JCAL02	1	0.8	2227.2	767.6	2994.8	19.2	28.1	62.1	19.4	152.9
JCAL03	2	1.4	5316.1	566.7	5882.8	51.3	78.3	124.7	16.9	429.6
JCAL04	2									
JCAL05	1									
JCAL06	2									
JCAL07	1	1.9	6822.0	1124.9	7946.9	73.7	87.4	156.4	29.8	505.7
JCAL08	2	0.8	5994.2	50.4	6044.6	59.3	92.7	133.5	1.4	908.6
JCAL09	1									
JCAL10	2	0.9	6273.6	20.4	6294.1	57.2	66.7	180.5	0.7	504.1
JCAL11	2	1.1	13704.4	862.0	14566.5	165.7	165.3	287.2	22.5	979.2
JCAL12	1									
JCAL13	2									
JCAL14	1	0.7	7284.7	1081.3	8365.9	78.0	111.3	150.3	30.0	553.6
JCAL15	2	2.1	17566.6	554.7	18121.3	211.6	175.8	404.9	16.4	1178.5
JCAL16	1									
JCAL17	2	1.0	5361.6	0.0	5361.6	53.4	68.2	133.3	0.0	625.3
JCAL18	1	1.3	22716.3	178.7	22895.0	241.6	401.4	412.5	5.2	1759.4
JCAL19	1									
JCAL20	2	0.7	7964.7	1584.2	9548.9	94.1	95.6	171.4	44.6	1559.0
JCAL21	2	1.8	13896.0	201.5	14097.5	172.8	168.1	278.3	5.7	1116.0
JCAL22	2									
JCAL23	1									
JCAL24	1	0.9	7107.9	0.0	7107.9	82.3	97.0	143.9	0.0	634.1
JCAL25	1									
JCAL26	1	1.1	4850.1	142.9	4993.0	54.6	54.6	114.1	4.2	654.4
JCAL27	2	0.9	6696.7	25.6	6722.3	67.7	83.0	164.3	0.9	698.6
JCAL28	2									
JCAL29	1	0.7	7297.8	35.8	7333.6	81.0	99.3	156.8	1.2	592.1

Appendix 3 Table 10 Baseline Responses from lifestyle questionnaire

ID	Group	Q1	Q2	Q3	Q4	Q5	Q5a	Q6	Q7	Q8
JCAL01	1	3	3	1	1	1		1	water	water/sport drink/energy drink
JCAL02	1	1	3	1	1	1		1	water	water
JCAL03	2	3	3	1	2	2	Bruised bone in heel of foot	2	nothing or energy drink	nothing/water/ sport drink/energy drink
JCAL04	2	2	3	1	1	1		1	Water	water
JCAL05	1	3	3	1	1	1		2	sports drink/energy drink	energy drink
JCAL06	2	3	3	1	2	2	ligament in ankle	1	water/sport drink/energy drink	water/sport drink/energy drink
JCAL07	1	3	2	1	2	2	Can't run	2	Sports drink	Sports drink
JCAL08	2	3	3	1	1	1		1	water	water
JCAL09	1	3	3	1	1	1		2	water/sport drink/energy drink	water
JCAL10	2	3	2	1	1	1		1	water	water
JCAL11	2	3	3	1	1	1		1	fruit juice	water
JCAL12	1	3	3	1	1	1		2	water	water
JCAL13	2	1	3	1	1	1		1	water	water
JCAL14	1	1	2	1	1	1		2	water/energy drink	water/energy drink
JCAL15	2	3	3	2	1	1		1	energy drink	nothing
JCAL16	1	3	3	1	1	1		2	water	water
JCAL17	2	2	3	1	1	1		1	coffee	water/energy drink
JCAL18	1	2	3	1	1	1		1	water/sport drink	water/sport drink/energy drink
JCAL19	1	3	3	1	1	1		1	tea/hot chocolate	nothing
JCAL20	2	3	3	1	2	1		4	up and go milk	water/energy/sport s drink
JCAL21	2	3	3	1	1	1		1	water	water
JCAL22	2	2	3	1	1	1		1	water/sport drink/energy/soft drink	water/sport drink/energy/soft drink
JCAL23	1	2	3	1	1	1		1	nothing	water/cordial
JCAL24	1	3	3	2	2	2	Apprentice school	2	sports drink	Sports drink
JCAL25	1	3	3	1	1	1		1	sports drink	water
JCAL26	1	3	3	1	1	1		1	water	water
JCAL27	2	3	3	1	1	1		1	beetroot juice	water/water and lemon
JCAL28	2	3	3	1	1	1		1	water/sport/energy /juice	water/sport/energy
JCAL29	1	3	3	1	1	1		1	water	water

Appendix 3 Table 10: Baseline Responses from lifestyle questionnaire cont'd

ID	Group	Q9	Q9a	Q10	Q11	Q11a	Q12	Q12a
JCAL01	1	2	baseball	3	1		2	20
JCAL02	1	1		1	1		2	7
JCAL03	2	1		1	1		1	0
JCAL04	2	1		1	1		1	0
JCAL05	1	1		1	1		1	0
JCAL06	2	1		1	1		1	0
JCAL07	1	2	OzTag	2	1		2	30
JCAL08	2	1		1	1		1	0
JCAL09	1	1		2	1		1	0
JCAL10	2	1		1	2	multivitamins, iron, calcium, magnesium	2	8
JCAL11	2	1		1	2	ventolin	1	0
JCAL12	1	2	golf, soccer	2	2	Akmin	2	5
JCAL13	2	1		1	1		1	0
JCAL14	1	2	touch football	3	1		1	0
JCAL15	2	1		1	2	borocca/ vitamins occasionally	2	12
JCAL16	1	1		1	1		2	5
JCAL17	2	2	training sessions/swimming	2	1		1	0
JCAL18	1	2	taekwondo	3	1		1	0
JCAL19	1	2	running	4	1		1	0
JCAL20	2	1		1	2	ventolin, seretide, vitamin D	1	0
JCAL21	2	1		1	1		1	0
JCAL22	2	1		1	1		1	0
JCAL23	1	1		1	2	multivitamins	1	0
JCAL24	1	2	running twice per week	4	2	nurofen/ multivitamins	1	0
JCAL25	1	1		1	1		1	0
JCAL26	1	1		1	1		2	15
JCAL27	2	2	triathlon	3	2	multivitamins, garlic, vitamin c	1	0
JCAL28	2	1		1	1		1	0
JCAL29	1	1		1	1		1	0

Appendix 3 Table 10: Baseline Responses from lifestyle questionnaire cont'd

ID	Group	Q13	Q13a	Q14	Q15	Q15a	Q16
JCAL01	1	2	5 to 6 times	7	2	skull and finger	1
JCAL02	1	2	2-3 times per week	4 to 5	1		1
JCAL03	2	2	1 to 2 times per week	7	2	Jaw	1
JCAL04	2	1	0	8	1		1
JCAL05	1	2	3-4 times	6	2	pelvis, collar bone, ribs, ankle, finger, leg	2
JCAL06	2	2	0-2 times per week	5 to 8	1		1
JCAL07	1	2	daily amount not specified	5 to 6	2	wrist, thumb, neck, collarbone	2
JCAL08	2	1	0	5	1		1
JCAL09	1	2	1 to 2 glasses per week	7 to 8	1		1
JCAL10	2	2	once a week	6	2	broken toes	1
JCAL11	2	2	< once a week	5	2	both tibia, L thumb, R Femur, L clavicle, L wrist	2
JCAL12	1	2	once a week	7			
JCAL13	2	1	0	4 to 5	2	ribs, ankle, finger	2
JCAL14	1	2	1 per day	7	2	wrist	1
JCAL15	2	1	0	7	2	L ankle	2
JCAL16	1	2	once a week	6	2	hand	2
JCAL17	2	1	0	8	1		1
JCAL18	1	2	1 per week	8 to 9	1		1
JCAL19	1	2	3-4 times	6	2	Jaw/Mandible	1
JCAL20	2	2	once a week	6 to 9	1		1
JCAL21	2	2	< once a week	6 to 8	1		1
JCAL22	2	2	3 to 4 times	7	2		2
JCAL23	1	1	0	8	2	collarbone, scaphoid, hand	2
JCAL24	1	2	< per week - rarely	7 to 8	2	2 x fracture to forearm, R tibia	2
JCAL25	1	1	0	5 to 6	1		1
JCAL26	1	2	1 to 2 per week	7	2	L collarbone	2
JCAL27	2	1	0	8	2	multiple, most recent sternum	2
JCAL28	2	2	3 to 4 times	7	1		1
JCAL29	1	2	< once per week	7 to 8	2	wrist, chipped knee cap	2

KEY:

Q1: 1 = less than 1; 2 = 1 to 2; 3 = 2 or more; Q2: 1 = less than 4; 2 = 4 to 5; 3 = 6 or more; Q3: 1 = yes; 0 = no; Q4: 1 = yes; 0 = no; Q5: 1 = yes; 0 = no; 5a. Explain yes answer; Q6: 0 = 0; 1 = 1 to 2; 2 = 2 to 3; 3 = 4 or more; Q7: record answer; Q8: record answer; Q9: 1 = yes; 0 = no; 9a. Explain yes answer; Q10: 0 = 0; 1 = 2 to 3; 2 = 4 to 5; 3 = 6 or more; Q11: 1 = yes; 0 = no; 11a. Explain yes answer; Q12: 1 = yes; 0 = no; 12a. Explain yes answer; Q13: 1 = yes; 0 = no; 13a. Explain yes answer; Q14: record answer; Q15: 1 = yes; 0 = no; 15a. Explain yes answer; Q16: 1 = yes; 0 = no

Appendix 3 Table 11: Six months Responses from lifestyle questionnaire

ID	Group	Q1	Q2	Q3	Q4	Q5	Q5a	Q6	Q7
JCAL01	1	3	3	2	1	1		1	water, coffee
JCAL02	1	2	3	1	1	1		1	water
JCAL03	2	3	3	1	1	1		1	water, energy drink
JCAL04	2								
JCAL05	1								
JCAL06	2								
JCAL07	1	3	3	1	1	1		4	energy drink
JCAL08	2	3	3	1	1	1		1	water, sports drink
JCAL09	1								
JCAL10	2	3	2	1	1	1		1	water
JCAL11	2								
JCAL12	1								
JCAL13	2								
JCAL14	1	1	2	1	1	1		1	water, sport drink, juice, energy drink
JCAL15	2	3	3	1	2	2	jarred finger	1	nothing
JCAL16	1								
JCAL17	2	3	3	1	1	1		1	energy drink
JCAL18	1	3	3	1	1	1		1	fruit juice, sport drink, water
JCAL19	1								
JCAL20	2	3	3	1	1	1		4	water
JCAL21	2	3	3	1	1	1		1	water
JCAL22	2								
JCAL23	1								
JCAL24	1	3	3	1	1	1		1	up and go
JCAL25	1								
JCAL26	1	3	3	1	1	1		1	water, sport drink
JCAL27	2	3	3	1	1	2	broken thumb, 5 weeks missed training as at measure date	4	water
JCAL28	2								
JCAL29	1	3	1	1	1	1		1	water, sports drink

Appendix 3 Table 11: Six months Responses from lifestyle questionnaire cont'd

ID	Group	Q8	Q9	Q9a	Q10	Q11	Q11a	Q12	Q12a
JCAL01	1	water	2	baseball	3	1	0	2	20
JCAL02	1	water	1		1	1	0	2	10
JCAL03	2	water, energy drink, sports drink, soft drink	1		1	1	0	2	5
JCAL04	2								
JCAL05	1								
JCAL06	2								
JCAL07	1	sports drink	1		1		0	2	20
JCAL08	2	water	1		1	1	0	1	0
JCAL09	1								
JCAL10	2	water	1		1	2	multivitamins, magnesium	2	6
JCAL11	2							1	
JCAL12	1								
JCAL13	2								
JCAL14	1								
JCAL15	2	nothing	1		1	1	0	2	10
JCAL16	1								
JCAL17	2	water	1		1	1	0	1	0
JCAL18	1	water, sport drink, energy drink	2	taekwondo, gym	3	1	0	1	0
JCAL19	1								
JCAL20	2	water, sport drink	1		1	2	ventolin, serotide, nasonex	1	0
JCAL21	2	water	1		1	1	0	1	0
JCAL22	2								
JCAL23	1								
JCAL24	1	water	1		1	2	multivitamins	1	0
JCAL25	1								
JCAL26	1	water, sports drink	1		1	2	multivitamins	2	8
JCAL27	2	water	2	gym	4	2	multivitamin, glucosamine, fish oil, vitamin c	1	0
JCAL28	2								
JCAL29	1	water, sports drink	1		1	1	0	1	0

Appendix 3 Table 11: Six months Responses from lifestyle questionnaire cont'd

ID	Group	Q13	Q13a	Q14	Q15	Q15a	Q16
JCAL01	1	2	daily	7	2	skull	1
JCAL02	1	2	2 to 3 times per week	4 to 5	1	0	1
JCAL03	2	2	once a month	6 to 7	2	jaw	1
JCAL04	2						
JCAL05	1						
JCAL06	2						
JCAL07	1	2	daily	5	2	wrist, collar bone	2
JCAL08	2	1	0	5	1	0	1
JCAL09	1						
JCAL10	2	2	once per week	6	2	foot	1
JCAL11	2	2			2	both tibia, L thumb, R Femur, L clavicle, L wrist	2
JCAL12	1						
JCAL13	2						
JCAL14	1						
JCAL15	2	2	once per week	5	2	ankle	2
JCAL16	1						
JCAL17	2	1	0	7	1	0	1
JCAL18	1	2	once a month	6 to 8	1	0	1
JCAL19	1						
JCAL20	2	2	2-3 times per week	6	2	fingers, neck	2
JCAL21	2	2	once a week	5 to 8	2	tailbone	2
JCAL22	2						
JCAL23	1						
JCAL24	1	1	0	7	2	arm (L), leg - R	2
JCAL25	1						
JCAL26	1	1	0	7	2	collarbone	2
JCAL27	2	1	0	8	2	thumb, nose, arm, ankle, sternum, wrist, humerus (left side injuries)	2
JCAL28	2						
JCAL29	1	2	once a week	8	2	wrist, knee cap	2

KEY:

Q1: 1 = less than 1; 2 = 1 to 2; 3 = 2 or more; Q2: 1 = less than 4; 2 = 4 to 5; 3 = 6 or more; Q3: 1 = yes; 0 = no; Q4: 1 = yes; 0 = no; Q5: 1 = yes; 0 = no; 5a. Explain yes answer; Q6: 0 = 0; 1 = 1 to 2; 2 = 2 to 3; 3 = 4 or more; Q7: record answer; Q8: record answer; Q9: 1 = yes; 0 = no; 9a. Explain yes answer; Q10: 0 = 0; 1 = 2 to 3; 2 = 4 to 5; 3 = 6 or more; Q11: 1 = yes; 0 = no; 11a. Explain yes answer; Q12: 1 = yes; 0 = no; 12a. Explain yes answer; Q13: 1 = yes; 0 = no; 13a. Explain yes answer; Q14: record answer; Q15: 1 = yes; 0 = no; 15a. Explain yes answer; Q16: 1 = yes; 0 = no

Appendix 3 Table 12: Baseline mineral mass by polar sector

ID	Group	0° - 10° (mg)	10° - 20° (mg)	20° - 30° (mg)	30° - 40° (mg)	40° - 50° (mg)	50° - 60° (mg)	60° - 70° (mg)	70° - 80° (mg)	80° - 90° (mg)	90° - 100° (mg)	100° - 110° (mg)	110° - 120° (mg)
JCAL01	1	15.35	17.79	21.63	20.86	14.61	12.24	9.32	6.91	5.37	4.71	4.46	4.65
JCAL02	1	14.11	14.25	13.86	13.76	12.35	10.79	8.73	6.94	6.01	5.33	5.11	5.49
JCAL03	2	14.06	12.70	9.22	8.91	9.56	9.77	8.36	6.97	6.08	5.13	4.66	4.74
JCAL04	2	12.23	13.07	12.96	11.63	9.83	7.46	6.50	6.09	5.87	5.65	5.70	5.63
JCAL05	1	14.87	15.24	12.59	10.01	8.60	7.17	6.45	6.56	7.00	6.66	6.61	6.52
JCAL06	2	12.95	14.47	12.75	10.57	8.68	7.38	6.34	5.47	4.93	4.85	4.48	4.71
JCAL07	1	16.15	14.39	10.58	8.93	9.51	9.74	9.67	9.50	8.56	7.36	6.51	6.29
JCAL08	2	11.29	10.05	8.55	8.25	8.13	8.17	7.77	7.78	7.15	6.51	5.67	5.39
JCAL09	1	12.09	12.88	12.84	12.84	11.52	10.13	9.98	9.45	8.39	8.01	8.02	6.38
JCAL10	2	14.70	12.08	11.73	10.75	10.86	11.17	8.71	7.82	8.04	8.56	8.95	6.93
JCAL11	2	7.40	7.22	6.40	8.13	9.47	7.82	5.95	4.83	3.82	3.19	2.91	2.61
JCAL12	1	17.12	17.09	14.92	13.05	11.12	9.21	8.38	7.49	6.61	6.24	6.03	6.34
JCAL13	2	14.18	15.85	15.98	15.04	12.81	10.87	9.47	8.14	7.64	7.64	7.38	7.54
JCAL14	1	17.12	18.36	15.25	12.28	9.38	7.87	5.99	5.16	5.16	4.84	5.09	5.54
JCAL15	2	12.72	12.22	11.02	10.26	9.53	8.70	7.85	7.14	6.19	5.90	5.98	6.56
JCAL16	1	11.43	10.92	11.72	11.33	10.04	9.68	9.20	8.29	6.63	6.17	5.80	5.54
JCAL17	2	14.04	12.15	11.51	10.75	10.16	8.92	7.49	6.22	5.50	4.96	4.66	4.73
JCAL18	1	14.38	14.07	12.12	11.99	11.85	10.11	8.83	7.51	6.43	5.88	5.67	5.68
JCAL19	1	12.87	14.10	13.85	12.37	10.18	9.04	8.35	7.32	6.39	5.92	5.62	5.60
JCAL20	2	16.66	17.02	15.16	13.77	12.31	10.54	8.94	7.54	6.74	6.08	5.99	6.27
JCAL21	2	12.62	14.42	14.26	12.11	10.42	9.01	8.32	7.97	7.61	7.15	5.96	5.20
JCAL22	2	13.13	13.19	11.87	10.64	9.98	8.94	7.73	7.36	6.82	6.58	6.50	6.59
JCAL23	1	13.32	12.75	12.24	11.99	11.47	9.69	7.78	5.85	5.21	4.76	4.41	3.97
JCAL24	1	12.88	13.05	12.00	9.84	9.18	8.16	6.89	6.13	6.00	5.61	4.58	4.32
JCAL25	1	13.53	13.36	8.93	7.58	7.54	8.49	8.85	8.24	6.90	5.97	5.58	5.35
JCAL26	1	12.79	12.96	12.04	11.51	10.45	8.87	7.40	6.36	5.20	4.50	4.30	4.56
JCAL27	2	18.77	19.30	14.64	11.48	9.63	8.28	8.00	8.11	7.33	6.75	6.49	6.45
JCAL28	2	17.56	16.51	13.25	12.04	11.16	9.37	8.67	8.21	7.34	6.41	5.64	5.54
JCAL29	1	13.36	11.81	10.47	10.53	9.31	8.43	7.72	6.84	6.38	5.83	5.56	5.35

Appendix 3 Table 12: Baseline mineral mass by polar sector cont'd

ID	Group	120° - 130° (mg)	130° - 140° (mg)	140° - 150° (mg)	150° - 160° (mg)	160° - 170° (mg)	170° - 180° (mg)	180° - 190° (mg)	190° - 200° (mg)	200° - 210° (mg)	210° - 220° (mg)	220° - 230° (mg)	230° - 240° (mg)
JCAL01	1	5.07	5.84	7.86	11.34	19.27	29.69	29.85	23.85	15.95	9.22	5.87	4.37
JCAL02	1	5.73	6.84	8.84	12.60	16.67	21.37	21.28	16.88	11.97	8.93	6.47	5.53
JCAL03	2	4.98	5.62	6.59	8.91	12.67	19.43	20.33	15.79	10.26	7.15	4.97	4.32
JCAL04	2	5.90	6.20	7.01	8.60	11.84	17.30	19.77	15.71	11.04	7.61	5.86	5.21
JCAL05	1	6.87	7.56	9.05	10.92	13.55	18.02	20.77	15.36	10.77	8.05	6.61	6.23
JCAL06	2	5.01	6.41	8.09	11.06	14.65	16.04	15.16	12.86	10.46	8.42	6.62	5.27
JCAL07	1	6.36	7.00	7.94	10.36	14.48	19.65	22.39	20.50	14.34	10.30	6.90	5.57
JCAL08	2	5.30	5.63	6.42	7.99	10.31	13.66	17.28	16.55	12.95	9.38	7.02	4.83
JCAL09	1	5.54	5.77	7.21	8.81	11.20	13.46	16.63	18.52	14.97	11.41	8.85	7.15
JCAL10	2	6.17	6.56	7.90	9.24	10.58	13.24	16.54	20.31	19.80	14.17	10.43	8.51
JCAL11	2	3.15	3.60	4.52	6.26	8.71	11.43	10.78	7.82	5.36	3.74	3.36	3.64
JCAL12	1	7.12	8.27	11.02	15.06	19.96	22.64	21.38	17.45	12.92	8.10	5.85	5.73
JCAL13	2	7.76	8.46	10.25	13.58	18.91	20.83	18.43	16.29	13.13	9.89	8.17	6.62
JCAL14	1	6.08	7.41	10.28	15.13	22.18	24.90	20.89	17.35	12.71	9.21	6.88	5.47
JCAL15	2	7.38	9.17	11.20	14.18	17.04	17.17	14.45	12.51	9.94	8.11	6.78	5.67
JCAL16	1	5.90	6.43	7.71	10.09	13.66	17.85	21.07	18.65	13.75	9.65	5.75	4.87
JCAL17	2	5.28	6.09	7.81	10.80	14.29	17.58	18.59	15.31	12.29	8.37	6.47	5.49
JCAL18	1	6.51	7.46	9.22	13.09	17.20	18.30	18.18	16.61	13.43	9.86	6.92	5.68
JCAL19	1	5.87	6.96	8.51	12.76	15.77	17.46	16.33	13.99	10.29	7.94	6.59	5.86
JCAL20	2	6.73	7.95	10.21	13.28	17.23	21.51	20.26	16.04	12.56	10.14	7.53	6.29
JCAL21	2	5.08	5.54	7.61	10.39	13.39	16.32	19.05	18.25	14.68	11.46	8.88	7.26
JCAL22	2	7.05	8.20	10.28	12.89	16.74	19.35	18.63	14.88	11.21	9.16	7.47	6.45
JCAL23	1	4.54	4.98	7.26	10.20	14.58	19.88	21.01	13.99	9.75	7.38	5.08	4.40
JCAL24	1	4.63	5.66	7.46	9.64	11.66	13.20	14.79	14.06	10.49	7.89	5.87	5.03
JCAL25	1	5.36	5.91	6.90	8.70	11.95	16.41	19.92	16.96	12.45	9.12	6.47	5.25
JCAL26	1	5.16	6.55	7.89	10.62	15.37	20.36	20.20	16.35	12.10	8.82	6.65	5.21
JCAL27	2	6.76	8.23	10.87	14.91	21.07	24.85	21.00	16.29	13.03	10.47	7.74	6.26
JCAL28	2	5.60	6.75	8.35	12.25	17.78	18.97	16.89	14.38	12.27	10.21	7.70	6.58
JCAL29	1	5.62	5.97	7.08	9.25	12.86	16.75	15.57	13.33	11.00	9.11	6.44	5.05

Appendix 3 Table 12: Baseline mineral mass by polar sector cont'd

ID	Group	240° - 250° (mg)	250° - 260° (mg)	260° - 270° (mg)	270° - 280° (mg)	280° - 290° (mg)	290° - 300° (mg)	300° - 310° (mg)	310° - 320° (mg)	320° - 330° (mg)	330° - 340° (mg)	340° - 350° (mg)	350° - 360° (mg)
JCAL01	1	3.91	4.31	5.14	6.15	10.06	9.76	9.69	10.35	10.44	8.71	11.19	12.57
JCAL02	1	5.33	5.57	6.68	7.82	7.61	7.85	9.01	8.75	10.76	14.40	13.98	12.30
JCAL03	2	3.87	4.25	5.18	5.20	5.62	6.21	6.96	7.42	7.65	11.06	13.44	12.99
JCAL04	2	5.05	5.60	6.09	6.33	6.99	7.83	9.47	10.41	11.02	13.88	14.84	12.74
JCAL05	1	5.92	6.36	7.17	8.50	8.57	8.51	8.68	9.10	9.04	10.93	11.59	12.47
JCAL06	2	5.01	4.98	5.06	5.48	5.97	6.68	5.86	8.24	10.16	10.03	10.27	10.65
JCAL07	1	4.53	4.56	4.75	5.26	7.51	11.15	10.65	10.62	10.79	12.38	13.49	15.94
JCAL08	2	4.11	4.19	5.06	6.16	6.62	6.81	7.54	8.96	10.33	10.67	11.98	12.43
JCAL09	1	6.31	5.86	5.82	5.85	6.46	7.13	7.59	8.56	8.93	8.70	9.72	11.54
JCAL10	2	7.16	6.33	6.34	6.34	6.00	5.96	6.34	7.32	10.09	13.06	15.08	15.74
JCAL11	2	3.62	3.85	5.17	6.02	5.94	6.13	6.42	6.62	6.98	7.17	7.52	7.37
JCAL12	1	5.75	6.76	6.93	7.15	7.90	8.29	8.91	10.03	12.18	13.20	12.61	13.53
JCAL13	2	6.52	6.92	8.15	8.40	8.47	8.75	9.94	11.88	12.78	13.41	13.46	13.33
JCAL14	1	6.22	7.28	6.96	6.72	6.78	7.01	8.20	8.26	9.51	13.28	15.00	15.26
JCAL15	2	5.84	6.80	8.23	7.69	7.19	6.64	9.01	8.90	8.71	9.68	10.74	12.11
JCAL16	1	4.26	4.90	6.02	8.23	7.34	7.69	8.61	10.39	13.51	14.76	13.62	11.71
JCAL17	2	4.82	5.11	5.80	5.43	5.37	6.14	6.89	8.33	9.97	12.51	14.97	14.37
JCAL18	1	5.72	6.03	7.27	8.02	7.61	7.54	8.19	9.86	11.82	14.60	14.47	13.99
JCAL19	1	5.93	6.21	7.59	8.99	9.59	9.92	10.16	9.46	9.66	10.33	10.36	11.21
JCAL20	2	5.48	5.70	6.74	8.93	9.68	9.25	9.79	10.41	9.48	12.11	13.39	14.83
JCAL21	2	6.21	5.54	5.33	5.30	5.64	6.64	7.75	8.89	9.86	10.09	11.06	11.27
JCAL22	2	6.22	6.44	7.26	8.24	8.22	8.54	9.71	10.16	11.94	12.95	12.41	12.15
JCAL23	1	4.02	4.31	5.66	7.55	7.76	8.21	9.51	9.60	10.15	11.63	11.77	12.29
JCAL24	1	4.79	4.67	4.90	5.49	6.21	6.97	7.66	7.22	6.31	6.86	8.78	11.20
JCAL25	1	4.54	4.60	4.34	5.44	6.78	8.72	8.87	9.37	10.38	10.90	11.71	12.53
JCAL26	1	4.74	4.78	5.79	7.63	7.12	7.25	8.32	9.68	10.96	10.96	11.07	11.89
JCAL27	2	5.73	5.55	6.40	6.76	6.61	6.58	4.95	9.30	10.98	12.23	11.93	14.69
JCAL28	2	6.06	6.24	7.41	7.03	6.38	6.75	7.59	8.43	10.69	14.04	14.77	18.26
JCAL29	1	4.17	4.52	4.94	6.75	7.98	7.92	8.56	7.90	6.49	9.53	11.15	15.44

Appendix 3 Table 13: Six months mineral mass by polar sector

ID	Group	0° - 10° (mg)	10° - 20° (mg)	20° - 30° (mg)	30° - 40° (mg)	40° - 50° (mg)	50° - 60° (mg)	60° - 70° (mg)	70° - 80° (mg)	80° - 90° (mg)	90° - 100° (mg)	100° - 110° (mg)	110° - 120° (mg)
JCAL01	1	15.27	18.98	22.16	20.41	14.32	11.52	8.80	6.34	5.53	4.86	4.43	4.60
JCAL02	1	14.49	14.20	13.96	13.44	12.49	10.70	8.57	7.00	6.08	5.45	5.40	5.36
JCAL03	2	13.47	13.87	12.58	9.96	8.68	7.70	6.64	6.09	5.96	5.98	4.83	4.65
JCAL04	2												
JCAL05	1												
JCAL06	2												
JCAL07	1	15.57	14.40	11.25	9.40	10.26	10.25	9.74	8.94	8.02	7.10	6.46	6.04
JCAL08	2	11.36	9.61	8.64	8.32	8.08	8.23	8.09	7.71	7.36	6.68	5.96	5.69
JCAL09	1												
JCAL10	2	14.59	12.19	11.23	10.22	10.53	11.12	8.56	7.86	8.25	8.63	9.33	7.10
JCAL11	2	7.71	8.12	6.94	8.28	9.30	7.63	5.88	4.70	3.77	3.27	2.93	2.83
JCAL12	1												
JCAL13	2												
JCAL14	1	17.69	16.71	13.46	11.78	9.26	8.73	7.10	5.96	5.50	5.31	5.22	5.39
JCAL15	2	12.64	12.38	11.23	10.03	9.58	8.65	7.94	7.23	6.23	5.72	6.01	6.61
JCAL16	1												
JCAL17	2	13.30	11.79	11.93	11.54	11.48	9.82	8.00	6.08	5.12	4.38	3.88	3.86
JCAL18	1	14.12	14.04	12.06	11.72	11.79	10.59	9.11	7.85	6.57	5.76	5.54	5.50
JCAL19	1												
JCAL20	2	16.67	17.21	15.26	13.71	12.22	10.55	8.79	7.67	6.74	6.14	6.03	6.24
JCAL21	2	12.83	14.75	14.74	12.26	10.78	9.03	8.29	7.93	7.78	7.23	5.79	5.06
JCAL22	2												
JCAL23	1												
JCAL24	1	14.62	12.88	9.52	9.34	9.79	8.87	8.05	6.76	6.03	5.14	4.72	4.87
JCAL25	1												
JCAL26	1	12.55	13.19	12.62	11.93	11.20	9.24	7.36	6.15	5.19	4.44	4.33	4.57
JCAL27	2	18.30	18.91	15.49	11.42	10.07	8.61	7.78	8.12	7.18	6.81	6.66	6.56
JCAL28	2												
JCAL29	1	13.50	11.62	10.62	10.46	9.58	8.25	7.83	6.65	6.48	5.73	5.68	5.54

Appendix 3 Table 13: Six months mineral mass by polar sector cont'd

ID	Group	120° - 130° (mg)	130° - 140° (mg)	140° - 150° (mg)	150° - 160° (mg)	160° - 170° (mg)	170° - 180° (mg)	180° - 190° (mg)	190° - 200° (mg)	200° - 210° (mg)	210° - 220° (mg)	220° - 230° (mg)	230° - 240° (mg)
JCAL01	1	5.08	6.09	7.88	12.14	20.04	29.35	28.52	22.02	16.10	9.93	6.21	5.12
JCAL02	1	5.76	6.69	8.95	12.44	16.89	21.44	21.93	17.34	12.15	8.84	6.59	5.72
JCAL03	2	5.05	6.13	7.97	10.11	11.97	12.98	14.30	13.68	10.79	8.15	6.32	5.48
JCAL04	2												
JCAL05	1												
JCAL06	2												
JCAL07	1	6.15	7.15	8.12	10.74	14.91	20.70	23.28	19.78	13.54	10.06	7.31	5.26
JCAL08	2	5.43	5.76	6.59	8.06	10.71	14.72	18.65	16.23	12.79	9.30	6.82	4.77
JCAL09	1												
JCAL10	2	6.50	6.77	7.90	9.03	10.39	13.26	16.55	20.35	19.80	14.29	10.38	8.23
JCAL11	2	3.33	3.79	4.72	6.46	9.22	12.70	12.45	8.81	5.73	4.31	3.97	3.34
JCAL12	1												
JCAL13	2												
JCAL14	1	5.98	7.58	9.93	14.67	22.73	26.46	21.62	15.97	11.31	8.38	6.34	5.71
JCAL15	2	7.49	9.05	11.41	14.01	16.90	17.10	14.44	12.30	9.90	8.54	6.99	5.87
JCAL16	1												
JCAL17	2	3.99	4.87	6.82	10.31	15.51	21.22	19.59	13.94	10.27	7.16	6.34	5.90
JCAL18	1	5.95	6.92	8.96	12.36	17.30	18.77	19.04	17.62	13.67	9.76	6.93	5.82
JCAL19	1												
JCAL20	2	6.97	8.05	10.27	13.07	17.13	21.50	20.11	16.15	12.23	9.70	7.43	5.83
JCAL21	2	5.15	5.99	7.87	10.33	13.05	15.79	18.50	19.36	15.06	11.82	9.48	7.47
JCAL22	2												
JCAL23	1												
JCAL24	1	5.27	5.87	6.77	9.16	13.18	18.71	19.03	15.09	10.39	7.63	5.47	4.37
JCAL25	1												
JCAL26	1	5.32	6.30	7.72	10.85	15.52	20.49	21.10	16.43	11.65	8.20	6.57	5.33
JCAL27	2	7.13	8.55	11.36	15.03	21.36	23.71	20.53	16.82	12.80	9.81	7.41	6.08
JCAL28	2												
JCAL29	1	5.52	5.99	7.13	9.36	12.93	16.62	15.47	13.46	11.28	8.83	6.10	5.13

Appendix 3 Table 13: Six months mineral mass by polar sector cont'd

ID	Group	240° - 250° (mg)	250° - 260° (mg)	260° - 270° (mg)	270° - 280° (mg)	280° - 290° (mg)	290° - 300° (mg)	300° - 310° (mg)	310° - 320° (mg)	320° - 330° (mg)	330° - 340° (mg)	340° - 350° (mg)	350° - 360° (mg)
JCAL01	1	4.46	4.37	5.05	6.22	10.48	10.78	9.98	10.03	9.65	8.58	11.38	12.31
JCAL02	1	5.38	5.71	6.71	7.93	7.61	7.92	8.67	8.67	10.63	14.65	14.29	12.78
JCAL03	2	4.98	4.92	5.20	5.78	6.33	6.87	7.39	7.12	6.49	6.97	9.13	11.37
JCAL04	2												
JCAL05	1												
JCAL06	2												
JCAL07	1	4.55	4.40	4.52	5.40	7.99	12.46	11.24	10.78	10.73	11.69	13.23	15.62
JCAL08	2	4.00	4.23	5.14	6.64	6.84	6.77	7.42	8.95	10.47	10.70	11.67	12.43
JCAL09	1												
JCAL10	2	7.41	6.54	6.45	6.46	6.36	6.10	6.45	7.53	10.22	13.23	15.39	15.86
JCAL11	2	3.57	3.83	4.74	6.00	6.06	6.19	6.58	6.87	7.14	7.16	7.15	7.50
JCAL12	1												
JCAL13	2												
JCAL14	1	6.16	6.46	6.24	6.66	7.36	7.98	8.88	8.06	11.15	14.96	14.47	14.68
JCAL15	2	5.86	6.60	8.08	8.08	7.52	6.70	8.69	8.84	8.75	9.71	10.84	12.01
JCAL16	1												
JCAL17	2	5.45	5.63	5.40	5.41	5.57	5.91	7.07	8.49	10.79	12.81	13.62	13.38
JCAL18	1	5.40	5.67	7.32	8.24	7.52	7.80	8.20	9.82	12.28	15.06	14.97	14.11
JCAL19	1												
JCAL20	2	5.52	5.68	6.91	8.64	9.51	9.18	9.88	10.79	9.20	12.31	13.73	14.86
JCAL21	2	6.39	5.79	5.53	5.64	6.02	6.80	7.58	8.78	9.66	10.29	11.42	11.43
JCAL22	2												
JCAL23	1												
JCAL24	1	3.98	4.24	5.71	5.22	5.59	6.05	6.87	7.47	7.53	10.76	13.27	12.77
JCAL25	1												
JCAL26	1	4.52	4.50	5.62	7.08	6.84	7.30	8.33	9.72	10.86	11.54	11.33	11.79
JCAL27	2	5.51	5.35	5.85	6.77	7.13	7.01	4.97	9.15	10.63	11.35	11.91	13.91
JCAL28	2												
JCAL29	1	4.37	4.21	4.94	6.51	7.69	8.02	8.60	7.88	6.92	9.70	11.33	15.22

Appendix 3 Table 14: Baseline and six months vBMD by Radial division

ID	Group	Radial division 0 vBMD [mg·cm^3]	Radial division 1 vBMD [mg·cm^3]	Radial division 2 vBMD [mg·cm^3]	Radial division 0 vBMD [mg·cm^3]	Radial division 1 vBMD [mg·cm^3]	Radial division 2 vBMD [mg·cm^3]
JCAL01	1	1157.96	1216.26	1204.97	1155.54	1223.28	1207.36
JCAL02	1	1152.30	1204.25	1207.06	1143.93	1202.00	1204.99
JCAL03	2	1135.71	1185.88	1181.24	1085.40	1136.30	1123.29
JCAL04	2	1115.78	1181.92	1171.07			
JCAL05	1	1114.72	1202.51	1205.71			
JCAL06	2	1112.56	1189.57	1185.99			
JCAL07	1	1114.21	1192.77	1172.26	1120.73	1199.70	1189.12
JCAL08	2	1096.91	1190.57	1182.07	1095.99	1206.09	1195.67
JCAL09	1	1140.64	1215.75	1212.97			
JCAL10	2	1135.97	1211.18	1207.32	1134.37	1222.66	1216.67
JCAL11	2	1129.72	1162.07	1154.02	1147.55	1189.79	1185.84
JCAL12	1	1152.26	1211.93	1185.12			
JCAL13	2	1123.02	1216.15	1217.13			
JCAL14	1	1084.36	1188.14	1172.30	1106.99	1212.24	1214.92
JCAL15	2	1153.34	1214.53	1209.34	1158.95	1220.97	1209.38
JCAL16	1	1156.70	1200.56	1189.88			
JCAL17	2	1091.87	1157.90	1144.09	1076.52	1169.25	1135.51
JCAL18	1	1129.21	1183.01	1179.94	1140.26	1193.42	1186.59
JCAL19	1	1068.98	1157.75	1165.46			
JCAL20	2	1157.78	1196.29	1193.64	1154.06	1200.23	1186.86
JCAL21	2	1124.65	1188.97	1178.12	1129.82	1193.48	1188.32
JCAL22	2	1094.31	1160.63	1147.64			
JCAL23	1	1101.53	1169.93	1163.83			
JCAL24	1	1065.71	1123.07	1102.59	1134.01	1185.10	1178.34
JCAL25	1	1051.86	1098.47	1074.29			
JCAL26	1	1140.24	1199.04	1185.95	1154.39	1202.53	1193.59
JCAL27	2	1148.18	1201.01	1191.07	1139.35	1200.88	1187.74
JCAL28	2	1153.29	1193.39	1175.14			
JCAL29	1	1111.42	1186.91	1185.44	1102.76	1185.62	1185.25

KEY: radial division 0 = endocortical; radial division 1 = mid-cortical, radial division 2 = pericortical

Appendix 3 Table 15: Baseline vBMD by polar sector

ID	Group	Polar sector 0 [mg·cm³]	Polar sector 1 [mg·cm³]	Polar sector 2 [mg·cm³]	Polar sector 3 [mg·cm³]	Polar sector 4 [mg·cm³]	Polar sector 5 [mg·cm³]	Polar sector 6 [mg·cm³]	Polar sector 7 [mg·cm³]	Polar sector 8 [mg·cm³]	Polar sector 9 [mg·cm³]	Polar sector 10 [mg·cm³]	Polar sector 11 [mg·cm³]
JCAL01	1	1218.45	1169.32	1209.20	1217.62	1192.92	1225.58	1243.10	1222.46	1241.04	1180.79	1225.10	1250.09
JCAL02	1	1191.55	1214.56	1220.53	1230.69	1246.04	1211.02	1234.64	1231.05	1205.87	1172.13	1175.50	1196.17
JCAL03	2	1152.04	1158.82	1178.13	1230.62	1260.88	1275.37	1237.93	1233.05	1198.84	1198.72	1153.54	1159.06
JCAL04	2	1092.49	1044.94	1181.48	1237.85	1229.00	1133.15	1105.57	1167.07	1161.49	1179.64	1170.09	1168.58
JCAL05	1	1162.74	1094.62	1172.49	1236.90	1222.81	1208.97	1134.89	1156.92	1212.42	1230.19	1186.49	1185.62
JCAL06	2	1135.81	1101.65	1167.65	1209.04	1199.91	1205.38	1190.17	1206.17	1173.93	1210.97	1204.61	1172.29
JCAL07	1	1206.99	1171.76	1193.06	1199.23	1190.27	1200.99	1202.28	1241.94	1267.68	1235.22	1210.02	1198.40
JCAL08	2	1160.76	1129.14	1121.59	1205.87	1225.78	1238.21	1186.02	1152.93	1135.26	1182.91	1140.57	1175.33
JCAL09	1	1224.03	1218.64	1210.60	1197.04	1215.96	1142.61	1165.12	1208.65	1237.26	1239.20	1238.23	1189.18
JCAL10	2	1169.66	1198.64	1206.44	1207.95	1198.41	1191.28	1163.89	1194.82	1180.96	1242.36	1238.10	1231.45
JCAL11	2	1123.60	1073.85	1129.84	1209.60	1232.20	1205.69	1179.78	1212.32	1146.06	1181.75	1144.85	1128.75
JCAL12	1	1168.73	1180.08	1225.14	1238.49	1190.84	1295.32	1288.37	1271.37	1322.89	1285.82	1241.40	1196.73
JCAL13	2	1136.91	1142.50	1171.81	1201.99	1189.34	1187.32	1188.81	1219.97	1215.30	1206.64	1215.41	1232.78
JCAL14	1	1110.48	1072.48	1183.01	1260.70	1246.98	1138.50	1029.54	998.29	1128.79	1147.66	1123.13	1154.42
JCAL15	2	1173.77	1212.24	1253.22	1234.22	1176.64	1176.65	1224.41	1250.27	1220.23	1211.64	1189.58	1208.29
JCAL16	1	1184.87	1182.97	1211.79	1229.86	1222.02	1214.52	1215.16	1238.81	1206.78	1187.00	1214.86	1189.23
JCAL17	2	1115.40	1134.41	1192.83	1220.61	1194.97	1179.17	1163.32	1154.39	1162.02	1135.57	1122.95	1124.74
JCAL18	1	1159.36	1159.89	1171.39	1205.77	1207.03	1196.62	1178.73	1178.18	1175.01	1183.47	1164.10	1160.32
JCAL19	1	1015.47	1084.85	1070.88	1149.88	1182.92	1195.51	1188.60	1166.60	1157.80	1170.03	1132.79	1122.88
JCAL20	2	1210.67	1173.00	1193.51	1211.84	1210.21	1186.06	1186.11	1174.81	1202.93	1190.38	1178.39	1185.84
JCAL21	2	1068.75	1127.96	1191.18	1166.11	1169.67	1181.39	1210.62	1165.97	1187.56	1188.07	1204.70	1192.81
JCAL22	2	1143.93	1150.52	1205.54	1223.30	1188.46	1169.07	1184.46	1181.36	1168.83	1134.14	1125.01	1108.94
JCAL23	1	1111.14	1064.74	1169.20	1223.74	1225.21	1236.81	1138.64	1131.92	1128.64	1130.43	1121.51	1081.51
JCAL24	1	1081.84	1069.88	1105.60	1021.50	1076.98	1167.37	1197.18	1184.80	1169.82	1119.31	1098.97	1093.71
JCAL25	1	1093.42	1068.45	1055.43	1080.11	1099.67	1103.21	1128.19	1127.17	1147.84	1064.43	1094.91	1105.99
JCAL26	1	1189.95	1206.59	1249.43	1242.61	1235.57	1180.21	1207.18	1199.17	1187.66	1173.06	1153.36	1135.35
JCAL27	2	1167.73	1188.77	1186.31	1249.16	1221.76	1201.86	1221.42	1239.50	1222.24	1205.90	1232.39	1217.37
JCAL28	2	1163.40	1214.56	1208.47	1181.53	1188.47	1183.14	1200.83	1278.00	1246.23	1207.18	1224.75	1165.68
JCAL29	1	1042.13	1062.30	1034.26	1189.88	1224.48	1213.16	1220.24	1195.23	1204.78	1213.14	1194.87	1178.30

Appendix 3 Table 15: Baseline vBMD by polar sector cont'd

ID	Group	Polar sector 12 [mg·cm³]	Polar sector 13 [mg·cm³]	Polar sector 14 [mg·cm³]	Polar sector 15 [mg·cm³]	Polar sector 16 [mg·cm³]	Polar sector 17 [mg·cm³]	Polar sector 18 [mg·cm³]	Polar sector 19 [mg·cm³]	Polar sector 20 [mg·cm³]	Polar sector 21 [mg·cm³]	Polar sector 22 [mg·cm³]	Polar sector 23 [mg·cm³]
JCAL01	1	1224.12	1186.94	1189.91	1142.37	1121.77	1177.55	1175.54	1172.22	1124.05	1113.49	1073.07	1082.64
JCAL02	1	1180.41	1171.34	1128.79	1171.69	1154.09	1153.90	1157.22	1130.65	1122.22	1145.12	1149.86	1163.42
JCAL03	2	1119.07	1128.41	1125.08	1119.56	1128.62	1119.54	1106.79	1065.08	1049.72	1155.67	1092.20	1134.48
JCAL04	2	1153.28	1133.84	1106.02	1120.99	1161.67	1096.71	1086.10	1009.01	1056.43	1059.16	1149.43	1165.03
JCAL05	1	1106.75	1119.23	1155.41	1134.16	1179.43	1120.09	1093.48	1117.96	1096.08	1157.79	1158.52	1196.22
JCAL06	2	1152.13	1144.43	1120.79	1081.47	1058.21	1066.91	1071.72	1081.65	1137.49	1169.17	1168.71	1164.17
JCAL07	1	1138.38	1130.09	1088.33	1114.83	1104.14	1094.49	1075.33	1052.83	1014.45	1079.60	1132.34	1112.82
JCAL08	2	1157.90	1161.62	1149.60	1125.81	1148.08	1052.80	1059.70	1016.66	1031.68	1114.93	1106.57	1115.67
JCAL09	1	1199.19	1203.81	1169.25	1123.27	1098.03	1078.54	1145.79	1154.50	1158.86	1145.77	1167.14	1143.64
JCAL10	2	1198.88	1186.74	1192.02	1160.33	1117.40	1127.79	1078.77	1051.51	1094.00	1109.23	1131.13	1188.85
JCAL11	2	1164.60	1096.82	1113.72	1076.04	1066.62	1012.33	980.91	999.80	981.59	986.59	1088.80	1190.72
JCAL12	1	1145.99	1108.40	1098.42	1134.55	1138.43	1153.46	1122.66	1138.90	1203.96	1140.66	1128.17	1242.14
JCAL13	2	1190.22	1194.29	1173.99	1174.98	1164.83	1169.95	1125.59	1141.56	1095.18	1147.95	1167.06	1159.60
JCAL14	1	1152.36	1181.43	1183.74	1161.97	1146.49	1103.37	1086.21	1085.98	1053.28	1092.31	1100.44	1131.12
JCAL15	2	1185.91	1184.32	1161.07	1154.02	1122.38	1104.84	1084.46	1115.82	1150.73	1166.59	1194.49	1205.67
JCAL16	1	1193.50	1174.18	1183.50	1173.94	1169.64	1122.12	1082.24	1093.20	1118.51	1163.33	1142.11	1142.60
JCAL17	2	1125.14	1121.10	1128.99	1089.25	1049.48	972.65	972.10	975.74	1022.62	1091.57	1168.38	1147.61
JCAL18	1	1177.00	1145.25	1108.33	1132.84	1111.19	1101.82	1078.70	1084.69	1076.47	1122.78	1145.24	1193.25
JCAL19	1	1124.21	1116.13	1106.73	1069.78	1054.06	1027.04	999.38	1045.06	1061.89	1043.48	1102.59	1146.96
JCAL20	2	1179.70	1178.93	1164.73	1161.16	1156.73	1141.20	1106.60	1100.52	1094.41	1138.89	1138.93	1166.14
JCAL21	2	1138.42	1114.91	1156.74	1188.56	1139.81	1082.96	1090.23	1074.01	1135.76	1165.63	1217.60	1191.15
JCAL22	2	1044.86	1029.40	1055.19	1046.19	1053.23	1024.12	1047.50	1083.02	1077.30	1103.75	1101.82	1123.10
JCAL23	1	1135.13	1123.15	1152.50	1131.92	1085.03	1073.37	1105.95	1041.53	1032.26	1056.99	1059.20	1099.92
JCAL24	1	1071.83	1059.02	1085.59	1047.24	1046.32	1032.56	1069.27	1009.14	993.84	1004.50	1014.91	1064.64
JCAL25	1	1028.58	1011.74	1029.20	1039.33	952.99	929.67	942.39	970.52	972.09	1044.98	1104.95	1090.16
JCAL26	1	1177.09	1147.62	1158.04	1093.10	1103.51	1075.29	1061.96	1071.50	1155.62	1171.80	1175.22	1151.05
JCAL27	2	1193.84	1177.40	1154.20	1152.27	1118.93	1110.66	1068.30	1034.04	1088.51	1176.05	1201.23	1185.49
JCAL28	2	1123.64	1164.02	1137.40	1154.50	1145.76	1168.63	1105.42	1044.19	1091.85	1204.17	1196.72	1179.14
JCAL29	1	1179.63	1135.50	1145.08	1135.75	1116.04	1066.93	1105.40	1115.71	1116.26	1123.44	1151.07	1138.74

Appendix 3 Table 15: Baseline vBMD by polar sector cont'd

ID	Group	Polar sector 24 [mg·cm³]	Polar sector 25 [mg·cm³]	Polar sector 26 [mg·cm³]	Polar sector 27 [mg·cm³]	Polar sector 28 [mg·cm³]	Polar sector 29 [mg·cm³]	Polar sector 30 [mg·cm³]	Polar sector 31 [mg·cm³]	Polar sector 32 [mg·cm³]	Polar sector 33 [mg·cm³]	Polar sector 34 [mg·cm³]	Polar sector 35 [mg·cm³]
JCAL01	1	1168.62	1201.79	1196.47	1177.63	1202.52	1185.21	1251.11	1264.62	1295.09	1248.84	1202.66	1176.42
JCAL02	1	1184.89	1201.68	1209.95	1207.36	1223.43	1240.96	1255.50	1196.30	1179.62	1180.46	1163.74	1160.95
JCAL03	2	1129.41	1107.68	1132.47	1195.42	1233.17	1213.94	1212.89	1226.77	1194.39	1225.68	1210.06	1170.92
JCAL04	2	1204.08	1217.87	1244.87	1282.14	1263.62	1199.83	1180.26	1187.23	1166.90	1171.31	1165.79	1172.23
JCAL05	1	1210.08	1248.55	1228.70	1239.12	1235.38	1199.13	1184.52	1201.77	1182.38	1172.71	1161.83	1170.94
JCAL06	2	1153.30	1162.85	1189.51	1216.37	1194.00	1204.00	1201.31	1185.10	1201.22	1213.60	1174.27	1167.44
JCAL07	1	1111.94	1154.75	1160.45	1149.39	1119.91	1160.34	1186.29	1226.27	1230.49	1222.83	1178.38	1194.40
JCAL08	2	1170.15	1173.02	1245.58	1269.51	1232.83	1206.03	1197.76	1181.93	1193.30	1172.48	1162.00	1134.60
JCAL09	1	1164.74	1188.67	1218.89	1225.24	1244.71	1210.75	1196.28	1222.25	1240.21	1222.73	1228.61	1194.87
JCAL10	2	1178.69	1154.76	1225.12	1230.67	1258.14	1195.56	1184.06	1248.01	1265.89	1241.80	1204.67	1205.74
JCAL11	2	1203.88	1223.10	1257.97	1279.99	1248.36	1236.74	1198.43	1174.43	1195.32	1204.57	1209.84	1190.25
JCAL12	1	1243.60	1116.88	1036.97	1002.14	1034.92	1089.03	1207.60	1261.44	1278.61	1250.35	1214.11	1195.22
JCAL13	2	1194.64	1248.15	1254.35	1272.85	1235.66	1184.93	1212.95	1208.41	1193.89	1176.67	1147.00	1132.15
JCAL14	1	1295.91	1399.36	1423.83	1280.12	1124.01	966.63	960.55	1074.23	1186.62	1171.12	1240.11	1142.31
JCAL15	2	1214.89	1206.72	1234.70	1214.71	1234.89	1252.12	1238.86	1241.29	1228.61	1163.92	1180.15	1154.32
JCAL16	1	1175.84	1180.70	1200.96	1233.41	1186.08	1205.03	1194.85	1172.41	1190.72	1184.22	1197.15	1187.55
JCAL17	2	1123.78	1151.74	1214.12	1178.26	1137.05	1181.36	1192.78	1201.06	1182.80	1127.13	1124.69	1146.61
JCAL18	1	1173.38	1192.95	1211.10	1214.25	1201.54	1196.05	1190.98	1203.51	1177.75	1198.31	1179.61	1148.99
JCAL19	1	1197.68	1210.95	1190.87	1130.76	1173.73	1225.67	1182.28	1191.73	1182.76	1204.75	1174.31	1105.24
JCAL20	2	1185.76	1212.85	1251.60	1268.87	1252.58	1203.04	1207.31	1204.50	1191.13	1186.17	1187.45	1189.64
JCAL21	2	1181.99	1162.60	1189.53	1156.44	1155.54	1185.38	1212.54	1249.44	1215.46	1181.43	1130.03	1129.89
JCAL22	2	1161.05	1183.85	1176.83	1197.42	1198.18	1182.73	1183.10	1155.22	1161.13	1188.37	1143.36	1126.71
JCAL23	1	1181.41	1166.16	1249.81	1264.15	1288.34	1232.40	1200.69	1174.92	1157.86	1174.59	1177.22	1095.46
JCAL24	1	1074.90	1100.00	1105.82	1123.25	1122.81	1168.37	1212.07	1221.91	1171.66	1114.52	1116.57	1078.86
JCAL25	1	1077.94	1145.65	1142.96	1142.16	1165.00	1165.71	1120.22	1104.09	1095.66	1069.54	1087.99	1093.17
JCAL26	1	1175.19	1131.12	1179.62	1242.76	1185.49	1209.42	1208.98	1255.03	1242.75	1194.06	1186.66	1189.76
JCAL27	2	1212.90	1181.79	1179.90	1161.06	1167.66	1136.19	1166.80	1235.89	1230.36	1228.20	1197.78	1169.18
JCAL28	2	1151.41	1145.65	1188.35	1104.41	1122.25	1139.28	1192.50	1224.27	1247.27	1223.29	1161.05	1184.35
JCAL29	1	1210.80	1190.75	1206.71	1214.16	1248.91	1245.13	1188.35	1177.15	1194.37	1168.83	1143.59	1114.21

Appendix 3 Table 16: Six months vBMD by polar sector

ID	Group	Polar sector 0 [mg·cm³]	Polar sector 1 [mg·cm³]	Polar sector 2 [mg·cm³]	Polar sector 3 [mg·cm³]	Polar sector 4 [mg·cm³]	Polar sector 5 [mg·cm³]	Polar sector 6 [mg·cm³]	Polar sector 7 [mg·cm³]	Polar sector 8 [mg·cm³]	Polar sector 9 [mg·cm³]	Polar sector 10 [mg·cm³]	Polar sector 11 [mg·cm³]
JCAL01	1	1196.87	1203.19	1214.18	1219.27	1220.64	1224.14	1218.26	1183.45	1199.12	1210.75	1193.74	1196.54
JCAL02	1	1202.21	1192.01	1221.47	1222.19	1246.18	1218.14	1181.84	1173.99	1197.17	1208.06	1188.08	1218.79
JCAL03	2	1108.90	1124.05	1157.18	1107.62	1117.92	1130.78	1130.15	1170.69	1171.71	1163.48	1138.69	1149.63
JCAL04	2												
JCAL05	1												
JCAL06	2												
JCAL07	1	1171.55	1149.97	1187.74	1212.09	1251.11	1229.93	1237.63	1215.18	1195.47	1192.19	1212.28	1198.98
JCAL08	2	1165.25	1099.71	1139.10	1232.02	1245.66	1234.61	1183.41	1169.90	1171.82	1208.48	1180.98	1184.56
JCAL09	1												
JCAL10	2	1183.51	1232.07	1182.99	1169.09	1188.97	1217.94	1195.05	1208.09	1213.42	1286.36	1273.15	1258.51
JCAL11	2	1120.05	1094.21	1144.08	1249.87	1263.20	1242.41	1259.01	1217.30	1207.70	1209.44	1200.78	1184.31
JCAL12	1												
JCAL13	2												
JCAL14	1	1084.97	1052.56	1169.18	1269.69	1256.55	1198.03	1169.62	1202.05	1194.02	1166.23	1123.06	1152.09
JCAL15	2	1173.46	1226.82	1281.03	1231.88	1164.72	1192.54	1235.03	1243.82	1214.02	1204.04	1188.64	1232.28
JCAL16	1												
JCAL17	2	1136.23	1115.43	1254.37	1257.99	1291.95	1358.57	1325.37	1308.91	1310.06	1173.25	1050.70	974.18
JCAL18	1	1144.60	1154.07	1180.09	1206.95	1216.33	1206.68	1217.91	1218.68	1198.01	1146.94	1164.34	1138.71
JCAL19	1												
JCAL20	2	1182.99	1176.54	1213.24	1199.51	1210.75	1227.55	1184.98	1202.49	1196.31	1214.90	1201.27	1165.00
JCAL21	2	1085.38	1133.36	1169.98	1167.58	1192.80	1164.94	1192.06	1207.66	1193.86	1181.91	1188.98	1200.24
JCAL22	2												
JCAL23	1												
JCAL24	1	1162.05	1143.96	1212.87	1247.36	1261.78	1222.58	1226.27	1201.41	1223.77	1181.59	1183.91	1171.46
JCAL25	1												
JCAL26	1	1183.57	1196.34	1229.44	1245.27	1232.78	1243.19	1211.80	1212.22	1226.82	1210.32	1181.38	1228.34
JCAL27	2	1188.94	1176.47	1163.38	1201.63	1215.90	1259.51	1206.44	1181.85	1199.29	1214.01	1207.70	1199.56
JCAL28	2												
JCAL29	1	1077.69	1076.07	1041.37	1179.76	1246.80	1225.64	1179.94	1195.27	1196.62	1210.55	1215.42	1208.76

Appendix 3 Table 16: Six months vBMD by polar sector cont'd

ID	Group	Polar sector 12 [mg·cm³]	Polar sector 13 [mg·cm³]	Polar sector 14 [mg·cm³]	Polar sector 15 [mg·cm³]	Polar sector 16 [mg·cm³]	Polar sector 17 [mg·cm³]	Polar sector 18 [mg·cm³]	Polar sector 19 [mg·cm³]	Polar sector 20 [mg·cm³]	Polar sector 21 [mg·cm³]	Polar sector 22 [mg·cm³]	Polar sector 23 [mg·cm³]
JCAL01	1	1206.29	1201.30	1181.54	1206.61	1164.96	1183.14	1159.74	1132.03	1116.23	1130.34	1123.05	1152.43
JCAL02	1	1190.81	1170.60	1147.89	1149.41	1146.22	1156.52	1152.82	1143.74	1121.05	1143.89	1163.39	1151.78
JCAL03	2	1145.26	1134.95	1109.01	1066.11	1045.49	1035.50	1033.99	1002.99	1005.15	1033.48	1027.33	1064.32
JCAL04	2												
JCAL05	1												
JCAL06	2												
JCAL07	1	1182.71	1157.78	1121.49	1139.59	1138.23	1112.43	1103.18	1055.05	1068.45	1141.60	1178.69	1074.67
JCAL08	2	1185.68	1164.59	1160.26	1138.56	1112.88	1112.32	1074.41	1022.97	1017.02	1090.21	1062.00	1126.31
JCAL09	1												
JCAL10	2	1196.58	1179.97	1168.95	1178.94	1120.92	1110.35	1101.58	1090.46	1078.86	1106.90	1148.05	1181.22
JCAL11	2	1146.52	1112.05	1122.06	1080.53	1067.99	1042.79	1070.53	1035.75	1075.40	1098.56	1103.92	1174.06
JCAL12	1												
JCAL13	2												
JCAL14	1	1170.01	1170.45	1160.04	1172.79	1196.14	1159.11	1126.35	1079.93	952.57	1088.45	1163.92	1232.08
JCAL15	2	1195.49	1209.32	1173.76	1168.41	1144.86	1110.58	1081.75	1141.19	1194.91	1184.35	1162.32	1180.55
JCAL16	1												
JCAL17	2	910.10	903.63	1004.53	1093.34	1105.16	1099.71	1043.02	889.53	971.67	1030.04	1086.81	1188.28
JCAL18	1	1146.40	1156.31	1158.22	1160.35	1142.18	1132.91	1113.37	1114.86	1106.65	1139.33	1143.57	1163.87
JCAL19	1												
JCAL20	2	1187.53	1182.23	1165.95	1149.65	1156.48	1135.23	1134.10	1107.01	1081.18	1109.16	1131.60	1143.40
JCAL21	2	1185.52	1166.08	1183.67	1178.35	1137.14	1096.38	1095.91	1099.54	1122.55	1170.87	1200.66	1185.22
JCAL22	2												
JCAL23	1												
JCAL24	1	1159.70	1142.98	1138.48	1145.97	1127.31	1079.47	1059.81	1049.87	1036.67	1130.25	1120.86	1088.46
JCAL25	1												
JCAL26	1	1173.80	1155.20	1143.42	1131.10	1090.60	1065.19	1051.39	1064.77	1103.26	1170.48	1164.41	1184.75
JCAL27	2	1197.50	1178.83	1169.86	1143.93	1111.88	1074.87	1052.67	1072.05	1096.00	1147.81	1163.40	1171.34
JCAL28	2												
JCAL29	1	1177.95	1158.45	1133.12	1089.98	1093.10	1086.00	1114.79	1098.23	1114.08	1095.63	1076.23	1143.27

Appendix 3 Table 16: Six months vBMD by polar sector cont'd

ID	Group	Polar sector 24 [mg·cm³]	Polar sector 25 [mg·cm³]	Polar sector 26 [mg·cm³]	Polar sector 27 [mg·cm³]	Polar sector 28 [mg·cm³]	Polar sector 29 [mg·cm³]	Polar sector 30 [mg·cm³]	Polar sector 31 [mg·cm³]	Polar sector 32 [mg·cm³]	Polar sector 33 [mg·cm³]	Polar sector 34 [mg·cm³]	Polar sector 35 [mg·cm³]
JCAL01	1	1186.10	1155.42	1194.75	1233.08	1301.00	1271.85	1234.74	1226.81	1233.83	1210.54	1173.18	1175.02
JCAL02	1	1164.44	1161.97	1189.71	1191.39	1218.63	1208.15	1195.79	1180.40	1186.72	1206.45	1205.82	1193.28
JCAL03	2	1096.94	1119.78	1146.81	1138.26	1162.06	1149.93	1168.49	1177.29	1149.28	1147.83	1170.44	1138.46
JCAL04	2												
JCAL05	1												
JCAL06	2												
JCAL07	1	1085.61	1112.68	1162.69	1170.59	1213.68	1247.32	1191.10	1225.25	1198.30	1207.23	1165.28	1206.83
JCAL08	2	1214.54	1218.06	1261.53	1300.61	1257.38	1217.34	1242.97	1225.23	1196.35	1145.56	1083.47	1127.17
JCAL09	1												
JCAL10	2	1172.25	1170.58	1223.60	1236.52	1249.83	1215.92	1218.90	1205.95	1273.59	1239.42	1206.20	1199.75
JCAL11	2	1157.79	1206.38	1256.50	1277.70	1250.82	1224.56	1243.41	1249.54	1258.14	1236.01	1208.31	1186.47
JCAL12	1												
JCAL13	2												
JCAL14	1	1271.41	1279.53	1354.20	1294.47	1247.83	1250.04	1211.49	1169.40	1194.50	1215.75	1141.50	1069.71
JCAL15	2	1224.99	1202.16	1229.29	1256.29	1249.41	1234.88	1216.87	1244.66	1199.68	1166.91	1173.79	1136.99
JCAL16	1												
JCAL17	2	1264.77	1219.55	1165.28	1143.00	1084.23	1148.83	1176.60	1239.68	1128.60	980.55	1017.97	1123.52
JCAL18	1	1158.08	1180.63	1210.37	1241.78	1240.41	1203.39	1187.14	1207.52	1197.76	1196.66	1179.19	1168.97
JCAL19	1												
JCAL20	2	1130.59	1166.81	1251.36	1243.82	1258.51	1212.54	1209.51	1221.60	1188.80	1190.08	1190.37	1170.74
JCAL21	2	1203.38	1215.86	1191.98	1196.64	1176.88	1162.25	1185.37	1186.72	1228.86	1179.70	1165.40	1145.76
JCAL22	2												
JCAL23	1												
JCAL24	1	1113.61	1147.15	1158.09	1158.81	1213.74	1222.88	1234.74	1219.88	1193.18	1200.42	1187.00	1201.03
JCAL25	1												
JCAL26	1	1186.41	1188.43	1182.24	1224.51	1229.53	1226.39	1201.38	1239.95	1228.35	1202.38	1197.17	1199.46
JCAL27	2	1176.93	1167.26	1167.31	1229.23	1240.45	1152.42	1219.80	1193.66	1218.82	1169.82	1214.75	1190.31
JCAL28	2												
JCAL29	1	1165.07	1144.03	1153.33	1179.63	1215.53	1239.41	1243.95	1211.53	1190.52	1212.92	1193.43	1099.54

Appendix 3 Table 17: Baseline Endocortical Radius by polar sector

ID	Group	0° - 10° (mm)	10° - 20° (mm)	20° - 30° (mm)	30° - 40° (mm)	40° - 50° (mm)	50° - 60° (mm)	60° - 70° (mm)	70° - 80° (mm)	80° - 90° (mm)	90° - 100° (mm)	100° - 110° (mm)	110° - 120° (mm)
JCAL01	1	9.06	8.38	8.39	8.73	8.62	9.37	8.85	8.09	7.66	7.12	7.38	7.31
JCAL02	1	8.67	9.50	9.94	9.54	8.94	7.82	7.04	6.61	6.24	5.95	5.92	5.88
JCAL03	2	9.92	10.46	10.63	10.41	9.78	8.99	8.38	7.71	7.29	7.10	7.17	6.85
JCAL04	2	10.90	10.84	11.44	11.42	10.92	9.96	9.02	8.13	7.57	7.31	7.04	7.05
JCAL05	1	11.14	11.25	12.20	12.03	11.42	10.97	10.26	9.51	8.68	8.29	8.02	8.15
JCAL06	2	9.36	9.17	9.75	9.63	9.31	8.66	8.01	7.26	7.10	6.59	6.72	7.03
JCAL07	1	8.98	9.00	9.18	9.14	9.09	8.82	8.40	8.05	7.26	7.02	6.68	6.47
JCAL08	2	8.92	8.26	8.62	8.93	8.83	8.92	8.93	8.32	7.70	7.38	7.10	7.01
JCAL09	1	8.52	8.03	7.72	7.16	7.68	7.59	7.89	8.20	8.45	8.65	8.21	8.28
JCAL10	2	12.39	12.32	11.78	11.84	11.06	9.87	9.80	9.92	10.11	10.63	10.76	10.19
JCAL11	2	11.01	10.85	10.94	9.89	8.94	8.15	7.46	6.87	6.54	6.41	6.45	6.32
JCAL12	1	10.71	12.19	12.97	12.15	11.27	10.39	9.42	8.65	8.36	8.09	7.88	7.90
JCAL13	2	7.59	7.71	8.49	9.34	9.17	8.67	7.90	7.59	7.17	6.75	6.57	6.53
JCAL14	1	10.10	10.59	11.29	11.70	11.91	11.16	10.30	9.88	9.09	8.56	8.17	8.20
JCAL15	2	9.21	9.89	10.49	10.81	10.25	9.64	8.84	8.11	7.80	7.65	7.73	7.60
JCAL16	1	9.53	9.97	10.41	10.91	10.86	10.56	9.81	9.06	8.54	8.00	7.73	7.69
JCAL17	2	11.45	11.90	12.00	11.88	11.13	10.16	9.05	8.40	7.87	7.77	7.73	7.99
JCAL18	1	8.44	9.16	9.51	9.39	8.49	8.37	7.18	6.70	6.52	6.33	6.10	6.06
JCAL19	1	11.39	11.72	12.26	12.49	12.55	11.80	11.16	10.36	9.63	9.41	9.08	9.35
JCAL20	2	8.33	8.39	8.98	9.05	9.15	8.72	8.37	7.69	7.09	6.85	6.86	6.63
JCAL21	2	9.51	9.37	9.41	9.34	9.20	8.91	8.67	8.22	8.16	7.99	7.83	7.70
JCAL22	2	9.27	9.98	10.37	10.52	10.46	9.99	9.73	9.16	8.34	8.02	7.37	7.19
JCAL23	1	10.61	11.04	11.89	11.74	11.44	10.64	9.62	8.74	8.18	7.56	7.47	7.76
JCAL24	1	7.48	7.20	7.20	7.34	7.50	7.47	7.09	7.09	6.91	6.77	6.65	6.44
JCAL25	1	9.54	10.07	10.30	10.40	10.12	9.96	9.47	9.12	8.51	8.00	7.78	7.83
JCAL26	1	9.86	10.67	11.11	10.88	10.35	9.40	8.53	7.93	7.41	7.39	7.30	7.41
JCAL27	2	10.41	10.82	11.49	11.44	10.66	10.15	9.52	8.56	7.74	7.42	7.13	7.17
JCAL28	2	10.91	11.18	11.27	11.41	11.04	10.56	9.65	8.92	8.23	7.91	7.82	7.62
JCAL29	1	10.65	10.97	11.07	11.09	10.60	10.20	9.42	8.75	8.11	7.99	7.57	7.66

Appendix 3 Table 17: Baseline Endocortical Radius by sector cont'd

ID	Group	120° - 130° (mm)	130° - 140° (mm)	140° - 150° (mm)	150° - 160° (mm)	160° - 170° (mm)	170° - 180° (mm)	180° - 190° (mm)	190° - 200° (mm)	200° - 210° (mm)	210° - 220° (mm)	220° - 230° (mm)	230° - 240° (mm)
JCAL01	1	7.58	7.93	8.21	8.75	9.28	9.87	10.00	9.13	8.55	8.01	7.16	6.48
JCAL02	1	6.31	6.58	6.71	7.46	8.08	8.86	9.66	9.99	9.47	7.99	6.78	6.32
JCAL03	2	7.15	7.29	8.41	8.81	9.44	9.74	10.54	10.65	10.51	9.41	8.18	7.53
JCAL04	2	7.51	7.95	8.71	9.83	11.13	11.72	11.63	12.00	11.44	10.08	8.71	7.78
JCAL05	1	8.32	8.89	9.54	10.48	11.62	11.16	10.76	12.50	11.77	10.53	9.24	8.80
JCAL06	2	7.18	7.58	7.97	8.40	8.29	9.00	9.12	9.65	9.72	8.86	8.09	7.40
JCAL07	1	6.42	6.46	6.68	7.35	8.10	8.31	9.12	9.29	9.31	8.95	8.02	6.90
JCAL08	2	7.08	7.32	7.62	7.90	8.74	8.72	9.14	8.82	9.49	9.22	7.95	7.45
JCAL09	1	8.21	8.41	8.88	9.52	9.75	9.50	9.47	8.82	8.38	7.61	6.97	6.52
JCAL10	2	9.93	9.97	10.84	11.62	12.36	12.67	12.21	11.79	11.76	11.16	10.30	9.52
JCAL11	2	6.59	7.14	8.02	8.89	9.91	10.50	10.69	10.59	9.74	8.41	7.51	6.64
JCAL12	1	8.20	8.61	9.33	10.17	11.10	11.72	11.66	12.17	11.67	10.12	9.17	8.86
JCAL13	2	6.57	6.85	6.89	7.20	7.05	7.15	7.66	7.89	7.95	8.74	7.76	6.91
JCAL14	1	8.50	8.88	9.54	9.65	10.18	10.67	10.85	11.03	11.21	10.75	9.94	9.69
JCAL15	2	7.71	8.07	8.24	8.69	9.01	9.22	9.84	10.42	10.19	9.18	8.90	8.87
JCAL16	1	7.88	8.17	8.79	9.21	9.89	10.74	10.92	11.26	11.29	9.72	8.79	8.04
JCAL17	2	8.22	9.10	9.79	10.23	10.95	11.11	11.77	12.64	12.06	11.06	9.43	8.49
JCAL18	1	6.34	6.58	6.93	7.38	7.81	8.39	8.55	8.74	8.54	8.17	7.53	6.97
JCAL19	1	9.99	10.20	11.03	11.34	11.83	11.61	11.45	11.99	12.67	11.95	10.89	9.94
JCAL20	2	6.80	7.25	7.30	7.60	8.16	8.34	8.15	8.93	8.79	8.11	7.47	7.11
JCAL21	2	7.81	8.64	9.43	10.61	11.06	10.18	10.02	9.70	9.25	8.43	7.93	7.51
JCAL22	2	7.07	7.13	7.32	8.03	8.88	9.25	9.80	10.20	10.09	9.88	9.12	8.60
JCAL23	1	8.07	8.38	9.04	10.04	10.68	11.32	12.02	12.43	11.44	9.83	8.78	7.75
JCAL24	1	6.52	7.07	7.68	7.69	8.11	8.30	7.95	7.25	7.03	6.45	6.33	6.11
JCAL25	1	7.75	7.90	8.61	9.44	9.79	10.68	11.53	11.65	11.16	10.17	8.83	8.04
JCAL26	1	7.62	7.83	8.42	8.99	9.92	10.58	10.91	11.33	10.88	9.18	8.31	8.07
JCAL27	2	7.47	7.77	8.15	8.97	9.57	10.39	10.89	11.27	11.20	10.26	9.18	8.27
JCAL28	2	8.03	8.64	9.36	10.16	10.54	10.99	10.96	11.08	10.92	10.19	9.50	8.86
JCAL29	1	7.99	8.19	9.04	9.71	10.77	11.14	11.94	11.97	11.21	9.80	8.61	7.88

Appendix 3 Table 17: Baseline Endocortical Radius by sector cont'd

ID	Group	240° - 250° (mm)	250° - 260° (mm)	260° - 270° (mm)	270° - 280° (mm)	280° - 290° (mm)	290° - 300° (mm)	300° - 310° (mm)	310° - 320° (mm)	320° - 330° (mm)	330° - 340° (mm)	340° - 350° (mm)	350° - 360° (mm)
JCAL01	1	6.10	5.92	6.33	6.69	7.65	8.16	8.73	8.90	8.98	9.47	9.28	9.30
JCAL02	1	6.10	6.07	6.23	6.30	6.55	6.78	6.99	7.27	7.66	7.80	7.96	8.17
JCAL03	2	7.53	7.33	7.37	7.50	7.08	7.06	7.40	7.76	8.17	8.69	9.28	9.56
JCAL04	2	8.01	7.61	7.67	7.77	7.93	8.30	8.47	9.30	9.50	10.01	9.83	10.69
JCAL05	1	8.54	8.37	8.78	9.36	9.57	9.58	9.56	9.61	9.51	9.32	9.86	10.76
JCAL06	2	6.97	7.03	6.98	7.10	6.90	6.88	7.00	7.41	8.00	8.44	8.56	9.21
JCAL07	1	6.41	6.30	6.39	6.69	7.16	7.50	7.81	8.37	7.98	8.14	8.46	8.58
JCAL08	2	7.21	6.84	6.89	7.20	7.27	7.56	7.86	8.42	8.95	9.04	9.28	8.72
JCAL09	1	6.14	6.34	6.42	6.69	7.17	7.62	8.72	9.60	9.97	9.81	9.29	8.72
JCAL10	2	8.86	8.50	8.41	8.58	9.00	9.43	10.44	11.25	11.66	11.92	11.93	11.70
JCAL11	2	6.45	6.46	6.67	7.13	7.26	7.17	7.28	7.64	8.12	8.63	9.00	9.94
JCAL12	1	8.90	9.07	9.11	9.42	9.18	9.25	9.21	8.91	9.20	9.36	10.26	10.19
JCAL13	2	6.75	6.88	6.92	7.09	7.30	7.53	7.78	7.44	7.89	7.69	6.78	7.33
JCAL14	1	9.37	9.36	9.17	9.06	9.17	9.37	8.97	9.31	9.05	9.58	9.71	9.69
JCAL15	2	8.82	8.72	8.81	8.42	8.21	8.25	8.19	8.50	8.57	8.18	8.57	8.45
JCAL16	1	7.67	7.76	8.09	8.74	9.08	9.07	9.55	9.76	9.69	9.86	9.56	9.38
JCAL17	2	8.21	8.31	8.63	8.32	8.33	8.69	8.95	9.48	10.16	10.46	10.92	11.28
JCAL18	1	6.69	6.61	6.50	6.31	6.30	6.49	6.52	6.61	6.89	7.43	7.85	8.01
JCAL19	1	9.40	9.18	9.87	10.17	10.20	10.48	10.90	10.86	10.82	10.85	10.93	11.21
JCAL20	2	7.00	7.08	6.99	7.46	7.80	7.81	7.65	7.49	7.24	7.71	8.13	7.99
JCAL21	2	7.27	7.08	7.10	7.19	7.49	7.81	8.23	8.88	9.57	10.05	10.04	10.14
JCAL22	2	8.37	8.06	8.20	8.05	7.89	7.99	7.96	7.98	8.32	8.41	8.75	9.08
JCAL23	1	7.51	7.80	7.98	8.44	9.07	9.37	9.47	9.56	9.90	10.23	10.57	10.36
JCAL24	1	6.10	6.17	5.95	6.16	6.40	6.96	7.15	7.31	7.50	7.63	7.85	7.58
JCAL25	1	7.57	7.42	7.71	7.68	7.75	8.27	8.59	9.10	9.13	8.96	9.36	9.47
JCAL26	1	7.73	7.56	7.81	8.23	8.22	8.12	8.50	8.83	9.39	9.57	9.60	9.67
JCAL27	2	8.14	8.10	8.31	8.41	8.29	8.33	8.55	8.55	8.94	8.97	9.69	9.80
JCAL28	2	8.45	8.61	8.53	8.61	8.52	8.69	9.04	9.33	9.49	9.82	10.27	10.48
JCAL29	1	7.48	7.33	7.41	8.11	8.83	9.23	9.36	9.13	8.96	9.39	10.26	10.89

Appendix 3 Table 18: Six months Endocortical Radius by sector

ID	Group	0° - 10° (mm)	10° - 20° (mm)	20° - 30° (mm)	30° - 40° (mm)	40° - 50° (mm)	50° - 60° (mm)	60° - 70° (mm)	70° - 80° (mm)	80° - 90° (mm)	90° - 100° (mm)	100° - 110° (mm)	110° - 120° (mm)
JCAL01	1	8.85	8.42	8.28	8.12	8.46	9.32	8.56	7.79	7.32	6.79	7.07	7.18
JCAL02	1	9.05	9.55	9.86	9.69	9.00	7.86	7.06	6.65	6.14	6.01	5.85	5.91
JCAL03	2	7.43	7.26	7.31	7.39	7.51	7.22	6.98	6.97	6.92	6.90	6.90	6.64
JCAL04	2												
JCAL05	1												
JCAL06	2												
JCAL07	1	8.84	8.54	8.89	9.09	9.04	8.64	8.29	7.70	7.22	6.81	6.67	6.52
JCAL08	2	8.69	8.37	8.52	8.87	8.75	8.75	8.78	8.36	7.79	7.42	7.07	7.36
JCAL09	1												
JCAL10	2	12.43	12.24	11.67	11.71	10.74	9.96	9.89	9.97	10.35	10.60	10.73	10.26
JCAL11	2	10.17	9.93	10.48	9.70	8.89	8.07	7.36	6.68	6.41	6.13	6.23	6.14
JCAL12	1												
JCAL13	2												
JCAL14	1	9.91	10.88	12.70	12.12	12.10	11.34	10.29	10.01	9.16	8.53	8.02	8.04
JCAL15	2	9.17	9.80	10.51	10.72	10.20	9.50	8.94	8.01	7.73	7.67	7.59	7.56
JCAL16	1												
JCAL17	2	11.28	11.54	11.63	11.57	10.87	10.04	8.89	8.45	7.90	7.55	7.46	7.56
JCAL18	1	8.24	8.97	9.51	9.46	8.74	8.18	7.21	6.85	6.39	6.11	5.92	5.86
JCAL19	1												
JCAL20	2	8.32	8.65	8.99	9.11	9.37	9.19	8.32	7.62	7.04	6.92	6.93	6.82
JCAL21	2	9.61	9.26	9.21	9.11	8.99	8.66	8.48	8.24	7.87	7.70	7.65	7.54
JCAL22	2												
JCAL23	1												
JCAL24	1	9.98	10.31	10.90	10.17	9.64	8.93	8.12	7.48	7.26	7.10	6.94	7.05
JCAL25	1												
JCAL26	1	10.43	10.77	11.21	11.20	10.34	9.50	8.69	8.02	7.62	7.53	7.47	7.87
JCAL27	2	10.29	10.84	10.92	11.33	10.47	10.00	9.27	8.50	8.02	7.52	7.11	7.13
JCAL28	2												
JCAL29	1	10.53	11.21	11.19	11.14	10.68	10.04	9.29	8.86	8.11	7.82	7.61	7.63

Appendix 3 Table 18: Six months Endocortical Radius by sector cont'd

ID	Group	120° - 130° (mm)	130° - 140° (mm)	140° - 150° (mm)	150° - 160° (mm)	160° - 170° (mm)	170° - 180° (mm)	180° - 190° (mm)	190° - 200° (mm)	200° - 210° (mm)	210° - 220° (mm)	220° - 230° (mm)	230° - 240° (mm)
JCAL01	1	7.31	7.70	8.08	8.82	9.23	9.58	9.68	9.05	8.51	8.15	7.44	6.91
JCAL02	1	6.22	6.61	6.79	7.64	8.14	9.01	9.85	9.90	9.62	8.13	7.11	6.70
JCAL03	2	6.70	7.16	7.42	8.10	8.42	8.46	8.28	7.82	7.22	6.71	6.22	6.04
JCAL04	2												
JCAL05	1												
JCAL06	2												
JCAL07	1	6.50	6.56	6.84	7.11	7.65	8.26	8.86	9.28	10.04	8.77	7.81	6.71
JCAL08	2	7.46	7.37	7.72	8.31	8.67	8.96	9.00	8.65	9.31	9.22	7.70	7.21
JCAL09	1												
JCAL10	2	9.71	9.90	10.69	11.60	12.37	12.49	12.35	11.95	11.44	11.23	10.28	9.57
JCAL11	2	6.36	6.79	7.82	8.49	9.39	10.03	10.34	10.16	9.10	7.99	6.94	6.43
JCAL12	1												
JCAL13	2												
JCAL14	1	8.60	8.80	9.22	10.04	10.49	11.24	11.44	11.28	11.01	10.73	9.83	9.57
JCAL15	2	7.80	8.05	8.16	8.53	8.96	9.03	9.40	10.76	10.51	9.25	8.95	8.81
JCAL16	1												
JCAL17	2	7.73	8.32	9.28	9.93	11.01	11.23	12.08	11.80	12.28	10.32	9.14	8.23
JCAL18	1	6.05	6.40	6.93	7.55	8.03	8.50	8.84	8.97	8.91	8.15	7.47	6.99
JCAL19	1												
JCAL20	2	7.10	7.28	7.28	7.90	8.27	8.25	8.57	8.88	9.07	8.11	7.82	7.42
JCAL21	2	8.08	8.44	9.48	10.42	11.05	10.76	10.57	9.75	9.50	8.51	7.85	7.51
JCAL22	2												
JCAL23	1												
JCAL24	1	6.94	7.23	8.00	8.91	9.09	9.85	10.26	10.54	10.19	9.21	7.94	7.23
JCAL25	1												
JCAL26	1	8.01	8.08	8.79	9.84	10.50	10.89	11.04	11.47	11.06	9.30	8.28	7.81
JCAL27	2	7.40	7.98	8.21	9.09	9.77	10.41	11.53	11.51	11.20	10.19	8.77	8.16
JCAL28	2												
JCAL29	1	7.88	8.15	9.11	9.53	10.78	11.51	12.31	12.08	10.90	9.37	8.53	7.69

Appendix 3 Table 18: Six months Endocortical Radius by sector cont'd

ID	Group	260° - 270° (mm)	270° - 280° (mm)	280° - 290° (mm)	290° - 300° (mm)	300° - 310° (mm)	310° - 320° (mm)	320° - 330° (mm)	330° - 340° (mm)	340° - 350° (mm)	350° - 360° (mm)
JCAL01	1	5.92	6.55	7.69	8.42	8.80	9.10	9.29	9.42	9.47	9.48
JCAL02	1	6.27	6.36	6.66	6.70	6.80	7.17	7.54	7.71	8.14	8.54
JCAL03	2	6.14	6.29	6.65	7.04	7.08	6.99	7.53	7.54	7.71	7.66
JCAL04	2										
JCAL05	1										
JCAL06	2										
JCAL07	1	6.49	6.83	6.99	7.63	7.76	7.75	7.64	7.74	7.92	8.49
JCAL08	2	6.78	6.93	7.34	7.59	7.86	8.42	8.76	8.86	8.53	8.49
JCAL09	1										
JCAL10	2	8.49	8.57	8.99	9.62	10.54	11.36	11.92	11.90	11.40	11.77
JCAL11	2	6.65	6.90	7.02	6.97	7.17	7.61	8.12	8.35	8.88	9.65
JCAL12	1										
JCAL13	2										
JCAL14	1	9.11	9.03	9.23	9.31	9.54	9.44	9.21	9.63	9.72	9.65
JCAL15	2	8.66	8.47	8.31	8.13	8.21	8.52	8.46	7.94	8.35	8.60
JCAL16	1										
JCAL17	2	8.04	8.16	8.15	8.43	8.63	9.46	9.38	9.31	9.91	10.49
JCAL18	1	6.76	6.48	6.62	6.56	6.78	6.59	6.80	7.46	7.71	8.18
JCAL19	1										
JCAL20	2	6.92	7.75	7.98	7.89	7.73	7.76	7.72	7.96	8.16	8.14
JCAL21	2	7.41	7.39	7.45	7.74	8.32	8.72	9.57	9.99	9.95	9.88
JCAL22	2										
JCAL23	1										
JCAL24	1	7.25	7.28	7.11	7.15	7.22	7.56	8.17	8.75	9.23	9.77
JCAL25	1										
JCAL26	1	7.91	8.36	8.19	8.10	8.27	8.73	9.28	9.57	10.01	9.75
JCAL27	2	8.34	8.37	8.16	8.25	8.30	8.35	8.77	8.93	9.58	9.80
JCAL28	2										
JCAL29	1	7.30	7.96	8.86	9.32	9.34	9.04	9.05	9.66	10.59	10.87

Appendix 3 Table 19: Baseline Pericortical Radius by sector

ID	Group	0° - 10° (mm)	10° - 20° (mm)	20° - 30° (mm)	30° - 40° (mm)	40° - 50° (mm)	50° - 60° (mm)	60° - 70° (mm)	70° - 80° (mm)	80° - 90° (mm)	90° - 100° (mm)	100° - 110° (mm)	110° - 120° (mm)
JCAL01	1	14.20	14.71	16.05	16.28	15.11	14.27	13.31	11.71	10.75	10.10	9.70	9.77
JCAL02	1	14.35	14.84	14.97	14.49	13.96	12.63	11.43	10.51	9.82	9.36	9.23	9.19
JCAL03	2	15.08	15.15	14.04	13.55	13.27	12.82	12.09	11.28	10.54	10.15	9.92	9.84
JCAL04	2	15.33	15.93	15.85	15.37	14.17	12.99	12.04	11.22	10.78	10.28	9.92	9.82
JCAL05	1	15.98	16.31	16.03	15.22	14.36	13.66	12.91	12.21	11.68	11.46	11.11	11.25
JCAL06	2	14.46	15.12	14.58	13.78	13.11	12.09	11.17	10.30	9.67	9.32	9.26	9.51
JCAL07	1	14.63	14.29	13.19	12.63	12.75	12.82	12.47	12.07	11.51	10.87	10.35	10.02
JCAL08	2	13.08	12.53	11.99	12.01	11.87	12.09	11.96	11.64	11.10	10.49	10.01	9.80
JCAL09	1	13.47	13.44	13.28	13.18	12.83	12.57	12.49	12.46	12.10	11.86	11.76	11.31
JCAL10	2	16.27	15.54	15.02	14.63	14.59	14.32	13.22	13.02	13.49	13.89	14.02	12.91
JCAL11	2	13.67	13.75	13.62	13.50	13.13	11.77	10.63	9.47	8.79	8.25	8.23	8.11
JCAL12	1	16.56	17.19	17.08	16.16	15.23	13.74	12.63	11.84	11.16	10.67	10.74	10.76
JCAL13	2	13.58	14.30	14.77	14.74	14.13	13.10	12.11	11.55	10.91	10.65	10.45	10.50
JCAL14	1	16.14	16.99	16.57	15.87	15.18	14.12	13.07	12.24	11.60	10.97	10.69	10.86
JCAL15	2	14.16	14.27	14.27	13.86	13.60	12.92	11.94	11.27	10.77	10.46	10.46	10.66
JCAL16	1	13.92	14.02	14.60	14.83	14.37	14.13	13.31	12.49	11.75	10.95	10.59	10.51
JCAL17	2	16.23	15.97	15.81	15.54	14.90	13.74	12.61	11.63	10.79	10.29	10.17	10.15
JCAL18	1	14.15	14.41	14.17	13.90	13.54	12.62	11.64	10.81	10.27	9.73	9.52	9.49
JCAL19	1	15.86	16.50	16.91	16.38	15.64	14.86	13.78	13.02	12.15	11.68	11.43	11.50
JCAL20	2	14.75	15.17	14.80	14.44	14.13	13.36	12.43	11.32	10.68	10.18	10.12	10.17
JCAL21	2	14.65	15.04	14.72	14.15	13.47	12.74	12.33	12.11	11.79	11.44	10.89	10.71
JCAL22	2	14.42	14.87	14.76	14.30	14.04	13.59	12.79	12.14	11.46	11.05	10.61	10.74
JCAL23	1	15.45	15.84	15.78	15.79	15.39	14.31	12.90	11.65	10.89	10.20	9.98	9.81
JCAL24	1	13.69	13.62	13.25	12.78	12.11	11.61	11.02	10.42	10.21	10.28	9.66	9.28
JCAL25	1	15.03	15.19	13.69	13.38	13.12	13.42	13.09	12.44	11.63	10.81	10.37	10.30
JCAL26	1	14.64	15.06	15.13	14.78	13.97	13.07	12.03	10.83	10.27	9.86	9.40	9.59
JCAL27	2	16.55	17.26	16.69	15.13	14.26	13.47	12.65	12.15	11.62	11.01	10.80	10.67
JCAL28	2	16.94	16.82	15.86	15.72	15.16	14.17	13.25	12.29	11.60	10.85	10.64	10.62
JCAL29	1	16.08	15.75	15.46	15.03	14.15	13.50	12.70	11.91	11.16	10.60	10.25	10.16

Appendix 3 Table 19: Baseline Pericortical Radius by sector cont'd

ID	Group	120° - 130° (mm)	130° - 140° (mm)	140° - 150° (mm)	150° - 160° (mm)	160° - 170° (mm)	170° - 180° (mm)	180° - 190° (mm)	190° - 200° (mm)	200° - 210° (mm)	210° - 220° (mm)	220° - 230° (mm)	230° - 240° (mm)
JCAL01	1	10.23	10.93	12.10	14.07	17.26	20.12	19.77	17.52	14.51	11.80	10.21	9.24
JCAL02	1	9.53	10.27	11.40	13.32	15.16	17.00	17.38	16.06	14.23	12.42	10.70	9.98
JCAL03	2	9.96	10.51	11.49	12.82	14.69	16.96	17.57	16.08	14.15	12.19	10.60	9.79
JCAL04	2	10.08	10.62	11.51	12.84	14.56	16.83	17.89	16.95	14.95	13.06	11.38	10.75
JCAL05	1	11.46	12.05	13.02	14.21	15.61	17.04	17.96	16.81	14.93	13.30	12.18	11.60
JCAL06	2	9.70	10.50	11.65	13.17	14.62	15.52	15.33	14.56	13.49	12.43	11.02	10.21
JCAL07	1	10.00	10.58	11.35	12.54	14.35	16.60	17.92	17.46	15.42	13.49	11.40	10.06
JCAL08	2	9.97	10.10	10.66	11.52	12.82	14.59	16.24	16.13	15.01	13.13	11.51	10.04
JCAL09	1	10.82	10.98	11.60	12.71	14.06	14.95	15.84	15.91	14.45	12.68	11.32	10.33
JCAL10	2	12.43	12.64	13.47	14.43	15.53	16.32	17.30	18.26	17.74	15.67	13.92	12.65
JCAL11	2	8.39	9.02	9.95	11.55	13.23	14.60	14.47	13.33	11.84	10.25	9.27	8.71
JCAL12	1	11.27	12.37	13.76	15.43	17.54	18.62	18.45	17.53	15.50	13.09	12.03	11.53
JCAL13	2	10.62	11.15	12.08	13.67	15.54	15.98	15.51	14.53	13.52	12.38	11.32	10.42
JCAL14	1	11.01	11.78	13.17	15.28	17.57	18.93	17.74	16.78	15.14	13.39	12.56	11.81
JCAL15	2	11.20	11.93	13.05	14.45	15.85	16.00	15.42	14.62	13.60	12.63	11.86	11.42
JCAL16	1	10.67	11.07	11.95	13.28	14.90	16.82	18.18	17.64	16.06	13.55	11.43	10.39
JCAL17	2	10.53	11.43	12.52	13.93	15.66	17.21	17.77	17.12	15.62	13.76	12.07	11.20
JCAL18	1	9.84	10.58	11.64	13.57	15.33	16.05	16.13	15.69	14.37	12.30	10.95	10.15
JCAL19	1	11.74	12.61	13.79	15.19	16.68	17.11	16.92	16.22	15.21	14.13	12.73	12.11
JCAL20	2	10.49	11.17	12.18	13.51	15.06	16.52	16.35	15.27	14.01	12.77	11.21	10.53
JCAL21	2	10.86	11.36	12.72	14.43	15.78	16.51	17.04	16.75	14.92	13.25	11.95	11.13
JCAL22	2	11.06	11.63	12.70	14.03	15.73	17.09	17.01	15.55	14.31	13.24	12.41	11.69
JCAL23	1	10.24	10.61	11.68	13.68	15.69	17.70	18.22	16.48	14.65	12.56	10.83	9.74
JCAL24	1	9.71	10.49	11.23	12.74	13.76	14.41	14.68	14.17	12.59	10.92	9.99	9.14
JCAL25	1	10.28	10.80	11.37	12.81	14.56	16.68	18.28	17.39	15.50	13.62	11.84	10.76
JCAL26	1	10.02	10.78	11.90	13.42	15.44	17.69	18.18	16.89	14.96	12.99	11.54	10.69
JCAL27	2	11.06	11.94	13.41	15.36	17.65	18.95	18.01	16.70	15.29	13.70	12.17	11.30
JCAL28	2	10.78	11.57	12.58	14.60	16.70	16.99	16.57	16.03	15.25	13.97	12.54	11.67
JCAL29	1	10.42	10.77	11.72	12.99	14.78	16.36	16.24	15.41	14.47	12.98	11.44	10.04

Appendix 3 Table 19: Baseline Pericortical Radius by sector cont'd

ID	Group	240° - 250° (mm)	250° - 260° (mm)	260° - 270° (mm)	270° - 280° (mm)	280° - 290° (mm)	290° - 300° (mm)	300° - 310° (mm)	310° - 320° (mm)	320° - 330° (mm)	330° - 340° (mm)	340° - 350° (mm)	350° - 360° (mm)
JCAL01	1	8.68	8.88	9.25	10.76	11.96	12.01	12.30	12.52	12.68	12.40	13.25	13.63
JCAL02	1	9.64	9.59	10.35	10.72	10.60	11.01	11.25	11.47	12.61	14.01	13.83	13.34
JCAL03	2	9.65	10.09	10.35	10.15	10.11	10.37	10.75	11.20	11.62	13.29	14.33	14.55
JCAL04	2	10.52	10.59	10.68	10.97	11.13	11.97	12.72	13.52	13.94	15.12	15.48	15.11
JCAL05	1	11.26	11.43	12.07	12.92	12.92	12.99	12.93	13.18	12.98	13.86	14.31	14.91
JCAL06	2	9.83	9.56	9.94	10.00	10.25	10.65	10.19	11.65	12.54	12.50	13.10	13.62
JCAL07	1	9.47	9.30	9.44	10.00	11.98	12.44	12.42	12.33	12.48	12.93	13.68	14.38
JCAL08	2	9.22	9.30	9.99	10.63	10.71	10.90	11.59	12.17	12.89	12.87	13.65	13.48
JCAL09	1	9.80	9.54	9.58	9.63	10.36	10.94	11.83	12.68	13.25	13.12	13.05	13.39
JCAL10	2	11.85	11.35	11.24	11.34	11.68	12.21	13.05	14.03	15.32	16.29	16.78	16.96
JCAL11	2	8.51	8.97	9.59	10.33	10.45	10.41	10.87	11.04	11.59	12.00	12.52	13.11
JCAL12	1	11.41	12.14	12.42	12.56	12.75	12.72	12.87	12.92	13.56	14.07	14.45	14.90
JCAL13	2	10.07	9.94	10.85	10.83	11.03	11.44	12.02	12.62	12.95	13.22	13.04	13.21
JCAL14	1	12.19	12.07	11.69	11.72	11.76	12.37	12.72	12.40	12.81	14.20	14.73	15.17
JCAL15	2	11.63	12.08	12.37	11.92	11.38	11.54	12.13	12.01	12.17	12.35	13.04	13.53
JCAL16	1	10.18	10.50	11.49	12.39	12.29	12.50	13.17	13.87	14.85	15.17	14.51	14.04
JCAL17	2	10.73	10.85	11.13	10.96	11.08	11.56	12.02	12.97	13.73	14.96	15.99	15.96
JCAL18	1	10.03	10.12	10.80	10.79	10.41	10.50	11.05	11.58	12.73	13.76	13.86	13.93
JCAL19	1	11.55	11.93	12.74	13.66	13.84	13.99	14.16	14.13	14.19	14.36	14.68	15.26
JCAL20	2	10.00	10.14	10.61	11.49	12.26	12.01	12.21	12.38	11.68	12.98	13.72	14.06
JCAL21	2	10.51	10.15	10.02	10.22	10.51	11.12	11.78	12.60	13.44	13.95	14.48	14.34
JCAL22	2	11.39	11.44	11.81	11.83	11.78	11.97	12.49	12.58	13.54	13.73	13.79	13.88
JCAL23	1	9.62	9.96	10.70	11.69	12.06	12.60	13.16	13.03	13.91	14.34	14.63	14.66
JCAL24	1	8.96	8.89	9.15	9.40	9.97	10.57	10.91	10.82	10.84	11.00	12.00	13.04
JCAL25	1	10.15	9.89	9.94	10.66	11.59	12.27	12.49	12.88	13.43	13.98	14.15	14.66
JCAL26	1	10.20	10.44	11.13	11.69	11.53	11.73	12.31	12.86	13.32	13.59	13.73	14.15
JCAL27	2	10.84	10.92	11.33	11.45	11.49	11.28	10.78	12.71	13.19	13.45	14.18	15.07
JCAL28	2	11.34	11.30	11.76	11.84	11.70	11.95	12.32	12.77	13.75	14.98	15.58	16.91
JCAL29	1	9.53	9.45	10.00	11.12	12.16	12.54	12.99	12.76	11.88	13.28	14.67	16.30

Appendix 3 Table 20: Six months Pericortical radius

ID	Group	0° - 10° (mm)	10° - 20° (mm)	20° - 30° (mm)	30° - 40° (mm)	40° - 50° (mm)	50° - 60° (mm)	60° - 70° (mm)	70° - 80° (mm)	80° - 90° (mm)	90° - 100° (mm)	100° - 110° (mm)	110° - 120° (mm)
JCAL01	1	14.13	14.88	16.01	16.19	15.00	14.10	12.98	11.46	10.45	9.80	9.45	9.68
JCAL02	1	14.58	14.78	14.85	14.53	13.69	12.71	11.53	10.42	9.83	9.47	9.26	9.33
JCAL03	2	13.88	13.78	13.33	12.75	12.15	11.33	10.71	10.31	10.22	10.15	9.86	9.47
JCAL04	2												
JCAL05	1												
JCAL06	2												
JCAL07	1	14.27	14.04	13.23	12.64	12.74	12.78	12.34	11.94	11.40	10.68	10.29	10.06
JCAL08	2	12.87	12.29	11.86	11.92	11.78	12.01	12.11	11.60	11.21	10.66	10.21	10.07
JCAL09	1												
JCAL10	2	16.33	15.57	14.91	14.76	14.54	14.11	13.33	13.04	13.29	13.75	13.84	13.05
JCAL11	2	13.40	13.42	13.34	13.18	12.65	11.65	10.27	9.42	8.50	8.27	7.99	7.96
JCAL12	1												
JCAL13	2												
JCAL14	1	16.40	16.92	16.44	15.97	15.04	14.38	13.40	12.35	11.66	11.12	10.85	10.91
JCAL15	2	14.13	14.40	14.40	13.88	13.64	12.87	12.07	11.31	10.70	10.45	10.38	10.64
JCAL16	1												
JCAL17	2	15.47	15.47	15.48	15.17	14.66	13.36	12.15	11.09	10.24	9.75	9.56	9.68
JCAL18	1	14.16	14.44	14.13	13.74	13.46	12.74	11.78	10.93	10.11	9.51	9.49	9.41
JCAL19	1												
JCAL20	2	15.04	15.40	14.84	14.50	14.12	13.32	12.39	11.40	10.68	10.12	10.01	10.15
JCAL21	2	14.62	14.82	14.88	14.16	13.44	12.81	12.27	12.00	11.62	11.56	10.90	10.49
JCAL22	2												
JCAL23	1												
JCAL24	1	15.23	15.29	14.17	13.63	13.32	12.71	12.00	11.14	10.52	10.03	9.73	9.73
JCAL25	1												
JCAL26	1	14.83	15.31	15.38	15.11	14.17	13.20	12.06	11.01	10.23	9.82	9.66	9.73
JCAL27	2	16.27	17.12	16.53	15.28	14.29	13.42	12.50	12.11	11.56	11.02	10.81	10.85
JCAL28	2												
JCAL29	1	15.81	15.57	15.47	14.99	14.14	13.46	12.68	11.85	11.07	10.64	10.26	10.07

Appendix 3 Table 20: Six months Pericortical radius cont'd

ID	Group	120° - 130° (mm)	130° - 140° (mm)	140° - 150° (mm)	150° - 160° (mm)	160° - 170° (mm)	170° - 180° (mm)	180° - 190° (mm)	190° - 200° (mm)	200° - 210° (mm)	210° - 220° (mm)	220° - 230° (mm)	230° - 240° (mm)
JCAL01	1	10.09	10.75	11.83	13.90	17.25	19.92	19.48	17.29	14.69	12.12	10.72	9.49
JCAL02	1	9.60	10.37	11.52	13.32	15.29	17.05	17.58	16.21	14.40	12.35	10.78	9.80
JCAL03	2	9.85	10.59	11.48	12.77	13.73	14.42	14.78	14.44	12.71	11.23	10.16	9.42
JCAL04	2												
JCAL05	1												
JCAL06	2												
JCAL07	1	10.08	10.47	11.22	12.52	14.46	16.86	18.07	17.32	15.05	13.15	11.23	9.89
JCAL08	2	9.98	10.22	10.78	11.61	13.09	14.86	16.51	16.01	14.86	12.97	11.29	9.95
JCAL09	1												
JCAL10	2	12.56	12.65	13.65	14.64	15.47	16.23	17.18	18.15	17.89	15.84	13.97	12.68
JCAL11	2	8.49	9.03	9.94	11.45	13.19	14.77	14.79	13.35	11.78	10.33	9.47	8.58
JCAL12	1												
JCAL13	2												
JCAL14	1	11.14	12.04	13.22	15.33	17.95	19.20	18.07	16.34	14.74	13.28	12.33	11.79
JCAL15	2	11.13	11.81	12.94	14.17	15.54	15.79	15.25	14.72	13.82	12.76	12.01	11.50
JCAL16	1												
JCAL17	2	9.91	10.47	11.54	13.58	15.76	18.07	18.13	16.81	15.39	13.10	11.95	11.31
JCAL18	1	9.70	10.25	11.28	13.01	15.09	16.10	16.37	15.91	14.59	12.74	11.23	10.21
JCAL19	1												
JCAL20	2	10.48	11.22	12.23	13.64	15.27	16.70	16.33	15.41	14.13	12.37	11.19	10.53
JCAL21	2	10.63	11.40	12.76	14.43	15.49	16.39	17.22	17.05	15.30	13.65	12.13	11.21
JCAL22	2												
JCAL23	1												
JCAL24	1	9.99	10.42	11.26	12.65	14.66	16.90	17.24	15.87	14.13	12.33	10.64	9.81
JCAL25	1												
JCAL26	1	10.18	10.86	12.14	13.66	15.83	18.04	18.44	17.17	15.02	12.71	11.56	10.39
JCAL27	2	11.25	12.21	13.55	15.45	17.94	18.99	18.21	16.98	15.25	13.61	12.17	11.04
JCAL28	2												
JCAL29	1	10.39	10.84	11.73	12.96	14.88	16.32	16.38	15.69	14.43	12.90	11.20	10.09

Appendix 3 Table 20: Six months Pericortical radius cont'd

ID	Group	240° - 250° (mm)	250° - 260° (mm)	260° - 270° (mm)	270° - 280° (mm)	280° - 290° (mm)	290° - 300° (mm)	300° - 310° (mm)	310° - 320° (mm)	320° - 330° (mm)	330° - 340° (mm)	340° - 350° (mm)	350° - 360° (mm)
JCAL01	1	8.84	8.88	9.20	10.67	12.17	12.36	12.57	12.70	12.71	12.63	13.39	13.47
JCAL02	1	9.54	9.69	10.46	10.73	10.66	10.93	11.53	11.46	12.73	14.03	13.79	13.76
JCAL03	2	9.16	9.04	9.21	9.55	10.20	10.28	10.69	10.89	10.85	11.31	12.04	13.13
JCAL04	2												
JCAL05	1												
JCAL06	2												
JCAL07	1	9.27	8.86	9.04	9.91	12.00	12.55	12.18	12.31	12.21	12.60	13.34	14.07
JCAL08	2	9.22	9.37	10.02	10.36	10.51	10.89	11.35	12.22	12.74	12.89	13.40	13.16
JCAL09	1												
JCAL10	2	11.96	11.28	11.22	11.35	11.68	12.27	13.19	14.03	15.37	16.28	16.82	16.90
JCAL11	2	8.31	8.66	9.28	10.03	10.22	10.48	10.56	11.09	11.31	11.86	12.12	13.02
JCAL12	1												
JCAL13	2												
JCAL14	1	11.83	11.71	11.63	11.76	12.15	12.40	12.76	12.58	13.45	14.53	14.76	15.30
JCAL15	2	11.52	11.89	12.21	11.98	11.52	11.43	12.16	12.03	12.21	12.37	13.01	13.63
JCAL16	1												
JCAL17	2	10.86	10.88	10.89	10.72	10.94	11.25	11.78	12.58	13.80	14.98	15.16	15.30
JCAL18	1	9.96	10.06	10.89	10.87	10.55	10.76	11.15	11.79	12.92	13.77	13.83	14.03
JCAL19	1												
JCAL20	2	10.22	10.24	10.74	11.84	12.02	12.20	12.29	12.57	12.03	13.35	13.92	14.37
JCAL21	2	10.57	10.25	10.12	10.40	10.53	11.41	11.78	12.49	13.36	14.00	14.34	14.33
JCAL22	2												
JCAL23	1												
JCAL24	1	9.57	9.93	10.45	10.19	10.16	10.33	10.67	11.25	11.53	13.22	14.23	14.40
JCAL25	1												
JCAL26	1	10.12	10.16	11.05	11.67	11.40	11.69	12.26	12.92	13.49	13.68	13.74	14.24
JCAL27	2	10.89	10.81	11.05	11.38	11.35	11.27	10.70	12.41	12.92	13.41	13.78	14.78
JCAL28	2												
JCAL29	1	9.75	9.42	10.01	11.09	12.10	12.55	12.89	12.65	11.76	13.40	14.71	16.53

Appendix 3 Table 21: Baseline Endocortical vBMD by sector

ID	Group	0° - 10° [mg·cm³]	10° - 20° [mg·cm³]	20° - 30° [mg·cm³]	30° - 40° [mg·cm³]	40° - 50° [mg·cm³]	50° - 60° [mg·cm³]	60° - 70° [mg·cm³]	70° - 80° [mg·cm³]	80° - 90° [mg·cm³]	90° - 100° [mg·cm³]	100° - 110° [mg·cm³]	110° - 120° [mg·cm³]
JCAL01	1	1178.33	1124.94	1151.62	1204.57	1143.60	1169.38	1220.90	1206.88	1217.10	1106.36	1223.19	1252.10
JCAL02	1	1130.92	1172.75	1226.75	1215.31	1229.64	1176.01	1190.39	1182.73	1171.53	1120.88	1160.22	1154.02
JCAL03	2	1111.87	1139.39	1123.84	1190.97	1261.08	1273.28	1226.42	1228.66	1185.56	1185.02	1117.70	1096.55
JCAL04	2	1008.27	840.40	1084.24	1263.82	1258.78	1108.84	1083.17	1181.04	1150.46	1180.75	1123.50	1113.90
JCAL05	1	1078.05	940.29	1081.08	1198.58	1186.58	1183.98	1069.17	1152.86	1177.36	1176.73	1138.61	1089.39
JCAL06	2	1010.05	942.63	1063.22	1175.94	1201.98	1193.83	1148.60	1142.98	1144.96	1126.25	1175.11	1135.01
JCAL07	1	1147.43	1093.19	1139.05	1162.88	1166.47	1164.05	1184.08	1252.66	1227.93	1200.71	1179.99	1168.14
JCAL08	2	1099.93	1004.46	1083.80	1203.08	1182.09	1200.21	1141.20	1103.21	1109.96	1147.27	1073.98	1062.78
JCAL09	1	1204.77	1188.17	1179.31	1123.07	1164.82	1172.24	1200.58	1177.18	1200.02	1216.78	1193.11	1167.75
JCAL10	2	1101.72	1177.01	1166.93	1193.95	1170.66	1164.98	1158.65	1190.58	1103.23	1234.40	1260.83	1220.12
JCAL11	2	1143.84	963.34	1060.26	1198.01	1240.97	1186.60	1162.94	1186.19	1101.58	1178.59	1155.06	1119.88
JCAL12	1	1098.69	1115.13	1156.43	1243.61	1143.98	1192.54	1256.41	1257.21	1344.48	1233.84	1170.18	1095.27
JCAL13	2	1022.77	1015.44	1075.19	1095.11	1061.44	1108.43	1090.41	1174.41	1190.32	1182.17	1160.91	1205.57
JCAL14	1	954.55	916.90	980.33	1125.50	1249.32	1077.85	1010.16	955.10	1099.81	1078.47	1018.76	1053.59
JCAL15	2	1120.03	1159.97	1255.31	1225.21	1127.90	1156.44	1202.99	1200.43	1155.53	1147.68	1139.41	1157.13
JCAL16	1	1137.87	1123.23	1153.12	1235.03	1213.84	1205.75	1181.39	1232.56	1184.89	1146.32	1150.02	1122.51
JCAL17	2	1023.84	1037.19	1128.45	1244.40	1171.34	1159.68	1120.82	1141.96	1128.51	1099.88	1112.39	1111.59
JCAL18	1	1051.73	1067.00	1130.52	1199.91	1171.02	1163.47	1152.92	1158.61	1153.20	1177.04	1153.97	1124.22
JCAL19	1	817.75	960.16	915.27	1019.49	1154.82	1187.49	1130.68	1152.24	1120.34	1132.28	1048.37	1093.43
JCAL20	2	1201.40	1143.83	1207.55	1214.15	1184.33	1162.26	1140.85	1145.38	1199.90	1152.50	1139.46	1115.03
JCAL21	2	1061.31	1027.64	1126.15	1137.72	1151.69	1205.94	1209.90	1156.38	1209.58	1182.46	1173.58	1213.23
JCAL22	2	1006.06	1028.66	1138.01	1176.09	1180.60	1130.38	1139.18	1185.37	1170.40	1140.90	1101.75	1056.53
JCAL23	1	975.34	902.61	1068.19	1158.60	1209.98	1185.42	1111.89	1104.69	1126.44	1091.22	1102.70	1070.91
JCAL24	1	1083.29	1101.55	1087.68	1062.75	1133.56	1157.98	1191.50	1158.72	1160.02	1059.74	1034.94	1067.27
JCAL25	1	1099.29	1070.46	1070.48	1082.30	1093.67	1085.00	1129.65	1085.73	1137.32	1028.25	1037.42	1065.57
JCAL26	1	1070.77	1159.44	1236.62	1226.23	1232.09	1154.63	1148.77	1190.47	1131.01	1146.73	1127.98	1119.13
JCAL27	2	1133.14	1164.92	1117.86	1234.81	1210.86	1162.77	1224.62	1238.26	1180.75	1201.24	1199.04	1188.91
JCAL28	2	1107.28	1167.42	1218.28	1176.59	1146.10	1136.25	1203.48	1298.82	1214.89	1182.84	1173.51	1069.67
JCAL29	1	862.59	867.63	916.64	1088.03	1184.26	1184.11	1196.32	1179.52	1191.12	1179.67	1103.96	1129.49

Appendix 3 Table 21: Baseline Endocortical vBMD by sector cont'd

ID	Group	120° - 130° [mg·cm³]	130° - 140° [mg·cm³]	140° - 150° [mg·cm³]	150° - 160° [mg·cm³]	160° - 170° [mg·cm³]	170° - 180° [mg·cm³]	180° - 190° [mg·cm³]	190° - 200° [mg·cm³]	200° - 210° [mg·cm³]	210° - 220° [mg·cm³]	220° - 230° [mg·cm³]	230° - 240° [mg·cm³]
JCAL01	1	1209.00	1162.37	1127.76	1049.94	1065.14	1180.15	1140.43	1118.76	1020.87	1038.36	1047.97	1034.75
JCAL02	1	1148.07	1137.59	1054.14	1107.91	1107.78	1109.50	1099.91	1056.90	996.32	1105.52	1114.93	1143.28
JCAL03	2	1022.87	1034.06	1081.59	1053.75	1058.45	1065.98	1035.90	958.02	972.60	1153.18	1094.93	1148.05
JCAL04	2	1102.30	1110.11	1061.50	1088.84	1122.80	973.06	974.16	896.95	979.87	1057.76	1118.34	1114.11
JCAL05	1	958.15	1000.35	1054.85	1075.83	1130.13	1016.87	964.81	1061.00	1009.47	1142.06	1164.24	1187.39
JCAL06	2	1118.43	1122.59	1071.38	982.05	946.86	991.45	973.22	992.34	1103.21	1161.05	1173.69	1126.82
JCAL07	1	1081.20	1069.07	1025.34	1054.05	1042.47	1041.55	1032.90	933.53	862.93	995.37	1124.81	1086.67
JCAL08	2	1116.55	1124.00	1077.12	990.39	1033.60	896.72	958.50	888.64	901.07	1076.84	1061.40	1112.39
JCAL09	1	1167.31	1182.76	1165.30	1041.98	932.15	963.38	1090.85	1124.14	1143.13	1102.75	1079.54	1049.97
JCAL10	2	1197.88	1189.30	1199.94	1078.19	1051.07	1065.44	967.94	910.86	948.74	983.80	1030.01	1132.00
JCAL11	2	1160.02	1095.30	1110.21	1039.29	1018.13	956.57	926.77	964.17	978.04	988.47	1096.42	1195.35
JCAL12	1	1058.74	1033.10	1034.46	1042.38	1050.97	1083.08	986.94	1009.10	1203.30	1140.57	1125.35	1203.99
JCAL13	2	1207.77	1155.40	1083.55	1095.11	1111.89	1118.14	1059.53	1117.89	1000.67	1055.32	1120.14	1156.26
JCAL14	1	1077.16	1093.96	1140.76	1026.75	1028.49	1026.51	996.42	943.65	938.30	1064.45	1082.96	1058.56
JCAL15	2	1125.80	1192.53	1134.28	1112.17	1066.95	1006.44	972.41	993.82	1089.33	1141.89	1181.79	1190.46
JCAL16	1	1149.39	1141.54	1143.21	1133.19	1132.61	1062.50	1056.48	1007.39	1057.72	1131.10	1148.67	1147.61
JCAL17	2	1080.11	1127.30	1106.76	993.40	971.69	845.73	841.78	867.79	930.94	1083.98	1154.86	1136.47
JCAL18	1	1139.31	1097.84	1081.53	1087.02	1053.29	1057.76	1016.02	1034.52	993.29	1055.24	1126.35	1202.27
JCAL19	1	1107.63	1073.57	1080.78	966.55	971.04	876.99	867.65	916.48	1003.28	1043.43	1109.95	1140.61
JCAL20	2	1120.85	1142.89	1141.91	1118.81	1144.14	1114.89	1044.54	1028.01	990.03	1068.29	1124.19	1176.42
JCAL21	2	1148.95	1146.80	1152.12	1146.14	1077.85	948.58	966.34	998.66	1103.47	1075.74	1191.44	1141.14
JCAL22	2	956.17	944.08	1005.14	1022.28	1054.34	1014.12	1045.27	1099.28	1065.43	1096.27	1090.85	1130.19
JCAL23	1	1112.32	1099.50	1137.71	1095.37	976.90	953.49	1044.83	950.21	988.80	1056.45	1075.01	1100.68
JCAL24	1	1057.19	1061.38	1038.47	972.58	969.92	990.19	1031.27	929.76	898.71	920.04	963.42	998.29
JCAL25	1	976.56	937.75	979.22	975.38	868.55	889.71	924.22	946.13	936.71	1017.17	1078.05	1072.56
JCAL26	1	1144.18	1075.00	1070.30	998.26	1031.71	975.93	977.93	1030.34	1133.10	1187.25	1152.69	1142.56
JCAL27	2	1182.70	1158.06	1112.77	1085.58	1058.07	1041.71	979.07	857.94	958.70	1135.08	1167.66	1156.97
JCAL28	2	1058.21	1130.00	1117.68	1131.16	1145.09	1158.84	1070.73	946.88	981.74	1169.94	1217.10	1199.70
JCAL29	1	1139.46	1058.00	1109.66	1094.63	1057.51	951.84	985.55	1058.61	1092.06	1114.77	1151.41	1138.17

Appendix 3 Table 21: Baseline Endocortical vBMD by sector cont'd

ID	Group	240° - 250° [mg·cm³]	250° - 260° [mg·cm³]	260° - 270° [mg·cm³]	270° - 280° [mg·cm³]	280° - 290° [mg·cm³]	290° - 300° [mg·cm³]	300° - 310° [mg·cm³]	310° - 320° [mg·cm³]	320° - 330° [mg·cm³]	330° - 340° [mg·cm³]	340° - 350° [mg·cm³]	350° - 360° [mg·cm³]
JCAL01	1	1145.17	1227.43	1191.85	1170.86	1177.21	1176.75	1228.53	1219.89	1261.53	1210.54	1184.64	1097.51
JCAL02	1	1170.76	1205.46	1247.48	1207.08	1187.35	1227.36	1212.60	1185.70	1194.21	1155.16	1102.24	1074.47
JCAL03	2	1131.49	1071.37	1076.42	1217.26	1206.08	1202.77	1219.51	1221.66	1216.60	1226.20	1167.07	1109.42
JCAL04	2	1207.49	1200.20	1247.14	1266.96	1220.85	1182.51	1142.03	1160.46	1117.59	1148.61	1125.26	1151.86
JCAL05	1	1151.36	1165.16	1201.12	1260.30	1216.05	1192.83	1174.42	1171.65	1119.25	1094.00	1051.70	1094.36
JCAL06	2	1163.51	1123.21	1148.33	1167.18	1161.89	1228.99	1178.44	1174.03	1187.78	1154.31	1083.71	1057.15
JCAL07	1	1092.97	1106.22	1139.45	1122.95	1115.69	1142.87	1160.24	1210.21	1228.98	1168.38	1125.99	1061.14
JCAL08	2	1170.21	1163.22	1241.96	1269.20	1168.39	1172.32	1161.77	1159.52	1130.61	1106.15	1111.53	984.81
JCAL09	1	1058.22	1106.62	1140.83	1162.52	1197.16	1183.04	1147.11	1193.62	1210.29	1185.60	1140.43	1106.56
JCAL10	2	1114.21	1076.36	1162.50	1168.52	1230.18	1098.18	1135.99	1235.51	1255.20	1192.83	1154.74	1172.55
JCAL11	2	1206.67	1216.98	1273.14	1295.84	1227.27	1193.13	1151.99	1137.22	1161.70	1224.49	1198.52	1156.91
JCAL12	1	1244.66	1187.07	1084.10	1061.37	1111.08	1153.82	1314.66	1333.48	1249.24	1174.87	1162.00	1125.38
JCAL13	2	1189.11	1195.04	1207.13	1243.04	1163.93	1103.41	1171.14	1148.71	1150.89	1102.61	1034.95	1055.01
JCAL14	1	1222.44	1402.44	1428.23	1235.64	1118.89	1045.54	1021.19	1081.47	1265.35	1234.15	1115.35	867.79
JCAL15	2	1194.72	1188.23	1229.28	1186.31	1230.23	1269.76	1226.81	1226.18	1191.76	1095.19	1134.44	1091.61
JCAL16	1	1189.05	1180.10	1192.66	1212.15	1161.91	1203.40	1211.88	1189.50	1166.09	1192.11	1197.39	1146.96
JCAL17	2	1127.75	1132.54	1206.73	1177.52	1166.93	1199.67	1191.82	1176.98	1153.33	1009.11	1045.30	1098.94
JCAL18	1	1158.75	1156.14	1199.87	1194.17	1141.18	1175.07	1217.42	1205.56	1142.35	1176.24	1157.50	1079.27
JCAL19	1	1193.51	1236.75	1202.35	1028.16	1110.94	1168.79	1183.10	1144.57	1124.05	1151.85	1074.17	974.75
JCAL20	2	1166.96	1220.29	1244.26	1257.65	1248.06	1192.96	1198.37	1157.72	1169.95	1213.57	1204.31	1184.54
JCAL21	2	1142.51	1131.85	1160.32	1129.91	1096.34	1151.65	1171.00	1253.53	1193.49	1080.21	983.38	1040.41
JCAL22	2	1152.48	1172.75	1189.30	1168.81	1149.90	1135.56	1122.13	1066.74	1101.79	1135.02	1006.79	1016.54
JCAL23	1	1142.42	1172.71	1226.34	1249.55	1298.97	1239.52	1180.30	1139.26	1132.59	1109.58	1136.29	928.17
JCAL24	1	1042.17	1065.56	1026.00	1037.00	1077.29	1152.42	1201.64	1187.43	1136.22	1069.59	1125.35	1114.77
JCAL25	1	1061.51	1122.83	1155.66	1185.26	1155.97	1140.48	1099.22	1119.12	1093.74	1053.49	1034.79	1057.85
JCAL26	1	1153.78	1110.96	1135.80	1214.47	1186.41	1227.53	1223.92	1261.33	1233.59	1165.85	1153.98	1117.80
JCAL27	2	1211.24	1206.19	1164.37	1145.73	1170.48	1105.63	1162.80	1237.01	1287.79	1233.60	1158.84	1099.19
JCAL28	2	1245.17	1179.98	1148.93	1039.12	1138.95	1150.57	1180.11	1251.16	1252.94	1187.81	1146.59	1174.75
JCAL29	1	1210.00	1175.47	1144.74	1202.31	1240.91	1228.11	1158.07	1199.08	1182.02	1156.73	1086.70	992.08

Appendix 3 Table 22: Six months Endocortical vBMD by sector

ID	Group	0° - 10° [mg·cm³]	10° - 20° [mg·cm³]	20° - 30° [mg·cm³]	30° - 40° [mg·cm³]	40° - 50° [mg·cm³]	50° - 60° [mg·cm³]	60° - 70° [mg·cm³]	70° - 80° [mg·cm³]	80° - 90° [mg·cm³]	90° - 100° [mg·cm³]	100° - 110° [mg·cm³]	110° - 120° [mg·cm³]
JCAL01	1	1125.37	1170.89	1152.75	1166.91	1134.48	1217.80	1198.42	1121.16	1147.35	1153.85	1152.23	1152.41
JCAL02	1	1168.38	1140.59	1195.22	1185.32	1224.97	1183.70	1138.39	1127.83	1134.75	1139.35	1124.25	1157.54
JCAL03	2	1120.26	1103.68	1166.86	1103.24	1117.46	1121.14	1151.97	1181.33	1188.53	1159.24	1151.74	1130.72
JCAL04	2												
JCAL05	1												
JCAL06	2												
JCAL07	1	1101.47	1063.90	1052.45	1170.27	1231.03	1229.78	1202.22	1161.02	1179.34	1186.31	1161.65	1143.60
JCAL08	2	1075.87	973.75	1028.39	1175.44	1204.46	1227.41	1145.06	1114.23	1126.67	1146.93	1101.39	1169.61
JCAL09	1												
JCAL10	2	1074.86	1180.29	1126.56	1133.72	1140.43	1157.65	1185.38	1209.79	1200.84	1240.65	1270.72	1236.47
JCAL11	2	1055.44	923.32	1043.22	1216.06	1229.41	1192.84	1236.34	1183.98	1203.03	1204.42	1185.60	1174.42
JCAL12	1												
JCAL13	2												
JCAL14	1	902.49	865.10	1044.08	1170.73	1270.21	1135.67	1123.03	1191.02	1156.92	1087.49	975.06	1018.99
JCAL15	2	1085.24	1162.16	1302.59	1210.58	1102.44	1160.32	1216.38	1201.11	1154.41	1175.24	1154.73	1196.97
JCAL16	1												
JCAL17	2	1022.89	1034.69	1201.62	1249.83	1200.80	1325.81	1398.03	1345.67	1280.77	1068.42	972.98	917.87
JCAL18	1	1054.38	1056.01	1143.91	1200.28	1182.67	1215.01	1182.18	1173.67	1177.44	1105.59	1106.59	1081.22
JCAL19	1												
JCAL20	2	1174.26	1163.09	1187.01	1159.73	1173.19	1208.93	1169.27	1192.94	1182.46	1178.07	1184.56	1099.64
JCAL21	2	1046.43	1008.86	1066.99	1145.96	1148.26	1143.92	1136.22	1189.39	1171.79	1180.38	1156.88	1171.55
JCAL22	2												
JCAL23	1												
JCAL24	1	1142.66	1085.52	1200.41	1262.01	1255.89	1220.47	1188.12	1219.83	1233.41	1159.94	1138.12	1114.35
JCAL25	1												
JCAL26	1	1146.02	1169.04	1170.38	1251.94	1212.04	1229.15	1203.85	1174.40	1181.71	1181.72	1157.99	1211.69
JCAL27	2	1138.63	1147.73	1048.32	1182.48	1231.21	1219.81	1170.69	1147.14	1226.83	1240.72	1178.30	1186.05
JCAL28	2												
JCAL29	1	905.88	875.92	896.54	1086.03	1224.09	1219.54	1131.20	1157.65	1181.37	1187.61	1181.50	1154.10

Appendix 3 Table 22: Six months Endocortical vBMD by sector cont'd

ID	Group	120° - 130° [mg·cm³]	130° - 140° [mg·cm³]	140° - 150° [mg·cm³]	150° - 160° [mg·cm³]	160° - 170° [mg·cm³]	170° - 180° [mg·cm³]	180° - 190° [mg·cm³]	190° - 200° [mg·cm³]	200° - 210° [mg·cm³]	210° - 220° [mg·cm³]	220° - 230° [mg·cm³]	230° - 240° [mg·cm³]
JCAL01	1	1176.29	1151.15	1116.55	1100.40	1121.38	1192.21	1147.24	1086.51	1039.34	1024.29	1107.86	1153.06
JCAL02	1	1145.95	1083.06	1062.40	1103.39	1101.95	1112.91	1117.96	1061.04	1068.04	1062.12	1169.63	1185.14
JCAL03	2	1137.76	1100.23	1049.51	996.70	951.94	996.82	1019.97	985.18	908.68	960.75	924.85	941.14
JCAL04	2												
JCAL05	1												
JCAL06	2												
JCAL07	1	1106.10	1047.91	1045.39	1042.38	1046.99	1059.72	1053.15	949.60	968.46	1049.05	1115.38	1055.60
JCAL08	2	1170.32	1072.67	1096.70	1061.25	968.05	955.06	902.11	908.09	908.72	1031.53	1000.22	1073.32
JCAL09	1												
JCAL10	2	1134.59	1141.80	1142.56	1161.57	1079.22	1035.42	1029.58	958.50	914.38	969.52	1036.18	1122.43
JCAL11	2	1148.05	1102.90	1126.41	1009.16	975.28	954.59	1015.63	967.26	1085.05	1091.48	1096.95	1169.09
JCAL12	1												
JCAL13	2												
JCAL14	1	1067.37	1059.65	1058.86	1114.26	1119.28	1086.79	1048.36	965.80	811.78	1055.41	1176.61	1188.01
JCAL15	2	1148.65	1191.68	1136.54	1132.61	1107.72	999.07	986.57	1068.33	1193.83	1131.89	1147.28	1160.16
JCAL16	1												
JCAL17	2	884.08	908.48	945.14	1037.61	1005.39	897.28	947.33	868.30	991.75	1017.15	1113.32	1201.11
JCAL18	1	1093.74	1129.50	1141.09	1139.30	1083.13	1068.28	1047.35	1080.80	1031.56	1118.36	1127.82	1168.40
JCAL19	1												
JCAL20	2	1118.43	1159.55	1113.49	1122.01	1135.42	1082.93	1073.69	1027.08	1027.16	1010.65	1119.86	1141.93
JCAL21	2	1161.33	1167.69	1223.41	1165.51	1085.38	1003.17	1015.43	1024.90	1098.48	1116.87	1142.82	1169.75
JCAL22	2												
JCAL23	1												
JCAL24	1	1079.23	1089.25	1091.21	1124.09	1087.53	1058.58	1017.44	933.60	912.27	1118.43	1101.76	1033.32
JCAL25	1												
JCAL26	1	1151.31	1090.10	1089.24	1092.73	1013.36	955.63	963.07	1018.44	1066.01	1146.60	1174.48	1198.36
JCAL27	2	1182.98	1165.47	1120.61	1110.15	1061.70	1022.35	1023.60	929.50	961.20	1120.42	1086.38	1153.61
JCAL28	2												
JCAL29	1	1097.16	1068.47	1062.13	1006.42	999.42	983.36	1004.21	1035.30	1071.34	1063.17	1069.50	1155.50

Appendix 3 Table 22: Six months Endocortical vBMD by sector cont'd

ID	Group	240° - 250° [mg·cm³]	250° - 260° [mg·cm³]	260° - 270° [mg·cm³]	270° - 280° [mg·cm³]	280° - 290° [mg·cm³]	290° - 300° [mg·cm³]	300° - 310° [mg·cm³]	310° - 320° [mg·cm³]	320° - 330° [mg·cm³]	330° - 340° [mg·cm³]	340° - 350° [mg·cm³]	350° - 360° [mg·cm³]
JCAL01	1	1207.26	1078.10	1161.30	1223.61	1294.78	1256.59	1190.53	1215.59	1213.60	1214.44	1135.72	1098.09
JCAL02	1	1171.29	1152.05	1216.42	1181.52	1157.44	1132.06	1141.10	1128.84	1173.34	1195.97	1187.28	1150.16
JCAL03	2	1053.76	1072.35	1111.75	1099.63	1117.75	1120.78	1129.15	1128.50	1140.45	1134.00	1152.92	1143.79
JCAL04	2												
JCAL05	1												
JCAL06	2												
JCAL07	1	1123.09	1113.25	1173.63	1176.47	1164.44	1211.24	1199.90	1254.91	1165.90	1134.33	1071.76	1134.48
JCAL08	2	1204.52	1113.98	1220.41	1279.84	1205.67	1199.03	1237.47	1199.84	1156.86	1096.59	940.42	963.62
JCAL09	1												
JCAL10	2	1092.88	1092.93	1131.10	1178.09	1221.86	1195.28	1168.57	1216.31	1266.16	1158.69	1089.60	1142.72
JCAL11	2	1160.47	1191.04	1264.55	1280.75	1247.99	1241.07	1250.32	1245.88	1242.34	1201.85	1216.70	1178.74
JCAL12	1												
JCAL13	2												
JCAL14	1	1235.44	1248.74	1350.06	1280.73	1258.83	1250.32	1181.97	1143.17	1165.60	1163.92	995.71	884.04
JCAL15	2	1209.47	1155.31	1176.64	1245.70	1256.91	1275.96	1222.74	1240.12	1146.62	1087.24	1119.37	1059.76
JCAL16	1												
JCAL17	2	1304.88	1241.27	1105.31	994.04	971.24	1060.85	1084.15	1289.06	1030.29	830.33	963.15	1043.94
JCAL18	1	1146.33	1167.71	1250.46	1278.27	1242.87	1187.30	1180.97	1171.08	1153.57	1173.36	1109.16	1080.15
JCAL19	1												
JCAL20	2	1122.03	1157.10	1239.83	1255.06	1232.56	1232.56	1193.61	1231.64	1215.76	1211.55	1128.36	1120.84
JCAL21	2	1189.63	1190.73	1163.79	1155.52	1147.85	1176.06	1145.06	1180.37	1218.51	1111.53	1026.63	1026.30
JCAL22	2												
JCAL23	1												
JCAL24	1	1071.62	1125.89	1122.21	1154.27	1203.19	1199.25	1194.54	1179.87	1183.97	1230.88	1116.81	1174.44
JCAL25	1												
JCAL26	1	1180.94	1165.93	1168.22	1243.67	1240.19	1220.04	1187.56	1228.15	1205.11	1155.62	1146.62	1166.69
JCAL27	2	1131.05	1146.21	1166.35	1232.47	1192.31	1151.69	1213.91	1195.29	1212.12	1151.02	1120.67	1047.53
JCAL28	2												
JCAL29	1	1163.58	1105.29	1121.39	1193.07	1241.41	1224.64	1196.06	1200.06	1164.78	1133.00	1176.07	962.67

Appendix 3 Table 23: Baseline Mid-cortical vBMD by sector

ID	Group	0° - 10° [mg·cm³]	10° - 20° [mg·cm³]	20° - 30° [mg·cm³]	30° - 40° [mg·cm³]	40° - 50° [mg·cm³]	50° - 60° [mg·cm³]	60° - 70° [mg·cm³]	70° - 80° [mg·cm³]	80° - 90° [mg·cm³]	90° - 100° [mg·cm³]	100° - 110° [mg·cm³]	110° - 120° [mg·cm³]
JCAL01	1	1238.10	1175.39	1232.15	1230.82	1232.55	1255.16	1265.15	1230.95	1254.72	1233.87	1236.21	1258.36
JCAL02	1	1200.82	1237.56	1221.24	1233.96	1250.56	1207.31	1265.79	1246.81	1210.53	1189.76	1185.13	1216.57
JCAL03	2	1165.86	1174.13	1199.91	1266.63	1279.10	1288.37	1255.18	1239.42	1202.89	1210.09	1160.25	1176.74
JCAL04	2	1123.98	1110.59	1225.26	1235.47	1236.66	1154.69	1110.56	1186.04	1173.15	1191.04	1194.78	1190.85
JCAL05	1	1193.79	1128.28	1217.07	1264.28	1247.72	1223.87	1171.32	1165.44	1225.39	1256.12	1205.54	1223.48
JCAL06	2	1185.11	1156.27	1238.94	1242.17	1212.13	1232.13	1212.30	1228.99	1185.59	1255.53	1230.03	1192.89
JCAL07	1	1238.23	1234.21	1217.47	1226.23	1210.48	1225.77	1206.20	1262.40	1322.64	1258.55	1234.36	1223.57
JCAL08	2	1197.69	1172.26	1148.62	1239.68	1249.37	1253.36	1223.84	1180.26	1167.36	1197.27	1166.63	1242.29
JCAL09	1	1245.01	1222.64	1229.90	1226.27	1254.10	1223.85	1225.69	1250.24	1259.45	1258.65	1255.36	1205.11
JCAL10	2	1202.25	1218.22	1240.60	1229.55	1215.47	1220.96	1196.05	1225.88	1217.48	1255.32	1241.12	1247.36
JCAL11	2	1127.09	1125.05	1168.42	1218.46	1249.26	1223.95	1200.38	1224.24	1163.19	1182.49	1147.04	1136.56
JCAL12	1	1186.01	1192.39	1263.56	1259.68	1194.01	1339.56	1313.46	1286.31	1344.74	1313.86	1296.16	1229.34
JCAL13	2	1201.05	1189.88	1223.01	1284.50	1257.58	1211.08	1202.69	1239.44	1225.46	1221.42	1219.82	1231.64
JCAL14	1	1160.16	1110.03	1236.40	1350.93	1256.76	1162.79	1064.27	1020.42	1162.57	1183.09	1145.49	1196.34
JCAL15	2	1178.58	1224.77	1248.93	1244.84	1196.93	1195.73	1237.53	1268.67	1247.33	1241.24	1212.70	1237.14
JCAL16	1	1202.93	1196.24	1232.31	1223.98	1229.62	1232.20	1228.20	1253.37	1224.65	1214.21	1233.59	1204.71
JCAL17	2	1159.78	1179.37	1250.36	1231.41	1237.72	1197.43	1184.80	1160.61	1176.26	1156.62	1128.72	1132.69
JCAL18	1	1189.62	1194.82	1179.84	1226.91	1210.65	1212.74	1191.37	1180.27	1201.50	1201.78	1177.22	1181.90
JCAL19	1	1054.63	1101.16	1106.80	1193.70	1205.09	1216.42	1214.41	1177.31	1169.80	1189.50	1164.36	1130.88
JCAL20	2	1219.99	1179.74	1207.60	1223.20	1215.52	1188.82	1206.30	1182.69	1211.83	1215.87	1200.04	1211.49
JCAL21	2	1054.02	1170.33	1219.22	1208.34	1190.90	1204.44	1222.66	1182.37	1198.29	1210.06	1239.48	1210.11
JCAL22	2	1193.49	1217.47	1240.44	1283.80	1200.82	1192.03	1207.06	1196.74	1173.96	1146.42	1146.70	1137.30
JCAL23	1	1167.97	1142.37	1219.19	1267.74	1262.58	1268.31	1149.85	1145.70	1150.03	1143.69	1134.19	1085.57
JCAL24	1	1090.89	1064.46	1131.94	1028.47	1091.14	1181.07	1237.88	1212.87	1187.33	1152.53	1113.16	1100.53
JCAL25	1	1114.18	1106.44	1095.89	1109.62	1128.87	1115.75	1152.90	1136.56	1159.72	1090.01	1120.04	1124.81
JCAL26	1	1242.90	1243.60	1270.47	1273.39	1249.95	1204.67	1245.05	1211.99	1204.21	1186.19	1164.45	1145.76
JCAL27	2	1189.55	1207.33	1224.94	1257.72	1240.02	1234.70	1225.58	1256.55	1237.35	1212.82	1255.02	1233.82
JCAL28	2	1162.46	1266.82	1239.97	1194.02	1235.22	1202.85	1190.61	1307.51	1272.91	1202.90	1228.76	1188.85
JCAL29	1	1068.62	1107.03	1034.09	1249.10	1237.61	1243.06	1229.56	1212.19	1221.13	1243.20	1227.52	1197.36

Appendix 3 Table 23: Baseline Mid-cortical vBMD by sector cont'd

ID	Group	120° - 130° [mg·cm³]	130° - 140° [mg·cm³]	140° - 150° [mg·cm³]	150° - 160° [mg·cm³]	160° - 170° [mg·cm³]	170° - 180° [mg·cm³]	180° - 190° [mg·cm³]	190° - 200° [mg·cm³]	200° - 210° [mg·cm³]	210° - 220° [mg·cm³]	220° - 230° [mg·cm³]	230° - 240° [mg·cm³]
JCAL01	1	1238.13	1203.79	1202.76	1146.31	1134.56	1189.03	1194.71	1183.18	1182.49	1181.80	1109.68	1102.92
JCAL02	1	1193.92	1181.75	1152.30	1195.03	1163.83	1170.41	1173.16	1160.81	1154.23	1175.29	1157.18	1182.61
JCAL03	2	1159.23	1167.59	1148.04	1149.26	1166.08	1133.54	1125.18	1108.45	1088.04	1155.93	1092.54	1141.48
JCAL04	2	1175.31	1154.60	1119.09	1154.11	1185.61	1144.74	1114.95	1041.39	1081.55	1068.46	1168.95	1185.10
JCAL05	1	1126.65	1169.40	1203.44	1153.13	1212.13	1138.01	1149.12	1165.24	1111.04	1166.02	1174.00	1215.95
JCAL06	2	1168.65	1155.70	1140.71	1116.98	1088.54	1091.40	1109.41	1119.72	1152.31	1182.46	1179.55	1195.36
JCAL07	1	1171.57	1175.53	1122.18	1148.40	1155.80	1142.62	1105.89	1124.30	1070.58	1103.03	1165.53	1134.84
JCAL08	2	1198.79	1186.00	1193.90	1187.03	1195.12	1121.55	1104.66	1057.01	1086.68	1137.97	1126.54	1121.90
JCAL09	1	1223.66	1205.08	1174.47	1136.17	1144.84	1115.60	1159.15	1172.31	1169.96	1166.44	1179.97	1152.07
JCAL10	2	1210.53	1213.43	1217.46	1199.91	1151.71	1157.01	1093.23	1078.86	1121.28	1145.11	1174.26	1217.75
JCAL11	2	1168.26	1100.06	1115.13	1090.35	1082.56	1014.00	987.54	1019.16	982.03	986.86	1086.74	1190.49
JCAL12	1	1175.21	1114.84	1134.26	1171.12	1174.62	1178.48	1154.66	1203.31	1234.20	1163.25	1153.75	1266.06
JCAL13	2	1188.86	1224.06	1226.16	1216.71	1201.23	1204.83	1147.01	1147.39	1092.89	1196.72	1194.26	1202.97
JCAL14	1	1205.71	1223.28	1210.63	1223.48	1174.11	1108.74	1111.80	1139.73	1111.25	1105.34	1122.67	1156.01
JCAL15	2	1208.15	1192.00	1182.38	1180.46	1173.68	1161.27	1131.01	1136.33	1175.86	1200.34	1208.98	1213.11
JCAL16	1	1214.42	1190.89	1198.95	1211.34	1197.17	1164.78	1091.34	1137.51	1166.02	1184.70	1166.29	1145.99
JCAL17	2	1149.73	1148.25	1153.07	1128.48	1075.35	1014.01	1016.44	1007.69	1049.94	1097.60	1183.91	1171.30
JCAL18	1	1203.67	1160.01	1120.44	1157.91	1145.63	1124.62	1106.41	1096.10	1117.57	1151.90	1160.15	1218.57
JCAL19	1	1138.08	1147.97	1126.58	1115.10	1087.34	1073.46	1034.57	1091.27	1100.20	1047.33	1100.53	1147.65
JCAL20	2	1194.40	1194.19	1179.78	1184.48	1156.23	1164.71	1139.28	1143.20	1110.47	1141.80	1131.32	1180.18
JCAL21	2	1150.25	1124.50	1178.27	1229.55	1189.22	1140.67	1131.45	1101.53	1146.96	1187.00	1232.96	1222.82
JCAL22	2	1092.66	1061.47	1069.64	1078.45	1063.32	1040.41	1065.11	1082.60	1084.29	1115.65	1114.60	1142.02
JCAL23	1	1143.18	1133.30	1160.18	1173.40	1137.79	1099.75	1132.19	1062.65	1057.24	1056.38	1061.66	1100.62
JCAL24	1	1088.71	1071.37	1102.75	1092.70	1089.61	1061.91	1114.87	1075.29	1033.12	1030.51	1045.62	1091.42
JCAL25	1	1044.22	1026.47	1051.77	1075.88	982.09	956.01	946.73	992.98	994.88	1059.08	1117.00	1102.00
JCAL26	1	1191.78	1164.12	1193.43	1134.53	1138.54	1100.26	1096.03	1091.66	1192.97	1185.26	1208.38	1164.07
JCAL27	2	1202.15	1179.90	1191.18	1180.97	1112.92	1128.25	1108.26	1066.71	1123.24	1199.85	1224.53	1217.61
JCAL28	2	1146.42	1172.44	1145.13	1183.39	1166.06	1201.96	1112.48	1086.28	1152.40	1245.30	1217.00	1191.59
JCAL29	1	1200.93	1168.99	1161.88	1160.38	1143.47	1096.29	1159.46	1152.94	1151.03	1149.03	1160.65	1138.63

Appendix 3 Table 23: Baseline Mid-cortical vBMD by sector cont'd

		240° - 250°	250° - 260°	260° - 270°	270° - 280°	280° - 290°	290° - 300°	300° - 310°	310° - 320°	320° - 330°	330° - 340°	340° - 350°	350° - 360°
ID	Group	[mg·cm³]	[mg·cm³]	[mg·cm³]	[mg·cm³]	[mg·cm³]	[mg·cm³]	[mg·cm³]	[mg·cm³]	[mg·cm³]	[mg·cm³]	[mg·cm³]	[mg·cm³]
JCAL01	1	1201.69	1246.11	1202.43	1179.51	1190.72	1198.60	1299.74	1306.69	1349.44	1287.20	1214.33	1196.17
JCAL02	1	1196.28	1218.77	1222.85	1223.31	1242.27	1270.30	1276.16	1217.79	1172.31	1187.98	1206.45	1191.83
JCAL03	2	1134.20	1132.77	1160.00	1204.41	1253.41	1226.43	1217.52	1242.40	1196.35	1242.80	1233.68	1193.86
JCAL04	2	1208.53	1276.41	1271.04	1305.11	1306.95	1256.39	1225.03	1195.06	1194.22	1199.49	1192.81	1190.99
JCAL05	1	1229.26	1274.66	1254.21	1245.99	1272.85	1210.76	1201.41	1239.28	1233.19	1221.00	1198.53	1202.76
JCAL06	2	1166.26	1176.41	1199.51	1246.33	1213.99	1249.95	1236.73	1204.69	1225.51	1238.85	1206.53	1186.83
JCAL07	1	1146.69	1195.32	1196.83	1165.08	1145.80	1170.21	1197.55	1240.77	1252.89	1260.48	1239.26	1248.64
JCAL08	2	1170.12	1186.34	1266.53	1274.49	1283.51	1216.23	1214.40	1204.69	1246.61	1227.45	1207.47	1206.74
JCAL09	1	1180.26	1226.35	1259.60	1244.11	1295.86	1226.12	1201.42	1237.28	1252.28	1246.28	1272.82	1268.51
JCAL10	2	1215.44	1194.18	1251.00	1260.84	1281.46	1228.28	1203.04	1262.33	1275.37	1269.42	1235.22	1235.23
JCAL11	2	1206.67	1239.91	1274.08	1297.90	1275.12	1251.42	1236.69	1179.74	1213.57	1223.48	1230.84	1215.85
JCAL12	1	1264.80	1154.71	1059.74	1013.54	1040.89	1128.82	1252.96	1362.44	1335.93	1267.21	1223.24	1182.49
JCAL13	2	1202.44	1259.54	1256.08	1300.99	1265.54	1237.52	1268.83	1260.74	1195.61	1219.05	1201.12	1163.15
JCAL14	1	1367.44	1448.18	1485.89	1314.40	1152.21	974.61	973.73	1121.35	1226.04	1216.52	1340.04	1210.62
JCAL15	2	1230.44	1226.71	1252.45	1218.69	1257.38	1275.42	1245.25	1264.81	1251.19	1200.46	1219.45	1183.03
JCAL16	1	1193.34	1195.37	1215.55	1251.07	1215.37	1231.15	1202.55	1181.99	1219.11	1185.47	1194.28	1189.53
JCAL17	2	1142.88	1161.59	1233.46	1193.87	1144.30	1194.18	1226.74	1260.39	1216.73	1187.60	1144.41	1186.74
JCAL18	1	1190.54	1208.29	1248.59	1246.62	1219.37	1180.41	1175.06	1214.97	1209.15	1231.70	1176.94	1174.98
JCAL19	1	1203.05	1241.04	1203.54	1181.29	1230.83	1257.52	1191.13	1216.12	1210.42	1236.40	1206.87	1166.62
JCAL20	2	1194.81	1224.88	1283.99	1262.97	1284.38	1214.88	1222.52	1234.11	1224.22	1172.72	1178.34	1185.41
JCAL21	2	1195.17	1172.10	1201.72	1171.43	1176.06	1204.42	1245.32	1269.58	1224.81	1226.40	1213.11	1157.30
JCAL22	2	1174.27	1216.86	1215.74	1215.48	1214.63	1215.26	1234.85	1195.06	1194.34	1215.74	1196.52	1147.64
JCAL23	1	1197.91	1185.64	1290.16	1295.88	1307.79	1231.13	1210.81	1203.87	1165.40	1213.98	1216.16	1143.16
JCAL24	1	1110.23	1121.86	1140.17	1165.80	1143.02	1193.95	1239.06	1244.86	1208.30	1123.00	1147.84	1102.38
JCAL25	1	1091.32	1171.44	1147.76	1167.09	1194.92	1214.10	1147.76	1103.90	1138.92	1079.26	1148.45	1136.10
JCAL26	1	1192.71	1148.26	1201.28	1267.91	1212.57	1246.98	1246.34	1260.98	1234.32	1227.38	1195.18	1227.84
JCAL27	2	1231.06	1205.29	1185.88	1182.45	1191.27	1162.15	1172.66	1293.24	1252.70	1221.32	1221.17	1206.20
JCAL28	2	1169.70	1136.68	1217.39	1132.04	1113.87	1128.93	1199.43	1239.58	1269.23	1260.07	1193.98	1187.69
JCAL29	1	1211.21	1197.19	1234.75	1225.37	1280.32	1263.42	1212.73	1194.01	1234.22	1193.16	1200.01	1168.36

Appendix 3 Table 24: Six months Mid-cortical vBMD by sector

ID	Group	0° - 10° [mg·cm³]	10° - 20° [mg·cm³]	20° - 30° [mg·cm³]	30° - 40° [mg·cm³]	40° - 50° [mg·cm³]	50° - 60° [mg·cm³]	60° - 70° [mg·cm³]	70° - 80° [mg·cm³]	80° - 90° [mg·cm³]	90° - 100° [mg·cm³]	100° - 110° [mg·cm³]	110° - 120° [mg·cm³]
JCAL01	1	1256.11	1197.66	1251.07	1268.81	1275.72	1268.45	1236.89	1198.92	1224.56	1246.05	1213.39	1209.12
JCAL02	1	1228.14	1206.88	1236.69	1251.95	1257.20	1235.61	1175.86	1161.93	1220.33	1234.01	1217.29	1249.73
JCAL03	2	1120.56	1148.11	1162.88	1135.66	1164.40	1119.83	1127.79	1178.46	1181.82	1164.21	1139.63	1168.64
JCAL04	2												
JCAL05	1												
JCAL06	2												
JCAL07	1	1221.50	1179.54	1251.80	1254.06	1259.95	1235.27	1264.13	1240.84	1213.87	1218.61	1256.19	1221.40
JCAL08	2	1207.03	1158.20	1204.58	1290.91	1279.28	1254.65	1237.60	1187.85	1206.59	1247.67	1208.23	1192.60
JCAL09	1												
JCAL10	2	1241.66	1269.73	1212.44	1193.13	1243.63	1263.60	1218.85	1228.38	1231.78	1318.84	1283.75	1284.96
JCAL11	2	1160.51	1134.50	1186.01	1266.53	1257.03	1265.24	1275.79	1251.60	1211.07	1215.48	1212.92	1193.16
JCAL12	1												
JCAL13	2												
JCAL14	1	1133.27	1097.62	1225.16	1327.12	1274.57	1220.38	1193.13	1208.80	1211.77	1184.53	1171.99	1214.60
JCAL15	2	1190.70	1275.37	1286.07	1250.73	1190.70	1216.62	1247.50	1271.54	1253.22	1236.74	1219.75	1257.13
JCAL16	1												
JCAL17	2	1179.50	1144.29	1297.13	1287.76	1323.83	1392.13	1330.40	1349.77	1359.99	1238.33	1075.38	994.96
JCAL18	1	1166.01	1187.86	1213.46	1227.32	1238.72	1206.64	1243.01	1228.79	1201.23	1160.87	1199.67	1186.84
JCAL19	1												
JCAL20	2	1195.72	1188.47	1253.49	1236.09	1216.15	1243.72	1167.09	1198.32	1222.99	1242.99	1224.01	1213.28
JCAL21	2	1085.83	1168.10	1217.36	1203.99	1229.46	1181.81	1224.56	1242.79	1217.36	1197.06	1216.12	1215.01
JCAL22	2												
JCAL23	1												
JCAL24	1	1166.01	1172.26	1240.68	1253.49	1271.27	1243.83	1253.84	1207.27	1237.99	1199.34	1200.55	1203.15
JCAL25	1												
JCAL26	1	1221.39	1211.04	1279.85	1257.81	1246.18	1262.28	1220.50	1244.26	1246.87	1223.86	1198.73	1243.73
JCAL27	2	1198.50	1216.55	1215.93	1230.95	1235.25	1289.15	1226.62	1213.09	1188.97	1230.73	1249.67	1223.36
JCAL28	2												
JCAL29	1	1090.53	1142.66	1097.45	1208.75	1272.85	1240.10	1201.06	1210.54	1210.91	1231.86	1226.93	1233.12

Appendix 3 Table 24: Six months Mid-cortical vBMD by sector cont'd

ID	Group	120° - 130° [mg·cm³]	130° - 140° [mg·cm³]	140° - 150° [mg·cm³]	150° - 160° [mg·cm³]	160° - 170° [mg·cm³]	170° - 180° [mg·cm³]	180° - 190° [mg·cm³]	190° - 200° [mg·cm³]	200° - 210° [mg·cm³]	210° - 220° [mg·cm³]	220° - 230° [mg·cm³]	230° - 240° [mg·cm³]
JCAL01	1	1224.78	1222.40	1193.84	1250.62	1175.49	1200.22	1169.69	1121.23	1124.37	1175.25	1145.37	1181.40
JCAL02	1	1217.63	1208.83	1181.64	1143.82	1153.21	1186.67	1174.98	1164.32	1157.20	1175.58	1178.53	1150.16
JCAL03	2	1158.62	1183.28	1143.27	1084.20	1063.25	1054.72	1053.65	1015.31	1039.04	1055.83	1060.68	1114.15
JCAL04	2												
JCAL05	1												
JCAL06	2												
JCAL07	1	1220.28	1197.05	1135.49	1191.23	1186.32	1151.32	1169.03	1133.21	1101.59	1174.84	1198.55	1079.07
JCAL08	2	1202.95	1206.85	1187.75	1168.40	1169.23	1196.71	1145.07	1082.03	1059.52	1115.90	1115.06	1169.42
JCAL09	1												
JCAL10	2	1239.19	1206.88	1186.88	1192.82	1124.13	1121.74	1130.18	1117.27	1132.06	1144.34	1200.52	1212.45
JCAL11	2	1160.08	1121.43	1120.94	1112.85	1101.27	1064.35	1083.41	1054.44	1075.00	1099.36	1110.71	1176.14
JCAL12	1												
JCAL13	2												
JCAL14	1	1219.68	1219.02	1187.52	1186.67	1209.63	1201.32	1200.91	1137.68	986.81	1099.39	1185.03	1260.11
JCAL15	2	1235.05	1227.98	1201.65	1199.32	1172.27	1159.34	1118.78	1173.78	1215.73	1220.64	1173.63	1186.11
JCAL16	1												
JCAL17	2	925.37	904.42	1022.18	1149.51	1157.81	1179.02	1122.22	923.85	981.46	1046.57	1128.55	1225.28
JCAL18	1	1180.23	1184.23	1164.59	1175.82	1165.35	1150.50	1157.60	1144.04	1143.25	1154.13	1134.02	1176.79
JCAL19	1												
JCAL20	2	1199.96	1176.11	1177.69	1172.75	1167.12	1149.14	1172.16	1156.58	1102.34	1158.93	1159.43	1161.42
JCAL21	2	1206.74	1174.78	1198.73	1202.09	1175.26	1138.26	1135.63	1126.67	1131.22	1193.52	1213.70	1200.59
JCAL22	2												
JCAL23	1												
JCAL24	1	1197.68	1167.37	1154.46	1157.29	1149.96	1066.11	1081.42	1074.78	1096.67	1144.49	1130.94	1096.27
JCAL25	1												
JCAL26	1	1187.32	1177.24	1173.15	1163.10	1116.41	1111.81	1075.56	1057.27	1116.84	1190.46	1168.79	1203.02
JCAL27	2	1207.27	1184.25	1183.70	1163.80	1125.82	1086.55	1051.79	1127.78	1176.00	1167.15	1187.22	1188.34
JCAL28	2												
JCAL29	1	1214.63	1190.64	1166.51	1118.24	1119.68	1119.16	1153.97	1128.14	1136.89	1126.44	1086.13	1151.60

Appendix 3 Table 24: Six months Mid-cortical vBMD by sector cont'd

ID	Group	240° - 250° [mg·cm³]	250° - 260° [mg·cm³]	260° - 270° [mg·cm³]	270° - 280° [mg·cm³]	280° - 290° [mg·cm³]	290° - 300° [mg·cm³]	300° - 310° [mg·cm³]	310° - 320° [mg·cm³]	320° - 330° [mg·cm³]	330° - 340° [mg·cm³]	340° - 350° [mg·cm³]	350° - 360° [mg·cm³]
JCAL01	1	1209.89	1242.52	1226.91	1251.45	1329.54	1305.62	1281.57	1251.17	1240.34	1247.50	1199.13	1220.93
JCAL02	1	1153.06	1163.83	1207.72	1199.22	1253.96	1244.92	1242.09	1196.02	1199.52	1218.44	1214.63	1210.43
JCAL03	2	1119.70	1151.53	1160.54	1157.45	1191.74	1163.47	1177.90	1204.49	1165.06	1167.97	1223.70	1185.37
JCAL04	2												
JCAL05	1												
JCAL06	2												
JCAL07	1	1103.43	1142.25	1182.05	1186.88	1218.40	1251.87	1198.59	1254.27	1237.66	1234.19	1193.43	1230.92
JCAL08	2	1239.42	1277.19	1313.49	1319.97	1282.30	1234.03	1262.05	1233.53	1235.89	1197.10	1153.23	1176.90
JCAL09	1												
JCAL10	2	1225.61	1205.55	1254.85	1263.65	1276.62	1242.35	1250.00	1203.61	1305.11	1287.98	1257.31	1243.97
JCAL11	2	1157.25	1221.48	1285.11	1292.49	1260.41	1226.06	1255.20	1275.97	1272.46	1251.89	1222.76	1201.54
JCAL12	1												
JCAL13	2												
JCAL14	1	1290.80	1304.93	1393.52	1317.95	1273.75	1262.02	1220.97	1185.63	1239.00	1234.44	1219.12	1141.78
JCAL15	2	1242.41	1231.15	1253.02	1287.60	1259.18	1228.10	1229.44	1264.70	1206.93	1196.55	1212.01	1163.63
JCAL16	1												
JCAL17	2	1332.64	1228.47	1190.95	1211.43	1131.86	1216.15	1247.32	1305.99	1251.31	1060.47	1024.42	1152.32
JCAL18	1	1161.79	1195.48	1234.42	1241.01	1268.87	1234.32	1210.14	1202.79	1214.25	1215.06	1207.77	1186.16
JCAL19	1												
JCAL20	2	1143.24	1171.11	1281.95	1270.89	1276.80	1212.56	1225.59	1220.88	1219.00	1204.30	1230.09	1195.70
JCAL21	2	1215.66	1221.86	1198.56	1204.44	1187.52	1169.65	1210.72	1184.00	1248.92	1242.89	1209.93	1174.66
JCAL22	2												
JCAL23	1												
JCAL24	1	1124.44	1174.39	1178.09	1166.91	1227.28	1232.55	1261.18	1261.13	1215.64	1207.90	1241.36	1205.69
JCAL25	1												
JCAL26	1	1205.91	1198.38	1191.47	1231.96	1264.14	1233.47	1211.04	1250.91	1249.50	1214.24	1205.06	1237.39
JCAL27	2	1201.63	1193.53	1190.37	1254.03	1267.33	1183.30	1231.85	1208.92	1239.09	1179.99	1264.91	1248.31
JCAL28	2												
JCAL29	1	1193.40	1164.44	1197.45	1191.11	1219.76	1249.08	1272.98	1232.21	1197.40	1264.37	1236.05	1185.39

Appendix 3 Table 25: Baseline Pericortical vBMD by sector

ID	Group	0° - 10° [mg·cm³]	10° - 20° [mg·cm³]	20° - 30° [mg·cm³]	30° - 40° [mg·cm³]	40° - 50° [mg·cm³]	50° - 60° [mg·cm³]	60° - 70° [mg·cm³]	70° - 80° [mg·cm³]	80° - 90° [mg·cm³]	90° - 100° [mg·cm³]	100° - 110° [mg·cm³]	110° - 120° [mg·cm³]
JCAL01	1	1238.93	1207.63	1243.84	1217.47	1202.62	1252.19	1243.26	1229.56	1251.31	1202.14	1215.91	1239.80
JCAL02	1	1242.90	1233.36	1213.61	1242.80	1257.91	1249.74	1247.74	1263.61	1235.54	1205.76	1181.14	1217.90
JCAL03	2	1178.39	1162.93	1210.64	1234.28	1242.45	1264.46	1232.19	1231.07	1208.08	1201.07	1182.68	1203.89
JCAL04	2	1145.24	1183.84	1234.94	1214.26	1191.55	1135.91	1122.96	1134.12	1160.87	1167.13	1191.99	1200.99
JCAL05	1	1216.38	1215.28	1219.31	1247.84	1234.13	1219.05	1164.18	1152.46	1234.51	1257.72	1215.33	1243.98
JCAL06	2	1212.26	1206.04	1200.79	1209.00	1185.62	1190.18	1209.60	1246.55	1191.23	1251.12	1208.71	1188.96
JCAL07	1	1235.31	1187.87	1222.68	1208.59	1193.85	1213.14	1216.56	1210.77	1252.49	1246.40	1215.70	1203.48
JCAL08	2	1184.64	1210.70	1132.36	1174.85	1245.88	1261.07	1193.00	1175.33	1128.46	1204.18	1181.11	1220.91
JCAL09	1	1222.31	1245.09	1222.58	1241.79	1228.96	1031.75	1069.09	1198.55	1252.29	1242.16	1266.24	1194.68
JCAL10	2	1205.01	1200.69	1211.80	1200.36	1209.10	1187.90	1136.97	1167.98	1222.16	1237.38	1212.36	1226.87
JCAL11	2	1099.86	1133.15	1160.84	1212.34	1206.36	1206.52	1176.03	1226.52	1173.42	1184.18	1132.46	1129.81
JCAL12	1	1221.50	1232.71	1255.43	1212.19	1234.52	1353.88	1295.25	1270.60	1279.44	1309.77	1257.85	1265.58
JCAL13	2	1186.90	1222.19	1217.23	1226.35	1249.02	1242.44	1273.34	1246.06	1230.13	1216.32	1265.49	1261.14
JCAL14	1	1216.74	1190.52	1332.31	1305.66	1234.87	1174.86	1014.19	1019.36	1123.99	1181.42	1205.13	1213.32
JCAL15	2	1222.69	1251.99	1255.41	1232.61	1205.08	1177.76	1232.72	1281.71	1257.82	1246.01	1216.61	1230.58
JCAL16	1	1213.81	1229.45	1249.94	1230.55	1222.60	1205.61	1235.88	1230.50	1210.79	1200.46	1260.97	1240.47
JCAL17	2	1162.59	1186.68	1199.68	1186.02	1175.86	1180.39	1184.35	1160.59	1181.30	1150.21	1127.74	1129.95
JCAL18	1	1236.73	1217.86	1203.82	1190.50	1239.43	1213.65	1191.91	1195.67	1170.32	1171.58	1161.10	1174.84
JCAL19	1	1174.03	1193.22	1190.57	1236.45	1188.84	1182.62	1220.70	1170.24	1183.24	1188.31	1185.64	1144.31
JCAL20	2	1210.61	1195.42	1165.39	1198.18	1230.77	1207.11	1211.20	1196.34	1197.06	1202.79	1195.68	1231.00
JCAL21	2	1090.92	1185.92	1228.19	1152.26	1166.41	1133.78	1199.30	1159.16	1154.83	1171.70	1201.05	1155.10
JCAL22	2	1232.24	1205.42	1238.17	1210.02	1183.97	1184.80	1207.13	1161.95	1162.14	1115.11	1126.57	1132.97
JCAL23	1	1190.11	1149.26	1220.22	1244.90	1203.06	1256.70	1154.17	1145.36	1109.45	1156.37	1127.64	1088.06
JCAL24	1	1071.35	1043.65	1097.17	973.27	1006.24	1163.07	1162.16	1182.81	1162.11	1145.65	1148.80	1113.35
JCAL25	1	1066.79	1028.45	999.92	1048.40	1076.46	1108.86	1102.03	1159.23	1146.48	1075.04	1127.26	1127.61
JCAL26	1	1256.17	1216.75	1241.19	1228.19	1224.67	1181.34	1227.72	1195.06	1227.77	1186.25	1167.63	1141.18
JCAL27	2	1180.50	1194.07	1216.14	1254.97	1214.41	1208.11	1214.07	1223.70	1248.61	1203.64	1243.10	1229.37
JCAL28	2	1220.47	1209.45	1167.17	1173.98	1184.09	1210.32	1208.39	1227.68	1250.88	1235.81	1271.98	1238.51
JCAL29	1	1195.18	1212.26	1152.05	1232.50	1251.58	1212.29	1234.85	1193.97	1202.09	1216.55	1253.14	1208.04

Appendix 3 Table 25: Baseline Pericortical vBMD by sector cont'd

ID	Group	120° - 130° [mg·cm³]	130° - 140° [mg·cm³]	140° - 150° [mg·cm³]	150° - 160° [mg·cm³]	160° - 170° [mg·cm³]	170° - 180° [mg·cm³]	180° - 190° [mg·cm³]	190° - 200° [mg·cm³]	200° - 210° [mg·cm³]	210° - 220° [mg·cm³]	220° - 230° [mg·cm³]	230° - 240° [mg·cm³]
JCAL01	1	1225.24	1194.65	1239.21	1230.87	1165.60	1163.45	1191.47	1214.70	1168.79	1120.31	1061.58	1110.25
JCAL02	1	1199.25	1194.67	1179.94	1212.12	1190.65	1181.78	1198.60	1174.24	1216.12	1154.55	1177.49	1164.38
JCAL03	2	1175.12	1183.59	1145.61	1155.66	1161.32	1159.11	1159.29	1128.76	1088.53	1157.89	1089.14	1113.89
JCAL04	2	1182.23	1136.81	1137.48	1120.02	1176.58	1172.33	1169.18	1088.70	1107.88	1051.26	1161.02	1195.89
JCAL05	1	1235.45	1187.94	1207.94	1173.53	1196.02	1205.40	1166.51	1127.63	1167.73	1165.30	1137.33	1185.32
JCAL06	2	1169.30	1155.00	1150.29	1145.37	1139.24	1117.88	1132.52	1132.89	1156.94	1164.00	1152.89	1170.33
JCAL07	1	1162.37	1145.67	1117.48	1142.05	1114.14	1099.29	1087.20	1100.67	1109.84	1140.40	1106.68	1116.96
JCAL08	2	1158.37	1174.87	1177.79	1200.02	1215.50	1140.12	1115.94	1104.34	1107.30	1129.98	1131.77	1112.73
JCAL09	1	1206.59	1223.58	1167.99	1191.65	1217.09	1156.63	1187.37	1167.04	1163.47	1168.12	1241.90	1228.88
JCAL10	2	1188.22	1157.49	1158.67	1202.89	1149.41	1160.93	1175.13	1164.81	1211.99	1198.79	1189.12	1216.81
JCAL11	2	1165.52	1095.09	1115.82	1098.46	1099.16	1066.41	1028.41	1016.08	984.71	984.45	1083.23	1186.33
JCAL12	1	1204.00	1177.26	1126.55	1190.14	1189.69	1198.82	1226.37	1204.30	1174.38	1118.18	1105.41	1256.38
JCAL13	2	1174.03	1203.41	1212.26	1213.12	1181.37	1186.90	1170.22	1159.40	1191.98	1191.80	1186.79	1119.58
JCAL14	1	1174.19	1227.05	1199.83	1235.68	1236.88	1174.87	1150.42	1174.55	1110.29	1107.14	1095.69	1178.79
JCAL15	2	1223.78	1168.42	1166.56	1169.44	1126.52	1146.83	1149.95	1217.33	1187.00	1157.54	1192.72	1213.44
JCAL16	1	1216.69	1190.10	1208.35	1177.28	1179.15	1139.09	1098.92	1134.69	1131.80	1174.20	1111.36	1134.20
JCAL17	2	1145.60	1087.75	1127.14	1145.88	1101.41	1058.21	1058.08	1051.75	1086.96	1093.12	1166.37	1135.07
JCAL18	1	1188.01	1177.90	1123.03	1153.60	1134.65	1123.08	1113.68	1123.44	1118.55	1161.20	1149.23	1158.90
JCAL19	1	1126.91	1126.86	1112.83	1127.70	1103.78	1130.66	1095.93	1127.42	1082.19	1039.69	1097.28	1152.61
JCAL20	2	1223.86	1199.70	1172.51	1180.20	1169.81	1143.99	1135.97	1130.37	1182.72	1206.58	1161.29	1141.82
JCAL21	2	1116.05	1073.42	1139.84	1189.98	1152.38	1159.62	1172.90	1121.83	1156.86	1234.16	1228.39	1209.48
JCAL22	2	1085.74	1082.65	1090.78	1037.85	1042.05	1017.82	1032.10	1067.18	1082.17	1099.35	1100.00	1097.09
JCAL23	1	1149.88	1136.65	1159.62	1126.97	1140.39	1166.87	1140.84	1111.72	1050.75	1058.15	1040.92	1098.48
JCAL24	1	1069.60	1044.32	1115.55	1076.43	1079.44	1045.58	1061.66	1022.36	1049.68	1062.93	1035.68	1104.19
JCAL25	1	1064.95	1070.99	1056.60	1066.72	1008.32	943.30	956.22	972.45	984.67	1058.69	1119.79	1095.93
JCAL26	1	1195.31	1203.75	1210.40	1146.52	1140.30	1149.67	1111.91	1092.49	1140.78	1142.89	1164.60	1146.51
JCAL27	2	1196.68	1194.24	1158.64	1190.28	1185.78	1162.01	1117.59	1177.46	1183.59	1193.21	1211.50	1181.90
JCAL28	2	1166.30	1189.63	1149.39	1148.95	1126.12	1145.10	1133.06	1099.42	1141.40	1197.28	1156.06	1146.13
JCAL29	1	1198.49	1179.53	1163.72	1152.23	1147.13	1152.66	1171.20	1135.59	1105.70	1106.51	1141.17	1139.41

Appendix 3 Table 25: Baseline Pericortical vBMD by sector cont'd

ID	Group	240° - 250° [mg·cm³]	250° - 260° [mg·cm³]	260° - 270° [mg·cm³]	270° - 280° [mg·cm³]	280° - 290° [mg·cm³]	290° - 300° [mg·cm³]	300° - 310° [mg·cm³]	310° - 320° [mg·cm³]	320° - 330° [mg·cm³]	330° - 340° [mg·cm³]	340° - 350° [mg·cm³]	350° - 360° [mg·cm³]
JCAL01	1	1159.00	1131.82	1195.12	1182.51	1239.61	1180.26	1225.05	1267.28	1274.29	1248.77	1209.00	1235.58
JCAL02	1	1187.62	1180.81	1159.50	1191.71	1240.68	1225.22	1277.74	1185.42	1172.34	1198.24	1182.54	1216.54
JCAL03	2	1122.55	1118.88	1160.98	1164.57	1240.03	1212.62	1201.66	1216.25	1170.22	1208.04	1229.43	1209.49
JCAL04	2	1196.22	1177.00	1216.45	1274.36	1263.07	1160.60	1173.72	1206.16	1188.89	1165.83	1179.30	1173.83
JCAL05	1	1249.60	1305.81	1230.77	1211.08	1217.23	1193.80	1177.74	1194.39	1194.69	1203.12	1235.25	1215.68
JCAL06	2	1130.13	1188.91	1220.69	1235.60	1206.13	1133.08	1188.77	1176.57	1190.38	1247.64	1232.56	1258.35
JCAL07	1	1096.18	1162.71	1145.07	1160.13	1098.25	1167.92	1201.09	1227.83	1209.59	1239.62	1169.89	1273.42
JCAL08	2	1170.12	1169.50	1228.26	1264.84	1246.60	1229.53	1217.13	1181.58	1202.68	1183.84	1167.00	1212.27
JCAL09	1	1255.74	1233.04	1256.24	1269.09	1241.13	1223.09	1240.31	1235.84	1258.07	1236.30	1272.57	1209.55
JCAL10	2	1206.40	1193.74	1261.85	1262.66	1262.77	1260.21	1213.14	1246.19	1267.11	1263.14	1224.05	1209.44
JCAL11	2	1198.31	1212.43	1226.70	1246.23	1242.68	1265.67	1206.61	1206.33	1210.69	1165.73	1200.15	1197.97
JCAL12	1	1221.32	1008.87	967.06	931.51	952.78	984.43	1055.17	1088.41	1250.65	1308.99	1257.08	1277.79
JCAL13	2	1192.36	1289.86	1299.84	1274.51	1277.50	1213.87	1198.88	1215.76	1235.17	1208.36	1204.92	1178.28
JCAL14	1	1297.86	1347.46	1357.36	1290.31	1100.94	879.73	886.73	1019.87	1068.46	1062.69	1264.95	1348.51
JCAL15	2	1219.50	1205.23	1222.36	1239.12	1217.06	1211.18	1244.53	1232.87	1242.89	1196.12	1186.56	1188.32
JCAL16	1	1145.12	1166.62	1194.66	1237.01	1180.97	1180.52	1170.13	1145.75	1186.97	1175.09	1199.78	1226.15
JCAL17	2	1100.70	1161.09	1202.18	1163.38	1099.93	1150.22	1159.79	1165.82	1178.35	1184.68	1184.35	1154.17
JCAL18	1	1170.84	1214.44	1184.84	1201.97	1244.08	1232.66	1180.45	1190.02	1181.75	1186.99	1204.39	1192.73
JCAL19	1	1196.49	1155.08	1166.71	1182.83	1179.43	1250.69	1172.60	1214.49	1213.81	1225.98	1241.89	1174.35
JCAL20	2	1195.49	1193.37	1226.55	1285.99	1225.30	1201.27	1201.04	1221.68	1179.22	1172.21	1179.69	1198.98
JCAL21	2	1208.28	1183.85	1206.55	1167.97	1194.22	1200.08	1221.29	1225.21	1228.09	1237.67	1193.61	1191.98
JCAL22	2	1156.40	1161.93	1125.43	1207.99	1230.00	1197.38	1192.33	1203.87	1187.27	1214.33	1226.77	1215.96
JCAL23	1	1203.91	1140.12	1232.93	1247.03	1258.28	1226.57	1210.97	1181.63	1175.58	1200.22	1179.20	1215.05
JCAL24	1	1072.28	1112.58	1151.30	1166.96	1148.11	1158.74	1195.51	1233.44	1170.46	1150.98	1076.54	1019.43
JCAL25	1	1080.99	1142.69	1125.47	1074.14	1144.12	1142.55	1113.68	1089.25	1054.32	1075.88	1080.73	1085.56
JCAL26	1	1179.07	1134.13	1201.77	1245.88	1157.49	1153.75	1156.67	1242.79	1260.34	1188.95	1210.82	1223.65
JCAL27	2	1196.39	1133.88	1189.43	1155.01	1141.22	1140.78	1164.95	1177.43	1150.60	1229.69	1213.34	1202.15
JCAL28	2	1039.36	1120.29	1198.72	1142.06	1113.93	1138.33	1197.96	1182.06	1219.65	1221.98	1142.57	1190.62
JCAL29	1	1211.18	1199.60	1240.63	1214.80	1225.49	1243.85	1194.26	1138.36	1166.88	1156.60	1144.06	1182.19

Appendix 3 Table 26: Six months Pericortical vBMD by sector

ID	Group	0° - 10° [mg·cm³]	10° - 20° [mg·cm³]	20° - 30° [mg·cm³]	30° - 40° [mg·cm³]	40° - 50° [mg·cm³]	50° - 60° [mg·cm³]	60° - 70° [mg·cm³]	70° - 80° [mg·cm³]	80° - 90° [mg·cm³]	90° - 100° [mg·cm³]	100° - 110° [mg·cm³]	110° - 120° [mg·cm³]
JCAL01	1	1209.14	1241.02	1238.72	1222.09	1251.73	1186.19	1219.49	1230.28	1225.44	1232.36	1215.61	1228.09
JCAL02	1	1210.11	1228.55	1232.48	1229.30	1256.38	1235.11	1231.26	1232.21	1236.44	1250.82	1222.71	1249.12
JCAL03	2	1085.87	1120.37	1141.79	1083.96	1071.89	1151.38	1110.69	1152.27	1144.79	1166.99	1124.70	1149.54
JCAL04	2												
JCAL05	1												
JCAL06	2												
JCAL07	1	1191.68	1206.46	1258.99	1211.92	1262.36	1224.74	1246.54	1243.68	1193.18	1171.65	1218.99	1231.93
JCAL08	2	1212.86	1167.17	1184.33	1229.72	1253.23	1221.76	1167.57	1207.61	1182.20	1230.84	1233.32	1191.45
JCAL09	1												
JCAL10	2	1234.02	1246.21	1209.98	1180.42	1182.86	1232.58	1180.91	1186.11	1207.65	1299.58	1264.97	1254.11
JCAL11	2	1144.21	1224.82	1203.00	1267.01	1303.16	1269.14	1264.89	1216.33	1209.00	1208.43	1203.82	1185.35
JCAL12	1												
JCAL13	2												
JCAL14	1	1219.15	1194.97	1238.29	1311.23	1224.88	1238.02	1192.69	1206.33	1213.35	1226.65	1222.12	1222.69
JCAL15	2	1244.44	1242.93	1254.42	1234.34	1201.00	1200.69	1241.21	1258.80	1234.43	1200.15	1191.44	1242.76
JCAL16	1												
JCAL17	2	1206.29	1167.31	1264.37	1236.37	1351.20	1357.78	1247.66	1231.28	1289.42	1212.99	1103.73	1009.70
JCAL18	1	1213.39	1218.33	1182.88	1193.24	1227.61	1198.40	1228.55	1253.57	1215.36	1174.35	1186.76	1148.06
JCAL19	1												
JCAL20	2	1178.99	1178.05	1199.23	1202.72	1242.91	1230.00	1218.58	1216.22	1183.48	1223.64	1195.22	1182.09
JCAL21	2	1123.87	1223.12	1225.59	1152.80	1200.68	1169.08	1215.39	1190.79	1192.43	1168.27	1193.94	1214.17
JCAL22	2												
JCAL23	1												
JCAL24	1	1177.47	1174.11	1197.52	1226.58	1258.17	1203.44	1236.84	1177.13	1199.90	1185.48	1213.06	1196.87
JCAL25	1												
JCAL26	1	1183.31	1208.93	1238.09	1226.07	1240.11	1238.13	1211.06	1218.00	1251.89	1225.36	1187.41	1229.61
JCAL27	2	1229.69	1165.12	1225.88	1191.47	1181.24	1269.56	1221.99	1185.34	1182.08	1170.58	1195.12	1189.28
JCAL28	2												
JCAL29	1	1236.68	1209.63	1130.13	1244.51	1243.45	1217.28	1207.57	1217.60	1197.59	1212.17	1237.82	1239.06

Appendix 3 Table 26: Six months Pericortical vBMD by sector cont'd

ID	Group	120° - 130° [mg·cm³]	130° - 140° [mg·cm³]	140° - 150° [mg·cm³]	150° - 160° [mg·cm³]	160° - 170° [mg·cm³]	170° - 180° [mg·cm³]	180° - 190° [mg·cm³]	190° - 200° [mg·cm³]	200° - 210° [mg·cm³]	210° - 220° [mg·cm³]	220° - 230° [mg·cm³]	230° - 240° [mg·cm³]
JCAL01	1	1217.81	1230.34	1234.23	1268.82	1198.02	1157.00	1162.28	1188.37	1184.99	1191.47	1115.91	1122.83
JCAL02	1	1208.86	1219.90	1199.61	1201.03	1183.50	1169.97	1165.52	1205.87	1137.90	1193.97	1141.99	1120.05
JCAL03	2	1139.38	1121.34	1134.26	1117.43	1121.29	1054.96	1028.36	1008.48	1067.73	1083.87	1096.47	1137.68
JCAL04	2												
JCAL05	1												
JCAL06	2												
JCAL07	1	1221.74	1228.37	1183.57	1185.15	1181.37	1126.26	1087.35	1082.33	1135.31	1200.90	1222.14	1089.33
JCAL08	2	1183.77	1214.24	1196.33	1186.02	1201.37	1185.20	1176.06	1078.79	1082.81	1123.21	1070.70	1136.19
JCAL09	1												
JCAL10	2	1215.95	1191.23	1177.41	1182.42	1159.42	1173.90	1144.97	1195.63	1190.13	1206.85	1207.45	1208.78
JCAL11	2	1131.44	1111.82	1118.83	1119.59	1127.42	1109.44	1112.56	1085.56	1066.16	1104.83	1104.09	1176.96
JCAL12	1												
JCAL13	2												
JCAL14	1	1223.00	1232.67	1233.75	1217.44	1259.50	1189.23	1129.77	1136.31	1059.11	1110.56	1130.12	1248.12
JCAL15	2	1202.76	1208.29	1183.07	1173.30	1154.60	1173.34	1139.90	1181.46	1175.15	1200.51	1166.05	1195.39
JCAL16	1												
JCAL17	2	920.84	898.01	1046.26	1092.88	1152.29	1222.83	1059.52	876.45	941.78	1026.39	1018.55	1138.45
JCAL18	1	1165.23	1155.20	1168.98	1165.93	1178.06	1179.93	1135.15	1119.73	1145.14	1145.50	1168.88	1146.43
JCAL19	1												
JCAL20	2	1244.21	1211.04	1206.67	1154.19	1166.88	1173.61	1156.44	1137.36	1114.03	1157.90	1115.51	1126.84
JCAL21	2	1188.50	1155.77	1128.85	1167.46	1150.78	1147.71	1136.68	1147.04	1137.94	1202.21	1245.44	1185.33
JCAL22	2												
JCAL23	1												
JCAL24	1	1202.20	1172.30	1169.76	1156.53	1144.44	1113.72	1080.58	1141.24	1101.07	1127.85	1129.89	1135.78
JCAL25	1												
JCAL26	1	1182.78	1198.27	1167.87	1137.48	1142.04	1128.14	1115.54	1118.59	1126.93	1174.39	1149.96	1152.88
JCAL27	2	1202.25	1186.79	1205.26	1157.86	1148.13	1115.71	1082.62	1158.87	1150.79	1155.88	1216.60	1172.08
JCAL28	2												
JCAL29	1	1222.05	1216.24	1170.73	1145.30	1160.21	1155.49	1186.18	1131.25	1134.03	1097.28	1073.07	1122.71

Appendix 3 Table 26: Six months Pericortical vBMD by sector cont'd

ID	Group	240° - 250° [mg·cm³]	250° - 260° [mg·cm³]	260° - 270° [mg·cm³]	270° - 280° [mg·cm³]	280° - 290° [mg·cm³]	290° - 300° [mg·cm³]	300° - 310° [mg·cm³]	310° - 320° [mg·cm³]	320° - 330° [mg·cm³]	330° - 340° [mg·cm³]	340° - 350° [mg·cm³]	350° - 360° [mg·cm³]
JCAL01	1	1141.13	1145.63	1196.06	1224.20	1278.69	1253.33	1232.12	1213.67	1247.53	1169.68	1184.69	1206.05
JCAL02	1	1168.97	1170.04	1145.00	1193.44	1244.48	1247.47	1204.17	1216.33	1187.29	1204.94	1215.54	1219.26
JCAL03	2	1117.37	1135.47	1168.13	1157.69	1176.69	1165.53	1198.43	1198.86	1142.32	1141.52	1134.69	1086.23
JCAL04	2												
JCAL05	1												
JCAL06	2												
JCAL07	1	1030.31	1082.54	1132.40	1148.43	1258.20	1278.87	1174.81	1166.56	1191.34	1253.16	1230.65	1255.10
JCAL08	2	1199.70	1262.99	1250.68	1302.02	1284.16	1218.95	1229.39	1242.33	1196.31	1142.98	1156.74	1240.99
JCAL09	1												
JCAL10	2	1198.27	1213.25	1284.83	1267.83	1251.00	1210.13	1238.13	1197.93	1249.51	1271.58	1271.68	1212.54
JCAL11	2	1155.64	1206.62	1219.82	1259.86	1244.05	1206.56	1224.70	1226.77	1259.61	1254.28	1185.48	1179.11
JCAL12	1												
JCAL13	2												
JCAL14	1	1287.97	1284.94	1319.02	1284.74	1210.91	1237.78	1231.54	1179.40	1178.90	1248.89	1209.68	1183.30
JCAL15	2	1223.09	1220.02	1258.21	1235.56	1232.14	1200.57	1198.44	1229.17	1245.50	1216.95	1189.99	1187.57
JCAL16	1												
JCAL17	2	1156.79	1188.90	1199.59	1223.54	1149.58	1169.48	1198.32	1123.99	1104.19	1050.84	1066.35	1174.30
JCAL18	1	1166.11	1178.70	1146.22	1206.07	1209.50	1188.56	1170.31	1248.70	1225.46	1201.55	1220.63	1240.60
JCAL19	1												
JCAL20	2	1126.50	1172.22	1232.31	1205.51	1266.17	1192.50	1209.33	1212.27	1131.64	1154.38	1212.65	1195.69
JCAL21	2	1204.85	1234.97	1213.60	1229.94	1195.26	1141.03	1200.32	1195.79	1219.15	1184.69	1259.64	1236.32
JCAL22	2												
JCAL23	1												
JCAL24	1	1144.78	1141.16	1173.98	1155.24	1210.74	1236.85	1248.52	1218.65	1179.93	1162.47	1202.84	1222.97
JCAL25	1												
JCAL26	1	1172.36	1200.98	1187.02	1197.90	1184.27	1225.67	1205.54	1240.80	1230.45	1237.27	1239.83	1194.31
JCAL27	2	1198.10	1162.05	1145.22	1201.18	1261.71	1122.28	1213.63	1176.77	1205.26	1178.46	1258.66	1275.09
JCAL28	2												
JCAL29	1	1138.25	1162.36	1141.14	1154.70	1185.41	1244.51	1262.81	1202.33	1209.39	1241.38	1168.16	1150.57

Appendix 4: Information statement

INFORMATION LETTER TO JOCKEYS
BONE DENSITY STUDY BEING UNDERTAKEN BY
AUSTRALIAN CATHOLIC UNIVERSITY

Dear Participant,

The Racing Industry and the Jockeys' Associations have approved this study to be undertaken by the Australian Catholic University [ACU] representatives Dr David Greene and Ms Leslie Silk. This study was started last year in NSW but could not be completed due to difficulties with equipment and apprentice attendance for testing. The study has the potential to confirm that there is a means to reduce the risk of bone breakage in falls by increasing bone density through the minor intervention of taking a calcium and vitamin D supplement. This study has also been approved by the Human Research Ethics Committee at Australian Catholic University.

You are invited to participate in this study profiling how bone structural properties change with calcium and vitamin-D supplementation over a 6-month period. The purpose is to look for changes in bone structural properties on two occasions:
At baseline, and After 6-months.

The study will also monitor reports of injury to see if there are any links between changes in bone structural properties and either injury incidence or injury prevention.

There is a minimal commitment on your behalf, when balanced against the potential benefits outlined above. You will be asked to take four (4) tablets per day for a period of 6-months. You will not be aware if the tablets contain the active ingredients of calcium and vitamin D or if you are taking a placebo. This will be revealed after the study has finished. You will be asked to complete a short (15 minute) questionnaire about your dietary habits, current lifestyle, and current level of physical activity. Then there will be a bone scan using peripheral quantitative computed tomography (pQCT). In addition, a 10 ml blood sample and a urine sample will be taken. The scan and related activity will take place at your Education & Training establishment; this should take no more than 40 minutes.

The timing of the baseline and follow up testing will be co-ordinated with your Training and Education program, so that any disruption to your routine is minimised. It is anticipated that this will commence in November 2013.

You should know that you are free to refuse consent altogether without having to justify that decision or to withdraw consent and discontinue participation in the study at any time without giving a reason. Withdrawal from the research will not prejudice your future as a jockey. Apprentices under the age of 18 will be required to have their parent or guardian sign a consent form to comply with legislative requirements.

Taking part in the study is likely to benefit you by having access to a hard-copy report of your own results. Because this is a research project, you should also know that results may be published or presented in scientific forums. However, data collected will be de-identified by giving you a number not a name, and number codes will only be known to the researcher. You have our assurance that individual data that could identify single participants will not be disclosed, because only group averages will be used for reports and publications. Furthermore, the Australian Racing Board (ARB) will not be aware of your participation in the project, nor will individual results be provided to the ARB.

If you have any questions regarding this project, they should be directed to either your Training & Education Co-ordinator or the NSWJA [Paul Innes on 02 9894 9629] or the VJA [Des O'Keefe on 0412 554 155].

We look forward to working with you on this important research project and thank you for your participation.

Ms Melissa Weatherly
Athlete Development and Industry
Careers Advisor, RVL

Ms Robyn Parkinson
Project Manager, Australian Racing &
Equine Academy, TAFE NSW

Des O'Keefe,
EO, VJA

Paul Innes,
Sec NSWJA

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ATTACHMENT I – FURTHER DETAILS OF STUDY

Bone Scanning Equipment

Peripheral Quantitative Computed Tomography (pQCT) bone scan is an easy, painless test. The only preparation required is the removal of any pieces of clothing and accessories that contain metal or thick plastics. All you need to do is sit in a chair for approximately 10 minutes. While you sit in a chair, you will be asked to place your lower leg into the scanner where an x-ray beam is passed through your body. After the leg scan, you will be re-positioned so that your lower arm is placed in the scanner where an x-ray beam is passed through your body. The scan involves exposure to a very small amount of radiation. The Australian Radiation Protection and Nuclear Safety Agency's Guidelines (http://www.arpansa.gov.au/pubs/rps/rps8.pdf) require us to communicate the following statement:

"This research study involves exposure to a very small amount of radiation. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about 2 to 3 millisieverts (mSv) each year. The effective dose from this study is about 0.003 mSv. At this dose level, no harmful effects of radiation have been demonstrated and the risk is negligible."

Appendix 5: Informed consent

TITLE OF PROJECT: Calcium and Vitamin-D supplementation on bone structural properties in young male jockeys: A randomized controlled trial.

(NAME OF) PRINCIPAL INVESTIGATOR: Dr David Greene

(NAME OF) STUDENT RESEARCHER: Ms Leslie Silk

I ..... *(the participant)* have read and understood the information provided in the Letter to jockeys. Any questions I have asked have been answered to my satisfaction. I agree to participate in this research project requiring me to take four (4) tablets per day for a period of 6-months. I also agree to answer questions about my eating habits, injuries, current level of physical activity, to be scanned using a peripheral quantitative computed tomography device, and to provide approximately 10 ml of blood and 20 – 30 ml of urine on two occasions, realising that I can withdraw my consent at any time without adverse consequences. I am aware that ionising radiation will be used. This research study involves exposure to a very small amount of radiation. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about 2 to 3 millisieverts (mSv) each year. The effective dose from this study is about 0.003 mSv. At this dose level, no harmful effects of radiation have been demonstrated and the risk is negligible.

I agree that research data collected for the study may be published or may be provided to other researchers in a form that does not identify me in any way.

If 18 years of age and over:
NAME OF PARTICIPANT: _____

SIGNATURE: _____ DATE: _____

SIGNATURE OF PRINCIPAL INVESTIGATOR: _____
DATE:_____

SIGNATURE OF STUDENT RESEARCHER:_____
DATE: _____

If under 18 years:
NAME OF PARENT/GUARDIAN:_____

NAME OF CHILD:_____

SIGNATURE OF PARENT:_____ DATE: _____

Appendix 6: Lifestyle questionnaire

ACU
AUSTRALIAN CATHOLIC UNIVERSITY

Apprentice Jockey Study
Australian Catholic University

Name:_____

Best time on a week day to call (if we have to clarify any information provided):

☐ Morning ☐ Afternoon ☐ Night

1. For how many years have you been training in this sport?

☐ Less than 1 ☐ 1 to 2 ☐ 2 or more

2. On average, how many hours per week do you currently train for this sport? (include all track work and any extra training outside of riding)

☐ Less than 4 ☐ 4 to 5 ☐ 6 or more

3. Do you suffer from any recurrent (ongoing or repeating) injuries?

☐ Yes ☐ No

4. Have you suffered an injury in the past two weeks?

☐ Yes ☐ No

5. Are there any other reasons that may cause you to miss a training session or impair your performance during training?

☐ Yes ☐ No

If "yes", please explain:_____

_____

6. How many training sessions have you missed in the past month due to injury?

☐ 0 ☐ 1 to 2 ☐ 2 to 3 ☐ 4 or more

7. What do you typically drink just *prior to* training sessions?

☐ Nothing	☐ Water	☐ Sports Drink	☐ Soft Drink
☐ Fruit Juice	☐ Cordial	☐ Energy Drink	☐ Other

If "other", please specify: _____

8. What do you typically drink *during* training sessions?

☐	Nothing	☐	Water	☐	Sports Drink	☐	Soft Drink
☐	Fruit Juice	☐	Cordial	☐	Energy Drink	☐	Other

If "other", please specify: _____

9. Are you currently participating in any organised sport or physical activity outside of riding?

☐ Yes ☐ No

If "yes", what is the sport or activity? _____

If "no", please go to question 11.

10. How many hours per week do you currently spend training for this sport or activity that is different of riding?

☐ 2 to 3 ☐ 4 to 5 ☐ 6 or more

11. Are you currently taking any medication(s) or supplement(s)?

☐ Yes ☐ No

If "yes", please list them:_____

_____

_____

12. Do you smoke?

☐ Yes ☐ No

If "yes", how many cigarettes per day?:_____

13. Do you drink alcohol?

☐ Yes ☐ No

If "yes", how often do you drink alcohol per week?_____

14. On average, how many hours sleep do you get per night? _____

15. Have you ever broken any bones?

☐ Yes ☐ No

If "yes", what bones were broken? _____

_____

16. Did the injury that caused a broken bone occur from riding?

☐ Yes ☐ No

Thank you for your participation!

Appendix 7: DQES form

Dietary Questionnaire

QUESTIONS ABOUT WHAT YOU USUALLY EAT AND DRINK

Please fill in the date you completed this questionnaire:

DAY	MTH	YEAR
	○ JAN	○ 2004
	○ FEB	○ 2005
0 0	○ MAR	○ 2006
1 1	○ APR	○ 2007
2 2	○ MAY	○ 2008
3 3	○ JUN	○ 2009
	4 ○ JUL	○ 2010
	5 ○ AUG	○ 2011
	6 ○ SEP	○ 2012
	7 ○ OCT	○ 2013
	8 ○ NOV	○ 2014
	9 ○ DEC	○ 2015

INSTRUCTIONS:

This questionnaire is about your **usual** eating habits **over the past 12 months**. Where possible give only **one answer per question** for the type of food you eat **most often.**
(If you can't decide which type you have most often, answer for the types you usually eat.)

- Use a soft pencil only, preferably 2B.
- Do not use **any** biro or felt tip pen.
- Erase mistakes fully.
- Make no stray marks.

Please MARK LIKE THIS: ○●○○

1. How many pieces of fresh fruit do you usually eat per day? *(Count 1/2 cup of diced fruit, berries or grapes as one piece.)*

- ○ I didn't eat fruit
- ○ less than 1 piece of fruit per day
- ○ 1 piece of fruit per day
- ○ 2 pieces of fruit per day
- ○ 3 pieces of fruit per day
- ○ 4 or more pieces of fruit per day

2. How many different vegetables do you usually eat per day? *(Count all types, fresh, frozen or tinned.)*

- ○ less than 1 vegetable per day
- ○ 1 vegetable per day
- ○ 2 vegetables per day
- ○ 3 vegetables per day
- ○ 4 vegetables per day
- ○ 5 vegetables per day
- ○ 6 or more vegetables per day

3. What type of milk do you usually use?

- ○ none
- ○ full cream milk
- ○ reduced fat milk
- ○ skim milk
- ○ soya milk

4. How much milk do you usually use per day? *(Include flavoured milk and milk added to tea, coffee, cereal, etc.)*

- ○ none
- ○ less than 250 ml *(1 large cup or mug)*
- ○ between 250 and 500 ml *(1-2 cups)*
- ○ between 500 and 750 ml *(2-3 cups)*
- ○ 750 ml *(3 cups)* or more

5. What type of bread do you usually eat?

- ○ I don't eat bread
- ○ high fibre white bread
- ○ white bread
- ○ wholemeal bread
- ○ rye bread
- ○ multi-grain bread

6. How many slices of bread do you usually eat per day? *(Include all types, fresh or toasted and count one bread roll as 2 slices.)*

- ○ less than 1 slice per day
- ○ 1 slice per day
- ○ 2 slices per day
- ○ 3 slices per day
- ○ 4 slices per day
- ○ 5-7 slices per day
- ○ 8 or more slices per day

7. Which spread do you usually put on bread?

- ○ I don't usually use any fat spread
- ○ margarine of any kind
- ○ polyunsaturated margarine
- ○ monounsaturated margarine
- ○ butter and margarine blends
- ○ butter

8. On average, how many teaspoons of sugar do you usually use per day? *(Include sugar taken with tea and coffee and on breakfast cereal, etc.)*

- ○ none
- ○ 1 to 4 teaspoons per day
- ○ 5 to 8 teaspoons per day
- ○ 9 to 12 teaspoons per day
- ○ more than 12 teaspoons per day

9. On average, how many eggs do you usually eat per week?

- ○ I don't eat eggs
- ○ less than 1 egg per week
- ○ 1 to 2 eggs per week
- ○ 3 to 5 eggs per week
- ○ 6 or more eggs per week

10. What types of cheese do you usually eat?

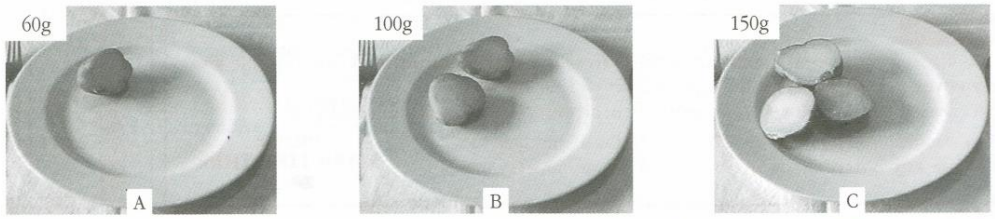
- ○ I don't eat cheese
- ○ hard cheeses, e.g. parmesan, romano
- ○ firm cheeses, e.g. cheddar, edam
- ○ soft cheeses, e.g. camembert, brie
- ○ ricotta or cottage cheese
- ○ cream cheese
- ○ low fat cheese

75515

DO NOT WRITE IN THIS AREA.

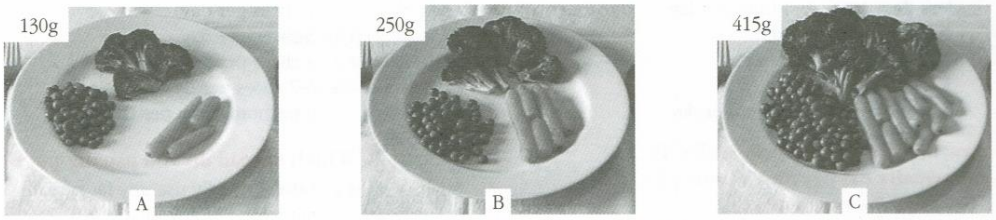
For each food shown on this page, indicate ***how much on average you would usually have eaten at main meals during the past 12 months.*** *When answering each question, think of the* ***amount*** *of that food you usually ate, even though you may rarely have eaten the food on its own.*
If you usually ate more than one helping, fill in the oval for the serving size closest to the ***total amount*** *you ate.*

11. When you ate potato, did you usually eat: ○ I never ate potato



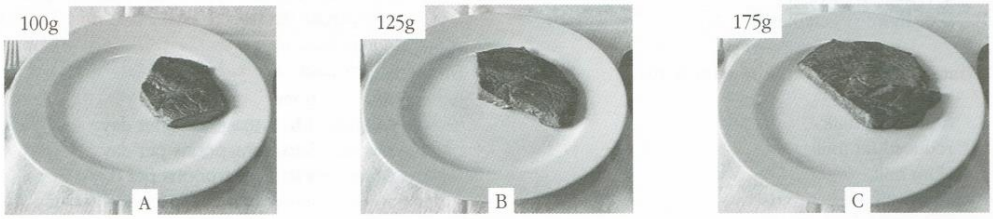
○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

12. When you ate vegetables, did you usually eat: ○ I never ate vegetables



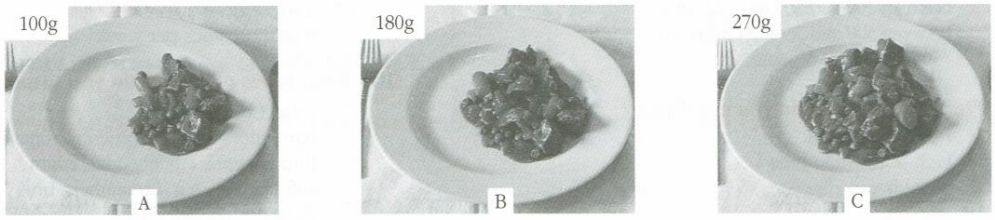
○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

13. When you ate steak, did you usually eat: ○ I never ate steak



○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

14. When you ate meat *or* vegetable casserole, did you usually eat: ○ I never ate casserole



○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

15. Over the last 12 months, on average, *how often* did you eat the following foods? *Please completely fill one oval in every line.*

Please MARK LIKE THIS: ⬭ NOT LIKE THIS: ✓ ✗ ⊖ ◯

Times You Have Eaten		NEVER	less than once	1 to 3 times	1 time	2 times	3 to 4 times	5 to 6 times	1 time	2 times	3 or more times
			per month		per week				per day		
CEREAL FOODS, SWEETS & SNACKS											
All Bran™	A1	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Sultana Bran™, FibrePlus™, Branflakes™	A2	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Weet Bix™, Vita Brits™, Weeties™	A3	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Cornflakes, Nutrigrain™, Special K™	A4	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Porridge	A5	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Muesli	A6	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Rice	A7	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Pasta or noodles (include lasagne)	A8	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Crackers, crispbreads, dry biscuits	A9	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Sweet biscuits	A10	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Cakes, sweet pies, tarts and other sweet pastries	A11	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Meat pies, pasties, quiche and other savoury pastries	A12	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Pizza	A13	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Hamburger with a bun	A14	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Chocolate	A15	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Flavoured milk drink (cocoa, Milo™, etc.)	A16	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Nuts	A17	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Peanut butter or peanut paste	A18	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Corn chips, potato crisps, Twisties™, etc.	A19	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Jam, marmalade, honey or syrups	A20	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Vegemite™, Marmite™ or Promite™	A21	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
DAIRY PRODUCTS, MEAT & FISH											
Cheese	B1	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Ice-cream	B2	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Yoghurt	B3	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Beef	B4	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Veal	B5	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Chicken	B6	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Lamb	B7	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Pork	B8	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Bacon	B9	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Ham	B10	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Corned beef, luncheon meats or salami	B11	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Sausages or frankfurters	B12	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Fish, steamed, grilled or baked	B13	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Fish, fried (include take-away)	B14	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Fish, tinned (salmon, tuna, sardines, etc.)	B15	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
FRUIT											
Tinned or frozen fruit (any kind)	C1	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Fruit juice	C2	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Oranges or other citrus fruit	C3	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Apples	C4	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Pears	C5	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Bananas	C6	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Watermelon, rockmelon (cantaloupe), honeydew, etc.	C7	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Pineapple	C8	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Strawberries	C9	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Apricots	C10	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Peaches or nectarines	C11	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Mango or paw paw	C12	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Avocado	C13	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭

Times You Have Eaten CONTINUED

		NEVER	less than once per month	1 to 3 times per month	1 time per week	2 times per week	3 to 4 times per week	5 to 6 times per week	1 time per day	2 times per day	3 or more times per day
VEGETABLES (INCLUDING FRESH, FROZEN AND TINNED)											
Potatoes, roasted or fried (include hot chips)	D1	○	○	○	○	○	○	○	○	○	○
Potatoes cooked without fat	D2	○	○	○	○	○	○	○	○	○	○
Tomato sauce, tomato paste or dried tomatoes	D3	○	○	○	○	○	○	○	○	○	○
Fresh or tinned tomatoes	D4	○	○	○	○	○	○	○	○	○	○
Peppers (capsicum)	D5	○	○	○	○	○	○	○	○	○	○
Lettuce, endive, or other salad greens	D6	○	○	○	○	○	○	○	○	○	○
Cucumber	D7	○	○	○	○	○	○	○	○	○	○
Celery	D8	○	○	○	○	○	○	○	○	○	○
Beetroot	D9	○	○	○	○	○	○	○	○	○	○
Carrots	D10	○	○	○	○	○	○	○	○	○	○
Cabbage or Brussels sprouts	D11	○	○	○	○	○	○	○	○	○	○
Cauliflower	D12	○	○	○	○	○	○	○	○	○	○
Broccoli	D13	○	○	○	○	○	○	○	○	○	○
Silverbeet or spinach	D14	○	○	○	○	○	○	○	○	○	○
Peas	D15	○	○	○	○	○	○	○	○	○	○
Green beans	D16	○	○	○	○	○	○	○	○	○	○
Bean sprouts or alfalfa sprouts	D17	○	○	○	○	○	○	○	○	○	○
Baked beans	D18	○	○	○	○	○	○	○	○	○	○
Soy beans, soy bean curd or tofu	D19	○	○	○	○	○	○	○	○	○	○
Other beans (include chick peas, lentils, etc.)	D20	○	○	○	○	○	○	○	○	○	○
Pumpkin	D21	○	○	○	○	○	○	○	○	○	○
Onion or leeks	D22	○	○	○	○	○	○	○	○	○	○
Garlic (not garlic tablets)	D23	○	○	○	○	○	○	○	○	○	○
Mushrooms	D24	○	○	○	○	○	○	○	○	○	○
Zucchini	D25	○	○	○	○	○	○	○	○	○	○

16. **Over the last 12 months, how often did you drink beer, wine and/or spirits?**

Times That You Drank

		NEVER	less than once a month	1-3 days per month	1 day per week	2 days per week	3 days per week	4 days per week	5 days per week	6 days per week	every day
Beer (low alcohol)	1	○	○	○	○	○	○	○	○	○	○
Beer (full strength)	2	○	○	○	○	○	○	○	○	○	○
Red wine	3	○	○	○	○	○	○	○	○	○	○
White wine (include sparkling wines)	4	○	○	○	○	○	○	○	○	○	○
Fortified wines, port, sherry, etc.	5	○	○	○	○	○	○	○	○	○	○
Spirits, liqueurs, etc.	6	○	○	○	○	○	○	○	○	○	○

When answering the next two questions, please convert the amounts you drank into glasses using the examples given below. For spirits, liqueurs, and mixed drinks containing spirits, please count each nip (30 ml) as one glass.

1 can or stubby of beer = 2 glasses
1 large bottle beer (750 ml) = 4 glasses
1 bottle wine (750 ml) = 6 glasses
1 bottle of port or sherry (750 ml) = 12 glasses

17. **Over the last 12 months, on days when you were drinking, how many glasses of beer, wine and/or spirits altogether did you *usually* drink?**

TOTAL NUMBER OF GLASSES PER DAY	1	2	3	4	5	6	7	8	9	10 or more
	○	○	○	○	○	○	○	○	○	○

18. **Over the last 12 months, what was the *maximum* number of glasses of beer, wine and/or spirits that you drank in 24 hours?**

MAXIMUM NUMBER OF GLASSES PER 24 HOURS	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19 or more
	○	○	○	○	○	○	○	○	○	○

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Thank You for completing this questionnaire

DO NOT WRITE IN THIS AREA.

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Appendix 8: Anthropometric Assessment Pro Forma

ANTHROPOMETRIC PROFORMA - APPRENTICE JOCKEY CALCIUM STUDY

Subject Name (first, last) _____ Subject ID#

	Day	Month	Year		
Date of Measurement				Measurer	
Date of Birth				Recorder	

	First measure	Second measure	Third measure
Body mass			
Stretch stature			
Sitting Height			
Triceps sf			
Subscapular sf			
Supraspinale sf			
Abdominal sf			
Front Thigh sf			
Medial Calf sf			
Head girth			
Arm girth relaxed			
Arm girth flexed and tensed			
Forearm girth (max. relaxed)			
Wrist girth (distal styloid)			
Chest girth (mesosternale)			
Waist girth (min.)			
Gluteal girth (max.)			
Thigh girth (1 cm dist. glut. line)			
Thigh girth (mid tro-tib lat)			
Calf girth (max.)			
Ankle girth (min.)			
Biacromial breadth			
Biiliocristal breadth			
Transverse chest breadth			
A-P Chest depth			
Humerus breadth (biepicondylar)			
Wrist breadth			
Femur breadth (biepicondylar)			
Ankle breadth			

Appendix 9: PRISMA Checklist for Systematic Review and Meta-Analysis

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	48
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	48
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	49
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	50-51
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	51
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	52
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	52
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	52
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	52-53

Section/topic	#	Checklist item	Reported on page #
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	53
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	52
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	53
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	53
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	53
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	54
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	54
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	56-65
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	56
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	65-68
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	67-68
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	55
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	67-68
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	68-70

Section/topic	#	Checklist item	Reported on page #
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	71-72
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	72-73
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	No funding received

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

Appendix 10: Consort Statement

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
TITLE AND ABSTRACT			
	1a	Identification as a randomised trial in the title	111
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	111
INTRODUCTION			
Background and objectives	2a	Scientific background and explanation of rationale	112-114
	2b	Specific objectives or hypotheses	114
METHODS			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	116
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	N/A
Participants	4a	Eligibility criteria for participants	115-116
	4b	Settings and locations where the data were collected	115
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	116
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	117-119
	6b	Any changes to trial outcomes after the trial commenced, with reasons	N/A
Sample size	7a	How sample size was determined	120
	7b	When applicable, explanation of any interim analyses and stopping guidelines	N/A
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	116
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	116
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	116
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	116
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	116

Section/Topic	Item No	Checklist item	Reported on page No
		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	N/A
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	120
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	120
RESULTS			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	78 (fig 4.1)
	13b	For each group, losses and exclusions after randomisation, together with reasons	120
Recruitment	14a	Dates defining the periods of recruitment and follow-up	120
	14b	Why the trial ended or was stopped	N/A
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	121
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	121
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	120-126
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	N/A
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	N/A
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	N/A
DISCUSSION			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	132
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	133
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	127-132
Other information			
Registration	23	Registration number and name of trial registry	116
Protocol	24	Where the full trial protocol can be accessed, if available	N/A
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	134

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.