Vitamin D and mood: effects of vitamin D supplementation on Brunel Mood Scale (BRUMS) score in semi-professional rugby union players

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Abstract

Background

Many New Zealanders have insufficient vitamin D status but the status of New Zealand athletes is unknown. Mood is important in the athletic environment as the response to training is partially dependent on psychological state, thus monitoring mood can help to prevent overtraining. The notion that vitamin D status affects mood is supported by animal studies but human research remains equivocal. There is an absence of randomised controlled trials exploring the relationship between vitamin D status and mood in athletes.

Objectives

- To investigate the relationship, if any, between baseline vitamin D status and Brunel Mood Scale score in Otago and Southland semi-professional male rugby union players.

- To investigate whether vitamin D supplementation for 11 – 12 weeks significantly affects Brunel Mood Scale score in Otago and Southland semi-professional male rugby union players.

Design

This thesis presents results from a secondary data analysis of a randomised blinded placebo-controlled intervention trial in 57 male semi-professional rugby union players from Otago and Southland, New Zealand. Participants were allocated either 50000 IU vitamin D supplements or placebo fortnightly for 11 or 12 weeks. The Brunel Mood Scale questionnaire was completed at baseline and study endpoint.

Results

At study endpoint, serum 25(OH)D was significantly greater in the treatment group (113.56 ± 18.55 nmol/L) than the placebo group (79.62 ± 21.34), although both groups were vitamin D replete. There was a small negative correlation of -0.387 ($p = 0.004$)
between BRUMS confusion and vitamin D concentrations at baseline. No other subscales were significantly correlated. There were no significant differences between treatment and placebo groups for change in Brunel Mood Scale subscale scores from baseline to weeks 11/12.

**Conclusion**

It is unlikely that vitamin D supplementation will affect mood in New Zealand semi-professional male rugby union players (who are vitamin D replete) and thus is unlikely to impact overtraining syndrome; thus supplementing with vitamin D for the sole purpose of bettering mood should be avoided. However, it remains unknown whether vitamin D supplementation would benefit those athletes with sub-optimal vitamin D status.
Preface

This thesis is based on a study by Dr Kirsty Fairbairn entitled “Vitamin D status and effect of supplementation on strength, speed and body composition in rugby union players”.

The text of this thesis is the original intellectual product of the thesis candidate, Lucy Carey. None of the text is taken from previously published articles. The candidate was responsible for writing the thesis and undertaking all statistical analyses.
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And to my amazing partner, Thomas – for always showing an interest, for keeping me stress-free, for your unwavering support and for simply being you.
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List of Abbreviations

% Percent
< Less than
> Greater than
≥ Greater than or equal to
/day Per day
°C Degrees Celsius
$1,25(\text{OH})_2\text{D}$ 1,25-dihydroxyvitamin D; calcitriol
$25(\text{OH})\text{D}$ 25-hydroxyvitamin D
95% CI Ninety-five percent confidence intervals
ANS08/09 2008/2009 New Zealand Adult Nutrition Survey
BDI Beck Depression Inventory
BMI Body mass index
BRUMS Brunel Mood Scale
CES-D Center for Epidemiological Studies – Depression Scale
CFA Confirmatory Factor Analysis
DEXA Dual-energy x-ray absorptiometry
DIS Diagnostic Interview Schedule
DSI Depressive Systems Inventory
e.g. For example
EFA Exploratory factor analysis
GDS Geriatric Depression Scale
GHQ General Health Questionnaire
HADS Hospital Anxiety and Depression Scale
InCHIANTI Invecchiare in Chianti, aging in the Chianti area
IU International units
kg  Kilograms
LC MS/MS  Liquid chromatography tandem mass spectroscopy
m  Metres
MCS  Mental Component Score
mg  Milligrams
Mg  Micrograms
mL  Millilitres
ng/mL  Nanograms per milliliter
nmol/L  Nanomoles per litre
NNS97  1997 National Nutrition Survey
NZ  New Zealand
OTS  Overtraining syndrome
PANAS  Positive and Negative Affect Schedule
PGI-I  Patient Global Impression Improvement
POMS  Profile of Mood States
POMS-A  Profile of Mood States – Adolescents
PTH  Parathyroid hormone
RAF/MAP  Rapidly Accelerated Fibrosarcoma/mitogen activated protein
RCT  Randomised controlled trial
rpm  Revolutions per minute
$r_s$  Spearman’s rho correlation coefficient
SAD  Seasonal Affective Disorder
SD  Standard deviation
SF-12  Short Form-12
SHPT  Secondary hyperparathyroidism
SIGH-SAD  Structured Interview Guide for the Hamilton Depression Rating Scale, SAD version
UK  United Kingdom
μL  Microlitres
USA  United States of America
UV  Ultraviolet
VDRs  Vitamin D receptors
vs  versus
WHO  World Health Organization
y  Years
1.0 Introduction

Vitamin D status in the general population has received much attention in recent years. The 2008/2009 New Zealand Adult Nutrition Survey (ANS08/09) revealed that 32% of New Zealanders aged 15 years and older had insufficient vitamin D status (serum 25(OH)D concentrations below 50 nmol/L) (1).

While there is some literature on vitamin D status in athletes, none deals specifically with New Zealand athletes (2). Of the few studies conducted overseas, serum 25(OH)D concentrations have varied drastically depending on geographical location, season and sport – those sports where the bulk of training occurs indoors have significantly lower serum 25(OH)D concentrations than those sports that train outdoors (3).

Mood has long been thought to be affected by one’s vitamin D status, primarily because of seasonal affective disorder (SAD) (4). Persons with this disorder become depressed during winter, when there are less sunlight hours (4). The notion that vitamin D affects mood is supported by the identification of vitamin D receptors (VDRs) in areas of the brain that regulate emotional behaviour (5, 6) and animal studies where mice with impaired VDRs exhibited increased anxiety (6). However, research looking specifically at athletes, who have different mood profiles than that of the general population (7, 8), has yet to be conducted.

A positive mood is a crucial element in the athletic environment because an athlete’s response to training depends not only on their physical condition but also their psychological state (9). The physical and psychological stress of training, insufficient recovery from training and stressors outside of training can contribute to an athlete developing overtraining syndrome (OTS) (10-12). Instead of positively adapting to training, athletes with OTS experience a performance decline (10) and a host of other negative side-effects, including but not limited to an apathetic mood, fear of competition,
low self-esteem, and a tendency to give up (13). Short questionnaires like the Brunel Mood Scale (BRUMS) assess an athlete’s mood profile, and have been used to monitor athlete’s responses to training, specifically to help prevent OTS (13, 14). If one can avoid overtraining they can train more consistently to attain their peak condition for competition.

As vitamin D status in the general population in New Zealand is often insufficient, New Zealand athletes are likely to display insufficiency also. If athletes are deficient in vitamin D and this is linked to mood, this could impact performance. Vitamin D status is easily improved with oral supplements, thus supplementation could be a simple and cost-effective way of improving mood. This raises the question of whether vitamin D status is linked to mood state in athletes, and if so could vitamin D supplementation improve mood state in athletes? To date there is a complete absence of randomised controlled trials (RCTs) looking at vitamin D and mood in athletes.
2.0 Literature Review

2.1 Aims and literature search methodology

The aims of this literature review were to:

- Investigate how OTS is defined, treated and prevented.
- Research the BRUMS’s development, validation and uses.
- Look at the evidence behind the proposed relationship between vitamin D and mood in healthy individuals and critique research conducted in this area.

In addition to these topics, this literature review will provide background information about the role of vitamin D in the body and vitamin D requirements of healthy individuals and athletes. Furthermore, this literature review will explain how vitamin D status is defined, and discuss training load of semi-professional rugby union players.

Literature for this review was acquired by conducting comprehensive searches of Medline, PsychInfo, SPORTDiscus and Web of Science (1990 - 2013). Key search terms used were vitamin D, molecular structure, requirement, healthy, status, New Zealand, mood, emotion, affect, mood states, athlete, sport, rugby, training, Brunel mood, BRUMS, overtraining, chronic fatigue syndrome and staleness. All searches were limited to those in the English language. In addition, reference lists from articles identified were manually searched for relevant articles.

2.2 Vitamin D background

2.2.1 Chemical structure

“Vitamin D” is a generic term that generally refers to either dietary vitamin D$_2$ (ergocaliferol) or vitamin D$_3$ (cholecaliferol) (15). Vitamin D is technically a prohormone with a secosteroid structure (16); the only structural differences between vitamins D$_2$ and D$_3$ are in the side chains (Figure 2.1).
Figure 2.1. Chemical structure of (a) vitamin D₃ or cholecalciferol, and (b) vitamin D₂ or ergocalciferol (17).

2.2.2 Synthesis, metabolism and excretion

An overview of the synthesis, metabolism and excretion of vitamin D is presented in Figure 2.2.

Vitamin D₂ is produced in plants when ultraviolet (UV) light irradiates the plant sterol ergosterol (15). Vitamin D₃ is produced in the skin when UVB radiation from sunlight (in the wavelength range 290 – 315 nm) converts 7-dehydrocholesterol to previtamin D₃, which is then quickly converted to the more thermodynamically stable vitamin D₃ (18). Excessive exposure to sunlight degrades previtamin D₃ and vitamin D₃ into inactive products, thus is it impossible for sunlight to cause vitamin D intoxication (18).
Figure 2.2. Synthesis, activation and excretion of vitamin D. Vitamin D₃ is produced from ultraviolet irradiation of 7-dehydrocholesterol in the skin; vitamin D₂ is produced from ultraviolet irradiation of ergosterol (not shown here). Both vitamins D₂ and D₃ can be sourced from the diet. Vitamin D binds to vitamin D binding protein in the circulation and is transported to the liver, where it is hydroxylated to 25-hydroxyvitamin D. 25-hydroxyvitamin D is hydroxylated again, chiefly in the kidneys, to produce the active from of vitamin D, 1,25-dihydroxyvitamin D. 1,25-dihydroxyvitamin D and 25-hydroxyvitamin D are broken down to calcitroic acid for excretion in the bile (19).
Vitamin D₃ is found in small quantities in a few foods, e.g. fatty fish, liver and eggs (15), while vitamin D₂ is restricted to fungi, e.g. mushrooms (20). In New Zealand very few foods are fortified with vitamin D and most adults obtain no more than 5-10% of their total vitamin D requirements through diet (15). Therefore, sunlight is the major determinant of vitamin D status (15).

Regardless of whether it is made in the skin or eaten, both vitamins D₂ and D₃ enter the circulation and are bound to vitamin D binding protein. This complex is then transported to the liver, where the enzyme vitamin D 25-hydroxylase converts them to 25-hydroxyvitamin D (25(OH)D) (15, 18, 19). This transport form of vitamin D circulates to the kidneys where the enzyme 25-hydroxyvitamin D-1-α-hydroxylase converts it to its active form, 1,25-dihydroxyvitamin D (1,25(OH)₂D; calcitriol) (15, 18, 19). Renal production of 1,25(OH)₂D is tightly regulated by plasma parathyroid hormone (PTH), serum calcium and serum phosphorus (18).

The enzyme 25-hydroxyvitamin D-1-α-hydroxylase found in the kidneys is also present in many other tissues such as the prostate, breast, colon, lung, and pancreatic cells (21), hence 1,25(OH)₂D can be produced locally in a paracrine fashion for cellular functions (22).

When there is adequate 1,25(OH)₂D, calcium and phosphorus, the enzyme 25-hydroxyvitamin D-24-hydroxylase converts 1,25(OH)₂D and 25(OH)D into the more water soluble calcitroic acid, which is excreted in the bile (19).

### 2.2.3 Biological function of vitamin D
Vitamin D has both genomic and non-genomic effects in the human body (23).

#### 2.2.3.1 Genomic effects
Upon entering the cell, 1,25(OH)₂D binds to VDRs at the nucleus (24). The vitamin D-VDR complex then binds with the retinoic acid X receptor to form a heterodimer complex
This complex binds to DNA sequences known as vitamin D-responsive elements; once bound an array of transcriptional factors also bind to it which either enhances or inhibits the transcription of vitamin D-responsive genes, such as calcium binding protein (24).

2.2.3.2 Non-genomic effects

As well as binding to VDRs at the nucleus to alter gene expression, vitamin D has also been found to bind to VDRs in the plasma membrane of target cells (16, 25). Binding of vitamin D to plasma VDRs may result in activation of one or more second messenger systems, such as phospholipase C, protein kinase C, G protein-coupled receptors, and phosphatidylinositol-3-kinase (16). Many outcomes are possible, such as opening calcium or chloride channels and activating second messengers, like phosphoproteins and the RAF/MAP kinase pathway (16). Some of the second messengers, like the RAF/MAP kinase cascade, alter gene expression by modifying the levels and activities of transcription factors (16).

2.3 Vitamin D Status

2.3.1 Vitamin D requirements and definition of vitamin D status

Although 1,25(OH)_2D is the active form of vitamin D, it should not be used as a marker of vitamin D status (24). It has a half-life of only about 4 hours and its concentration is about 1000 times less than 25(OH)D (24). When a person is vitamin D deficient, 1,25(OH)_2D concentrations are maintained in the normal range or even increased due to PTH secretion increasing, which stimulates the kidneys to produce more 1,25(OH)_2D (24). Instead, 25(OH)D (made up of both 25(OH)D_2 and 25(OH)D_3) is used as a marker of vitamin D status as it has a half-life of about two weeks in circulation and decreases as a person becomes vitamin D deficient (24).
However, there is no consensus as to what constitutes sufficient levels of 25(OH)D in humans (26). Generally values below 50 nmol/L are considered to be insufficient or deficient, while concentrations above 75 nmol/L are considered more optimal. The 75 nmol/L cut-off was noted by Bischoff-Ferrari to optimise bone mineral density, fracture prevention, cancer prevention and function of the lower extremities (27). Many recent publications exploring vitamin D status in athletes have used the cut-off of 75 nmol/L to reflect optimal vitamin D status (refer to Table 1.).

However, a 2010 review by Larson-Meyers and Willis (2) looking specifically at athletes uses generally higher values: deficient <50 nmol/L, insufficient 50 – 80 nmol/L, sufficient 100 – 175 nmol/L and intoxication >374 nmol/L. The cut-off for deficiency was based on the approximate concentration where PTH would begin to rise; the cut-off for insufficiency is the approximate concentration where calcium absorption is maximized; and the optimum levels are based on the hypothesis that the human genome evolved with concentrations between 100 and 175 nmol/L (2).

The following terminology is used in New Zealand for healthy adults (1) and will be used henceforth given the lack of conclusive data in athletes:

- Deficient <25 nmol/L
- Suboptimal <50 nmol/L
- Sufficient 50 – 75 nmol/L
- Optimal >75 nmol/L
- Intoxication >125 nmol/L

Unlike vitamin D deficiency, vitamin D intoxication has received little attention in the literature. The term “vitamin D intoxication” was coined by Holick, who suggested that toxicity could result from ingestion of excessively high supplement doses, leading to
hypercalciuria and hypercalcaemia in the general population (15, 18, 28, 29). Garcia and Guisado’s study in professional basketball players in Spain used 375 nmol/L as the cut-off point for vitamin D intoxication (30), based off Holick’s 2007 review (18). Although inter-seasonal variation, assay differences and inconsistent evidence (31) mean no safe upper limit has been established, the New Zealand Ministry of Health uses the much lower and conservative value of 125 nmol/L (1, 31). This value is used because the long term safety above 125 nmol/L is unknown (32).
<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Severely deficient (nmol/L)</th>
<th>Mildly deficient (nmol/L)</th>
<th>Insufficient (nmol/L)</th>
<th>Sufficient (nmol/L)</th>
<th>Optimal (nmol/L)</th>
<th>Intoxication (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Close et al. 2013 (33)</td>
<td>30 club-level athletes; UK.</td>
<td>&lt;12</td>
<td>12 – &lt;30</td>
<td>30 – 50</td>
<td>&gt;50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Close et al. 2013 (34)</td>
<td>61 male full time athletes (rugby league, soccer, flat jockeys and 30 healthy controls; UK.</td>
<td>&lt;12.5</td>
<td>12.5 – 30</td>
<td>30 – 50</td>
<td>&gt;50</td>
<td>&gt;100</td>
<td></td>
</tr>
<tr>
<td>Morton et al. 2012 (35)</td>
<td>20 male professional soccer players; varying nationalities.</td>
<td>&lt;50</td>
<td></td>
<td>≥50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Halliday et al. 2011 (3)</td>
<td>41 college athletes; USA.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>≥100</td>
<td></td>
</tr>
<tr>
<td>Garcia et al. 2011 (30)</td>
<td>21 male professional basketball players; Spain.</td>
<td>&lt;12.5</td>
<td>12.5 – 25</td>
<td>25 – 50</td>
<td>&gt;50</td>
<td>75 – 150</td>
<td>&gt;375</td>
</tr>
<tr>
<td>Galan et al. 2011 (36)</td>
<td>34 professional soccer players; Spain.</td>
<td>&lt;50</td>
<td>50 – 74</td>
<td>≥75</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Where values were given in ng/mL, they have been converted to nmol/L using a conversion factor of 2.496.
<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>SeVERELY deficient (nmol/L)</th>
<th>MILDLY deficient (nmol/L)</th>
<th>Insufficient (nmol/L)</th>
<th>Sufficient (nmol/L)</th>
<th>Optimal (nmol/L)</th>
<th>Intoxication (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ducher et al. 2011 (37)</td>
<td>18 male ballet dancers (aged 10 to 18); Australia.</td>
<td>&lt;25</td>
<td>25 – 50</td>
<td>&gt;50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamilton et al. 2010 (38)</td>
<td>93 males of varying sports; Middle-East.</td>
<td>&lt;25</td>
<td>25 – 50</td>
<td>50 – 75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lovell 2008 (39)</td>
<td>18 female elite artistic gymnasts; Australia.</td>
<td>&lt;75</td>
<td>≥75</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.3.2 Factors that affect vitamin D status

The amount of vitamin D made in the skin is affected by those factors that alter the amount of 7-dehydrocholesterol or the number of UVB photons that penetrate the skin (24). These factors include: age; sunscreen use; exposure to sunlight; skin colour; obesity; physical activity; time of day; season and geographic location (15, 24). Some of these factors are explained in more detail below.

2.3.2.1 Exposure to sunlight

Minimal exposure of the skin to sunlight because of clothing or being indoors is very common, especially as window glass blocks UVB rays (15). Halliday et al. (3) found that serum 25(OH)D concentrations were significantly higher in outdoor athletes compared to indoor athletes during autumn.

2.3.2.2 Skin colour

Skin colour also affects vitamin D synthesis. People with darker skin have more melanin in their skin. Melanin is a natural sunscreen that absorbs UVB rays, thus people with darker skin make less vitamin D when exposed to the same amount of sunlight as lighter skinned people (24).

2.3.2.3 Physical activity

Although research is not conclusive, physical activity may increase vitamin D concentrations independently of sun exposure. It is postulated that the increased heat production due to exercise increases the conversion of previtamin D$_3$ to vitamin D$_3$, as this reaction is dependent on temperature (40).

2.3.2.4 Time of day, season and geographical location

Time of day, season and latitude all affect the angle of the sun (24). If one is farther away from the equator or during winter, early morning or late afternoon, the sun’s rays hit the
Earth at a more oblique angle (24). This means UVB photons have farther to travel through the ozone layer, so more are absorbed in the ozone and less reach the Earth’s surface (24).

### 2.3.3 Prevalence of suboptimal vitamin D status in New Zealanders

The 2008/2009 New Zealand Adult Nutrition Survey (ANS08/09) results showed that most (68.1%) New Zealanders aged 15 years and older were within the sufficient category for vitamin D status but 32% had concentrations below 50 nmol/L:

- 4.9% (95% CI 4.0 – 5.9) were deficient (<25 nmol/L)
- 27.1% (95% CI 24.7 – 29.5) suboptimal (25 – 50 nmol/L)
- 68.1% (95% CI 65.6 – 70.5) sufficient (≥50 nmol/L)
- 1.7% (95% CI 1.0 – 2.8) had very high concentrations (≥125 nmol/L) (1).

There were significant differences between ethnicities and geographical regions.

Of the major ethnic groups in New Zealand, Māori and Pacific Islanders tend to have darker skin than New Zealand Europeans, putting them at increased risk of deficiency (41). However, as ethnicity is self-defined and there is much intermarriage between cultures, the correlation between ethnicity and skin colour may have weakened over the years (42). The ANS08/09 found Māori women had significantly lower values than non-Māori women but no significant differences were found between Māori men and non-Māori men (1). Pacific men and women both had significantly lower values than non-Pacific men and women (1). This is in line with the 1997 National Nutrition Survey (NNS97) trend that mean values of New Zealand Europeans were higher than Māori and Pacific Islanders (51 (99% CI 49 – 53) vs. 42 (38 – 46) and 37 (33 – 42) nmol/L respectively (p < 0.01)) (41). Caution must be used when comparing ANS08/09 results with NNS97 results, as the NNS97 measured
serum 25(OH)D levels using a radioimmunoassay kit and the ANS08/09 used high-performance liquid chromatography, which is now considered the ‘gold standard’ (1).

The ANS08/09 also found significant differences between geographic locations. When the country was divided into northern, central and southern regions based on district health board areas, there were significant differences in the mean annual concentrations of serum 25(OH)D after adjusting for sex, age and ethnicity (1). The mean for the northern regions (Northland, Waitemata, Auckland and Counties Manukau district health boards) was 65.1 nmol/L (95% CI 62.2 – 68.0), the central regions (Waikato, Bay of Plenty, Lakes, Taranaki, Tairawhiti, Hawke’s Bay, Whanganui, MidCentral, Wairarapa, Hutt, Capital and Coast and Nelson Marlborough district health boards) 62.6 nmol/L (95% CI 60.8 – 64.4), and the southern regions (Canterbury, South Canterbury, West Coast and Southern district health boards) 60.5 nmol/L (95% CI 56.1 – 65.0) (1). Although the prevalence of deficiency (< 25 nmol/L) was not significantly different between the regions, there was a trend for southern regions to have more deficiency than central and northern regions (1). The clinical significance of these findings is questionable however as the differences are small. Indeed the NNS97 found no significant difference between the North and South Islands (41).

2.3.4 Vitamin D status in athletes

While vitamin D status in the general population has received much attention, very few studies have focused on athletes (2) (see Table 2.2.). Several studies in athletes and military personnel reported differences between darker-skinned participants and lighter-skinned participants, with darker-skinned participants having lower vitamin D status in every case (30, 35, 43, 44). Athletes who complete most of their training indoors also have lower mean serum 25(OH)D concentrations than those who train outside (3).
It has been suggested that athletes may be at higher risk of vitamin D deficiency during winter if they have low body fat, as vitamin D is stored in adipose tissue (45). This argument is credited by the greater seasonal variation in 25(OH)D concentrations in lean subjects compared to fatter subjects in the elderly (46).

Several studies did not report dietary vitamin D intake (33-35, 39, 44) and one study of male ballet dancers only measured intake in six participants (37). Of the few studies that did report dietary vitamin D intake, country-specific guidelines for intakes were often not met (43, 47). However, under-reporting has long been known to be a problem in dietary assessments (48).

While Garcia et al. (30) found a strong association between vitamin D intake and 25(OH)D concentrations in 21 basketball players in Spain (r = 0.65, p <0.001), Halliday et al. (3) reported no correlation in 41 college athletes in the USA. This may be due to the much higher concentrations of 25(OH)D in Halliday et al.’s participants as generally dietary vitamin D intake has little effect on vitamin D status.

Supplementation with vitamin D alone was not overly common in studies conducted thus far and few participants took multivitamins regularly. Halliday et al. (3) reported a positive correlation between multivitamin supplementation and 25(OH)D concentrations in 41 college athletes during winter (r=0.39).

Overall, vitamin D studies in athletes have been widely varied, having been conducted in many countries, in many seasons and focused on many sports. Vitamin D supplementation has not always been reported; when it was supplementation with vitamin D was rare. Similarly, dietary vitamin D intake was not always calculated; when it was it was usually measured by FFQ and normally fell short of that country’s specific guidelines for intake.
Table 2.2. Summary of studies reporting vitamin D status of athletes

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Location</th>
<th>Season</th>
<th>Mean serum 25(OH)D (nmol/L) (mean ± SD)</th>
<th>Supplementation or dietary intake of vitamin D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Close et al. 2013</td>
<td>25 club-level athletes.</td>
<td>UK</td>
<td></td>
<td>51 ± 24</td>
<td>No participants were taking vitamin D or fish oil supplements.</td>
</tr>
<tr>
<td>Close et al. 2013²</td>
<td>11 male professional soccer players.</td>
<td>UK</td>
<td>Winter</td>
<td>29 ± 25 in treatment group 53 ± 29 in placebo group</td>
<td>No participants were taking vitamin D supplements.</td>
</tr>
<tr>
<td>Close et al. 2013</td>
<td>61 male full time athletes (rugby league, soccer, flat jockeys, jump jockeys)</td>
<td>UK</td>
<td>Winter</td>
<td>Means only presented graphically. Estimated means from Figure 1 as follows: Rugby league: 70 Soccer: 38 Flat jockeys: 23 Jump jockeys: 30</td>
<td>No participants were taking vitamin D, fish oil or multivitamin supplements.</td>
</tr>
<tr>
<td>Morton et al. 2012</td>
<td>20 male professional soccer players.</td>
<td>England</td>
<td>Summer</td>
<td>104.4 ± 21.1 51.0 ± 19.0</td>
<td>Not reported.</td>
</tr>
<tr>
<td>Lutz et al. 2012</td>
<td>71 female soldiers during basic combat training.</td>
<td>USA</td>
<td>Winter</td>
<td>64.1 ± 3.8 at baseline 60.4 ± 2.9 at week 3 60.7 ± 2.6 at week 6 63.2 ± 2.6 at week 9</td>
<td>Quantitative FFQ at baseline and week 9 to estimate usual dietary intake. Mean vitamin D intake was 3.9 ± 0.4 μg, which falls short of the recommended dietary allowance of 15 μg). Military protocol does not permit dietary supplements.</td>
</tr>
</tbody>
</table>

² This paper reports the results of two studies, which have been presented separately in this table.
Table 2.2. Summary of studies reporting vitamin D status of athletes continued

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Location</th>
<th>Season</th>
<th>Mean serum 25(OH)D (nmol/L) (mean ± SD)</th>
<th>Supplementation or dietary intake of vitamin D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halliday et al. 2011 (3)</td>
<td>41 college athletes.</td>
<td>USA</td>
<td>Autumn</td>
<td>122.3 ± 41.4</td>
<td>FFQ in autumn, winter and spring.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Winter</td>
<td>76.1 ± 23.5</td>
<td>Mean estimated vitamin D intake (food + supplements) was 553 ± 471 IU/day in autumn, 683 ± 610 IU/day in winter, and 489 ± 456 IU/day in spring. Vitamin D intake was not significantly correlated with 25(OH)D concentrations at any point. 25(OH)D concentrations were significantly ( (p &lt; 0.05) ) correlated with multivitamin intake in the winter ( (r = 0.39) ).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Spring</td>
<td>104.6 ± 36.4</td>
<td></td>
</tr>
<tr>
<td>Garcia et al. 2011 (30)</td>
<td>21 male professional basketball players.</td>
<td>Spain</td>
<td>Spring</td>
<td>47.8 ± 21.8</td>
<td>FFQ; mean vitamin D intake was 139 ± 78 IU/day including supplements. Intake was significantly correlated with serum 25(OH)D concentrations ( (r = 0.65, p &lt;0.001) ).</td>
</tr>
<tr>
<td>Gibson et al. 2011 (47)</td>
<td>33 female adolescent elite soccer athletes.</td>
<td>Canada</td>
<td>Spring</td>
<td>75.4 ± 18.5</td>
<td>Four day food record; mean intake (SD) was 163.3 IU/day (94.7). 100% of participants did not meet the 2010 Institute of Medicine Dietary Reference Intake for vitamin D for females 14-18 years old (400 IU/day). Two participants took multivitamins regularly.</td>
</tr>
<tr>
<td>Ducher et al. 2011 (37)</td>
<td>18 adolescent male ballet dancers.</td>
<td>Australia</td>
<td>Winter</td>
<td>50.5</td>
<td>FFQ administered by phone ( (n=6) ) (not validated in adolescents or children). Vitamin D intake ranged from 55 to 505 IU/day. One participant took multivitamins regularly.</td>
</tr>
<tr>
<td>Study</td>
<td>Population</td>
<td>Location</td>
<td>Season</td>
<td>Mean serum 25(OH)D (nmol/L) (mean ± SD)</td>
<td>Supplementation or dietary intake of vitamin D</td>
</tr>
<tr>
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<td>---------------------------------------------</td>
</tr>
<tr>
<td>Andersen et al. 2010 (44)</td>
<td>74 female soldiers during basic combat training.</td>
<td>USA</td>
<td>Summer</td>
<td>72.9 ± 30.0</td>
<td>Not reported.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Autumn</td>
<td>63.3 ± 19.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lovell 2008 (39)</td>
<td>18 female adolescent elite artistic gymnasts.</td>
<td>Australia</td>
<td>Autumn</td>
<td>56</td>
<td>Not reported.</td>
</tr>
</tbody>
</table>
2.4 Training

2.4.1 Training definition

Athletes train to maximize their performance in competitive events. Training disrupts an athlete’s homeostasis, leading to short-term fatigue and performance improvement after recovery. Temporary increases in training load will fatigue the athlete and briefly decrease their performance, but with adequate recovery this results in “supercompensation” – the athlete adapts to the increased training load and their performance improves compared to baseline. This short-term intensified training is termed ‘functional overreaching’ (10) and is the basis of many effective training programmes (10, 13).

2.4.2 Training for rugby union

Semi-professional rugby union players are one group that use ‘functional overreaching’ to prepare for competition. However, as they compete year-round in multiple competitions, players have restricted time to prepare between competitions (49). Two to six weeks before weekly competitions resume, athletes undergo ‘pre-season training’ to condition their bodies for the demands of the competitive season (49). Pre-season training typically entails high-volume, high-intensity conditioning work in order to build lean mass and decrease fat mass, increase speed, strength, power, aerobic fitness and anaerobic fitness (49). When the competitive season begins the amount of conditioning training generally decreases to allow more rugby-specific training, such as skill and tactical sessions (49). Therefore, most functional overreaching and supercompensation occurs primarily during pre-season training (50). Nicholls et al. (51) found that many of the professional rugby union players in their study started the competitive season in a fatigued state indicative of overtraining, suggesting they did not have sufficient time to recover before the competitive season began. In a follow up study also conducted by Nicholls (50), players perceived
training volume, sleep and training structure (the schedule of a training day), as the top three sources of stress.

2.4.3 Overtraining syndrome (OTS)

In this section OTS in athletes will be defined and methods to monitor athletes’ responses to training explained, including the development, validation and use of the BRUMS.

2.4.3.1 Overtraining syndrome definition

If intensified training is maintained without adequate rest, the athlete may not supercompensate and instead may see negative symptoms, such as psychological and hormonal disturbances (10). This is termed ‘non-functional overreaching’ and it may take the athlete anywhere from several days to several weeks to fully recover (52). There is a continuum between high intensity training involving functional overreaching, non-functional overreaching and OTS. The point where an athlete crosses the boundary from non-functional overreaching into OTS will be different for each individual athlete (9). OTS has not been well defined in the literature but is generally considered to require a longer recovery period than non-functional overreaching, anywhere from several weeks to months (52). Thus, a diagnosis of OTS is dependent on the recovery period of the athlete and is made retrospectively, after all other explanations have been ruled out, e.g. inadequate nutrition, illness, psychosocial stressors and sleep disorders (10). As it is unethical to knowingly overtrain athletes, there are very few prospective studies. The exact cause of OTS remains unknown, although it is thought to result from a combination of the stress of training, inadequate recovery and stressors outside of training (11, 12).

Athletes suffering from OTS not only see a decline in performance, they are also susceptible to environmental stress, making them more likely to become ill (e.g. develop upper respiratory tract infections) (10, 52) or get injured (52). Athletes may also
experience signs and symptoms such as a depressed mood, reduced concentration, diminished self-esteem, become easily distracted, afraid of competition, apathetic and liable to giving up (13).

Treatment for OTS is either complete rest or low-intensity training, depending on the athlete’s symptoms (53). However, the negative signs and symptoms of OTS described above and the long rest period required for recovery means prevention of OTS is highly preferable to treatment.

2.4.3.2 Preventing overtraining syndrome

There is no single agreed upon marker to monitor an athlete’s response to training. Different markers have been suggested, such as performance testing (10), perceived exhaustion (53), biochemical (10, 53), hormonal (10, 53), immunological (10, 53), physiological (10) and psychological markers (9, 10, 14).

Meeusen et al. (10) stated that the ideal marker for the onset of OTS should be sensitive to training load and not affected by other factors like dietary intake. Acute changes in the marker in response to exercise should be distinguishable from chronic changes, the marker should be easy to measure and inexpensive, and of course changes in the marker should precede the onset of OTS so OTS can be prevented. This elite list presents a challenge to researchers in the field.

Performance tests are the gold standard for monitoring training but they must be sport-specific and sustained until exhaustion (10) or complete a set amount of physical work in a given time frame in highly standardised conditions. However, this method may be affected by the athlete’s motivation at the time and may increase the risk of injury near competitions (53). The large grey area between functional overreaching and non-functional
overreaching and overtraining is such that performance testing alone is unlikely to be useful in preventing OTS, although it may be of use in diagnosis (10).

Perceived exhaustion at a given training load can also be used to monitor training. An early sign of non-functional overreaching and OTS is the athlete feeling that more effort is required to perform the training (54).

The usefulness of biochemical markers has been questioned (53). Urea is a marker of protein catabolism so can be used to evaluate training load and recovery; however, during high intensity exercise the rise in lactate concentrations suppresses urea production (55). Creatine kinase is elevated with muscle damage so could be an indirect sign of non-functional overreaching but it cannot distinguish between athletes who tolerate their training load and athletes who do not (53). Maximal blood lactate concentrations have been shown to be reduced in endurance athletes suffering from OTS but the differences can be so small they lie within normal measurement error, and no lactate changes have been reported in strength athletes (10). While glutamine falls with increased training load, low plasma glutamine has not been consistently reported in athletes with OTS (10).

Hormonal markers such as cortisol and testosterone can reflect training volume and intensity (53); cortisol rises as exercise duration and intensity increases, testosterone rises with heavy-resistance exercise (55). However, many factors can influence results, for example, food intake, menstrual cycle phase, the act of either exercising or resting, and diurnal and seasonal variations of the hormone (10). Hormonal markers are difficult to use: they are expensive (10), baseline comparison is needed (53) and hospital or laboratory access is required (53).

The immune system is sensitive to stress, so markers of immune function could be used to assess stress levels in response to training (10). However, while sensitive to training load,
these changes do not distinguish between athletes who successfully adapt to overreaching and those who go on to develop OTS (10). Also, the presence of infection may confound markers (10).

Other physiological markers such as heart rate variability have shown inconsistent results and it is virtually impossible to differentiate between changes caused by functional overreaching, non-functional overreaching and OTS (10).

Training can also be monitored by assessing the athlete’s mood (53); this will be discussed in more detail in the section below.

2.4.3.3 Mood markers to monitor training

Overtraining is consistently related to changes in mood; mood markers such as the Profile of Mood States (POMS) and the Brunel Mood Scale (BRUMS) are sensitive to training load (56). In fact, mood changes have been reported as being more consistent than physiological markers for detecting overtraining (13).

There are several limitations of using mood markers to assess training response: baseline measures are needed for comparison, performance declines or losses in games could cause a depressed mood instead of the other way around, the timing of measurement is important as there may be differences before and after exercise, and the results are dependent on the athlete being honest (10).

However, there are several distinct advantages to psychological markers as well: results are available immediately, it is quick (9, 10), non-invasive (9), inexpensive (9, 10), and psychological changes coincide with physiological and performance changes (10).
2.4.4 Brunel Mood Scale (BRUMS)

While there are many options for monitoring training load, very few have shown consistency across different sports (9). The most consistent relationships have been found with psychological self-report measures like the POMS and the BRUMS (9).

2.4.4.1 Development and validation of the Brunel Mood Scale

The BRUMS is a 24-item self-administered questionnaire that asks how one feels and the respondent rates their answers on a five point likert scale, from 0 (“not at all”) to 4 (“extremely”). In this way the BRUMS assesses one’s mood profile via six factors: anger, confusion, depression, fatigue, tension and vigour (57). The BRUMS is derived from the 65-item POMS (58), originally developed by McNair, Lorr and Droppleman in 1971 (59) and validated in university students and psychiatric outpatients (60).

Despite not being validated in athletes, POMS was used extensively in the context of sport, at first principally by William Morgan and his colleagues throughout the 1970s (61-63). Morgan highlighted the differences in mood profile between athletes and non-athletes, particularly that athletes showed consistently higher vigour scores and lower anger, confusion, depression, fatigue and tension scores (7) – a pattern Morgan coined the “iceberg profile” and used as the basis of his Mental Health Model (8). The Model purports that positive mental health in terms of the iceberg profile is associated with successful sports performance (8). POMS has since been used to predict athletic success, with varying results (64, 65).

Subsequently, Morgan suggested that POMS could be used to assess athletes for mood changes that could be forerunners of OTS (14). This proposition was echoed by other researchers (13); although Hollander, Meyers and LeUnes (13) recognized the time consuming nature of this suggestion. Indeed, POMS had been previously critiqued as
taking too long and many shortened versions had already been spawned (58, 66). Briefness is especially important in sporting environments, where the questionnaire is often administered before a competition, thereby disruption to the athlete should be minimised (60, 66).

This was one of the reasons Terry et al. (60) validated a shortened version of POMS to use with adolescents in 1999, naming the new version the Profile of Mood States – Adolescents (POMS-A). The 24-item POMS-A was rigorously tested for validity. Content validity was assessed using a panel of experts and a group of school children to ensure the language used was age-appropriate and that the items actually measured the theoretical construct they were designed to measure (60). Preliminary factorial validity was assessed to test whether POMS-A was consistent with the mood construct. This was done via confirmatory factor analysis (CFA). After removing the three weakest items from each scale, analyses were acceptable (60). The model was then tested among a new sample of school children and young athletes; multi-sample CFA showed that the hypothesized relationships remained consistent in this new group, despite multivariate analysis of variance showing that the two groups were significantly different (60).

Criterion validity was assessed against the Positive and Negative Affect Schedule (PANAS) and the State-Trait Anger Expression Inventory.

While the PANAS had been previously validated in both athletes and children, the State-Trait Anger Expression Inventory had only been validated in children; this presents a limitation of the analyses. Despite this, POMS-A correlated well with the other questionnaires (60). The authors suggested that POMS-A be used to screen young athletes for mood disturbances, to prevent overtraining syndrome (60).
In 2000, Terry and Lane (59) developed normative data for the POMS-A in athletic samples. Sport researchers had previously been using the original POMS normative data, despite this being derived from university students and psychiatric outpatients (59). Morgan had already demonstrated that normal values for athletes were different than non-athletes (the iceberg profile) (8), so athletic normative data was desperately needed.

Terry and Lane’s athletic normative data was based on 2086 athletes at the international, club and recreational levels in Great Britain, including rugby union players (59). Data was gathered from 1990 to 1995 in one of three situations: pre-competition/exercise, post-competition/exercise and away from the athletic environment (at least 48 hours after competition/exercise) (59). A limitation of the study is that some data were collected for other purposes, such as for the Olympic Games, therefore it was impossible in these situations to control the collection procedures. This meant that some factors that have been shown to affect mood scores, e.g. training load (14), could not be controlled. However, this normative data is continually updated and refined.

For years, shortened POMS questionnaires were used not only with adolescents but with adults as well, despite not being validated specifically for them (67-71). Then in 2003, Terry, Lane and Fogarty (57) showed that POMS-A is equally relevant for adult athletes, after which it was re-named the Brunel Mood Scale (BRUMS) (72).

Terry, Lane and Fogarty (57) tested its validity in four samples of people: adult athletes, adolescent athletes, adult students and adolescent students. They argued that POMS-A was essentially a shorter and simpler version of POMS and would therefore be suitable for adults as well as adolescents.

Firstly, single sample CFA was used. All aspects were within acceptable ranges.
Factor loadings were significant (72% above 0.70), indicating strong relationships between the items on the questionnaire and the hypothesized latent factors of anger, confusion, depression, fatigue, tension and vigour. The direction and size of correlations between items was consistent with values published by Terry et al. in 1999 (60) and with the hypothesized model (57). For example, Terry, Lane and Fogarty found that confusion and anger were correlated in adolescent students at 0.613 (57) while in 1999 Terry et al. found the same correlation of 0.613 between confusion and anger in school children (60).

Next, multi-sample CFA was used to test the strength of the factor solution in all four samples simultaneously. Although results showed the factor structure was adequate when constrained to be equal across samples, it was only marginal when factor loadings and factor co-variances were also constrained to be equal (57). Subsequent exploratory factor analysis (EFA) suggested that allowing the item ‘uncertain’ to load on ‘tension’ and ‘confusion’ instead of just ‘confusion’ would improve the model. Overall, the authors reported that results from the CFA and EFA supported factorial validity as the hypothesized factor structure of POMS-A could be replicated in all sample groups, despite the groups being dissimilar (57).

Criterion validity was assessed against the original POMS, the PANAS, the State-Trait Anger Expression Inventory and the Hospital Anxiety and Depression Scale (HADS).

POMS-A and POMS were highly correlated, except for vigour, which only correlated at 0.67. The authors argued that while POMS vigour included items such as ‘cheerful’ and ‘carefree’, POMS-A had a narrower definition of vigour, only using items such as ‘active’, and ‘alert’ (57). POMS-A anger strongly correlated with State-Trait Anger Expression Inventory anger; POMS-A vigour strongly correlated with PANAS ‘positive affect’ scores; and POMS-A anger, confusion, depression, fatigue and tension strongly correlated with PANAS ‘negative affect’ scores. POMS-A depression only correlated with HADS
depression at 0.57; however this was expected as HADS measures clinical depression, whereas POMS-A measures depressed mood at one point in time (57).

Overall, POMS-A demonstrated sufficient validity to be used with adults (57), thus it became known as the BRUMS (72). The validity of the BRUMS in other groups of athletes has also been investigated. Hashim et al. (73) examined the validity of a Malay language version of the BRUMS for use with Malaysian athletes and Lan et al. (72) confirmed its validity in another sample of 1485 Malaysian athletes competing in the Malaysia Games. Lane et al. (74) investigated validity in UK, Hungarian and Italian athletes using confirmatory factor analysis; all indices were close to the 0.90 criterion for acceptable fit.

**2.4.4.2 Uses of the Brunel Mood Scale to date**

The 24-item BRUMS has been critiqued as focusing too much on negative mood states, thus the BRUMS-32 was developed. The BRUMS-32 added items that assess the factors ‘happiness’ and ‘calmness’ to give a more balanced mood profile (75).

Use of the BRUMS and the BRUMS-32 has not been limited to sport. Research has focused on university students, using the BRUMS to identify relationships between mood and assessments, examinations and goal setting (75-77).

The BRUMS has also been used to assess mood in a variety of athletes, both cross-sectionally and longitudinally. This has included water-skiers (78), wakeboarders (79), gymnasts (80), tennis players (81), soccer players (82), jockeys (83), ultra-endurance runners (84) and cyclists (85).

Galambos et al. (86) showed that BRUMS scores have a modest ability to predict injury in elite athletes, while Lane, Thelwell and Devonport (87) demonstrated that mood states are associated with sports performance in university student athletes, for example, higher
confusion scores after dysfunctional compared to functional sports performance (mean 1.35 (SD 0.96) compared to 0.7 (0.65)), although the BRUMS was administered retrospectively in this study.

The effects of other substances on BRUMS scores have also been looked at. Duncan and Oxford (88) showed that caffeine ingestion significantly decreased fatigue and increased vigour scores in men competing at national level in team sports, performing bench presses at 60% one repetition maximum to failure.

The BRUMS has also been used as the basis of other assessment models. Main and Grove (9) created a model for monitoring training distress in athletes based off the 10-item version of the Perceived Stress Scale, a checklist of 19 symptoms reported by Fry et al. in their study of overtraining in military personnel and the 24-item BRUMS.

2.4.4.3 Conclusions

The BRUMS is one of the most widely used mood assessment tools in the sporting world. It’s validity in a variety of athletes has been demonstrated and normative data generated. The BRUMS remains one of the quickest, simplest and most consistent ways to assess mood and explore risk of OTS in an athletic environment.

2.5 Vitamin D and Mood

2.5.1 Plausible mechanisms of how vitamin D affects mood

Vitamin D receptors (VDRs) are found in several areas of the human brain, including the cortex, cerebellum and limbic system (6). The enzymes vitamin D 25-hydroxylase and 25-hydroxyvitamin D-1-α-hydroxylase have also been isolated in various areas of the brain, suggesting that the brain can locally convert 25(OH)D to its active form 1,25(OH)\(_2\)D (89). It seems vitamin D in these areas is involved in the control of neurotransmitters that have a role in regulating emotional behaviour, e.g. acetylcholine and catecholamines (5). This
theory is supported by studies in mice with genetically impaired VDRs (“knockout mice”). These mice exhibit noticeable differences in behaviour, including increased anxiety (6). It is therefore plausible that the vitamin D status of an individual could directly affect their mood.

Furthermore, changes in mood according to season are well documented. It appears there is a continuum from those who do not report any changes with the seasons at one extreme to seasonal affective disorder (SAD) at the other (4). SAD is a major disorder characterised by depression over the winter months (4). Disrupted circadian rhythms have been hypothesized to explain this phenomenon (90-92).

Hormones including thyroid hormones, sex steroids, glucocorticoids and melatonin have been shown to modify circadian rhythms (91) – all of these hormones are affected by vitamin D. Cross-talk between vitamin D and glucocorticoids has been demonstrated in the hippocampus region of the brain and disrupted glucocorticoid signaling is related to depression (93). Suppressed melatonin secretion has occurred in SAD sufferers exposed to bright light, raising questions about the role of melatonin in SAD (92). An endocrine organ in the brain called the pineal secretes melatonin under conditions of darkness. The pineal receives input from several nuclei, all of which are immunoreactive to a calcium binding protein, calbindin-D28k. Calbindin-D28k is dependent on vitamin D$_3$ (94), therefore changes in vitamin D$_3$ will affect calbindin-D28k, which will affect the input to the pineal, which will alter melatonin secretion and thus induce changes in one’s circadian rhythm. Thus vitamin D may indirectly affect mood.

2.5.2 Influence of vitamin D status on mood

Key studies exploring the relationship between vitamin D and mood in non-depressed persons are presented in Table 2.3. Cross-sectional observational studies vary drastically.
Participants ranged from just 21 to 3262, in countries all over the world (England, China, the Netherlands, Norway, USA, Lithuanian and Ireland); some undertaken in the general population and some in select groups of patients with fibromyalgia, secondary hyperparathyroidism, dementia or obesity. A variety of questionnaires were used to assess mood (the Geriatric Depression Scale (GDS), the Center for Epidemiological Studies – Depression Scale (CES-DS), the Diagnostic Interview Schedule (DIS), the Beck Depression Inventory (BDI), the Hospital Anxiety and Depression Scale (HADS) and a clinician’s diagnosis using the Depressive Systems Inventory (DSI)) and overall results vary. There were significant positive associations between serum 25(OH)D and mood in 21 patients with secondary hyperparathyroidism and their controls ($p = 0.01$) (5) and when those with serum 25(OH)D concentrations <25 nmol/L were compared to those >25 nmol/L in 80 demented and non-demented patients and 75 fibromyalgia patients ($p = 0.02$ and $p < 0.05$ respectively) (95, 96). However, these studies had small sample sizes and focused on participants with some kind of disorder. In larger studies in the general population and those with obesity, the relationship between serum 25(OH)D and mood is attenuated by controlling for confounding factors, or no relationship has been found (97-99). The exceptions are Hoogendijk et al.’s (100) study undertaken in 1282 elderly participants in the Netherlands where a significant association was reported ($p = 0.03$) and Lašaite at al.’s study in young Lithuanian males where lower serum 25(OH)D concentrations were associated with higher POMS confusion-bewilderment scores ($p = 0.032$). However, cross-sectional studies cannot infer causality.

Prospective cohort studies have also shown mixed results. Harris and Dawson-Hughes’s (101) study in 250 postmenopausal women showed no relationship between 25(OH)D and mood despite POMS scores declining during winter – this of course raises the possibility that depressed mood during winter may not be due to insufficient or deficient vitamin D.
status. On the other hand, Milaneschi et al. (102) demonstrated that those participants with lower 25(OH)D status were more likely to be depressed. However, while the study was large (n = 954), the questionnaire used to assess mood was a self-report measure (the CES-D) and serum 25(OH)D was only measured at baseline and may have changed over the extensive six year follow up period.

Randomised controlled trials (RCTs) also demonstrate mixed results, and are difficult to compare directly due to the variety of questionnaires used to assess mood. Even serum 25(OH)D concentrations after supplementation appears to be a factor in some studies. One RCT that did not increase their participants’ serum 25(OH)D concentrations beyond the ‘sufficient’ category saw no difference in mood scores compared to placebo (103), while trials that gave adequate supplementation to increase scores to ‘optimal’ tended to show an effect (97, 104). However, this finding is disputed by Sanders et al. (93) and Gloth et al. (105). Sanders et al. (93) ran a reasonably large trial in elderly women (n = 102) with high doses of vitamin D (500000 IU/year for 3-5 years). The researchers found no significant differences in mood compared to the placebo group at any time point despite the vitamin D group increasing serum 25(OH)D from 53 to ~90 nmol/L three months post-dose. Gloth et al. (105) compared phototherapy to vitamin D supplementation in a very small group of SAD patients and found significant improvements in mood in the vitamin D group. However, both the vitamin D group and the phototherapy group remained in the ‘insufficient’ category (<50 nmol/L). While serum 25(OH)D increased more in the vitamin D group, the phototherapy group had higher levels at baseline so there was very little difference between groups at study end point. Also, serum 25(OH)D was measured at baseline and one week but the mood questionnaires only administered at baseline and one month, therefore further changes in vitamin D status may have occurred but were not measured.
Some RCTs did not measure serum 25(OH)D, so vitamin D status cannot be looked at (90, 106). Dumville (106) demonstrated no significant differences in mental component score between a supplementation group taking 1000 mg calcium and 800 IU vitamin D/day for six months and a group taking no supplements. However, serum 25(OH)D was not measured, the trial was unblinded, not placebo-controlled, had a low dose of vitamin D and it is questionable whether the mental component score used is a marker for mood, as this score primarily measures quality of life. Contradicting Dumville’s findings, Lansdowne and Provost (90) conducted a small trial in 44 healthy university students. Participants were allocated either: 10000 IU vitamin A and placebo, 9000 IU vitamin A and 400 IU vitamin D3 or 8000 IU vitamin A and 800 IU vitamin D3, every day for five days. After five days the vitamin D supplemented groups had significantly higher positive affect scores on the PANAS compared to the vitamin A and placebo group but no significant differences in negative affect scores. However, the follow up period of five days was short, the vitamin D dose low, the small differences in vitamin A doses cannot be conclusively ruled out as having an effect and, as aforementioned, serum 25(OH)D was not measured. Overall, findings are mixed, study designs are often very limited and no studies have been conducted in athletes. Robustly designed, double blind, placebo-controlled RCTs in athletic populations are needed.
### Table 2.3. Studies exploring the relationship between serum 25(OH)D concentrations and mood and/or examining the effect of vitamin D supplementation on mood

**Randomised Control Trials**

<table>
<thead>
<tr>
<th>Study</th>
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<th>Methods</th>
<th>Results</th>
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<tbody>
<tr>
<td>Sanders et al. 2011 (93)</td>
<td>118 women &gt;70 years from the Vital D study (61 placebo, 57 vitamin D$_3$); Australia.</td>
<td>Double blind, placebo controlled RCT.</td>
<td>Randomised to either annual oral dose of 500000 IU vitamin D$_3$ in the autumn/winter for 3 – 5 years or matched placebo. World Health Organisation (WHO) Well-Being Index and Patient Global Impression Improvement (PGI-I) scale completed and vitamin D status assessed: 12 months after last study medication, 1 month after next dose, 3 months after next dose.</td>
<td>No statistical differences were found between the vitamin D and placebo groups for scores from the WHO Well-Being questionnaires ($p = 0.47$) or the PGI-I scale ($p = 0.52$) at any point, despite vitamin D concentrations significantly increasing in the vitamin D group.</td>
<td>Primary endpoint of the Vital D study was fracture risk, thus women were selected if they had an identified risk factor for hip fracture and/or at high risk of low vitamin D status or osteoporosis. Only a subgroup of the study population completed mood questionnaires and had their serum vitamin D status checked.</td>
</tr>
</tbody>
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3 Where values were given in ng/mL, they have been converted to nmol/L using a conversion factor of 2.496
Table 2.3. Studies exploring the relationship between serum 25(OH)D concentrations and mood and/or examining the effect of vitamin D supplementation on mood *continued*

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Zhang et al. 2011(103)</td>
<td>32 hospitalised patients; Canada.</td>
<td>Double blind RCT.</td>
<td>Randomised to either 2000 IU vitamin D/day or 1000 mg vitamin C/day for 5 – 10 days. POMS administered before and after supplementation.</td>
<td>Vitamin D therapy significantly increased plasma 25(OH)D concentrations from a baseline average of 51.2 ± 20.0 nmol/L to 61.8 ± 17.2 nmol/L ($p = 0.0004$) but there was no significant effect on mood profile.</td>
<td>The trial was very short and the dose of vitamin D quite low. Patients already supplementing with vitamin D while in hospital were allocated to the vitamin C group, which could confound results. The baseline average of 51 nmol/L is considered sufficient by our standards and the final average of 61.8 did not reach the target of 75 nmol/L.</td>
</tr>
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</table>
Table 2.3. Studies exploring the relationship between serum 25(OH)D concentrations and mood and/or examining the effect of vitamin D supplementation on mood continued

<table>
<thead>
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<tr>
<td>Jorde et al. 2008(97)</td>
<td>441 volunteers with a body mass index (BMI) between 28.0 and 47.0 kg/m², mean age 47 years; Norway.</td>
<td>Double blind, placebo controlled RCT.</td>
<td>All participants given 500 mg calcium daily. Group DD – 40000 IU vitamin D per week; group DP – 20000 IU vitamin D per week; group PP – placebo, for one year. BDI conducted at baseline and the end of the study. Serum 25(OH)D taken every third month.</td>
<td>334 participants completed the study. Vitamin D status in the DD group significantly increased from 55.3 nmol/L at baseline to 112.1 nmol/L after 12 months; in the DP group the mean significantly increased from 52.2 to 87.8 nmol/L; the placebo group remained unchanged from baseline. Total BDI score significantly improved in both treatment groups but not in the placebo group.</td>
<td>Good compliance (~95% in all groups) but a high dropout rate (~25% of participants in each group). The treatment groups reached serum 25(OH)D levels &gt;75 nmol/L. Subjects all overweight and obese, which may affect their vitamin D requirements (107). Results are for vitamin D plus calcium versus calcium alone, not vitamin D versus placebo. Intention-to-treat analysis.</td>
</tr>
<tr>
<td>Dumville 2006(106)</td>
<td>1621 women ≥70 years old with one or more risk factors for hip fracture, mean age 77 years; UK.</td>
<td>RCT.</td>
<td>Women allocated to take 1000 mg calcium and 800 IU vitamin D per day or no supplements for six months. Mental component score (MCS) calculated from Short Form-12 (SF-12) questionnaire at baseline and 6 months.</td>
<td>No significant difference between groups.</td>
<td>Not placebo-controlled or blinded; serum 25(OH)D not measured. MCS may not be an appropriate marker of mood as the SF-12 measures quality of life.</td>
</tr>
</tbody>
</table>

4 This study had both cross-sectional and randomised control trial components. The methods, results and comments for the randomised control trial component are presented here and results for the cross-sectional study presented below.
Table 2.3. Studies exploring the relationship between serum 25(OH)D concentrations and mood and/or examining the effect of vitamin D supplementation on mood continued

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Vieth et al.</td>
<td>46 participants (Study 1) and 66 participants (Study 2) in an outpatient endocrinology clinic with summer 25(OH)D concentrations &lt;61 nmol/L in Study 1 and &lt;51 nmol/L in Study 2; USA.</td>
<td>Blinded RCT (participants blinded, researchers unblinded).</td>
<td>Participants randomised to receive either 4000 IU vitamin D$_3$ or 600 IU vitamin D$_3$ per day for 6 months. Authors developed their own questionnaire to assess wellbeing with six questions in Study 1 and 16 questions in Study 2.</td>
<td>Study 1: 25(OH)D increased significantly in both groups (46 ± 9 nmol/L to 79 ± 30 nmol/L in the lower dose group and from 49 ± 9 nmol/L to 112 ± 41 nmol/L in the higher dose group). Higher dose group had significantly lower (better) scores than the low dose group (1.1/6 compared to 2.3/6). Study 2: Participants scored significantly lower (better) in the wellbeing questionnaire after supplementation but no significant difference between the high dose and low dose groups. Study 1 and 2 data combined: the correlation between months on vitamin D and wellbeing showed significant improvement in wellbeing in the high dose group but no correlation in the low dose group.</td>
<td>Not placebo-controlled as it would be unethical not to give vitamin D supplements to these vitamin D insufficient participants, thus a placebo effect may have occurred. Questionnaire was not validated and therefore the clinical significance of results is impossible to conclude. Statistical significance for Study 1 results was one-tailed, which is not conventional.</td>
</tr>
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</table>
Table 2.3. Studies exploring the relationship between serum 25(OH)D concentrations and mood and/or examining the effect of vitamin D supplementation on mood continued

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<tr>
<td>Gloth et al. 1999 (105)</td>
<td>15 SAD patients (14 females, 1 male); USA.</td>
<td>RCT.</td>
<td>Participants randomly allocated to receive one dose of 100000 IU vitamin D$_2$ or phototherapy for two hours per day. Serum 25(OH)D measured at baseline and after 1 week of therapy. Hamilton Depression scale, Structured Interview Guide for the Hamilton Depression Rating Scale, SAD version (SIGH-SAD), SAD-8 depression scale administered at baseline and after 1 month of therapy.</td>
<td>Serum 25(OH)D significantly increased from 27.5 ± 9.0 nmol/L to 47.7 ± 20.5 nmol/L in the vitamin D group and 34.2 ± 7.7 nmol/L to 46.4 ± 6.2 nmol/L in the phototherapy group. All test scores significantly improved in the vitamin D group; no significant changes in the phototherapy group. The post-therapy scores in the vitamin D group were significantly better than the phototherapy group.</td>
<td>Not placebo-controlled. Very small sample size. At baseline the phototherapy group had higher serum 25(OH)D concentrations than the vitamin D group so their status did not change by much, which may contribute to the lack of effect seen.</td>
</tr>
<tr>
<td>Lansdowne and Provost 1998(90)</td>
<td>44 healthy student volunteers, mean age 22 years; Australia.</td>
<td>Double blind, placebo controlled RCT.</td>
<td>Allocated either 0 IU vitamin D$_3$ and 10000 IU vitamin A per day; 400 IU vitamin D$_3$ and 9000 IU vitamin A; or 800 IU vitamin D$_3$ and 8000 IU vitamin A, for five days. The authors did not state why vitamin A differed between the arms. PANAS was administered after the five days.</td>
<td>$p&lt;0.001$ for difference in positive affect scores between un-supplemented and supplemented groups (supplemented groups higher). No significant difference between scores for negative affect, although a non-significant trend for lower scores in the supplemented groups was observed.</td>
<td>The authors assumed the minor differences in vitamin A had no effect but one cannot conclusively rule out the effects of vitamin A. No serum 25(OH)D taken so vitamin D status unknown.</td>
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</table>
Table 2.3. Studies exploring the relationship between serum 25(OH)D concentrations and mood and/or examining the effect of vitamin D supplementation on mood continued

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<tbody>
<tr>
<td>Milaneschi et al. 2010(102)</td>
<td>531 women and 423 men &gt;60 years enrolled in the InCHIANTI Study (Invecchiare in Chianti, aging in the Chianti area), mean age women 75 years, mean age men 74 years; Italy.</td>
<td>Prospective cohort study.</td>
<td>Serum 25(OH)D measured at baseline. CES-D administered at baseline and 3 years and 6 years to assess depressed mood.</td>
<td>Women with serum 25(OH)D &lt;50 nmol/L had CES-D scores significantly higher (worse) at 3 y ($p = 0.02$) and 6 y ($p = 0.04$) than those women &gt;50 nmol/L; they also had significantly higher risk of developing depressive mood over the follow up period (hazard ratio = 2.0, 95% CI = 1.2-3.2, $p = 0.005$). Similarly, men with serum 25(H)D &lt;50 nmol/L had CES-D scores slightly higher (worse) than those men &gt;50 nmol/L, which was significant at 3 y ($p = 0.01$) but not 6 y ($p = 0.20$). They also tended to have a higher risk of developing depressed mood over the follow up period but this was not significant (hazard ratio = 1.6, 95% CI = 0.9-2.8, $p = 0.1$). When analyses were restricted to healthy participants, results were similar.</td>
<td>Vitamin D status may have changed over the 6 year follow up period yet it was only measured at baseline. The only significant changes were in women and men at 3 y, although the clinical significance of these changes in CES-D is undetermined.</td>
</tr>
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Table 2.3. Studies exploring the relationship between serum 25(OH)D concentrations and mood and/or examining the effect of vitamin D supplementation on mood continued

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<tr>
<td>Harris and Dawson-Hughes 1993(101)</td>
<td>250 white, postmenopausal women enrolled in a vitamin D supplementation study, mean age 62 years; USA.</td>
<td>Prospective cohort study.</td>
<td>POMS conducted four times over a one year period. All participants received 377 mg of calcium during the trial and 400 IU of vitamin D per day.</td>
<td>POMS scores were significantly worse during winter months; this relationship remained after those women ever treated for depression were excluded from the analyses. However, there was no relationship between either 1,25(OH)₂D or 25(OH)D and mood, and changes in vitamin D status were not correlated with changes in mood scores.</td>
<td>Study limited to middle-aged and older white women. No placebo group to follow over time to determine effect of supplementation.</td>
</tr>
</tbody>
</table>
Table 2.3. Studies exploring the relationship between serum 25(OH)D concentrations and mood and/or examining the effect of vitamin D supplementation on mood continued

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<tr>
<td>Lašaite et al. 2011 (108)</td>
<td>130 men aged 18 – 26 years; Lithuania.</td>
<td>Cross-sectional study.</td>
<td>Serum 25(OH)D concentrations and the POMS and HADS used to assess mood. Data were collected in winter.</td>
<td>When divided into vitamin D deficient (&lt;30 nmol/L), insufficient (30 – 50 nmol/L) and sufficient (&gt;50 nmol/L), lower serum 25(OH)D concentrations were associated with significantly higher scores for POMS confusion-bewilderment ($p = 0.32$). Study limited to young male medical students or military conscripts. There was a non-significant trend for higher scores for POMS depression-dejection with lower serum 25(OH)D concentrations.</td>
<td></td>
</tr>
<tr>
<td>Stewart et al. 2010(98)</td>
<td>2070 participants in the 2005 Health Survey for England, mean age 74 years; England.</td>
<td>Cross-sectional study.</td>
<td>Serum 25(OH)D concentrations and the 10-item GDS used to assess depressive symptoms. Data were collected throughout the year.</td>
<td>Results presented in three parts: &lt;25, &lt;50 and &lt;75 nmol/L. The OR of depressive symptoms in &lt;25 nmol/L=1.46 after adjusting for age, sex, social class, season, vitamin D supplement intake, smoking status, BMI, long-standing limiting illnesses and subjective general health (95% CI = 1.02–2.08, $p = 0.04$). When grouped into &lt;50 nmol/L and &lt;75 nmol/L, significant associations were attenuated by adjustment for confounders, particularly subjective general health. The dose-response relationship between serum 25(OH)D and depressive symptoms was strongly significant (B-value = -1.94, 95% CI = -2.67 – -1.20).</td>
<td>The sample was large and nationally representative of older people in England.</td>
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<tbody>
<tr>
<td>Pan et al. 2009(99)</td>
<td>3262 community residents aged 50 – 70; China.</td>
<td>Cross-sectional study (Nutrition and Health of Aging Population in China).</td>
<td>Serum 25(OH)D concentrations and CES-D. Data was collected in spring and summer (109).</td>
<td>The correlation between low 25(OH)D status and depressive symptoms was attenuated by controlling for confounding factors, and disappeared after adding geographic location to the analyses.</td>
<td>Healthy population of middle-aged and elderly Chinese. The 20-item CES-D had been previously validated in Chinese populations.</td>
</tr>
<tr>
<td>Hoogendijk et al. 2008(100)</td>
<td>1282 community residents aged 65 – 95 years; the Netherlands.</td>
<td>Cross-sectional study (in the Longitudinal Aging Study Amsterdam cohort).</td>
<td>Serum 25(OH)D measured, along with CES-D to measure depression. Those participants scoring ≥ 16 on the CES-D (the generally accepted cut-off point for clinically relevant depressive symptoms) were approached to undergo the DIS to determine if they had minor depression or major depressive disorder. Data were collected throughout the year.</td>
<td>Mean serum 25(OH)D was 52 ± 25 nmol/L. 25(OH)D was 14% lower in participants with minor depression (mean 47 nmol/L) and 14% lower in participants with major depressive disorder (mean 47 nmol/L) compared to controls (mean 55 nmol/L) (p &lt; 0.001). Depression severity as assessed by CES-D score was significantly associated with decreased serum 25(OH)D concentrations, after adjustment for age, sex, BMI, smoking status, number of chronic conditions and urbanisation (p = 0.03).</td>
<td>Study limited to elderly.</td>
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</table>
Table 2.3. Studies exploring the relationship between serum 25(OH)D concentrations and mood and/or examining the effect of vitamin D supplementation on mood continued

<table>
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<tr>
<td>Jorde et al. 2008⁵(97)</td>
<td>441 volunteers with a BMI between 28.0 and 47.0 kg/m², mean age 47 years; Norway.</td>
<td>Cross-sectional study.</td>
<td>BDI conducted at baseline and the end of the study (one year). Serum 25(OH)D taken every third month.</td>
<td>Cross sectional: Serum 25(OH)D was not significantly related to BDI score; however, when participants were divided into those &lt;40 nmol/L and &gt;40 nmol/L, those &lt;40 nmol/L scored significantly higher (more depressed) on the BDI.</td>
<td>Good compliance (95% in all groups). The effect of physical activity cannot be ruled out as a confounder. Subjects all overweight and obese.</td>
</tr>
</tbody>
</table>

⁵ This study had both cross-sectional and randomised control trial components. The methods, results and comments for the cross sectional component only are presented here and results for the randomised control trial presented above.
Table 2.3. Studies exploring the relationship between serum 25(OH)D concentrations and mood and/or examining the effect of vitamin D supplementation on mood  

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<tbody>
<tr>
<td>Jorde et al.</td>
<td>21 participants with secondary hyperparathyroidism (SHPT) and 63 age- and sex-matched controls, mean age 62 years; Norway.</td>
<td>Nested case-control (Tromsø cohort).</td>
<td>Serum 25(OH)D taken at baseline and on a separate day BDI and the General Health Questionnaire (GHQ) administered.</td>
<td>Baseline 25(OH)D was significantly lower in the SHPT group compared to control group (52.9 ± 15.0 nmol/L vs 67.3 ± 18.3 nmol/L). Controls had significantly more favourable scores than SHPT group for ‘depressed mood’ (BDI [1-13] score) but were not significantly different in total BDI score. Controls also had significantly better results for the GHQ factor ‘difficulty in coping’ but there were no significant differences in the other four factors and overall mental health status score. However, serum 25(OH)D was significantly associated with BDI [1-13] score ($p = 0.01$) and BDI total score ($p = 0.04$). When the cohort was split into quartiles of 25(OH)D there was a trend for those with higher vitamin D to score more favourably on the BDI [1-13].</td>
<td>As this study is cross-sectional, causality cannot be inferred. Did not adjust for confounding factors as sample size was too small. The difference in scores was small and may not be clinically important.</td>
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</table>
Table 2.3. Studies exploring the relationship between serum 25(OH)D concentrations and mood and/or examining the effect of vitamin D supplementation on mood continued

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<tbody>
<tr>
<td>Wilkins et al.</td>
<td>80 participants (40 with mild dementia and 40 non-demented), all participating in studies at the Alzheimer’s Disease Research Center, mean age 75 years; USA.</td>
<td>Cross-sectional study.</td>
<td>Mood assessed by a clinician, using the DSI (a nine-item inventory used to diagnose depression). This was completed by both the participant and an informant (usually a spouse or adult child). Serum 25(OH)D was collected at the same time.</td>
<td>Mean serum 25(OH)D: 46 ± 19 nmol/L. Serum levels &lt;25 nmol/L associated with active mood disorder (OR compared with &gt;50 ng/mL = 11.69, 95% CI = 2.04–66.86), serum levels between 25 and 50 nmol/L associated with active mood disorder (OR compared with &gt;50 ng/mL = 2.56, 95% CI = 0.63–10.51); after adjusting for age, race, gender and season of vitamin D determination.</td>
<td>87.5% of participants were white, therefore results may not be generalizable to other ethnicities. Cross sectional analysis cannot infer causality.</td>
</tr>
<tr>
<td>Armstrong et al.</td>
<td>75 white Caucasian sufferers of fibromyalgia (70 female, 5 male), mean age 50 years; Ireland.</td>
<td>Cross-sectional study.</td>
<td>HADS questionnaire and serum 25(OH)D concentrations measured during winter.</td>
<td>Patients were separated into those vitamin D deficient (&lt;25 nmol/L), insufficient (25–49.9 nmol/L) and normal (≥50 nmol/L). The deficient group had significantly higher (worse) HADS scores than the insufficient and normal groups (p &lt; 0.05).</td>
<td>55% of participants were taking antidepressants but no relationship between antidepressant use and HADS score was found. Causality cannot be inferred. Fibromyalgia patients with anxiety and depression may be less exposed to sunlight; vegetarian and vegan diets with little vitamin D are popular in fibromyalgia patients.</td>
</tr>
</tbody>
</table>
2.6 Conclusions

It is vital that athletes have a balance between training and recovery so that OTS can be prevented. The many negative side-effects of OTS and the long recovery period required to treat it makes monitoring responses to training a priority for athletes and coaches. This is especially true during pre-season training for athletes such as semi-professional rugby union players, as most high-volume, high-intensity conditioning work occurs during this period. Mood questionnaires such as the BRUMS have consistently monitored training across different sports and can be used to help prevent OTS.

It is unclear whether a person’s vitamin D status significantly affects their mood. Studies in mice with impaired VDRs demonstrate a plausible mechanism for the connection but research in humans is equivocal. Prospective cohort studies have shown mixed results; smaller cross-sectional observational studies have tended to show an association between 25(OH)D concentration and mood, however participants in these small studies all had a medical or an health disorder. In larger observational studies in the general population, associations have generally been attenuated by controlling for confounding factors.

Randomised controlled trials remain inconclusive. This is perhaps because studies were limited in design and difficult to compare: different mood questionnaires were used, some were originally designed to measure quality of life instead of mood; all studies were carried out in different geographical areas, making the effect of sunlight exposure difficult to determine; serum 25(OH)D was not measured in some studies, so the relationship, if any, between vitamin D status and mood cannot be seen; and while vitamin D supplementation is likely to significantly affect one’s vitamin D status, very few studies reported supplement use. Furthermore, RCTs have focused on the elderly, the hospitalised, the overweight and those with SAD. Just one RCT was undertaken in a general healthy population attending university, yet this study did not measure serum 25(OH)D
concentrations. No RCTs have been specifically carried out in athletes, even though athletes have been shown to have a different mood profile than the general population – this indicates a substantial gap in the literature.
3.0 Objective Statement

While the ANS08/09 showed that many New Zealand adults had serum 25(OH)D concentrations below 50 nmol/L (insufficient vitamin D status), there is limited literature on the vitamin D status of athletes and none specifically on New Zealand athletes.

A positive mood is important for athletes because their ability to respond to training depends on both their physical and their psychological states. The BRUMS questionnaire has been used to consistently assess athletes’ mood profiles across different sports, specifically in an effort to prevent overtraining.

The notion that vitamin D status affects mood seems plausible, when considering that VDRs have been isolated in areas of the brain that have a role in emotional behaviour. However, human research has been ambivalent. Prospective cohort studies have shown mixed results and any associations between vitamin D status and mood seen in large observational studies have been attenuated by controlling for confounders, such as geographical location. RCTs are also inconclusive. Plagued by design limitations, they have utilised different mood questionnaires, have been conducted in many locations with varied sun exposure, some have neglected to measure serum 25(OH)D and very few studies have investigated supplement use. RCTs have been focused on specialized populations including the elderly, the hospitalised, the overweight and those with SAD. The complete lack of RCTs carried out in athletes indicates a substantial gap in the sports nutrition literature.

Study objectives were established based on the above. The objectives are as follows:

- To investigate the relationship, if any, between baseline vitamin D status and BRUMS mood score in Otago and Southland semi-professional male rugby union players.
- To investigate whether vitamin D supplementation for 11 – 12 weeks significantly affects BRUMS mood score in Otago and Southland semi-professional rugby union players.
4.0 Subjects and Methods

The results presented in this thesis constitute a secondary data analysis from a randomised blinded placebo-controlled intervention trial entitled “Vitamin D status and effect of supplementation on strength, speed and body composition in rugby union players” (Australian New Zealand Clinical Trials Registry Number: ACTRN12612001233819). The following sections report the study design of the main RCT unless otherwise specified.

4.1 RCT study design

The study took place in Otago and Southland, New Zealand from March 2011 to August 2011, during the pre-season of the ITM Cup.

4.1.1 Participants

Participants were recruited from the Otago and Southland provincial ITM Cup Championship Rugby Union Squads from late March to early May 2011. Inclusion criteria were:

- a member of the Otago or Southland provincial ITM Cup Championship pre-season training squad;
- able to complete standardised rugby performance tests during pre-season training.

Exclusion criteria were:

- aged under 18 years;
- taking vitamin D supplements;
- currently diagnosed with a chronic disease (e.g. heart disease, diabetes, cancer);
- currently diagnosed with any condition that affects hormone metabolism;
- current smoker.
All participants provided written informed consent in accordance with the Helsinki Declaration (Appendix A). Study visits were conducted at the Department of Human Nutrition, University of Otago in Dunedin and at the Rugby Southland offices in Invercargill. Approval for the study was granted by the University of Otago Human Research Ethics Committee (Appendix B).

### 4.1.2 Sample size

The primary endpoint for the main RCT was 30 m sprint. Performance testing data from the Otago and Southland ITM Cup Squads over the previous two years was analysed to establish usual variance, and the 30 m sprint time selected as the primary outcome variable. Both strength and conditioning coaches indicated that 30 m sprint time is comparable between players of different body weights, and that a difference of 0.08 s between the two treatment groups would be clinically significant (mean 30 m sprint time in the previous two years was 4.14 s). To have 80% power to detect a change in 30 metre sprint performance of 0.08 seconds, using a two-sided 0.05 level of significance, 28 participants were needed in each group; a total of 56 participants.

Secondary outcome variables for the main RCT included serum 25(OH)D concentrations (nmol/L), body mass (kg), 10 m sprint time (seconds), BRUMS score, and predicted one repetition maximum bench pull (kg), weighted reverse grip chin-up (kg) and bench press (kg).

The secondary data analysis presented in this thesis investigates the change in BRUMS scores over the course of the RCT.

### 4.1.3 Randomisation

Randomisation was stratified by location (either Otago or Southland) and training group (strength, power or muscle hypertrophy). Half of those in each training group were
allocated to vitamin D and half to placebo. An independent off-site statistician used computerized sequence generation to allocate either treatment 1 (placebo) or treatment 2 (vitamin D) to each participant.

4.1.4 Blinding

Allocations from the independent statistician were sent directly to an independent University of Otago staff member, who placed the treatments into white containers with each participant’s initials on them. These containers were then provided to each squad’s Head Strength and Conditioning Coach. In this way, participants, coaches and researchers were blinded.

Recruitment bias was addressed by having an independent person in-house recruit (strength and conditioning coaches). Although voluntary, strength and conditioning coaches strongly encouraged everyone to participate and the majority of players did.

Treatment bias was limited by stratifying according to training group.

4.1.5 RCT intervention

Participants received either 50000 IU cholecalciferol every fortnight (pharmaceutical grade: Cal.D.Forte, PSM Healthcare, Auckland, NZ) or placebo (NZ Nutritionals, Christchurch, NZ) for either 11 weeks (Otago squads) or 12 weeks (Southland squad) in the form of a single oral tablet. The vitamin D tablet equated to 3571 IU/day or 89 μg/day.

The different treatment durations existed as the trial was designed to coincide with scheduled performance testing dates and these dates differed between squads.

The Institute of Medicine recommends an upper limit for vitamin D intake of 4000 IU/day (110) and a dietary survey of Otago ITM Cup rugby players completed in 2009 concluded that the mean vitamin D intake in this population was 300 IU/day (111). The dietary
estimate combined with the dose administered (3871 IU/day) should still therefore fall within a safe level of intake.

Every participant had their own pill box containing six tablets. Once every fortnight, after taking their tablet, participants ticked off a schedule and trainers counted remaining tablets to monitor compliance.

4.2 Data collection

All participants completed a screening form, mood questionnaire, provided fasting blood samples and underwent anthropometric testing, dual-energy x-ray absorptiometry (DEXA), performance testing and a reaction time test at baseline, weeks 5/6 (Otago/Southland) and weeks 11/12 (Otago/Southland). Due to its potential as a confounding factor, data was also collected on sun exposure and sun protection habits via a sun exposure questionnaire. Body composition data was collected at baseline and weeks 11/12 via DEXA.

Only those data collection procedures relevant to the secondary data analysis for this thesis are presented in detail below.

4.2.1 Screening form

The screening form collected demographic information: date of birth, age, self-defined ethnicity, playing position, years playing rugby union, number of years playing at representative level, supplement use, and pre-season training goals.

4.2.2 Mood questionnaire

Participants completed the 24-item BRUMS questionnaire in the morning (57). Participants were asked to cross the circle that best described “how you feel right now” in regards to the items and answered on a 5-point likert scale (0 = “not at all”, 1 = “a little”, 2 = “moderately”, 3 = “quite a lot”, 4 = “extremely”). This assessed six subscales: anger,
confusion, depression, fatigue, tension and vigour. The BRUMS has been validated in adult athletes and takes one to two minutes to complete (57). See Appendix C.

### 4.2.3 Blood collection

A registered nurse from the Department of Human Nutrition at the University of Otago collected fasted blood samples from all Otago participants. Six mL of blood was collected in red-top (no anticoagulant) Vacutainer Rapid Serum Tubes (Becton Dickinson, Plymouth, UK), allowed to clot at room temperature for at least 60 minutes then centrifuged at 3000 rpm for 15 minutes. Five aliquots of 400 µL of serum per participant were placed in plastic micro-centrifuge tubes (Labcon, California, USA) and held on ice until stored at -80°C until analyses. One aliquot was used for analysis of vitamin D.

### 4.2.4 Laboratory analysis of serum 25-hydroxyvitamin D

All serum 25(OH)D samples were analysed after completion of the study. Total serum 25(OH)D concentrations were determined using liquid chromatography tandem mass spectroscopy (LC MS/MS) according to the methods published by Maunsell et al (112) at Canterbury Health Laboratories, Christchurch, New Zealand. LC MS/MS measures 25(OH)D₂ and 25(OH)D₃ concentrations, however no 25(OH)D₂ was detected in any sample so results are for 25(OH)D₃ concentrations only.

Canterbury Health Laboratories have calculated assay sensitivity and inter-assay coefficients of variation in 885 samples. Assay sensitivity is 1 nmol/L and the control standard sample was within 98.8 – 100.7% of the expected value. The inter-assay coefficients of variation were acceptable at 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.
4.2.5 Sun exposure questionnaire

The sun exposure questionnaire was a modified version of the New Zealand Sun Exposure Survey developed in 2009 and conducted every three years in New Zealand by the Health Sponsorship Council in co-operation with the Cancer Society of New Zealand (113). The modified version assessed sunlight exposure in the week prior to blood sample collection, time spent outdoors, sunburn, outside training hours in daylight, skin type, recent travel and sun protection habits including clothing and sunscreen. The original survey included this information but also assessed such things as skin cancer knowledge and attitudes to getting a tan (113). It was not validated against a similar survey as no such survey existed at the time. However, an expert reference group involved in skin cancer research provided advice regarding content (113).

4.3 Statistical analyses

The statistical methods employed in the secondary data analysis for this thesis are presented here. The analysis was performed on an intent-to-treat basis.

Statistical analyses were conducted using IBM SPSS Statistics (version 21, International Business Machines Corporation).

Correlations were run to answer the first research question regarding the relationship between vitamin D status and BRUMS mood score. Normal distribution was assessed via Shapiro-Wilks test. Where data were normally distributed Pearson’s product-moment correlations were run to assess the relationship between BRUMS scores and serum 25(OH)D concentrations at baseline. Where data were not normally distributed, the non-parametric Spearman’s rank-order correlations test was run. Potential confounders were also tested to see if they correlated with serum 25(OH)D or BRUMS scores.
To answer the second research question, does vitamin D supplementation significantly affect BRUMS mood score in Otago and Southland male semi-professional rugby union players, between group comparisons were made. Where data were normally distributed independent samples t-tests were used; where data were not normally distributed both independent samples t-tests and Mann-Whitney U tests were run in a sensitivity analysis. Homogeneity of variances was assessed by Levene's Test for Equality of Variances and outliers identified via visual inspection of box plots.

The BRUMS scores for each mood subscale were converted to T-scores to standardise against other adult athletes. The normative data used to do this was obtained from the BRUMS user guide (114). This uses the following formula to convert scores to a standardised score with a mean of 50 and a standard deviation of 10:

$$T = 50 + 10((n - m)/s)$$

where $n =$ raw score, $m =$ mean, and $s =$ standard deviation (59).

All analyses were run using both raw and standardised BRUMS scores.

Change in BRUMS scores were calculated by subtracting baseline scores from weeks 11/12 scores for each mood subscale. A positive value represented more intense mood at weeks 11/12, a negative value indicated less intense mood and a value of zero showed no change.

A p-value of <0.05 was considered significant. All data are presented as mean ± SD unless otherwise indicated.
5.0 Results

5.1 Participant flow, losses and exclusions

A total of 58 participants met inclusion criteria and were randomised to treatment or placebo groups (see Figure 5.1). There were seven withdrawals and two participants were non-compliant (less than 80% compliance) but provided data. For further information on withdrawals please refer to Figure 5.1.

![Figure 5.1. Participant flow, losses and exclusions](image-url)

<table>
<thead>
<tr>
<th></th>
<th>Assessed for eligibility (n=78)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excluded (n=20)</td>
<td>Declined to participate (n=20)</td>
</tr>
<tr>
<td></td>
<td>Randomised (n=58)</td>
</tr>
<tr>
<td>Allocated to treatment (n=29)</td>
<td>Received treatment (n=28)</td>
</tr>
<tr>
<td></td>
<td>Did not receive treatment</td>
</tr>
<tr>
<td></td>
<td>(declined to participate) (n=1)</td>
</tr>
<tr>
<td>Lost to follow up (personal reasons and injury) (n=3)</td>
<td>Discontinued intervention (non-compliant with treatment) (n=2)</td>
</tr>
<tr>
<td>Analysed (n=28)</td>
<td>(intent-to-treat analysis)</td>
</tr>
<tr>
<td></td>
<td>Excluded from analysis (n=0)</td>
</tr>
</tbody>
</table>

Allocated to placebo (n=29) | Received placebo (n=29) |
Lost to follow up (personal reasons and injury) (n=2) | Discontinued intervention (relocation) (n=1) |
Analysed (n=29) | (intent-to-treat analysis)      |
|                 | Excluded from analysis (n=0)    |
5.2 Recruitment

Eligible participants were recruited from the Otago and Southland provincial ITM Cup Championship Rugby Union Squads in two recruitment drives. The first drive was from March 21 to April 8, 2011, with randomisation occurring in the same period; the second drive was to recruit from an extended pool of players to improve participant numbers, it was conducted from May 2 to May 6, 2011 with randomisation occurring in the same period. Following randomisation, participants were allocated to either placebo or vitamin D, to take every fortnight for 11 weeks (Otago squads) or 12 weeks (Southland squad).

Baseline demographic and clinical characteristics of participants are presented in Table 5.1. Participants had a mean (SD) serum 25(OH)D concentration of 94.26 (17.92) nmol/L at baseline, which was considered optimal (>75 nmol/L).
Table 5.1. Baseline demographic and clinical characteristics*

<table>
<thead>
<tr>
<th></th>
<th>Vitamin D group (N = 28)</th>
<th>Placebo group (N = 29)</th>
<th>Total (N=57)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>21.54 (2.78)</td>
<td>20.86 (2.84)</td>
<td>21.19 (2.81)</td>
</tr>
<tr>
<td>Ethnicity:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Zealand European</td>
<td>14 (50%)</td>
<td>21 (72%)</td>
<td>35 (61%)</td>
</tr>
<tr>
<td>Māori</td>
<td>10 (36%)</td>
<td>4 (14%)</td>
<td>14 (25%)</td>
</tr>
<tr>
<td>Pacific</td>
<td>4 (14%)</td>
<td>4 (14%)</td>
<td>8 (14%)</td>
</tr>
<tr>
<td>Years playing rugby union (years)</td>
<td>12.1 (4.7)</td>
<td>12.5 (4.4)</td>
<td>12.3 (4.5)</td>
</tr>
<tr>
<td>Years playing rugby union at a representative level (years)</td>
<td>5.5 (3.6)</td>
<td>5.4 (3.4)</td>
<td>5.5 (3.4)</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>97.1 (10.2)</td>
<td>96.0 (12.7)</td>
<td>96.5 (11.4)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.85 (0.06)</td>
<td>1.84 (0.08)</td>
<td>1.85 (0.07)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.2 (2.2)</td>
<td>28.3 (2.1)</td>
<td>28.2 (2.1)</td>
</tr>
<tr>
<td>Sum of 8 skinfolds (mm)</td>
<td>75.3 (29.8)</td>
<td>81.5 (22.8)</td>
<td>78.6 (26.2)</td>
</tr>
<tr>
<td>Percent body fat (%)</td>
<td>14.4 (4.2)</td>
<td>14.9 (5.1)</td>
<td>14.6 (4.6)</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>14.2 (4.7)</td>
<td>14.5 (5.9)</td>
<td>14.3 (5.3)</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>79.2 (8.6)</td>
<td>76.6 (9.3)</td>
<td>77.9 (8.9)</td>
</tr>
<tr>
<td>Serum 25(OH) D (mmol/L)</td>
<td>93.4 (17.9)</td>
<td>95.1 (17.4)</td>
<td>94.3 (17.9)</td>
</tr>
</tbody>
</table>

* Data are means (SD), except ethnicity which is numbers (%).

5.3 Analyses

5.3.1 Baseline BRUMS scores

Baseline BRUMS scores for the whole group were positively skewed toward zero for anger, confusion, depression and tension; whereas fatigue and vigour were not skewed (Figure 5.2). Most participants answered in the “Not at all” categories for anger,
confusion, depression and tension, thus receiving raw scores of zero for those subscales.

Any participants that answered “A little”, “Moderately”, “Quite a lot” or “Extremely” thus became statistical outliers, greatly altering the mean BRUMS scores compared to the medians (Table 5.2.). As they were not true anomalies, all outliers were kept in the subsequent analyses and non-parametric tests were employed.

Table 5.2. Baseline BRUMS scores for whole group (n=57)

<table>
<thead>
<tr>
<th>Baseline BRUMS score</th>
<th>Mean (SD)</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRUMS anger</td>
<td>0.69 (1.514)</td>
<td>0.00</td>
<td>0-7</td>
</tr>
<tr>
<td>BRUMS confusion</td>
<td>0.84 (1.288)</td>
<td>0.00</td>
<td>0-5</td>
</tr>
<tr>
<td>BRUMS depression</td>
<td>0.40 (0.784)</td>
<td>0.00</td>
<td>0-3</td>
</tr>
<tr>
<td>BRUMS fatigue</td>
<td>4.45 (3.144)</td>
<td>4.00</td>
<td>0-14</td>
</tr>
<tr>
<td>BRUMS tension</td>
<td>0.98 (1.727)</td>
<td>0.00</td>
<td>0-7</td>
</tr>
<tr>
<td>BRUMS vigour</td>
<td>7.27 (2.997)</td>
<td>7.00</td>
<td>1-14</td>
</tr>
<tr>
<td>Standardised BRUMS anger</td>
<td>49.58 (9.946)</td>
<td>45.00</td>
<td>45-91</td>
</tr>
<tr>
<td>Standardised BRUMS confusion</td>
<td>45.35 (5.150)</td>
<td>42.00</td>
<td>42-62</td>
</tr>
<tr>
<td>Standardised BRUMS depression</td>
<td>47.64 (5.104)</td>
<td>45.00</td>
<td>45-64</td>
</tr>
<tr>
<td>Standardised BRUMS fatigue</td>
<td>55.78 (11.103)</td>
<td>54.00</td>
<td>40-89</td>
</tr>
<tr>
<td>Standardised BRUMS tension</td>
<td>39.95 (5.180)</td>
<td>37.00</td>
<td>37-58</td>
</tr>
<tr>
<td>Standardised BRUMS vigour</td>
<td>47.78 (7.894)</td>
<td>47.00</td>
<td>32-65</td>
</tr>
</tbody>
</table>
Figure 5.2. Distribution of baseline BRUMS scores for whole group

Outliers (greater than or equal to the third quartile plus 1.5 times the interquartile range) are presented by circles. Extreme outliers (greater than the third quartile plus 3 times the interquartile range) presented by asterisks.

5.3.2 Correlations between BRUMS, serum 25-hydroxyvitamin D and hours of sun exposure at baseline

Results are presented in Table 5.3. According to Cohen’s effect sizes (115), there was a small negative correlation between BRUMS confusion scores and serum 25(OH)D, $r_s(53) = -0.387$, $p = 0.004$ for both raw and standardised BRUMS confusion scores. In contrast, baseline BRUMS anger, depression, fatigue, tension and vigour were not significantly correlated with baseline serum 25(OH)D concentrations.
Table 5.3. Correlations between baseline BRUMS scores and baseline serum 25(OH)D concentrations for whole group¹

<table>
<thead>
<tr>
<th></th>
<th>Spearman’s rho correlation</th>
<th>p-value</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Raw scores for subscales</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anger</td>
<td>0.105</td>
<td>0.446</td>
<td>55</td>
</tr>
<tr>
<td>Confusion</td>
<td>-0.387</td>
<td>0.004</td>
<td>55</td>
</tr>
<tr>
<td>Depression</td>
<td>-0.024</td>
<td>0.860</td>
<td>55</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0.142</td>
<td>0.301</td>
<td>55</td>
</tr>
<tr>
<td>Tension</td>
<td>-0.071</td>
<td>0.605</td>
<td>55</td>
</tr>
<tr>
<td>Vigour</td>
<td>0.015</td>
<td>0.915</td>
<td>55</td>
</tr>
<tr>
<td><strong>Standardised scores for subscales</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anger</td>
<td>0.105</td>
<td>0.446</td>
<td>55</td>
</tr>
<tr>
<td>Confusion</td>
<td>-0.387</td>
<td>0.004</td>
<td>55</td>
</tr>
<tr>
<td>Depression</td>
<td>-0.024</td>
<td>0.860</td>
<td>55</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0.142</td>
<td>0.301</td>
<td>55</td>
</tr>
<tr>
<td>Tension</td>
<td>-0.071</td>
<td>0.605</td>
<td>55</td>
</tr>
<tr>
<td>Vigour</td>
<td>-0.007</td>
<td>0.957</td>
<td>55</td>
</tr>
</tbody>
</table>

¹Spearman’s rank-order correlations

Spearman’s rank-order correlation was also run to assess whether any relationship existed between hours of sun exposure, serum 25(OH)D and BRUMS mood scores. No significant correlations were identified; therefore hours of sun exposure was not considered to be a confounder in subsequent analyses.

5.3.3 Longitudinal changes in serum 25-hydroxyvitamin D by treatment group

At study endpoint, serum 25(OH)D was greater in the treatment group (113.56 ± 18.55 nmol/L) than the placebo group (79.62 ± 21.34), a statistically significant difference of 33.95 nmol/L (95% CI, 22.68 to 45.21), p < 0.0005. This is presented in Figure 5.3. There
were no outliers in the data, serum 25(OH)D concentrations in each group were normally distributed and there was homogeneity of variances ($p = 0.752$).

![Graph showing serum 25(OH)D concentrations](image)

**Figure 5.3. Mean serum 25(OH)D concentrations and standard error from baseline to weeks 11/12 as per treatment allocation (vitamin D group n = 28; placebo group n = 29)**

### 5.3.4 Change in BRUMS scores between baseline and study endpoint by treatment group

Due to the skewed data, there were outliers in anger, confusion, depression, fatigue, tension and vigour as assessed by inspection of boxplots; outliers were kept in the analyses. There was homogeneity of variances ($p > 0.05$). Only changes in raw and standardised fatigue and vigour BRUMS scores were normally distributed ($p > 0.05$) but results from the non-parametric Mann-Whitney U test agreed with results from the independent-samples t-test.

Between group comparisons found that there were no statistically significant differences between groups for change in any of the BRUMS scores; this is presented in Table 5.4 and
**Figure 5.4.** By looking at the change in BRUMS scores instead of BRUMS scores at weeks 11/12, personality differences between participants were accounted for.

**Table 5.4. Differences in change in BRUMS score between placebo (n = 29) and treatment (n = 28) groups from baseline to weeks 11/12**

<table>
<thead>
<tr>
<th>Raw scores for subscales</th>
<th>Median difference of placebo group</th>
<th>Median difference of treatment group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anger</td>
<td>0.0</td>
<td>0.0</td>
<td>0.184</td>
</tr>
<tr>
<td>Confusion</td>
<td>0.0</td>
<td>0.0</td>
<td>0.120</td>
</tr>
<tr>
<td>Depression</td>
<td>0.0</td>
<td>0.0</td>
<td>0.944</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0.0</td>
<td>0.5</td>
<td>0.379</td>
</tr>
<tr>
<td>Tension</td>
<td>0.0</td>
<td>0.0</td>
<td>0.309</td>
</tr>
<tr>
<td>Vigour</td>
<td>-1.0</td>
<td>-2.0</td>
<td>0.118</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Standardised scores for subscales</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anger</td>
</tr>
<tr>
<td>Confusion</td>
</tr>
<tr>
<td>Depression</td>
</tr>
<tr>
<td>Fatigue</td>
</tr>
<tr>
<td>Tension</td>
</tr>
<tr>
<td>Vigour</td>
</tr>
</tbody>
</table>

1Mann-Whitney U test
Figure 5.4. Difference in mean standardised BRUMS scores from baseline to weeks 11/12 and standard error as per treatment allocation (placebo n = 29; vitamin D n = 28)
Figure 5.4. Difference in mean standardised BRUMS scores from baseline to weeks 11/12 and standard error as per treatment allocation (placebo n = 29; vitamin D n = 28) continued
6.0 Discussion and Conclusions

6.1 Key findings

BRUMS scores for the subscales anger, confusion, depression and tension were skewed toward zero, reflecting a generally positive mood profile. However, fatigue scores were higher and vigour scores lower than expected.

There was a small negative correlation ($r_s(53) = -0.387, p = 0.004$) between baseline BRUMS confusion and serum 25(OH)D concentrations. No other subscales were significantly correlated with serum 25(OH)D concentrations.

While vitamin D supplementation resulted in significantly higher serum 25(OH)D concentrations in the treatment group compared to placebo group at weeks 11/12, there were no significant differences between treatment and placebo groups for change in any of the BRUMS subscale scores from baseline to weeks 11/12.

6.2 Possible mechanisms and explanations

*BRUMS scores for anger, confusion, depression and tension were all skewed toward zero.*

Previous studies of mood in athletes have also found this, along with low fatigue scores and high vigour scores (7) – a finding termed the “iceberg profile” by Morgan and colleagues that distinguishes athletes from the general population (who have higher anger, confusion, depression, fatigue and tension scores, and lower vigour scores) (8). Terry and Lane (59) suggested three possible reasons for the iceberg profile: firstly, that those people involved in sport and exercise simply have better mental health; secondly, that athletes may develop strategies to self-regulate their mood in order to perform at their best; and thirdly, that zero scores could represent a social desirability bias for athletes.
Fatigue scores were higher and vigour scores lower than expected.

In this study, vigour scores were lower and fatigue scores higher than previous studies in athletes (79, 81). This may be because in this study BRUMS questionnaires were filled out prior to pre-season training sessions, instead of prior to competition like other studies (79, 82, 84, 114). As pre-season training involves high-volume, high-intensity conditioning work (49), with most functional overreaching and supercompensation occurring during this period (50), it is likely that participants would have been more physically fatigued than before a competition, when athletes’ training loads are tapered prior to a competitive event (49). Therefore, while anger, confusion, depression and tension were all as expected, fatigue and vigour scores meant the participants in this study did not have typical iceberg profiles (see Figure 6.1.).
Figure 6.1. BRUMS profile of (a) participants, compared to (b) typical iceberg profile

(8)

This could represent a normal shift in mood that is not clinically significant or it could indicate that the majority of athletes were overtraining. Of note, a Wilcoxon signed-rank test showed that mood did not significantly change in the placebo group over the course of the pre-season training period (Figure 6.2). As the last BRUMS questionnaire completed
could be considered to be no longer pre-season and more the beginning of the season, this does lend credit to the theory that these players were overtraining.

However, the BRUMS mean score of a group is not sufficient to diagnose overtraining, especially as it can only be compared against normative data that is not specific to pre-season training for rugby union. An individual mood profile for each athlete (i.e. each athlete would have taken the BRUMS many times) is needed to know if this represents a significant mood disturbance or if this is a normal response to the training load for that particular athlete. This is a limitation of the current study.

![BRUMS profile of placebo group at baseline and at study endpoint](image)

Figure 6.2. BRUMS profile of placebo group at baseline and at study endpoint

*The only statistically significant Spearman's rank-order correlation between BRUMS subscales and serum 25(OH)D was that of confusion (raw and standardised), $r_s(53) = -0.0387$, $p = 0.004$.*
According to Cohen’s classification for behavioural sciences this can be considered a small effect (115). As no other BRUMS subscales were significantly correlated with serum 25(OH)D, one must ask the question why confusion?

This finding is similar to that of Lašaite et al.’s study in Lithuanian men (108) which found a significant association between lower serum 25(OH)D concentration and higher POMS confusion-bewilderment scores but no other subscales. Lašaite et al. offered no explanation as to why only confusion-bewilderment was significant and not the other subscales of the POMS, although they did note a trend for higher depression-dejection scores with lower serum 25(OH)D.

Lašaite et al.’s study was conducted in healthy men aged 18 – 26 years, making their population similar to this study. However, these men were medical students or military conscripts, not athletes, and the mean serum 25(OH)D concentration was far lower than the rugby players of this study (32.45 ± 13.23 vs 94.26 ± 17.92 nmol/L).

Perhaps there is a confounder that is responsible for the association between confusion and vitamin D status, as a simple correlation does not account for any potential confounders; or the finding is simply a type 1 error that occurred by chance and mood is not related to vitamin D status at all.

Another possible explanation is that vitamin D status is only related to mood when one is vitamin D deficient, then once past a certain threshold mood is no longer dependent on vitamin D status. The vitamin D status threshold for confusion could be greater than the threshold for the other subscales, which could explain the finding.

Of note, both Lan et al. (72) and Lane et al. (74) argued that ‘confusion’ is a symptom of anxiety and not a mood state in itself, and therefore should be removed from the BRUMS questionnaire.
There were no significant differences between treatment and placebo groups in terms of change in BRUMS subscale scores from baseline to weeks 11/12.

This finding would indicate that there is no association between serum 25(OH)D concentrations and mood in healthy male athletes after confounders are controlled for (in this instance by random treatment allocation). Given that this population was vitamin D replete, it is possible that there may be an association between serum 25(OH)D and mood in healthy male athletes but only when serum 25(OH)D concentrations are less than optimal. Mean serum 25(OH)D concentrations were higher in the treatment group at weeks 11/12 (113.6 ± 18.6 nmol/L) but the placebo group retained ‘optimal’ status (defined as serum 25(OH)D concentrations above 75 nmol/L) with concentrations of 79.6 ± 21.3 nmol/L.

Previous studies in athletes have shown great diversity in vitamin D status, depending on geographical location, season and sport. As this study was conducted during autumn, the participants had residually high vitamin D concentrations from the summer months. Without supplementation, the placebo group’s mean serum 25(OH)D concentrations were declining. It is likely that during winter the players’ vitamin D status would drop further. Future study is needed to determine whether less than optimal vitamin D status in athletes affects mood.

6.4 Strengths and limitations of the present study

The main strengths of this study include the study design as a randomised placebo controlled trial that stratified randomisation by location and training regime – this minimized allocation bias and balanced both the known and unknown potential confounders between the treatment and placebo groups. Allocation concealment and the blinding of coaches, participants and researchers to the treatment received also prevented
bias. In particular, participants filling out the BRUMS could have been easily biased if their treatment was known as the BRUMS is a subjective measure of mood.

Further strengths included having an independent person (strength and conditioning coach) recruit participants to reduce recruitment bias; the extensive validation of the BRUMS questionnaire in adult athletes (see section 2.4.4.1); the reasonably large sample size of 57 participants compared to other studies in sports nutrition; and using LC-MS/MS to analyse serum 25(OH)D. LC-MS/MS is considered a more sensitive and specific method to analyse serum 25(OH)D than traditional immunoassays (112) and allows the measurement of both 25(OH)D$_2$ and 25(OH)D$_3$ metabolites (116).

Despite these strengths there are also several limitations of the study, including the possibility of type 1 and 2 errors and the reasonably short timeframe of 11 or 12 weeks. Greatly limiting in answering the objectives is the fact that there were no vitamin D deficient participants, so one cannot say if vitamin D supplementation affects mood when a person is deficient. Furthermore, standardised BRUMS scores are based on normative data. Normative data for athletes has been generated from data collected approximately one hour before competition ($n = 621$) while data for the current study was collected on the mornings of pre-season training, thus the normative data may not be relevant to the current study (57). To minimise this limitation raw BRUMS scores as well as standardised scores were used in all analyses.

The RCT conducted in these rugby union players was powered appropriately to determine clinically significant changes in performance (28 participants in each group to detect a 0.08 second difference in 30 m sprint time). While the subject numbers are relatively large compared to other studies in sports nutrition globally, we cannot be certain that this study was adequately powered to detect significant changes in BRUMS scores in this population.
6.5 Conclusions

It is unlikely that vitamin D supplementation will affect mood in rugby players who are not vitamin D deficient, and thus will not contribute to a better response to training.

Future research in this area should attempt to recruit vitamin D deficient athletes to investigate whether mood is affected by vitamin D supplementation those athletes.
7.0 Application to Dietetic Practice

Dietitians may come across athletic clients presenting with symptoms of OTS, such as a depressed mood, reduced concentration, diminished self-esteem and a fear of competition. Nutritional factors such as energy and fluid intake should be explored and the client encouraged talk to their coach about revisions to their training schedule.

It is appropriate for dietitians to work with medical colleagues to screen athletes to positively identify vitamin D deficiency before prescribing vitamin D supplements. In the case of sufficient serum 25(OH)D concentrations, the results of this project indicate that vitamin D supplementation is not warranted in order to improve mood. If mood disturbances are suspected, the dietitian must remain within the scope of their professional practice by referring the individual on to an appropriate health professional, such as their general practitioner or sports psychologist, so that other factors affecting mood can be addressed.
8.0 References

9.0 Appendices
Appendix A – Informed Consent
“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.

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PO Box 56, Dunedin 9054, New Zealand
Tel 64 3 479 7959 • Fax 64 3 479 7958
Email human-nutrition@otago.ac.nz • Web https://www.otago.ac.nz/humannutrition/
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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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 1/10th 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 Pre-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.

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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
Department of Human Nutrition
University Telephone Number: 479 5359
Email: kirsty.fairbairn@otago.ac.nz

Ingrid Ceelen
Department of Human Nutrition
University Telephone Number: 479 8369
Email: ingrid.ceelen@otago.ac.nz

Or

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
"Vitamin D Status and Effect of Supplementation on Pre-season Performance in Rugby Union"

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) x 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.

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DUNEDIN • CHRISTCHURCH • WELLINGTON • HAMILTON • AUCKLAND
“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.

Ethics Approval No. 11/064 22nd March 2011
Appendix B – University of Otago Human Research Ethics Committee 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 Forms 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 in principle.

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

Yours sincerely,

Mr Gary Wilts
Manager, Academic Committees
Tel 479 5393
Email gary.wilts@otago.ac.nz

cc. Emeritus Professor L J Holloway Head Department of Human Nutrition
Appendix C – The Brunel Mood Scale
The Brunel Mood Scale: Vitamin D Status and Effect of Supplementation on Strength, Speed and Body Composition in Rugby Union Players

Player ID number: ____________
Date: ____________
Session: Morning/Afternoon

Below is a list of words that describe feelings. Please read each one carefully. Then cross the circle that best describes HOW YOU FEEL RIGHT NOW. Make sure you answer every question.

<table>
<thead>
<tr>
<th>Word</th>
<th>Not at all</th>
<th>A little</th>
<th>Moderately</th>
<th>Quite a lot</th>
<th>Extremely</th>
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<tbody>
<tr>
<td>1 Panicky</td>
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<td>2 Lively</td>
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<td>3 Confused</td>
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<td>4 Worn out</td>
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<td>5 Depressed</td>
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<td>6 Downhearted</td>
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<td>7 Annoyed</td>
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<td>8 Exhausted</td>
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<td>9 Mixed-up</td>
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<td>10 Sleepy</td>
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<td>11 Riled</td>
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<td>12 Unhappy</td>
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<td>13 Anxious</td>
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<td>14 Worried</td>
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<td>15 Energetic</td>
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<td>16 Miserable</td>
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<td>17 Muddled</td>
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<td>18 Nervous</td>
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<td>19 Angry</td>
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<tr>
<td>20 Active</td>
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<tr>
<td>21 Tired</td>
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<td>22 Bad-tempered</td>
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<td>23 Alert</td>
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<td>24 Uncertain</td>
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