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THE EFFECTS OF A VITAMIN D
RANDOMISED CONTROLLED TRIAL ON
MUSCLE STRENGTH AND POWER IN
FEMALE ADOLESCENT ATHLETES

A Thesis presented in partial fulfillment of the requirements for the degree of

Master of Science in

Human Nutrition

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Abstract

Background:

Vitamin D deficiency is widespread in the general public and emerging evidence has revealed it is common in athletic populations, particularly those who train indoors. Recent studies suggest that vitamin D deficiency is correlated with impaired skeletal muscle function; however there is limited evidence from randomised controlled trials that vitamin D supplementation can improve muscle strength and power in trained athletes.

Objective:

To investigate the effects of vitamin D₃ supplementation on serum 25(OH)D concentrations and muscle strength and power in female adolescent athletes training predominantly indoors.

Methods:

Female adolescent dancers, gymnasts, and swimmers ($n = 61$) who trained regularly for at least five hours per week participated in this randomised double blind placebo controlled trial. Participants were stratified to receive 50,000 IU vitamin D₃ or placebo every month for six months. Serum 25(OH)D concentrations, muscle strength (handgrip and isokinetic knee extensor and flexor torque), power (vertical jump), and anthropometric measurements were assessed at baseline and endpoint ($n = 54$).

Results:

At baseline, the median 25(OH)D concentration was 77.5 [63.5,92] nmol/L for the vitamin D group and 74 [64.5,88.5] nmol/L for the placebo group. Following six

months of supplementation, serum 25(OH)D concentrations increased significantly in the vitamin D group (16.5 [7,46] nmol/L) ($P = 0.001$), but not the placebo group (-6.25 [-21,44] nmol/L). Peak torque (Nm) of the knee extensors in concentric and eccentric extension increased significantly for both groups ($P < 0.05$), and there was no significant difference in change in peak torque between groups. After controlling for change in 25(OH)D and baseline 25(OH)D separately, supplementation with vitamin D was not associated with any of the strength or power variables.

Conclusions:

Supplementation of 50,000 IU of vitamin D₃ per month improved vitamin D status but did not improve the chosen measures of muscle strength and power in this group of female adolescent athletes. This may be due in part to the small sample size and high baseline serum 25(OH)D concentrations seen in this cohort.

Keywords: skeletal muscle strength, skeletal muscle power, athletes, dancers, gymnasts, vitamin D, 25(OH)D

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List of Abbreviations

1,25(OH) ₂ D	1,25- hydroxyvitamin D
24-OHase	25-hydroxyvitamin D-24-hydroxylase
25(OH)D	25-Hydroxyvitamin D
7-DHC	7-Dehydrocholesterol
AI	Adequate intake
BMD	Bone Mineral Density
BMI	Body Mass Index
Ca ²⁺	Calcium ion
CDPKII	Calmodulin-dependent protein kinase II
CYP27B1	25-hydroxyvitamin D-1 α -hydroxylase
DXA	Dual-energy X-ray absorptiometry
ES	Endocrine Society
IBP	Intracellular binding protein
IGF1	Insulin-like growth factor-1
IGFBP-3	Insulin-like growth factor binding protein-3
IOM	Institute of Medicine

IU	International Units
MED	Minimal Erythematol Dose
MOH	Ministry of Health
nm	nanometer
Nm	Newton metre
nmol/L	nanomoles per liter
ng/ml	nanograms per milliliter
PI3-K	Phosphoinositide 3-kinase
PLA	Placebo (group)
PLC	Phospholipase C
PKA	protein kinase A
PKC	protein kinase C
PTH	Parathyroid Hormone
RXR	Retinoid X receptor
RCT	Randomised controlled trial
SR	Sarcoplasmic reticulum
UV	Ultraviolet
UVB	Ultraviolet B

VD	Vitamin D (group)
VDKO	Vitamin D receptor knockout (mouse)
VDBP	Vitamin D binding protein
VDR	Vitamin D Receptor
VDRE	Vitamin D response element
VO _{2max}	Maximal oxygen consumption

CHAPTER 1 – INTRODUCTION

Vitamin D is more accurately described as a steroid hormone, sourced from synthesis in the skin through exposure to the sun's Ultraviolet B (UVB) radiation and absorbed in the intestine from some limited dietary sources. The effect of vitamin D on multiple physiological systems has in recent years become a focal point of intense interest, thanks in no small part to the recognition that vitamin D deficiency is now considered a worldwide pandemic; it is estimated that vitamin D deficiency affects one billion people worldwide (Holick, 2007). Vitamin D is more traditionally known for its role in the regulation of calcium and phosphate metabolism, raising the blood levels of these minerals via intestinal absorption and renal reabsorption to facilitate bone mineralisation (Hausler et al., 2013). However, research exploring the novel actions of vitamin D on physiological systems outside skeletal health is rapidly accumulating, such as in the autoimmune (Haroon & FitzGerald, 2012), immune (Adams & Hewison, 2008), cardiovascular (Anderson et al., 2010), and neurological (DeLuca, Kimball, Kolasinski, Ramagopalan, & Ebers, 2013) systems, to name a few. The proposed role of vitamin D in muscle has been comprehensively scrutinised, however certain aspects remain controversial (Girgis, Clifton-Bligh, Hamrick, Holick, & Gunton, 2012).

The purported presence of the vitamin D receptor (VDR) in skeletal muscle, as well as the rise of *in vitro*, histological, and functional studies have supported a pivotal role for vitamin D in maintaining muscle structure and function. The majority of functional studies have largely investigated the elderly; sarcopenia is a well-known consequence of ageing, and serum 25(OH)D concentrations decrease with age (Visser, Deeg, & Lips, 2003). Several large clinical studies have attempted to establish a causal link between

low vitamin D status and reduced muscle strength and incidence of falls in the elderly (Bischoff-Ferrari et al., 2004; Visser, et al., 2003). Evidence from these studies tend to support an effect of muscle strength and function in the elderly, although meta-analyses of muscle function indicate that any beneficial effect is limited to those with low baseline serum 25(OH)D concentrations (Rejnmark, 2011). Postural control and balance have been linked with vitamin D status, where numerous studies have examined this in relation to falls in the elderly (Bischoff-Ferrari et al., 2006; Boersma et al., 2012). Meta-analyses have acknowledged a stronger correlation between greater serum 25(OH)D concentrations and reduced incidence of falls, again seen best in those with vitamin D deficiency (Bischoff-Ferrari et al., 2009). A growing number of studies have recently explored the relationship between vitamin D deficiency and muscle function in otherwise healthy young adults and adolescents (Grimaldi et al., 2012; Ward et al., 2009), the outcome from which have also been mixed. These studies have subsequently promoted speculation on whether such an association might be found in athletes.

Should vitamin D deficiency negatively impact muscle function in otherwise healthy athletes, such a condition has the potential to diminish peak athletic performance (Angeline, Gee, Shindle, Warren, & Rodeo, 2013). In contrast to the widely published data concerning the vitamin D status in the general and elderly populations, there is a scarcity of research on the effect of vitamin D in athletic groups, despite the importance of maintaining peak muscular function in most sports. Recently however, athletic populations are beginning to receive more consideration from the scientific community, particularly in regard to their vitamin D status. Several studies have documented a surprisingly high proportion of athletes who present with vitamin D insufficiency and

deficiency. These include both male and female athletes training in a wide range of disciplines such as gymnastics (Lovell, 2008), ballet (Ducher et al., 2011), football (Hamilton, Whiteley, Farooq, & Chalabi, 2013), and basketball (Constantini, Arieli, Chodick, & Dubnov-Raz, 2010). A cross-sectional study recently published on the association between serum 25(OH)D concentrations and muscle function in professional footballers found that body mass and lean mass was significantly higher in those with serum 25(OH)D concentrations higher than 50 nmol/L (Hamilton, et al., 2013).

Athletes who train primarily indoors, such as gymnasts and dancers, have a reduced likelihood of obtaining sufficient vitamin D from sunlight. For highly competitive or professional athletes a significant proportion of their daily time is spent indoors for training and competition; as such, the prevalence of vitamin D insufficiency for athletes who participate in indoor sports is found to be higher than athletes who train and compete outdoors (Halliday et al., 2011). A study of elite female gymnasts in Australia determined that 83% of the subjects had serum 25(OH)D concentrations below the recommended guidelines (75 nmol/L), and a third had concentrations under 50 nmol/L (Lovell, 2008). Therefore indoor athletes such as gymnasts and dancers are at risk for suboptimal vitamin D status, which could impact the maximum level of muscle strength and power achieved by these athletes.

Adolescent dancers and gymnasts must also accommodate both the physical demands of competitive training as well as the accelerated rate of growth associated with adolescence. Furthermore, these sports emphasize leanness (Ravaldi et al., 2006), which may result in reduced dietary intake of all nutrients including vitamin D, as well as

erroneous nutritional choices that can negatively impact optimal musculoskeletal health necessary for success in these sports (Van Durme, Goossens, & Braet, 2012).

In view of the fact that gymnasts and dancers are at a high risk for vitamin D deficiency, and that the necessary strength required for performance in these sports may be compromised, there is a demand for a well-designed, placebo-controlled intervention study. Upon the commencement of the present study, there had been no randomised controlled trials published on the direct effect of vitamin D supplementation on muscle function in any athletic population. Some researchers have hypothesised that the creation of a double-blind, placebo-controlled, multiple-dose crossover study with long washout periods using high doses - such as 2000, 4000, and 6000 IU of vitamin D per day - could determine if peak athletic performance levels exist for any particular serum 25(OH)D concentration (Cannell, Hollis, Sorenson, Taft, & Anderson, 2009). Alternatively, participants could receive the dose required to increase their own serum 25(OH)D concentrations to 100 nmol/L, theoretically regarded to be associated with peak neuromuscular performance (Bischoff-Ferrari, Dietrich, Orav, Hu, et al., 2004). However, there are ethical concerns in identifying without treating a vitamin D deficient control group, as well as the practical difficulties concerning time and participant numbers.

The aim of the present thesis is to determine the effect of a double-blind, placebo controlled six-month vitamin D supplementation trial on serum 25(OH)D concentrations, muscle strength and power, and postural balance in adolescent female dancers and gymnasts.

1.1 Hypotheses

The major null hypotheses (H0) for this research were:

H01: Adolescent female dancers and gymnasts will have sufficient serum concentrations of vitamin D.

H02: Six months of vitamin D supplementation will not affect strength and power measures in hand grip or proximal leg muscles in female dancers and gymnasts.

H03: Six months of vitamin D supplementation will not affect isometric postural stability during a one-legged static balance in female dancers and gymnasts.

1.2 Overview

This thesis is presented in five chapters that describe the effect of vitamin D supplementation on a collection of dynamic muscle strength and power, and isometric postural control tests in a cohort of adolescent female dancers and gymnasts. Chapter 2 is a review of the literature of vitamin D as it pertains to its effect on muscle strength. Chapter 3 is a description of the methodology used for the study. Chapter 4 contains the results, and Chapter 5 is a critical analysis of the data as they relate to the literature.

CHAPTER 2 – REVIEW OF THE LITERATURE

The objective of this review is to provide a thorough investigation of the literature on the relationship between vitamin D and skeletal muscle. The topics that will be reviewed in detail are the prevalence of vitamin D deficiency, specifically the evidence of vitamin D status in athletic groups; the proposed actions of vitamin D on skeletal muscle; and the literature to date from observational studies and randomised trials that have investigated the relationship between vitamin D status and muscle strength, power and postural balance with particular regard to the athletic population.

2.1 Physiology of vitamin D

2.1.1 Sources

Vitamin D is a fat-soluble vitamin more accurately described as a secosteroid hormone. Vitamin D has two forms; vitamin D₂ also called ergocalciferol, and vitamin D₃ also known as cholecalciferol, and several metabolites (Holick et al., 2011). Vitamin D₂ is obtained from the UV irradiation of the yeast sterol ergosterol and is found naturally in sun-exposed mushrooms. Vitamin D₃ is synthesised in the skin when exposed to UVB rays and is present in oil-rich fish (Table 1).

2.1.1.1 Exposure to sunlight

The primary source of vitamin D for most humans is endogenous synthesis from sunlight (Wacker & Holick, 2013). During exposure of the skin to sunlight, UVB radiation (290–315 nm) is absorbed by 7-dehydrocholesterol in the skin to form previtamin D₃ (MacLaughlin, Anderson, & Holick, 1982). Previtamin D₃ is then rapidly

converted to vitamin D₃. Once formed, it is transferred from the skin cell out into the extracellular space. Vitamin D-binding protein (VDBP) pulls it into the dermal capillary bed, thereby entering circulation. Vitamin D obtained through the diet (as either vitamin D₂ or D₃) is assimilated into chylomicrons, which are absorbed into the lymphatic system and enter the venous blood (Holick, et al., 2011) (Figure 1).

2.1.1.2 Diet

Dietary sources of vitamin D in New Zealand are limited. Those foods where it is found in reasonable amounts are in oily fish such as salmon, sardines, herring, and tuna, followed by eggs, butter, and liver (Chen et al., 2007). Other food sources include cod liver oil and fortified foods such as margarines, milks and yoghurts, although in New Zealand it is not mandatory to fortify food with vitamin D (NZNF, 2013). Consequently, dietary intake of vitamin D contributes only toward a fraction of our overall requirements.

2.1.1.3 Supplements

Both cholecalciferol and ergocalciferol are used for production of supplements. In New Zealand, the maximum permitted over-the-counter dose of vitamin D is 1000 IU as a dietary supplement, and some multivitamin preparations contain vitamin D of up to 200 IU per dose. However, dietary supplements in New Zealand do not require strict regulation and a recent analysis demonstrated a large variation in the percentage label claim of vitamin D (Garg et al., 2013). Prescription formulations of 50,000 IU are available which are more strictly regulated (Medsafe, 2013).

2.1.2 Metabolism of vitamin D

Once in the bloodstream, vitamin D₃ or D₂ is transported to fat cells where it is stored, or to the liver where it is hydroxylated to 25-hydroxyvitamin D (25(OH)D), also known

as calcidiol. Although biologically inactive, this is the major circulating form of vitamin D, the concentration of which is used for the clinical measurement of vitamin D status (Glade, 2012). Calcidiol is then metabolised in the kidneys by 25-hydroxyvitamin D-1 α -hydroxylase (CYP27B1), to produce the circulating active form 1,25-dihydroxyvitamin D [1,25(OH)₂D], also known as calcitriol (Holick, 2007). Renal production of 1,25(OH)₂D regulates calcium and phosphorus metabolism, and is in turn

Table 1 Natural sources of vitamin D₂ and D₃. Modified from Wacker & Holick, 2013

Natural Sources	Approximate quantity of vitamin D
Cod liver oil	~400-1000 IU/tsp vitamin D ₃
Egg yolk	~20 IU/yolk vitamin D ₃ or D ₂
Salmon, canned	~300-600 IU/100 g vitamin D ₃
Salmon, fresh farmed	~100-250 IU/100 g vitamin D ₃
Tuna, canned	236 IU/100 g vitamin D ₃
Sardines, canned	~300 IU/100 g vitamin D ₃
Shitake mushrooms, fresh	~100 IU/100 g vitamin D ₂
Shitake mushrooms, sun dried	~1600 IU/100 g vitamin D ₂
Sunlight/UVB radiation	~20,000 IU equivalent to exposure to 1 minimal erythemal dose (MED) in a bathing suit

regulated by several factors such as serum phosphorus, calcium, fibroblast growth factors (FGF-23), and itself (Holick, 2007). 1,25(OH)₂D feedback regulates its own synthesis, as well as decreasing the synthesis of parathyroid hormone (PTH) in the parathyroid glands. Calcitriol can induce its own elimination by increasing the expression of 25-hydroxyvitamin D-24-hydroxylase (24-OHase) to break down

1,25(OH)₂D to the water-soluble biologically inactive calcitroic acid which is then excreted in bile (Holick, 2007). The enzyme CYP27B1 is also expressed in various other tissues enabling 1,25(OH)₂D to be produced locally to exert auto- or paracrine effects (Wacker & Holick, 2013). Recent identification of CYP27B1 activity in skeletal muscle cells and adult mouse muscle lends support to the direct action of 1,25(OH)₂D in muscle (Srikuea, Zhang, Park-Sarge, & Esser, 2012).

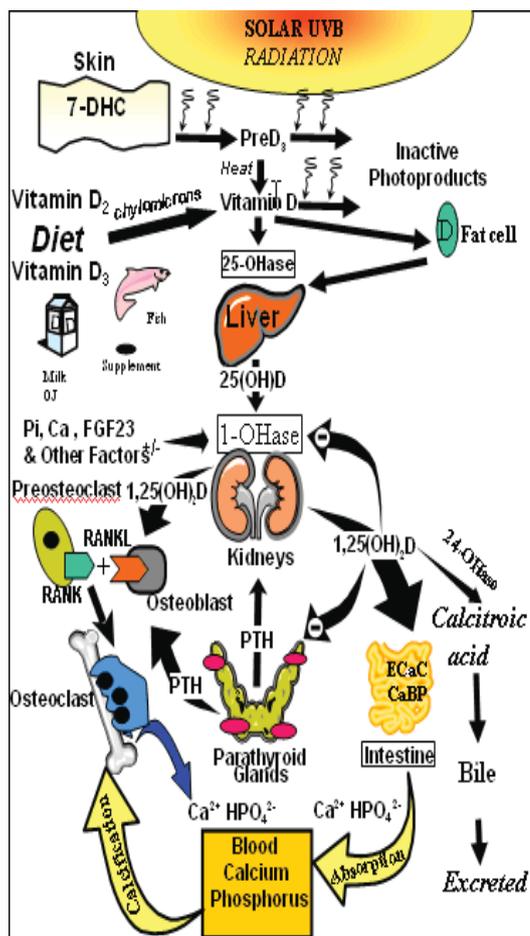


Figure 1 Schematic diagram of the synthesis and metabolism of vitamin D. Reproduced from Wacker & Holick, 2013.

2.1.3 Functions of vitamin D

The physiological role of vitamin D as it is traditionally known is to increase intestinal absorption of dietary calcium and phosphorus. Calcitriol interacts with the VDR in the intestine, promoting the expression of an epithelial calcium channel which increases the efficacy of calcium absorption by around 10% - 40% (Christakos, 2012). Vitamin D is essential for bone growth, density, and remodeling, and without adequate amounts, bone loss will occur (DeLuca, 2004). However, vitamin D is a pleiotropic hormone, influencing various other physiological systems. Since the discovery of the vitamin D receptor in other tissues such as smooth muscle, cardiac muscle, liver, lung, colon, and skin, vitamin D has been implicated in a number of biological processes and diseases (Wacker & Holick, 2013). In particular, there is accumulating evidence that vitamin D is directly involved in skeletal muscle function and strength (Girgis, et al., 2012).

2.1.4 Vitamin D recommendations (desirable levels and intake)

2.1.4.1 Definitions of vitamin D status

The definition of vitamin D deficiency, insufficiency, and sufficiency is currently under debate. The New Zealand Ministry of Health (MOH) defines serum 25(OH)D concentrations under 25 nmol/L as deficient and concentrations over 50 nmol/L as sufficient, as does the Institute of Medicine (IOM) (IOM, 2011; Ministry of Health, 2006). These conclusions are based on what is regarded as sufficient to ensure skeletal health, as there is currently inadequate evidence to support greater serum concentrations of 25(OH)D for other non-skeletal health conditions (IOM, 2011). This stance has been vigorously disputed (Heaney & Holick, 2011). Holick and colleagues (2011) have defined vitamin D deficiency as 25(OH)D concentrations below 50 nmol/L, and severe vitamin D deficiency as concentrations under 25 nmol/L. Vitamin D insufficiency is

defined as 25(OH)D concentrations between 51–74 nmol/L, 25(OH)D concentrations over 75 nmol/L is considered sufficient, and levels between 100–150 nmol/L have been proposed to be within the preferred range (Holick, et al., 2011; Wacker & Holick, 2013).

The rationalisation for serum 25(OH)D concentrations to be maintained around or as a minimum of 100 nmol/L is proposed from several sources. It is asserted that the rate of conversion from 25(OH)D to 1,25(OH)₂D plateaus at 100 nmol/L (Hollis, Wagner, Drezner, & Binkley, 2007). Also, 25(OH)D levels are inversely associated with parathyroid hormone (PTH) levels until 25(OH)D concentrations reach 75-100 nmol/L, where PTH concentrations level off (Holick, 2007). Other important biomarkers continue to improve when serum 25(OH)D concentrations are above 80 nmol/L such as bone mineral density (Bischoff-Ferrari, Dietrich, Orav, & Dawson-Hughes, 2004), intestinal calcium absorption (Devine, Wilson, Dick, & Prince, 2002), and muscle strength (Visser, et al., 2003). The Longitudinal Ageing Study investigated the relationship between low serum 25(OH)D concentrations with hand-grip strength and muscle mass in adults (average age of 74 years) (Visser, et al., 2003). They found that participants with serum 25(OH)D concentrations between 50-74.9 nmol/L were twice as likely to experience loss of grip strength compared to those with concentrations over 75 nmol/L, suggesting that 75 nmol/L may be the threshold for gaining greater muscle strength (Visser, et al., 2003). A more recent controlled trial using athletes has suggested serum 25(OH)D concentrations need to exceed 100 nmol/L for optimal athletic performance (Close et al., 2013).

2.1.4.2 Requirements

There are diverging viewpoints for the requirements of vitamin D, and a consensus is still lacking for a definitive definition of vitamin D deficiency, insufficiency, and sufficiency. The two most authoritative reports on the optimal requirements for vitamin D are conflicting – the IOM and the Endocrine Society (ES). The IOM recommends that any individual aged up to 70 years old should receive 600 IU of vitamin D per day and adults over 70 years should receive 800 IU vitamin D per day (IOM, 2011). In contrast, the ES proposes that 600-1000 IU is required for children over one year of age, and 1500-2000 IU is recommended for adults aged 18 years and over (Holick, et al., 2011). Conversely, the adequate intake (AI) suggested by the Ministry of Health is 200 IU for all individuals aged up to 50 years, increasing to 400 IU for those between 51-70 years old, and 500 IU for those aged over 70 years (Ministry of Health, 2006). This rationale is founded on the quantity of vitamin D required to maintain serum 25(OH)D concentrations at 27.5 nmol/L, in order to prevent deficiency.

The IOM report has been declared by some leading researchers to be too conservative in its recommendations, the basis of which is ostensibly for the prevention of deficiency rather than for achieving optimal health (Glade, 2012; Heaney & Holick, 2011). For most children and adults without adequate sun exposure, most experts agree that at least 1000 IU per day is required, although more is deemed necessary if serum 25(OH)D concentrations are deficient (Bischoff-Ferrari, Giovannucci, Willett, Dietrich, & Dawson-Hughes, 2006; Holick, 2007). It is proposed that the general rule of thumb for increasing serum 25(OH)D concentrations is for every 100 IU of vitamin D ingested or absorbed, serum 25(OH)D concentrations increase by approximately 2.5 nmol/L (Holick, 2011). By this rule, an intake of 600 IU/day would not be enough to produce a

value of even 25 nmol/L, let alone achieve or maintain sufficiency (Heaney & Holick, 2011). Others have indicated that for serum 25(OH)D concentrations to exceed 100 nmol/L, a total vitamin D intake of 4000 IU per day is required (Vieth, 1999).

2.2 Vitamin D deficiency

2.2.1 Factors affecting vitamin D status

Vitamin D status can be compromised by various factors that limit the amount of quality sun exposure to bare skin. For example, distance from the equator (Webb, 2006). At latitudes of over 33° north or south of the equator radiation from the sun becomes less effective because the solar zenith angle of the sun results in diminished UVB wavelengths. During the winter months, the zenith angle of the sun increases early morning and late afternoon resulting in a longer path for the solar UVB photons to travel through the ozone layer, which absorbs them. This explains why humans who live above and below approximately 33° latitude will synthesise little if any vitamin D₃ endogenously during the winter months (Hosseini-Nezhad & Holick, 2013). Atmospheric interference such as the ozone, cloud cover, and suspended particles can decrease UVB radiation. Skin colour is yet another determining factor for vitamin D status. Melanin, a pigment found in the skin which is the primary determinant for skin colour, blocks UVB photons from reaching 7-dehydrocholesterol. Consequently, individuals with darker skin have a comparatively lower vitamin D status than those with lighter skin living in the same regions, particularly when they reside in far northern or southern latitudes (Armas et al., 2007). Other factors inhibiting adequate vitamin D₃ production include regular sunscreen use, clothing, and time spent indoors (Binkley, Ramamurthy, & Krueger, 2012). Advanced age is also associated with a lower vitamin

D status due to a reduced capacity of older skin to synthesise vitamin D₃ under the influence of UVB rays, and also partly due to reduced sunshine exposure caused by decreased mobility, particularly in the frail elderly (Visser, et al., 2003).

2.2.2 Prevalence

Deficiency in Vitamin D is considered to be epidemic; it has been estimated that it currently affects one billion people worldwide (Holick, 2007). In the general population, data from the USA has presented a significant increase in vitamin D insufficiency over the last 30 years, where over 77% of Americans are considered to be vitamin D insufficient (Ginde, Liu, & Camargo Jr, 2009). In New Zealand, data has revealed that 5% of adults 15 years of age and older are deficient (less than 25 nmol/L), and 27% are insufficient (25-50 nmol/L) in the general population (MoH, 2012). There is also a high prevalence of vitamin D insufficiency in New Zealand children (28% below 37.5 nmol/L), where seasonality, gender and ethnicity are strong determinants (Rockell et al., 2005). Even in sunny countries widespread vitamin D deficiency has been established. A study in the Middle East and Africa has demonstrated that vitamin D deficiency affects 30-90% of children and adults (Bassil, Rahme, Hoteit, & Fuleihan, 2013). In Australia, there is evidence from certain sub-populations that up to 8% of adults have serum 25(OH)D concentrations below 28 nmol/L, and around 30% have concentrations under 50 nmol/L (McGrath, Kimlin, Saha, Eyles, & Parisi, 2001; Vasikaran, Sturdy, Musk, & Flicker, 2000).

Athletes are a sub-population who are sensitive to the risks for vitamin D deficiency (Willis, Peterson, & Larson-Meyer, 2008); across the world an increasing number of studies have identified sub-optimal vitamin D status in various athletic populations. A review of the literature has located 12 studies to date that report on the vitamin D status

of athletic populations, nine of which were published after 2010. These are summarised in Table 2. Athletes who train and compete indoors such as dancers, gymnasts, basketball players, martial artists, and swimmers have been proposed to be at greater risk of suboptimal vitamin D status. For example, a recent observational study compared the vitamin D status of indoor versus outdoor athletes, and discovered that indoor athletes had significantly ($P < 0.05$) lower serum 25(OH)D concentrations (90 ± 28 nmol/L) compared to athletes who trained outside (131 ± 35 nmol/L) (Peeling, Fulton, Binnie, & Goodman, 2013).

Research has now identified that athletes such as dancers and gymnasts may be at increased risk for low serum 25(OH)D concentrations. Wolman and colleagues (2013) found within a small cohort of professional ballet dancers in the UK that all were either insufficient or deficient (<75 nmol/L) during the winter. During the summer months, 84% of the group remained vitamin D insufficient or deficient (Wolman, et al., 2013). Male adolescent ballet dancers in Australia have also been reported to have low serum 25(OH)D concentrations during the winter months (Ducher, et al., 2011). This pilot study found that 12.5% of the participants exhibited vitamin D deficiency, 44% with insufficiency, and 44% with sufficient levels. However the authors' defined sufficiency as serum 25(OH)D concentrations over 50 nmol/L, therefore if one was to follow the guideline for sufficiency levels to be above 75 nmol/L, an even higher percentage in this study would be insufficient. Another study in Australia reported insufficient (<75 nmol/L) serum 25(OH)D concentrations in 15 out of 18 elite female gymnasts, six of those having concentrations under 50 nmol/L (Lovell, 2008).

Athletes who train and compete outdoors are also not exempt from sub-optimal vitamin D status. Often these athletes will train in the early hours of the morning or during the

evening when there is little opportunity to absorb sufficient vitamin D from the sunlight. Athletes involved in sports such as football (Morton et al., 2012), athletics (Hamilton, Grantham, Racinais, & Chalabi, 2010), and road cycling (Maimoun et al., 2006) to name a few, have been found to be at risk for insufficiency, particularly during winter and spring (Halliday, et al., 2011; Lombardi, Colombini, Freschi, Tavana, & Banfi, 2011), and particularly if they are dark-skinned (Hamilton, et al., 2010).

Table 2 Vitamin D status in athletic populations

Reference	n	Ethnicity	Subject group	Location	Season	25(OH)D concentration (nmol/L)*
Wolman et al., 2013	19 (6M/13F)	Caucasian	Elite ballet dancers from an international touring company	52.3°N UK	Winter Summer	37 59.5
Peeling, 2013	72 (43M/29F)	Caucasian & Asian	Elite-level athletes from a range of sporting programs	31°S Western Australia	Summer	111 ± 37
Morton, 2012	20M	Not stated	Premier league soccer players	53°N UK	Winter	51.0 [22-86]
Garcia, 2011	21M	Not stated	Elite basketball players	40.7°N Spain	Winter	47.8 ± 21.8
Ducher, 2011	16M	Not stated	Ballet dancers from the Australian Ballet School	37°S Australia	Winter	50.5 [20.8-94.3]
Lombardi et al., 2011	14F	Caucasian	Alpine Skiers	46°N- 50°N Italy	Winter Spring	53.5 ± 9.6 40.3 ± 10.2
Halliday et al., 2011	41 (18M/23F)	Not stated	College athletes in football, running, dance, wrestling, swimming, basketball	41.3°N WY-USA	Autumn Winter Spring	122.5 ± 41.5 104.8 ± 14 76.3 ± 23.5
Constantini, 2010	98 (52M/46F)	Israeli	Dancers, basketball players, Tae Kwon Do fighters	31.8°N Israel	Not stated	63.1 ± 20.7
Hamilton, 2010	449M	Middle Eastern	Amateur to professional athletes in football, athletics, handball, shooting, squash, cycling, martial arts, body building	35.4°N Qatar	Not stated	27.5 [<17.5-70]
Lovell, 2008	18F	Caucasian	Elite gymnasts from the Australian Institute of Sport	35.3°S Australia	Autumn	56 [29-84]
Maimoun et al., 2006	7M	Not stated	Road cyclists	43.6°N France	Spring- Summer	81.3 ± 16
Lehtonen-Veromaa et al., 1999	66F 65F	Caucasian	Gymnasts Runners	60.4°N Finland	Winter Summer	34 ± 14 63 ± 15

*25(OH)D concentration is reported as mean ± SD or as mean [range]; M=male, F=female

2.3 Effect of vitamin D on skeletal muscle

2.3.1 Skeletal muscle contraction

Skeletal muscle is made up of myocytes or “muscle fibres” which are in turn made up of thousands of myofibrils (Figure 2). Myofibrils are composed of small units called sarcomeres made up of actin and myosin filaments, and the sarcomere is the fundamental functional unit of the muscle fibre (McArdle, Katch, & Katch, 2010). Actin and myosin filaments overlap, allowing for contraction to occur. Excitation-contraction coupling is the physiological mechanism that enables skeletal muscle function, and is initiated by an action potential from the nervous system. The action potential stimulates calcium (Ca^{2+}) release from the sarcoplasmic reticulum (SR). The release of Ca^{2+} from the SR is vital to muscle contraction. Ca^{2+} diffuses into the actin-myosin complex, binding to troponin. This causes a conformational change of the actin filament exposing the myosin binding site, and therefore allowing actin and myosin to interact (Exeter & Connell, 2010).

2.3.2 Myopathy

The relationship between vitamin D and muscle function was first observed in children with rickets and adults with osteomalacia who presented with concurrent myopathy. It was initially believed that this muscle weakness was secondary to osteomalacia or disuse, however this has been challenged (Glerup et al., 2000). These authors examined the effect of vitamin D supplementation in a subject group with osteomalacia (mean age 63.1 ± 5.3 years, mean serum $25(\text{OH})\text{D}$ 7.0 ± 0.7 nmol/L). Muscle strength was assessed with an isokinetic dynamometer before and after treatment with alfacalcidol (an analogue of vitamin D) and vitamin D_2 for three months. They found that muscle

strength increased significantly in all of the muscle groups assessed ($P < 0.03$), with the most significant increases in the knee extensors and flexors (Glerup, et al., 2000).

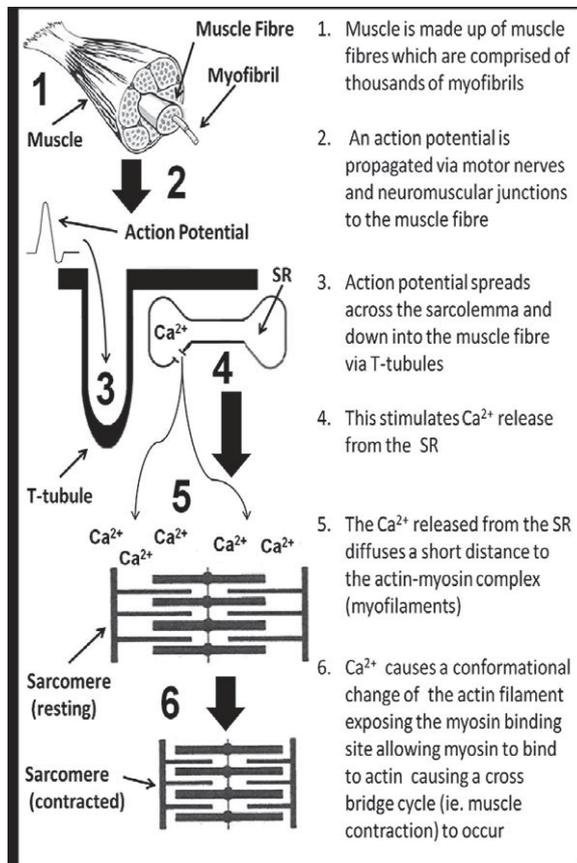


Figure 2 Overview of skeletal muscle contraction, reproduced from Hazell et al., 2012

2.3.3 Morphology

Type II (fast twitch) muscle fibres are predominantly used in anaerobic and power activity (McArdle, et al., 2010). Vitamin D deficiency has been associated with selective atrophy of type II fibres (Sato, Iwamoto, Kanoko, & Satoh, 2005). Sato and colleagues (2005) randomised 96 vitamin D-deficient elderly women to take either 1000 IU vitamin D₂ daily for two years or a placebo. The treated group experienced an increase of 96.5% in the diameter of type II muscle fibres of the *m. vastus lateralis*,

whereas the untreated group experienced a decrease (22.5%) in diameter. The diameter of type II muscle fibres was also found to be correlated with serum 25(OH)D concentrations.

Additionally, an earlier study found that treatment in elderly women with alphacalcidol increased the relative number and size of type II muscle fibres within three months of treatment (Soerensen et al., 1979). Furthermore, a significant improvement in muscle strength and an increase of type II muscle fibres of the quadriceps was established after vitamin D supplementation in a group of 12 osteomalacic patients (Young, 1981).

A recent observational study has supported these findings with a positive association of lean body mass with serum 25(OH)D concentrations (Hamilton, et al., 2010).

2.3.4 The vitamin D receptor

The active vitamin D metabolite, 1,25-(OH)₂D, is the ligand for a transcription factor and intracellular receptor called the vitamin D receptor (VDR). The VDR is expressed in virtually all tissues of the body, including skeletal muscle cells. It co-factors with another transcription factor, retinoid X receptor (RXR), to activate vitamin D response elements in the promoter regions of target genes (Haussler, et al., 2013).

The VDR was first identified in skeletal muscle cells in 1985 (Simpson, Thomas, & Arnold, 1985). Cultured rat (H-9c2) and mouse (G-8) myoblast cells were shown to possess receptors that were characterised as having a high affinity (<0.1 nM) for 1,25-(OH)₂D. It was first isolated from human skeletal muscle by Bischoff and colleagues (2001). A number of studies since have supported the presence of the VDR on both the nucleus and plasma membrane of skeletal muscle cells *in vitro* (Buitrago & Boland, 2010; Garcia, King, Ferrini, Norris, & Artaza, 2011), and in chick skeletal muscle cells

(Boland et al., 2002). However, a study recently challenged the findings of Bischoff and colleagues, claiming the monoclonal antibody 9A7 they used to detect VDR in human skeletal muscle binds not only VDR but other proteins as well (Wang & DeLuca, 2011). Research from Wang and DeLuca (2011) investigated multiple immunoassays to detect VDR in mouse muscle tissues. The results demonstrated that the VDR was not present in muscle tissue, suggesting that the only effect of $1,25(\text{OH})_2\text{D}$ on muscle function is most likely indirect. Conversely, a contemporaneous study went on to prove that the same antibodies used by Wang and DeLuca (i.e. the D-6 Santa Cruz monoclonal antibody) did in fact detect the VDR in muscle biopsy tissue from older female subjects (Ceglia et al., 2010).

Despite the controversy, VDR knockout (VDRKO) mice models have provided persuasive evidence of the existence of the VDR in skeletal muscle. Research has revealed that VDRKO mice had 20% smaller muscle fibre sizes in the quadriceps muscle group than wild-type mice (Endo et al., 2003). The VDRKO also displayed increased expression of myogenic transcription factors myf5, E2A and myogenin compared with normal mice as well as inappropriate expression of embryonic and neonatal type myosin heavy chain (Endo, et al., 2003).

2.3.4.1 Genomic actions

The genomic pathway is slow to act, but long-lasting. $1,25(\text{OH})_2\text{D}$ binds to a vitamin D binding protein (DBP) in the plasma membrane. It is then transported by way of an intracellular binding protein (IBP) to the nucleus where it binds to the nuclear VDR, which is heterodimerised with the RXR. This complex binds to vitamin D response elements (VDREs) in the promoter region of $1,25(\text{OH})_2\text{D}$ responsive genes, ultimately resulting in modified gene expression (Hamilton, 2010). This increases the synthesis of

proteins required for muscle function, such as calmodulin, calbindin, and insulin-like growth factor binding protein 3 (IGFBP-3). Calmodulin and calbindin are Ca^{2+} binding proteins, which help regulate Ca^{2+} uptake by enhancing activity of calcium pumps in the SR and sarcolemma, thereby affecting the acquisition of intracellular calcium (Ceglia, 2009). In vitamin D deficient rats, Ca^{2+} uptake into the SR is impaired, as well as the amount of Ca^{2+} that is released in response to an action potential (Hazell, DeGuire, & Weiler, 2012). IGFBP-3 binds to insulin-like growth factor 1 (IGF-1), which itself induces skeletal muscle hypertrophy and differentiation (Duan, Ren, & Gao, 2010).

2.3.4.2 Non-genomic actions

Cellular $1,25(\text{OH})_2\text{D}$ also elicits rapid (within seconds to minutes) non-genomic responses mediated by the activation of a plasma membrane-bound VDR, and via activation of several interacting intercellular pathways (Lanteri, Lombardi, Colombini, & Banfi, 2013). For instance, $1,25(\text{OH})_2\text{D}$ activates phospholipase C (PLC) and phosphoinositide 3-kinase (PI3-K), generating 1,4,5-triphosphate (IP3) and diacylglycerol (DAG), together with an increase in cyclic AMP (cAMP) levels (De Boland & Boland, 1994). This leads to the activation of protein kinase A (PKA) and C (PKC), resulting in the release of calcium from intracellular stores, and activation of voltage-dependent calcium channels (Ceglia, 2009). The coupling of DAG and PKC stimulates an influx of extracellular Ca^{2+} via store-operated calcium channels. Increased concentrations of intracellular Ca^{2+} activates calmodulin and calmodulin-dependent protein kinase II (CDPKII), which further activates PKC (Hazell, et al., 2012).

2.3.5 Interactions with other hormones

Vitamin D interacts with the sex hormones testosterone and oestrogen. Testosterone and oestrogen are both associated with changes in lean mass and vitamin D concentrations,

and the measurement of these hormones should be taken into consideration when analysing the effect of vitamin D on muscle function. For example, serum testosterone increased in healthy males after vitamin D supplementation in a RCT (Pilz et al., 2011). In this study, participants were randomised to either 1000 IU per day of vitamin D or a placebo for one year. Serum 25(OH)D concentrations significantly increased in the supplemented group, as did testosterone ($P < 0.001$). Another study reported a significant effect of testosterone supplementation on lean body mass and strength (leg press and chest press) in healthy young males (Bhasin et al., 2012). Therefore, vitamin D supplementation may increase muscle mass and strength indirectly by increasing testosterone levels in males.

The relationship between oestrogen and muscle strength in females is not well understood. It has been suggested that oestrogen does benefit muscle strength as demonstrated in both postmenopausal women and oestrogen-deficient rodents. For example, in mice with their ovaries removed and the subsequent loss of oestradiol, the force-generating capacity of the hind-limb muscles decreased, but strength was returned after oestradiol replacement (Moran, Nelson, Landisch, Warren, & Lowe, 2007). The mechanism underlying oestrogen's effect on muscle strength is thought to be from the action of nuclear oestrogen receptors that cause an improvement in the function of myosin. (Lowe, Baltgalvis, & Greising, 2010). There is evidence to suggest that oestrogen may up regulate CYP27B1 activity, increasing the circulating level of 1,25(OH)₂D (Pike, Spanos, Colston, MacIntyre, & Haussler, 1978).

2.4 Evidence for the effect of vitamin D on muscle strength

The relationship between muscle function and vitamin D has been explored in human and animal studies, often with conflicting results. In the last decade, the bulk of research on the effect of vitamin D on muscle strength, postural stability and physical function has been performed on older individuals, as the ageing process results in sarcopenia – muscle atrophy followed by loss of strength and power - as well as diminished vitamin D status. Due to an increasing prevalence of vitamin D deficient younger adults and adolescents, the body of research in this area is growing outside the confines of the elderly population. Despite the significance of optimal muscle function in sport performance and the high rates of vitamin D deficiency seen in some athletes, the relationship between vitamin D and athletic performance is yet to be fully elucidated (Angeline, et al., 2013). Unfortunately, the studies are often difficult to compare, as they employ a wide range of study designs, study intervals, protocols, age groups, supplementation methods (dose and combination with calcium), ethnicities, muscle strength and function tests, and contexts (e.g. community dwelling versus institutionalised, or vitamin D deficient versus sufficient).

2.4.1 Association of vitamin D and muscle strength

2.4.1.1 Elderly

From a small study in the early 1980s that improved quadriceps muscle function in older osteomalacic women with vitamin D supplementation (Young, 1981), many cross-sectional studies have since demonstrated a positive relationship between serum 25(OH)D concentrations and used multiple measures of muscle function such as handgrip strength, proximal leg strength, six-minute walk distance, and sit to stand tests

in older populations (Bischoff-Ferrari, Dietrich, Orav, Hu, et al., 2004; Houston et al., 2007; Mowe, Haug, & Bohmer, 1999; Wicherts et al., 2007).

Two of the more recent studies demonstrate conflicting outcomes. Evaluation of serum 25(OH)D concentrations and muscle function strength was conducted in 54 post-menopausal females, measuring proximal leg muscle strength with a manual dynamometer, and function with walking-speed test, standing balance, and sit-to-stand tests (Mastaglia et al., 2011). Serum 25(OH)D concentrations over 50 nmol/L were found to be associated with better muscle function and strength. Those with 25(OH)D levels greater than 50 nmol/L scored higher on muscle function tests ($P = 0.003$), and had stronger knee extensor ($P = 0.03$) and hip abductor ($P = 0.04$) muscles.

In contrast a recent cross-sectional and longitudinal study conducted over four years in China measured grip strength, six-minute walking speed, step length in a six-minute walk, and time to complete five chair stands in community dwelling older males (Chan et al., 2012). Serum 25(OH)D concentrations of over 50 nmol/L were found in 94.1% of participants. After adjustment for potential confounding factors, serum 25(OH)D concentrations were not associated with baseline or four year change in physical performance measures.

Unfortunately, one of the weaknesses of cross-sectional studies is that they are unable to demonstrate a causal effect. In the elderly who have reduced mobility, decreased daily activity in combination with reduced exposure to sunlight hours can simultaneously lead to decreased muscle strength and power and decreased serum 25(OH)D concentrations independently of each other.

2.4.1.2 Younger Adults and Adolescents

A cross-sectional relationship between low vitamin D concentration and poor muscle strength in adolescents and younger adults has been suggested in some (Grimaldi, et al., 2012; Valtueña et al., 2013; von Hurst, Conlon, & Foskett, 2012; Ward, et al., 2009) but not all (Ceglia, Chiu, Harris, & Araujo, 2011) studies. These are summarised in Table 3.

In a cross-sectional study of 99 females aged between 12-14 years, a positive association between serum 25(OH)D concentrations and muscle power, velocity and jump height assessed by jumping mechanography was reported (Ward, et al., 2009). At a median serum 25(OH)D concentration of 21.3 nmol/L, there was a positive relationship between 25(OH)D and jump velocity ($P = 0.002$), jump height ($P = 0.005$), power ($P = 0.003$), and force ($P = 0.05$). In addition, PTH had an inverse relationship with jump velocity ($P = 0.04$). It was suggested that this may have been due to increased muscle mass in those with higher performance scores, although body mass was not measured. The potential influence of physical activity was also not assessed in this study.

Another study examined the relationship between serum 25(OH)D concentrations and muscle strength in a cohort of 419 healthy males and females (Grimaldi, et al., 2012). Isometric and isokinetic strength of the arms and legs was measured with a handgrip dynamometer and a Biodex System 3 dynamometer, respectively. Serum 25(OH)D concentrations were positively associated with all strength variables except handgrip when controlling for age and gender ($P < 0.05$), however when controlling for variables such as physical activity, BMI, and cardio respiratory fitness, serum 25(OH)D concentrations were no longer related to isokinetic leg strength (Grimaldi, et al., 2012).

A comprehensive cross-sectional study recently published findings on a relationship between vitamin D status and parameters of physical performance in European adolescents (Valtueña, et al., 2013). In males there was a significant positive association between maximal oxygen consumption (VO_{2max}) and serum 25(OH)D concentrations ($P < 0.05$), and in females, handgrip strength was positively associated with serum 25(OH)D concentrations ($P < 0.01$). However, those adolescents with low BMI and high fitness levels also presented with significantly higher serum 25(OH)D concentrations. Again, it is problematic to assume a causal relationship where vitamin D status precedes and predicts muscle strength, when those who have higher serum 25(OH)D concentrations might do so due to their higher fitness levels under greater sun exposure. Conversely, no significant association ($P > 0.05$) between serum 25(OH)D concentration and hand grip strength, functional tests such as chair stand and walking speed, or lean body mass was reported in a large observational study in the USA of adult males, 42.2% of whom had serum 25(OH)D concentrations of under 75 nmol/L, and 19.1% under 50 nmol/L (Ceglia, et al., 2011).

2.4.1.3 Athletes

Despite the scarcity of contemporaneous research examining the association between serum 25(OH)D concentrations and muscle strength or power in athletes, it is no novel concept that vitamin D is considered beneficial to athletic performance in sport. Original research relating to vitamin D and athletic performance dates back to the early twentieth century. Russian and German researchers investigated the positive effects of UV light irradiation on aspects of athletic performance, such as improved sprint times, cardiovascular fitness on cycle ergometers and muscle strength (Cannell, et al., 2009). In the 1960's, researchers in the USA determined that a single large dose of UV

radiation tended to improve strength, speed, and endurance performance of college-aged females (Cannell, et al., 2009). Despite the positive results from UV irradiation studies, they are largely descriptive, and do not determine causation; that can only be possible with randomised controlled trials.

In recent years, only one observational study has emerged regarding the association between serum 25(OH)D concentrations and tests of muscle strength. A cross-sectional clinical trial in Qatar assessed lower limb isokinetic performance and its relationship between serum 25(OH)D concentrations in professional football players (Hamilton, et al., 2013). Eighty-four percent had serum 25(OH)D concentrations under 75 nmol/L, and 12% were classified as severely deficient (<25 nmol/L). However, no association was found between their chosen strength measurements and serum 25(OH)D concentrations when adjusting for total body mass, although total body mass and lean mass were significantly higher in those players who had 25(OH)D concentrations greater than 50 nmol/L.

Table 3 A summary of cross-sectional studies on the association of vitamin D and muscle strength in athletic and healthy non-athletic populations

Reference	<i>n</i>	Gender	Age (yrs)	Study Population	Location & Latitude	Season	25(OH)D Status (nmol/L)	Findings
Ward et al., 2009	99	F	12-14	Post-menarchal females	Manchester, UK 53°N	Not stated	21.3 [2.5-88.5]	Positive relationship between 25(OH)D and two-legged jump velocity, power, & height
Von Hurst et al., 2012	137	F	19-29	Young NZ females	Auckland, NZ 36.8°S	Late winter	54±28	Hand-grip strength positively associated with 25(OH)D levels
Valtuena et al., 2013 (HELENA)	1006	M,F	12.5 - 17.5	European adolescents (470 males)	Europe 59-35°N	Winter, Spring, Autumn	Males: 57.4±22.7 Females: 59.9±23.4	VO _{2max} positively associated w. 25(OH)D in males; handgrip strength positively associated w. 25(OH)D in females
Grimaldi et al., 2013	419	M,F	20-76	Healthy adults enrolled in the STOMP study	CT & MA, USA 40-42°N	All	84	25(OH)D levels positively associated w. isometric and isokinetic leg strength when controlling for age and gender (P <0.05). After controlling for physical activity, BMI, and cardio respiratory fitness, 25(OH)D levels were no longer related to isokinetic leg strength
Ceglia et al., 2011	1219	M	30-79	Healthy adults enrolled in the BACH study	MA, USA 42.3°N	Not stated	78 [51.9,106.7]	No association between serum 25(OH)D concentration and lean body mass, muscle strength or physical function
Hamilton et al., 2013	342	M	24.4±8.3	Professional footballers	Qatar 35.4°N	Summer	84% under 75 12% under 25	No association between 25(OH)D levels and lower limb isokinetic peak torque. Body mass and lean mass were significantly higher in those with 25(OH)D levels higher than 50nmol/L

2.4.2 Intervention trials of vitamin D and muscle strength

2.4.2.1 Elderly

There are many reports of randomised controlled intervention studies on the effect of vitamin D supplementation alone or in combination with calcium on various measures of muscle function in the elderly but the evidence has often found to be contradictory. A meta-analysis of 17 randomised controlled trials involving 5,072 individuals concluded that there was no significant effect of vitamin D supplementation on grip strength or proximal lower limb strength in adults with serum 25(OH)D concentrations over 25 nmol/L (Stockton, Mengersen, Paratz, Kandiah, & Bennell, 2011).

2.4.2.2 Younger adults and adolescents

Randomised controlled studies of healthy adolescents or younger adults are summarised in Table 4. In 69 postmenarchal adolescent females with poor vitamin D status, those who were randomised to receive 150,000 IU once every four months for one year demonstrated a significant improvement in movement efficiency (a composite of jump height and velocity), measured by jumping mechanography, compared to baseline ($P = 0.02$). However, jumping velocity and height measured separately were not significantly improved (Ward et al., 2010). Moreover, physical activity was not reported for this group, despite its likely effect on the measures of strength.

Another cohort of 170 adolescent girls with low vitamin D status (average 34 nmol/L) were randomised to receive 1400 IU or 14,000 IU per week or a placebo for one year (El-Hajj Fuleihan et al., 2006). Although the primary outcome for this study was to investigate for improvement in bone markers, handgrip strength was also measured. They found that although lean mass increased significantly in both treatment groups ($P < 0.05$), handgrip strength did not improve.

Thirty healthy male and female subjects (aged between 25-35 years) with an average baseline 25(OH)D concentration of 80 nmol/L, were randomised to receive 200 IU or 4000 IU of vitamin D per day, or a placebo for 28 days during the winter (Barker et al., 2012). Single-leg strength tests were performed with a horizontal ply-press force plate. Serum 25(OH)D concentrations increased in both treatment groups ($P < 0.05$), and although serum 25(OH)D correlated with muscle strength ($P < 0.05$), neither dose improved leg muscle strength in this study. The lack of a significant increase in strength may be due to the short length of the intervention and high serum 25(OH)D concentrations of the group at baseline (63.3% of the group over 75 nmol/L).

The effect of vitamin D together with calcium supplementation on handgrip strength, isokinetic strength of the *m. triceps surae*, and pinch-grip strength was measured in 40 healthy males and females (average age 31.5 years) (Gupta et al., 2010). They were randomised to either a placebo or six months of vitamin D (60,000 IU/week for two months, then 60,000 IU/fortnight for four months) and calcium supplementation (1000 mg/day for six months). During the trial the treatment group significantly improved handgrip strength ($P = 0.001$), *m. triceps surae* strength ($P = 0.04$), and pinch-grip strength ($P = 0.06$) compared to the placebo group. The findings in this study are contrary to a more recent study conducted by the same research team, who measured handgrip, pinch-grip, and distance walked in six minutes (Goswami et al., 2012). This study adopted a two by two factorial design: participants were randomised to a double placebo, calcium-placebo, vitamin D-placebo or vitamin D-calcium for six months. The primary difference between this and the preliminary study was that the participants in the latter study were all female (21.7 ± 4.4 years) and the sample size was considerably larger ($n = 173$). Although this group provided a more homogeneous sample than the

previous study (same gender, and no significant differences in age, BMI, and activity), and serum 25(OH)D concentrations were significantly improved after six months in the supplemented group ($P < 0.001$), no significant change was found in any of the strength or function measurements. This may have been due to several reasons. The participants who were randomised into the vitamin D groups increased their serum 25(OH)D concentrations to an average of 74.6 nmol/L (vitamin D group) and 67.4 nmol/L (vitamin D-calcium group), which may not have been a significant enough increase to begin noticing measurable improvements in muscular strength, which, as previously discussed could start at concentrations of at least 100 nmol/L (Close, et al., 2013). Also, there may be a gender-specific effect of vitamin D supplementation – the previous study included males and females. After the researcher team had sex-stratified the analysis, males still demonstrated significant improvements in handgrip strength ($P = 0.001$), but the increase was no longer significant for females ($P = 0.071$).

Table 4 A summary of RCTs on the effect of vitamin D supplementation on muscle strength in healthy non-athletic populations

Reference	n	Location	Study Population	Study duration	Dose	Strength assessors	Outcomes
Barker et al., 2012	30 (15M/15F)	Utah, USA	Healthy adults, 25-35 yrs	28 days	200 IU D3/day; 4000 IU/day; or placebo	Single-leg peak isometric force	Supplementation did not improve muscle strength
Goswami et al., 2012	153 F	New Delhi	153 students, 21.7±4.4yr. Grouped in: Ca & vit D (n=44), vit D & placebo (n=43), Ca & placebo (n=43), double placebo (n=43)	6 months	60,000IU D3/wk for 8 wks, followed by 60,000IU/fortnight for 4 months. 1000mg Ca/day for 6 months; or placebo	Hand grip, pinch grip strength, six min walk test	No significant difference between the four groups
Wischerts et al., 2011	211 (53M/158F)	The Netherlands	211 non-western immigrants, 41.3±11.6 yrs	3 months	Sunlight group: at least ½ hour/day; 800IU D3/day; 100,000IU D3/month. No placebo group	Hand grip, chair-stand test	No significant differences between groups. High drop-out rate in sunlight group
Gupta et al., 2010	40 (26 M/16 F)	New Delhi	Vitamin D deficient otherwise healthy adults, 31.5±5 yrs	6 months	60,000IU D3/wk for 8 wks, followed by 60,000IU/fortnight for 4 months. 1000mg Ca/day for 6 months; or placebo	Hand grip, pinch grip strength, gastro-soleus dynamometry, six min walk test	Treatment group scored significantly greater in all strength tests
Ward et al., 2010	69 F	Manchester, UK	Post-menarchal girls 12-14 yrs	1 year	150,000IU once every 4 months; or placebo	Handgrip; Jump height, power, velocity	Movement efficiency increased in the treatment group. No change in jump performance

2.4.2.3 Athletes

There are few intervention trials that examine the effect of vitamin D supplementation on muscle function in athletes; the only three controlled trials published to date have been within the last year. Prior to this, publications have been limited to enquiries into the vitamin D status of athletes and speculative reviews.

In the UK, a research team performed a randomised controlled trial on vitamin D supplementation and physical performance in a small ($n = 10$) group of professional male soccer players (Close et al., 2012). This study compared the effect of 5000 IU/day of vitamin D₃ supplementation for eight weeks with a placebo-matched group. Exercise tests included the 1-RM bench press and back squat, vertical jump height, and 10 and 30 metre sprint tests. A significant increase was observed in 10 metre sprint times ($P = 0.008$) and vertical jump ($P = 0.008$) in the vitamin D group whereas the placebo group produced no significant change over time. These results are at variance with a subsequent study conducted by the same authors (Close, et al., 2013). Club-level soccer and rugby players ($n = 25$) were block-randomised into one of three groups that received either 20,000 IU, or 40,000 IU of vitamin D₃ per week, or a placebo for 12 weeks. Muscle strength and power was measured with the 1-RM bench press and 1-RM leg press, and vertical jump height. They found that supplementation with both doses of vitamin D enhanced serum 25(OH)D concentrations, but this time neither dose significantly improved their chosen measures of physical performance ($P > 0.05$) (Close, et al., 2013). The difference in the performance outcomes may be due to the final serum 25(OH)D concentrations – the authors speculated that the threshold for optimal neuromuscular function might begin at serum 25(OH)D concentrations of 100 nmol/L, a level that, despite the high dosages, the participants did not reach.

A third controlled trial investigated the effect of vitamin D supplementation on muscle strength in elite ballet dancers ($n = 24$ males and females) (Wyon, Koutedakis, Wolman, Nevill, & Allen, 2013). The research team had previously measured the participants serum 25(OH)D concentrations a year prior to ensure that all who participated in the study were either vitamin D insufficient or deficient (Wolman, et al., 2013). In order to form a control group the researchers were ethically obliged to ask their subjects to volunteer, whereupon at the conclusion of the intervention period the control group volunteers would receive appropriate vitamin D supplementation. The intervention group received 2000 IU per day over a period of four months. All participants were measured for their maximal isometric quadriceps strength and vertical jump height at baseline and again at four months. Significant increases were observed in the intervention group for isometric strength (18.7%, $P < 0.01$) and vertical jump height (7.1%, $P < 0.01$). It is possible that the participant numbers for each group (control = 7, intervention = 17) might have influenced the results in this case. The change in serum 25(OH)D concentrations in the intervention group was not known as a blood analysis was not included in this four month interventional study.

The common limitation in these studies was the small sample size. This was explained for differing reasons. Close and colleagues noted that the rise of personal vitamin D supplementation in athletes prevented them from recruiting more subjects, while Wyon and colleagues explained that in order to control for training load and improve homogeneity, recruiting more participants was not possible.

2.4.2 Postural control

There is evidence to suggest that vitamin D deficiency is associated with impaired postural control. Such lines of evidence come from several studies conducted in the elderly in relation to falls, and VDRKO mice models. To date, there is no information on what effect if any vitamin D deficiency may have on the postural control of younger adults or trained athletes.

2.4.2.1 Intervention trials in the elderly

There is consistent evidence of a protective effect of vitamin D for falls in older people. A prolific researcher on the topic, Bischoff-Ferrari and colleagues first composed an RCT on the effect of vitamin D supplementation on muscle function and falls (Bischoff et al., 2003). In a cohort of 122 females (average age of 85.3 years), supplementation of 800 IU/day in combination with 1200 mg of calcium for three months reduced the risk of falling by 49%. In another randomised, controlled multi-dose study, supplementation of 800 IU/day lowered the incidence of falls by 72% compared to those taking a placebo over five months (Broe et al., 2007). It is proposed that the increase in type II muscle fibres after vitamin D supplementation may be a mechanism for improvements seen in such studies, as type II fibres are the first to be recruited to prevent falling (Ceglia, 2009).

Postural instability was also studied in a group of adults aged 65 years and older, specifically measuring limits of stability, centre of pressure, and visio-vestibular stimulation (Boersma, et al., 2012). Those individuals with serum 25(OH)D concentrations below 30 nmol/L performed significantly more poorly in all tests. Additionally, a relationship between vitamin D deficiency and low postural control was

identified even when muscle function tests such as grip strength remained unaffected, suggesting a role for vitamin D in neuromuscular control (Boersma, et al., 2012).

Vitamin D was observed in another study to be a significant independent variable for postural stability measured by postural sway (Dhesi et al., 2002). It was determined that individuals with serum 25(OH)D concentrations below 30 nmol/L exhibited greater postural sway than those with higher concentrations, suggesting that deficiency needs to be reasonably pronounced to affect isometric postural balance.

A recent meta-analysis of eight randomised controlled trials of elderly people revealed that vitamin D supplementation of 700–1000 IU per day or a serum 25(OH)D concentration of over 60 nmol/L reduced the risk of falls by 19% and 23% respectively (Bischoff-Ferrari, et al., 2009). Doses of supplemental vitamin D of less than 700 IU or serum 25(OH)D concentrations of less than 60 nmol/L did not reduce the risk of falling among older individuals (Bischoff-Ferrari, 2012). To date, the association between falls and vitamin D status remains as one of the strongest relationships between serum 25(OH)D concentrations and muscular function.

2.4.2.2 Mice models

It is proposed that vitamin D may have an effect on the vestibular system. VDRKO mice exhibited several vestibular dysfunctions such as a shorter latency to fall from a rotating rod, smaller fall angle in the tilting box test, and aberrant poor swimming (Minasyan et al., 2009). Another study ruled out the vestibular system as a mechanism, suggesting that the poor motor performance manifested in VDR knockout mice might in fact be due to dysregulation of calcium homeostasis (Kalueff, Lou, Laaksi, & Tuohimaa, 2004).

2.4.2.3 Athletes

It is currently unknown if vitamin D supplementation has an effect on postural control in well-trained athletes. In dancers and gymnasts, isometric strength and balance is an integral aspect of their performance. Dancing in particular has been described as an intermittent exercise form, with transitory movements at a range of intensities requiring explosive bursts of power and precision (Twitchett, Koutedakis, & Wyon, 2009). Such activity would rely on optimal activation of type II muscle fibres, which have been demonstrated to improve in size and number with vitamin D supplementation (Sato, et al., 2005). The literature suggests that improvements in postural balance are manifest only in subjects who are initially vitamin D deficient; thus, given that at least some of these indoor athletes could present with vitamin D deficiency as evidenced from previous studies (Ducher, et al., 2011; Lehtonen-Veromaa et al., 1999; Lovell, 2008; Wolman, et al., 2013), future research in this area may be relevant.

2.5 Summary

It has been well demonstrated that vitamin D has an effect on skeletal muscle function. Although the precise mechanisms are still being elucidated, research in rodent models and functional studies in vitamin D deficient humans have supported a relationship between serum 25(OH)D and parameters of muscular strength, power, and postural control. The majority of controlled trials however provided conflicting results. This is likely due to the heterogeneity of study protocols, doses, measures of muscle function, study length, definitions of vitamin D sufficiency, and baseline serum 25(OH)D concentrations. It may be viable that vitamin D supplementation could improve muscle

strength and power in vitamin D depleted athletes, however research in this area is lacking. Future RCT's with sufficient power are necessary to establish whether such a causal link exists.

CHAPTER 3 - METHODOLOGY

3.1 Study Design

This study was a randomised placebo-controlled double-blind intervention trial. All participants visited the Vitamin D Research Centre at Massey University, Albany on two occasions six months apart. During the first visit, all participants completed demographic and medical history forms. At both visits, they performed a selection of muscle strength and power tests and provided blood samples. The participants received a six month supply of a vitamin D supplement or placebo and continued with their usual training schedule.

3.2 Research Ethics

The study was approved by the Health and Disability Human Ethics Committee, Northern Y (Application NTY/12/02/013), clinical trial registration number ACTRN1261000031864. All participants were screened via a general questionnaire that ensured they were physically healthy and were able to take part in the study. All participants were informed of the study requirements, benefits and risks (Appendix 1), before providing written consent (Appendix 2). A parental consent form was completed by a guardian for those participants who were aged under 16 years (Appendix 3). Participation in this study was restricted to athletes who were involved in regular training of their chosen sport (at least five hours per week) who were also not at an increased risk of discomfort or injury during the prescribed exercise tests. All

participants were assigned a unique study identification number, which was used on all questionnaires and data forms. These forms are stored in a locked filing cabinet in a locked office within the Human Nutrition Research Unit, which is a restricted access building. The electronic data is stored on computers and servers, which are protected by passwords within the same facility.

3.3 Intervention

The subjects were randomised at baseline to either the cholecalciferol group or the placebo group. The randomisation was age-matched and performed by a faculty member not involved in the study. The supplement was Cal.D.Forte (1.25 mg cholecalciferol, or 50,000 IU), which is typically prescribed in New Zealand when vitamin D deficiency is suspected. It has been shown that whole body exposure to 10–15 minutes of midday sun in summer (one minimal erythemal dose [MED], or the amount of sun exposure which produces a faint pinkness of skin) is comparable to ingesting up to 25 000 IU of vitamin D₃ orally (Lagunova et al., 2013). It has also been demonstrated that this dose, administered over 12 months, does not produce toxicity (Binkley et al., 2007). The placebo was in the form of oral tablet identical in appearance to the intervention tablet but with no active ingredients. Participants were given a full six months' supply (six tablets) at the first appointment. They were instructed to take the first tablet that day, and the subsequent tablets on the same day of every month until finished. Monthly supplements were selected over daily supplements in order to facilitate adherence. Participants were reminded to take the supplement on the given date by text message and/or email prompts from the researcher.

The study duration was set for six months. Vitamin D treatment has been shown to increase muscle strength within three months as seen in osteomalacic patients (Young, 1981). However, it has also been shown that it can take four to five months to reach a steady state serum 25(OH)D concentration at high vitamin D inputs (Heaney, Davies, Chen, Holick, & Barger-Lux, 2003). Therefore six months was considered to be an appropriate length to observe a demonstrable result.

3.4 Participants and the Recruitment process

The participants were healthy adolescent girls aged between 13-18 years old, involved in dancing, gymnastics, or swimming. Initially, only ballet dancers were targeted as candidates for this study to promote homogeneity. However, during the recruitment process it became apparent that there was insufficient interest from dancers in this age bracket to make up the calculated sample size. Therefore the invitation to participate was extended to other female athletes involved in the sports of gymnastics and indoor swimming in order to boost participant numbers. Participants were recruited from ballet schools, gymnasiums, pilates studios, swimming halls, secondary schools, and universities within the greater Auckland area. The research project was advertised by distributing posters and information at these centers (Appendices 4 & 1), as well as discussing with teachers, coaches and parents the nature of the research. The study was also publicised through the media in local and national newspapers (i.e. North Shore Times and the New Zealand Herald), internet news pages (e.g. www.scoop.co.nz), the Massey University newsletter, Listener magazine, 1ZB newstalk radio, and through social media (Facebook). Interested individuals were contacted by phone or email, and

were asked to fill in an online screening questionnaire (Appendix 5). Should they fit the criteria for the study, an invitation to join was extended by email. Recruitment commenced in March 2012 and concluded September 2012. Baseline data was collected as participants were recruited. Baseline testing for all participants was originally intended to be completed in autumn. Data collection was chosen for this period to control for seasonal fluctuations of serum 25(OH)D concentrations (Wacker & Holick, 2013). However, the recruitment process took longer than anticipated to reach sufficient participant numbers and so baseline testing continued on until the end of winter.

3.4.1 Sample Size

A power calculation based on data from a previous study conducted at the Vitamin D Research Centre at Massey University, Albany was used to discover a significant difference in hand-grip strength (von Hurst, et al., 2012). The literature suggests that a meaningful increase in hand-grip strength is 4kg. In the previous study the standard deviation of a group of young women was 6.3kg. Based on these values, 39 subjects were required for each group to achieve a power of 80%, and 5% significance. Therefore the goal was to recruit 100 participants to allow for up to 20% lost to follow-up during the six month study.

3.4.2 Inclusion Criteria

3.4.2.1 Gender

Females were chosen for homogeneity; they also dominate the participation of dance and gymnastic sports in Auckland. Gender-variant patterns become apparent during adolescence, reflecting the different musculoskeletal effects of testosterone and oestrogen in males and females. Vitamin D supplementation has been found to have a positive effect on testosterone in males, possibly confounding the results (Pilz, et al.,

2011). Oestrogen levels can also have an effect on muscle strength, allowing for inclusion in the analysis (Lowe, et al., 2010).

3.4.2.2 Physical activity

Individuals were invited to enlist if they participated in any form of dance, (and later gymnastics and swimming) as training and competing in these sports are performed predominantly indoors. All participants were required to have been training a minimum of five hours per week in their sport.

3.4.3 Exclusion Criteria

Exclusion criteria included any disabilities, chronic illness or injuries that would prevent optimal performance on all physical tests. Those with a history of kidney stones were excluded due to the association of prolonged vitamin D supplementation and urinary stone formation (Jackson et al., 2006). Participants must also have had no recent history (within the two months preceding the study) of supplementation with vitamin D or cod liver oil beyond what is normally found in a multivitamin (up to 400 IU/day). Should an individual present with hemoglobin levels below 115 g/L measured at baseline, they were removed from the study.

3.5 Description of tests and measures

3.5.1 Performance tests

This section will describe the assessment of skeletal muscle strength and postural balance at baseline and six months. Three methods were used to measure muscle function – handgrip strength, vertical jump, and maximal torque of the knee extensors and flexors. Postural stability was measured by static one-legged balance on a force

plate. The tests were completed in the same order for all subjects at baseline and endpoint:

<i>First</i>	Balance
<i>Second</i>	Handgrip
<i>Third</i>	Vertical jump
<i>Last</i>	Maximal isokinetic knee extensor and flexor torque

This sequence was selected to reduce the possibility of fatigue in those muscles necessary for each subsequent test. All equipment was supplied and used within the Sport and Exercise Science Laboratory at Massey University.

3.5.1.1 Warm up

All participants were required to complete a short warm up prior to testing. An ex-Royal Ballet dancer created a warm up program specific to ballet dancers and all dancers were asked to follow this (Appendix 6). Those participants who were swimmers or gymnasts with no knowledge of ballet completed a five minute warm up at their own pace on a cycle ergometer.

3.5.1.2 Hand-grip strength

Muscle strength of the anterior compartment of the forearm was assessed using a dynamometer (Smedley's Dynamometer, 100 kg, TTM, Tokyo, Japan) (Figure 3). Handgrip strength has been one of the most common methods to test muscle strength in vitamin D studies. The participants were asked to complete two maximal efforts with both their dominant and non-dominant hands; the best attempt was recorded (kg). The participants stood with the test arm abducted so that it was parallel to the floor.

Standardised verbal instruction, demonstration, and pictures were provided before the participant attempted the test (Appendix 7).



Figure 3 Hand grip dynamometer used in the study

3.5.1.3 Vertical Jump

Functional power of the leg muscles was tested by a countermovement jump on a flight-time calculating jump mat. (Just Jump, Probotics Inc., Huntsville AL, USA). The validity of using Just Jump for the vertical jump has been tested (Leard et al., 2007), and also used in a previous cross-sectional study (von Hurst, et al., 2012). This mat measures airtime and from this data calculates jump height. The participants were instructed to stand upright with feet shoulder-width apart and hands placed on the hips where they remained throughout the test. When ready, they were required to perform a maximal vertical jump. Three trials were attempted and the best jump height (cm) was recorded and used for analysis. Verbal instruction, demonstration, and pictures were provided before the participant attempted the test (Appendix 8).

3.5.1.4 Strength of the knee extensors and flexors

Peak torque of the knee extensors (quadriceps) and flexors (hamstrings) was measured using an isokinetic dynamometer (Biodex Multi-joint system, PRO). This method has been previously used in a cross-sectional study examining the association between vitamin D and muscle strength (Grimaldi, et al., 2012). In the present study, no participants had previously undergone any form of isokinetic testing.

The participant sat upright in the Biodex chair with the hip and knee flexed at 90 degrees. The seating position was adjusted for individual height and limb length. The axis of the dynamometer was aligned with the axis of rotation of the knee joint. The test leg was firmly strapped to the lever just above the malleoli of the ankle, with the thigh strapped to the seat, minimising any unnecessary movement (Figure 4). Calibration was performed before each test, in which angles for joint range of motion were set individually.

The protocol consisted of a passive bilateral knee flexion and extension in concentric and eccentric contraction. Trial one (concentric contraction) and trial two (eccentric contraction) for each leg consisted of three maximal reciprocal isokinetic knee extensions and flexions at a speed of 30°/s. The highest peak torque (Nm^{-1}) from the three efforts was recorded and used for analysis. Peak torque was determined from the Biodex software (Biodex Advantage Software, V.4X).

The right leg was tested first in all subjects for standardisation. Each trial was explained in full and prior to recording every trial the participants were instructed to perform a sub-maximal effort trial for familiarisation. Trial one and two were separated by a rest period of at least one minute.

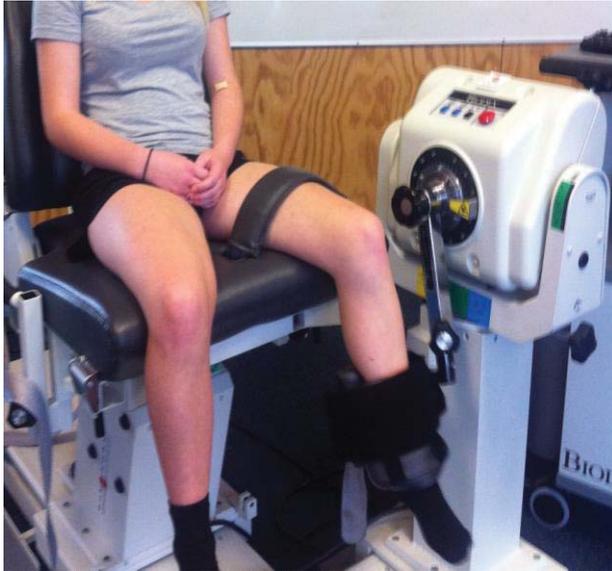


Figure 4 Set up for the isokinetic knee flexion and extension test (Biodesx)

3.5.1.5 Postural stability

Static one-legged balance was measured to determine isometric postural strength using a AccuGait force platform (Advanced Medical Technology Inc., Watertown, MA, USA) at a sampling rate of 200Hz. This system analyses input from strain gauges under a force platform and thereby calculates the center of pressure. Force plate data was collected using data collection software (AMTI Netforce 2.0), which was the standard software that the manufacturer provided with the force plate. Anterior-posterior and medio-lateral centre of pressure (COP) oscillations were measured to calculate the standard deviations (SD) and maximal displacement from the centroid.

The participants were instructed to look straight ahead with arms in the first position and one leg up to r etire position. When ready, they were to rise up to demi-pointe. This position requires the body to be supported by the toes and metatarsal heads of the feet in a plantar flexed position of the ankle joint. Those subjects unfamiliar with this classic ballet pose were asked to present as close an approximation as they could. This pose was then replicated for the six month visit. The subjects were instructed to hold the

position for 30 seconds, on both the left and right foot. Verbal instruction, demonstration, and pictures were provided before the participant attempted the test (Appendix 9).

3.5.2 Anthropometric measurements

Body mass (kg) was measured to the nearest 0.1kg barefoot using electronic scales (Tanita, THD-646, Japan). Height (M) was measured to the nearest 0.1cm using a stadiometer (Holtain LTD, England). The research team ensured that the participants' heels were against the heel board and an upright posture was assumed before the measurement was taken. These measurements were taken at baseline and endpoint using the same equipment.

BMI was calculated from their measured height and weight [BMI = weight divided by height squared (kg/m^2)].

Lean body mass and body fat percentage were determined by Dual-energy X-ray absorptiometry (DXA), obtained on the Hologic Discovery A densitometer (Hologic, Inc., Waltham, MA, USA). A trained technologist performed the scan. This measurement was taken during baseline testing only.

3.5.3 Dietary Assessment

Daily dietary intake was assessed at baseline and estimated from a four-day food diary. Participants were instructed on how to complete a food diary at home during their first appointment. The completed food diaries were posted back to the Vitamin D Research Center. The diets were analysed for total energy, macro- and micro-nutrient intake, particularly calcium and vitamin D intakes using Food Works version 7 (Xyris Software, Australia 2009).

3.5.4 Physical Activity

The characteristics of training (years of training, number of sessions per week, and duration of the sessions) were documented as part of a general questionnaire developed by the research team (Appendix 10).

For the duration of their participation in the study, participants were also required to complete a weekly online training diary powered by Survey Monkey (www.surveymonkey.com). This was devised as a method for monitoring activity levels (Appendix 11). Activity levels were estimated by the following procedure:

Didn't train – assigned a value of 0

Less than one hour – assigned a value of 1

One to four hours – assigned a value of 2

More than four hours – assigned a value of 4

Daily answers were converted to these values, and the sum of these values became the total estimated training hours for that participant for the week. For analysis, only the average weekly training hours of the first four weeks (baseline) and the last four weeks (endpoint) were used to investigate change.

Compliance was frequently monitored, and email and text message reminders were sent to those who missed entries.

3.5.5 Blood sampling and analysis

3.5.5.1 Sampling

A qualified phlebotomist collected a non-fasting venous blood sample at baseline and at six months, using a sterile Vacutainer Flashback Precisionglyde needle and needle holder.

Blood was collected into a 10ml EDTA vacutainer for plasma, and a 10ml plain tube for serum. After collection they were stored at room temperature for at least 30 minutes but within two hours before being centrifuged. Blood samples were centrifuged at 3500 rpm for 10 minutes at 4°C prior to being dispensed into eppendorf tubes. Aliquots of at least 0.5ml of serum and plasma were measured into nine (five serum and four plasma) labeled 2ml eppendorf tubes for the measurement of vitamin D, calcium, albumin, oestradiol, and intact parathyroid hormone (PTH), and stored at -80 °C. At the conclusion of the study all samples were sent to Clinical Health Laboratories, North Shore Hospital, Auckland for analysis.

3.5.5.2 Analysis

Serum 25-OH Vitamin D Assay:

Serum 25(OH)D was used to assess vitamin D status (Binkley, et al., 2012). Serum 25(OH)D was determined using an automated immunoassay (ADVIA Centaur Vitamin D Total assay, Siemens Healthcare Diagnostics Inc, Il, USA). The intra-assay precision was <7.1% and the inter-assay CV <11.2%. (Farrell et al., 2012). The lower detection limit of the assay was 9.3 nmol/L. Serum 25(OH)D concentrations were described using the most commonly accepted definitions as summarised in Table 5 (Holick, 2007).

Table 5 Definition of serum 25(OH)D status

25(OH)D concentration (nmol/L)	Classification
< 25	Severely deficient
25-49	Deficient
50-74	Insufficient
>75	Sufficient

Intact parathyroid hormone (PTH) Assay:

Plasma levels of intact PTH were determined with a two-site sandwich immunoassay using direct chemiluminescence technology (ADVIA Centaur intact PTH-serum assay, Siemens Healthcare Diagnostics Inc, IL, USA). Analytical sensitivity was 0.265 pmol/L. The intra-assay CV was between 2.2 and 3.5%. The lower detection limit of the assay was 0.265 pmol/L.

Calcium and albumin Assay:

Serum calcium and serum albumin were measured using the CA method and ALB method respectively, with the Dimension Vista System (Siemens Healthcare Diagnostics Inc, IL, USA). Serum concentrations of calcium were adjusted for albumin.

Oestrogen Assay:

Serum levels of oestradiol were estimated by a competitive chemiluminiscent assay (ADVIA Centaur CP System; Siemens Healthcare Diagnostics Inc, IL, USA). The sensitivity was 36.7 pmol/L and the inter-assay CV was 5%. This assay measures oestradiol concentrations up to 11,010 pmol/L with a lower detection limit of 43.6 pmol/L.

3.6 Statistical analysis

The variables were tested for normality using the Shapiro Wilk test and for homogeneity using Levene's test. Where possible, non-normally distributed data were

log transformed to obtain normality. Normally distributed data were expressed as mean \pm SD or as geometric mean (95%CI) if log transformed and data not normally distributed were expressed as median [25th, 75th percentiles]. A *P* value less than 0.05 was considered to be significant. For variables that showed statistically significant differences between groups, effect size was calculated to provide an objective measure of the importance of the effect by using the following formulas: for the Mann-Whitney U test, effect size = Z/\sqrt{n} ; for the independent t-test, $\sqrt{t^2/(t^2+df)}$. An effect size value of 0.1 indicated a small effect, a value of 0.3 indicated a medium effect and a value of ≥ 0.5 indicated a large effect (Field, 2009). Comparisons were made between the vitamin D and placebo group at baseline using the independent t-test for parametric data, the Mann-Whitney test for non-parametric data, and the Chi-square test for categorical data. Comparisons were made within groups between baseline and endpoint measures using the dependent t-test for parametric data and the Wilcoxon-signed rank test for non-parametric data. The independent t-test and Mann-Whitney test were used to compare the change in muscle strength and power from baseline to endpoint between the vitamin D and placebo group. Finally, analysis of covariance (ANCOVA) tested differences between the groups, where the covariates were serum 25(OH)D at baseline and change in serum 25(OH)D between baseline and endpoint. All statistical analyses were performed using SPSS for Windows Version 20 (SPSS Inc, Chicago, IL).

CHAPTER 4 – RESULTS

Sixty-one female adolescents who were dancers, gymnasts, and swimmers were recruited for this study. A total of 54 completed the study (vitamin D = 26, placebo = 28). Figure 5 shows the CONSORT diagram for participant flow from enrolment to the final analysis.

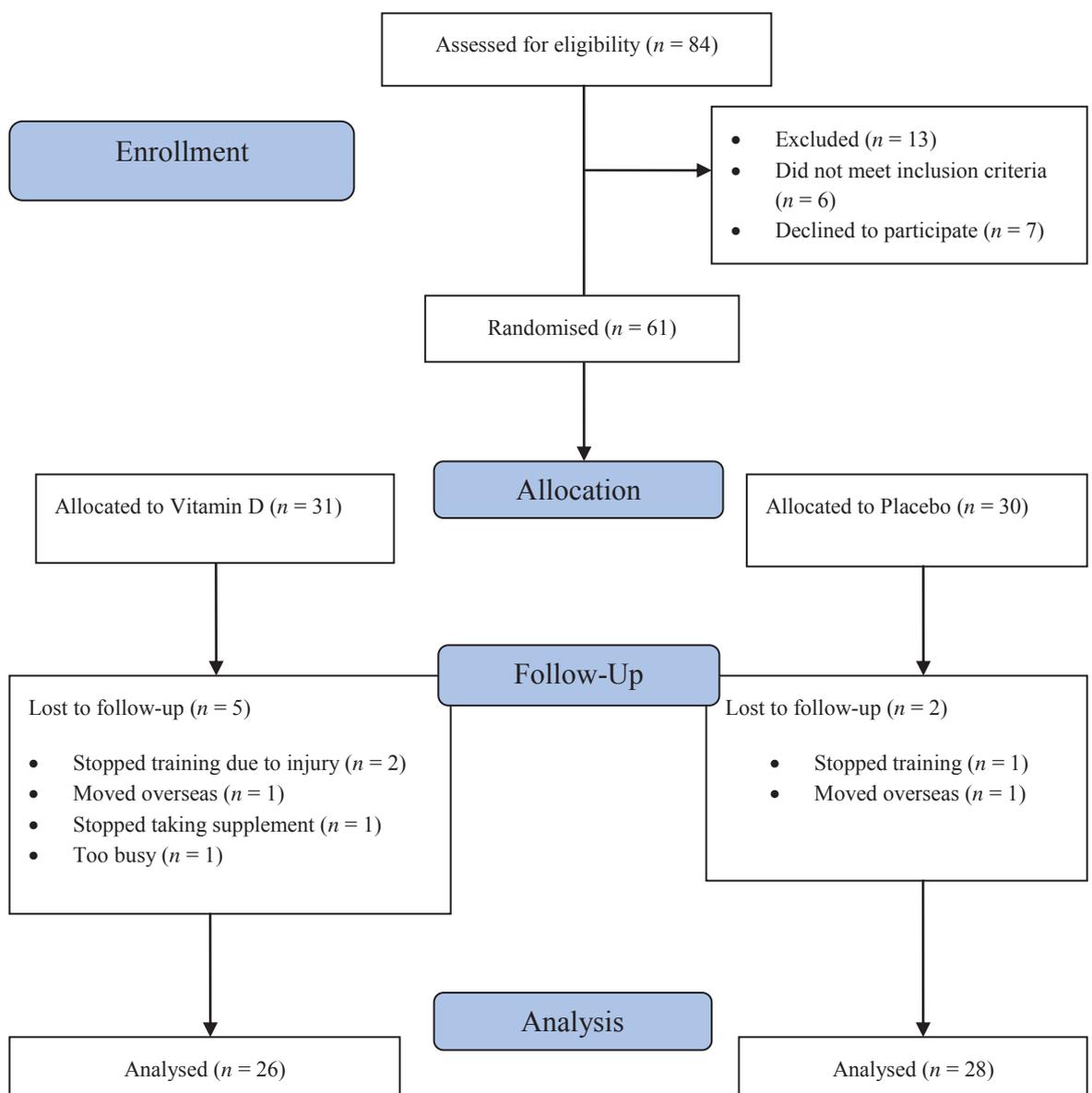


Figure 5 CONSORT diagram demonstrating participant flow

4.1 Baseline characteristics

At baseline, there were no significant differences between groups for age, BMI, body fat percentage, years of training, volume of training, or dietary intake. The data for these characteristics are listed in Table 6.

There were no significant differences in sport participation between groups ($P = 0.252$). In the vitamin D (VD) group, 22 girls (84.6%) listed their major sport as ballet, two girls (7.7%) as gymnastics, and two girls (7.7%) as swimming. In the placebo group (PLA), 20 (71.4%) listed their major sport as ballet, four (14.3%) as modern or contemporary dance, three (10.7%) as gymnastics, and one (3.6%) as swimming.

There was no significant difference in ethnicity between groups ($P = 0.512$). Of the participants in the VD group 23 identified as Caucasian (88.4%), two as Asian (7.4%), and one as Maori (3.8%). Twenty-seven (96.5%) of the participants in the PLA group identified as Caucasian, and one (3.6%) as Asian.

Those participants who were lost to follow up ($n = 7$) were not significantly different in any of the baseline measurements compared to those who completed the study ($P > 0.05$).

Table 6 Participant characteristics and dietary intake at baseline

Characteristic		Vitamin D (n = 26)	Placebo (n = 28)	P ^a
Age (years) ^b		14 [13,15]	14 [13,16]	0.866
BMI (kg/m ²) ^c		19.5±2.2	20.2±2.5	0.273
Total Body fat (%) ^b		23[4,9]	22.3[20,25]	0.712
Years of training (years) ^c		9±3.2	9.1±2.2	0.865
Volume of training (hours/week) ^b		11 [5,24.5]	10.6 [3,17]	0.829
Dietary Intake ^d	Total energy (kj/day) ^c	8118.4 ± 1919.7	8507.7 ± 2086.8	0.499
	Protein (g/kg/day) ^c	1.6 ± .5	1.5 ± 0.4	0.428
	Fat (g/kg/day) ^c	1.5 ± .5	1.5 ± 0.6	0.658
	Carbohydrate (g/kg/day) ^c	4.9 ± 1.2	4.9 ± 1.5	0.994
	Vitamin D (µg/day) ^c	1.2 ± .4	1.4 ± 0.5	0.156
	Calcium (mg/day) ^c	914.9 ± 339.9	922.3 ± 303.3	0.936

^aDifference between groups (independent t-test or Mann-Whitney test); ^bValues are medians; 25th, 75th percentiles in parentheses; ^cValues are means ± SD; ^dThree participants from the vitamin D group and one participant from the placebo group did not complete a food diary.

4.2 Blood analysis data

Blood samples were obtained from a total of 52 participants (VD group = 26, PLA group = 26). Two subjects from the PLA group refused blood sampling due to venipuncture phobia. The data from the blood analysis is described in Table 7. Mean values for the main outcome variable, serum 25(OH)D, were approximately 75 nmol/L at baseline for both groups. At baseline, a total of 34 individuals (57.7%) had sufficient (>75 nmol/L) concentrations of serum 25(OH)D. Fourteen (30.8%) participants had serum 25(OH)D concentrations between 50 and 75 nmol/L (insufficient), and four (11.5%) participants had concentrations below 50 nmol/L (deficient). No participants in this study presented with severely deficient baseline serum 25(OH)D concentrations

(<25 nmol/L). One participant had a baseline serum 25(OH)D value above 100 nmol/L.

4.2.1 Serum 25(OH)D concentration

There was no significant difference in serum 25(OH)D concentrations between groups at baseline ($P = 0.615$). Serum 25(OH)D concentrations significantly increased between baseline and endpoint in the VD group ($P = 0.001$) although the effect size was small ($r = 0.1$). There was no change in the PLA group ($P = 0.310$). The difference in change between the VD group and the PLA group was appreciable, but did not reach significance ($P = 0.088$). The change in serum 25(OH)D concentrations between baseline and endpoint is illustrated in Figure 6.

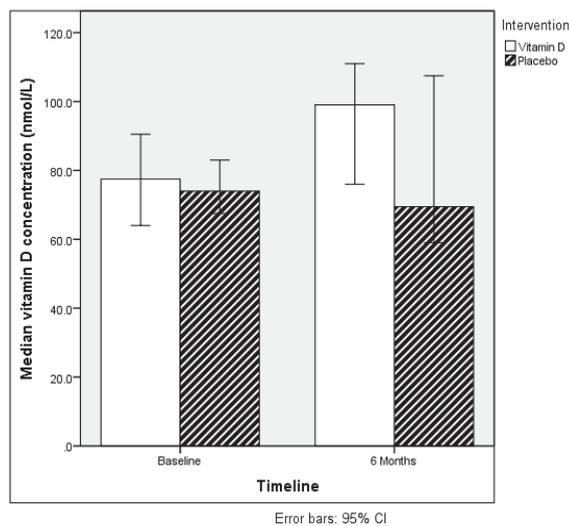


Figure 6 Bar graph illustrating the differences in median serum 25(OH)D concentrations between groups at baseline and endpoint.

Following supplementation, 46.2% of the subjects in the PLA group had serum 25(OH)D concentrations over 75 nmol/L, of which 34.6% were over 100 nmol/L. In the VD group, 76% were over 75 nmol/L, 48% of which were over 100 nmol/L. Deficiency disappeared in the VD group (Figure 7).

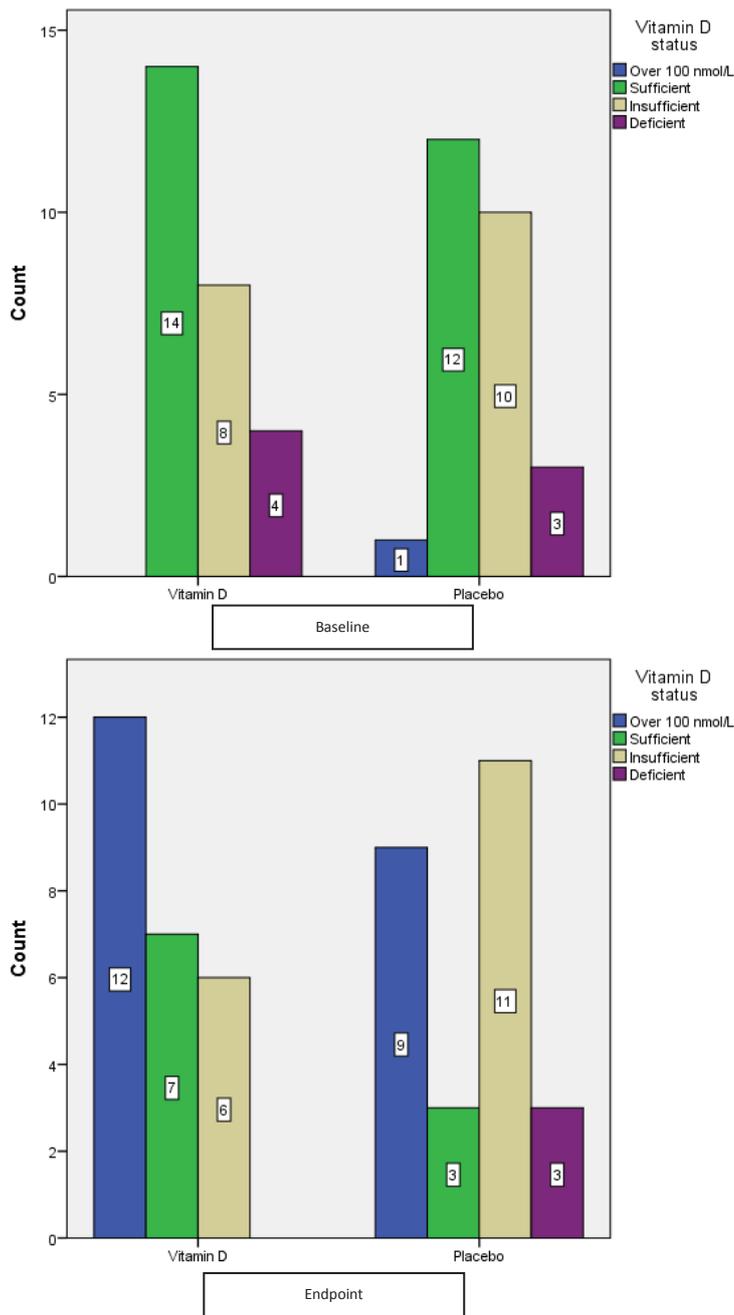


Figure 7 Bar graphs illustrating the distribution of vitamin D status within groups at baseline and endpoint

4.2.2 Serum calcium, albumin, oestradiol, and intact PTH concentrations

There was no significant difference in any of the other blood parameters between groups at baseline (Table 7). In the VD group, there were also no significant differences in the change between baseline and endpoint. In the PLA group, albumin concentrations

decreased significantly ($P = 0.039$). PTH levels also tended to decrease in the PLA group between baseline and endpoint, but not significantly ($P = 0.060$). There were no significant differences in the change between groups for any of the blood parameters.

Table 7 Baseline, endpoint, and change values for serum 25(OH)D, calcium, albumin, oestradiol, and intact parathyroid hormone for vitamin D and placebo groups

	Vitamin D (n = 26)	Placebo (n = 26*)	P ^a
Serum 25(OH)D (nmol/L)^b			
Baseline	77.5 [63.5,92]	74 [64.5,88.5]	0.615
End	99 [75.5,112.5]	69.5 [58,108]	
Change ^c	16.5 [7,46]	-6.3 [-21,44]	0.088
P ^d	0.001	0.310	
Calcium (mmol/L)^b adjusted for albumin			
Baseline	2.3 [2.3,2.4]	2.3 [2.3,2.3]	0.188
End	2.3 [2.3,2.4]	2.3 [2.3,2.4]	
Change ^c	0.1 [-0.1,0.5]	0.0 [-0.5,0.5]	0.790
P ^d	0.930	0.948	
Albumin (g/L)^e			
Baseline	43.1±2.5	43.2±1.8	0.898
End	42.3±2.6	42.4±1.7	
Change ^c	-0.8±2.1	-0.8±1.9	0.989
P ^d	0.072	0.039	
Oestradiol (pmol/L)^f			
Baseline	195 (150.4,252.7)	204.6 (167.4,250.2)	0.720
End	200.5 (142.1,283)	217.9 (161,294.9)	
Change ^c	94.4 (-65.2,254)	55.9 (-33.2,145.1)	0.667
P ^d	0.879	0.658	
Intact parathyroid hormone (PTH) (pmol/L)^e			
Baseline	2.7±1.3	2.9±1.4	0.567
End	2.7±1.8	2.3±1.3	
Change ^c	-0.1±2.2	-0.6±1.6	0.326
P ^d	0.866	0.060	

*Two participants did not provide blood samples due to fear of venipuncture; ^aDifference between groups (independent t-test or Mann-Whitney test); ^bValues are medians; 25th, 75th percentiles in parentheses; ^cChange: End value – Baseline value; ^dDifference between baseline and end (dependent t-test or Wilcoxon signed-rank test); ^eValues are means ± SD; ^fValues are geometric means; 95% CI in parentheses

4.3 Training data

All participants were instructed to complete an online weekly training log. Four of the participants (VD group = 2, PLA group = 2) did not complete a weekly training diary, therefore their contribution was omitted from the average results of each group. There was no significant difference between groups in self-reported level of training hours at baseline ($P = 0.527$), nor was there a significant change between groups ($P = 0.906$) (Table 8).

Table 8 Self-reported weekly training hours

Weekly training (hrs)	Vitamin D ($n = 24$)	Placebo ($n = 26$)	P^a
Baseline	10.3±4.3	11.2±3.9	0.527
Endpoint	10.1±3.9	10.8±5.3	
Change ^b	-0.2±3.4	-0.3±3.9	0.906
P^c	0.775	0.681	

**All values are means ± SD; ^aDifference between groups (independent t-test); ^bChange: End value – Baseline value; ^cDifference between baseline and end (dependent t-test)*

4.4 Strength and power data

All data for the performance tests are shown in Table 9. Values are listed for both dominant and non-dominant maximal handgrip strength; maximal vertical jump; and peak torque of the extensors and flexors of the right and left knee in eccentric and concentric contraction. Data for isometric postural strength from static one-legged balance is not included in the analysis due to a malfunction of the recording equipment.

At baseline, there were no significant differences in any of the performance tests between the VD and PLA groups.

Handgrip strength (in both dominant and non-dominant hands) did not change significantly in either group.

Vertical jump height increased in both groups between baseline and endpoint, although only in the PLA group did this reach significance ($P = 0.042$). There was no significant difference in the change between groups ($P = 0.811$).

There was a trend for knee extensor strength in both concentric and eccentric contraction to increase for both groups. Isokinetic knee extensor strength increased significantly in the VD group for the right leg in concentric ($P = 0.016$) and eccentric ($P = 0.007$) contraction, and for the left leg in eccentric contraction ($P = 0.002$). The PLA group demonstrated significant increases in knee extensor strength of the left leg in concentric contraction ($P = 0.041$) and eccentric contraction ($P < 0.001$), and for the right leg in knee eccentric extension ($P = 0.042$).

There were no significant changes in any of the isokinetic knee flexion tests for either group.

A one-way analysis of covariance (ANCOVA) was conducted on all outcome measures. After controlling for change in vitamin D concentrations and baseline vitamin D concentrations, Vitamin D supplementation had no remaining significant effect on the change in any of the strength variables.

Table 9 Baseline, endpoint, and change values for maximal handgrip strength, vertical jump height, and isokinetic peak torque of the knee extensors and flexors of the vitamin D and placebo groups

Strength variable		Vitamin D (n = 26)	Placebo (n = 28)	P ^a
Handgrip (kg)	Dominant			
	Baseline	27.1±5.3	27.9±6.1	0.611
	End	27.6±5.7	27.7±5.2	
	Change ^b	0.4±2.5	-0.3±3.7	0.421
	P ^c	0.384	0.706	
	Non-dominant			
	Baseline	26.2±5.9	26.1±5.3	0.976
	End	26.2±5.7	26.7±5.4	
	Change ^b	0.02±3.4	0.6±2.5	0.474
	P ^c	0.977	0.216	
Vertical Jump (cm)	Baseline	36.3±4.9	37±6.1	0.658
	End	37.7±3.7	38.6±6.1	
	Change ^b	1.4±3.9	1.6±3.9	0.811
	P ^c	0.091	0.042	
Isokinetic peak torque right leg (Nm)	Knee concentric extension			
	Baseline	119.3±29.8	119.7±29.6	0.967
	End	130.1±30.1	123.6±29.6	
	Change ^b	10.8±21.3	3.95±15.7	0.185
	P ^c	0.016	0.195	
	Knee concentric flexion			
	Baseline	62.5±12.6	61.9±14	0.867
	End	62±13.4	63.3±13	
	Change ^b	0.5±12.1	1.35±10.9	0.543
	P ^c	0.814	0.518	
	Knee eccentric extension			
	Baseline	149.1±43.1	135±46.2	0.254
	End	167.4±42.8	151.2±52.3	
	Change ^b	18.3±31.7	16.2±40.1	0.829
	P ^c	0.007	0.042	
	Knee eccentric flexion			
	Baseline	74.2±16.9	74.5±17.3	0.952
	End	73.8±16.4	73.8±12.3	
	Change ^b	0.4±12.2	0.7±8.6	0.933
	P ^c	0.860	0.685	
Isokinetic peak torque left leg (Nm)	Knee concentric extension			
	Baseline	115±26.5	116.4±28.5	0.856
	End	126.6±39	123.9±23.8	
	Change ^b	11.6±29.7	8.7±20.9	0.678
	P ^c	0.058	0.041	
	Knee concentric flexion			
	Baseline	55.8±14.3	59.5±13.9	0.337
	End	57.1±12.8	59.8±12.6	
	Change ^b	1.3±9	0.3±6.9	0.638
	P ^c	0.460	0.829	
	Knee eccentric extension			
	Baseline	152.2±42.4	139.5±40.2	0.267
	End	173.1±41.8	164.9±40.3	
	Change ^b	20.8±31.1	25.4±31.3	0.595
	P ^c	0.002	<0.001	
	Knee eccentric flexion			
	Baseline	66.9±13.5	71.2±16.3	0.302
	End	69.4±13.3	71.4±14.3	
	Change ^b	2.45±6.6	0.2±9.6	0.330
	P ^c	0.070	0.909	

*All values are means ± SD; ^aDifference between groups (independent t-test or Mann-Whitney test); ^bChange: End value – Baseline value; ^cDifference between baseline and end (dependent t-test or Wilcoxon signed-rank test)

CHAPTER 5 – DISCUSSION

The purpose of this study was to determine if there was a significant effect on muscle function after vitamin D₃ supplementation in a group of well-trained dancers, gymnasts, and swimmers who spend the majority of their training time indoors. The rationale was based on research that has revealed a high prevalence of vitamin D deficiency in athletes particularly in those who trained indoors, and that this deficiency could result in impaired performance.

The main finding of this study was that vitamin D supplementation of 50,000 IU per month significantly increased serum 25(OH)D concentrations from 77.5 nmol/L [63.5,92] to 99 nmol/L [75.5,112.5]. Although the change (16.5 nmol/L [7,46]) was significant, the effect was small as the change itself was not large. Previous studies that have used comparable doses of vitamin D supplementation have yielded greater changes in serum 25(OH)D concentrations. El-Hajj Fuleihan and colleagues (2006) gave school-aged children 2000 IU/day of vitamin D for 12 months, increasing the average serum 25(OH)D concentrations from 35 nmol/L to 95 nmol/L. In postmenarchal girls, four doses of 150,000 IU over one year - equivalent to 50,000 IU per month - increased average 25(OH)D concentrations from 18 nmol/L to 56 nmol/L (Ward, et al., 2010).

In keeping with null hypothesis 2, vitamin D supplementation did not increase muscle strength or power compared to the placebo group, despite significantly increasing serum 25(OH)D concentrations in the VD group over time. Although this finding is in agreement with previous reports that also found no effect of vitamin D supplementation

on muscle strength (Barker, et al., 2012; Ward, et al., 2010), there were methodological and physiological limitations to this study that may explain the lack of effect.

Baseline concentrations of serum 25(OH)D for the group as a whole were surprisingly high. In the VD group, baseline serum 25(OH)D concentrations prior to supplementation were on average 75 nmol/L. This is contradictory to reports in previous studies on athletes (refer to Table 2) and young women in Auckland (von Hurst, et al., 2012) who were found to be predominantly vitamin D insufficient or deficient. Under Holicks' (2011) definition of sufficiency, insufficiency and sufficiency, over half of the participants had sufficient 25(OH)D concentrations at baseline, and only four were deficient. The elevated 25(OH)D concentrations observed in this subject group may be indicative of a greater than originally predicted time spent outdoors - sunlight exposure provides 80-90% of serum 25(OH)D stores (Holick, 2011). Previous research with dancers have demonstrated that those participants with serum 25(OH)D concentrations in the deficient range spent on average 38 hours per week training (Wolman, et al., 2013; Wyon, et al., 2013). Despite efforts to control for outdoor exercise by recruiting indoor athletes in the present study, the average weekly training hours for the participants in their sport was comparatively low (on average 10 hours per week).

Vitamin D deficiency or insufficiency results in muscular weakness (Wicherts, et al., 2007). In vitamin D deficient patients, supplementation with vitamin D improved muscle strength in the elderly (Verhaar et al., 2000), as well as stroke (Sato, et al., 2005) and osteomalacia patients (Glerup, et al., 2000). It is possible that vitamin D sufficient individuals may not have the same magnitude of improvement in muscle strength, power,

or function as those who are deficient at baseline. In a group of healthy males and females with average baseline 25(OH)D concentrations of 80 nmol/L, there was no significant increase in strength over time (Barker, et al., 2012).

It is currently unknown what the optimal range of serum 25(OH)D is for improving neuromuscular function. Cannell and Hollis (2008) have observed that healthy populations living close to the equator have serum 25(OH)D concentrations ranging from 100-174 nmol/L, and Heaney supports an optimal range between 120-225 nmol/L, based on the observation that pathology can present at concentrations below 100 nmol/L (Heaney, 2011). On this basis, Close (2012) speculated that the threshold for improvement in muscle function is above 100 nmol/L. Participants in their trial did not improve in any of their strength assessments, possibly because their serum 25(OH)D concentrations did not exceed 100 nmol/L.

In the current study, the VD group and the PLA group had 14 and nine subjects respectively in excess of 100 nmol/L at endpoint (Figure 7). Consequently, even without supplementation a third of the PLA group had serum 25(OH)D concentrations considered optimal for muscle function at endpoint. Indeed, the lack of a significant change in serum 25(OH)D concentrations between groups ($P = 0.088$) is reflective of the number of subjects in the placebo group with high endpoint concentrations.

Season is a strong predictor of serum 25(OH)D concentrations, such that concentrations are highest in late summer and early autumn and lowest in late winter and early spring (Bolland et al., 2007). Previous research studies have endeavored to recruit subjects within a limited time frame to control for these fluctuations (Barker, et al., 2012; Close,

et al., 2013; Wyon, et al., 2013). In this study, baseline measurements were taken from early April to early September, resulting in a wide range of baseline serum 25(OH)D concentrations as the seasons progressed. For example, the number of individuals in the PLA group who had baseline 25(OH)D values of over 100 nmol/L went from one participant to nine at endpoint. The increase in 25(OH)D concentrations was due to the contribution of those whose endpoint measurements were taken during mid-summer.

An observation of this study was that there was a general trend in both groups to improve in knee extensor strength and vertical jump height. Contributing factors are possibly due to increased lean mass as expected from growing adolescents, and perhaps a learning effect, although improvements were not seen in any of the flexion tests. Differences in lean mass between the participant groups were not calculated as body composition data was not collected at the second visit. These results contradict other studies that have assessed proximal leg strength in healthy subjects. Serum 25(OH)D concentrations of 75nmol/L and under were related to poor performance of quadriceps isometric extension ($P = 0.020$) and hamstring flexion ($P = 0.032$) measured with a Biodex in women (average age of 75 years) (Gerdhem, Ringsberg, Obrant, & Akesson, 2005). Grimaldi (2012) also found a positive association between isometric knee strength and vitamin D status in younger adults. Wyon (2013) observed a significant increase in isometric knee strength in their treatment group compared to the control group, although that could be due to the relatively small size of the control group. Barker (2012) did not find a significant improvement in proximal leg strength after supplementation; as with the present study the participants were found to be vitamin D sufficient at baseline.

In this study, no significant changes were observed in maximal handgrip strength after supplementation with vitamin D. Handgrip strength is a test frequently employed in studies of vitamin D studies and muscle strength, but with variable results. Grip strength was shown to be related to vitamin D status in a previous study (von Hurst, et al., 2012). The inconsistency could be explained by the difference in subject numbers compared to the present study; the previous study had a total of 137 participants which may have been sufficient to observe a significant difference. The present study fell short of the desired sample size calculated to detect a significant effect. Nonetheless, a recent meta-analysis based on the pooled findings of 17 RCTs found that vitamin D supplementation did not improve grip strength (Stockton, et al., 2011).

This is the first RCT of athletes in a vitamin D study of whom all were female. Prior controlled trials with athletes have used males only (Close, et al., 2013; Close, et al., 2012) or a combination of males and females (Wyon, et al., 2013). Comparison of studies using males versus females may be debatable because of the effect vitamin D supplementation has on raising testosterone levels in males (Pilz, et al., 2011). It was observed in a previous vitamin D study that males and females seemed to have different physical responses in terms of their vitamin D status – VO_{2max} and handgrip strength were positively associated with 25(OH)D levels in men and women, respectively (Valtueña, et al., 2013). Furthermore, in two similar studies with the same strength assessments, males exhibited improvements in strength, notably handgrip strength after supplementation with vitamin D (Gupta, et al., 2010), whereas there were no significant improvements in strength for females, even with a larger sample size (Goswami, et al., 2012).

Vitamin D supplementation in older adults tends to show improvements in muscle strength and function tests only when vitamin D deficiency is present (Stockton, et al., 2011). In younger active individuals, it is plausible that the benefit of regular training may outweigh the negative impacts of vitamin D deficiency. Increasing concentrations of 25(OH)D in healthy young athletes may not induce an identifiable improvement in muscle function as is demonstrated in the elderly (Bischoff-Ferrari, Dietrich, Orav, Hu, et al., 2004). Therefore it may be appropriate to utilise more sensitive measures of muscular strength, such as single muscle fibre analysis.

There were some methodological strengths to this study. Chiefly, the sample was homogenous. The participants had similar characteristics, and so there was little to control for. In other similar studies, variables such as BMI, age, and gender reduced the significance of the results (Grimaldi, et al., 2012; Gupta, et al., 2010). In addition, physical activity levels were monitored throughout the study. Changes in physical activity can be the most powerful predictor for changes in muscle strength. The present study found no significant difference in hours spent training between groups over time.

5.1 Limitations

There were a number of limitations within this study that should be addressed for future research:

- The primary weakness of the study was the high baseline serum 25(OH)D concentrations of the participants. To avoid this, screening for vitamin D status

would be necessary to minimise the number of participants who were vitamin D sufficient. However, a control group is required to ascertain the effect of vitamin D supplementation on any outcome, and knowingly randomising vitamin D deficient individuals into a control group is ethically questionable.

- The study lacked power. It was calculated that in order to detect a significant difference in muscle function tests, a minimum of 39 subjects per group were required. Potential candidates were difficult to recruit for several reasons: the high injury rate in dancers and gymnasts led to many being ineligible for participation; fear of venipuncture was a common reason for declining to participate; and reluctance to commit to the study length – the present study being part of a 12 month research project.
- A fault was discovered in the force plate after collection of the baseline data, so that isometric postural stability data could not be analysed and reported.
- Time of day for strength testing was not standardised between visits. This was due to the logistical difficulties of coordinating the available time of the required technical staff, availability of university facilities, and time donated by the participants and their caregivers. Research has shown that time of day and quality of sleep prior to testing can have a considerable effect on strength. Muscle strength can also be influenced by a variety of other factors such as mood, food and drink intake, and previous activity which could not always be controlled for.

5.2 Conclusion

This study demonstrated that active dancers, gymnasts, and swimmers in the Auckland area who train on average for 10 hours per week may not be at risk for vitamin D deficiency, and that vitamin D supplementation of 50,000 IU per month does not contribute to increases in muscle strength.

Until recently, there has been a lack of randomised controlled trials to support the association studies regarding the neuromuscular benefits of vitamin D in young healthy individuals and athletes. Existing evidence has so far not sufficiently proven the efficacy of vitamin D supplementation on athletic performance in active individuals due to numerous methodological constraints. Currently, the optimal concentration of serum vitamin D for muscle is still unknown. Nevertheless, it is important to establish the threshold for optimal vitamin D status as it relates to neuromuscular health. Athletes who train long hours indoors may be at risk for vitamin D deficiency, and should have their serum vitamin D levels checked regularly. Further research in this field should consider recruitment of large samples, documentation of all lifestyle factors that can affect vitamin D status, and consider varying doses of vitamin D supplementation to determine the optimal dose for muscle function.

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Appendices

Appendix 1 – Information Sheet

Appendix 2 – Consent Form

Appendix 3 – Parental Consent Form

Appendix 4 – Recruitment Poster

Appendix 5 – Online Screening Questionnaire

Appendix 6 – Warm Up Instruction

Appendix 7 – Handgrip Dynamometer Instruction

Appendix 8 – Vertical Jump Instruction

Appendix 9 – Balance Test Instruction

Appendix 10 - Participant Information and Medical History Form

Appendix 11 - Online Weekly Training Diary

APPENDIX 1



MASSEY UNIVERSITY
COLLEGE OF SCIENCES
TE WĀHANGA PŪTAIAO

Vitamin D and muscle strength in female dancers and gymnasts The Sunflower Study

INFORMATION SHEET

We are looking for adolescent female dancers or gymnasts to take part in the Sunflower Study. This study aims to investigate whether taking a vitamin D supplement improves muscle strength and bone health of female adolescent athletes.

Researcher(s) Introduction

The researchers involved in this study are Dr Pamela von Hurst, Kathryn Beck, Dr Andrew Foskett, Dr Mark Fulcher and Sarah Mitchell. Pamela von Hurst and Kathryn Beck are staff members in the Institute of Food Nutrition and Human Health at Massey University. Andrew Foskett is a staff member in the School of Sport at Massey University. Mark Fulcher is Sports Physician who practices at Unisports Sports Medicine Clinic. Sarah Mitchell has postgraduate qualifications in sport and exercise science and is completing her Masters degree in Human Nutrition as part of this project.

Study contacts:

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Participant's Rights

You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- decline to answer any particular question;
- withdraw from the study at any time;
- ask any questions about the study at any time during participation;
- provide information on the understanding that your name will not be used unless you give permission to the researcher;
- be given access to a summary of the project findings when it is concluded.

Project Description and Invitation

It is thought that vitamin D, which we mostly make from sun exposure, may improve the muscle strength and bone mineral density of female adolescent athletes including dancers. Studies undertaken in the elderly have shown a positive relationship between vitamin D levels in the body and muscular strength. A few studies in young people have found positive relationships between vitamin D status and measures of performance such as grip strength, jump height and leg strength, as well as lean body mass.

Athletes spending hours training indoors, such as dancers and gymnasts, are at risk of vitamin D deficiency and therefore may not be performing to their potential. Dancers and gymnasts are also often at risk of iron deficiency, and decreased bone mass due to reduced energy (and nutrient)

Vitamin D and muscle strength in female dancers. V1, 24/01/12.

intakes and delayed menstruation. These deficiencies potentially impact short and long-term health as well as ability to achieve optimal performance.

For this study we would like to recruit 100 female ballet dancers or gymnasts aged between 13 and 18 years. Half of the group will receive a vitamin D supplement to take once a month for 12 months. The other half of the group will receive a placebo supplement (a supplement that looks the same as the vitamin D supplement, but doesn't contain vitamin D) for 12 months. We will measure muscle strength and power at the beginning, after 6 months and after 12 months. We will also measure bone mineral density, body composition, vitamin D and iron levels in the blood and dietary intake.

Who are we looking for?

We are looking for 100 female adolescent gymnasts or dancers (ballet or contemporary) to participate in this study.

To take part in this study you should:

- Be between 13 and 18 years of age
- Be actively involved in ballet or contemporary dance or gymnastics
- Training/practicing in these sports for an average of at least 1 hour per day, 5 days per week
- Not pregnant nor intending to get pregnant in next 12 months
- Have no known health problems
- Be responsible and committed to the project
- Not be taking or have not taken vitamin D supplements including cod liver oil in the past 2 months

Project Procedures

Participant involvement - what is going to happen?

If you decide to participate in the study, it will involve three visits to the Human Nutrition Research Unit at Massey University in Auckland. Following the first visit and for the next 12 months half of the group will take a vitamin D supplement once per month. The other half of the group will take a placebo tablet every month. This will be decided randomly using a computer programme designed for this task. Each week we will email participants a simple form to complete regarding training levels and health. This form will take no more than 10 minutes to complete. Following your first visit we will ask you to record everything that you eat for a period of 4 days.

During your first visit, you will be asked to sign a consent form having considered this information sheet. This visit will take approximately 2 hours.

During your first visit you will:

- Be given the opportunity to ask any questions and to complete a form regarding personal details (e.g. contact details, etc)
- Be asked to complete a questionnaire with questions on age, ethnicity, training levels, medical history, medication and supplement use and smoking habits
- Have your height and weight measured. Your body composition (lean body mass and percentage body fat) will be measured using the DXA.
- Have a DXA scan to measure the density of your bones.
- Complete some simple muscle strength tests.
- Be asked questions about your diet including filling out an online questionnaire.
- Be asked questions about blood loss through an online questionnaire.
- Have a blood test taken by a trained phlebotomist. We will be measuring vitamin D and iron levels.

The second visit will occur approximately 6 months after the first visit and will take approximately 1 hour. During this visit you will:

Vitamin D and muscle strength in female dancers. V1, 24/01/12.

- Have your height and weight measured.
- Complete some simple muscle strength tests.
- Have a blood test taken by a trained phlebotomist to measure vitamin D levels.

The third visit will occur approximately 12 months after the first visit and will take approximately 1½ hours. During this visit you will:

- Have your height and weight measured. Your body composition (lean body mass and percentage body fat) will be measured using the DXA.
- Have a DXA scan to measure the density of your bones.
- Complete some simple muscle strength tests.
- Have a blood test taken by a trained phlebotomist to measure vitamin D levels.

What are the benefits and risks of taking part in this study?

There will be no charges made for any of the tests that you undertake. You will receive information regarding your body composition measurements, vitamin D levels and you will be checked for iron-deficiency anaemia. We will provide you with some useful resources on nutrition for your sport. The principal benefit of taking part in this study is that you will contribute to a study and our understanding of how vitamin D supplementation improves muscle strength in dancers and gymnasts. We will also provide you with information on how to maintain a healthy vitamin D status through careful sun exposure or supplementation. Vitamin D supplements are available both by prescription from your GP, or over the counter in chemists and health food shops.

We will use the Hologic DEXA machine to estimate bone mineral density and bone mineral content of your hip and femur. The DXA has X-ray beams at 2 different energies and while no dose of radiation is harmless this dose is very low and unlikely to cause harm. The total effective dose of radiation to which you will be exposed to is 10 microsieverts (μSv), which is much lower than the range normally used in medical diagnostics. To place in perspective, the amount of radiation you are exposed to during a flight to the United Kingdom return is 100 μSv and from a dental X-ray 50 μSv . The room is private and you can enter the DXA room in complete privacy. We will provide you with a gown to wear during this measurement. The staff who do this are certified and the bone scan will be assessed and approved by our consultant Radiologist. If there is a problem with your bone density, you will receive a letter of referral to your GP from the Radiologist.

If at any stage during the study you suffer from bone pain which could be attributed to a stress fracture, and which causes you to miss three or more training/lesson/rehearsal sessions, you will be offered a free consultation with the study doctor for a clinical assessment.

During your first visit we will take a total of 10 mL (2 tsp) of blood. At the 2nd and 3rd visit 6ml (1 tsp) of blood will be taken. Some people may have a fear of having a blood sample taken or experience discomfort when the blood samples are taken. Occasionally a slight bruising will result. The bruising usually disappears within a day or two. Blood samples will be taken by a trained phlebotomist. There may be social or cultural discomfort from having body composition measurements done or a blood sample taken, however, privacy will be ensured, and you will be treated with respect. You may also be accompanied by a support person if preferred.

The total time that you will have to invest in this research project is approximately 14.5 hours including the three visits; the time spent recording your food intake; and the time taken to answer simple questions regarding your training and health status.

All participants will be provided with \$30 worth of petrol vouchers for completing the study.

Data Management

The data will be used only for the purposes of this project and no individual will be identified. Only the investigators and administrators of the study will have access to personal information and this will be kept secure and strictly confidential. Participants will be identified only by a study identification number. Results of this project may be published or presented at conferences or seminars. No individual will be able to be identified.

Vitamin D and muscle strength in female dancers. V1, 24/01/12.

At the end of the study the list of participants and their study identification number will be disposed of. Any raw data on which the results of this project depend will be retained in secure storage for 10 years after participants have turned 16 years of age, after which it will be destroyed. All participants will be sent a summary of the project findings at the conclusion of the study.

Support Processes

If we find that your blood results are outside normal parameters you will be advised to talk to your medical practitioner or at your request, we can send your results directly to them. If as a result of this you require additional blood tests you may be liable for any costs.

Project Contacts

If you have any further questions or concerns about the project either now or in the future, please contact either Sarah Mitchell or Pamela von Hurst.

Committee Approval Statement

This project has been reviewed and approved by the Health and Disability Human Ethics Committee, Northern Y: Application NTY/12/02/013.

Compensation for Injury

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic and your claim will be assessed by ACC in accordance with the Accident Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury.

If your ACC claim is not accepted you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

APPENDIX 2



MASSEY UNIVERSITY
COLLEGE OF SCIENCES
TE WĀHANGA PŪTAIAO

Vitamin D and muscle strength in female dancers The Sunflower Study

PARTICIPANT CONSENT FORM – INDIVIDUAL (16 years and over)

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I agree to participate in this study under the conditions set out in the Information Sheet.

Signature:

Date:

.....

Full Name - printed

.....

Vitamin D and muscle strength in female dancers. V1, 24/01/12.

APPENDIX 3



MASSEY UNIVERSITY
COLLEGE OF SCIENCES
TE WĀHANGA PŪTAIAO

Vitamin D and muscle strength in female dancers The Sunflower Study

PARENTAL CONSENT FORM - INDIVIDUAL (for participants under the age of 16 years)

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I agree to allow my child to participate in this study under the conditions set out in the Information Sheet.

Signature: _____ **Date:** _____

Full Name - printed _____

Name of child _____

APPENDIX 4



MASSEY UNIVERSITY

**Are you a female dancer or
gymnast ?**
**Training for at least one-hour
5x/week?**
Are you aged 13-18 years old ?

Then Participate in the
Vitamin D and muscle strength in female dancers & gymnasts

Sunflower Study

What you would need to do:

- Visit Massey University on three occasions
- Keep a record of your diet for four days
- Take a vitamin D supplement or a placebo for 6 -12 months
- Answer some simple questions about your training and health status

INTERESTED ??

What you will gain from taking part:

- Individual results on your body composition, bone density and diet
- A \$30 petrol voucher



CONTACT US

Sarah Mitchell 021 160 5949
Dr Pamela von Hurst (09) 414 0800 extension 41205

Email sunflower@massey.ac.nz



www.facebook.com/sunflowerstudy

Vitamin D and muscle strength in female dancers. V1, 24/01/12.

Human Nutrition Research, Institute of Food, Nutrition and Human Health,
Massey University, Albany, Auckland, New Zealand

APPENDIX 5

Sunflower - screening questionnaire

[Exit this survey](#)

Sunflower Study

We are looking for female dancers and gymnasts between the ages of 13 to 18 years old. If you would like to find out more about the study, please complete this online questionnaire. We will contact you to tell you more about the study but this doesn't mean you have to take part.

*1. Please complete the following details

First name
Family name
Email
Contact phone number

2. Does your parent agree for you to take part in the study

Yes No Don't know

*3. What is your date of birth?

DOB DD / MM / YYYY

4. Do you currently train in

- Ballet
 Modern/contemporary
 Both ballet and modern
 Gymnastics

Other (please specify)

5. How often do you train?

Less than 1 hour per week 1-2 hours per week 3-4 hours per week 5 hours or more per week

Other (please specify)

6. Are you able to remove all jewellery and piercings.

Yes

No

7. Do you have any medical conditions or injuries which may affect you taking part?

Yes

No

Please tell us about your medical condition or injury

8. Are you taking any vitamin D supplements or cod liver oil? (or you have taken any within the last 2 months please tick not sure)

Yes

No

Not sure

9. Would you like to be notified about future research studies within the Institute of Food, Nutrition and Human Health?

Yes

No

Thanks for completing the questionnaire

We will be in contact to tell you more about the study

In the meantime check out our page on facebook

Done

Powered by **SurveyMonkey**
Check out our [sample surveys](#) and create your own now!

APPENDIX 6

WARM UP

2 minutes to perform any warm up activity and stretches of your choice.

Then carry out the following exercises at the barre:

1. Two demi pli  and two grande pli  in first, second, fourth and fifth positions. Repeat on other side.
2. Two tendu front, side and back. Repeat on the other side.
3. Four ronde de jame en dehors and en dedans. Repeat on the other side.
4. Two slow grande battement front, side, back and side. Repeat on the other side.
5. Balance on demi pointe first position of the feet, arms in fifth position for 16 counts.

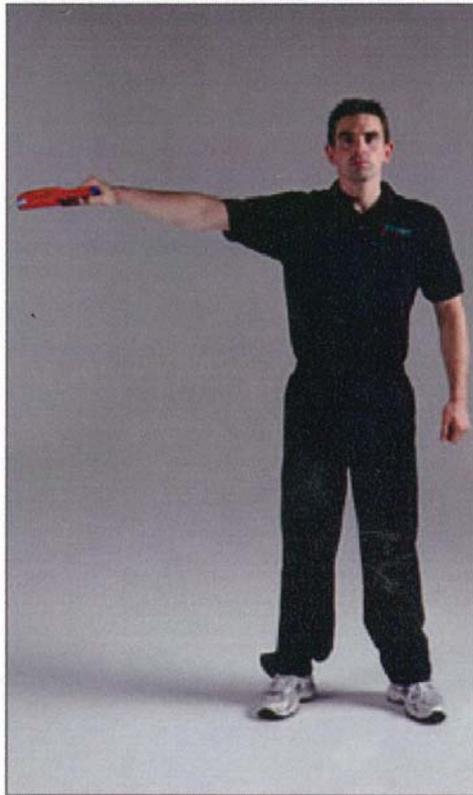
A further 2 minutes to perform any warm up activity and stretches of your choice.

APPENDIX 7

HAND GRIP DYNAMOMETER

Give the following instructions to the participant.

- Hold the dynamometer in your RIGHT hand (record which hand is dominant)
- Hold your arm out perpendicular to the body (see picture) and squeeze the handle as tightly as you can
- Relax
- You will perform 2 attempts and we will record your highest score.
- Repeat for your LEFT hand



APPENDIX 8

COUNTER MOVEMENT JUMP

Give the following instructions to the participant.

- Stand upright on the mat with your weight evenly distributed over both feet.
- Place hands on the hips, where they must remain throughout the test.
- When ready, squat down and then immediately jump vertically as high as possible, landing back on the mat on both feet at the same time.
- The take-off must be from both feet, with no initial steps or shuffling.
- Do not pause at the base of the squat or tuck your legs up.
- You will perform 3 attempts and we will record your highest score



APPENDIX 9

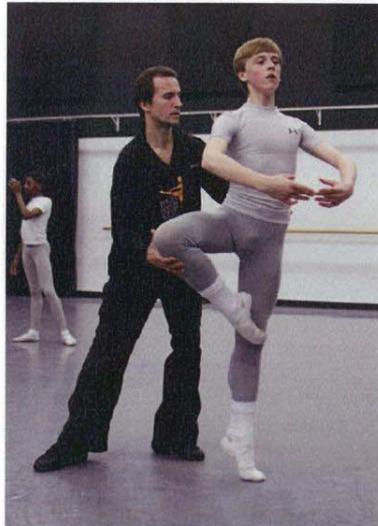
BALANCE TEST

Set up force plate (see below)

Give the following instructions to the participant.

- Stand on the force plate with the ball of the foot of your RIGHT leg over the cross.
- When you are ready bring your arms to first position and bring your other leg up to retiré position
- When you are ready raise up to demi-pointe
- You are to hold the position with best form for 30s
- The time will start when you move into the demi-pointe position

Repeat for LEFT leg.



Set up

Have subject stand OFF force plate.

Click Start, Tare and then Arm.

Subject can then proceed as above.

Click SPACE BAR to begin recording. Data will be recorded for exactly 30s (see progress bar across top of screen)

APPENDIX 10



MASSEY UNIVERSITY
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TE WAHANGA PŪTAIAO

Participant ID number:.....

SUNFLOWER STUDY Participant Information form and medical history

Section 1: Demographics and lifestyle

1) Are you aged between 13 to 18 years?

Yes

No

If the answer is no please speak to a researcher

2) Which ethnic group do you belong to? Tick whichever applies to you (you may tick more than one box).

- New Zealand European
- Maori
- Samoan
- Cook Island Maori
- Tongan
- Niuean
- Chinese
- Indian
- Other *Please state which ethnicity* _____

3) Which country were you born in?

4) If you live in New Zealand but were not born here, when did you first arrive to live in New Zealand?

Month (e.g. February) _____ Year (e.g.2000) _____

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5) How would you describe your eating pattern?

Eat a variety of all foods, including animal products

Eat eggs, dairy, fish and chicken but avoid other meats

Eat eggs and dairy products but avoid all meats and fish

Eat eggs but avoid dairy products, all meats and fish

Eat no animal products

Other

Please specify _____

6) Do you follow any diet for cultural or religious reasons?

Yes No

If yes, what type of diet do you follow? _____

7) Have you dieted strictly in the last year?

Yes No

Please comment _____

9) Do you smoke?

Yes No

11) Do you have any food intolerances or allergies?

Yes No

Please specify

Section 3: Training

1) Please describe your usual weekly training?

	Details of type of training	Time
example	Lesson, rehearsal, training run	2 hours 30 minutes
Monday		
Tuesday		
Wednesday		
Thursday		
Friday		
Saturday		
Sunday		

2) On average, how much of your daily training time is spent outdoors?

Section 3: Health

1) Do you have or have you ever suffered from any acute or chronic illness (e.g. asthma, Crohn's disease, frequent colds)?

Yes No

Details on diagnosis, including when and by whom, and any treatment received

2) Have you ever suffered from low iron stores, iron deficiency or iron deficiency anaemia?

Yes No

Details on diagnosis, including when and by whom, and any treatment received

3) Do you have or have you had any medical condition which has resulted in blood loss?

Yes No

If yes, please describe and give approximate dates

4) Have you had any blood loss (other than your periods or nose bleeds) such as wounds, regular scratches from contact sports, blood in stools or urine in the past year?

Yes No

If yes, please describe

5) Have you ever suffered from stress fractures?

Yes No

(Details on diagnosis (including when and by whom, severity and any treatment received)

6) Does anyone in your immediate family (that is, blood relatives) have osteoporosis that you are aware of?

Yes No

If yes, please tell us the gender of the person, and the age at which they were diagnosed and their relationship to you?

7) Do you currently have periods?

Yes No

If yes, at what age did you start menstruating? When did your last period start?

If no, have you ever menstruated before? (please provide details regarding when this occurred, for how long, and the age at which you first started menstruating)

8) Are you currently taking any form of contraception, either oral or by injection?

Yes No

If yes:

What is the name of the contraceptive? _____

How long have you been taking it? _____

Your reason for taking it – tick one or more of the following:

- Contraception
- To regulate your periods
- For skin health
- To control dysfunctional bleeding

9) Are you currently taking any medication (including traditional or homeopathic medication; but not including contraception or nutritional supplements)?

Yes No

If yes, please state what medication you are taking, how long you have been taking this medication for and the reason for taking it

Section 4: Supplements

1) Did you take any vitamin and/or mineral capsules/tablets at any time during the past year? Yes No

If yes, please list the brand name of the supplement, the type of supplement, the number taken, the frequency of intake and the dose (including units) and the reason for taking?

eg. Healtheries Iron & vitamin C, 1 taken every 2nd day, ferrous gluconate (170mg) providing elemental iron (20mg) and vitamin C (40mg), taken because I was feeling tired

If you can't remember the details of the supplements, please send us an email with the brand, type and dose so we can record these on your file.

Email requested Email received

2) Did you take any sports supplements at any time during the past year (including sports drinks, sports gels sports bars, liquid meal supplements, protein supplements) ? Yes No

If yes, please list the brand name / type of sports supplement, the frequency of intake and the dose (including units), and the reason for taking.

eg. Powerade (750ml) taken every day during evening training (5x/week) for the past 3 years

3) Did you take any other dietary supplements during the past year? (for example, omega-3 tablets, evening primrose oil, performance enhancers)

Yes No

If yes, please list the brand name of the supplement, the type of supplement, the number taken and the frequency of intake and the dose (including units)?

eg. Omega 3 supplements, 1 capsule taken per day for the past 3 months, contains 180mg eicosapentanoic acid & 120mg docosahexaenoic acid 1000mg, took for aching joints

APPENDIX 11

Sunflower training diary

[Exit this survey](#)

Sunflower Training Diary

Thanks for doing this!

Please feel free to contact us at anytime and for any reason – we appreciate your help with this study and we want to support you.

Contacting us

Sarah Mitchell on 021 160 5949
Dr Pamela von Hurst on (09) 414 0800 ext 41205

or email us on:
sunflower@massey.ac.nz

* 1. What is your Study ID? (eg Lizzie007)

* 2. Please complete

Week beginning DD MM YYYY
 / /

* 3. Please record the amount of time spent training (this should include ballet lessons, practice and related exercise eg pilates)

	Didn't train	Less than 1 hour	1 to 4 hours	More than 4 hours
Monday	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Tuesday	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Wednesday	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Thursday	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Friday	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Saturday	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sunday	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

If you want to tell us anything else about your training you can provide the details here