Vitamin D Status and Performance in Semi-Professional Male Rugby Union Players: A Cross-Sectional Analysis

Lisa Daniels

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Abstract

Background: Low vitamin D status is common amongst the athletic population internationally. Sufficient vitamin D is important for bone health and recent research also suggests an importance for physical performance, with associations found between serum 25-hydroxyvitamin D concentrations and muscle strength and physical performance in healthy populations. However, these associations have not been assessed in New Zealand athletes.

Objectives: The objectives were to 1) evaluate the vitamin D status of semi-professional male rugby union players in Otago and Southland, New Zealand; 2) within this group, to assess associations between serum 25-hydroxyvitamin D concentrations and specific athletic performance measures; and 3) to identify potential predictors of serum 25-hydroxyvitamin D concentrations in this group of New Zealand athletes.

Design: Cross-sectional secondary data analysis of a randomised, placebo-controlled, double-blinded intervention study.

Methods: Fifty-seven semi-professional male rugby union players residing in Otago and Southland, New Zealand (latitude: 45-47° S) completed baseline measures including: a demographic questionnaire, sun exposure questionnaire, serum 25-hydroxyvitamin D analysis and standard New Zealand Rugby Union performance tests during the months of autumn (March to May), 2011.

Results: The mean serum 25-hydroxyvitamin D concentration was 94 nmol/L (range, 57-131 nmol/L). No participant had a serum 25-hydroxyvitamin D concentration of <50 nmol/L. There were no significant associations between serum 25-hydroxyvitamin D concentrations and the specific athletic performance measures after adjusting for body mass and training group: Yo-Yo Intermittent Recovery Test ($p = 0.36$), 30m sprint ($p = 0.11$), predicted one repetition maximum (1RM) bench pull ($p = 0.09$), predicted 1RM weighted reverse-grip chin-up ($p = 0.11$) and predicted 1RM bench pull ($p = 0.14$). Those of self-identified Pacific ethnicity had significantly lower serum 25-hydroxyvitamin D concentrations compared to New Zealand European ($p <0.001$) and Māori ($p = 0.003$). Looking at potential predictors of
serum 25-hydroxyvitamin D concentrations including: sun exposure, body mass, BMI, percent body fat, ethnicity and sunscreen use, self-identified Pacific ethnicity was a predictor of serum 25-hydroxyvitamin D concentrations \( (p = 0.001) \). The final regression model of the aforementioned potential predictors of serum 25-hydroxyvitamin D concentrations explained 34\% \( (p = 0.01) \) of the variability in circulating 25-hydroxyvitamin D.

**Conclusion:** This study concludes that this group of semi-professional male rugby union players from the southernmost parts of New Zealand (Otago and Southland) were vitamin D replete during autumn, based on the current New Zealand Ministry of Health cut off of >50 nmol/L. There were no associations between serum 25-hydroxyvitamin D concentrations and the specific measures of athletic performance, suggesting that athletic performance is not associated with circulating 25-hydroxyvitamin D. Participants of self-identified Pacific ethnicity had significantly lower serum 25-hydroxyvitamin D concentrations compared to those of self-identified New Zealand European and Māori ethnicities. Self-identified Pacific ethnicity was a significant predictor of serum 25-hydroxyvitamin D concentrations.

**Key Words:** 25(OH)D, elite athletes, New Zealand, predictors, sun exposure
Preface

A Randomised Controlled Trial (RCT) titled “Vitamin D Status and Effect of Supplementation on Strength, Speed and Body Composition in Rugby Union Players” was conducted in the Department of Human Nutrition at the University of Otago, Dunedin, New Zealand during 2011. Dr Kirsty Fairbairn and Dr Tracy Perry were responsible for the overall design of the study, conducting the research and obtaining ethical approval.

The initial study aim for the current candidate Lisa Daniels (LD) was to examine secondary data from the above study and determine whether vitamin D status is associated with serum insulin-like growth factor-1 (IGF-1) concentrations, in both cross-sectional and longitudinal analyses (following 10 weeks of vitamin D supplementation) in male rugby union players. LD completed the literature review on this topic; however, the study was unable to be carried out due to the funding application for the analysis of IGF-1 being unsuccessful.

The new topic conceived for this thesis consisted of cross-sectional secondary analysis of vitamin D status in male rugby union players, from the same previously completed RCT. Therefore; the candidate (LD) was responsible for the following work:

- Cleaning up baseline data ready for analysis.
- Conducting and interpreting all statistical analyses using SPSS (version 21), with advice from Dr Jill Haszard (Biostatistician, Department of Human Nutrition, University of Otago) and Claire Cameron (Biostatistician, Department of Preventative and Social Medicine, University of Otago).
- Drafting an approval letter for the inclusion of ethnicity data in this thesis. Sent to the ethics committee on April 5th, 2013.
- Writing up of this thesis.
- Primary responsibility for the final content of this thesis.

The candidate presented aspects of this thesis at the Department of Human Nutrition seminar on May 30th, 2013.
Acknowledgements

Thank you to the following people, who helped me to enjoy research and made this thesis possible:

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Dr Tracy Perry for your experience, guidance and support and for having your door always open for me when things were tough.

Dr Jill Haszard for your advice and support with my statistics and for being very understanding with my nature to ensure everything is perfect.

The Participants, although your involvement was long before I even knew this was my thesis topic, thank-you for partaking in this study.

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>1RM</td>
<td>One repetition maximum</td>
</tr>
<tr>
<td>1,25(OH)$_2$D</td>
<td>1,25-dihydroxyvitamin D</td>
</tr>
<tr>
<td>25(OH)D</td>
<td>25-hydroxyvitamin D</td>
</tr>
<tr>
<td>AI</td>
<td>Adequate intake</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ANS08/09</td>
<td>2008/09 New Zealand Adult Nutrition Survey</td>
</tr>
<tr>
<td>BMC</td>
<td>Bone mineral content</td>
</tr>
<tr>
<td>BMD</td>
<td>Bone mineral density</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CLIA</td>
<td>Chemiluminescence immunoassay</td>
</tr>
<tr>
<td>CPBA</td>
<td>Competitive protein binding assay</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual-energy X-ray absorptiometry</td>
</tr>
<tr>
<td>EAR</td>
<td>Estimated average requirement</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Insulin-like growth factor -1</td>
</tr>
<tr>
<td>IOM</td>
<td>Institute of Medicine</td>
</tr>
<tr>
<td>ITM Cup</td>
<td>Independent Merchants Co-operative Ltd. Cup (highest level of New Zealand domestic professional rugby union competition)</td>
</tr>
<tr>
<td>IU</td>
<td>International units</td>
</tr>
<tr>
<td>Kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>Kg/m$^2$</td>
<td>Kilogram per square metre</td>
</tr>
<tr>
<td>L</td>
<td>litre</td>
</tr>
<tr>
<td>LC-MS/MS</td>
<td>Liquid chromatography tandem mass spectrometry</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>LD</td>
<td>Lisa Daniels</td>
</tr>
<tr>
<td>M</td>
<td>Mean</td>
</tr>
<tr>
<td>mL</td>
<td>mililitre</td>
</tr>
<tr>
<td>MoH</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>ng</td>
<td>nanogram</td>
</tr>
<tr>
<td>nmol</td>
<td>nanomol</td>
</tr>
<tr>
<td>NHRMC</td>
<td>National Health and Research Medical Council</td>
</tr>
<tr>
<td>NNS97</td>
<td>1997 New Zealand National Nutrition Survey</td>
</tr>
<tr>
<td>NRV</td>
<td>Nutrient reference value</td>
</tr>
<tr>
<td>NZ European</td>
<td>New Zealand European</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomised controlled trial</td>
</tr>
<tr>
<td>RDI</td>
<td>Recommended dietary intake</td>
</tr>
<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SPF</td>
<td>Sun protection factor</td>
</tr>
<tr>
<td>SZA</td>
<td>Solar zenith angle</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>UVA</td>
<td>Ultraviolet A</td>
</tr>
<tr>
<td>UVB</td>
<td>Ultraviolet B</td>
</tr>
<tr>
<td>VDR</td>
<td>Vitamin D receptor</td>
</tr>
<tr>
<td>VDRE</td>
<td>Vitamin D response element</td>
</tr>
<tr>
<td>Yo-Yo test</td>
<td>Yo-Yo Intermittent Recovery Test</td>
</tr>
<tr>
<td>µg</td>
<td>microgram</td>
</tr>
<tr>
<td>µL</td>
<td>microlitre</td>
</tr>
</tbody>
</table>
## Glossary

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>Months of March, April and May.</td>
</tr>
<tr>
<td>Baseline</td>
<td>Initial time period of the study (point of reference), in this thesis the months of March, April and May.</td>
</tr>
<tr>
<td>Serum 25(OH)D</td>
<td>Serum 25-hydroxyvitamin D concentrations from blood analyses. Measured in nanomols per litre (nmol/L).</td>
</tr>
<tr>
<td>Ultraviolet (UV)</td>
<td>Electromagnetic radiation of wavelengths between 5 to 400 nm.</td>
</tr>
<tr>
<td>Ultraviolet B (UVB)</td>
<td>Medium wavelengths of between 280 to 320 nm. Assists with synthesis of vitamin D₃.</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>A group of secosterols, referred to as calciferol. Both vitamin D₂ and Vitamin D₃ are the major forms.</td>
</tr>
<tr>
<td>Vitamin D₂</td>
<td>Ergocalciferol, found in plant extracts.</td>
</tr>
<tr>
<td>Vitamin D₃</td>
<td>Cholecalciferol, found in very small amounts in animal-based foods and the main source is through skin synthesis from 7-dehydrocholesterol after UV exposure.</td>
</tr>
</tbody>
</table>

### Defining vitamin D status

<table>
<thead>
<tr>
<th>Status</th>
<th>Serum 25-hydroxyvitamin D concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficient vitamin D status</td>
<td>&lt; 25 nmol/L</td>
</tr>
<tr>
<td>Insufficient vitamin D status</td>
<td>&lt; 50 nmol/L</td>
</tr>
<tr>
<td>Sufficient/replete vitamin D status</td>
<td>&gt; 50 nmol/L</td>
</tr>
</tbody>
</table>
1 Introduction

Vitamin D is required for mineral homeostasis of calcium and phosphate, and optimal bone health (1). Vitamin D availability from food sources is scarce, as very few foods naturally contain vitamin D and fortification of vitamin D in foods in New Zealand is not mandated (2). Therefore, the major source of vitamin D is sun exposure (3). Vitamin D is produced in the skin through the conversion of 7-dehydrocholesterol to cholecalciferol when sun exposure is adequate (4). However, when there is little sun exposure, this leads to a reduction in cutaneous synthesis of vitamin D and may cause deficiency. Circulating 25-hydroxyvitamin D (25(OH)D) concentration is the best indicator of vitamin D status (5) and is influenced by many factors other than sun exposure, including: geographic location, season, skin pigmentation, age, body composition and food intake.

To date, there has been little global agreement on what constitutes sufficient vitamin D status, as well as the cut-offs for defining vitamin D deficiency, optimal vitamin D status and toxicity. Some international researchers suggest a 25(OH)D concentration of 75 nmol/L is sufficient (6,7). The New Zealand Ministry of Health recommends that individuals have a circulating 25(OH)D concentration of at least 50 nmol/L (8). It is unknown whether the cut-offs for defining vitamin D status in athletes are different to the general public and whether there is an optimal vitamin D status for athletes for musculoskeletal performance.

There is a considerable amount of research demonstrating a high prevalence of vitamin D insufficiency (using the current Ministry of Health cut-off of <50 nmol/L) in both indoor and outdoor athletes internationally (9-14). These reports suggest that those at greatest risk of vitamin D insufficiency are athletes who: receive limited amounts of sun exposure either during the winter season or by training indoors, live at high latitudes, have dark skin pigmentation or have a high percent body fat.

Cannell et al. (15) cites that, in the early 20th century it was believed that training under artificial ultraviolet B (UVB) radiation was beneficial to athletic performance (15). Since then,
and with the recent identification of the vitamin D receptor (VDR) on human muscle cells (16), several review papers have aimed to investigate the relationship between vitamin D and muscle tissue and therefore, whether vitamin D influences athletic performance (15,17-19). To date, there is a paucity of evidence supporting the assumption that higher circulating 25(OH)D concentrations improves performance in athletes (20,21).

Most of the current research on physical performance is in elderly populations, demonstrating that those with low 25(OH)D concentrations perform less well in measures of physical performance than those with higher 25(OH)D concentrations (22-24). Little research has been conducted to determine whether there is an association between low 25(OH)D concentrations and reduced muscle strength or performance in athletes and if there is, which specific performance variables are likely to be associated with circulating 25(OH)D concentrations (strength, speed, endurance or reaction time).

Athletes primary concern is their performance. Currently, athletes worldwide perceive nutritional supplements to have performance enhancing effects (25). However, many still believe that a balanced diet will maintain a nutritionally adequate diet (25). Therefore, it is important to determine any beneficial effect of higher 25(OH)D concentrations on athletic performance before any initiative to enhance the vitamin D status of athletes is deemed necessary.

The purpose of the current study is to examine the vitamin D status of New Zealand athletes (rugby union players, who train both indoors and outdoors) on the basis of serum 25-hydroxyvitamin D concentrations and determine whether there is any association between serum 25(OH)D concentrations and athletic performance (including strength, speed, endurance and reaction time). Examining factors that influence serum 25(OH)D concentrations could additionally assist with predicting which athletes are most at risk of vitamin D deficiency or insufficiency.
2 Literature Review

2.1 Introduction

The aim of this literature review is to:

1) Define vitamin D nomenclature: sources, structure and function, including consequences of vitamin D deficiency or toxicity.

2) Examine whether there is any evidence of an association between vitamin D status and athletic performance.

3) Examine the different methods of vitamin D analysis.

4) Identify factors that influence serum 25(OH)D concentrations and report the current vitamin D status of New Zealanders and athletes.

5) Report the current recommendations for vitamin D intake in healthy adults, including athletes.

6) Investigate vitamin D intakes of the non-athletic and athletic population.

7) Introduce the sport of rugby union.

2.1.1 Literature Search Methods

Literature searches were regularly conducted between the time points of December 2012 and May 2013 and were performed using Medline (Ovid MEDLINE(R) 1946 to present with daily update), Web of Science (Web of knowledge), ScienceDirect and SPORTDiscus databases. Key terms used for searching were chosen based on the relevance of the topic, which were: vitamin D, vitamin D deficiency, athletes, vitamin D receptor, VDR, sports, muscle mass, sports performance, 25-hydroxyvitamin D, bone mineral density, healthy humans and vitamin D intake. Main keyword combinations were also used, which included the main term “Vitamin D” combined with terms; “athletes” OR “sports” OR “New Zealand” OR “performance” OR “muscle strength”.

No limits were applied to the literature search, although only articles written in the English language were referenced. Relevant references were also obtained from the reference lists of the articles retrieved.
Figure 2.2. Conversion of vitamin D$_2$ and D$_3$ in the body from sunlight and diet.
2.2 Vitamin D

2.2.1 Structure
Vitamin D is a fat-soluble vitamin with a secosteroid structure (4). There are two main forms of vitamin D: vitamin D\(_3\) and D\(_2\). They are metabolized in similar ways in humans, yet are structurally different (Figure 2.1) (26).

![Figure 2.1](image.png)

Figure 2.1. Chemical structure of vitamin D\(_3\) (cholecalciferol) and vitamin D\(_2\) (ergocalciferol) from Rao et al. (27).

Vitamin D\(_3\) (cholecalciferol) is produced in the skin after UVB radiation from the sun (4). The vitamin D precursor, 7-dehydrocholesterol (present in the skin) is converted into vitamin D\(_3\) with the action of direct sunlight (4). Once vitamin D\(_3\) enters the circulation it is converted to 25-hydroxyvitamin D (25(OH)D) in the liver (28). While 25(OH)D is the main circulating and storage form of vitamin D, it needs to be converted in the kidney to the biologically active form 1,25-dihydroxyvitamin D (1,25(OH)\(_2\)D), before functions of vitamin D can be carried out in the body (28). Vitamin D\(_2\) (ergocalciferol) from plant tissue is consumed in the diet in small amounts (as well as small amounts of vitamin D\(_3\)), which is then hydroxylated once in the circulation, to the active form of vitamin D following the same two processes in the body, as described above for vitamin D\(_3\) (Figure 2.2) (4). Although vitamin D\(_2\) is the more common dietary form, both vitamin D\(_2\) and vitamin D\(_3\) can be found in the diet through natural and fortified products (29).
2.2.2 Sources
There are three main sources of vitamin D (3,7,30):

1) Cutaneous synthesis of vitamin D from sun exposure.

2) Foods naturally containing vitamin D₃ (such as: fatty fish, for example: salmon, mackerel, sardines) and vitamin D₂ in plant extracts.

3) Fortification of food and/or supplementation of vitamin D.

2.2.3 Biological Function

2.2.3.1 Vitamin D Receptor
Vitamin D receptors (VDRs) are steroid hormone receptors, binding the active form of vitamin D (1,25(OH)₂D) with high affinity (7). The VDR mediates all functions of vitamin D via its DNA-binding domains and also acts through the vitamin D response elements (VDREs), for the regulation and expression of genes (31-33). VDRs are found in many of the body’s cells, as well as skeletal, heart and liver muscle (16,17,31,34,35). However, the expression of VDR in human skeletal muscle significantly decreases with age (36).

The expression of VDRs has recently been confirmed to have an important role during regeneration and repair of skeletal muscle tissue in mice (37). It is likely that VDR is important in athletes, due to its presence in parts of the body affecting sports performance, including skeletal muscle, heart and liver (35). The binding of 1,25(OH)₂D on VDR may play a pivotal role on muscle structure and function and physical performance.

In the body’s cells, 1,25(OH)₂D induces genomic and non-genomic mechanisms mediated through an interaction with the VDR (29,38,39). These mechanisms of vitamin D are being investigated because of their proliferating and differentiating effects and because of the potential to enhance sports performance (7).

2.2.3.2 Genomic Mechanisms
Genomic effects occur when 1,25(OH)₂D binds to its nuclear receptor (VDR) through intracellular binding protein (40) and is a relatively slow process (19). This binding allows for changes in messenger ribonucleic acid (RNA) and the activation of protein transcription (increased cell protein synthesis) (40). This pathway has also been found to influence calcium and phosphate metabolism, as well as proliferation and differentiation of muscle cells (17).
2.2.3.3 Non-genomic Mechanisms
The non-genomic mechanism of vitamin D in muscle tissue is still being explored (18). Non-genomic actions do not result from gene transcription and produce rapid effects (39). More research is needed to clarify the effect of 1,25(OH)₂D on this pathway, however, it is thought that the non-genomic effects are mediated through the cell surface via membrane-bound VDRs (17,39,41). Several second messenger pathways transmit signals to the cytoplasm, which are activated by 1,25(OH)₂D and influence calcium metabolism (42).

2.2.3.4 Mineral Homeostasis
One of the most important functions of vitamin D in the body is the maintenance and regulation of calcium and phosphate concentrations, which optimises bone health and muscle function (3).

2.3 Hypovitaminosis D – Deficiency
Vitamin D deficiency can be caused by factors including a lack of sun exposure, certain medications or diseases associated with malabsorption (43) and its consequences include skeletal disorders such as rickets in children, and secondary hyperparathyroidism leading to osteoporosis in adults (1,7,43-45). Vitamin D deficiency also plays a direct role in influencing musculoskeletal effects, for example, stress fractures and injury (38,46) and has been associated with skeletal muscle myopathy, displaying as muscle weakness (17,38,41,47,48). Therefore, it is possible that treatment of vitamin D deficiency in athletes to correct existing muscle weakness, may improve performance as well, by increasing muscle strength (15).

2.3.1 Importance of Vitamin D on Bone Health
Abnormalities in both calcium and phosphate metabolism occur in vitamin D deficiency (1). Decreased serum 25(OH)D and serum calcium concentrations increase parathyroid hormone (PTH) secretion, resulting in higher bone turnover and increased bone resorption (49). As calcium is tightly regulated in the body, small increases in PTH can have potentially harmful effects on bone health (50). In a large study by Chapuy et al. (50) it was demonstrated that PTH concentrations began to increase when serum 25(OH)D concentrations were equal to or lower than 78 nmol/L, therefore, concentrations above this value are considered to have a
more positive effect on bone mass and decrease the risk of fractures (50). Other researchers have also concluded that higher serum 25(OH)D concentrations (~90-100 nmol/L) are likely to be more advantageous for bone health (51).

Cross-sectional studies have found significant associations between serum 25(OH)D concentrations and bone mineral density (BMD) in various groups (51-55). However, due to the cross-sectional design of these studies, it cannot be determined whether serum 25(OH)D concentrations cause changes in BMD. Other observational studies (11,56-59) and experimental studies (60) have found no association between vitamin D status and BMD, even when confounders (such as age) were included in the analyses.

A large population-based observational survey by Hannan et al. (61) revealed that the relationship between vitamin D status and BMD is strongly affected by ethnicity, with a strong positive correlation between serum 25(OH)D concentration and BMD found in white men, but this was not found to be significant in black or Hispanic men (61). This association could be explained by differing rates in bone turnover in different ethnicities (61).

Low BMD is also associated with increased risk of stress fractures (62,63). A case-control study by Myburgh et al. (63) found that athletes with stress fractures in the lumbar spine and proximal femur had lower BMDs (both $p = 0.02$) than those without stress fractures (63). However, only vitamin D intake was measured and there was no significant difference in vitamin D intake of injured versus non-injured athletes, suggesting a lack of association of vitamin D intake on fracture risk (63). In contrast a more recent study by Nieves et al. (64) found that increases in BMD at the hip and spine were related to vitamin D intake, although this was not significant for whole body BMD (64).

### 2.4 Hypervitaminosis D - Toxicity

Toxicity does not occur from prolonged sun exposure, as excess exposure causes degradation of previtamin D$_3$ and vitamin D$_3$ into inactive photoproducts (26,65,66). However, toxicity can occur from accidental or intentional supplemental intake (7,26,67). Chronic toxicity causes increases of calcium in the urine (hypercalciuria), as well as increases in serum calcium concentrations (hypercalcemia) (68). Hypercalcemia can cause soft tissue
calcification, hypertension and cardiovascular damage, with classic signs and symptoms including: fatigue, forgetfulness, back pain, nausea and vomiting (67). Currently, it is still uncertain what serum 25(OH)D concentration leads to toxicity.

It is thought that as serum 25(OH)D concentrations rise in the circulation above 125 nmol/L, signs of hypercalcemia (as above) become evident (68,69). To add to the debate, some researchers are suggesting toxicity occurs when serum 25(OH)D concentrations are greater than 250 nmol/L (65,66,70). The Institute of Medicine (5) states that “supplementation exceeding 125 nmol/L is not recommended because of unknown long-term effects at these levels” (5).

2.5 Vitamin D, Muscle Strength and Physical Performance

Observational studies demonstrate that elderly individuals with insufficient or deficient vitamin D status\(^1\) suffer from impaired muscle strength (measured as either, hand grip strength, leg extension power or thigh strength) and physical performance (for example, balance, gait, sit-to-stand test) (22-24,47). It has been suggested that this age related decrease in muscle function could be due to the decrease in VDRs during ageing (36,38).

Although most evidence on the relationship between vitamin D status and skeletal muscle function is reported in the elderly, a British cross-sectional study looking at postmenarchal girls aged 12-14 years, with a median serum 25(OH)D concentration of 21 nmol/L, found that vitamin D status was significantly associated with muscle power and force (measured by jumping mechanography, which includes jumping power, height and velocity) (71). In contrast, a large RCT looking at a similar age group (girls aged 10-17 years), found no significant increase in muscle strength (measured by hand grip strength) in the vitamin D supplementation group, compared to the placebo group \( (p = 0.16) \) (72).

Recent studies have investigated muscle strength and serum 25(OH)D concentrations in young healthy adults. Marantes et al. (73) looked at an age-stratified random sample of community adult men and women, including young adults (age range: 21-97 years) with mean

\(^1\) Please refer to glossary terms for definitions of vitamin D status.
serum 25(OH)D concentrations of 57.5 nmol/L in men and 55.3 nmol/L in women (73). No association between serum 25(OH)D concentrations and muscle mass (by dual-energy X-ray absorptiometry) or muscle strength (handgrip force and isometric knee extension) was found, when adjusted for confounders such as: age, physical activity, fat mass and season (73). In contrast, a large cross-sectional study by Grimaldi et al. (74) looking at healthy men and women (aged 20-76 years) with serum 25(OH)D concentrations ranging from <25 - >75 nmol/L, found that higher serum 25(OH)D concentrations were associated with arm and leg muscle strength when adjusted for age and gender (74).

2.6 Vitamin D and Athletic Performance

Given the associations between vitamin D deficiency/insufficiency and muscle weakness, there is increasing interest in the association between vitamin D status and sports performance and the potential role of vitamin D on muscle tissue (18). However, to date there is minimal evidence of the potential impact vitamin D has on muscle tissue strength, especially in young healthy adults and athletic populations. Currently, the only research looking at associations between serum 25(OH)D concentrations and performance in athletes is through randomised and prospective studies using supplementation of vitamin D.

Recently, a two phase study by Close et al. (20) assessed male athletes in different sports (rugby league, soccer, flat jockeys and jump jockeys) (n=61) and made comparisons with a healthy non-athletic male control group (n=30) (20). Phase one of the study, only looked at serum 25(OH)D concentrations and found that the median serum 25(OH)D concentration in athletes was 50 nmol/L and <50 nmol/L in the non-athletic control group (20). There were statistically significant differences in vitamin D status across the five groups ($p = 0.001$), with the highest serum 25(OH)D concentrations in rugby league players (20). Phase two included only 14 soccer players (for unknown reasons) in the performance testing, however, four players were excluded due to fear of venepuncture and injury (20). Participants were randomised into a vitamin D supplementation group (n=5) and placebo group (n=5) to investigate effects of vitamin D on performance, for the following tests: 10m sprint, 30m sprint, vertical jump, 1-RM bench press, 1-RM back squat and Illinois Agility Test (20). At
baseline, there were no significant differences in any measurements between the vitamin D supplementation group and the placebo group (20). After intervention, there was a significant time-by-group difference in performance of the 10m sprint time and vertical jump height in the vitamin D supplemented group \( (p = 0.008 \text{ and } p = 0.008, \text{ respectively}) \) compared to the placebo group \( (10m \text{ sprint time } (p = 0.587) \text{ and vertical jump height } (p = 0.204)) \) (20). A limitation of the study was its small sample size.

A small controlled prospective cohort study by Wyon et al. (21) looked at a group of male and female ballet dancers, where participants voluntarily split themselves into the intervention \( (n=17) \) or control group \( (n=7) \) (21). The reason for the voluntary split was because a previous study revealed that these ballet dancers had insufficient or deficient vitamin D status and therefore the authors realised the importance of all the ballet dancers receiving vitamin D supplementation (21). They reported significant improvements in muscular strength (quadriceps contraction) \( (p <0.001) \) and muscular power (standing vertical jump) \( (p <0.001) \) in the intervention group, post supplementation of vitamin D (21). Whereas no significant improvement was seen in the control group (21). However, this study is likely confounded by its small sample size and lack of appropriate randomisation into groups.

Based on the above findings, it is proposed that increased serum 25(OH)D concentrations is associated with muscle strength in all age groups. However, there is limited information regarding this topic and more research is needed to confirm this assumption.

### 2.7 Vitamin D Analysis

The standard clinical measure to determine vitamin D status is the concentration of serum or plasma 25-hydroxyvitamin D \( (25(OH)D) \), as this is an accurate measure of both cutaneous synthesis and dietary intake \( (5,7,75,76) \). Serum 25(OH)D has a long circulating half life of 2-3 weeks, as opposed to 4-6 hours for the biologically active 1,25-dihydroxyvitamin D \( (1,25(OH)_2D) \), therefore 25(OH)D gives a more accurate indication of vitamin D status \( (77) \). Serum 25(OH)D can be measured in values of ng/mL \( (\mu g/L) \) and nmol/L \( (5) \). To convert nmol/L into ng/mL a conversion factor of 2.5 is used \( (\text{for example, } \text{nmol/L} / 2.5 = \text{ng/mL} (\mu g/L)) \) \( (5) \).
In many places, routine clinical measurement of 25(OH)D is uncommon, as the assays used for assessment are challenging and expensive (3,29,38). There are many different types of assays used, which has caused controversy amongst the scientific community, as there is no standard universal method of measurement and different assays have been shown to produce very different intra- and inter-assay results (77-79). The many assays include (5,80):

- Immunoassays, such as: radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), chemiluminescence immunoassay (CLIA).
- Liquid chromatography based methods, such as: high-performance liquid chromatography (HPLC) and liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Currently, antibody based methods are the most commonly used for clinical measurement of 25(OH)D (81). However, recent tests for quality control have shown fluctuations among different assays (82). All these methods measure both 25(OH)D$_2$ and 25(OH)D$_3$ (77,83), however, they are also found to measure other vitamin D metabolites, leading to an overestimation of approximately 10 - 20% (77).

The newer LC-MS/MS method is described as faster, more cost-effective, more reliable and more robust than the antibody-based assays (84). LC-MS/MS is now considered to be the ‘gold standard’ for determination of 25(OH)D, because of its high sensitivity of detection and specificity (81,83).

A recent study by Lai et al. (85) compared two commonly used assay methods (CLIA and LC-MS/MS), at three different laboratories (Lab A and B = CLIA, Lab C = LC-MS/MS), using 813 samples, where they reported substantial variations in both methods (85). They reported that measurements of 25(OH)D at Lab A and B (both using the CLIA method), were lower than Lab C using the LC-MS/MS method (26.05 nmol/L and 11.61 nmol/L lower respectively) (85). The authors concluded that these methods of vitamin D analysis are not adequate for determining vitamin D deficiency (85). Also, more research is needed, using a larger sample of laboratories to make these results more generalisable (85).

For the purpose of this project, the assay method chosen was the LC-MS/MS, as it is now considered the ‘gold standard’ for 25(OH)D measurement.
### Table 2.1.

**Definition of vitamin D cut-offs**

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Population characteristics</th>
<th>Vitamin D cut-offs (nmol/L)</th>
<th>Deficiency</th>
<th>Insufficiency</th>
<th>Sufficiency</th>
<th>Optimal</th>
<th>Potential Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapuy et al., 1997 (50)</td>
<td>Healthy adults</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&gt;78</td>
<td>-</td>
</tr>
<tr>
<td>Thomas et al., 1998 (86)</td>
<td>General medical ward patients</td>
<td></td>
<td>&lt;20</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Malabanan et al., 1998 (44)</td>
<td>Adults</td>
<td></td>
<td>-</td>
<td>-</td>
<td>50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Looker et al., 2002 (87)</td>
<td>Adolescents and adults</td>
<td></td>
<td>&lt;17.5</td>
<td>&lt;37.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lips, 2004 (88)</td>
<td>Adults and elderly</td>
<td></td>
<td>&lt;25</td>
<td>25 – 50</td>
<td>&gt;50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dawson-Hughes et al., 2005 (89)</td>
<td>Older men and women</td>
<td></td>
<td>-</td>
<td>-</td>
<td>50 – 80</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bischoff-Ferrari et al., 2006 (6)</td>
<td>Young adults and adults</td>
<td></td>
<td>-</td>
<td>-</td>
<td>&gt;75</td>
<td>90 – 100</td>
<td>-</td>
</tr>
<tr>
<td>Holick, 2006 (7)</td>
<td>Children, adults and elderly</td>
<td></td>
<td>-</td>
<td>&lt;50</td>
<td>&gt;75</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Institute of medicine, 2011 (5)</td>
<td>Adults</td>
<td></td>
<td>&lt;30</td>
<td>30 – 50</td>
<td>50 – 125</td>
<td>-</td>
<td>&gt;125</td>
</tr>
<tr>
<td>Ministry of Health, 2012 (8)</td>
<td>Adults</td>
<td></td>
<td>&lt;25</td>
<td>25 – 49.9</td>
<td>&gt;50</td>
<td>-</td>
<td>&gt;125</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>&lt;25</td>
<td>25 – 50</td>
<td>51 – 75</td>
<td>76 – 125</td>
<td>&gt;125</td>
</tr>
</tbody>
</table>
2.8 Vitamin D Status

There is no consensus in the literature for defining vitamin D status (65). This makes it complicated when comparing data from different studies, as different reference ranges are used. Vitamin D deficiency has been defined by some, as a serum 25(OH)D concentration of <25 nmol/L (8,88). A summary of values proposed to define vitamin D insufficiency (also known as suboptimal), sufficiency, optimal status and potential toxicity is presented in Table 2.1. A serum 25(OH)D concentration of between 25-50 nmol/L is defined as insufficient and concentrations between 51-75 nmol/L as sufficient (Table 2.1). The New Zealand Ministry of Health recommends that individuals have a serum 25-hydroxyvitamin D concentration of at least 50 nmol/L (8). In the absence of well-defined cut-offs, results of current studies should be interpreted with caution.

2.8.1 New Zealand Status

Sixty-eight percent of the New Zealand adult population have sufficient concentrations of 25-hydroxyvitamin D (serum 25(OH)D >50 nmol/L), with a mean serum 25(OH)D concentration of 63 nmol/L (8). However, this still leaves a third of the New Zealand adult population with either insufficient or deficient vitamin D status (8). In New Zealand, Māori and Pacific people have lower serum 25(OH)D concentrations than New Zealand European (75). Ten percent of Pacific men and women were classified as having vitamin D deficiency (<25 nmol/L) in the most recent New Zealand Nutrition Survey (ANS08/09) (8). The two strongest factors in New Zealand influencing vitamin D status are reported to be season and ethnicity (75).

2.8.1.1 Otago and Southland

Residents of the South Island of New Zealand are more likely to be deficient than those residing in the North Island because of the higher latitude in the north and therefore, greater UV radiation (90,91). Recent research shows that the three main regions of New Zealand (northern, central and southern) do not significantly differ in the prevalence of deficiency, even after adjusting for age, sex and ethnicity (8). However, southern New Zealand residents are more prone to vitamin D deficiency during the late winter and early spring months (August – October) of the year, than residents living in northern New Zealand (8).
2.8.2 Vitamin D Status Among Athletes

Internationally, there is much research looking at the vitamin D status of athletes (Table 2.2). The percentage of athletes overseas who have serum 25(OH)D concentrations of <50 nmol/L and <75 nmol/L suggests a cause for concern, as many athletes are found to be vitamin D deficient or insufficient, as presented in Table 2.2. Overall, these studies demonstrate that serum 25(OH)D concentrations vary depending on the population and generally the percentage of athletes with serum 25(OH)D concentrations <50 and <75 nmol/L is higher in the winter compared to summer or autumn. The worst vitamin D status was in Finnish gymnasts and runners where 100% of the athletes had serum 25(OH)D concentrations of <50 nmol/L during the winter; however, these athletes live at a very high latitude of 61° N (9). There has been no research reported within New Zealand looking at the vitamin D status of athletes.

There are issues with comparing this data (Table 2.2), as there is a lack of agreement of what serum 25(OH)D concentration is considered sufficient for athletes. Also, there are currently several different assays used to measure 25(OH)D and as the sensitivity and specificity of these assays vary, this makes it difficult to compare studies using different assays. Serum 25(OH)D concentrations are also influenced by several other factors (refer to Section 2.9), which are often not reported in studies. There is also no clear separation between indoor and outdoor sports, with many studies classifying a specific sport as either indoor or outdoor, without measurement. This makes comparison between studies difficult, as many sports may be considered outdoor sports, however, they also undertake considerable amounts of training indoors (e.g. rugby).
### Table 2.2.

Prevalence of 25-hydroxyvitamin D concentrations <50 and 50-75 nmol/L in athletes internationally

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Study Design</th>
<th>Location</th>
<th>Month (season)</th>
<th>Below 50 nmol/L n (%)</th>
<th>50-75 nmol/L n (%)</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lehtonen-Veromaa et al., 1999 (9)</td>
<td>Design: One year follow-up study n=191 Female athletes (gymnasts and runners) and 60 non-athletic controls 9 - 15 years of age Ethnicity: Finnish 25(OH)D Assay: RIA Inter-assay CV: 8.3% for 35.3 nmol/L</td>
<td>Finland</td>
<td>Feb - Mar (Winter)</td>
<td>186 (100)</td>
<td>0</td>
<td>Young sample group only. Females only. Only 186 completed the trial, but it was not recorded whether the dropouts were athletes or controls. Skin colour of the ethnic group not reported. No measurements of sun exposure reported.</td>
</tr>
<tr>
<td>Guillemant et al., 2001 (10)</td>
<td>Design: Randomised Controlled Trial n=54 Male athletes (jockeys) 13 - 16 years of age Ethnicity: Caucasian 25(OH)D Assay: CPBA Inter-assay CV: 7 - 10%</td>
<td>Le Moulin à Vent, Gouvieux, France</td>
<td>1st year Mar (Winter) 2nd year Mar (Winter)</td>
<td>39 (72) 37 (68)</td>
<td>-</td>
<td>Small sub sample of the original study done in the year 1999. Young sample group only. Males only. No measurements of sun exposure reported.</td>
</tr>
<tr>
<td>Lovell et al., 2008 (92)</td>
<td>Design: Cross-sectional Study n=18 Female athletes (gymnasts) 10 - 17 years of age 25(OH)D Assay: RIA</td>
<td>Australia</td>
<td>May (Autumn)</td>
<td>6 (33) 9 (50)</td>
<td></td>
<td>Small and young sample. Females only. Previous stress injuries had occurred in 12 of the athletes. Ethnicity or skin colour not reported.</td>
</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>Location</td>
<td>Season</td>
<td>Mean (SD)</td>
<td>CV (%)</td>
<td>Note</td>
</tr>
<tr>
<td>---------------------</td>
<td>-------------------------</td>
<td>----------------</td>
<td>-----------------</td>
<td>-----------</td>
<td>--------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>Hamilton et al., 2010 (11)</td>
<td>Cross-sectional Study</td>
<td>Doha, Qatar</td>
<td>Apr – Oct</td>
<td>85 (91)</td>
<td>8 (9)</td>
<td>Wide screening period from April to October. Males only. 3% of athletes were taking multivitamin supplements containing vitamin D. 34% of athletes were taking multivitamins, for which the vitamin D content could not be determined.</td>
</tr>
<tr>
<td>Constantini et al., 2010 (93)</td>
<td>Cross-sectional Study</td>
<td>Israel</td>
<td>May - Oct</td>
<td>25 (26)*</td>
<td>47 (48)*</td>
<td>Blood samples were taken from individuals at different times in the year. Not all subjects had blood analysed in the winter months (n=53). Ethnicity or skin colour not reported. No measurement of sun exposure reported.</td>
</tr>
<tr>
<td>Halliday et al., 2011 (57)</td>
<td>Longitudinal Study</td>
<td>University of Wyoming, USA</td>
<td>Sep - Oct</td>
<td>20 (38)*</td>
<td>26 (49)*</td>
<td>Actual ages of athletes not reported. Ethnicity or skin colour not reported. High dropout rate in the spring (n=16).</td>
</tr>
</tbody>
</table>

**Note:** If data was not reported in the study, it was not included in the table e.g. ethnicity, latitude, inter-assay CV.

**Abbreviations:** °N, degrees north of the equator; °S, degrees south of the equator; 25(OH)D, 25-hydroxyvitamin D; CLIA, chemiluminescence immunoassay; CPBA, competitive protein binding assay; CV, coefficient of variation; HPLC, high performance liquid chromatography; RIA, radioimmunoassay; UK, United Kingdom; USA, United States of America.
Table 2.2. Continued

Prevalence of 25-hydroxyvitamin D concentrations <50 and 50-75 nmol/L in athletes internationally

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Study Design</th>
<th>Location</th>
<th>Month (season)</th>
<th>Below 50 nmol/L n (%)</th>
<th>50-75 nmol/L n (%)</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bescos Garcia &amp; Rodriguez Guisado, 2011 (12)</td>
<td>Design: Cross-sectional Study n=21 Male athletes (basketball players) Average age 25 years Ethnicity: White Caucasian (n=6) and African American (n=5) 25(OH)D Assay: CLIA Inter-assay CV: 8%</td>
<td>Barcelona, Spain Latitude: 41° N</td>
<td>Apr (Spring)</td>
<td>12 (57)</td>
<td>7 (33)</td>
<td>Small sample size. Males only. Athletes were at different latitudes prior to blood samples being analysed.</td>
</tr>
<tr>
<td>Morton et al., 2012 (13)</td>
<td>Design: Cross-sectional Study n=20 Male athletes (soccer players) Average age 26 years Ethnicity: Varying nationalities (English, Danish, Spanish, Scottish, Australian, Serbian, Dutch, Greek, Brazilian and French) 25(OH)D Assay: RIA</td>
<td>Liverpool, United Kingdom Latitude: 53° N</td>
<td>Dec (Winter)</td>
<td>13 (65)</td>
<td>-</td>
<td>Small sample size. Actual ages not recorded. Males only. Only two participants had reported dark skin colour.</td>
</tr>
<tr>
<td>Willis et al., 2012 (94)</td>
<td>Design: Pilot Study n=19 Male and female athletes (runners) 19 - 45 years of age 25(OH)D Assay: CLIA</td>
<td>University of Wyoming, USA Latitude: 30° N</td>
<td>Not recorded</td>
<td>2 (11)</td>
<td>8 (42)*</td>
<td>Small sample size. Ethnicity or skin colour not reported. Neither month nor season of blood collection was reported. No measurement of sun exposure reported.</td>
</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>n</td>
<td>Gender</td>
<td>Age (years)</td>
<td>Ethnicity</td>
<td>25(OH)D Assay</td>
</tr>
<tr>
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</tr>
<tr>
<td>Galan et al., 2012 (95)</td>
<td>Cross-sectional Study</td>
<td>28</td>
<td>Male</td>
<td>26.7</td>
<td>Caucasian</td>
<td>CLIA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pollock et al., 2012 (35)</td>
<td>Cross-sectional Study</td>
<td>63</td>
<td>Male and female</td>
<td>24.9</td>
<td>Varying nationalities (Japanese, English, Southern European, American)</td>
<td>CLIA or HPLC</td>
</tr>
<tr>
<td></td>
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<tr>
<td>Close et al., 2012 (20)</td>
<td>Cohort study</td>
<td>61</td>
<td>Male</td>
<td>23.8</td>
<td></td>
<td>HPLC</td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**Note:** If data was not reported in the study, it was not included in the table e.g. ethnicity, latitude, inter-assay CV.

**Abbreviations:** °N, degrees north of the equator; °S, degrees south of the equator; 25(OH)D, 25-hydroxyvitamin D; CLIA, chemiluminescence immunoassay; CPBA, competitive protein binding assay; CV, coefficient of variation; HPLC, high performance liquid chromatography; RIA, radioimmunoassay; UK, United Kingdom; USA, United States of America.
Table 2.2. Continued

Prevalence of 25-hydroxyvitamin D concentrations <50 and 50-75 nmol/L in athletes internationally

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Study Design</th>
<th>Location</th>
<th>Month (season)</th>
<th>Below 50 nmol/L n (%)</th>
<th>50-75 nmol/L n (%)</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilson et al., 2013 (14)</td>
<td>Design: Cross-sectional Study n=37¹</td>
<td>United Kingdom Latitude: 53° N</td>
<td>Jan – Apr (Winter)</td>
<td>28 (78)</td>
<td>-</td>
<td>Small sample size. Ethnicity or skin colour not reported. No measurement of sun exposure reported.</td>
</tr>
<tr>
<td></td>
<td>Male athletes (flat and jump jockey’s) Average age 26 years 25(OH)D Assay: HPLC Inter-assay CV: &lt;10% for 2.5 – 625 nmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Close et al., 2013 (96)</td>
<td>Design: Randomised dose-response study n=30</td>
<td>United Kingdom Latitude: 53° N</td>
<td>Jan – Apr (Winter)</td>
<td>17 (57)</td>
<td>-</td>
<td>Small sample size. Unreported if athletes are male or female. Ethnicity or skin colour not reported. No measurement of sun exposure reported.</td>
</tr>
<tr>
<td></td>
<td>Athletes (university athletics clubs including football and rugby) Average age 21 years 25(OH)D Assay: HPLC Inter-assay CV: &lt;10% for 2.5 – 625 nmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: If data was not reported in the study, it was not included in the table eg. ethnicity, latitude, inter-assay CV.

¹ Serum 25(OH)D concentrations reported in 36 of the 37 participants.

Abbreviations: °N, degrees north of the equator; °S, degrees south of the equator; 25(OH)D, 25-hydroxyvitamin D; CLIA, chemiluminescence immunoassay; CPBA, competitive protein binding assay; CV, coefficient of variation; HPLC, high performance liquid chromatography; RCT, randomised controlled trial; RIA, radioimmunoassay; UK, United Kingdom; USA, United States of America.
2.9 Factors Influencing Serum 25-hydroxyvitamin D Concentrations

There are multiple factors influencing the amount of UVB radiation reaching the skin and which therefore have the potential to reduce cutaneous synthesis of vitamin D. These include environmental factors such as season and latitude and behavioural factors such as sun exposure, clothing and sunscreen use (97,98). Individual factors such as skin pigmentation, age and body composition are also key factors influencing serum 25(OH)D concentrations (97,98).

2.9.1 Sun Exposure

Sun exposure is the most important source of vitamin D (1,3,45,99). However, excessive exposure to sunlight (causing sunburn) can lead to an increased risk of skin cancer and therefore, a balance is necessary to ensure enough sun exposure is obtained to maintain sufficient vitamin D status without increasing the risk of skin cancer (3,100).

Researchers have suggested that twice weekly exposure to sunlight (arms and legs, or hands, arms and face) for 5 to 30 minutes (depending on season, latitude and skin colour) between the hours of 10am and 3pm is often adequate for an acceptable vitamin D status (1,7,65,101). However, according to a position statement by the American Academy of Dermatology (102) “there is no scientifically validated level of sun exposure that allows for maximum vitamin D uptake without increasing risk of skin cancer” (102).

Athletes who train indoors are more likely to exhibit vitamin D deficiency or insufficiency than those training outdoors (15,57,93). Those training outdoors early in the morning or late in the afternoon are also at risk of vitamin D insufficiency (67,103,104).

A longitudinal study in the USA (latitude: 41° N) was conducted by Halliday et al. (57) in athletes (n=41) from different sports (classified as indoor or outdoor) (57). The results demonstrated that athletes training indoors had lower overall circulating 25(OH)D concentrations than those training outdoors, but this was only found to be significant in the autumn season ($p < 0.05$) and not in spring or winter (57). Although Halliday et al. (57) created a sun exposure questionnaire, including questions such as: frequency of leisure time
spent outdoors, frequency of sunscreen application, type of clothing worn outdoors, this was not validated, which could impact on the outcome of the results (57).

A recent RCT by Lewis et al. (60) in the Southeastern United States (latitude: $38^\circ$ N), found all indoor athletes ($n=32$, swimmers and divers) to have sufficient vitamin D status at baseline ($>80$ nmol/L) and that leisure time spent in the sun was a significant predictor of serum 25(OH)D concentrations at baseline ($p = 0.001$) (60). However, these results were from the summer season where the amount of UVB exposure is higher compared to the winter and early spring.

### 2.9.2 Clothing
The type of clothing worn by individuals can determine how much sun exposure the skin receives, as clothing acts as a barrier to UVB radiation (105). The effectiveness of clothing, as a barrier to UVB radiation, depends on the type and colour of the fabric (105-107). Black clothes have been shown to completely (100%) exclude UVB radiation (45). Glass and plastic are also barriers to the harsh UVB radiation, excluding 100% of UVB rays (45).

A study conducted in South Queensland, Australia by Kimlin et al. (108) assessed the use of protective clothing on vitamin D status and found that participants who usually or almost always wore protective clothing, such as long sleeved shirts or long trousers, had lower serum 25(OH)D concentrations than those who never wore long sleeves or trousers ($p = 0.046$ and $p = 0.010$ respectively) (108). The results from this study are likely confounded by recall bias or response burden from the 7-page self-administered, non-validated, sun exposure questionnaire.

### 2.9.3 Sunscreen Use
Sunscreen is designed to block UVB and some ultraviolet A (UVA) radiation from reaching the skin by absorbing the ultraviolet rays for the prevention of sunburn (1,5,109,110).

An early small in vivo study by Matsuoka et al. (111) found that chronic sunscreen use significantly affects cutaneous synthesis of vitamin D (111). The application of sunscreen with a internationally recognised sun protection factor (SPF) of 8 has been shown to reduce cutaneous vitamin D synthesis by 95%, whereas sunscreen with a SPF of 15 can reduce it by
98% (1,111). Observational studies have found females significantly more likely to apply sunscreen than males (110,112) and have also found that higher vitamin D status is associated with increased sunscreen application (108,112), suggesting that sunscreen use is a potential indicator of overall sun exposure.

Although observational research is limited in this area, because of the difficulty in accurately measuring this behaviour, improper application of sunscreen may prevent full protection from UVB radiation and therefore may not be compromising vitamin D status as much as previously thought, though sunscreen use is found to be effective at preventing sunburn (5,113). To ensure efficient cutaneous synthesis of vitamin D, unprotected skin should be exposed to ultraviolet rays (97).

2.9.4 Solar Zenith Angle: Season and Latitude
Mean concentrations of serum 25(OH)D are strongly influenced by season (90). Several studies have found that serum 25(OH)D concentrations differ in individuals between the summer and winter months, with serum 25(OH)D concentrations higher during the summer season (13, 75, 114, 115, 116). A national New Zealand study demonstrated, that changes in serum 25(OH)D concentrations were significantly different between spring and summer, (difference = 31 nmol/L and 28 nmol/L in women and men respectively) (90). Similar results have been found in both indoor and outdoor athletes, where serum 25(OH)D concentrations are at their lowest in the winter months and improve over the spring, summer and autumn (9, 57, 93, 95, 117).
In the winter months and at latitudes greater than 35°, when the solar zenith angle (SZA) of the sun is increased, photons must travel longer distances through the ozone layer (Figure 2.3) (99,101,104). This decreases the amount of UVB radiation projected from the sun, down to the earth's surface and therefore reduces the body’s ability to synthesise vitamin D in the skin (101).

![Diagram of Solar Zenith Angle]

**Figure 2.3.** Demonstration of the SZA, which influences the amount of radiation received on a horizontal surface and is the angle between the centre of the sun and the point on the Earth's surface.

The SZA at the equator is the smallest, producing higher ultraviolet (UV) levels (118). The further away we get from the equator, the greater the reduction in UV radiation (118). New Zealand sits at a high latitude of 35-47° south, with Dunedin (Otago) and Invercargill (Southland) sitting at the higher end of this range between 45° and 47° south. The amount of UVB radiation is reduced at these high latitudes (91), impacting on the vitamin D status of residents living in southern New Zealand, particularly during winter (90). Yet, Southern and Northern Hemisphere latitudes are not always found to be equivalent, as it has been estimated...
that in New Zealand at a latitude of 45° the UV levels are up to 13-50% higher than similar latitudes north of the equator because of differences in atmospheric turbidity (119).

Rockell et al. (90) reported that women living in the South Island (~40–47° S) of New Zealand had a mean serum 25(OH)D concentration of 43 nmol/L, which was significantly lower than women in the North Island (~35–40° S) with mean serum 25(OH)D concentration of 49 nmol/L ($p<0.01$) (90). However, latitude was not found to be significant in men (90), potentially because of the lower percentage of men included in this study. In contrast, a meta-analysis of cross-sectional international studies concluded that there was no influence of latitude on vitamin D status (120). Variations in cultural and genetic factors may have caused or influenced this lack of association.

2.9.5 Ethnicity or Skin Pigmentation
Genetically determined skin types affect the amount of vitamin D synthesised in the skin (97). It is well known that individuals with higher concentrations of melanin pigmentation (dark skinned races) have reduced quantities of vitamin D produced under the skin at any given dose of UVB radiation (1,5,90,97,121). This protects the skin from harsh sunlight similar to sunscreen (1). It has been suggested that those with darker skin tones need up to 10 times the amount of exposure to UVB radiation than their lighter skinned peers to increase serum 25(OH)D concentrations (1).

In New Zealand, the three main ethnic groups are New Zealand European (NZ European), Māori and Pacific. As both Māori and Pacific people generally have darker skin colour than NZ European people, they may be at a greater risk of vitamin D deficiency (90).

Results from the 1997 New Zealand National Nutrition Survey (NNS97) reported by Rockell et al. (90) demonstrated that mean serum 25(OH)D concentrations in both men and women of either Māori or Pacific descent were lower than NZ European men and women, when adjusted for age, region, season and body mass index (BMI) category (90). This finding was repeated in the more recent 2008/09 New Zealand Adult Nutrition Survey (ANS08/09) (8), where Pacific men and women had significantly lower serum 25(OH)D concentrations (49.6 nmol/L and 46.0 nmol/L respectively) than non-Pacific men and women (63.6 nmol/L
and 62.4 nmol/L respectively) (8). This was also significant for Māori women (57.2 nmol/L) but not men (60.9 nmol/L) (8).

Studies in athletes from different countries have found associations between low serum 25(OH)D concentrations and dark skin colour or dark skinned ethnicities (12,13,35). It was unreported as to how skin colour or ethnicity was assessed in these studies, whether it was measured using equipment (eg. reflectance colorimetry) or self-reported, which could impact on the accuracy of the results. In contrast, a study by Hamilton et al. (11) found no significant association between serum concentrations of 25(OH)D and nurse reported participant skin colour (dark, olive or fair) (11).

2.9.6 Age
Increasing age is associated with decreased serum 25(OH)D concentrations, as concentrations of previtamin D₃ (7-dehydrocholesterol) decrease due to changes in skin morphology (122,123). It has been established that after 20 years of age, skin thickness decreases linearly with age (124). MacLaughlin and Holick (122) conducted an in vitro study comparing human skin samples from two different age groups (8-18 and 77-82 years of age) and found that skin samples from the older age group had a two-fold lower ability to produce vitamin D₃ in the skin (122). Similarly, a cross-sectional study by Kimlin et al. (108) found an inverse relationship between age and vitamin D status; however, this was not statistically significant ($p = 0.279$) (108).

2.9.7 Body Composition
Vitamin D is stored in adipose tissue (as it is a fat-soluble vitamin) and can be released during the winter months when vitamin D synthesis is low (1). Those with either a high or low percent body fat may be at increased risk of vitamin D inadequacy (67).

Concentrations of serum 25(OH)D are found to be lower in overweight and obese individuals (BMI >25-30 kg/m²) when compared to normal weight individuals (BMI <25-30 kg/m²) (5,8,108,112,125-127). This may be explained by the altered release of vitamin D₃ into the circulation from adipose tissue, causing a limited ability to convert vitamin D₃ to 25(OH)D in those who are overweight or obese (128). Differences in lifestyle (such as:
reduced physical activity levels, lower quality of the diet and reduced hours of sun exposure) could also be contributing to the lower serum 25(OH)D concentrations found in overweight and obese individuals (112,127).

In contrast, some studies have shown that percent body fat does not correlate with vitamin D status (57,58), but many confounders could be contributing to this lack of association such as: small ranges of body fat comparisons, differences in physical activity levels and small sample sizes. The majority of studies show that vitamin D status is lower in those who are overweight or obese (5,8,108,112,125-127).

It is thought that those with a low percentage of body fat are likely to be at risk of vitamin D inadequacy because of a decreased ability to store vitamin D₃ in adipose tissue (67,103,128), although no research was found to verify this.

### 2.10 Vitamin D Recommendations for Intake

Recommendations for dietary vitamin D intake are different between countries and are based on the clinical measure of serum 25(OH)D.

The Nutrient Reference Values (NRVs) for vitamin D in Australia and New Zealand are set by the National Health and Medical Research Council (NHRMC) and the Ministry of Health (MoH) (129). The recommendations assume no or minimal sun exposure and are based on the amount of dietary vitamin D to yield a serum 25(OH)D concentration of at least 27.5 nmol/L (129). NHRMC and MoH (129) cite that vitamin D deficiency occurs in young children when serum 25(OH)D concentrations are less than 27.5 nmol/L, also that mild deficiency occurs in young adults when serum 25(OH)D concentrations are between 25-50 nmol/L, therefore these values were used when determining reference values for vitamin D (129).

The NRV used for vitamin D is an Adequate Intake (AI), which is a value used when an Recommended Dietary Intake (RDI) cannot be determined (129). The definition of an AI is “The average daily nutrient intake level based on observed or experimentally-determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate” (129). For males and females aged 1 – 50 years the
AI is 5 μg (200 International Units (IU)) per day for males and females aged 51 – 70 years it is 10 μg (400 IU) per day and for those greater than 70 years it is 15 μg (600 IU) per day (129). The American Institute of Medicine (IOM) has an Estimated Average Requirement (EAR) of 400 IU (10 μg) per day, for males and females aged 1 - >70 years, which also assumes individuals have minimal sun exposure (5). This is a useful reference value for estimating the prevalence of inadequate intakes within a group (5). This level was established on the basis that this would be a sufficient amount to achieve a serum 25(OH)D concentration of between 40-50 nmol/L (5). The IOM based their reference values for vitamin D on the amount of vitamin D needed for bone maintenance (5). They state that at serum 25(OH)D concentrations between 30-50 nmol/L calcium absorption is at its maximum, with no increase above this amount (5).

To further add to the debate, Holick (130) is suggesting children receive 1,000 IU (25 μg) per day and adults 2,000 IU (50 μg) per day to maintain a serum 25(OH)D concentration of at least 75 nmol/L, the concentration suggested to be required for maximal bone health (130).

2.10.1 Athlete Recommendations for Vitamin D Intake
Although athletes partake in large volumes of physical activity, the recommendations for vitamin D intake are the same for all adults. Based on studies looking at the vitamin D status of athletes, there is currently no evidence to suggest they have different or higher vitamin D needs than the non-athletic population (67).

2.11 Vitamin D Intake
Achieving sufficient vitamin D status is challenging through diet alone (3). Particularly in New Zealand it is difficult to reach sufficient status by diet alone, as fortification of staple foods with vitamin D is not mandated (2) (as it is in countries such as; Canada, the United States of America (131) and Australia (2)) and therefore, very few food products are fortified with vitamin D (3,69,129). Estimates of vitamin D intakes of New Zealanders are limited, as local food databases lack data on the vitamin D content of many foods (129).
2.11.1 Intake of Athletes

Similar problems that occur in the estimation of vitamin D intakes in the non-athletic population also occur in athletes. There is a lack of research assessing the relationship between serum 25(OH)D concentrations and vitamin D intake in athletes (12).

As shown in Table 2.3, athletes overall intake of vitamin D is far less than both recommendations made by Australia and New Zealand (129) and the American IOM (5). Despite athletes increased energy needs, due to large volumes of physical activity, they are still found to be consuming insufficient intakes of vitamin D. This is likely due to the limited foods naturally containing vitamin D and lack of mandatory food fortification in most countries.
### Table 2.3.
**Vitamin D intake of athletes internationally**

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Study design</th>
<th>Assessment method</th>
<th>Vitamin D intake (IU/day)</th>
<th>Main findings</th>
<th>Limitations of study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farajian et al., 2004 (132)</td>
<td>Design: Cross-sectional Study Country: Greece n=58 Male and female athletes (swimming and waterpolo) 16 - 32 years of age</td>
<td>24 hour diet recall and FFQ.</td>
<td>240 ± 148 (males) 160 ± 96 (females)</td>
<td><strong>Australia &amp; New Zealand (AI) outcome</strong> Mean intakes of males are above the AI but not for females. <strong>American IOM (EAR) outcome</strong> 39% males and 67% females are below the EAR.</td>
<td>Data reported on a group of athletes whom are likely to restrict energy intake (aesthetic sports). 14% (10% males, 19% females) classified as under-reporters.</td>
</tr>
<tr>
<td>Swinbourn, 2009 (133)</td>
<td>Design: Cross-sectional Study Country: New Zealand n=40 Male athletes (rugby union players) Average age 23.7 years</td>
<td>4 day food record.</td>
<td>300 ± 720</td>
<td><strong>Australia &amp; New Zealand (AI) outcome</strong> Mean intake is above the AI. 15 participants (38%) are below the AI. <strong>American IOM (EAR) outcome</strong> Mean intake is below the EAR.</td>
<td>Small sample size. Males only. Under-reporting and recall bias is common with this type of assessment method.</td>
</tr>
<tr>
<td>Nieves et al., 2010 (64)</td>
<td>Design: Prospective Cohort Study Country: USA n=125 Female athletes (distance runners) 18 - 20 years of age</td>
<td>Self-administered FFQ.</td>
<td>231 ± 145</td>
<td><strong>Australia &amp; New Zealand (AI) outcome</strong> Mean intake is above the AI. <strong>American IOM (EAR) outcome</strong> Mean intake is below the EAR.</td>
<td>Females only. Data reported on a group of athletes whom are likely to restrict energy intake (aesthetic sport). Under-reporting was not assessed. Under-reporting and recall bias is common with this type of assessment method.</td>
</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>Country</td>
<td>Sample</td>
<td>Age Range</td>
<td>Methodology</td>
</tr>
<tr>
<td>-------------------------------------------</td>
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<td>-----------------------------------------------------------------------------</td>
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</tbody>
</table>
| Bescos García & Rodríguez Guisado, 2011   | Cross-sectional     | Spain        | 21     | 25 years           | 4 day non-consecutive food record and FFQ.                                  | Outcome: Mean intake is below the AI. Four participants (19%) are above the AI.  
   | Study                      |                     |          |                    |                                                                             | American IOM (EAR) outcome: No participant has a value above the EAR.    |
|                                           |                     |              |        |                    |                                                                             | Small sample size. Males only. Under-reporting was not assessed.        |
|                                           |                     |              |        |                    |                                                                             | Underreporting and recall bias is common with this type of assessment method. |
| Ducher et al., 2011                       | Pilot Study         | Australia    | 16     | 10-19 years        | Interview administered FFQ.                                               | Outcome: Unknown how many participants are above the AI.               |
|                                           |                     |              |        |                    | Range (55 – 505)                                                           | Australian IOM (EAR) outcome: Unknown how many participants are above the EAR. |
|                                           |                     |              |        |                    |                                                                             | Small sample size. Males only. Not all participants agreed to do the questionnaire. |
|                                           |                     |              |        |                    |                                                                             | Only the range of intake is reported, therefore it is hard to determine how many participants are meeting the recommendations. |
|                                           |                     |              |        |                    |                                                                             | The FFQ was not validated for children and adolescents (participants of this study). |
|                                           |                     |              |        |                    |                                                                             | Data reported on a group of athletes likely to restrict energy intake (aesthetic sport). |

1 Values are means ± standard deviation in IU per day, unless otherwise indicated. Conversion of any data recorded in µg/day, was worked out on the basis that 5 µg/day is equal to 200 IU/day.

2 Recommendations for adults = AI = 200 IU/day (1 – 50 years) – Australia and New Zealand, EAR = 400 IU/day (1 >70 years) – American IOM.

Abbreviations: AI, Adequate Intake; EAR, Estimated Average Requirement; FFQ, food frequency questionnaire; IOM, Institute of Medicine; USA, United States of America.
Table 2.3. Continued

*Vitamin D intake of athletes internationally*

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Study design</th>
<th>Assessment method</th>
<th>Vitamin D intake (IU/day)¹</th>
<th>Main findings²</th>
<th>Limitations of study</th>
</tr>
</thead>
</table>
| Gibson et al., 2011 (134)  | Design: Cross-sectional Study         | 4 day non-consecutive food record.     | 163.3 ± 94.7               | Australia & New Zealand (AI) outcome
  Mean intake below the AI.  
  American IOM (EAR) outcome
  Mean intake is below the EAR. | Small sample size.  
  Females only.  
  Under-reporting was not assessed.  
  Underreporting and recall bias is common with this type of assessment method. |
|                            | Country: Canada  
  n=33  
  Female athletes (soccer)  
  14.6 - 17.3 years of age |                                                        |                             |                |                                                                                     |
| Wilson et al., 2013 (14)   | Design: Cross-sectional Study         | 7 day food record.                     | Flat Jockey’s 62.8 ± 64    | Australia & New Zealand (AI) outcome
  Mean intake of both groups is below the AI.  
  American IOM (EAR) outcome
  Mean intake of both groups is below the EAR. | Small sample size.  
  Males only.  
  Only 70% of the participants completed the 7 day food record.  
  Data reported on a group of athletes likely to restrict energy intake (aesthetic sport).  
  Under-reporting was not assessed.  
  Underreporting, high respondent burden and recall bias is common with this type of assessment method. |
|                            | Country: United Kingdom  
  n=37  
  Male athletes (flat and jump jockey’s)  
  Average age 26 years |                                                        | Jump Jockey’s 93.4 ± 61    |                |                                                                                     |

¹Values are means ± standard deviation in IU per day, unless otherwise indicated. Conversion of any data recorded in µg/day, was worked out on the basis that 5 µg/day is equal to 200 IU/day.

²Recommendations for adults = AI = 200 IU/day (1 – 50 years) – Australia and New Zealand, EAR = 400 IU/day (1 - >70 years) – American IOM.

Abbreviations: AI, Adequate Intake; EAR, Estimated Average Requirement; FFQ, food frequency questionnaire; IOM, Institute of Medicine; USA, United States of America.
2.12 Description of Rugby Union

For the purpose of this study, the focus is now on the sport of rugby union. During the pre-season training, during autumn (time point of the current study) athletes are semi-professional, as players maintain other occupations during the workday and training during this time occurs both outdoors and indoors. Therefore, at this time of the year, this team field sport should not be solely classified as an outdoor sport.

Rugby union is a team game of high physical and tactical demand, with many instances of physical contact including tackling, rucks, mauls and scrums (135). The game includes periods of moderate to high intensity sprints interspersed with periods of lower intensity or rest (136,137). Player positions can be loosely grouped into forwards and backs (135).

Over the years rugby union players are becoming heavier and stronger, due to the increases in training load (135). Recently, it was discovered that world cup teams with heavier forwards and taller backs were performing better than other world cup teams (135). A heavier body mass in forwards players is important for the physical contact with other teams during the scrum, whereas the backs need speed to gain territory and score tries (136). The sport demands speed, strength and power from its players to ensure physical dominance in confrontation and therefore, the importance of muscle size and strength is vital for rugby union game performance (135).

2.13 Summary

Sufficient vitamin D status is important for athletes, as vitamin D maintains calcium and phosphate metabolism and bone health (1). A higher vitamin D status has the potential to increase muscle power and strength through the vitamin D receptor (7), which may lead to the enhancement or maintenance of athletic performance. What is not yet clear is the exact mechanism by which vitamin D has the potential to affect muscle strength and mass; however, this is outside the scope of this thesis.

Recent findings in healthy populations, including athletes, are demonstrating significant associations between higher vitamin D concentrations and increased muscle strength and
power (20,21,71,74). However, the current evidence is limited and therefore this study aims to add to the limited amount of research in this area.

The competitive nature of athletes means many are seeking supplementation, as a way to enhance performance (138). There is increasing interest in vitamin D supplementation (138), yet too much vitamin D can have negative effects, such as hypercalcemia (69). It is therefore important to only supplement when necessary. Athletes at risk of vitamin D deficiency need to be provided with suitable recommendations around the safe use of vitamin D supplementation.

Factors such as: limited amounts of sun exposure (athletes training indoors) (57), excessive clothing (108), the winter season (75), dark skin pigmentation (75) and high percent body fat (112) are associated with low vitamin D status. These factors influencing 25(OH)D concentrations are often not reported in studies, which makes it difficult to accurately assess the vitamin D status of individuals. As New Zealand has no mandatory fortification of vitamin D in food products (2) and intakes of foods rich in vitamin D are shown to be insufficient, sun exposure is necessary to maintain sufficient vitamin D status. The increasing evidence from international research suggests that vitamin D deficiency and insufficiency (serum 25(OH)D concentrations of <50 nmol/L) are common in the athletic population (9-14,20,35,57,58,92-96,117).

There have been no previous studies reporting the vitamin D status of New Zealand athletes, nor has there been any research investigating any associations between serum 25(OH)D concentrations and performance or potential predictors of serum 25(OH)D concentrations.
3 Objective Statement

Internationally, low vitamin D status has been reported in different athletic populations (9-14,20,35,57,58,92-96,117). There is evidence to suggest that this may impact on athletic performance, as recent publications indicate that significant associations exist between higher 25(OH)D concentrations and muscle strength and performance in healthy populations, including athletes (20,71,74). However, these relationships have yet to be explored in New Zealand athletes. Similarly, while several potential predictors of serum 25(OH)D concentrations have been suggested in New Zealand (75,121,139,140) and internationally (97,98), it remains to be determined whether these predictors also apply in a New Zealand athletic group.

In this current study, data from semi-professional male rugby union players in the Otago and Southland region of New Zealand is presented. The main aim of the study “Vitamin D Status and Effect of Supplementation on Strength, Speed and Body Composition in Rugby Union Players” was to investigate the effect vitamin D supplementation had on specific aspects of sports training and physiological parameters in this athletic population.

The objectives of this part of the cross-sectional sub study are to:

a. Evaluate the vitamin D status in this cohort of semi-professional male rugby union players in New Zealand, during autumn.

b. Determine whether associations between serum 25-hydroxyvitamin D concentrations and athletic performance occur in this cohort of semi-professional male rugby union players in New Zealand.

c. Investigate whether factors identified in the literature as potential predictors of serum 25-hydroxyvitamin D concentrations also apply in this cohort of semi-professional male rugby union players in New Zealand.
4 Methods

4.1 Study Design
This cross-sectional secondary data analysis is part of a randomised, placebo-controlled, double-blinded intervention study looking at the effect of vitamin D supplementation on strength, speed and performance, as well as vitamin D status, serum insulin-like growth factor 1 concentration, bone mineral density, body composition and mood. The Department of Human Nutrition at The University of Otago, Dunedin, New Zealand, conducted the study. The University of Otago, New Zealand, Human Ethics Committee approved the study (Appendix A and B). Only the baseline data is used for the purpose of this secondary data sub study. The methodology has been described by Fairbairn et al. (141) and is summarised below.

4.2 Methodology Employed in the Randomised Controlled Trial “Vitamin D Status and Effect of Supplementation on Strength, Speed and Body Composition in Rugby Union Players”

4.2.1 Participants
Semi-professional male rugby union players from either Otago or Southland Provincial Rugby Unions (latitude: 45-47° S) were eligible to participate in the study. Players were included if they participated in the pre-season training programme for either the Otago or Southland ITM Cup squads or a contributing academy/development squad. Participants were excluded if they were: younger than 18 years of age, a smoker, taking vitamin D supplements or if they had any underlying diagnosed disease such as: heart disease, diabetes, cancer or a condition that affects hormone metabolism. Fifty-eight rugby union players were eligible to complete the study, however, only 57 were included in the final analyses due to the late withdrawal of one participant.
Participants were fully informed of the purpose and procedures of the study before completing a written informed consent, in accordance with the Helsinki Declaration (Appendix C).

### 4.2.2 Data Collection

During the months of autumn (March to May) 2011, participants had fasting blood samples taken, underwent a Dual-energy X-Ray Absorptiometry (DXA) scan and completed performance tests, aligning with the pre-season Rugby Union testing period (Figure 4.1). Due to the potential effect of individual confounders on vitamin D status, participants also completed a demographic screening questionnaire and a sun exposure questionnaire during this time. Height and weight measurements were also collected and used to calculate body mass index (BMI = weight (kg) divided by height squared (m²)). Southland players had their weight measured using calibrated scales (3600SC bench scale, Avery Weigh-Tronix, West Midlands, UK) and self-reported their height. Otago players had their height measured using a calibrated stadiometer (Harpenden Stadiometer, Holtain Ltd., Pembrokeshire, UK) and weight measured using calibrated scales (Seca Alpha 770, Seca Corp, Hamburg, Germany), at the University of Otago, Department of Human Nutrition research clinic.

**Figure 4.1.** Baseline data collection time points for the different Rugby Unions (Otago ITM Cup, Southland ITM Cup and Otago Academy). March to May 2011.
4.2.3 Screening Questionnaire
A self-administered screening questionnaire (Appendix D) was completed by all (n=57) participants, which included data such as: age and date of birth, self-identified ethnicity, occupation, playing position, years playing rugby union and years playing at representative level, use of supplements and goals of pre-season training.

4.2.4 Sun Exposure Questionnaire
A self-administered sun exposure questionnaire (Appendix E) was completed by 55 participants, which included questions regarding outdoor activities (including outside training and other outdoor activities between the hours of 11am and 4pm, for at least 15 minutes), sun protection habits and recent travel. It focused on sun exposure relating to the week prior to the fasting blood sample being taken. The questionnaire was a modified version of the New Zealand Sun Exposure Survey (SES), developed by the Health and Sponsorship Council and the Cancer Society of New Zealand (142). The SES is conducted by interviewers via telephone every three years and assesses behaviours and attitudes around sun exposure (142). The modified questionnaire included information specific to athletes, such as, time spent outdoors during training and excluded information, such as; weather perception, advertising of sun behaviour and skin cancer knowledge.

4.2.5 Performance Tests
Performance tests were conducted, as per usual squad routines, during the pre-season training period for both the Otago and Southland squads. The strength, speed and repeated sprint ability tests performed were standard New Zealand Rugby Union (NZRU) fitness tests and therefore, all participants were familiar with the testing protocols. The strength and conditioning coaches recorded test results on paper. Cognitive function was also included as a performance test.

4.2.5.1 Strength
Predicted One Repetition Maximum (1RM) box squat (Otago), squat (without box) (Southland), predicted 1RM bench pull, predicted 1RM bench press and predicted 1RM weighted reverse-grip chin-up (over-hand grip) were assessed, as the strength performance tests. Predicted 1RM tests are the most frequently used test for evaluating muscle strength.
(143). Because of the injury risk inherent in performing a single repetition of maximum weight, the values for 1RM are a predicted score using lesser weights (143).

4.2.5.2 Speed
A 10m sprint and a 30m sprint were completed using a computerized timing system with timing lights.

4.2.5.3 Repeated Sprint Ability
Participants also performed the Yo-Yo Intermittent Recovery Test (Yo-Yo test), which involved a 20m shuttle run marked out by cones, a quick turn around and a full 20m run back with a 10m active recovery turn around before the next shuttle is to be completed, which was controlled by audio using a compact-disc player (Figure 4.2) (144). Each section of the test is performed at the sound of a beep and there is a decrease in the length of time before the next beep, as participants get further into the test (144). The number of levels completed by each participant was recorded.

![Diagram](image)

**Figure 4.2.** Schematic of the Yo-Yo Intermittent Recovery Test.

4.2.5.4 Cognitive Function
Professor Liz Franz of the Psychology Department at the University of Otago designed a reaction time test for use in this study. Fifty-five of the participants completed the test, which
was performed individually via a laptop computer at the site of the performance testing. As soon as a green dot appeared on the screen, participants were to hit the enter/return key on the keyboard. If participants hit enter too early by anticipating the green dot appearing, the computer would display an error message and results of this error were recorded (error rate of the reaction time test (number of errors)). Any green dots missed were also recorded as errors. The main outcome of the reaction time test was the mean time (s) it took participants to hit enter/return after the green dot appeared on the screen.

4.2.6 Body Composition

Results regarding body composition are reported elsewhere (145). Briefly, 50 participants underwent a whole body DXA scan to determine body composition and bone mineral density (BMD). Participants located in Otago had their DXA scan done at the Dunedin Hospital using a Lunar Prodigy scanner (Lunar Corporation, Madison, WI, USA) with standard Lunar software package (enCORE, version 13.6) and using the procedures recommended by the manufacturer with this particular scanner.

The scanner was regularly monitored to meet international drug trial stability requirements for quality control and a single trained technician (trained in the operation and positioning of the subjects) conducted the scan on participants lying supine with thumbs facing up and palms facing legs after removing any metal objects such as belts, metal buttons, jewellery, coins and other items left in their pockets. For participants taller than 196cm (maximal length of the scanner), participants were positioned with a portion of the head outside of the scanner to ensure feet were within view, reducing any affect on body composition measures (the head has lower fat mass and lean tissue mass). Southland participants also underwent DXA scans using the same model scanner at the Southland Branch of Otago Radiology.

Outcome measures for DXA were total fat mass (kg), bone-free lean tissue mass (kg), BMD (g/cm²), bone mineral content (BMC) (kg) and percent body fat (%), which was calculated as (total fat mass / (total fat mass + bone-free lean tissue mass + bone mineral content) x 100). The Z-score for BMD was also calculated, which is expressed as a standard deviation of the mean of a normal distribution of an age-matched control population. The
coefficients of variation (CVs) for repeat scans in adults for whole body BMC (g) and BMD (g/cm²) are 1.61% and 1.96%, respectively. CV values were 1.8% for total fat mass, 1.0% for bone-free lean tissue mass and 1.8% for percentage fat.

4.2.7 Blood Sample Collection

Venous blood was collected from all participants in the original study after an overnight fast. Otago players had their blood collected in the clinic at the Department of Human Nutrition, University of Otago by an experienced research nurse. The same research nurse travelled to Invercargill (Southland) with research staff to collect blood samples from the 23 Southland players.

Standard 6mL ‘red top’ Vacutainer Rapid Serum Tubes (Becton Dickinson, Plymouth, UK) with no anti-coagulant were used for collecting venous blood samples. The samples were left to stand at room temperature immediately after collection for at least 60 minutes, to allow the samples to clot. The blood samples were then centrifuged for 15 minutes at 3,000 RPM. Each of the participants serum samples were split into five aliquots of 400µL, pipetted into plastic micro-centrifuge tubes with lids (Labcon, California, USA) and held on ice before being stored at -80°C, prior to analysis. All serum blood samples were collected and stored until the end of the study period, when all samples could be analysed together. One of the samples was used for the analysis of 25(OH)D and the remaining samples were for the determination of parathyroid hormone, insulin-like growth factor 1 and insulin-like growth factor binding proteins 1-7 (currently not analysed).

4.2.8 Biochemical Analysis of Serum 25-hydroxyvitamin D

Serum 25(OH)D concentration was determined by the ‘gold standard’ liquid chromatography tandem mass spectroscopy (LC-MS/MS). Samples were delivered to The Canterbury Health Laboratories in Christchurch, New Zealand, for analysis according to the methods for LC-MS/MS, published by Maunsell et al. (146). LC-MS/MS determines both 25(OH)D₂ and 25(OH)D₃ (77), however, during the analysis of samples, no results were detected for 25(OH)D₂ and therefore, the results reflect only 25(OH)D₃.
The Canterbury Health Laboratories calculated the sensitivity and inter-assay CV in 885 samples. The assay sensitivity was 1 nmol/L. The inter-assay CV’s for 25(OH)D were 10.9% for the 24.2 nmol/L standard, 14.4% for the 52.8 nmol/L standard, 9.2% for the 101.9 nmol/L standard and 6.5% for the 194.8 nmol/L standard. The control sample was within 98.8-100.7% of the expected value.

4.2.9 Sample Size Estimation

The size of the study population was based on the number of participants needed to provide statistical power to detect a difference in the primary outcome variable - 30m sprint, measured in seconds, which is an indicator of speed and acceleration that is directly comparable among players with differing body weights, according to the strength and conditioning coaches. A difference between the two treatment groups of 0.08 seconds was identified as clinically significant (historical data showing a mean 30m sprint time of 4.14 seconds). The study had 80% power to detect a difference of 0.08 in the 30m sprint time, using a two-sided 0.05 level of significance. A total of 28 participants were needed in each treatment group. Therefore the sample size was not estimated for all the comparisons presented in this thesis.

4.3 Statistical Analysis (Employed for this Secondary Data Sub Study)

Statistical analyses were performed using the Statistical Package for Social Science (SPSS, version 21) for Mac. P values of <0.05 were considered statistically significant for all tests.

Descriptive analyses of the data were completed, including means and standard deviations for continuous variables (eg. age, body mass, height) and number and percentages for categorical data (eg. ethnicity). Ethnicity was collapsed into three categories: NZ European, Māori and Pacific, where NZ European was considered a priority group, as it was the dominant ethnic group in this population. Sunscreen use was also collapsed into two categories: yes or no.

Individual serum 25(OH)D concentrations were grouped into categories to demonstrate the percentage of overall vitamin D status of the participants by category. Five categories were created: <25 nmol/L “deficient”, 25-50 nmol/L “insufficient”, 51-75 nmol/L “sufficient”, 76-125 nmol/L “optimal” and >125 nmol/L “potential toxicity”. Two of the categories <25
nmol/L and 25-50 nmol/L were not included in the final analysis, as no participant had serum 25(OH)D concentrations within these ranges.

### 4.3.1 Performance Tests

The following variables were examined for associations with serum 25(OH)D concentrations: Yo-Yo test (level attained), 30m sprint (s), predicted 1RM bench press (kg), predicted 1RM bench pull (kg) and predicted 1RM weighted reverse-grip chin-up (kg). The predicted 1RM box squat/squat (kg) was eliminated, as different methodologies were used in the different training squads. The 10m sprint (s) was eliminated, as the 30m sprint was the primary outcome of the original study.

Bivariate Pearson’s correlation analyses were used to determine any associations between performance tests (dependent variable) and serum 25(OH)D concentrations. Scatterplots were generated with lines of best-fit, $R^2$, correlation coefficient ($r$) and $p$ value for each of the performance tests.

To evaluate associations between performance tests and serum 25(OH)D concentrations, a linear regression model was used. Unstandardised regression coefficients ($B$), 95% confidence intervals and $p$ values were calculated individually for each performance variable in an unadjusted analysis. As body mass was significantly related to all performance variables, it was considered a confounder, along with training group. These confounders were included in the final multivariate analysis to give the adjusted values, as unstandardised regression coefficients ($B$), 95% confidence intervals and $p$ values. The residuals of the adjusted regression analyses were plotted and assessed to be normal. There were no problems with multicollinearity in this linear regression model.

Median reaction time test (s) and reaction time error rate (number of errors) were analysed for associations with serum 25(OH)D concentrations, using spearman’s rho analyses, as this resolved issues with non-normality in the residuals.

### 4.3.2 Potential Predictors of Serum 25-hydroxyvitamin D Concentrations

When exploring potential predictors of serum 25(OH)D concentrations, again the evidence base of literature was referred to in identifying factors influencing 25(OH)D concentrations.
Factors identified included: season, latitude, sun exposure, clothing, sunscreen use, ethnicity, age, body composition (BMI, percent body fat) and BMD. Season, latitude and clothing were disregarded in the analyses, as these factors remained constant within the participants of this study. Age was also disregarded, as the age range was very narrow in this population (18 to 27 years). Recent travel was also reported, however, as only two participants had recently travelled, this data was not included in the analyses. While BMD is an unlikely predictor of serum 25(OH)D concentrations, significant associations between vitamin D status and BMD occur; therefore this factor was included in the correlation analysis only. Thus, it was decided \textit{a priori} to include sun exposure, body mass, BMI, percent body fat, ethnicity and sunscreen use in a multivariate linear regression model.

Initially Pearson’s correlations were plotted using scatterplots for the continuous variables to demonstrate their associations with serum 25(OH)D concentrations (dependent variable). These were generated with lines of best-fit, \textit{R}-squared linear, correlation coefficient \( (r) \) and \( p \) value for each continuous variable.

Because self-identified ethnicity was a categorical variable, a one-way between-groups analysis of variance (ANOVA) was used to investigate any significant associations between serum 25(OH)D concentrations and ethnicity. Inspection of the skewness, kurtosis and Shapiro-Wilk statistics indicated that the assumption of normality was supported for each of the three categories (NZ European, Māori and Pacific). Levene’s test was non-significant \( (F (2, 54) = 1.29, p = 0.28) \) and therefore, the assumption of homogeneity of variance was not violated. A post hoc analysis with Bonferroni correction for multiple comparisons was used to determine any between-groups effects.

Sunscreen use was also categorical, so an independent samples t-test was used to compare sunscreen use (yes/no) with serum 25(OH)D concentrations. Levene’s test for equality of variances was not significant for this test \( (F = 0.33, p = 0.569) \), therefore, equal variances could be assumed.

Subsequently, all variables were included in univariate (unadjusted) linear regression analyses, excluding BMD, as it is an unlikely predictor of serum 25(OH)D concentrations.
Unstandardised regression coefficients ($B$), 95% confidence intervals and $p$ values were calculated individually for each potential predictor variable in an unadjusted analysis. All potential predictor variables were considered important confounders for inclusion into the final multivariate regression model to create a final model of potential predictors associated with serum 25(OH)D concentrations. Unstandardised regression coefficients ($B$), 95% confidence intervals and $p$ values were calculated adjusting for all potential predictors, presented as adjusted values. Prior to interpretation of analysis, the residuals were plotted and assessed to be normal. There were no problems with multicollinearity in this linear regression model.
Table 5.1.

Participant characteristics at baseline

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) n=57</td>
<td>21.2 (2.81)</td>
</tr>
<tr>
<td>Body mass (kg) n=57</td>
<td>96.5 (11.40)</td>
</tr>
<tr>
<td>Height (m) n=57</td>
<td>1.9 (0.07)</td>
</tr>
<tr>
<td>BMI (kg/m²) n=57</td>
<td>28.2 (2.11)</td>
</tr>
<tr>
<td>Years playing rugby union (years) n=55</td>
<td>12.3 (4.51)</td>
</tr>
<tr>
<td>Years representing (years) n=52</td>
<td>5.5 (3.43)</td>
</tr>
<tr>
<td>Percent body fat (%) a n=50</td>
<td>14.6 (4.62)</td>
</tr>
<tr>
<td>Bone-free lean tissue mass (kg) a n=50</td>
<td>77.9 (9.92)</td>
</tr>
<tr>
<td>Bone mineral density (g/cm²) a n=50</td>
<td>1.5 (0.80)</td>
</tr>
<tr>
<td>Bone mineral density (Z-score) a n=50</td>
<td>2.8 (0.92)</td>
</tr>
<tr>
<td>Bone mineral content (kg) a n=50</td>
<td>4.4 (0.48)</td>
</tr>
<tr>
<td>Sun exposure per week (hrs) n=55</td>
<td>7.6 (7.21)</td>
</tr>
<tr>
<td>Total serum 25(OH)D (nmol/L) b n=57</td>
<td>94.3 (17.92)</td>
</tr>
<tr>
<td>Ethnicity n (%)</td>
<td></td>
</tr>
<tr>
<td>NZ European</td>
<td>35 (61)</td>
</tr>
<tr>
<td>Māori</td>
<td>14 (25)</td>
</tr>
<tr>
<td>Pacific</td>
<td>8 (14)</td>
</tr>
<tr>
<td>Training squad n (%)</td>
<td></td>
</tr>
<tr>
<td>Otago ITM Cup</td>
<td>26 (46)</td>
</tr>
<tr>
<td>Otago Academy</td>
<td>8 (14)</td>
</tr>
<tr>
<td>Southland ITM Cup</td>
<td>23 (40)</td>
</tr>
<tr>
<td>Training group n (%)</td>
<td></td>
</tr>
<tr>
<td>Strength-power (Otago)</td>
<td>10 (18)</td>
</tr>
<tr>
<td>Strength-speed (Otago)</td>
<td>10 (18)</td>
</tr>
<tr>
<td>Strength (Otago)</td>
<td>10 (18)</td>
</tr>
<tr>
<td>Hypertrophy (Otago academy)</td>
<td>4 (7)</td>
</tr>
<tr>
<td>Power (Southland)</td>
<td>6 (10)</td>
</tr>
<tr>
<td>Hypertrophy (Southland)</td>
<td>10 (18)</td>
</tr>
<tr>
<td>Uni-lateral strength (Southland)</td>
<td>7 (12)</td>
</tr>
</tbody>
</table>

Note: Descriptive statistics of study population. Results presented as mean (SD) or number (percent).

a Percent body fat, bone-free lean tissue mass, bone mineral density and bone mineral content measured by DXA (Dual-energy X-Ray absorptiometry).

b 25(OH)D samples all taken during the same season (Autumn).

Abbreviations: BMI, body mass index; ITM Cup, Independent merchants co-operative Ltd. Cup; NZ European, New Zealand European; 25(OH)D, 25-hydroxyvitamin D.
5 Results

Fifty-seven participants completed the randomised, placebo-controlled, double-blinded intervention study “Vitamin D Status and Effect of Supplementation on Strength, Speed and Body Composition in Rugby Union Players”. All participants had serum 25(OH)D concentrations measured and were included in the final analysis. Of the participants, 55 completed the sun exposure questionnaire, completing data on hours of sun exposure per week and sunscreen use. Due to existing injury not all participants completed all measures of performance testing (Yo-Yo Intermittent Recovery Test, n=37; 30m sprint, n=24; predicted 1RM bench pull, n=38; predicted 1RM weighted reverse-grip chin-up, n=38; predicted 1RM bench press, n=39). Due to equipment failure all 23 Southland players were unable to complete the 30m sprint test.

5.1 Participant Characteristics

Demographic characteristics of the study participants are shown in Table 5.1. The mean age was 21 years (range, 18 to 27 years) and most participants self-identified as NZ European ethnicity (61%). The participants had a mean BMI (weight (kg) divided by height squared (m²)) of 28 kg/m². Mean percent body fat of the participants was 14.6%. Sun exposure per week averaged 7.6 hours (range, 0-33 hours per week). Thirty-four (60%) of the participants were from Otago and 23 (40%) were from Southland, New Zealand.

5.2 Evaluation of Vitamin D Status

The mean serum 25(OH)D concentration was 94.3 nmol/L, which ranged from 57-131 nmol/L, therefore, no participant had insufficient or deficient vitamin D status when defined by serum 25(OH)D concentrations of <50 nmol/L (8). Seven participants (12%) had serum 25(OH)D concentrations between 50-75 nmol/L (sufficient), of these, four self-identified as Pacific ethnicity. The majority of the participants (n=46, 81%) had serum 25(OH)D
concentrations of between 76-125 nmol/L (optimal) and four participants (7%) had a serum 25(OH)D concentration of greater than 125 nmol/L (potential toxicity) (Figure 5.1).

**Figure 5.1.** Vitamin D status of rugby union players from Otago and Southland, New Zealand (latitude: 45-47° S).

### 5.3 Performance Tests

#### 5.3.1 Correlations between Serum 25-hydroxyvitamin D Concentrations and Strength, Speed and Repeated Sprint Ability

There were no associations between serum 25(OH)D concentrations and strength, speed and repeated sprint ability performance tests ($p > 0.05$) (Figures 5.2-5.6), suggesting that these measures of performance were not linearly related to serum 25(OH)D concentrations.
**Figure 5.2.** Scatterplot demonstrating unadjusted bivariate correlation between Yo-Yo Intermittent Recovery Test (level) and serum 25-hydroxyvitamin D concentration (nmol/L).

![Scatterplot](image)

$R^2$ Linear = 0.018  
Pearson correlation  
$r = -0.13$  
$p = 0.43$

**Figure 5.3.** Scatterplot demonstrating unadjusted bivariate correlation between 30m sprint (s) and serum 25-hydroxyvitamin D concentration (nmol/L).

![Scatterplot](image)

$R^2$ Linear = 0.049  
Pearson correlation  
$r = 0.22$  
$p = 0.30$
Figure 5.4. Scatterplot demonstrating unadjusted bivariate correlation between predicted 1RM bench pull (kg) and serum 25-hydroxyvitamin D concentration (nmol/L).

Figure 5.5. Scatterplot demonstrating unadjusted correlation between predicted 1RM weighted reverse-grip chin-up (kg) and serum 25-hydroxyvitamin D concentration (nmol/L).
Figure 5.6. Scatterplot demonstrating unadjusted bivariate correlation between predicted 1RM bench press (kg) and serum 25-hydroxyvitamin D concentration (nmol/L).

$R^2$ Linear = 0.018
Pearson correlation
$r = -0.13$
$p = 0.42$
Table 5.2.

Associations between serum 25-hydroxyvitamin D concentrations and performance tests

<table>
<thead>
<tr>
<th>Performance variable</th>
<th>n (%)</th>
<th>Serum 25-hydroxyvitamin D (nmol/L)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Unadjusted(^a)</td>
<td>Adjusted(^b)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B (95% CI)</td>
<td>p value</td>
<td>B (95% CI)</td>
</tr>
<tr>
<td>Yo-Yo Test (level attained)</td>
<td>37 (65)</td>
<td>-0.01 (-0.03, 0.01)</td>
<td>0.43</td>
<td>-0.01 (-0.02, 0.01)</td>
</tr>
<tr>
<td>30m Sprint (s)</td>
<td>24 (42)</td>
<td>0.00 (-0.00, 0.01)</td>
<td>0.30</td>
<td>0.00 (-0.00, 0.01)</td>
</tr>
<tr>
<td>Predicted 1RM Bench Pull (kg)</td>
<td>38 (67)</td>
<td>-0.12 (-0.30, 0.06)</td>
<td>0.18</td>
<td>-0.13 (-0.28, 0.02)</td>
</tr>
<tr>
<td>Predicted 1RM Bench Press (kg)</td>
<td>39 (68)</td>
<td>-0.14 (-0.49, 0.21)</td>
<td>0.42</td>
<td>-0.25 (-0.57, 0.06)</td>
</tr>
<tr>
<td>Predicted 1RM Wt Reverse Chin-Up (kg)</td>
<td>38 (67)</td>
<td>-0.07 (-0.34, 0.20)</td>
<td>0.61</td>
<td>-0.15 (-0.35, 0.05)</td>
</tr>
</tbody>
</table>

\(^a\) Unadjusted regression coefficients using linear regression, statistical significance at \(p < 0.05\).

\(^b\) Adjusted for training group and body mass. Regression coefficients using multiple linear regression, statistical significance at \(p < 0.05\).

Training group involves 7 categories; Otago = Strength-power, Strength-speed, Strength; Otago academy = Hypertrophy; Southland = Power, Unilateral strength, Hypertrophy.

Regression coefficient (\(B\)) represents the change in serum 25-hydroxyvitamin D concentration per unit change in performance test.

Abbreviations: \(B\), unstandardized regression coefficient; CI, confidence interval; Wt reverse chin-up, weighted reverse-grip chin-up; Yo-Yo test, Yo-Yo Intermittent Recovery Test; 1RM, one repetition maximum.
5.3.1 Regression Analysis of Serum 25-hydroxyvitamin D Concentrations on Strength, Speed and Repeated Sprint Ability

Strength, speed and repeated sprint ability performance tests were not associated with serum 25(OH)D concentrations in the unadjusted analysis (Table 5.2). Although the strength of associations between serum 25(OH)D concentrations and performance variables did improve with the inclusion of confounders in the multivariate analysis, none of these associations reached statistical significance ($p > 0.05$). The relationship between serum 25(OH)D concentrations and predicted 1RM bench pull tended towards significance when confounders were included in the multivariate analysis ($p = 0.09$). However, the effect size remained small.

5.3.2 Associations between Serum 25-hydroxyvitamin D Concentrations and Cognitive Performance

No association was found between serum 25-hydroxyvitamin D concentrations and the median reaction time test ($p = 0.21$) or the error rate of the reaction time test ($p = 0.66$).

5.4 Potential Predictors of Serum 25-hydroxyvitamin D Concentrations

5.4.1 Correlations between Serum 25-hydroxyvitamin D Concentrations and Continuous Potential Predictors

No associations were found between serum 25(OH)D concentrations and factors identified in the literature, as influencing circulating 25(OH)D, including sun exposure, body mass, percent body fat and BMD ($p > 0.05$) (Figures 5.7-5.10). The association between serum 25(OH)D concentrations and sun exposure tended towards significance ($p = 0.09$) (Figure 5.7).
Figure 5.7. Scatterplot demonstrating unadjusted bivariate correlation between 25-hydroxyvitamin D concentration (nmol/L) and sun exposure (hrs of sun per week).

Figure 5.8. Scatterplot demonstrating unadjusted bivariate correlation between 25-hydroxyvitamin D concentration (nmol/L) and body mass (kg).
Figure 5.9. Scatterplot demonstrating unadjusted bivariate correlation between 25-hydroxyvitamin D concentration (nmol/L) and percent body fat (%).

Figure 5.10. Scatterplot demonstrating unadjusted bivariate correlation between 25-hydroxyvitamin D concentration (nmol/L) and whole body bone mineral density (g/cm²).
Self-identified Pacific ethnic group was significantly lower in serum 25-hydroxyvitamin D compared to self-identified NZ European ($p < 0.001$) and Māori ($p = 0.003$).

* Figure 5.11. Bar graph demonstrating mean serum 25-hydroxyvitamin D concentrations (nmol/L) in different self-identified ethnic groups ($n=57$).
5.4.1 Associations between Serum 25-hydroxyvitamin D Concentrations and Categorical Potential Predictors

Ethnicity and sunscreen use were also identified in the literature as factors influencing serum 25(OH)D concentrations.

5.4.1.1 Self-identified Ethnicity
Serum 25(OH)D concentrations differed between the three self-identified ethnicities ($F (2, 54) = 9.83, p <0.001, \eta^2 = 0.27$). The post hoc analysis demonstrated that mean (SD) serum 25(OH)D concentrations in both self-identified NZ European (98.77 (16.14)) and Māori (95.86 (16.83)) ethnicities were significantly higher than those of self-identified Pacific ethnicity (71.75 (9.62)), $p <0.001$ and $p = 0.003$ respectively (Figure 5.11). However, there was no difference in serum 25(OH)D concentrations between self-identified NZ European and self-identified Māori ethnicities ($p = 1.00$).

5.4.1.2 Sunscreen Use
No association was found between sunscreen use (yes/no) and serum 25(OH)D concentrations ($p = 0.48$).
## Table 5.3.

**Predictors of serum 25-hydroxyvitamin D concentrations**

<table>
<thead>
<tr>
<th>Variable</th>
<th>n (%)</th>
<th>Serum 25-hydroxyvitamin D (nmol/L)</th>
<th>Unadjusted&lt;sup&gt;a&lt;/sup&gt;</th>
<th>p value</th>
<th>Adjusted&lt;sup&gt;b&lt;/sup&gt;</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>B (95% CI)</strong></td>
<td></td>
<td><strong>B (95% CI)</strong></td>
<td></td>
</tr>
<tr>
<td>Sun exposure (hours)</td>
<td>55 (96)</td>
<td>0.58 (-0.09, 1.25)</td>
<td>0.09</td>
<td></td>
<td>0.52 (-0.18, 1.21)</td>
<td>0.14</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>57 (100)</td>
<td>0.08 (-0.35, 0.50)</td>
<td>0.71</td>
<td></td>
<td>0.06 (-0.63, 0.75)</td>
<td>0.86</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>57 (100)</td>
<td>0.36 (-1.94, 2.66)</td>
<td>0.75</td>
<td></td>
<td>1.41 (-2.49, 5.31)</td>
<td>0.47</td>
</tr>
<tr>
<td>Percent Body fat (%)</td>
<td>50 (88)</td>
<td>-0.20 (-1.33, 0.93)</td>
<td>0.72</td>
<td></td>
<td>-0.18 (-1.42, 1.06)</td>
<td>0.77</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NZ European</td>
<td>35 (61)</td>
<td>11.68 (2.34, 21.02)</td>
<td><strong>0.02</strong></td>
<td></td>
<td>0 (Reference)</td>
<td></td>
</tr>
<tr>
<td>Māori</td>
<td>14 (25)</td>
<td>1.06 (-4.51, 6.63)</td>
<td>0.71</td>
<td></td>
<td>0.66 (-5.05, 6.37)</td>
<td>0.82</td>
</tr>
<tr>
<td>Pacific</td>
<td>8 (14)</td>
<td>-8.73 (-12.69, -4.77)</td>
<td><strong>&lt;0.001</strong></td>
<td></td>
<td>-8.57 (-13.63, -3.52)</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Sunscreen use</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5 (9)</td>
<td>-5.99 (-22.88, 10.90)</td>
<td>0.48</td>
<td></td>
<td>0 (Reference)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>50 (91)</td>
<td>2.67 (-4.60, 9.95)</td>
<td>0.47</td>
<td></td>
<td>5.51 (-3.31, 14.32)</td>
<td>0.21</td>
</tr>
</tbody>
</table>

<sup>a</sup> Unadjusted regression coefficients using linear regression, statistical significance at p <0.05.

<sup>b</sup> Adjusted for sun exposure, body mass, BMI, percent body fat, ethnicity and sunscreen use. Multiple linear regression, statistical significance at p <0.05. Regression coefficient (B) represents the change in serum 25-hydroxyvitamin D concentration per unit change in the relevant predictor.

Abbreviations: B, unstandardized regression coefficient; BMI, body mass index; CI, confidence interval; NZ European, New Zealand European.
5.4.1 Regression Analysis to Identify Potential Predictors of Serum 25-hydroxyvitamin D Concentrations

There were significant associations found between serum 25-hydroxyvitamin D concentrations and both self-identified NZ European and Pacific ethnicities \( (p = 0.02 \text{ and } p < 0.001, \text{ respectively}) \) (Table 5.3). Those of self-identified Pacific ethnicity were predicted to have a serum 25(OH)D concentration of 8.73 nmol/L lower than those of other self-identified ethnicities (NZ European and Māori). When controlling for other potential predictors in the adjusted multivariate model the association was attenuated, but remained statistically significant when compared to the reference group of self-identified NZ European \( (p = 0.001) \).

The association between serum 25(OH)D concentrations and sun exposure tended towards significance \( (p = 0.09) \) (Table 5.3). Sun exposure is well recognised as the biggest contributor to circulating 25(OH)D (99). Therefore, a bootstrap analysis of 1000 samples (taken from this sample) was conducted to investigate if the lack of significance in this study might be due to the sample size. However, this analysis did not indicate any significant relationship between serum 25(OH)D concentrations and sun exposure in this group, as it was identified as a predictor for less than 26% of the regression (results not shown).

The \( R^2 \) for the final model was 0.34 \( (p = 0.01) \). Suggesting that 34% of the total variance in serum 25-hydroxyvitamin D concentrations was explained by the predictors in the final model (including; sun exposure, body mass, BMI, percent body fat, ethnicity and sunscreen use).
6  Discussion

This is the first study to report the vitamin D status of New Zealand athletes, to assess associations between serum 25-hydroxyvitamin D concentrations and athletic performance in rugby union players and to determine potential predictors of serum 25-hydroxyvitamin D concentrations in these New Zealand athletes.

6.1 Main Findings

In this cross-sectional study, we found that this group of semi-professional male rugby union players, residing in Otago and Southland, New Zealand (latitude: 45-47° S) had a mean (SD) serum 25(OH)D concentration of 94 (18) nmol/L, from samples taken in autumn. No participant had a serum 25(OH)D concentration of less than 50 nmol/L, and only seven participants (12%) had serum 25(OH)D concentrations of ≤75 nmol/L. There were no significant associations between serum 25(OH)D concentrations and any measurements of athletic performance including: strength, speed, repeated sprint ability and cognitive tests (p >0.05). Self-identified ethnicity was a significant predictor of serum 25(OH)D concentration in this population. Those self-identifying as having Pacific ethnicity had serum 25(OH)D concentrations that were on average 8.57 nmol/L lower than those in the reference group of self-identified NZ European (p = 0.001).

6.2 Participant Characteristics

The participants were well-trained semi-professional male rugby union players, aged 18-27 years, who were studied during the autumn season. The mean BMI of the group was 28 kg/m² (healthy: 18.5 - 24.9), which could classify them as overweight, according to the World Health Organisation (147). However, BMI is not an indicator of adiposity in this athletic group and does not reflect body fat stores or health risk. Athletes have a high muscle mass (148) and low mean percent body fat (14.6% in the current study group) compared to the non-athletic population.
BMI is used in clinical sports nutrition, as an indicator of body mass, which reflects their muscle mass (as a result of resistance training (135)) that is relative to their height (personal communication, Dr. K Fairbairn, June 2013). If BMI is used in clinical sports nutrition, it is used to assess lean body mass and would be used alongside a measure of body fat (sum of 8 skin folds or percent body fat) (personal communication, Dr. K Fairbairn, June 2013). The average BMI is different for each playing position in rugby union, with forwards having a higher average BMI of 28-29 kg/m² than backs with a BMI of 25-26 kg/m² (135).

6.3 The Adequacy of Vitamin D Status

The mean serum 25(OH)D concentration (94 nmol/L) suggests that sufficient serum 25(OH)D concentrations are achievable in semi-professional rugby union players even at this high southern latitude (45-47° S). However, the high mean serum 25(OH)D concentration during autumn could reflect the accumulation of vitamin D during the summer (8), where the ability to spend leisure and training time outdoors is greater during the daylight hours where cutaneous synthesis of vitamin D is most effective (Figure 6.1).

![Figure 6.1. Average number of sunshine hours for each month of the year, data from historical averages (from 1749) in Dunedin (Otago), New Zealand (149). Sunshine hours are at their highest during the summer months (December, January and February) and lowest during the winter months (June, July and August).]
The results of mean serum 25(OH)D concentrations in this group were in contrast to much of the existing literature, which suggests a high prevalence of vitamin D deficiency and insufficiency (<50 nmol/L) in athletes worldwide (refer to Table 2.2, Section 2.8). However, much of this literature reports the vitamin D status of athletes during the winter months, when serum 25(OH)D concentrations tend to be lower because of reduced UV radiation and therefore, reduced cutaneous synthesis of vitamin D (101).

Consistent with other longitudinal and cross-sectional studies in athletes during the autumn season, only a small (12%) proportion of the participants in the current study had serum 25(OH)D concentrations of less than 75 nmol/L. At the University of Wyoming (USA), male and female athletes from different sports (residing at a similar latitude to the current study with the exception of being at a northern latitude (41° N)), found that 9.8% of the participants had serum 25(OH)D concentrations <75 nmol/L (57). Galan et al. (95) also assessed vitamin D status during the autumn and found that 7% of male football players residing in Spain (37° N) had serum 25(OH)D concentrations of less than 75 nmol/L (95).

Of note, different biochemical methods were used to assess serum 25(OH)D concentrations in the aforementioned studies, which could prevent direct comparisons. Biochemical method RIA was used by Halliday et al. (57), CLIA, by Galan et al. (95) and the biochemical method LC-MS/MS was used in the current study. These methods have different sensitivities for 25(OH)D and a recent study by Lai et al. (85) found that 25(OH)D concentrations measured by CLIA were lower than when measured by LC-MS/MS (85). The LC-MS/MS method is the current ‘gold standard’ for serum 25(OH)D analysis.

In contrast to our findings, a cross-sectional study of female Australian gymnasts reported that 50% of the participants had serum 25(OH)D concentrations of <75 nmol/L, during the autumn season (92). However, this could be because gymnasts train indoors over the summer season, reducing the amount of sun exposure in this group. Furthermore, females have been found to have lower serum 25(OH)D concentrations when compared with males in non-athletic (75) and athletic populations (35).
No rugby player in the current study had a serum 25(OH)D concentration of <50 nmol/L, suggesting that no participant had clinically insufficient or deficient vitamin D status (8). However, New Zealand research suggests that vitamin D concentrations tend to be higher in the autumn and only decrease during the winter and spring months (8,90). It is a limitation of the current study that serum 25(OH)D concentrations were only investigated during autumn. It would be interesting to see if the 25(OH)D concentrations of these rugby players declines during the winter months, as observed previously in other athletic populations internationally (57,93,95,117).

A small number of participants (n=4) in the current study had serum 25(OH)D concentrations >125 nmol/L (129-131 nmol/L), which could indicate toxicity (68,69). However, other researchers suggest that toxicity occurs when serum 25(OH)D concentrations are >250 nmol/L (65,66,70). These amounts are arbitrary, as there is a lack of adequate data assessing the consequences and long term effects of these high vitamin D concentrations and currently no safe upper level of vitamin D (3,5). Therefore, the use of these cut-offs as a target for toxicity in this population group should be interpreted with caution.

None of the participants in the current study were taking vitamin D supplements; therefore, the reason for these high serum 25(OH)D concentrations (>125 nmol/L) is uncertain at this time. Other diagnostic or clinical tests would be needed to investigate this further. However, the cause could be naturally high 25(OH)D concentrations. As serum calcium was not measured, we were unable to determine whether the high 25(OH)D concentrations caused hypercalcemia in these rugby players. No participant in the current study had any underlying diagnosed disease that could have caused high serum 25(OH)D concentrations. The potential adverse effects of high serum 25(OH)D concentrations from sun exposure and food intake compared to supplementation needs further investigation, as the effects may differ between sources of vitamin D (5).
6.4 Relationship between Serum 25-hydroxyvitamin D Concentrations and Performance Tests

There were no significant correlations between serum 25(OH)D concentrations and rugby
union standard performance tests: Yo-Yo Intermittent Recovery Test ($p = 0.43$), 30m sprint ($p = 0.30$), predicted 1RM bench pull ($p = 0.18$), predicted 1RM weighted reverse-grip chin-up ($p = 0.61$) and predicted 1RM bench pull ($p = 0.42$). When adjusting for training group and body mass in the regression model, the strength of associations improved from the unadjusted model, however, remained non significant (refer to Table 5.2, Section 5.3). After adjustment, there was a tendency towards significance for the predicted 1RM bench pull ($p = 0.09$), but this effect size was very small.

It is likely that if any decline in athletic performance occurs with a fall in vitamin D status, it would not occur until serum 25(OH)D concentrations drop ($<50$ nmol/L) towards the end of winter and early spring (8,15,90). It would be of interest to see whether a greater range of serum 25(OH)D concentrations (from deficiency to potential toxicity) within our study group, would have produced a greater variation in the athletic performance outcomes. It has been reported that improvements in performance are less likely to occur when vitamin D status is already sufficient (15), and as this group were sufficient, this could be a reason why improvements in athletic performance were not seen.

Very little evidence exists in the literature investigating the relationship between vitamin D status and performance outcomes in athletes. The results of the current study are in contrast to two cross-sectional studies, which report significant associations between vitamin D status and measures of physical performance in non-athletic populations (71,74). The ranges included excessively low 25(OH)D concentrations in both these studies; 2.5 - 88.5 nmol/L (71) and $<25 - >75$ nmol/L (74), compared to the current study of 57 – 131 nmol/L. Most studies, finding significant improvements in physical performance with higher vitamin D status, have been conducted in either the elderly or in children, which may reflect differences in protein synthesis, muscle function and growth during those life stages. On the other hand, a recent cross-sectional study by Grimaldi et al. (74) found a significant relationship between greater muscle strength and higher vitamin D status (which was
collected over all four seasons), across a broader age range of non-athletic participants (age range, 20-76 years) (74). This finding suggests that vitamin D status improves performance in healthy young adults and is not limited to elderly and children.

Both of the aforementioned cross-sectional studies cannot conclude that higher vitamin D concentrations caused improved performance, because associations cannot imply causality. It is also difficult to compare the current study findings to these studies, due to the differences in measures of performance used and the different age groups.

A randomised dose-response study in a group of 30 athletes from different sports was conducted in the UK (latitude: 53° N) by Close et al. (96). Findings indicate that there were no baseline associations between performance and serum 25(OH)D concentrations (96). Furthermore, they found no significant changes in performance measures (1-RM bench press (kg), 1-RM leg press (kg), vertical jump (cm) and 20-m sprint (s)) over time with vitamin D supplementation (either 20,000 or 40,000 IU/week of oral vitamin D₃), during a 12 week period (96). This study had many limitations including: not reporting many factors known to influence vitamin D status, such as; sun exposure and skin pigmentation and not powered to detect significant changes in performance.

Our findings in rugby players are consistent with that of another large (n=667) cross-sectional study by Marantes et al. (73) of vitamin D replete non-athletic adults (21-97 years of age), from the USA, where no association was seen between serum 25(OH)D concentrations and any measurements of skeletal muscle mass or strength, even after adjustment for potential confounders including age, physical activity, season and fat mass (73). This study did find that lower serum concentrations of the biologically active form of vitamin D (1,25(OH)₂D) were associated with lower muscle mass in those under 65 years of age (73). The exact reason for this finding was unknown and therefore still needs exploring further. What is known is that VDR’s bind 1,25(OH)₂D with high affinity (7), which may demonstrate a more direct role of vitamin D on muscle mass and strength. We did not assess 1,25(OH)₂D in the current study, as it is not an accurate measure of vitamin D status, because of its short circulating half life of only 4-6 hours compared to 2-3 weeks for serum 25(OH)D (77).
6.5 Predictors of Serum 25-hydroxyvitamin D Concentrations

There was a significant difference in serum 25(OH)D concentrations between the three self-identified ethnic groups (NZ European, Māori and Pacific). Mean serum 25(OH)D concentrations in those of self-identified Pacific ethnicity were on average 24 nmol/L lower than those of self-identified Māori ethnicity and on average 27 nmol/L lower than those of self-identified NZ European ethnicity. This amount could be the difference between deficient and sufficient vitamin D status. It is well known that skin pigmentation affects the amount of vitamin D that is synthesised in the skin, as those with darker skin pigmentation have significantly lower concentrations of circulating 25(OH)D (5,97,121). Although many studies use ethnicity as a proxy for skin pigmentation, there are issues with assessing ethnicity only, as it is often self-identified and therefore, it is important to note the wide range of variability in natural skin colour among individuals of the same ethnicity, as well as between different ethnicities. Unfortunately, we were unable to objectively measure skin pigmentation in the current study.

Self-identified ethnicity was found to be a significant predictor of serum 25(OH)D concentrations in this study population. When adjusted for confounders, those of self-identified Pacific ethnicity had serum 25(OH)D concentrations on average 8.57 nmol/L lower than the reference group of self-identified NZ European ($p = 0.001$). This finding is in agreement with three previous studies in New Zealand adults and children, where those of Pacific ethnicity had lower mean (range) serum 25(OH)D concentrations of 43 (36-56) nmol/L, when compared to NZ Europeans: 60 (51-75) nmol/L (75,90,150). This finding is also in agreement with others investigating athletes internationally, where researchers found that skin pigmentation or ethnicity were associated with low serum 25(OH)D concentrations (12,13,35).

Latitude and season are known determinants of vitamin D status. As these factors were constant in our study group (all participants resided at a similar latitude (45-47° S) and all serum 25(OH)D analysis was conducted during the same season), they were therefore not assessed for associations with serum 25(OH)D concentrations in the current study.
Research suggests that sun exposure is the main contributor towards serum 25(OH)D concentrations (1,3). The total mean sun exposure hours in the current study, during autumn, was 7.6 hours per week, which was not associated with serum 25(OH)D concentrations ($p = 0.09$). A bootstrap analysis of 1000 samples was conducted to determine whether this insignificance could be due to the sample size, however this did not indicate any significant association between serum 25(OH)D concentrations and sun exposure. The reason for this lack of association could be because of limitations with the collection of sun exposure data (by questionnaire), as it relied on memory recall. Although the questionnaire in the current study was based on a validated sun exposure survey in non-athletes (142), it was a modified version and therefore, the rugby players may have interpreted the question about “other outdoor activities” differently to the non-athletic population (e.g. other activities may have been interpreted as a combination of training time and other outdoor activities).

Halliday et al. (57) looked at 41 male and female athletes from varying sports, residing at a similar latitude (41° N) and reported sun exposure hours for training and competition to be 7.8 hours per week, plus 4.5 hours per week for leisure time outdoors, also during the autumn season (57). The collection of data was also by questionnaire and was an estimate using a frequency scale of time spent outdoors (57). Halliday et al. (57) found that time spent outdoors during training and competition and total time spent outdoors correlated with vitamin D status (57), which is in contrast to the current study. The differences in findings between these studies could be due to a multitude of factors, including: differences in the groups of athletes compared, limitations in using a questionnaire to recall sun exposure (the frequency scale used in Halliday et al. (57) could have under or overestimated sun exposure hours), and differences in statistical analyses used to determine associations (Spearman’s rank (57), compared to Pearson’s correlation in the current study).

In the current study, BMI and percent body fat were not associated with serum 25(OH)D concentrations. This is consistent with other studies in athletes, which report that vitamin D status is not influenced by adiposity (57,58). However, is in contrast to studies in non-athletes, which report that adiposity is associated with low vitamin D status (108,112,127). The discrepancies between athletes (in the current study and others) and non-
athletes are likely due to differences in body composition between the two populations (athletes have a higher muscle mass and lower percent body fat).

Sunscreen use was not significantly associated with serum 25(OH)D concentrations in this group of male rugby union players. However, only a very small (n=5) sample of the participants reported using sunscreen. Research demonstrates that males are less likely to use sunscreen than females (110,112).

The final $R^2$ for the predictor model investigating potential predictors of serum 25(OH)D concentrations showed that 34% ($p = 0.01$) of the total variance in serum 25(OH)D concentrations was explained by the predictor variables used in the model including: sun exposure, body mass, BMI, percent body fat, ethnicity and sunscreen use. This percentage demonstrates that the final model is good at predicting the outcome of serum 25(OH)D concentrations. To our knowledge, there has been no research reporting a similar predictor model in athletes, although one study investigating New Zealand children (aged 12-22 months) reported that 36.3% of the variance in serum 25(OH)D concentrations was explained by predictors in their final multivariate regression model (including: season, sex, ethnicity, skin colour, education of caregiver, exposure to cigarette smoke, breastfeeding, age and BMI) (151).

While BMD is not a likely predictor of serum 25(OH)D concentrations, in the non-athletic population, vitamin D status has been significantly associated with BMD in that those with low vitamin D status tend to have low BMD (52,54,55). In our study population no such significant association existed ($p = 0.59$). This is in agreement with other research in athletic populations (11,57,58). The lack of association in this group of athletes (in the current study) could be due their high mean serum 25(OH)D concentration (94 nmol/L) or because of their high BMD and BMC, which is common in male athletes who engage in weight-bearing and high impact sports, such as rugby (148).
6.6 Strengths and Limitations of the Study

6.6.1 Strengths
The data collected for this cross-sectional analysis was from a well-designed double-blinded randomised placebo-controlled trial. There was a reasonable sample size of 57 participants compared to other studies investigating vitamin D status in athletic populations internationally of: 18 athletes (92), 41 athletes (57), 16 athletes (58), 21 athletes (12), 20 athletes (13), 19 athletes (94), 28 athletes (95), 19 athletes (117), 37 athletes (14) and 30 athletes (96).

There was homogeneity amongst this group in factors that influence serum 25(OH)D concentrations. For example, the blood samples were all assayed using the same method (LC-MS/MS, which is the ‘gold standard’ method for accurate measure of serum 25(OH)D status) and samples were also taken during the same season, which eliminated any confounding effect of season in the current results. However, this might also be perceived as a limitation, as vitamin D status could decrease in later months, which may have provided differing results. No participant was taking vitamin D supplementation; therefore this did not affect the overall vitamin D status of the participants.

The current study used standard New Zealand Rugby Union performance tests that were designed to replicate what rugby players would experience during training or competition and therefore were sport specific. The testing protocols were well known to the participants and this strengthens the applicability of this study. The results from the performance tests were recorded by the strength and conditioning coaches, which reduced any potential bias.

6.6.2 Limitations
The limitations of the current study include the cross-sectional nature of the study, which means that causation cannot be inferred. This study included only male rugby union players and reportedly athletic males have higher serum 25(OH)D concentrations compared to females (35). Therefore, these findings might not be transferable to female rugby players or female athletes in other team field type sports.
Vitamin D intake was not measured. However, the evidence suggests that vitamin D intake contributes very little to serum 25(OH)D concentrations (3). Additionally, unlike other countries, there is currently no mandatory fortification of vitamin D in food products within New Zealand (2).

Some participants were unable to complete the performance tests due to injury and equipment failure, which may have reduced our ability to detect any significant associations between serum 25(OH)D concentrations and performance.

The current study was limited by reliance on self-reported questionnaires for the measurement of sun exposure. This may have contributed to under or overestimation of actual UVB exposure. Although the participants were asked about certain behaviours for the week prior to blood collection, such as time spent outdoors, sunscreen use, clothing cover and recent travel, it is still hard to get accurate descriptions of these behaviours (refer to section 6.4). Also, atmospheric conditions, such as the thickness of cloud cover (which can reduce UV irradiiances by about 50%) and atmospheric turbidity (119) have not been taken into account but can have an impact on total sun exposure (119). No information was collected on indoor training and therefore, we could not solely classify the sport of rugby union as outdoors.

Another limitation was that we did not objectively measure skin pigmentation and it is well known that natural skin colour varies considerably within and between each ethnic group (121).
6.7 Directions for Future Research

As the current study was based on cross-sectional data, we cannot establish the nature of any relationships seen. However, this sub study was part of an RCT looking at vitamin D supplementation and athletic performance in rugby union players. Based on the results of the RCT, we are unable to confirm a benefit of vitamin D supplementation on athletic performance (personal communication, Dr K. Fairbairn, June 2013).

Further research should focus on:

- Confirming what 25(OH)D concentration is considered “sufficient” for athletes, as the current research is complicated by variations in definitions of what constitutes sufficient, insufficient and deficient vitamin D status. It is also important to determine what serum 25(OH)D concentration is considered “optimal”, including what concentration (if any) is deemed beneficial when it comes to athletic performance enhancement and what/if any negative effects occur when serum 25(OH)D concentrations are greater than 125 nmol/L, as seen in the current study.

- Conducting a longitudinal study to measure variations in serum 25(OH)D concentrations over the year to see if these variations are associated with changes in athletic performance. The research should be extended to include female rugby players and could also include males and females from different team field sports.

- Assessing the biologically active form of vitamin D (1,25(OH)_2D) and any potential improvements in athletic performance associated with this form of vitamin D, as this may demonstrate a more direct role of how vitamin D affects skeletal muscle. Serum samples would need to be taken directly after performance tests, as 1,25(OH)_2D has a short half life of 4-6 hours (77).

- Improving methodological aspects for collecting sun exposure data. For example: a questionnaire more suited to athletes to ascertain training time spent outdoors versus leisure time outdoors, as well as information on training time indoors for classification of outdoor or indoor sport; use of a daily log book or personal UV monitoring device, to
assess day-to-day variations in UV exposure, as this would include weather conditions, occupational sun exposure as well as leisure, training and competition sun exposure.

Assessing skin pigmentation objectively (by reflectance colorimetry) in a larger sample of ethnically diverse athletes within New Zealand, to see if the association found in the current study between darker skin pigmentation and lower 25(OH)D concentrations remains in a larger sample group.
6.8 Conclusions

This is the first study to assess the vitamin D status of a group of New Zealand athletes. Much research demonstrates high rates of vitamin D deficiency and insufficiency in athletes internationally (9-14,20,35,57,58,92-96,117). Therefore, it was interesting to note that when using the current Ministry of Health cut-offs (sufficient concentrations >50 nmol/L) (8), the male rugby union players in this study are considered vitamin D replete during autumn, even though these participants reside in the southernmost parts of the country (latitude: 45-47° S). Recent international research suggests that vitamin D concentrations between 75 nmol/L and 125 nmol/L are considered “optimal” (6,99), which would mean that seven (12%) participants in the current study do not have “optimal” vitamin D status, as they have serum 25(OH)D concentrations of <75 nmol/L.

Many athletes are currently taking multiple nutritional supplements with the thought that they will help with “improving performance” (25). However, the results from this study suggest that having higher than adequate vitamin D concentrations and therefore status is not going to influence athletic performance in semi-professional male rugby union players. Therefore, based on the results of this current study, in a group of vitamin D replete athletes, vitamin D supplementation is unlikely to be required to improve athletic performance, at least during the autumn.

Self-identified ethnicity was a significant predictor of serum 25(OH)D concentrations in the current study. Participants that self-identified as Pacific ethnicity had serum 25-hydroxyvitamin D concentrations that were on average 8.57 nmol/L lower than the reference group of self-identified NZ European. This finding supports that of other studies in athletes, finding a significant association between darker-skinned ethnicities and low vitamin D status (12,13,35).
7 Application to Dietetic Practice

The results of the current study show that rugby players from Otago and Southland, New Zealand are vitamin D replete during autumn. Therefore, sports dietitians need not be concerned about the vitamin D status of rugby players during this time. This study also suggests that athletic performance during autumn is unlikely affected by serum 25(OH)D concentrations. Further to this, it is unlikely that vitamin D concentrations in this group will become low enough to recommend vitamin D supplementation during other seasons.

Because of the conjecture regarding the possible links between higher vitamin D status and athletic performance (15,18,19), many in the sports nutrition world are considering supplementation for their athletic clients. Vitamin D concentrations are expensive to measure (3), making it tempting to move directly to supplementation, as a precautionary measure. However, this may be inappropriate for this group of male rugby union players who have already sufficient serum 25(OH)D concentrations and in some cases (n=4) their concentrations exceed the recommendations (>125 nmol/L).

Despite the Ministry of Health stating that if someone is at risk of vitamin D deficiency they should be prescribed supplements without testing (3), results from the current study suggest that even those at risk of deficiency (eg. Pacific ethnicity) had sufficient vitamin D status. Vitamin D supplementation is not recommended for those without risk of vitamin D deficiency or a specific medical condition (3). The Institute of Medicine states that higher concentrations of vitamin D have no shown benefit, instead they have been linked to negative health consequences (152). Therefore, supplementation in a group of replete athletes could be detrimental to their health, specifically those rugby players with already high serum 25(OH)D concentrations of 129-131 nmol/L, where it is unknown what/if any long term effects occur at these high concentrations. Furthermore, there is no evidence to suggest an ergogenic effect of vitamin D supplementation in those who have sufficient vitamin D status at the outset.
If medical and dietetic staff were interested in screening their athletes to assess vitamin D status, knowing what factors predict serum 25(OH)D concentrations in New Zealand athletes could help to prioritise the expense of screening. In the current study we found that athletes of self-reported Pacific ethnicity are at significantly higher risk of having low serum 25(OH)D concentrations. This result compares to the findings of previous studies in the New Zealand population where those of Pacific ethnicity were found to have lower circulating 25(OH)D concentrations than New Zealand Europeans (75,90,150). Sports dietitians interested in determining the vitamin D status of their athletes should discuss this with medical colleagues first.

On the basis of this research, there is no evidence to suggest that any male rugby union player living in southern New Zealand would have insufficient vitamin D status, at least in autumn.

7.1 Screening and Prevention of Vitamin D Deficiency in Athletes

The Nutrition Care Process (NCP) is the process of describing how dietitians provide care to patients, which uses four key steps Assessment, Nutrition Diagnosis, Intervention, Monitoring and Evaluation (AnDIME) (153). During individual assessments, sports dietitians could incorporate these key points if concerned about the vitamin D status of their athletic clientele, to inform their decision-making.

Assessment: Sports dietitians should follow standard practice of collecting self-identified ethnicity in the initial assessment, along with dietary assessment. It is important to note that dietary intake contributes little towards vitamin D status (3), due to the limited amounts of foods naturally containing vitamin D and lack of mandatory fortification in New Zealand (2,3). Vitamin D status is however, significantly influenced by sun exposure (3). Key questions regarding sun exposure could be included during the assessment, such as:

- How many hours of sun exposure do you get during the hours of 11am-4pm?
- What are your current sun protection habits including: sunscreen use and clothing?

Following the assessment, if still concerned, the sports dietitian should refer clients to their general practitioner for biochemical assessment of 25(OH)D. Testing 25(OH)D
concentrations during the winter and spring will be most informative when determining whether vitamin D concentrations are sufficient year round, as concentrations tend to be lower at this time of the year (75). It is however important to note that there is considerable debate around the appropriate cut-offs for vitamin D status, therefore appropriate cut-offs should be agreed with medical colleagues in advance, following consultation with good quality evidence-based literature.

*Intervention:* In this step, sports dietitians would ideally use the ‘food first’ approach, which assumes that an adequate nutritional status can be achieved with a balanced diet. However, little vitamin D is obtained from food sources (3). Instead, adequate, safe, sun exposure year round is needed to ensure sufficient vitamin D status (3). Cannell et al. (68) proposes that 10-15 minutes of UVB exposure during peak hours of sunlight provides around 10,000 IU of vitamin D (68). In New Zealand, one would have to eat 25 litres of yoghurt (100g contains 40 IU – only certain brands who choose to fortify) or take 50 multivitamin tablets (200 IU per tablet) to achieve this amount of vitamin D orally. Oral supplementation of vitamin D, in combination with adequate sun exposure year round, may be necessary to correct vitamin D deficiency. Especially in southern New Zealand during the winter season, when vitamin D deficiency is more likely to occur.

Advice regarding vitamin D supplementation should be given to athletes only following screening, as they may well be vitamin D replete. Athletes should not be supplementing with vitamin D without a biochemical assessment of their vitamin D status first.

*Monitoring and Evaluation:* The sports dietitian has a relatively minor role to play in monitoring and evaluating ones vitamin D status, as this is best done via biochemical, rather than dietary assessment.

As part of the nutritional assessment, sports dietitians should be assessing athletes for risk factors of vitamin D deficiency and make appropriate recommendations that will help athletes to achieve an adequate vitamin D status year round.
References


133. Swinbourn S. Evaluating macro- and micro-nutrient intakes of elite rugby union players against current sports nutrition recommendations [MSc Thesis]. Dunedin, New Zealand: University of Otago; 2009.


140. Logan V. Predictors of vitamin D status in New Zealand adults and the effect of vitamin D₃ and vitamin D₃ supplementation on 25-hydroxyvitamin D and parathyroid hormone concentrations [MSc Thesis]. Dunedin, New Zealand: University of Otago; 2011.


145. Ceelen I. Comparison of sum of 8 skinfolds against DXA scan percent body fat in elite rugby union players [MSc Thesis]. Dunedin, New Zealand: University of Otago; 2011.


Appendices

Appendix A: Ethical Approval
Appendix B: Ethics Letter and Confirmation Letter for Inclusion of Ethnicity Data
Appendix C: Information Sheet and Consent Form
Appendix D: Screening Questionnaire
Appendix E: Sun Exposure Questionnaire
Appendix A: Ethical Approval
Dr K Fairbairn  
Department of Human Nutrition  
Division of Sciences

23 March 2011

Dear Dr Fairbairn

I am again writing to you concerning your proposal entitled "Vitamin D Status and Effect of Supplementation on Strength, Speed and Body Composition in rugby union players". Ethics Committee reference number 11/064.

Thank you for your letter outlining the changes that have been made to the application as per the Committee’s requests. We note that you have included information regarding the ultimate fate of the blood samples, and the dosage of Vitamin D to be administered, and are grateful to receive copies of the amended Information Sheet and Consent Form with this information and other minor changes made.

On the basis of this response, I am pleased to confirm that the proposal now has full ethical approval to proceed.

Approval is for up to three years. If this project has not been completed within three years from the date of this letter, re-approval must be requested. If the nature, consent, location, procedures or personnel of your approved application change, please advise me in writing.

Yours sincerely,

[Signature]

Mr Gary Witte  
Manager, Academic Committees  
Tel: 479 8256  
Email: gary.witte@otago.ac.nz

c.c. Emeritus Professor L J Holloway  Head  Department of Human Nutrition
Ngāi Tahu Research Consultation Committee
Te Komiti Rakahau ki Kāi Tahu

19/04/2011 - 21
Tuesday, 19 April 2011

Dr Fairbairn
Human Nutrition
Dunedin

Tēnā koe Dr Fairbairn

Title: Vitamin D Status and Effect of supplementation on Strength, Speed and Body Composition in rugby union players.

The Ngāi Tahu Research Consultation Committee (The Committee) met on Tuesday, 19 April 2011 to discuss your research proposition.

By way of introduction, this response from the Committee is provided as part of the Memorandum of Understanding between Te Rūnanga o Ngāi Tahu and the University. In the statement of principles of the memorandum, it states "Ngāi Tahu acknowledges that the consultation process outlined in this policy provides no power of veto by Ngāi Tahu to research undertaken at the University of Otago". As such, this response is not "approval" or "mandate" for the research, rather it is a mandated response from a Ngāi Tahu appointed committee. This process is part of a number of requirements for researchers to undertake and does not cover other issues relating to ethics, including methodology; they are separate requirements with other committees, for example the Human Ethics Committee, etc.

Within the context of the Policy for Research Consultation with Māori, the Committee base consultation on that defined by Justice McGechan:

"Consultation does not mean negotiation or agreement. It means: setting out a proposal not fully decided upon; adequately informing a party about relevant information upon which the proposal is based; listening to what the others have to say with an open mind (in that there is room to be persuaded against the proposal); undertaking that task in a genuine and not cosmetic manner. Reaching a decision that may or may not alter the original proposal."

The Committee considers the research to be of importance to Māori health.

As this study involves human participants, the Committee strongly encourage that ethnicity data be collected as part of the research project. That is the questions on self-identified ethnicity and descent, these questions are contained in the 2006 census.

The Committee suggests including in the research team a researcher with expertise in analysing and interpreting data by ethnicity.

The Committee suggests researchers consider the Southern District Health Board's Tikaka Best Practice document, in particular patient engagement. The document also covers the
collection, storage and disposal of blood and tissue samples. This document is available on the
Southern District Health Board website.

The Committee suggests dissemination of the research findings to Māori sports organisations
regarding this study.

We wish you every success in your research and the Committee also requests a copy of the
research findings.

This letter of suggestion, recommendation and advice is current for an 18 month period from
Tuesday, 19 April 2011 to 19 October 2012.

The recommendations and suggestions above are provided on your proposal submitted
through the consultation website process. These recommendations and suggestions do not
necessarily relate to ethical issues with the research, including methodology. Other
committees may also provide feedback in these areas.

Nahaku noa, nā

Mark Brunton
Kaitakawaenga Rangahau Māori
Facilitator Research Māori
Research Division
Te Whare Wānanga o Otāgo
Ph: +64 3 479 8738
email: mark.brunton@otago.ac.nz
Web: www.otago.ac.nz

The Ngāi Tahu Research Consultation Committee has membership from:

Te Rūnanga o Ōtākou Incorporated
Kāti Huia Rūnanga ki Paheterekākū
Te Rūnanga o Māori

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Appendix B: Ethics Letter and Confirmation Letter for Inclusion of Ethnicity Data
Mr Gary Witte  
Manager, Academic Committees  
Academic Services  
University of Otago

5th April 2013

Dear Mr Witte,

Re: “Vitamin D Status and Effect of Supplementation on Strength, Speed and Body Composition in rugby union players”, Reference code 11/064.

Currently, one of my Master’s of Dietetics students (Lisa Daniels) is investigating cross-sectional baseline data for the above study. The literature suggests that many factors affect vitamin D status including; season, latitude, sun exposure, age, body composition, sunscreen use, clothing, ethnicity and skin colour. Initial analyses of the baseline data have revealed that there are significant differences in baseline serum vitamin D status and self-reported ethnicity (see below). This reflects the findings of the 2008/09 New Zealand Adult Nutrition Survey (ANS), where those of Pacific ethnicity had significantly lower serum vitamin D status than non-Pacific participants.

*Pacific ethnic group significantly lower in serum 25-hydroxyvitamin D than NZ European (p = <0.001) and Maori (p = 0.003) ethnic groups.

Department of Human Nutrition  
PO Box 56, Dunedin 9054, New Zealand  
Tel 64 3 479 7959 • Fax 64 3 479 7958  
Email human-nutrition@otago.ac.nz • Web http://www.otago.ac.nz/humannutrition/
In our Ethics Application for this study, we indicated that while we would be collecting data on self-identified ethnicity, we did not intend to compare results between ethnic groups. However, since the release of the ANS data it is apparent that vitamin D status may be influenced by ethnicity in the NZ population. Given the differences noted above in our study, we feel it is prudent to describe this finding, and to control for ethnicity when analysing relationships between vitamin D status and other variables (for example indicators of sports performance). We are employing the expertise of a consulting statistician within the department of Human Nutrition, who is assisting with the interpretation of the results of Lisa’s project.

These results will be important for local Sports Dietitians when working with rugby players (a large number of whom identify themselves as of Pacific Island ethnicity), as they will be able to make informed decisions around the potential need to supplement these players with vitamin D in New Zealand.

In light of these findings, we would like to present results regarding ethnic variations in serum vitamin D status in this students Masters of Dietetics thesis.

Please do not hesitate to contact us should you have any further concerns or queries.

Warm regards,

Dr Kirsty Fairbairn
Lecturer
Sports Nutrition and Exercise Metabolism Group
Department of Human Nutrition

Cc: Mr Mark Brunton, Kaitakawaenga Rangahau Maori, Research Division, Te Whare Wananga o Otago.
Dr K Fairbairn  
Department of Human Nutrition  
Division of Sciences

Dear Dr Fairbairn,

I am again writing to you concerning your proposal entitled “Vitamin D Status and Effect of Supplementation on Strength, Speed and Body Composition in rugby union players”, Ethics Committee reference number 11/064.

Thank you for your letter dated 5 April 2013 requesting an amendment to allow for comparing results by ethnicity. We note that you are employing the expertise of a consulting statistician from within the Department for this task. Thank you for keeping the Committee informed, we confirm that this amendment is approved.

Your proposal continues to be fully approved by the Human Ethics Committee. If the nature, consent, location, procedures or personnel of your approved application change, please advise me in writing. I hope all goes well for you with your upcoming research.

Yours sincerely,

Mr Gary Witte  
Manager, Academic Committees  
Tel: 479 8256  
Email: gary.witte@otago.ac.nz

cc. Emeritus Professor L J Holloway  Head Department of Human Nutrition
Appendix C: Information Sheet and Consent Form
“Vitamin D Status and Effect of Supplementation on Pre-season Performance in Rugby Union”

INFORMATION SHEET FOR PARTICIPANTS

Thank you for showing an interest in this project. Please read this information sheet carefully before deciding whether or not to participate. Please feel free to discuss this project with your family and whānau. We are happy to answer their questions. If you decide to participate we thank you. If you decide not to take part there will be no disadvantage to you of any kind and we thank you for considering our request.

What is the Aim of the Project?

Vitamin D status (blood concentrations of vitamin D) has been linked to muscle function and performance in sports nutrition and exercise research literature. Additionally, the risk of low vitamin D status is higher in locations that are far south, like Otago and Southland. Therefore, the aim of this project is to determine whether Otago and Southland Rugby Union players have low vitamin D status, and whether supplementing with Vitamin D improves strength, speed and fitness adaptations during your pre-season training.

We will be randomly allocating you to receive EITHER a Vitamin D supplement OR Placebo (dummy pill), in the form of one tablet to be taken every 2 weeks, for the duration of the study. Your Strength and Conditioning coach will give out your tablet to you. Neither you, your Strength and Conditioning Coach or the University of Otago research staff will know which treatment you are receiving until we ‘unblind’ the study after we finish.

If you are allocated to receive Vitamin D, you will be provided with 50,000 International Units (IU) or 1.25 milligrams of Vitamin D in the form of Cholecalciferol, once a fortnight. This equates to a daily dose of 3280 IU or 82 micrograms per day. The US Institute of Medicine recommends that adults over 18 years of age require 400 IU/day, and they specify an Upper Level of Intake of 4000 IU/day. This level should not be exceeded due to higher risk of harm. Very high intakes of Vitamin D (over 10,000 IU/day) have been associated with kidney and tissue damage.

Previous surveys of Otago ITM Cup and Academy squads have indicated an average daily dietary intake of Vitamin D (from food only) of 300 IU/day. Thus we expect that the consumption of the Vitamin D supplement along with your usual dietary intake should not exceed the Upper Level of Intake, while also maximising any likely performance benefits to you.

Department of Human Nutrition
PO Box 56, Dunedin 9054, New Zealand.
Tel 64 3 479 7959 • Fax 64 3 479 7958
Email human-nutrition@otago.ac.nz • Web http://www.otago.ac.nz/humannutrition/

DUNEDIN • CHRISTCHURCH • WELLINGTON • HAMILTON • AUCKLAND
**Vitamin D Status and Effect of Supplementation on Pre-season Performance in Rugby Union**

**Information Sheet for Study Participants**

**What Type of Participants are being sought?**

We are hoping to recruit players from the North Otago, Otago, and Southland ITM cup and Heartland Championship squads. You need to be male, aged over 18 years of age, a non-smoker, not taking any Vitamin D supplements, and participating in the pre-season training and performance testing with these squads. Whether you are able to complete full training loads or not (eg. due to an injury) does not matter for this project – we would still appreciate your participation.

If you are aged less than 18 years of age, are a smoker, are currently taking Vitamin D supplements or have a chronic disease such as heart disease, diabetes, cancer or any condition that affects hormone metabolism then you should not take part in this research study.

**What will Participants be Asked to Do?**

All information you provide to us will be coded by a ‘Study ID Number’, so that your name will not be directly associated with any information you provide. Only one study investigator (Ingrid Ceelen) will be able to match Study Numbers to Names.

Should you agree to take part in this project, you will be asked to:

- Complete a short screening questionnaire regarding your age, height, weight, playing position, years playing at representative level, ethnicity and contact details, which will take 2 minutes of your time.

- Because Vitamin D is made in your skin during exposure to sunlight, we will also ask you to complete another questionnaire about time spent outdoors, and sun protection habits (clothing worn, use of sunscreen etc). This will take approximately 10 minutes.

- Because Vitamin D can be related to mood, we will also ask you to complete a one page Mood Questionnaire, so that we can track whether the Vitamin D supplementation affects the mood states you report. This will take approximately 2 minutes.

- Provide two 4mL tubes of blood BEFORE eating breakfast, in concert with your pre-season testing weeks, three times, six weeks apart (Week 0, Week 6, Week 12). We expect to start with your testing week at the start of April, so that is when the first sample will be taken. The next sample will be taken to coincide with your next testing week 5-6 weeks later, and the last one with your testing week 5-6 weeks following that. Three blood samples will be collected in total, over a 10-12 week period. At the end of the study we can dispose of these bloods for you, or you can request to have them returned to you. We can also perform a karakia (blessing) for them prior to disposal if you wish – you can indicate whether you want this on the Consent Form.

- Undergo a Dual X-ray Absorptiometry (DEXA) scan. This is a painless scan, that gives us a more reliable indicator of your muscle mass and body fat stores than skinfold measurements that you may have had done by your Nutritionist before. This will take approximately 40 minutes including set-up and scanning time.

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Ethics Approval No. 11/064

22nd March 2011
Vitamin D Status and Effect of Supplementation on Pre-season Performance in Rugby Union

Information Sheet for Study Participants

When collecting blood samples, there is a risk of minor pain, discomfort and or bruising at the sampling site. The DEXA scan is completely painless, but does involve exposure to a small amount of X-ray radiation (2uSV). This is equivalent to approximately \(1/10^{th}\) of that resulting from a chest X-ray, and typically New Zealanders are exposed to 2000uSV due to background radiation each year.

You will be provided with a $20 grocery voucher as a gesture of thanks for participating in this project. We will also provide breakfast following your fasting blood sample collection.

Please be aware that if you decide not to take part in the project, there will be no disadvantage to yourself of any kind. The services provided to you by the medical, nutrition, coaching and management staff of your Rugby Union would not be affected in any way.

**Can Participants Change their Mind and Withdraw from the Project?**

You may withdraw from participation in the project at any time and without any disadvantage to yourself of any kind.

**What Data or Information will be Collected and What Use will be Made of it?**

Your blood samples will be stored at the Department of Human Nutrition at the University of Otago, under a unique Study ID number to protect your identity. Laboratory staff at the Department of Human Nutrition, the University of Otago, will do the analysis of the blood samples, again using Study ID numbers so that your identity is protected.

All study data will be recorded using your Study ID Number. We will also ask your Strength & Conditioning Coach for access to your pre-season strength, speed and fitness results, which will be coded by a single investigator (Ingrid Ceelen) to study number before the other investigators see that information. Your Pr-season Performance data will then be related to the Vitamin D concentration in your blood, your sun exposure questionnaire information, your body composition information and your Mood scores, using your Study ID Number. We need your consent to obtain your Performance Testing results.

The data collected will be securely stored, using Study ID Numbers, in such a way that only those mentioned below will be able to gain access to it. At the end of the project any personal information will be destroyed immediately except that, as required by the University’s research policy, any raw data on which the results of the project depend will be retained in secure storage for five years, after which it will be destroyed. Reasonable precautions will be taken to protect and destroy data gathered by email. However, the security of electronically transmitted information cannot be guaranteed. Caution is advised in the electronic transmission of sensitive material.

Only Ingrid Ceelen will have access to the Study ID number and Names list, so that she can ‘unblind’ the blood vitamin D information and provide feedback to you about your vitamin D status at the end of the study. We will also provide you with an Individual report of your results at the end of the study.

Ethics Approval No. 11/064

22nd March 2011
Vitamin D Status and Effect of Supplementation on Pre-season Performance in Rugby Union
Information Sheet for Study Participants

The data from this project will be written up and submitted for publication in a sports nutrition and exercise research journal, however all data is presented anonymously to protect your identity. The results from this project may also be presented at sports nutrition and exercise conferences both in New Zealand and possibly overseas, and will also be available in the University of Otago Library (Dunedin, New Zealand) but every attempt will be made to preserve your anonymity.

My Results:
You will be provided with a copy of your individual results plus the group results of the project (the data of your home squad, and that of the three squads combined, presented anonymously).

What if Participants have any Questions?
If you have any questions about our project, either now or in the future, please feel free to contact either:-

Dr Kirsty Fairbairn or Ingrid Ceelen
Department of Human Nutrition
University Telephone Number: 479 5359
Email: kirsty.fairbairn@otago.ac.nz

Dr Tracy Perry
Department of Human Nutrition
University Telephone Number: 479 7508
Email: tracy.perry@otago.ac.nz

Many thanks for considering taking part in this research.

The University of Otago Human Ethics Committee has reviewed and approved this project. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (ph 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.

Ethics Approval No. 11/064

22nd March 2011
CONSENT FORM FOR PARTICIPANTS

I have read the Information Sheet concerning this project and understand what it is about. All of my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:-

1. My participation in the project is entirely voluntary.
2. I am free to withdraw from the project at any time without any disadvantage.
3. I will be allocated a Study ID Number, and all data will be recorded using that number. I understand that personal identifying information will be destroyed at the conclusion of the project but any raw data on which the results of the project depend will be retained in secure storage for five years, after which they will be destroyed. I understand that at the completion of this research project my blood samples will be disposed of by standard biohazard methods, unless I request otherwise (see below). I may also request a karakia (blessing) for my blood samples prior to whichever method of disposal I choose.
4. I will be asked to provide six (6) 4mL blood samples for the purposes of this project. The first two in concert with Pre-season performance testing in late March/early April 2011, the second two 5-6 weeks after that, and the third two 5-6 weeks after that.
5. I will be asked to undergo two Dual X-ray Absorptiometry (DEXA) scans taking approximately 40 minutes each. These scans are painless but will expose me to a low level of exposure to radiation (about 1/10th of that from a Chest X-ray).
6. I will be asked to fill out a short screening questionnaire about my demographic information at the start of the study (which will take approximately 2 minutes), a short Mood States questionnaire around each Performance Testing week (which will take approximately 2 minutes) and a questionnaire about my sun exposure and sun protection habits at the same time (which will take approximately 10 minutes).
7. I may experience slight pain, discomfort and/or bruising as a result of the drawing of a blood sample, and that an experienced research nurse will be used for this to minimise any side effects.
8. The results of the project may be published and will be available in the University of Otago Library (Dunedin, New Zealand) but every attempt will be made to preserve my anonymity.
9. I will be given a copy of my individual results at the end of the study, with the results of my squad and the entire study provided for comparison.
10. If I complete the study in its entirety I will receive a $20 grocery voucher as a gesture of thanks.
“Vitamin D Status and Effect of Supplementation on Pre-season Performance in Rugby Union”

CONSENT FORM FOR PARTICIPANTS

Checklist Item #1:
Please indicate whether you wish to have your blood sample disposed of by standard methods, or if you would prefer to have your blood samples returned to you for disposal:

- I elect to have my blood samples disposed of by standard biohazard disposal methods  ☐
- OR
- I elect to have my blood samples returned to me for disposal  ☐

Checklist Item #2:
Please indicate whether you would like to have an appropriate karakia for your blood sample, regardless of the method of disposal you choose, at the end of the study:

- I wish to have any remaining blood samples blessed with the appropriate karakia at the end of the study: - YES / NO

Checklist Item #3:
I appreciate that I will be randomly allocated to receive either 50,000IU of Vitamin D, or a placebo tablet, once every two weeks during this research project

- YES / NO

Checklist Item #4:
I consent to the researchers obtaining copies of my strength, speed and fitness testing results done as part of my ITM Cup/Heartland Cup campaign.

- YES / NO

I agree to take part in this project.

Name: ..........................................................................................................

Signature: .......................................................... Date: .....................................

(Signature of participant)

The University of Otago Human Ethics Committee has reviewed and approved this project. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (ph 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.
Appendix D: Screening Questionnaire
Screening Page: Vitamin D Status and Effect of Supplementation on Strength, Speed & Body Composition in Rugby Union Players

Player Study ID Number: _________________________
Date of Birth: ___________________ Age: ____________

Self-identified Ethnicity:
- [ ] New Zealand European
- [ ] Niuean
- [ ] Maori
- [ ] Chinese
- [ ] Samoan
- [ ] Indian
- [ ] Cook Island Maori
- [ ] Other…………………..
- [ ] Tongan

Home Union: _______________ Position: _______________
Years Playing: _______________ Years at Rep Level: ____________________________

Weight: _______________ Height: _______________ BMI: ______

Goals of Pre-season Training:
- [ ] Increase Strength
- [ ] Increase Power
- [ ] Increase Speed
- [ ] Increase Acceleration
- [ ] Increase fitness
- [ ] Increase Muscle Mass
- [ ] Increase Body Weight
- [ ] Decrease Body Weight
- [ ] Decrease Body Fat
- [ ] Increase Speed
- [ ] Increase Acceleration
- [ ] Increase fitness

Supplements currently being taken:

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
Appendix E: Sun Exposure Questionnaire
SUN EXPOSURE QUESTIONNAIRE

READ OUT:
Thank you for agreeing to take part in this research.
There are no right or wrong answers - we just ask about what you think.
OUTDOOR ACTIVITIES

"We would like to ask about your outdoor activities, on the week just passed."

Q1. "On which days, if any, were you out of doors for longer than 15 minutes between 11am and 4pm? By out of doors we mean not in a building and not in a covered vehicle."
(MARK. If intermittently outdoors: "Would you say you were actually out of doors for longer than 15 minutes in total?")

Monday □
Tuesday □
Wednesday □
Thursday □
Friday □
Saturday □
Sunday □
No days □

Go to Q2.

Q2. "On which days, if any, did you get sunburnt on the week just passed? By sunburnt we mean any amount of reddening (shades of pink as well) of the skin after being in the sun."
(MARK. CHECK THAT DAY(S) SUNBURNT AGREES WITH DAY(S) OUTDOORS)

Monday □
Tuesday □
Wednesday □
Thursday □
Friday □
Saturday □
Sunday □
No days □

Go to Q3.

Q3. Which parts of your body got sunburnt? (FOR EXAMPLE: SHOULDERS OR FACE)

Q4. "On which days did you train outside the last week?"

Monday □
Tuesday □
Wednesday □
Thursday □
Friday □
Saturday □
Sunday □
Q5. "About how much time in total did you spend outdoors for training per day?"  
(MARK. NOTE THIS IS ANY TIME DURING THE DAY)

<table>
<thead>
<tr>
<th>Time</th>
<th>Mon</th>
<th>Tues</th>
<th>Wed</th>
<th>Thurs</th>
<th>Fri</th>
<th>Sat</th>
<th>Sun</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
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</tr>
</tbody>
</table>

Q6. "About what time did you start and finish this outdoor training?"

<table>
<thead>
<tr>
<th>Day</th>
<th>Started</th>
<th>Finished</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monday</td>
<td></td>
<td></td>
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<tr>
<td>Tuesday</td>
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<tr>
<td>Wednesday</td>
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<tr>
<td>Saturday</td>
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<tr>
<td>Sunday</td>
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</tr>
</tbody>
</table>

Q7. "Was there another activity (besides training) that you were doing outdoors this week?"  
(MARK. IF MORE THAN ONE PER DAY MENTIONED, ASK: "During which activity did you spend the most time in the sun?")

<table>
<thead>
<tr>
<th>Activity</th>
<th>Mon</th>
<th>Tues</th>
<th>Wed</th>
<th>Thurs</th>
<th>Fri</th>
<th>Sat</th>
<th>Sun</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water activities</td>
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<tr>
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<tr>
<td>Jobs at home</td>
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<tr>
<td>Other recreation</td>
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</tbody>
</table>
Q8. "About how much time in total did you spend outdoors per day doing this other activity?"
(MARK. NOTE THIS IS ANY TIME DURING THE DAY)

<table>
<thead>
<tr>
<th></th>
<th>Mon</th>
<th>Tues</th>
<th>Wed</th>
<th>Thurs</th>
<th>Fri</th>
<th>Sat</th>
<th>Sun</th>
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</thead>
<tbody>
<tr>
<td>15 minutes</td>
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<td>30 minutes</td>
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<td>1 hr 45 min</td>
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<tr>
<td>Don't know</td>
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</tbody>
</table>

Q9. "About what time did you start and finish this activity?"

<table>
<thead>
<tr>
<th></th>
<th>Started</th>
<th>Finished</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monday</td>
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<tr>
<td>Tuesday</td>
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<td>Saturday</td>
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<tr>
<td>Sunday</td>
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</tbody>
</table>

Q10. "Could you please tell me which of the following parts of your body were covered or shaped by a hat, cap or visor most of the time while you were outside?"
(READ OUT EACH BODY PART AND MARK ALL MENTIONED)

<table>
<thead>
<tr>
<th></th>
<th>Mon</th>
<th>Tues</th>
<th>Wed</th>
<th>Thurs</th>
<th>Fri</th>
<th>Sat</th>
<th>Sun</th>
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</thead>
<tbody>
<tr>
<td>Face</td>
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<tr>
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<td>Scalp</td>
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<tr>
<td>Ears</td>
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<tr>
<td>Neck</td>
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<tr>
<td>Shoulders</td>
<td></td>
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</tr>
</tbody>
</table>
Q11. "Which of the following parts of your body were covered or shaded by clothing most of the time while you were outside? Clothing includes towels, scarves and covered shoes, but not hats" (READ OUT EACH BODY PART AND MARK ALL MENTIONED)

<table>
<thead>
<tr>
<th></th>
<th>Mon</th>
<th>Tues</th>
<th>Wed</th>
<th>Thurs</th>
<th>Fri</th>
<th>Sat</th>
<th>Sun</th>
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</thead>
<tbody>
<tr>
<td>Face</td>
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<tr>
<td>Nose</td>
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<tr>
<td>Scalp</td>
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<td>Shoulders</td>
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<td>Chest</td>
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<tr>
<td>Stomach</td>
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<tr>
<td>Arms - above</td>
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<tr>
<td>Arms - below</td>
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</tr>
<tr>
<td>Legs - above</td>
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</tr>
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<td>Legs - below</td>
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<tr>
<td>Feet</td>
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</tr>
</tbody>
</table>

Q12. "Which of the following parts of you body were covered with sunscreen most of the time you were outside?" (READ OUT EACH BODY PART AND MARK ALL MENTIONED)

<table>
<thead>
<tr>
<th></th>
<th>Mon</th>
<th>Tues</th>
<th>Wed</th>
<th>Thurs</th>
<th>Fri</th>
<th>Sat</th>
<th>Sun</th>
</tr>
</thead>
<tbody>
<tr>
<td>Face</td>
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<td></td>
</tr>
<tr>
<td>Nose</td>
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<td>Scalp</td>
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<tr>
<td>Ears</td>
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<tr>
<td>Shoulders</td>
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<tr>
<td>Chest</td>
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<td></td>
</tr>
<tr>
<td>Stomach</td>
<td></td>
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<tr>
<td>Back</td>
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<td></td>
</tr>
<tr>
<td>Arms - above</td>
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<tr>
<td>Arms - below</td>
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<tr>
<td>Legs - above</td>
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<td>Legs - below</td>
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<tr>
<td>Feet</td>
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</tr>
</tbody>
</table>
Q13. "How many times did you apply sunscreen during the day?"  
(MARK ONE PER DAY)

<table>
<thead>
<tr>
<th></th>
<th>Mon</th>
<th>Tues</th>
<th>Wed</th>
<th>Thurs</th>
<th>Fri</th>
<th>Sat</th>
<th>Sun</th>
</tr>
</thead>
<tbody>
<tr>
<td>Once</td>
<td></td>
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<tr>
<td>Twice</td>
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<tr>
<td>Three times</td>
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<tr>
<td>Four times</td>
<td></td>
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<tr>
<td>More than four</td>
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<tr>
<td>Don't know</td>
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</tbody>
</table>

Q14. "Did you travel recently?" (MARK)

No □
Yes □ Please specify…………………………………………………………………………………………

SKIN TYPE AND LIFE TIME SUNBURN

Q15. "We are interested in your skin colour so we can understand how likely people are to burn. How would you describe your natural, untanned colour at the end of the winter?"  
(MARK ONE)

Very fair □
Fair □
Medium □
Olive □
Dark □
Very dark or black □
Other □ Please specify………………………………………………

Q16. "Suppose your untanned skin was exposed to strong sunshine at the beginning of summer using no sun protection at all. If you stayed in the sun for 30 minutes, would your untanned skin...?"  
(READ OUT. MARK ONE ONLY)

"Just burn and not tan afterwards" □
"Burn first, then tan afterwards" □
"Not burn at all, just tan" □

Q17. "Apart from this week just finished, have you ever been sunburnt so badly you got blisters or were in pain for two or more days?" (MARK ONE)

Yes □
No □
Don't know □