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Influence of simulated sunlight and oral supplementation

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VITAMIN D AND EXERCISE PERFORMANCE: INFLUENCE OF SIMULATED SUNLIGHT AND ORAL SUPPLEMENTATION

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Summary

Vitamin D is synthesised endogenously within the epidermal layer of the skin on exposure to ultraviolet B radiation (UVB). Therefore, inadequate exposure to UVB results in vitamin D insufficiency, for example during winter at high latitudes when the availability of solar UVB is negligible, or due to living indoors for the majority of sunlight hours and heeding recommendations to cover-up from the sun. Avoiding low vitamin D status (serum 25-hydroxyvitamin D (25(OH)D)) is accepted as important for bone health because the primary physiological function of vitamin D is to maintain calcium and phosphorus homeostasis. The identification of the vitamin D receptor in various tissues (including the cardiovascular system and skeletal muscle), has led to recent evidence suggesting vitamin D may also affect exercise performance. This thesis set out to investigate the potential role of vitamin D in optimising exercise performance among young healthy adults. First, in a prospective cohort study (n = 967; **Chapter 3**), it was demonstrated that vitamin D insufficiency was prevalent among male and female military recruits during winter (91% of males and 64% of females: 25(OH)D <50 nmol·L^{-1}), and vitamin D status was positively associated with endurance exercise performance ($P < 0.01$, ΔR^2 = 0.03–0.06; 1.5-mile run time was ~half-a-second faster for every 1 nmol·L^{-1} increase in 25(OH)D). No significant effects on muscular strength or power emerged ($P > 0.05$). Next, to restore and then maintain vitamin D sufficiency from its winter nadir (n = 33), closely matched vitamin D supplementation protocols using safe simulated sunlight or oral vitamin D_3 were demonstrated to be effective in a 12-week randomised placebo controlled trial (89% at week 5 and 100% at week 12: 25(OH)D ≥50 nmol·L^{-1}; **Chapter 4**). Finally, in a randomised placebo controlled trial among 137 male military recruits, despite 12-weeks simulated sunlight in accordance with recommendations on safe sunlight exposure and oral vitamin D_3 supplementation restoring and then maintaining vitamin D sufficiency in almost all (97%), there was no effect on exercise performance ($P > 0.05$; **Chapter 5**). This lack of beneficial effect was observed despite the majority of supplemented individuals achieving 25(OH)D ≥75 nmol·L^{-1}, which has been proposed to be optimal. These findings suggest vitamin D does not directly affect exercise performance.

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Publications

The following publication arose from work presented within this thesis

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Conference proceedings

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Table of contents

Thesis format

This thesis contains a literature review (**Chapter 1**) which starts by outlining the background and main research aims. The general methods section (**Chapter 2**) then provides details of the procedures and measures that have been used in two or more of the subsequent experimental chapters. Three experimental studies form the main focus of the thesis. The first field-based study examines the relationship between vitamin D status and exercise performance in 967 young, healthy male and female military recruits (**Chapter 3**). With vitamin D insufficiency widespread among otherwise healthy adults, the second study investigates the effect of safe simulated sunlight and oral vitamin D_3 supplementation in restoring and then maintaining vitamin D sufficiency from its winter nadir, in a laboratory-based randomised placebo controlled trial **(Chapter 4)**. The third study then investigates the effect of vitamin D supplementation on exercise performance in 137 young healthy male military recruits, using safe simulated sunlight or oral vitamin D_3 in a field-based randomised placebo controlled trial (**Chapter 5**). Finally, the general discussion (**Chapter 6**) summarises the main findings and aims to critically analyse these with consideration of the recognised limitations and proposed areas for future research. A list of abbreviations, tables and figures are included before **Chapter 1**. **Bold text** is used to refer to chapters or sections within this thesis.

List of tables

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List of abbreviations

°C	degree Celsius
°N	degree north
°S	degree south
Δ	change
λ	wavelength
1,24,25$(OH)_3$D	1,24,25-trihydroxyvitamin D
1,25$(OH)_2$D	1,25-dihydroxyvitamin D
24,25$(OH)_2$D	24,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
ACSM	American College of Sports Medicine
ANOVA	analysis of variance
BMI	body mass index
CAMK	calmodulin-dependent protein kinase
cAMP	cyclic adenosine monophosphate
CI	confidence interval
cm	centimetre
CV	coefficient of variation
DNA	Deoxyribonucleic acid
DXA	dual-energy x-ray absorptiometry
EFSA	European Food Safety Authority
FFQ	food frequency questionnaire
g	gram
g	gravitational acceleration
HPLC	high pressure liquid chromatography
IGF1	insulin-like growth factor 1
IGFBP-3	insulin-like growth factor binding protein 3

IOM	Institute of Medicine
IU	international unit
J	joule
kg	kilogram
km	kilometre
L	litre
LC-MS/MS	liquid chromatography-tandem mass spectrometry
m	metre
mmHg	millimetre of mercury
MAPK	mitogen-activated protein kinase
MED	minimal erythemal dose
nm	nanometre
nmol	nanomole
NO	nitric oxide
ORAL	oral vitamin D_3
ORAL-P	oral placebo
ORAL→P	restoration phase oral vitamin D_3 then maintenance phase placebo
PKA	protein kinase A
pmol	picomole
PSF	polysulphone
PTH	parathyroid hormone
RXR	retinoid X receptor
s	second
SACN	Scientific Advisory Committee on Nutrition
SD	standard deviation
SED	standard erythemal dose
SSR	simulated sunlight

SSR-P	placebo simulated sunlight
SSR→P	restoration phase simulated sunlight then maintenance phase placebo
UK	United Kingdom of Great Britain and Northern Ireland
USA	United States of America
UV	ultraviolet
UVA	ultraviolet A radiation
UVB	ultraviolet B radiation
UVR	ultraviolet radiation
VDR	vitamin D receptor
VDRE	vitamin D response elements
VEGF	vascular endothelial growth factor
$\dot{V}O_{2max}$	maximal oxygen consumption
$\dot{V}O_{2peak}$	peak oxygen consumption
W	watt
y	year

CHAPTER ONE

Literature review

1.1 Discovery of vitamin D: a brief history

At the beginning of the 20th century, rickets had become widespread throughout industrialised Europe and North America, with 80–90% of children reported to have this juvenile bone disease; now known to be caused by vitamin D deficiency (Holick, 2006). With cod-liver oil the preferred medicinal treatment for rickets, it was during the early 1900s that the existence of an essential dietary factor within cod-liver oil was first proposed, and initially named fat-soluble factor A (McCollum and Davis, 1914). The elimination of fat-soluble factor A from cod-liver oil did not inhibit cod-liver oil's anti-rachitic ability, hence it was concluded that fat-soluble factor A must consist of at least two entities: one later named vitamin A and the other vitamin D (McCollum et al., 1922). Also during the early 20th century, it was observed that sunlight was as effective as cod-liver oil in curing rickets (Chick et al., 1922), and in 1925 it was first hypothesised that solar rays and artificial radiation could convert cholesterol within the skin into an anti-rachitic factor (Hess et al., 1925). In 1937 it was confirmed that dermal 7-dehydrocholesterol could indeed be transformed by irradiation to an anti-rachitic substance, named cholecalciferol (vitamin D_3; Windaus and Bock, 1937). Towards the end of the 20th century, Michael Holick and colleagues were the first to describe the exact sequence of steps leading to the photo-production of vitamin D_3 in the skin (Holick et al., 1980). It is now known the primary physiological function of vitamin D is to maintain calcium and phosphorus homeostasis, and therefore support bone mineralisation (Holick, 2006).

1.2 Sources, synthesis and metabolism of vitamin D

Endogenous synthesis within the epidermal layer of the skin and dietary intake are the two sources of vitamin D for humans (Holick, 2007), with the former providing ~90% of a typical

human's total intake (Macdonald, 2013). Within the epidermis, solar ultraviolet B radiation (UVB; wavelength (λ): 290–315 nm) promotes photolytic cleavage of 7-dehydrocholesterol into pre-vitamin D_3, and via a heat dependent process, inert pre-vitamin D_3 then undergoes isomerisation to form vitamin D_3 (Figure 1.1; Holick, 1994). To prevent overproduction and toxicity, UVB itself regulates vitamin D_3 synthesis by converting pre-vitamin D_3 into one of two biologically inactive photoisomers (lumisterol or tachysterol). Thus, whether exposure of 7-dehydrocholesterol to UVB is for ~30-minutes or several hours, no more than 10–15% of the initial cutaneous concentration is converted to pre-vitamin D_3 (Holick, 1995). Similarly, once vitamin D_3 is synthesised in the skin, if it does not escape to the circulation it is converted by UVB into one of three biologically inert photoisomers (5,6-trans-cholecalciferol, supersterol I or supersterol II; Holick, 1994). Two forms of vitamin D can be obtained from dietary sources: vitamin D_2 (ergocalciferol), present in some plants and fungi; and vitamin D_3 which is found in foods of animal origin, and fortified cereals and dairy products (Holick, 2007). Over-the-counter oral vitamin D supplements may be in either form, with vitamin D_2 and D_3 typically manufactured through the ultraviolet (UV) irradiation of ergosterol in yeast, and 7-dehydrocholesterol in lanolin, respectively. Dietary vitamin D is lipid soluble and absorbed with long-chain triglycerides in the small intestine (Haddad et al., 1993). Ingested vitamin D is incorporated into chylomicrons within the enterocytes and transported by the lymphatic system into the venous circulation (Holick, 2007).

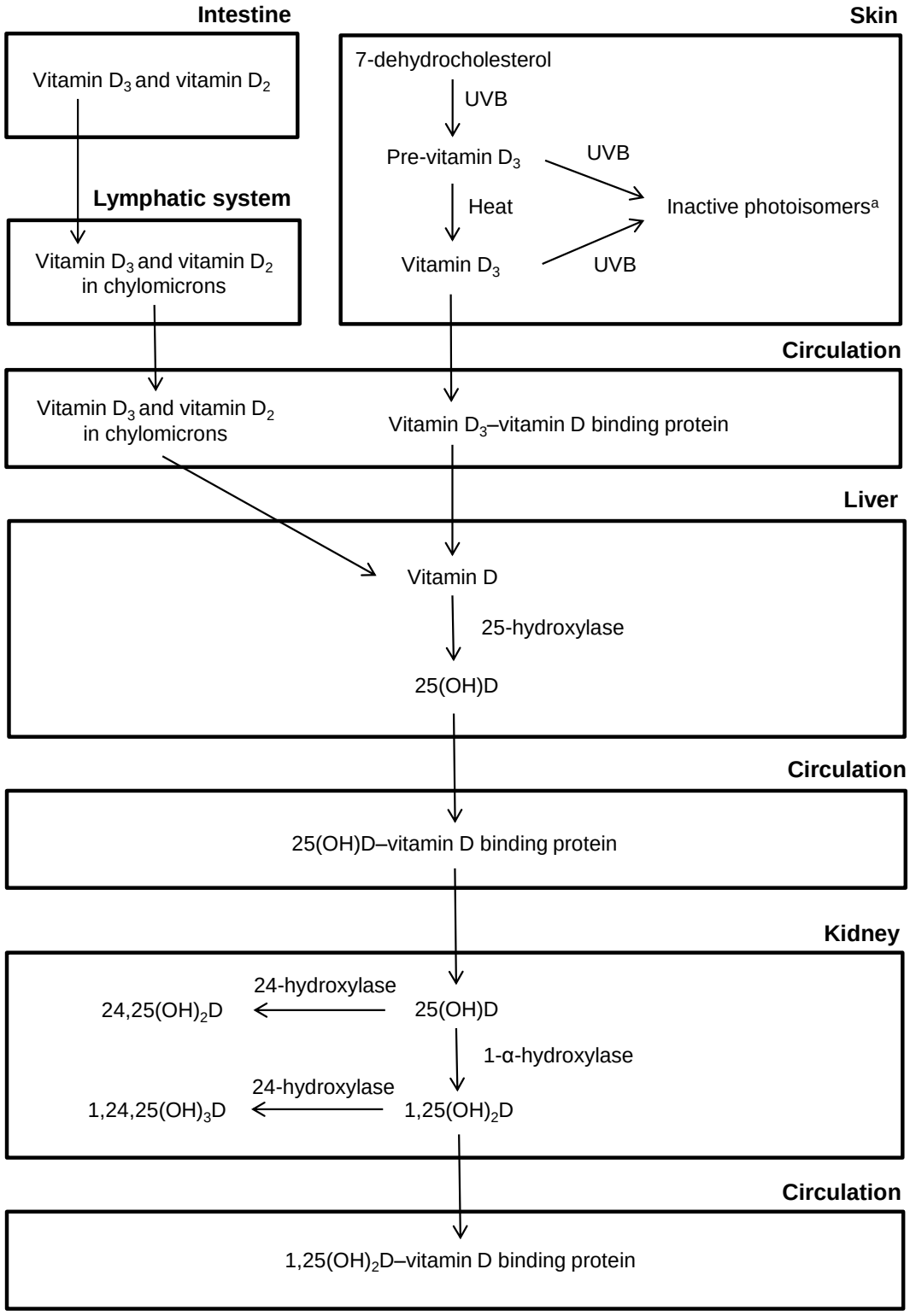

Figure 1.1. Vitamin D synthesis and metabolism. UVB, ultraviolet B radiation; 25(OH)D, 25-hydroxyvitamin D; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; $24,25(OH)_2D$, 24,25-dihydroxyvitamin D; $1,24,25(OH)_3D$, 1,24,25-trihydroxyvitamin D; [a] lumisterol, tachysterol, 5,6-trans-cholecalciferol, supersterol I and supersterol II.

Following cutaneous synthesis or dietary intake, vitamin D bound to vitamin D-binding protein or within chylomicrons, respectively, is transported to the liver via the circulation (Figure 1.1; Holick et al., 1980). Vitamin D must undergo two successive hydroxylations, first within the liver in the presence of the enzyme 25-hydroxylase to form 25-hydroxyvitamin D (25(OH)D); and then in the kidney catalysed by 1-α-hydroxylase to form the biologically active 1,25-dihydroxyvitamin D ($1,25(OH)_2D$; Holick, 1981). Circulating $1,25(OH)_2D$ has a half-life of ~4 hours (Zerwekh, 2008). With a half-life of ~3 weeks, 25(OH)D is the major circulating metabolite of vitamin D, and the primary indicator of vitamin D status (Zerwekh, 2008; Heaney, 2011). During times of normo- or hyper-calcaemia, 24-hydroxylase catalyses the conversion of 25(OH)D to the biologically inactive 24,25-dihydroxyvitamin D ($24,25(OH)_2D$), the concentration of which is often expressed in ratio to 25(OH)D (Holick et al., 1972). To limit excessive vitamin D activity and reduce the likelihood of hypercalcaemia, $1,25(OH)_2D$ concentrations are tightly regulated in a negative feedback loop. Circulating $1,25(OH)_2D$ induces 24-hydroxylase, which (i) metabolises $1,25(OH)_2D$ to the biologically inactive trihydroxyvitamin D ($1,24,25(OH)_3D$); and (ii) inhibits renal 1-α-hydroxylase, to reduce further production of the active vitamin D metabolite. The renal production of $1,25(OH)_2D$ is also tightly regulated by circulating parathyroid hormone (PTH), calcium and phosphorus concentrations (Holick, 2007). Outside the kidney, the enzyme 1-α-hydroxylase is also expressed by tissues including the skin, pancreas, brain, and colon (Zehnder et al., 2001); cells of the immune system (Hewison, 2010); and skeletal muscle (Srikuea et al., 2012). Therefore, 25(OH)D can be converted to $1,25(OH)_2D$ locally, and act in an autocrine or paracrine fashion.

The classical function of vitamin D, in regulating calcium homeostasis and promoting bone health, is achieved by augmenting the absorption of calcium within the duodenum; promoting

renal calcium reabsorption; and stimulating osteoclast differentiation and bone resorption to release calcium into the circulation (Holick, 2007). Following the identification of the vitamin D receptor (VDR) in numerous tissues and cells throughout the body (Rosen et al., 2012), evidence suggests vitamin D can also regulate aspects of innate and acquired immunity (Hewison, 2012), cardiovascular health (Judd and Tangpricha, 2009), and skeletal muscle metabolism (Girgis et al., 2013). To exert its effects (reviewed in **section 1.8** with a focus on mechanisms relevant to exercise performance), 1,25$(OH)_2$D passes through the plasma membrane of target cells and binds with the VDR, a nuclear receptor and ligand-activated transcription factor. The VDR bound with 1,25$(OH)_2$D then forms a heterodimer with retinoid X receptor (RXR) and translocates to the nucleus, binding with vitamin D response elements (VDRE) to induce the expression of vitamin D responsive genes, of which there are more than 900 (Kongsbak et al., 2013). Some actions of 1,25$(OH)_2$D are more immediate, mediated by a membrane bound VDR and therefore follow non-genomic pathways (Rosen et al., 2012).

1.3 Measurement of vitamin D metabolites

As the major circulating vitamin D metabolite, serum or plasma 25(OH)D concentrations are widely used in research and clinical practice to quantify vitamin D status. The concentration of 25(OH)D in serum samples is stable given reliable measurements have been made even when stored at room temperature and after up-to four freeze-thaw cycles (Antoniucci et al., 2005; Wielders and Wijnberg, 2009). The concentration of 25(OH)D can be measured using immunoassay; competitive protein binding assay; high pressure liquid chromatography (HPLC); or liquid chromatography-tandem mass spectrometry (LC-MS/MS; Fraser and Milan, 2013). Unlike most commercial immunoassays, LC-MS/MS can accurately measure 25$(OH)D_2$ and 25$(OH)D_3$ simultaneously (total 25(OH)D = 25$(OH)D_2$ + 25$(OH)D_3$) and is

therefore the gold standard method of assessing vitamin D status (de la Hunty et al., 2010). Vitamin D metabolites, 24,25$(OH)_2D_2$ and 24,25$(OH)_2D_3$ can also be reliably quantified using LC-MS/MS. Biologically active 1,25$(OH)_2D$ is typically measured using enzyme-linked immunosorbent assay or chemiluminescent immunoassay, with its measurement particularly challenging because circulating concentrations are a thousand-fold lower than its precursor 25(OH)D (Fraser and Milan, 2013).

1.4 Factors that affect 25(OH)D concentrations

1.4.1 Exogenous

Latitude, season, time-of-day, altitude, ozone layer density, pollution, and cloud cover all influence the quantity of solar UVB photons reaching the earth's surface and consequently the potential for cutaneous vitamin D_3 synthesis (Holick, 2007). With increasing distance from the equator towards the polar regions, solar UVB photons must travel a greater distance to reach the earth as the solar zenith angle increases. Therefore, at high latitudes the probability of absorption or scattering of UVB is increased, reducing the availability of UVB photons that can penetrate the skin (Engelsen, 2010). A larger solar zenith angle during winter *vs.* summer, and at dawn or dusk *vs.* midday, results in a decreased availability of UVB at the skin and reduced potential for cutaneous vitamin D synthesis (Holick, 1995). For example, at 52°N the photosynthesis of pre-vitamin D_3 has been shown to cease from October to April (Webb et al., 1988). The intensity of solar ultraviolet radiation (UVR) is estimated to increase by ~7% for every 1,000 m above sea level because at high altitude the thinner atmosphere filters less UVR (Engelsen, 2010). Therefore the intensity of UVB and potential for vitamin D_3 synthesis increases with altitude. The stratospheric ozone layer absorbs solar UVB, thus in areas where the ozone layer is depleted, a larger number of high-energy UVB photons can be transmitted to the earth's surface, increasing the potential for

vitamin D_3 synthesis (Norval et al., 2011). Finally, air pollution and cloud can absorb UVB photons, reducing their availability for cutaneous photosynthesis of vitamin D_3 (Holick, 1995). The aforementioned factors affect the number of UVB photons reaching the earth. Crucially however, clothing (long trousers and sleeves), sunscreen (applied thickly and frequently) and behaviour such as seeking shade, all reduce the skin surface area available for penetration by solar UVB and limit subsequent cutaneous vitamin D_3 synthesis (Matsuoka et al., 1987; Matsuoka et al., 1992).

1.4.2 Endogenous

Melanin absorbs UVB in competition with 7-dehydrocholesterol; consequently, individuals with more skin pigment have a reduced capacity to synthesise vitamin D_3 (Loomis, 1967; Clemens et al., 1982; Armas et al., 2007). For example, 6-weeks of simulated summer sunlight (also known as solar simulated radiation (SSR)) increased serum 25(OH)D in south Asian participants (sun-reactive skin type V; +11 $nmol \cdot L^{-1}$) by less than half the concentration observed in white-skinned individuals (sun-reactive skin type I–IV; +26 $nmol \cdot L^{-1}$; Farrar et al., 2011). The epidermal structural protein filaggrin is the main endogenous UVB filter in individuals with light skin pigmentation, filtering by a process mediated by trans-urocanic acid production. Therefore, similar to melanin, individuals with increased expression of filaggrin have a reduced capacity to synthesise vitamin D_3 (Thyssen et al., 2014). With age, the availability of 7-dehydrocholesterol in the epidermis is reduced and circulating vitamin D binding protein concentrations are lower; hence the capacity to synthesise vitamin D_3 decreases with age (Holick et al., 1989; MacLaughlin and Holick, 1985; Pop et al., 2015).

With the liver and kidney the site of key vitamin D hydroxylases, liver dysfunction or failure impedes the synthesis of 25(OH)D; and crucially, chronic kidney disease reduces the synthesis and availability of biologically active $1,25(OH)_2D$ (Holick, 2007). Heritable disorders can also affect vitamin D status, for example mutations in genes CYP2R1 and CYP27B1, which code for 25-hydroxylase and 1-α-hydroxylase, respectively (Bu et al., 2010), can cause conditions such as rickets via reduced renal $1,25(OH)_2D$ synthesis (Kitanaka et al., 1998). Variants in vitamin D binding protein caused by single nucleotide polymorphisms can also can affect vitamin D status (Fu et al., 2009). In addition, acquired disorders such as primary hyperparathyroidism and hyperthyroidism both lead to increased metabolism of 25(OH)D resulting in lower 25(OH)D concentrations (Holick, 2007).

Body composition also affects vitamin D status, with total body fat inversely associated with serum 25(OH)D and $1,25(OH)_2D$ (Snijder et al., 2005; Parikh et al., 2004). Wortsman et al. (2000) demonstrated the percentage conversion of cutaneous 7-dehydrocholesterol to pre-vitamin D_3 was not different between obese and non-obese individuals (9.4% *vs.* 9.6%, respectively in young; and 7.6% *vs.* 7.3%, respectively in older individuals). However, 24 hours after exposure to UVB irradiation, obese individuals' increase in serum vitamin D_3 was 57% less than non-obese individuals. Therefore, obesity does not affect the capacity of the skin to synthesise vitamin D_3, but may impede the release of vitamin D_3 into the circulation. Oral supplementation of vitamin D deficient rats with radio-labelled vitamin D_3 demonstrated 80% of administered vitamin D was rapidly deposited in adipose tissue, and is therefore the major storage site for vitamin D_3 (Rosenstreich et al., 1971). It has been hypothesised that subcutaneous fat sequesters more cutaneous synthesised vitamin D_3 in obese than non-obese individuals due to larger pools of adipose tissue in obese individuals (Wortsman et al., 2000).

1.4.3 Dietary

Given the seasonal variation in the availability of UVB at temperate latitudes (between the tropics and polar regions), the proportion of vitamin D gained from diet will vary throughout the year. For example, even if diet remains unchanged, dietary intake will contribute a greater proportion of vitamin D intake when the availability of ambient UVB is diminished during winter. Few foods naturally contain vitamin D, with the best sources oily or fatty fish (for example, ~600–1,000 IU·100 g^{-1} salmon) and eggs (~20 IU·$yolk^{-1}$; Holick, 2007). Fortified foods and vitamin D supplements including cod-liver oil also contribute to the proportion of vitamin D obtained from diet (Macdonald, 2013). Independent of the foods and supplements an individual consumes, intestinal fat malabsorption syndromes can impair the absorption of vitamin D from diet and increase the risk of vitamin D deficiency (Holick, 2007).

1.5 Vitamin D status

1.5.1 Definition

Definitions of deficiency, sufficiency and potentially optimal vitamin D status remain subject to debate. No global consensus exists due to the large number of diverse outcome measures, and lack of empirical evidence to support thresholds for specific outcomes, such as immune function or exercise performance. However, the Institute of Medicine (IOM) and European Food Safety Authority (EFSA) agree that avoiding low serum 25(OH)D is essential for musculoskeletal health, and both recommend maintaining serum 25(OH)D ≥50 nmol·L^{-1} (Institute of Medicine, 2011; European Food Safety Authority, 2016). The IOM judge there to be sufficient evidence to categorise vitamin D status (serum 25(OH)D) as deficient, insufficient, and sufficient with reference to skeletal health (Table 1.1; with these thresholds used in **Chapters 3–5**). In their 2011 report, the IOM conclude there to be no benefit of achieving serum concentrations of 25(OH)D above 75 nmol·L^{-1}. In contrast, the Endocrine

Society recommend achieving 25(OH)D concentrations ≥75 nmol·L^{-1} to maximise the effect of vitamin D on calcium, bone and muscle metabolism (Holick et al., 2011). The American College of Sports Medicine (ACSM) reported vitamin D sufficiency as 25(OH)D >75 nmol·L^{-1}, with concentrations as high as 125 nmol·L^{-1} potentially beneficial for optimal training induced adaptations (Thomas et al., 2016). However, the ACSM's position stand concludes that further research is required to determine the potentially important role of vitamin D for exercise performance, before optimal vitamin D thresholds can be determined, and supplementation recommendations be refined (Thomas et al., 2016). Some authors have proposed higher circulating 25(OH)D concentrations should be considered optimal (for example ≥100 nmol·L^{-1}; Zitterman, 2003; or ≥120 nmol·L^{-1}; Heaney, 2011); however, these recommendations lack supporting evidence and have been made simply because individuals living and working outdoors in sun rich environments sometimes report 25(OH)D concentrations above these thresholds (Zittermann, 2003). Instead, the IOM (2011) recommend avoiding concentrations of serum 25(OH)D above 125 nmol·L^{-1}.

Table 1.1. Vitamin D status classification recommended by Institute of Medicine (2011) and possible optimal status (Holick et al., 2011; Thomas et al., 2016).

Circulating 25(OH)D concentration (nmol·L^{-1})	**Status**
<30	Deficient
<50	Insufficient
≥50	Sufficient
≥75	Optimal

1.5.2 Prevalence of vitamin D insufficiency

At latitudes above 42°N, cutaneous vitamin D synthesis contributes little to serum 25(OH)D concentrations during winter (Webb et al., 1988); hence circulating serum 25(OH)D has a

distinct seasonal pattern, peaking in summer and dropping to its nadir in winter (Hypponen and Power, 2007). Vitamin D insufficiency (25(OH)D concentration <50 nmol·L^{-1}) is therefore common during winter and spring (Cashman et al., 2016). Vitamin D intake may also be inadequate throughout the year because people live indoors for the majority of sunlight hours and heed recommendations to cover-up from the sun. Among 55,844 children, adolescents, and adults living across Europe, 40% were vitamin D insufficient (25(OH)D <50 nmol·L^{-1}) and 13% were deficient (25(OH)D <30 nmol·L^{-1}). In participants sampled during an extended winter period (October–March), the proportion that were vitamin D deficient increased to 18% (Cashman et al., 2016). A similar prevalence of vitamin D insufficiency (25(OH)D <50 nmol·L^{-1}) has also been reported in the United States of America (42% of 4,495 adults; Forrest and Stuhldreher, 2011), and Australia (31% of 11,247 adults; Daly et al., 2012). Furthermore, 91% UK-based adults and 73% of adults living in Australia had 25(OH)D concentrations <75 nmol·L^{-1}, with data collection spread across seasons (Cashman et al., 2016; Daly et al., 2012).

1.6 Vitamin D supplementation

1.6.1 Oral supplementation

As a result of the lack of consensus on an agreed threshold for vitamin D sufficiency, contrasting recommended daily oral vitamin D intakes exist. In the UK, a vitamin D reference nutrient intake of 400 IU·day^{-1} has been recommended for all aged ≥4 years, with an aim to protect musculoskeletal health by avoiding 25(OH)D <25 nmol·L^{-1} (Scientific Advisory Committee on Nutrition, 2016). Similarly, the IOM promote a recommended daily allowance of 600 IU·day^{-1} for individuals aged 1–70 years.

Supplementation with vitamin D_2 or D_3 increases circulating 25(OH)D concentrations (Seamans and Cashman, 2009). Vitamin D_3 has been demonstrated to be 1.87 times more potent than vitamin D_2 in raising and maintaining circulating 25(OH)D concentrations and is the preferred option for correcting vitamin D deficiency (Heaney et al., 2011). However, disagreement exists as to whether both forms are equally effective at elevating and maintaining sufficient 25(OH)D concentrations (Logan et al., 2013; Swanson et al., 2014; Holick et al., 2008; Houghton and Vieth, 2006). To enable a comparison of oral vitamin D and UV irradiation (endogenous vitamin D_3 synthesis) supplementation protocols, only studies that have supplemented participants with oral vitamin D_3 will be reviewed here. Among healthy adults, the effect of oral vitamin D_3 supplementation on 25(OH)D concentrations, using a range of doses and durations, have been characterised in numerous randomised controlled trials (Table 1.2). Only studies including winter data collection and at high latitudes were included to minimise the influence of ambient UVB on 25(OH)D concentrations. These data suggest a non-linear response of circulating 25(OH)D above baseline concentrations to doses of vitamin D_3. A steeper rise in 25(OH)D concentrations has been observed when vitamin D_3 supplementation is <1,000 IU·day^{-1}, with a slower, more flattened response observed with doses ≥1,000 IU·day^{-1}. For example, among healthy adults, increases in 25(OH)D with vitamin D_3 doses of 400, 1,000 and 2,000 IU·day^{-1} were 2.1, 0.8 and 0.5 nmol·L^{-1} per 40 IU, respectively (Smith et al., 2009). Additionally, baseline 25(OH)D concentrations predict the magnitude of change in 25(OH)D in response to vitamin D supplementation, with an inverse correlation consistently reported (Mazahery and von Hurst, 2015; Trang et al., 1998). To achieve vitamin D sufficiency (25(OH)D ≥50 nmol·L^{-1}) an estimated daily intake of ~1,000 IU·day^{-1} vitamin D_3 is required based on previous predictive modelling (Cashman et al., 2008).

Table 1.2. A summary of randomised controlled studies examining the effect of oral vitamin D_3 supplementation on circulating 25(OH)D among healthy adults.

Study	**Study design, duration, seasons, latitude**	**Participants**	**Vitamin D_3 daily dose**	**Change in 25(OH)D ($nmol \cdot L^{-1}$)**
Cashman et al. (2008)	RCT-P DB, 22-weeks, autumn/winter/spring, 51 and 55°N	*n* = 215 M and F, Age = 30 ± 6 (20–40) y	200 IU, *n* = 48 400 IU, *n* = 57 600 IU, *n* = 53 Control, *n* = 57	200 IU: ↓ 10 to 50 400 IU: ↓ 12 to 60 600 IU: ↓ 8 to 69 Control: ↓ 28 to 37
Viljakainen et al. (2009)	RCT-P DB, 6-months, autumn/winter/spring, 60°N	*n* = 48 M, Age = 29 ± 7 (21–49) y	400 IU, *n* = 16 800 IU, *n* = 16 Control, *n* = 16	400 IU: ↑ 15 to 75 800 IU: ↑ 28 to 90 Control: ↓ 13 to 52
Smith et al. (2009)	RCT DB, 5-months, winter/spring, 78°S	*n* = 62 M and F, Age = 42 (39–44) y	400 IU, *n* = 18 1,000 IU, *n* = 19 2,000 IU, *n* = 18 Control, *n* = 7	400 IU: ↑ 13 to 57 1,000 IU: ↑ 19 to 63 2,000 IU: ↑ 26 to 71 Control: ↓ 2 to 34

Backx et al. (2016)	RCT DB, 12-months, spring/summer/autumn/ winter, 52°N	*n* = 61 M and F, Age = 22 ± 3 (18–32) y	400 IU, *n* = 16 1,100 IU, *n* = 14 2,200 IU, *n* = 20 Control, *n* = 11	400 IU: ↑ 28 to 81 1,100 IU: ↑ 25 to 76 2,200 IU: ↑ 50 to 100 Control: ↑ 4 to 96
Harris and Dawson-Hughes (2002)	RCT, 8-weeks, winter/spring, 42°N	*n* = 27 M, Age = 18–35 y	800 IU, *n* = 14 Control, *n* = 13	800 IU: ↑ 23 to 82 Control: ↓ 5 to 54
Biancuzzo et al. (2010)	RCT-P DB, 11-weeks, winter/spring, 42°N	*n* = 35 M and F, Age = 40 (22–81) y	1,000 IU, *n* = 20 Control, *n* = 15	1,000 IU: ↑ 23 to 70 Control: ↓ 4 to 45
Heaney et al. (2003)	RCT, 5-months, autumn/winter/spring, 49°N	*n* = 67[a] M, Age = 39 ± 11 y	1,000 IU 5,000 IU 10,000 IU Control	1,000 IU: ↑ 5 to 77[b] 5,000 IU: ↑ 81 to 150[b] 10,000 IU: ↑ 146 to 212[b] Control: ↓ 18 to 52[b]

Li-Ng et al. (2009)	RCT-P DB, 12-weeks, winter/spring/summer, 41°N	*n* = 148 M and F, Age = 59 ± 13 (18–80) y	2,000 IU, *n* = 78 Control, *n* = 70	2,000 IU: ↑ 24 to 89 Control: ↓ 2 to 61
He et al. (2016)	RCT-P DB, 14-weeks, winter/spring, 53°N	*n* = 39 M, Age = 20 ± 2 y	5,000 IU, *n* = 20 Control, *n* = 19	5,000 IU: ↑ 71 to 126 Control: ↓ 25 to 33

Studies are listed in order of increasing dose. Order of seasons indicates beginning and end of data collection. RCT-P, randomised placebo controlled trial; RCT, randomised controlled trial; DB, double-blind; M, males; F, females; age, mean ± SD (min–max) where reported; ↑ increase; ↓ decrease; [a] *n* per group not reported; [b] estimated from figure.

1.6.2 Vitamin D intoxication

Acute and chronic excess intake of vitamin D can cause a state of vitamin D intoxication or hypervitaminosis D; with resultant hypercalcaemia, demineralisation of bone, soft tissue calcification, and renal and cardiovascular damage possible (Scientific Advisory Committee on Nutrition, 2016; Institute of Medicine, 2011). Hypercalcaemia is regarded as the most appropriate endpoint on which to base upper intake limits for vitamin D, i.e. the level above which the risk for harm begins to increase. Derived in-part from observational data and using animal models, the IOM established an upper limit of 4,000 IU·day^{-1} for individuals aged ≥9 years (Institute of Medicine, 2011). Likewise, EFSA recommend a tolerable upper intake limit of 4,000 IU·day^{-1} for individuals aged ≥11 years (European Food Safety Authority, 2012). Few studies have assessed the safety of moderate to high doses of vitamin D; to date, only two studies have used one or more doses ≥4,000 IU·day^{-1} for ≥2 months (Heaney et al., 2003; Barger-Lux et al., 1998). This paucity of evidence is likely due to the ethical issues associated with conducting clinical trials designed to examine the adverse effects of a substance. There remains a lack of global consensus on the intake levels at which vitamin D may cause harm; indeed, the IOM reports doses below 10,000 IU·day^{-1} are unlikely to result in symptoms of toxicity (Institute of Medicine, 2011). However, there is a need to proceed cautiously, rather than assume intakes of vitamin D below those expected to cause hypervitaminosis D are harmless. The risk of harm may have been underestimated due to existing studies being of relatively short duration; and adverse outcomes not sufficiently monitored, or lacking adequate statistical power for detection. In summary, given the uncertainty about the progressive health effects of regular ingestion of moderate to high doses of vitamin D (>4,000 IU·day^{-1}); abiding by the 4,000 IU·day^{-1} upper intake limit recommended by the IOM (2011) and EFSA (2012), derived from the best available evidence, appears to be prudent.

1.6.3 Ultraviolet irradiation

A number of studies have examined the efficacy of UV irradiation to increase or maintain circulating 25(OH)D concentrations (Table 1.3). Irradiation protocols, mostly using uncontrolled study designs, have manipulated the dose and frequency of exposures; the size of skin surface area exposed; and the duration and time-of-year of supplementation. To minimise inter-individual variation in vitamin D synthesis due to differences in skin pigmentation (Armas et al., 2007; Clemens et al., 1982; Farrar et al., 2011; Loomis, 1967), the studies included in Table 1.3 are limited to participants with sun-reactive skin type I–IV (Fitzpatrick, 1988). In addition, only studies including winter data collection and at a high latitudes were included to minimise the effect of ambient UVB on vitamin D synthesis. In physical terms, exposure to UVR is measured in units of $J \cdot m^{-2}$; because joule = watt x second, these units of irradiance are for a given area over a given time. However, to measure UVR action on the skin and quantify dose, the physical dose is weighted by the erythema action spectrum, using units of standard erythemal dose (SED), where 1 SED is equivalent to 100 $J \cdot m^{-2}$ (Bogh, 2012). Among light-skinned Caucasians, 2–3 SED is equal to ~1 minimal erythemal dose (MED), with 1 MED the specific dose that causes skin erythema (or pinkness) in a given individual (Macdonald, 2013). The erythemal effectiveness of irradiation can be quantified and compared between study protocols using SED units; however, the potential to synthesise vitamin D will vary according to the exact wavelength of light emitted by irradiation bulbs, meaning direct comparisons between study protocols are somewhat difficult to make.

Table 1.3. A summary of studies examining the effect of ultraviolet irradiation on circulating 25(OH)D among healthy adults.

Study	**Study design, duration, number (N) (frequency) of irradiations, season(s), latitude**	**Participants**	**Irradiation protocol: cumulative dose (D), wavelength (λ), skin surface area (SA)**	**Change in 25(OH)D ($nmol \cdot L^{-1}$)**
Bogh et al. (2011b)	RT 4 groups, 1-week, N = 4 ($1x \cdot 2–3days^{-1}$), winter/spring, 56°N	*n* = 55 M and F Age = 32 ± 9 (18–51) y Skin-type: I–IV	D = 1.5 SED, *n* = 10 D = 3 SED, *n* = 10 D = 6 SED, *n* = 15 D = 12 SED, *n* = 20 λ = 280–360 nm SA = 24%	1.5 SED: ↑ 14 to 45[a] 3 SED: ↑ 20 to 51[a] 6 SED: ↑ 19 to 50[a] 12 SED: ↑ 25 to 56[a]
Datta et al. (2012)	UT 2 groups, 10-days, N = 4 ($1x \cdot 2–3days^{-1}$), winter/spring, 56°N	*n* = 19[b] M and F Age = 40 (19–68) y Skin-type: I–IV	D = 3 SED, *n* = 15 D = 6 SED, *n* = 14 λ = 290–365 nm SA = 8.5%	3 SED: ↑ 3 to 62 6 SED: ↑ 12 to 66

Bogh et al. (2011a)	RT 3 groups, 1-week, N = 4 (1x·2–3days^{-1}), winter/spring, 56°N	*n* = 92[c] M and F Age = 33 ± 11 (18–62) y Skin-type: I–IV	D = 3 SED D = 6 SED D = 12 SED λ = 280–360 nm SA = 24%	3 SED: ↑ 20 to 56[a] 6 SED: ↑ 20 to 56[a] 12 SED: ↑ 25 to 61[a]
Bogh et al. (2012b)	RCT 4 groups, 16-weeks, N = 5–17 (1x·1–4weeks^{-1}), autumn/winter, 56°N	*n* = 55 M and F Age = 36 ± 11 (20–60) y Skin-type: I–IV	D = 5 SED, N = 5 (1x·4-weeks^{-1}), *n* = 12 D = 9 SED, N = 9 (1x·2-weeks^{-1}), *n* = 14 D = 17 SED, N = 17 (1x·week^{-1}), *n* = 15 λ = - SA = 88% Control, *n* = 14	5 SED: ↓ 9 to 48 9 SED: ↓ 5 to 67 17 SED: ↑ 13 to 85 Control: ↓ 25 to 40
Vahavihu et al. (2010)	UT 4 groups, 1-week, N = 7 (1x·day^{-1}), winter/spring, 67°N	*n* = 53 F Age = 41 (21–61) y Skin-type: II–III	D = 13 SED SA = 10.5%, λ = 295–400[d] nm, *n* = 11 SA = 9%, λ = 280–400[d] nm, *n* = 14 SA = 18.5%, λ = 280–400[d] nm, *n* = 9 SA = whole-body, λ = 280–400[d] nm, *n* = 19	10.5%: ↑ 4 to 42 9%: ↑ 4 to 39 18.5%: ↑ 11 to 49 Whole-body: ↑ 11 to 56

Rhodes et al. (2010)	UT 1 group, 6-weeks, N = 18 (3x·week^{-1}), winter, 54°N	*n* = 109 M and F Age = 20–60 y Skin-type: I–IV	D = 23.4 SED λ = 290–400 nm SA = 35%	23.4 SED: ↑ 26 to 70
Lagunova et al. (2013)	UT 1 group, 5-weeks, N = 10 (2x·week^{-1}), winter/spring, 59°N	*n* = 11 M and F Age = 40 (23–59) y Skin-type: I–III	D = 23.8 SED λ = 'including' 280–320 nm SA = whole-body	23.8 SED: ↑ 20 to 70
Karppinen et al. (2016)	RCT 2 groups, 24-weeks, N = 13 (1x·2-weeks^{-1}), autumn/winter/spring, 61°N	*n* = 34 M and F Age = 36 (20–61) y Skin-type: II–IV	D = 25 SED, *n* = 16 λ = - SA = whole-body Control, *n* = 18	25 SED: ↑ 12 to 89 Control: ↓ 11 to 66

Ala-Houhala et al. (2012)	UT 1 group, 4-weeks, N = 12 (3x·week^{-1}), winter/spring, -	*n* = 33 M and F Age = 44 (23–59) y Skin-type: II–IV	Mean D = 48.4 SED λ = - SA = whole-body	48.4 SED: ↑ 41 to 94
Bogh et al. (2012a)	UT 1 group, 6-weeks, N = 18 (3x·week^{-1}), winter/spring/autumn, 56°N	*n* = 16 M and F Age = 42 ± 15 y Skin-type: II–IV and VI[e]	Mean D = 54.9 SED λ = 85% at 311 nm SA = whole-body	54.9 SED: ↑ 56 to 75

Studies are listed in order of increasing cumulative dose. Order of seasons indicates beginning and end of data collection. UT, uncontrolled trial; RT, randomised trial; RCT, randomised controlled trial; N, total number (frequency) of irradiations; -, not reported; M, males; F, females; age, mean ± SD (min–max) where reported; skin-type, sun-reactive skin type (Fitzpatrick, 1988); D, cumulative dose of ultraviolet radiation; λ, wavelength of ultraviolet radiation (UVB = 290–315 nm); SA, ~skin surface area; ↑ increase; ↓ decrease; [a] baseline 25(OH)D mean of all groups; [b] *n* = 10 participated twice; [c] *n* = 92 randomised, *n* per irradiation protocol not reported; [d] estimated from figure; [e] *n* = 2 skin type VI.

Unlike oral vitamin D_3 supplementation, there have been no reports of vitamin D intoxication by UV irradiation alone (Institute of Medicine, 2011); however, for safety, exposure to UVR for vitamin D synthesis should be sub-erythemal because skin erythema or sunburn increases skin cancer risk (Holick, 2007). UK guidelines on safe sunlight exposure for vitamin D synthesis recommend ~15-minutes-per-day of summer sunlight exposure to ~33% skin surface area to achieve 25(OH)D ≥50 nmol·L^{-1} in the majority of white-skinned individuals (Advisory Group on Non-ionising Radiation, 2017). Using simulated summer sunlight, Rhodes and colleagues (2010) demonstrated that wintertime exposure to 1.3 SED 3 times-a-week whilst wearing shorts and T-shirt was effective at restoring 25(OH)D to ≥50 nmol·L^{-1} in the majority of white-skinned individuals without causing skin erythema. This dose is equivalent to ~15-minutes, midday summer sun exposure 6 times-a-week for a casually dressed individual in northern England, and taking account of pre-vitamin D irradiance at different latitudes can be related to exposure times at other world locations (Webb et al., 2011). For example, the equivalent exposure in Philadelphia, Pennsylvania, USA (40°N) would be ~12-minutes; and that for Oslo, Norway (60°N) would be ~18-minutes. Despite recognising sunlight is the major source of vitamin D for most people, the Scientific Advisory Committee on Nutrition (SACN, 2016) did not quantify how much sunlight is required during summer to maintain 25(OH)D ≥25 nmol·L^{-1} during the following winter. The absence of this advice from their 2016 report was reportedly due to the complexity of factors that affect endogenous vitamin D synthesis. The IOM (2011) state that vitamin D requirements cannot be based on a recommended level of sun exposure, due to concerns about sun exposure and skin cancer risk.

1.6.4 Ultraviolet irradiation vs. oral supplementation

To date, two studies have compared the effect of UV irradiation *vs.* oral vitamin D_3 supplementation on 25(OH)D concentrations in healthy adults. Among participants with baseline 25(OH)D <25 nmol·L^{-1}, Bogh et al. (2012a) compared the effect of whole-body UVB 3 times-a-week for 6-weeks (Table 1.3) with a daily dose of 1,600 IU vitamin D_3. The increase in 25(OH)D was greater (P < 0.05) in the UVB group (increasing from 19 to 75 nmol·L^{-1}) compared with the oral vitamin D_3 group (increasing from 23 to 61 nmol·L^{-1}). Ala-Houhala et al. (2012) compared whole-body UV irradiation 3 times-a-week for 4-weeks (Table 1.3) with an oral vitamin D_3 dose of 800 IU·day^{-1}. Among participants with baseline 25(OH)D <75 nmol·L^{-1} (mean 53 nmol·L^{-1}), increases in 25(OH)D were not well matched between methods, with increases of +41 nmol·L^{-1} using UVR and +20 nmol·L^{-1} using oral vitamin D_3 (P < 0.001; Ala-Houhala et al., 2012). Both studies are limited by an absence of a control group. Unfortunately, these two studies lack real-world meaningfulness because participants were irradiated whilst not wearing clothes and using UVR doses in excess of typical summertime ambient UVR, for which the long-term safety is unclear (Macdonald et al., 2011). Future investigations comparing UV irradiation with oral vitamin D_3 supplementation should use safe doses of simulated summer sunlight, closely matched to natural sun exposure; and expose skin surfaces areas whilst wearing summer attire, because the findings from such studies will have real-world practicability.

1.7 Vitamin D and exercise performance

Long before the discovery that UVB was essential for endogenous vitamin D synthesis; the sun's rays were valued as a source of physical strength and vitality among ancient Egyptians and Greek Olympians (Girgis et al., 2013). In recent years, an increasing body of research has suggested that vitamin D may indeed have a role in enhancing exercise performance (Owens

et al., 2018; Todd et al., 2015). Correcting vitamin D deficiency has been shown to increase strength in elderly adults (Stockton et al., 2011); and positive associations between vitamin D status and exercise performance have been reported in studies including elderly participants (Ardestani et al., 2011; Grimaldi et al., 2013; Annweiler et al., 2009). Intriguingly, vitamin D supplementation in a small cohort of athletes with low baseline serum 25(OH)D (~30 $nmol \cdot L^{-1}$) has also been shown to improve musculoskeletal performance (Close et al., 2013b); however this positive effect has not been repeated, as reviewed in **section 1.7.2**. The widespread expression of VDR in a range of tissues throughout the body provides a means for vitamin D to modify muscle and cardiovascular function (Rosen et al., 2012). As reviewed in **section 1.8**, vitamin D stimulates skeletal muscle protein synthesis, via VDR mediated signalling (Girgis et al., 2013), and may improve cardiac structure and endothelial function (Allison et al., 2015; Tarcin et al., 2009). Hence, avoiding low 25(OH)D concentrations might be important for strength and endurance exercise performance (Thomas et al., 2016).

1.7.1 Observational studies

The majority of observational studies investigating the relationship between vitamin D status and musculoskeletal performance have used elderly persons, with most reporting a positive association (Annweiler et al., 2009; Cannell et al., 2009). The association between vitamin D status and exercise performance among young healthy adults remains unclear because observational studies have presented conflicting results (Table 1.4). Findings from studies that have included elderly participants cannot be applied to young healthy adults due to comorbidities and lower vitamin D status among elderly persons, and the use of measures of physical function rather than exercise performance. The failure to control for covariates known to influence exercise performance (i.e. age, sex, body composition, smoking, physical

activity and season; Mattila et al., 2007; Kok et al., 2012; Song et al., 1998; Maughan et al., 1983; Cannell et al., 2009) also limit the meaningfulness of several significant associations reported to date. Small sample sizes preclude an appropriate statistical approach to control for such confounding variables in a number of studies. To investigate the influence of 25(OH)D using multiple linear regression requires a minimum sample size = 104 + number of predictors (Green, 1991). Finally, single season study designs limit inter-individual variation in participants' vitamin D status; likely decreasing the chances of observing a relationship.

Table 1.4. A summary of observational studies examining the association between circulating 25(OH)D and exercise performance among healthy adults and adolescents.

Study	**Study design, season(s)**	**Participants**	**Mean 25(OH)D ($nmol \cdot L^{-1}$)**	**Covariates**	**Association between 25(OH)D and exercise performance (+, positive; –, negative; NS, no significant association)**
Marantes et al. (2011)	Cross-sectional, -	*n* = 667 M and F Age = 57 (21–97) y	57[a]	Age, height, physical activity, fat mass and season	NS grip or quadriceps strength.
Grimaldi et al. (2013)	Cross-sectional, spring/summer/ autumn/winter	*n* = 419 M and F Age = 44 (20–76) y	84	Age, sex, heart rate, blood pressure, BMI, $\dot{V}O_{2max}$, physical activity and season	+ arm strength ($P < 0.05$). + leg strength ($P < 0.05$; 2 out of 4 measures). NS grip strength.
Hamilton et al. (2014)	Cross-sectional, summer	*n* = 342 M athletes Age = 24 y	52	Age, body mass and leg lean mass	NS leg strength. Greater leg strength among participants with 25(OH)D >75 and 50–75 *vs.* <25 $nmol \cdot L^{-1}$ ($P < 0.05$; 3 out of 10 measures)[b].

Ardestani et al. (2011)	Cross-sectional, spring/summer/ autumn/winter	*n* = 200 M and F Age = 40 (20–73) y	85	Age, sex, BMI and physical activity	+ $\dot{V}O_{2max}$ ($P < 0.05$). Greater $\dot{V}O_{2max}$ among participants with 25(OH)D >75 *vs.* ≤50 nmol·L^{-1} ($P < 0.01$)[b].
von Hurst et al. (2013)	Cross-sectional, winter/spring	*n* = 137 F Age = 24 (19–29) y	54	Physical activity	+ grip strength ($P < 0.05$). NS vertical jump.
Hildebrand et al. (2016)	Cross-sectional, autumn	*n* = 103 M and F athletes Age = 20 y	91[a]	Lean mass	+ vertical jump ($P < 0.001$), + triple hop ($P < 0.001$), + shuttle sprint test ($P = 0.001$) and back squat ($P < 0.001$).
Koundourakis et al. (2014)	Longitudinal (6-weeks, baseline and follow-up), summer	*n* = 67 M athletes Age = 26 y	Baseline: 86 Follow-up: 118	None	Baseline and follow-up: + vertical jump ($P < 0.001$). + $\dot{V}O_{2max}$ ($P < 0.01$). – 10 and 20 m sprint ($P < 0.001$).
Mowry et al. (2009)	Cross-sectional, summer/autumn	*n* = 59 F Age = 20 (16–24) y	115	None	+ $\dot{V}O_{2max}$ ($P < 0.05$).

Fitzgerald et al. (2014)	Cross-sectional, spring/summer	*n* = 52 M athletes Age = 20 (18–23) y	89	None	NS $\dot{V}O_{2peak}$.
Fitzgerald et al. (2015)	Cross-sectional, spring/summer	*n* = 52 M athletes Age = 20 (18–23) y	89	Fat free mass, fat mass, physical activity and playing experience	+ grip strength ($P < 0.05$). NS vertical jump or Wingate.
Forney et al. (2014)	Cross-sectional, summer	*n* = 39 M and F Age = 23 (20–38) y	87	None	+ $\dot{V}O_{2max}$ ($P < 0.05$). NS vertical jump, Wingate, or arm and leg strength. Greater $\dot{V}O_{2max}$ among M only with 25(OH)D >88 *vs.* <88 nmol·L^{-1} ($P < 0.05$).
Wilson et al. (2013)	Cross-sectional, spring/summer/ autumn/winter	*n* = 5,392 M and F adolescents and adults Age = 29 (12–49) y	62	Age, ethnicity, BMI, physical activity and season	NS $\dot{V}O_{2max}$. Greater $\dot{V}O_{2max}$ among M with 25(OH)D ≥50 *vs.* <50 nmol·L^{-1} ($P = 0.001$).

Carson et al. (2015)	Cross-sectional, spring/summer/ autumn/winter	*n* = 1,013 M and F adolescents Age = (12–15) y	41[a]	Physical activity, season, sexual maturation stage and dietary protein	Greater grip strength among M aged 15 y with 25(OH)D >51 *vs.* <32 nmol·L^{-1} ($P < 0.001$). NS vertical jump or $\dot{V}O_{2max}$.
Valtuena et al. (2013)	Cross-sectional, spring/summer/ autumn/winter	*n* = 1,006 M and F adolescents Age = 15 (12–17) y	59	Age, latitude and season	+ $\dot{V}O_{2max}$ among M ($P < 0.01$). + grip strength among F ($P < 0.01$). NS standing long jump.
Dong et al. (2010)	Cross-sectional, spring/summer/ autumn/winter	*n* = 559 M and F adolescents Age = 16 (14–18) y	73	Age, sex, ethnicity, height, season and sexual maturation stage	+ $\dot{V}O_{2max}$ ($P < 0.05$).
Foo et al. (2009)	Cross-sectional, -	*n* = 301 F adolescents Age = 15 y	34	Body mass, sexual maturation stage, physical activity, and dietary calcium and vitamin D	Greater grip strength among participants with 25(OH)D >50 *vs.* <25 and 25–50 nmol·L^{-1} ($P < 0.05$).

Ward et al. (2009)	Cross-sectional, -	*n* = 99 F postmenarchal Age = 14 (12–14) y	29	Body mass	+ vertical jump height, power and velocity ($P < 0.01$).

Studies among adults are listed first, in order of decreasing *n*. M, males; F, females; age, mean (min–max) where reported; BMI, body mass index; $\dot{V}O_{2max}$, maximal oxygen consumption; $\dot{V}O_{2peak}$, peak oxygen consumption; - not reported; + positive, – negative, or NS, no significant association between 25(OH)D and given performance measure; [a] estimated from male and female data; [b] without control for covariates.

1.7.2 Vitamin D supplementation studies

Randomised placebo controlled trials are necessary for conclusions regarding a causal relationship between vitamin D and exercise performance to be reached. The effect of vitamin D supplementation on exercise performance among young healthy adults remains unclear; however, most studies have reported non-significant effects (Table 1.5). No studies have demonstrated a beneficial effect of supplementation with oral vitamin D_2 (Shanely et al., 2014; Ward et al., 2010; Nieman et al., 2014). Only one study has assessed aerobic capacity (Todd et al., 2017) and none endurance performance, with the remainder focusing on muscular strength (typically grip strength) and/or power. Current literature is also limited by some studies including adolescent participants (El-Hajj Fuleihan et al., 2006; Dubnov-Raz et al., 2015; Shanely et al., 2014; Ward et al., 2010); possible inadequate sample sizes (for example ≤10 participants per intervention group; Wyon et al., 2014; Close et al., 2013b); not measuring 25(OH)D (Wyon et al., 2014; El-Hajj Fuleihan et al., 2006); and not using a placebo control group (Wyon et al., 2014). Many of the vitamin D supplementation studies shown in Table 1.5 are also limited because participants were already vitamin D sufficient at baseline (25(OH)D >50 $nmol \cdot L^{-1}$), or used oral vitamin D_3 doses greater than the tolerable upper intake recommended by the IOM and EFSA (4,000 $IU \cdot day^{-1}$; Institute of Medicine, 2011; European Food Safety Authority, 2012). Performance benefits outlined by pioneering UV irradiation studies are difficult to interpret as they were not placebo controlled; did not assess 25(OH)D concentrations (Allen and Cureton, 1945; Ronge, 1952; Hettinger and Seidl, 1956); and in at least one case used unsafe UVR doses which resulted in sun burn (Allen and Cureton, 1945). Further empirical data using safe doses of oral vitamin D_3 or UVR to correct vitamin D insufficiency are required to clearly elucidate the potential beneficial effect of vitamin D on exercise performance.

Table 1.5. A summary of controlled intervention studies examining the effect of vitamin D supplementation on exercise performance among young healthy adults and adolescents.

Study	**Study design, duration, season(s)**	**Participants**	**Vitamin D daily dose, D_3 or D_2**	**Change in 25(OH)D ($nmol \cdot L^{-1}$)**	**Results (↑ increase; ↓ decrease; ND, no significant differences between vitamin D and control groups)**
Barker et al. (2012)	RCT-P DB, 4-weeks, winter/spring	*n* = 30 M and F Age = 28 y	200 IU, *n* = 10 4,000 IU, *n* = 10 Control, *n* = 10 D_3	200 IU: ↑ 5 to 95[a] 4,000 IU: ↑ 45 to 125[a] Control: ↓ 7 to 63[a]	ND in leg press.
Wyon et al. (2014)	CT, 4-months, winter/spring	*n* = 24 M and F athletes Age = 28 y	2,000 IU, *n* = 17 Control, *n* = 7 D_3	-	↑ vertical jump (+7%; $P < 0.01$) and ↑ quadriceps strength (+19%; $P < 0.01$) in vitamin D_3 group.
Close et al. (2013a)	RCT-P DB, 12-weeks, winter/spring	*n* = 30 M athletes Age = 21 ± 2 y	≡ 2,857 IU, *n* = 10 ≡ 5,714 IU, *n* = 10 Control, *n* = 10 D_3	2,857 IU: ↑ 31 to 84[a] 5,714 IU: ↑ 39 to 90[a] Control: ↓ 12 to 40[a]	ND in 20 m sprint, vertical jump, bench or leg press.

Todd et al. (2017)	RCT-P DB, 12-weeks, winter/spring	*n* = 42 M and F Age = 20 ± 2 y	3,000 IU, *n* = 22 Control, *n* = 20 D_3	3,000 IU: ↑ 37 to 84 Control: ↑ 6 to 49	ND in $\dot{V}O_{2max}$, vertical jump, or grip strength.
Close et al. (2013b)	RCT-P DB, 8-weeks, winter	*n* = 10 M athletes Age = 18 ± 5 y	5,000 IU, *n* = 5 Control, *n* = 5 D_3	5,000 IU: ↑ 73 to 103[a] Control: ↑ 21 to 73[a]	↓ 10 m sprint (–2%[a]; *P* = 0.008) and ↑ vertical jump (+7%[a]; *P* = 0.008) in vitamin D_3 group. ND in 30 m sprint, bench press, back squat or Illinois Agility Test.
Jastrzebska et al. (2016)	RCT-P DB, 8-weeks, winter/spring	*n* = 36 M athletes Age = 18 ± 0.6 y	~5,000 IU, *n* = 16 Control, *n* = 20 D_3	~5,000 IU: ↑ 58 to 106 Control: ↓ 4 to 44	ND in 5, 10, 20, or 30 m sprint, vertical jump, or Wingate.
Goswami et al. (2012)	RCT-P DB, 6-months, autumn/winter/ spring	*n* = 76 F Age = 22 y	≡ 8,571 (month 1-2) and 3,945 IU (month 3-6), *n* = 39 Control, *n* = 37 D_3	8,571 and 3,945 IU: ↑ 52 to 75 Control: ↓ 2 to 20	ND in grip or pinch strength, or 6-minute walk test.

Owens et al. (2014)	RCT-P DB, 12-weeks, winter/spring	*n* = 22 M Age = 23 ± 3 y	10,000 IU, *n* = 14[b] Control, *n* = 15[b] D_3	10,000 IU: ↑ 79 to 120[a] Control: ↓ 15 to 25	ND in quadriceps or hamstrings strength.
El-Hajj Fuleihan et al. (2006)	RCT-P DB, 12-months, -	*n* = 134 F postmenarchal Age = 14 ± 2 y	200 IU, *n* = 46 2,000 IU, *n* = 41 Control, *n* = 47 D_3	-	ND in grip strength.
Dubnov-Raz et al. (2015)	RCT-P DB, 12-weeks, winter	*n* = 47 M and F adolescents Age = 14 ± 2 y	2,000 IU, *n* = 25 Control, *n* = 22 D_3	2,000 IU: ↑ 13 to 74 Control: ↓ 11 to 51	ND in grip strength.
Shanely et al. (2014)	RCT-P DB, 6-weeks, winter	*n* = 33 M Age = 16 y	600 IU, *n* = 17 Control, *n* = 16 D_2	600 IU: ↑ 6 to 69[a] Control: ↓ 4 to 63[a]	ND in vertical jump or dead-lift.
Ward et al. (2010)	RCT-P DB, 12-months, winter/spring/ summer/autumn	*n* = 65 F postmenarchal Age = 14 ± 1 y	≡ 1,644 IU, *n* = 33 Control, *n* = 32 D_2	1,644 IU: ↑ 38 to 56 Control: ↓ 2 to 16	ND in vertical jump or grip strength.

Nieman et al. (2014)	RCT-P DB, 6-weeks, winter	*n* = 28 M Age = 27 ± 1 y	3,800 IU, *n* = 13 Control, *n* = 15 D_2	3,800 IU: ↓ 1 to 95[a] Control: ↓ 15 to 95[a]	ND in vertical jump, grip strength, dead-lift, bench press, or Wingate.

Studies among adults, using vitamin D_3 supplementation are listed first, in order of increasing dose. Order of seasons indicates beginning and end of data collection. D_3, vitamin D_3; D_2, vitamin D_2; RCT-P, randomised placebo controlled trial; DB, double-blind; CT, controlled trial; M, males; F, females; Age, mean ± SD where reported; ≡ equivalent to; - not reported; $\dot{V}O_{2max}$, maximal oxygen consumption; ↑ increase; ↓ decrease; ND, no significant differences between vitamin D and control groups; [a] estimated from figure; [b] assigned to treatment.

1.8 Vitamin D and exercise performance mechanisms

Figure 1.2 summarises the influence of vitamin D in skeletal muscle, blood vessels, heart, liver, intestine and kidneys, with outcomes relevant to exercise performance. Supporting evidence and details of potential mechanisms, predominantly from studies using cellular and animal models, are reviewed in **sections 1.8.1 and 1.8.2**. Studies using exogenous treatment of skeletal muscle progenitor cells and myocytes in culture have often used supra-physiological doses of vitamin D. How well these results translate to whole tissue (human) physiology remains unclear.

1.8.1 Muscle form, function and metabolism

The presence of VDR within skeletal muscle provides a means for active vitamin D metabolites to act via genomic and non-genomic pathways, and have a direct influence on skeletal muscle form, function and metabolism (Girgis et al., 2013; Ceglia and Harris, 2013). Genomic effects arise from $1,25(OH)_2D$-VDR-RXR heterodimer binding to VDRE within target deoxyribonucleic acid (DNA) promoter regions. Subsequent upregulation of gene transcription and expression of contractile proteins and transcription factors (for example myogenic differentiation antigen and myogenin; Girgis et al., 2014; Garcia et al., 2011) can positively influence muscle development. Myostatin is a negative regulator of muscle mass, causing enhanced protein degradation and reduced protein synthesis. Vitamin D may promote skeletal muscle synthesis by reducing myostatin expression. The addition of $1,25(OH)_2D$ to mouse myoblasts has been shown to increase the expression and nuclear translocation of the VDR and decrease the expression of myostatin (Garcia et al., 2011).

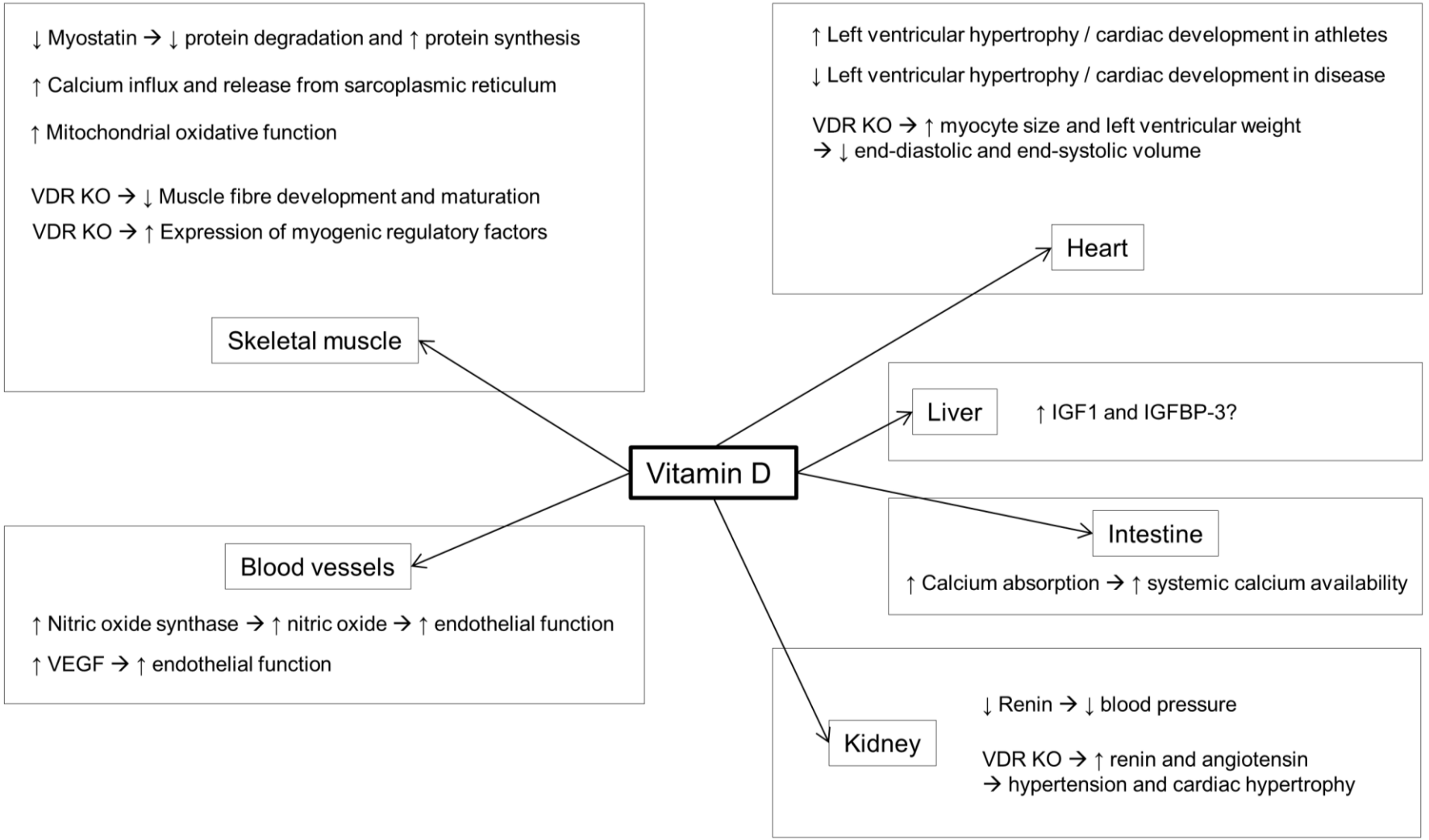

Figure 1.2. Summary of the influence of vitamin D in skeletal muscle, blood vessels, heart, liver, intestine and kidneys, with outcomes relevant to exercise performance. VDR KO, vitamin D receptor knockout mouse or rat; VEGF, vascular endothelial growth factor; IGF1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; ↑ increase; ↓ decrease; → results in.

Bound to a non-nuclear VDR, $1,25(OH)_2D$ can initiate the formation of secondary messengers or phosphorylation of intracellular proteins including protein kinase A/cyclic adenosine monophosphate (PKA/cAMP); calmodulin-dependent protein kinase (CAMK); and mitogen-activated protein kinase (MAPK). Activation of these non-genomic pathways has been demonstrated to lead to rapid cellular effects, including calcium influx (Dirks-Naylor and Lennon-Edwards, 2011). Within minutes of vitamin D treatment, increases in intracellular calcium concentrations via calcium release from intracellular stores and subsequent entry through voltage-gated calcium channels have been demonstrated in cultured myocytes (Girgis et al., 2013). For example, chick sarcolemma vesicles treated with vitamin D *in vitro* showed improved calcium and phosphate uptake; increased calcium-ATPase activity; and mitochondria an enhanced ability to accumulate calcium compared with vitamin D deficient chicks (de Boland and Boland, 1987). Furthermore, these effects were not blocked by inhibitors of protein or RNA synthesis, demonstrating this mechanism was independent of gene transcription. Increases in intracellular calcium were inhibited by calcium channel blockers, and therefore dependent upon calcium flux through calcium channels. Consequently, it appears calcium mediated muscle function and metabolism, including contraction, insulin signalling and fuel handling can be directly affected by vitamin D.

Using VDR knockout mice (Li et al., 1997), the role of vitamin D in muscle function and skeletal muscle fibre development and maturation has been investigated. Compared with wild-type mice, animals without VDR are characterised by a reduced body size and weight; impaired motor coordination; reduced muscle fibre diameters; and behavioural phenotypes such as poor swimming ability (Burne et al., 2005). In addition, locomotive ability has been demonstrated to improve in wild-type mice exercised and treated with vitamin D; whereas

exercised wild-type mice treated with a control vehicle had inferior locomotive ability; and no locomotive enhancement was observed in exercised VDR knockout mice, despite treatment with vitamin D (Sakai et al., 2015). Examining the skeletal muscle of VDR gene deleted mice, muscle fibres are smaller than wild-type mice, and accompanied by abnormally high and persistent expression of myogenic regulatory factors (normally down-regulated during muscle differentiation when VDR is expressed; Endo et al., 2003). Vitamin D appears to play a role in normal muscle development because these regulatory factors (for example myogenic factor 5 and E2A) with critical roles in myoblast differentiation and skeletal muscle development, were down-regulated in wild-type myoblasts treated with $1,25(OH)_2D$ (Endo et al., 2003). VDR knockout mice are also characterised by secondary biochemical abnormalities in calcium, phosphate and PTH concentrations that can negatively affect muscle metabolism and function. Phosphate homeostasis may be of some importance, because muscle weakness in rachitic rats with very low or zero serum 25(OH)D has been shown to be reversible following vitamin D supplementation, but not without a phosphorus-containing diet (Schubert and DeLuca, 2010).

Whether the aforementioned mechanisms investigated using cellular and animal models translate to humans *in vivo* remains unknown. The presence of the VDR within human skeletal muscle has been doubted by some investigators because the VDR has been shown to be undetectable in skeletal, cardiac and smooth muscle (Wang and DeLuca, 2011). However, differences in experimental conditions, tight binding of VDR to DNA, or possible low levels of VDR expression in resting muscle may explain the apparent absence of VDR reported by some (Girgis et al., 2014). Alternatively, vitamin D may influence muscle metabolism through VDR signalling in tissues other than skeletal muscle and via proteins not directly related to calcium metabolism, including insulin-like growth factor 1 (IGF1), IGF-binding

protein 3 (IGFBP-3), and testosterone. Mice without VDR have lower circulating IGF1 concentrations and vitamin D_3 supplementation has been demonstrated to increase the concentration of circulating IGF1 among obese humans (Ameri et al., 2013); however, such an affect has not been demonstrated among healthy individuals. With VDR found within the testes, vitamin D could promote testosterone synthesis (Lundqvist et al., 2011; Blomberg, 2012); however, evidence for a positive relationship between 25(OH)D and circulating testosterone concentrations is limited to elderly or obese males (Wehr et al., 2010; Nimptsch et al., 2012), and has not been observed among healthy males (Valimaki et al., 2004). As such, a mechanism for vitamin D to affect muscle metabolism involving testosterone appears unlikely.

In human patients presenting with 25(OH)D <15 nmol·L^{-1}, vitamin D_3 supplementation (increasing serum 25(OH)D to a mean 114 nmol·L^{-1}) has been shown to improve muscle mitochondrial oxidative function (Sinha et al., 2013). Using ^{31}P magnetic resonance spectroscopy, phosphocreatine and adenosine diphosphate recovery kinetics after exercise were significantly improved compared with a placebo group. Therefore, vitamin D could enhance endurance exercise performance through inhibition of skeletal muscle fatigue. However, randomised controlled trials in participants without fatigue and myopathy, and investigating whether 25(OH)D concentrations need be as high as >100 nmol·L^{-1} are warranted before translation of these results can be made to healthy adults.

1.8.2 Cardiovascular function

More than 30-years ago, an association between 25(OH)D and cardiovascular function was first observed in vitamin D deficient rats (Weishaar and Simpson, 1987). Since then, in humans and using animal models, sufficient vitamin D status has been linked to superior

cardiovascular function, with subsequent improved aerobic performance possible via augmented delivery of oxygenated blood to the musculature. Firstly, via improved endothelial function: which is positively associated with maximal aerobic capacity; an important predictor of endurance performance (Montero, 2015). Flow mediated dilatation has been shown to be higher in young healthy adults with a mean 25(OH)D concentration of 75 $nmol \cdot L^{-1}$ compared with <25 $nmol \cdot L^{-1}$ (Tarcin et al., 2009). Furthermore, high dose vitamin D_3 supplementation increasing 25(OH)D to 117 $nmol \cdot L^{-1}$ has also been shown to be effective at enhancing endothelial function in asymptomatic adults (Tarcin et al., 2009). The vasodilator nitric oxide (NO) may mediate the relationship between vitamin D and endothelial function, because mice without active VDR are characterised by reduced NO and NO synthase availability and subsequent endothelial dysfunction (Andrukhova et al., 2014). Vitamin D induced improvements in endothelial function may be mediated by vascular endothelial growth factor (VEGF), an endothelial specific growth factor with angiogenic activity (Ferrara and Henzel, 1989). Treating cultured vascular smooth muscle cells with $1,25(OH)_2D$ has been shown to stimulate the expression and release of VEGF (Cardus et al., 2006), a process potentially induced by direct binding of the activated VDR to the VEGF promoter region (Cardus et al., 2009).

Secondly, sufficient vitamin D status has been linked to normotension and therefore potentially superior exercise capacity in otherwise healthy adults (Forman et al., 2010; Lim et al., 1996). A consistent inverse association between 25(OH)D and risk of hypertension has been observed within large longitudinal studies (Forman et al., 2007). Mechanistically, vitamin D appears to be an endogenous inhibitor of the renin angiotensin system (Forman et al., 2010). In VDR knockout mice, augmented renin expression and plasma angiotensin II synthesis have been observed, resulting in hypertension and cardiac hypertrophy. In further

support, inhibition of 1,25$(OH)_2$D synthesis in wild-type mice also increased renin expression, with injection of 1,25$(OH)_2$D resulting in a suppression of renin synthesis (Li et al., 2002).

Finally, individuals with vitamin D deficiency have been found to have increased left ventricular mass and left ventricular hypertrophy (Patange et al., 2013; Kulah et al., 2007; Ky et al., 2013). These observational studies and the majority of evidence examining relationships between vitamin D status and cardiac structure and function are from individuals with chronic disease. Diseased individuals with low vitamin D status may therefore have impaired exercise capacity because pathological left ventricular hypertrophy can impair filling capacity, ejection fraction and stroke volume. Using VDR knockout rats to investigate possible mechanisms, Chen et al. (2011) reported VDR signalling appears to conduct anti-hypertrophic activities in cardiac myocytes (for example by supressing pro-hypertrophic calcineurin/nuclear factor of activated T-cells/modulatory calcineurin inhibitory protein 1 pathways). VDR knockout rats are also characterised by increased myocyte size and left ventricular mass, and lower end-diastolic and -systolic volumes compared with wild-type animals (Chen et al., 2011). In contrast to diseased individuals, observational data collected using echocardiograms from healthy athletes revealed men with 25(OH)D <25 nmol·L^{-1} had smaller atrial and ventricular structures than those with 25(OH)D >50 nmol·L^{-1} (Allison et al., 2015). Furthermore, athletes with 25(OH)D <25 nmol·L^{-1} had smaller left ventricular mass than those with 25(OH)D >75 nmol·L^{-1}. Therefore, 25(OH)D <25 nmol·L^{-1} does not appear to contribute to left ventricular hypertrophy in athletic populations. Speculatively, these findings suggest inferior cardiac development, rather than cardiac hypertrophy accounts for differences in cardiac function between athletes with vitamin D sufficiency and deficiency. At present, the mechanisms whereby vitamin D may influence cardiac structure

and function in health and disease remain unclear, with future trials to establish causality required.

1.9 Thesis objectives

The aims of this thesis were:

1. To examine the seasonal variation of three major vitamin D metabolites (serum 25(OH)D, 24,25$(OH)_2$D and 1,25$(OH)_2$D), and their relationship with endurance, strength and power exercise performance among young healthy adults.

2. To investigate the effectiveness of simulated summer sunlight (in accordance with recommendations on safe sunlight exposure) and oral vitamin D_3 supplementation during winter, to restore and then maintain vitamin D sufficiency over 12-weeks, until the availability of ambient UVB increases during spring.

3. In light of the positive association between vitamin D status and endurance exercise performance, and prevalence of vitamin D insufficiency during winter; a final aim was to explore the effect of 12-weeks vitamin D supplementation, by either safe simulated sunlight or oral vitamin D_3 on serum vitamin D metabolites, exercise performance and resting blood pressure.

CHAPTER TWO

General methods

2.1 Participants. In **Chapters 3 and 5** participants were military recruits joining the British Army; aged 17–33 years; and had passed a physician-screened medical assessment. Males (**Chapters 3 and 5**) were located at Infantry Training Centre Catterick, UK (latitude 54°N) and females (**Chapter 3**) were located at Army Training Centre Pirbright, UK (51°N). All military recruits were studied during Basic Military Training that follows a generic syllabus of basic military skills including physical training, weapon handling, map reading and field craft. The progressive, structured, physical training programme included: endurance training, typically involving running in groups, with and without load carriage; circuit training, consisting of high-repetition, low force exercises using all major muscle groups; agility based gymnasium work using benches and ropes; and assault course practice. Marching with various loads while on military exercise and military drill were also undertaken. Participants in **Chapter 4** were male and female students or staff at Bangor University, UK (53°N) aged 19–29 years, and completed a medical questionnaire to confirm they were healthy (Appendix A).

2.2 Ethical approval. Ethical approval was obtained from the Ministry of Defence (UK) Research Ethics Committee (**Chapters 3 and 5**) or from the local Ethics Committee (School of Sport, Health and Exercise Sciences, Bangor University; **Chapter 4**). All protocols were conducted in accordance with the Declaration of Helsinki (2013). The nature and purpose of each study was fully explained in writing and verbally to each participant. All participants voluntarily agreed to participate and provided fully informed written consent (Appendix B and C).

2.3 Anthropometry. At baseline in all studies (**Chapters 3–5**), height was measured using a stadiometer and body mass was determined using a digital platform scale (Seca, Hamburg, Germany).

2.4 Exercise performance. Participants completed endurance, strength and power exercise performance assessments in **Chapters 3 and 5**. Participants wore Army shorts and T-shirt for all exercise performance assessments, and trainers for the 1.5-mile run.

2.4.1 Endurance exercise performance. After an ~800 m warm-up run led by a military Physical Training Instructor, a 1.5-mile (2.4 km) time trial run was completed on an outdoor course. Time to complete the course was recorded to the nearest second. The 1.5-mile run is used widely among military personnel, with performance indicative of an individual's maximal aerobic capacity (Friedl et al., 2015). Participants were highly motivated because their best effort was required for progression in their military careers.

2.4.2 Muscular strength. Maximum dynamic lift strength was assessed using an incremental lift machine that simulates the power clean weight lifting movement, as described previously (Nindl et al., 2007). The device consisted of a vertically moving carriage with handgrips 0.3 m above the ground. Without shoes, participants started the incremental lifts with a mass of 20 kg, with the weight lifted to a point where the handgrips were 1.45 m from the ground; replicating the floor height of an Army four tonne truck. With each successful lift, the mass was increased by 5 kg with 1 minute rest between attempts. The test was terminated when participants failed to lift the weight on their second attempt.

2.4.3 Muscular power output. Maximum vertical jump height was determined using a digital jump meter (Takei Scientific Instruments, Tokyo, Japan), as described previously (Fortes et al., 2011). A belt was fitted around the waist of each participant and secured to a rubber mat. Without shoes and after three warm-up jumps, participants were instructed to jump as high as possible three times, with hands placed on hips to prevent upper limb assistance. Where an increase in jump height occurred across jumps 1 to 3, indicative of a learning effect, a fourth jump was made. Maximum vertical jump height was recorded as the highest score achieved. Muscular power output was calculated from maximum jump height and body mass using a validated equation reflecting instantaneous power output (Sayers et al., 1999):

$$\text{Muscular power output (W)} = (51.9 \times \text{jump height (cm)}) + (48.9 \times \text{body mass (kg)}) - 2007$$

2.5 Simulated sunlight. In accordance with UK guidelines on safe sunlight exposure for vitamin D synthesis (Advisory Group on Non-ionising Radiation, 2017), participants receiving the simulated sunlight intervention (**Chapters 4 and 5**) were exposed to UV irradiations 3 times-a-week during a 4-week restoration phase, and once-a-week during an 8-week maintenance phase. A constant UVR dose of 1.3 SED was delivered during each irradiation using an investigator controlled whole-body irradiation cabinet (Hapro Jade, Kapelle, The Netherlands) fitted with Arimed B fluorescent tubes (Cosmedico, Stuttgart, Germany), as described previously (Rhodes et al., 2010). The fluorescent tubes emitted a UVR spectrum similar to sunlight (λ: 290–400 nm; 95% ultraviolet A (UVA): 320–400 nm, 5% UVB: 290–320 nm) which was characterised using a spectroradiometer (USB2000+, Ocean Optics BV, Duiven, The Netherlands), radiometrically calibrated with traceability to UK national standards. A constant UVR dose was maintained throughout each study by monitoring irradiance using a spectroradiometer (USB2000+, Ocean Optics BV) and

adjusting for any decrease in measured irradiance emitted by increasing exposure time where necessary, as described (Rhodes et al., 2010; mean exposure time in **Chapter 4**: 186 s; and **Chapter 5**: 222 ± 23 s). Investigators controlled the exposure time by using an electronic timer on the irradiation cabinet. Participants wore standardised V-neck T-shirt and knee-length shorts to expose 37% skin surface area in **Chapter 4**, and standard issue Army T-shirt and mid-thigh shorts to expose 40% skin surface area in **Chapter 5**. The UVR transmission through clothing was measured using a spectroradiometer and shown to be minimal, with 98% of UVR blocked by the shorts and T-shirt fabric. Exposures for placebo participants were of the same duration as simulated sunlight and were delivered using an identical whole-body irradiation cabinet, but with the panels containing the fluorescent tubes covered with transparent UVR blocking film (DermaGard UV film, SunGard, Woburn, Massachusetts, USA). Spectroradiometry confirmed the UVR blocking film was effective at preventing transmission of 99.9% of UVR. Participant compliance was determined by investigator counts of simulated sunlight and placebo exposures. Investigators were blinded to simulated sunlight and placebo irradiation cabinets by a medical physicist not involved in data collection (**Chapter 5**).

2.6 Oral vitamin D_3. Participants receiving the oral vitamin D_3 intervention consumed a vitamin D_3 capsule daily, using a 1,000 and 400 IU dose during a 4-week restoration phase and 8-week maintenance phase, respectively (**Chapters 4 and 5**; Pure Encapsulations, Sudbury, Massachusetts, USA). The restoration dose was considerably less than the tolerable upper intake recommended by EFSA and IOM (European Food Safety Authority, 2012; Institute of Medicine, 2011), and maintenance dose was in accordance with UK government guidelines (Scientific Advisory Committee on Nutrition, 2016). Placebo participants consumed a cellulose placebo capsule daily (**Chapter 4:** Biotech Pharmacal Inc.,

Fayetteville, Arkansas, USA; **Chapter 5:** Almac Group, Craigavon, County Armagh, UK). Participant compliance was estimated by counting the number of unused capsules returned each week. Oral vitamin D_3 and placebo capsules were independently analysed for total vitamin D content using HPLC (by NSF International Laboratories, Ann Arbor, Michigan, USA; **Chapter 5**). The vitamin D_3 content of the 1,000 and 400 IU capsules was found to be 1,090 and 460 IU, respectively, with vitamin D_2 undetectable. Analysis confirmed the placebo capsules did not contain vitamin D.

2.7 Blood collection and handling. Whole blood samples were collected by venepuncture from an antecubital vein into plain vacutainers (Becton Dickinson, Oxford, UK; **Chapters 3–5**). Samples were left to clot at room temperature for 1 hour and then spun at 1500*g* for 10 minutes in a refrigerated centrifuge (4°C). Serum was aliquoted into universal tubes, and immediately frozen at -80°C for later analysis.

2.8 Vitamin D metabolites. Total serum 25(OH)D (**Chapters 3–5**) and 24,25$(OH)_2$D (**Chapters 3 and 5**) were analysed by LC-MS/MS using a Micromass Quattro Ultima Pt mass spectrometer (Waters Corp., Milford, Massachusetts, USA), as described (Tang et al., 2017). Serum 1,25$(OH)_2$D was measured using the DiaSorin LIAISON XL 1,25$(OH)_2$D chemiluminescent immunoassay method (DiaSorin, Stillwater, Minnesota, USA; **Chapters 3 and 5**). Analyses were performed in a Vitamin D External Quality Assurance Scheme certified laboratory (Bioanalytical Facility, University of East Anglia, Norwich, UK).

2.9 Factors that affect 25(OH)D concentrations. During daylight hours, participants wore polysulphone (PSF) badges at the sternum on their outermost layer of clothing to record total erythemal dose (**Chapters 4 and 5**); a marker of exposure to UVB responsible for dermal

vitamin D_3 synthesis (Webb et al., 2010). The total erythemal dose was relativised to the number of days badges were worn (recorded by participants in a diary) to give a daily dose, and did not include that received from the simulated sunlight intervention. Participants also completed a food frequency questionnaire (FFQ) to record their habitual daily dietary intake of vitamin D (**Chapters 4 and 5**; Appendix D). Dietary vitamin D intake was calculated excluding that which participants assigned to oral vitamin D_3 supplementation received from their intervention.

2.10 Skin type. In **Chapters 4 and 5**, sun-reactive skin type was assessed using a questionnaire (Appendix E; Fitzpatrick, 1988), with participants categorised by a professor of experimental dermatology (Prof. Lesley Rhodes, University of Manchester, UK).

2.11 Statistical analysis. Data in the text and tables are presented as mean ± SD, unless otherwise stated and statistical significance was accepted at $P < 0.05$. Statistical analyses were conducted using SPSS Statistics 22.0 (IBM, Armonk, New York, USA). Data were checked for sphericity, with Greenhouse-Geisser adjustments to the degrees of freedom made where necessary. Data were checked for normality, with any skewed variables transformed to achieve normal distribution. Where significant interactions were found using mixed model analysis of variance (ANOVA), simple main effects were explored using one-way repeated measures ANOVA or paired-sample *t*-tests, and one-way ANOVA or independent Student's *t*-tests, where applicable. Where significant interactions were not observed, statistically significant main effects were explored using pairwise comparisons. One-way ANOVA were used to compare participant characteristics between intervention groups. Bonferroni correction was used for *post hoc* analyses. Where reported, Cohen's *d* effect sizes were

calculated for the difference between two means. Cohen's *d* effect sizes ≥0.2, 0.5 and 0.8 represent small, medium, and large effects, respectively (Cohen, 1988).

CHAPTER THREE

Vitamin D status predicts endurance exercise performance in young healthy military recruits

3.1 SUMMARY

Reports investigating an association between vitamin D status and exercise performance are conflicting. The aim of this prospective cohort study was to investigate the seasonal variation in vitamin D metabolites (serum 25(OH)D, 24,25$(OH)_2$D and 1,25$(OH)_2$D); and determine if an association exists between these metabolites and exercise performance in young healthy adults, with control for covariates. Recruited from all seasons, 967 military recruits (age 22 ± 3 years; 64% male, 36% female) completed a 1.5-mile run, maximum dynamic lift, and maximum vertical jump to assess endurance, strength and power, respectively. Body composition was measured by DXA. These measurements were repeated in a cohort after 12-weeks military training (n = 331; 51% male, 49% female). Serum 25(OH)D, 24,25$(OH)_2$D (measured by LC-MS/MS) and 1,25$(OH)_2$D (measured by immunoassay) were lowest during winter, and highest during summer ($P < 0.01$). Serum 25(OH)D and 24,25$(OH)_2$D were positively associated with endurance performance after controlling for fat mass, smoking and season ($P < 0.001$). For every 1 nmol·L^{-1} increase in 25(OH)D, 1.5-mile run time was ~half-a-second faster. Following 12-weeks of structured military training, 25(OH)D and 24,25$(OH)_2$D were once again positively associated with endurance performance after controlling for fat mass, smoking, incomplete training days, and season ($P \leq 0.01$). Serum 1,25$(OH)_2$D was not associated with 1.5-mile run time ($P \geq 0.12$). No vitamin D metabolites were associated with muscle strength or power ($P \geq 0.09$). In conclusion, all 3 major vitamin D metabolites demonstrated a seasonal variation, and were lowest during winter. Serum 25(OH)D and 24,25$(OH)_2$D were positively associated with endurance but not strength or power exercise performance in young healthy military recruits.

3.2 INTRODUCTION

Vitamin D can be obtained from dietary sources, such as oily fish, eggs and fortified cereals; however, vitamin D is primarily synthesised endogenously by exposing the skin to solar UVB. Following conversion of cutaneous 7-dehydrocholesterol to pre-vitamin D and subsequently vitamin D_3, or after dietary intake, vitamin D is hydroxylated to 25(OH)D in the liver. Principally within the kidney, 25(OH)D then undergoes a further hydroxylation step to form the biologically active $1,25(OH)_2D$, with production and degradation tightly regulated (St-Arnaud and Glorieux, 1998). A third major circulating vitamin D metabolite, $24,25(OH)_2D$, is a product of 25(OH)D catabolism and is thought to have limited biological activity (DeLuca, 2004; Holick, 2007). At latitudes >42°N, cutaneous synthesis contributes little serum 25(OH)D during winter when the intensity of solar UVB is reduced and hours of sunlight are shortened; hence circulating 25(OH)D has a distinct seasonal pattern, peaking in summer and dropping to its nadir in winter (Webb et al., 1988). Vitamin D insufficiency (25(OH)D <50 $nmol \cdot L^{-1}$) is therefore common during winter and spring (Cashman et al., 2016). Avoiding low 25(OH)D is important for musculoskeletal health, hence EFSA (2016) and IOM (2011) guidelines recommend maintaining serum 25(OH)D ≥50 $nmol \cdot L^{-1}$.

As reviewed in **Chapter 1** (**section 1.8**), vitamin D might enhance strength and endurance exercise performance by stimulating skeletal muscle protein synthesis via VDR mediated signalling (Girgis et al., 2013). Furthermore, vitamin D may expand the cardiovascular system's ability to transport oxygenated blood, and therefore endurance performance, for example via improved endothelial function or cardiac structure (Tarcin et al., 2009; Allison et al., 2015). Results from observational studies investigating the relationship between vitamin D status and exercise performance have been conflicting (Hildebrand et al., 2016; Koundourakis et al., 2014; Marantes et al., 2011; Ardestani et al., 2011; Grimaldi et al., 2013;

Fitzgerald et al., 2015; Fitzgerald et al., 2014; Forney et al., 2014; Hamilton et al., 2014); hence the importance of vitamin D for exercise performance remains unclear (Thomas et al., 2016). Currently, observational studies in young adults are limited by small sample size, single-sex recruitment, poor spread of 25(OH)D concentrations, or failure to control for confounding variables (Hildebrand et al., 2016; Fitzgerald et al., 2015; Fitzgerald et al., 2014; Forney et al., 2014; Koundourakis et al., 2014). To date, studies examining the association between vitamin D status and exercise performance in young adults have only measured serum 25(OH)D and have not assessed endurance performance *and* controlled for covariates known to influence exercise performance (for example: age, sex, body composition, smoking, physical activity and season; Mattila et al., 2007; Kok et al., 2012; Song et al., 1998; Maughan et al., 1983; Cannell et al., 2009).

Whether a seasonal variation exists in $24,25(OH)_2D$ and $1,25(OH)_2D$ remains unexplored in healthy, UK adults. Furthermore, the relationship between vitamin D metabolites and exercise performance is yet to be clearly determined in young adults. Therefore, the purpose of this study was to investigate the seasonal variation in vitamin D metabolites in a large sample of young healthy adults; and determine if an association exists between serum 25(OH)D, $24,25(OH)_2D$ or $1,25(OH)_2D$ and endurance, strength or power exercise performance, after controlling for covariates known to influence performance. These aims were achieved by assessing vitamin D metabolites, body composition and exercise performance in military recruits, during all seasons. Associations were analysed at the start of military training and after 12-weeks of structured training. It was hypothesised that a seasonal variation in 25(OH)D and $24,25(OH)_2D$, but not $1,25(OH)_2D$ would be seen; and that 25(OH)D and $24,25(OH)_2D$, but not $1,25(OH)_2D$, would be associated with exercise performance.

3.3 METHODS

Participants. Nine-hundred and sixty-nine male and female military recruits (age 22 ± 3 years; 95% white ethnicity; *n* = 621 males: body mass 75.0 ± 10.0 kg; height 178 ± 6 cm; body mass index (BMI) 23.8 ± 2.8 $kg \cdot m^{-2}$; body fat 19.8 ± 5.3%; current smokers 45%; *n* = 346 females: body mass 63.9 ± 7.9 kg; height 165 ± 6 cm; BMI 23.4 ± 2.4 $kg \cdot m^{-2}$; body fat 30.2 ± 4.9%; current smokers 25%) participated in this study between January 2014 and September 2015.

Study design and experimental procedures. In this prospective cohort study, participants were recruited throughout the year to maximise the spread of seasonal variation in vitamin D metabolites (Figure 3.1). During week 1 of military training, participants completed baseline measurements for three exercise performance tests (1.5-mile run, maximum dynamic lift, and maximum vertical jump; as described in **Chapter 2, section 2.4**); and a blood sample was collected (as described in **Chapter 2, section 2.7**) for the analysis of vitamin D metabolites (serum 25(OH)D, 24,25$(OH)_2$D and 1,25$(OH)_2$D; see **Chapter 2, section 2.8** for details). Participants also completed anthropometric measurements (as described in **Chapter 2, section 2.3**). Whole-body body composition was assessed by fan-beam dual-energy x-ray absorptiometry (DXA; Lunar iDXA, GE Healthcare, Buckinghamshire, UK), and whole-body fat mass and lean body mass were calculated. Participants were instructed to lie motionless in the supine position for the duration of the DXA scan, with straps around the lower limbs to minimise movement. Males wore underwear only and females light clothing. Participants answered questions on ethnicity (white, black, Asian, Chinese, Mixed or other) and smoking history (current, former, or never). After 12-weeks of military training, a cohort of 331 participants (170 males, 161 females) randomly selected throughout the year to provide a full seasonal range of follow-up measurements, repeated baseline measurements

(Figure 3.1). Time-of-day for maximum dynamic lift and vertical jump tests were matched at baseline and follow-up (± 78 (0–150) minutes (± mean (min–max)). Medical records were accessed to calculate the number of incomplete training days due to illness or injury for each participant.

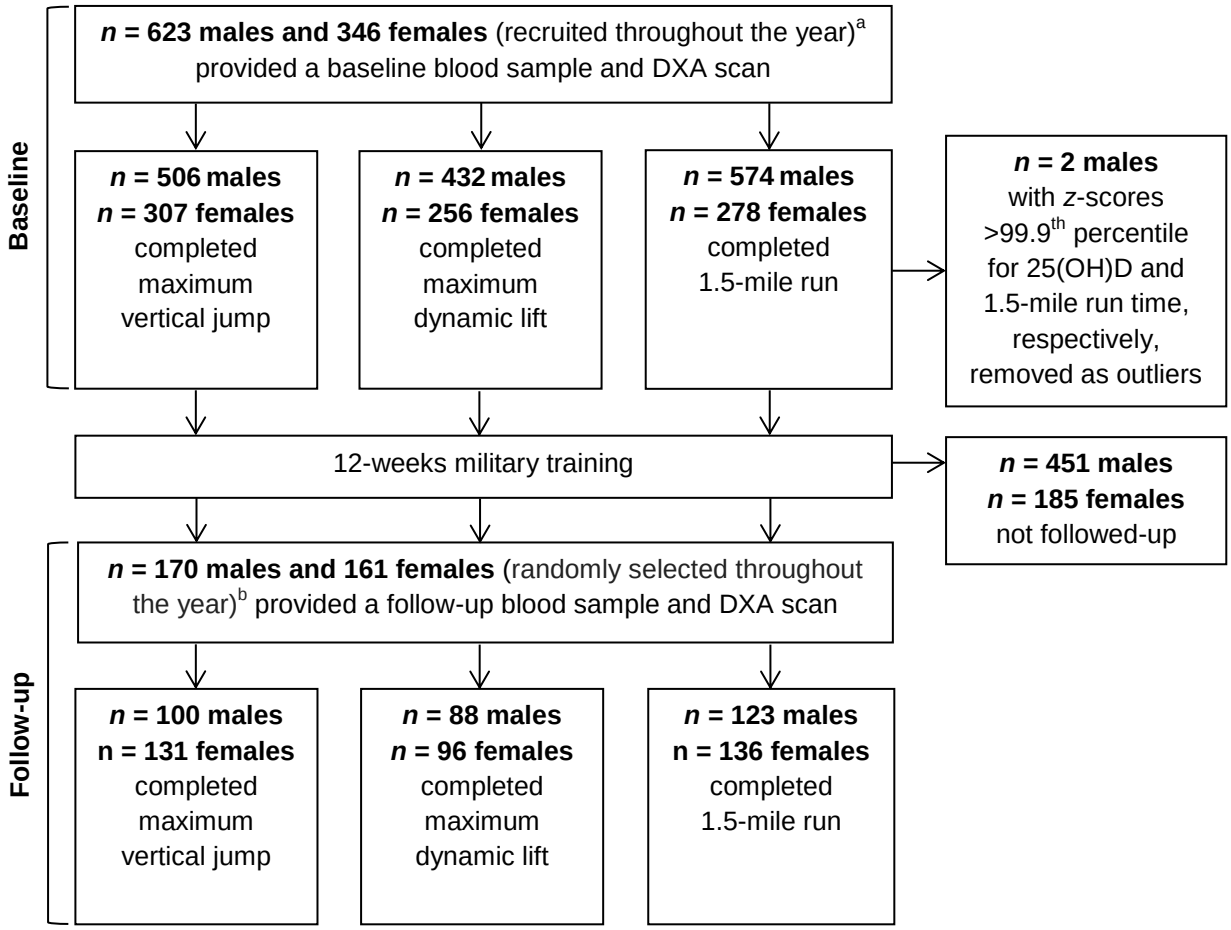

Figure 3.1. Study flow diagram. Participants (*n* = 969) were recruited from Infantry Training Centre Catterick (*n* = 623 males) and Army Training Centre Pirbright (*n* = 346 females). A subsample of participants randomly selected from each season repeated measurements at follow-up. Blood samples were analysed for serum 25(OH)D, 24,25$(OH)_2$D and 1,25$(OH)_2$D. DXA, dual-energy x-ray absorptiometry. [a] Baseline recruitment: spring 94 (15%) males, 92 (27%) females; summer 252 (41%) males, 42 (12%) females; autumn 132 (21%) males, 109 (31%) females; winter 143 (23%) males, 103 (30%) females. [b] Follow-up recruitment: spring 18 (11%) males, 33 (21%) females; summer 57 (34%) males, 39 (24%) females; autumn 45 (26%) males, 36 (22%) females; winter 50 (29%) males, 53 (33%) females.

Statistical analysis. Hierarchical multiple linear regression was used to determine the association between vitamin D metabolites and each measure of exercise performance at baseline and follow-up, whilst controlling for covariates. Covariates were entered into regression models based on established influences on exercise performance. For the 1.5-mile run, covariates were fat mass, smoking and season (Mattila et al., 2007; Song et al., 1998; Cannell et al., 2009). For maximum dynamic lift strength and muscular power output, covariates were lean body mass, smoking, height, and season (Maughan et al., 1983; Kok et al., 2012; Cannell et al., 2009). Follow-up regression models included these covariates with the addition of number of incomplete training days to control for injury and illness. The assumption of homoscedasticity was met, and multicollinearity not present between predictor variables. To correct the positive skew of variables not normally distributed, fat mass, lean body mass, 25(OH)D, 24,25$(OH)_2$D and 1,25$(OH)_2$D were log or square root transformed where necessary. As required, the negative skew of 1.5-mile run was corrected using a cube transformation. To facilitate meaningful interpretation of multiple regression beta coefficients, all models where 25(OH)D was a significant predictor of performance were repeated using non-transformed variables. For each regression model, Cohen's f^2 effect size for vitamin D metabolites was calculated, whereby Cohen's $f^2 \geq 0.02$, 0.15 and 0.35 represent small, medium and large effect sizes, respectively (Cohen, 1988). For multiple regression analyses, a minimum sample size of 155 was estimated using effect sizes from previous studies (Fitzgerald et al., 2015; von Hurst et al., 2013) and standard formula ($n \geq (8/f^2)$ + (number of predictors – 1) (Green, 1991). Analyses were completed on 967 participants after removing 2 male outliers with *z*-scores $\geq 99.9^{th}$ percentile for baseline 1.5-mile run time and 25(OH)D. One-way ANOVA was used to compare exercise performance between baseline serum 25(OH)D quartiles and to investigate seasonal differences in vitamin D metabolites. Meteorological seasons were defined as spring, March–May; summer, June–August; autumn,

September–November; and winter, December–February. Independent Student's *t*-tests and paired-sample *t*-tests were used to identify differences in exercise performance between sexes, and baseline to follow-up changes, respectively.

3.4 RESULTS

Seasonal variation in vitamin D metabolites

A seasonal variation in all vitamin D metabolites ($P < 0.001$; Figure 3.2A–C) and the relationship between metabolites, expressed as ratios ($P < 0.001$; Figure 3.2D–F) was observed in males and females.

During winter, only 9% of males and 36% of females were vitamin D sufficient (25(OH)D ≥50 nmol·L^{-1}); and 53% of males and 16% of females were vitamin D deficient (25(OH)D <30 nmol·L^{-1}; Figure 3.3). In contrast, during summer, 81% of males and 90% of females were vitamin D sufficient; and 4% of males and no females were vitamin D deficient. Serum 25(OH)D changed minimally during 12-weeks of training. For those who completed measures at baseline and follow-up, 27 (16%) males and 3 (2%) females increased 25(OH)D concentration from <30 to ≥50 nmol·L^{-1}; 12 (7%) males and 7 (4%) females increased from <50 to ≥75 nmol·L^{-1}; and 3 participants (3 male, 0 female) increased from <30 to ≥75 nmol·L^{-1}.

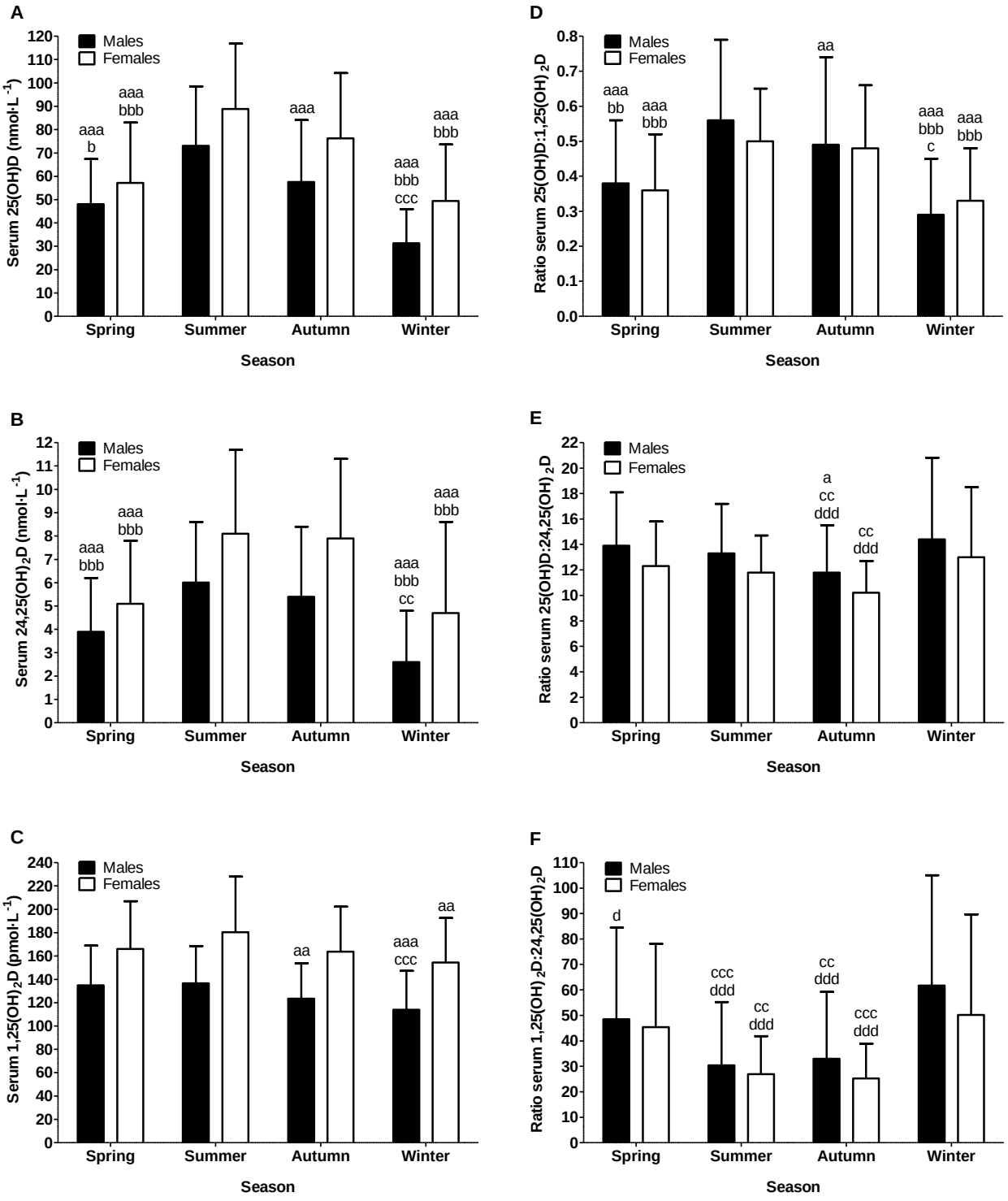

Figure 3.2. Seasonal variation in baseline serum vitamin D metabolites: (A) 25(OH)D, (B) 24,25$(OH)_2$D, and (C) 1,25$(OH)_2$D; and the relationships between vitamin D metabolites expressed as ratios: (D) ratio of 25(OH)D to 1,25$(OH)_2$D, (E) ratio of 25(OH)D to 24,25$(OH)_2$D, and (F) ratio of 1,25$(OH)_2$D to 24,25$(OH)_2$D (n = 967; 621 males, 346 females). Data are mean ± SD: a $P < 0.05$, aa $P < 0.01$ and aaa $P < 0.001$ lower than summer; b $P < 0.05$, bb $P < 0.01$ and bbb $P < 0.001$ lower than autumn; c $P < 0.05$, cc $P < 0.01$ and ccc $P < 0.001$ lower than spring; d $P < 0.05$ and ddd $P < 0.001$ lower than winter.

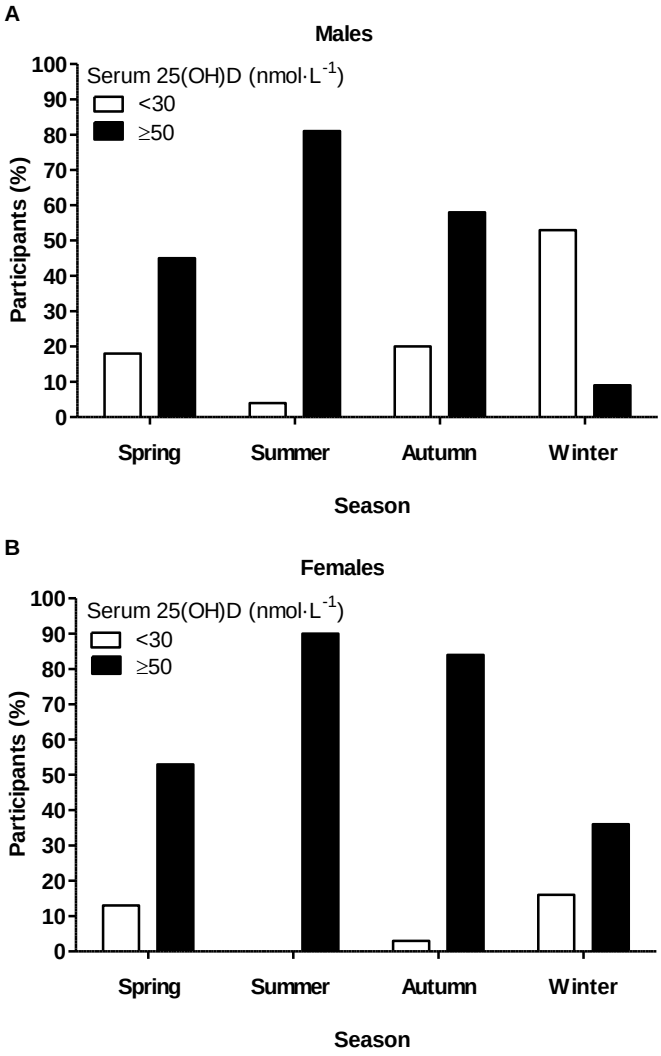

Figure 3.3. Percentage of (A) males and (B) females categorised as vitamin D deficient (25(OH)D <30 nmol·L^{-1}) and sufficient (25(OH)D ≥50 nmol·L^{-1}) during each season at baseline.

Exercise performance

Males performed better than females in all performance tests (P < 0.001). For participants who completed measures at baseline and follow-up, 1.5-mile run time was faster at follow-up (males 627 ± 48 *vs*. 578 ± 31 s; females 699 ± 54 *vs*. 667 ± 44 s; P < 0.001). Maximum dynamic lift strength decreased in males (71 ± 12 *vs.* 68 ± 11 kg; P < 0.01), and did not change in females (43 ± 9 *vs.* 44 ± 9 kg; P = 0.13). From baseline to follow-up, muscular power output decreased in males (3868 ± 619 *vs*. 3797 ± 573 W; P < 0.01), and increased in females (2766 ± 465 *vs*. 2840 ± 436 W; P < 0.01).

Endurance exercise performance

Serum 25(OH)D was positively associated with endurance exercise performance after controlling for fat mass, smoking and season, with baseline 25(OH)D accounting for 4% of the variance in male 1.5-mile run time and 6% of the variance in female 1.5 mile run time (ΔR^2 = 0.04 and 0.06, respectively; Table 3.1). Every 1 nmol·L^{-1} increase in 25(OH)D translated into 0.42 ± 0.16 s faster (± 95% CI) 1.5-mile run time in males and 0.57 ± 0.25 s faster 1.5-mile run time in females. These relationships were not reliant on participants with high or low 25(OH)D concentrations because positive associations remained after removing males and females with 25(OH)D ≥75, or <30 nmol·L^{-1} (P < 0.05). Once more at follow-up, after 12-weeks of military training, 25(OH)D was again positively associated with endurance exercise performance, explaining 6% of the variance in male 1.5-mile run time and 3% of variance in female 1.5-mile run time, after controlling for fat mass, smoking, season, and incomplete training days (ΔR^2 = 0.06 and 0.03, respectively; Table 3.1). At follow-up, every 1 nmol·L^{-1} increase in 25(OH)D translated into 0.34 ± 0.23 s faster 1.5-mile run time in males and 0.47 ± 0.35 s faster 1.5-mile run time in females. Serum 24,25$(OH)_2$D was also positively associated with endurance exercise performance after controlling for fat mass, smoking and season, with baseline 24,25$(OH)_2$D accounting for 3% of variance in male and female 1.5-mile run time (ΔR^2 = 0.03; Table 3.1). The positive association between 24,25$(OH)_2$D and 1.5-mile run time remained at follow-up, with serum 24,25$(OH)_2$D explaining 7% of the variance in male 1.5-mile run time and 4% of female 1.5-mile run time after controlling for fat mass, smoking, season, and incomplete training days (ΔR^2 = 0.07 and 0.04, respectively; Table 3.1). Serum 1,25$(OH)_2$D was not associated with 1.5-mile run time in males or females at baseline (P = 0.12 and 0.51, respectively) or follow-up (P = 0.12 and 0.85, respectively). Using a simple one-way ANOVA, i.e. without control for fat mass, smoking and season, 1.5-mile run time was fastest among males with baseline 25(OH)D in

the highest quartile (≥75 nmol·L^{-1}, $P < 0.05$, Cohen's *d* effect size = 0.4); a similar trend was observed in females ($P = 0.09$, Cohen's *d* effect size = 0.4; Figure 3.4).

Table 3.1. Serum 25(OH)D and 24,25$(OH)_2$D as predictors of 1.5-mile run time after controlling for covariates including: fat mass, smoking and season at baseline, plus incomplete training days at follow-up.

	Vitamin D metabolite	R^2 overall	ΔR^2	Sig F-change	Standardised beta	Cohen's f^2
Males						
Baseline[a]	25(OH)D	0.20	0.04	< 0.001	-0.24	0.04
	24,25$(OH)_2$D	0.20	0.03	< 0.001	-0.22	0.04
Follow-up[b]	25(OH)D	0.30	0.06	0.002	-0.33	0.09
	24,25$(OH)_2$D	0.30	0.07	0.001	-0.32	0.10
Females						
Baseline[c]	25(OH)D	0.34	0.06	< 0.001	-0.26	0.08
	24,25$(OH)_2$D	0.32	0.03	< 0.001	-0.20	0.05
Follow-up[d]	25(OH)D	0.39	0.03	0.01	-0.22	0.05
	24,25$(OH)_2$D	0.40	0.04	0.007	-0.24	0.06

[a] $n = 572$; [b] $n = 123$; [c] $n = 278$; [d] $n = 136$. Cohen's f^2 ≥0.02, ≥0.15 and ≥0.35 represent small, medium and large effect sizes, respectively (Cohen, 1988).

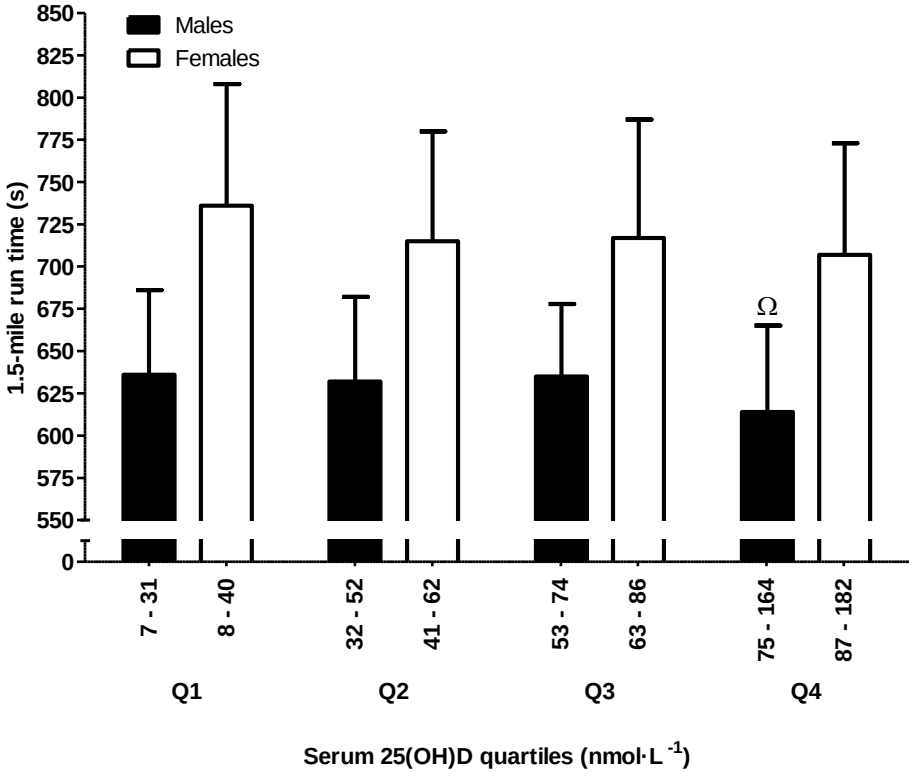

Figure 3.4. One-and-a-half-mile run time by baseline serum 25(OH)D quartiles. Data are mean ± SD: Ω $P < 0.05$ faster than quartiles 1, 2 and 3.

Muscular strength and power performance

Serum 25(OH)D was not associated with maximum dynamic lift strength in males ($n = 432$, $P = 0.23$) or females ($n = 256$, $P = 0.09$); or muscular power output in males ($n = 506$, $P = 0.25$) or females ($n = 307$, $P = 0.91$) when controlling for lean body mass, smoking, height and season. Similarly at follow-up, serum 25(OH)D was not associated with maximum dynamic lift strength in males ($n = 88$, $P = 0.30$) or females ($n = 96$, $P = 0.62$); or muscular power output in males ($n = 100$, $P = 0.67$) or females ($n = 131$, $P = 0.63$). Additionally, neither serum 24,25$(OH)_2$D nor 1,25$(OH)_2$D were associated with maximum dynamic lift strength or muscular power output in males at baseline or follow-up ($P \geq 0.09$); or females at baseline or follow-up ($P \geq 0.16$). Analysing quartiles of baseline serum 25(OH)D using simple one-way ANOVA, there were no differences in maximum dynamic lift strength or muscular power output in either sex ($P > 0.05$).

3.5 DISCUSSION

As the first study to examine the relationship between three major vitamin D metabolites and exercise performance in young healthy men and women, these data demonstrate serum 25(OH)D and 24,25$(OH)_2$D concentrations were positively associated with endurance exercise performance. Contrary to the hypotheses, neither 25(OH)D nor 24,25$(OH)_2$D were associated with muscle strength or power. Aligned with the hypotheses, serum 1,25$(OH)_2$D was not associated with any measure of exercise performance. These novel findings from 967 military recruits can be considered robust as they were observed at the start of training (assuming varied lifestyle, diet and activity before enrolment) after controlling for body composition, smoking and season and in both men *and* women; and again after 12-weeks of military training (assuming similar lifestyle, diet and physical activity), and with control for incomplete training days due to illness or injury (Table 3.1). In terms of practical significance, the magnitude of association between serum 25(OH)D and endurance performance can be considered relatively small (Cohen's f^2 effect sizes <0.15). Nevertheless, in real-world-terms 1.5-mile run time was ~half-a-second faster for every 1 nmol·L^{-1} increase in 25(OH)D in males and females. To illustrate, a male with the mean summer 25(OH)D concentration of 73 nmol·L^{-1} would be predicted to run 1.5-miles 18 s faster than a male with the mean winter 25(OH)D of 31 nmol·L^{-1}; whilst a female with the mean summer 25(OH)D concentration of 89 nmol·L^{-1} would be predicted to run 1.5-miles 23 s faster than a female with the mean winter 25(OH)D of 49 nmol·L^{-1}. As hypothesised, a seasonal variation was observed in serum 25(OH)D and 24,25$(OH)_2$D, and unexpectedly 1,25$(OH)_2$D. All three major vitamin D metabolites were lowest during winter and highest during summer.

Vitamin D metabolites

As anticipated, a variation in serum 25(OH)D was observed between seasons, reflecting expected differences in the availability of ambient UVB throughout the year (highest during summer and negligible during winter in the UK; Hypponen and Power, 2007). A similar seasonal variation in the ratio of 25(OH)D to $1,25(OH)_2D$ was also seen (Figure 3.2). Given $24,25(OH)_2D$ (a product of 25(OH)D catabolism) has been shown previously to be strongly correlated with 25(OH)D concentrations (Cashman et al., 2015; Tang et al., 2017), a similar seasonal variation in serum $24,25(OH)_2D$ was observed; hence the relationships observed for serum 25(OH)D and $24,25(OH)_2D$ with exercise performance were not dissimilar (Table 3.1). The tight regulation of $1,25(OH)_2D$ production and degradation to maintain calcium and phosphorus homeostasis meant serum $1,25(OH)_2D$ was not expected to vary between seasons (Chesney et al., 1981); however, $1,25(OH)_2D$ concentration was lowest during winter. A similar observation has been made in hospital patients (Moan et al., 2009b). The magnitude of $1,25(OH)_2D$ seasonal differences in the present study were less than those observed for 25(OH)D and $24,25(OH)_2D$. Although $1,25(OH)_2D$ is the biologically active form of vitamin D, its short ~4 hour half-life and normal or elevated production during vitamin D deficiency mean it is not the ideal marker for vitamin D status (Zerwekh, 2008; Holick, 2009). These characteristics may explain the lack of association between $1,25(OH)_2D$ and exercise performance, especially because in many cases performance assessments were made >4 hours after blood was collected. The ratio of 25(OH)D to $24,25(OH)_2D$, which may serve as a marker of vitamin D catabolic status and be predictive of the 25(OH)D response to supplementation, was lowest during autumn suggesting a higher catabolic rate of 25(OH)D towards its $24,25(OH)_2D$ metabolite at this time (Wagner et al., 2011). The ratio of $1,25(OH)_2D$ to $24,25(OH)_2D$ was lowest during summer and autumn, which indicates

25(OH)D metabolism was driven to produce 24,25$(OH)_2$D rather than 1,25$(OH)_2$D at this time, when 25(OH)D concentrations peak.

Endurance exercise performance

These findings expand upon previous literature that have reported bivariate correlations between circulating 25(OH)D and aerobic performance in small samples of young adults (*n* = 39–67; Fitzgerald et al., 2014; Forney et al., 2014; Koundourakis et al., 2014). In contrast to the present study but including children, Wilson et al. (2013) found no association between 25(OH)D and maximal oxygen consumption ($\dot{V}O_{2max}$) after controlling for BMI (*n* = 5,392, 12–49 years). In agreement with the present study but including elderly participants, Ardestani et al. (2011) found a positive association remained between 25(OH)D and $\dot{V}O_{2max}$ after controlling for BMI (*n* = 200, 20–73 years). Vitamin D status is affected by body composition because excess adipose tissue sequesters vitamin D; hence individuals with high fat mass have lower 25(OH)D concentrations (Wortsman et al., 2000). High fat mass impairs exercise performance in young adults (Mattila et al., 2007); consequently, high body fat and low availability of vitamin D may be responsible for poor performance in individuals with insufficient 25(OH)D concentrations. Therefore, the influence of vitamin D on performance may be overestimated in studies that do not control for body composition. Both Wilson et al. (2013) and Ardestani et al. (2011) attempted to account for body composition by controlling for BMI. However, BMI is a measure of relative body mass that does not distinguish between fat and lean mass, especially in active individuals (Flegal et al., 2009). The present study is the first to control for body fat in regression models investigating the relationship between vitamin D status and endurance exercise performance.

Several plausible mechanisms may explain the positive association observed between vitamin D metabolites and endurance performance. As reviewed in **Chapter 1** (**section 1.8.2**), higher 25(OH)D concentrations may improve endurance performance via enhanced delivery of oxygenated blood to the musculature through: (i) improved endothelial function mediated by the vasodilator nitric oxide (Andrukhova et al., 2014; Tarcin et al., 2009); (ii) maintenance of normotension by inhibiting the renin angiotensin system (Forman et al., 2010); or (iii) superior cardiac structures, including greater left ventricular mass and volume (Allison et al., 2015). In addition to cardiovascular mechanisms, vitamin D may improve endurance performance by enhancing mitochondrial oxidative function and inhibiting skeletal muscle fatigue (Sinha et al., 2013). However, cardiovascular and mitochondrial function were not measured in the present study, hence these mechanisms require further investigation. Interestingly, the positive relationships observed here between vitamin D and endurance performance were not dependent on participants with high or low vitamin D status because associations remained after removing those with 25(OH)D $\geq$75, or <30 nmol·L^{-1}.

Alternatively, the positive associations observed in the current study between vitamin D status and endurance performance could be explained by reverse causation, such that fitter, more physically active individuals spend more time outdoors, exposed to sunlight and hence have higher concentrations of vitamin D metabolites. A limitation of the present study was not including physical activity levels as a covariate in regression models. However, after participants had completed 12-weeks of the same structured military training programme, the association between vitamin D status and endurance performance remained. Physical activity levels were likely similar between participants at follow-up and therefore unlikely to account for the relationships observed. Nevertheless, not measuring physical activity levels to confirm this assumption remains a limitation of these data.

Muscular strength and power performance

The lack of association between vitamin D metabolites and muscular strength or power in the present study are in agreement with previous results from young adult males (mean age 24 years; Hamilton et al., 2014) and men and women aged 21–97 years (Marantes et al., 2011), with both of these studies controlling for body composition. In contrast to the results of the present study, 25(OH)D has been shown to be associated with muscle strength among participants from across a wide age range (20−76 years; Grimaldi et al., 2013). These participants completed isolated muscle assessments rather than the functional strength assessments used in the present study, and absolute body composition was not controlled for.

Conclusion

Both serum 25(OH)D and 24,25$(OH)_2$D were positively associated with endurance exercise performance, but not muscle strength or power, in young healthy military recruits after controlling for confounding variables, including body composition, smoking and season. In contrast, serum 1,25$(OH)_2$D was not associated with exercise performance. A seasonal variation was observed in all three major vitamin D metabolites, with 25(OH)D, 24,25$(OH)_2$D and 1,25$(OH)_2$D lowest during winter, and highest during summer. Future studies should investigate the effect of vitamin D supplementation on exercise performance so cause and effect can be established; for example, during winter when 80% of military recruits have insufficient vitamin D status (Figure 3.3).

CHAPTER FOUR

Simulated sunlight and oral vitamin D_3 supplementation to restore and maintain vitamin D sufficiency

4.1 SUMMARY

Vitamin D insufficiency (25(OH)D <50 nmol·L^{-1}) is prevalent during winter and spring. To investigate the effectiveness of safe simulated sunlight and oral vitamin D_3 supplementation in restoring vitamin D sufficiency, 33 healthy males and females aged 20 ± 2 years received simulated sunlight (SSR: 1.3 SED, 3x·$week^{-1}$), oral vitamin D_3 (ORAL: 1,000 IU·day^{-1}) or their respective placebos (SSR-P or ORAL-P) for 4-weeks during winter. Then, to maintain vitamin D sufficiency, participants either continued SSR (1x·$week^{-1}$) or ORAL (400 IU·day^{-1}); ceased active interventions and received placebo (SSR→P or ORAL→P); or continued placebo interventions (SSR-P or ORAL-P) for 8-weeks until the availability of ambient UVB increased during spring. Serum 25(OH)D concentrations were measured at week 1, 5, 9 and 12 using LC-MS/MS. Natural sunlight exposure and dietary vitamin D intake were not different between groups ($P \geq 0.17$). From week 1–5, serum 25(OH)D increased +28.2 nmol·L^{-1} in SSR, +38.6 nmol·L^{-1} in ORAL participants ($P < 0.001$), and did not change in placebo groups ($P \geq 0.05$). At week 5, 89% of SSR and ORAL participants were vitamin D sufficient; with no difference in 25(OH)D between active interventions ($P = 0.26$). From week 5–12, 25(OH)D did not change in SSR ($P = 0.05$), SSR→P ($P = 0.83$) or ORAL→P ($P = 0.42$) groups. Serum 25(OH)D increased in ORAL participants (+6.4 nmol·L^{-1}; $P < 0.01$); and in SSR-P and ORAL-P groups due to ambient UVB ($P < 0.01$). At week 12, all SSR and ORAL participants were vitamin D sufficient; whereas 25% of SSR→P and ORAL→P participants were insufficient. In conclusion, SSR and ORAL were effective strategies to restore vitamin D sufficiency, with similar increases in serum 25(OH)D. Continued SSR and ORAL interventions were necessary to maintain vitamin D sufficiency in all.

4.2 INTRODUCTION

Exposure to UVB is required for cutaneous vitamin D_3 synthesis and is the body's main source of vitamin D (Hollis, 2005). Seasonal changes in the availability of ambient UVB results in a variation in vitamin D status, typically reaching a peak in summer and its nadir during winter (**Chapter 3, Figure 3.2A**). Vitamin D insufficiency (25(OH)D <50 nmol·L^{-1}, Institute of Medicine, 2011; European Food Safety Authority, 2016) is therefore prevalent during winter and spring (Cashman et al., 2016). In the absence of ambient UVB during winter, oral vitamin D_3 supplementation or exposure to simulated sunlight are two possible ways to restore vitamin D sufficiency. For the latter, UVR can be closely matched to that of summer sunlight (λ = 290–400 nm; 95% UVA and 5% UVB), and be delivered to an individual wearing casual clothing using a whole-body irradiation cabinet; thereby mimicking safe, casual summer sun exposures (Rhodes et al., 2010; Webb et al., 2011; Advisory Group on Non-ionising Radiation, 2017). Alternatively, daily oral vitamin D_3 supplementation can be used to increase 25(OH)D from a winter low, and subsequently maintain vitamin D sufficiency (Heaney et al., 2003; Vieth et al., 2001).

In healthy men and women, 4-weeks oral vitamin D_3 supplementation (1,000 IU·day^{-1}) has been demonstrated to increase serum 25(OH)D by ~ +20 nmol·L^{-1} (Vieth et al., 2001). Similarly, 4-weeks simulated sunlight has been shown to increase 25(OH)D by ~ +25 nmol·L^{-1} in healthy, white-skinned individuals (1.3 SED to 35% skin surface area, 3x·$week^{-1}$; Rhodes et al., 2010). To maintain 25(OH)D at summer concentrations and prevent a decline during winter, an estimated daily dose of 500 IU vitamin D_3 has been deemed necessary (Heaney et al., 2003). Indeed, 400 IU·day^{-1} oral vitamin D_3 has been demonstrated to maintain 25(OH)D concentrations in the absence of ambient UVB (Macdonald et al., 2013). How much simulated sunlight is necessary to maintain vitamin D sufficiency (serum

25(OH)D ≥50 nmol·L^{-1}) remains unknown; however, UVB irradiation once-a-fortnight (1 SED to 88% skin surface area) has been shown to be adequate to maintain summer 25(OH)D concentrations during winter (Bogh et al., 2012b). A protocol of simulated sunlight to match changes in serum 25(OH)D achieved via oral vitamin D_3 supplementation remains unexplored in a single study. Matching the changes in 25(OH)D concentration using simulated sunlight with oral vitamin D_3 supplementation will enable future studies to investigate the effect of these supplementation strategies on physiological functions thought to be influenced by vitamin D; such as, immune function and exercise performance (Owens et al., 2018). Simulated sunlight has the potential to be used as a method to restore and maintain vitamin D sufficiency in the absence of ambient UVB. Simulated sunlight data could also be used to make recommendations for natural sunlight exposures, and thus has real world practicability.

Therefore, the purpose of this study was to investigate the effect of simulated sunlight and oral vitamin D_3 supplementation on serum 25(OH)D concentrations, using protocols intended to restore and subsequently maintain vitamin D sufficiency. Serum 25(OH)D was intended to change by a similar magnitude using either simulated sunlight or oral supplementation. In a 12-week randomised, placebo controlled trial among young healthy adults; this aim was achieved in two phases. The primary aim was to restore vitamin D sufficiency (25(OH)D ≥50 nmol·L^{-1}). To investigate this aim, participants received either simulated sunlight (1.3 SED to 37% skin surface area, 3x·week^{-1}), oral vitamin D_3 (1,000 IU·day^{-1}), or their respective placebos for 4-weeks during winter (restoration phase). Subsequently, the secondary aim was to maintain vitamin D sufficiency. To investigate this aim, participants received simulated sunlight once-a-week; a lower dose of oral vitamin D_3 (400 IU·day^{-1}); or their respective placebos for 8-weeks (maintenance phase), until the availability of ambient UVB increased

during spring. It was hypothesised that the simulated sunlight and oral vitamin D_3 supplementation protocols would increase 25(OH)D by a similar magnitude during the restoration phase, achieving vitamin D sufficiency. Continued simulated sunlight or oral supplementation were hypothesised to be required to maintain vitamin D sufficiency.

4.3 METHODS

Participants. Thirty-eight young, healthy, white Caucasian adults volunteered to participate in the study (32 males and 6 females; age 20 ± 2 years; height 178 ± 8 cm; body mass 73.0 ± 11.1 kg; BMI 23.0 ± 2.9 $kg \cdot m^{-2}$). Participants had sun-reactive skin type I–IV (assessed as described in **Chapter 2, section 2.10**); were not currently taking vitamin D supplements, fish oils, multivitamins containing vitamin D, or photoactive medication; had not used a sun bed or travelled to a sunny climate in the 3-months before the study; were not pregnant or breast feeding; and were without a history of skin cancer, photosensitivity or systemic lupus erythematosus. For the duration of the study (February–May 2015), participants refrained from taking supplements containing vitamin D, using sun beds (other than their allocated intervention) or travelling to sunny climates.

Study design. In a 12-week randomised, single-blind placebo controlled trial; participants were randomly allocated to one of four intervention groups for the restoration phase (weeks 1–4; Figure 4.1): 1) simulated sunlight (SSR); 2) placebo simulated sunlight (SSR-P); 3) oral vitamin D_3 (ORAL); or 4) oral placebo (ORAL-P). Based on their allocated restoration phase group, participants were then randomly allocated to one of six intervention groups for the maintenance phase (weeks 5–12). Restoration phase SSR participants were allocated to either continue simulated sunlight (SSR) or instead receive placebo simulated sunlight (SSR→P). Restoration phase ORAL participants were allocated to either continue oral vitamin D_3 supplementation (ORAL) or instead receive oral placebo (ORAL→P). Restoration phase SSR-P and ORAL-P participants continued to receive placebo interventions (SSR-P and ORAL-P, respectively). Thirty-three participants commenced the intervention after 5 of the original 38 withdrew from the study, due to a reluctance to commit to the study's time requirements. All 33 participants completed the restoration phase. During the maintenance

phase, 4 participants no longer wished to participate (due to an unwillingness to commit to the study's time requirements), hence a total of 29 participants completed the maintenance phase; with all achieving a compliance ≥75%. At the end of the restoration and maintenance phases, participants were asked to guess which intervention they thought they had been receiving (i.e. active or control).

Experimental procedures. Three times-a-week during the restoration phase, participants in the SSR group were exposed to simulated sunlight (1.3 SED to 37% skin surface area), and participants in the SSR-P group were exposed to placebo simulated sunlight (as described in **Chapter 2, section 2.5;** Figure 4.1). Once-a-day during the restoration phase, participants in the ORAL group consumed a 1,000 IU capsule of vitamin D_3, and participants in the ORAL-P group consumed a placebo capsule (as described in **Chapter 2, section 2.6**). Once-a-week during the subsequent maintenance phase, SSR participants were exposed to simulated sunlight; whilst SSR→P and SSR-P participants were exposed to placebo simulated sunlight. Once-a-day during the maintenance phase, participants in the ORAL group consumed a 400 IU capsule of vitamin D_3; whilst ORAL→P and ORAL-P group consumed a placebo capsule. Participants in the SSR group were exposed to a cumulative dose of 15.6 and 10.4 SED during the restoration and maintenance phases, respectively. Participants provided a blood sample at week 1, before the restoration phase; week 5, before the maintenance phase; week 9, mid-way through the maintenance phase; and week 12, at the conclusion of the maintenance phase (as described in **Chapter 2, section 2.7**; Figure 4.2). Blood samples were analysed for total serum 25(OH)D (see **Chapter 2, section 2.8** for details). Participants completed a FFQ at week 1, and wore PSF badges during weeks 4, 8 and 12, preceding the collection of blood samples (as described in **Chapter 2, section 2.9**).

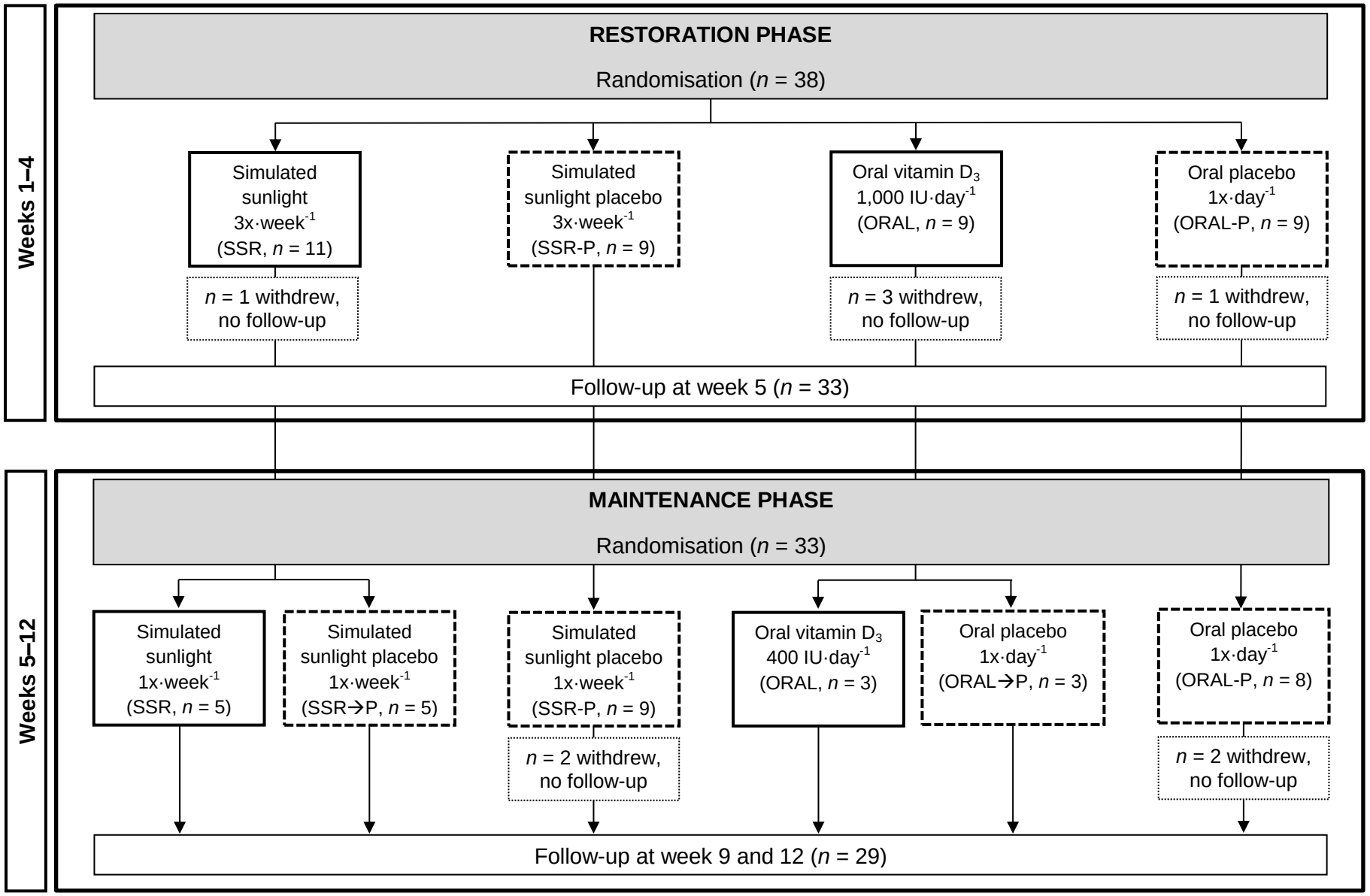

Figure 4.1. Flow diagram of participants through restoration (weeks 1–4; four groups) and maintenance (weeks 5–12; six groups) phases.

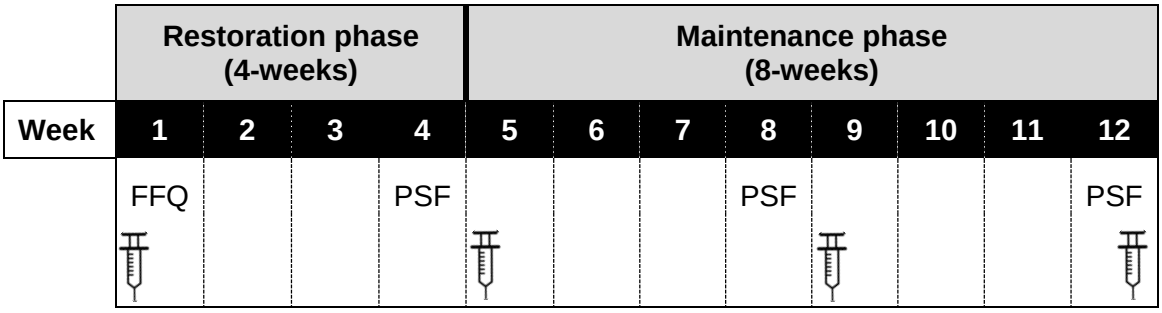

Figure 4.2. Schematic of study restoration (weeks 1–4) and maintenance (weeks 5–12) phases. FFQ, food frequency questionnaire; PSF, polysulphone badge; syringe icon represents blood sample.

Statistical analysis. The primary analysis was a 4 x 2 mixed model ANOVA to compare serum 25(OH)D between SSR and ORAL groups and their respective placebos during the restoration phase. A sample size estimation for this analysis indicated that 5 participants per group were required to produce an 80% chance of obtaining statistical significance at the 0.05 level, based upon a large effect size (f = 0.40) of vitamin D supplementation (Smith et al., 2009) and correlation between repeated measures of 25(OH)D (r = 0.58) determined from winter data from **Chapter 3** (G*Power, version 3.1.9.2). A total of 38 participants were recruited and randomised between the 4 intervention groups to allow for drop-outs. As a secondary analysis, a 6 x 3 mixed model ANOVA was used to compare serum 25(OH)D between intervention groups during the maintenance phase, and individual participants' changes in 25(OH)D were examined. Kruskal-Wallis one-way ANOVA were used to compare non-parametric sunlight exposure between groups, and these data are presented as medians. Missing data (serum 25(OH)D concentration at week 9) for one SSR-P participant (y) was replaced with a value calculated using the formula:

$$\text{y } 25(\text{OH})\text{D at week } 9 =$$

$$\text{y } 25(\text{OH})\text{D at week } 5 + \left(\frac{\text{SSR}-\text{P group } \Delta 25(\text{OH})\text{D week 5 to 9}}{\text{SSR}-\text{P group } \Delta 25(\text{OH})\text{D week 5 to 12}} \times \text{y } \Delta 25(\text{OH})\text{D week 5 to 12}\right)$$

4.4 RESULTS

Participant characteristics

There were no differences in participant characteristics between intervention groups ($P \geq 0.17$; Table 4.1). Dietary vitamin D intake was not different between groups, and indicated the vitamin D gained from this source was limited and similar for all intervention groups. Before commencing the intervention, only 15% of participants were vitamin D sufficient (25(OH)D ≥50 nmol·L^{-1}) and ~half (49%) were vitamin D deficient (25(OH)D <30 nmol·L^{-1}). Participants were adequately blinded to the intervention since 61% of participants correctly guessed their allocated intervention during the restoration phase, and 50% correctly guessed their intervention group during the maintenance phase.

Restoration phase: effect of simulated sunlight and oral vitamin D_3 on serum 25(OH)D

Simulated sunlight and oral vitamin D_3 supplementation increased 25(OH)D concentrations such that at week 5, serum 25(OH)D in the SSR and ORAL groups were higher than placebo groups (group x time interaction: $F(3,29) = 44.5$, $P < 0.001$; Figure 4.3). Serum 25(OH)D in SSR participants increased by +28.2 ± 11.6 nmol·L^{-1}, reaching 58.8 ± 10.5 nmol·L^{-1} at week 5 ($P < 0.001$). Among ORAL participants, serum 25(OH)D increased by +38.6 ± 10.8 nmol·L^{-1}, reaching 73.7 ± 11.2 nmol·L^{-1} at week 5 ($P < 0.001$). There was no difference in SSR and ORAL participants' 25(OH)D concentrations at week 5 ($P = 0.26$). Serum 25(OH)D did not change among placebo group participants during the restoration phase (SSR-P: $P = 0.05$ and ORAL-P: $P = 0.90$). Cohen's *d* effect size (Δ25(OH)D) = 3.5 and 4.7 for SSR *vs.* SSR-P and ORAL *vs.* ORAL-P, respectively. After 4 weeks of supplementation, 80% of SSR and 100% of ORAL participants were vitamin D sufficient (25(OH)D ≥50 nmol·L^{-1}) and none were vitamin D deficient (25(OH)D <30 nmol·L^{-1}). No participants within the placebo groups increased 25(OH)D concentrations to become vitamin D sufficient at week 5. Sunlight

exposure during week 4 was very low and not different between intervention groups (SSR: 0.06; SSR-P: 0.10; ORAL: 0.07; ORAL-P: 0.08 SED·day^{-1}; P = 0.51), indicating the vitamin D gained from ambient UVB was minimal and similar for all intervention groups.

Table 4.1. Participant characteristics for the 4 intervention groups and combined data (all groups) at week 1.

	SSR n = 10	SSR-P n = 9	ORAL n = 6	ORAL-P n = 8	All groups n = 33	P value[a]
Sex: male, female	8, 2	7, 2	5, 1	7, 1	27, 6	-
Sun-reactive skin type: I, II, III, IV	1, 3, 6, 0	0, 4, 4, 1	0, 2, 4, 0	0, 2, 5, 1	1, 11, 19, 2	-
Age (years)	21 ± 3	19 ± 1	20 ± 1	20 ± 1	20 ± 2	0.20
Height (cm)	175 ± 10	177 ± 10	181 ± 5	179 ± 3	177 ± 8	0.56
Body mass (kg)	72.0 ± 11.6	77.6 ± 11.3	72.7 ± 11.3	74.5 ± 9.3	74.3 ± 10.7	0.72
BMI (kg·m^{-2})	23.5 ± 2.3	24.8 ± 3.2	22.3 ± 3.4	23.3 ± 2.6	23.6 ± 2.9	0.39
Dietary vitamin D intake (IU·day^{-1})	84 ± 36	120 ± 68	120 ± 68	160 ± 88[b]	116 ± 68	0.17
Serum 25(OH)D (nmol·L^{-1})	30.7 ± 11.8	36.4 ± 13.6	35.1 ± 8.7	31.8 ± 17.6	33.3 ± 13.1	0.79

[a] One-way ANOVA between groups; [b] Dietary vitamin D data missing for 2 participants. BMI, body mass index; SSR, simulated sunlight; SSR-P, placebo simulated sunlight; ORAL, oral vitamin D_3; ORAL-P, oral placebo. Data are mean ± SD or n.

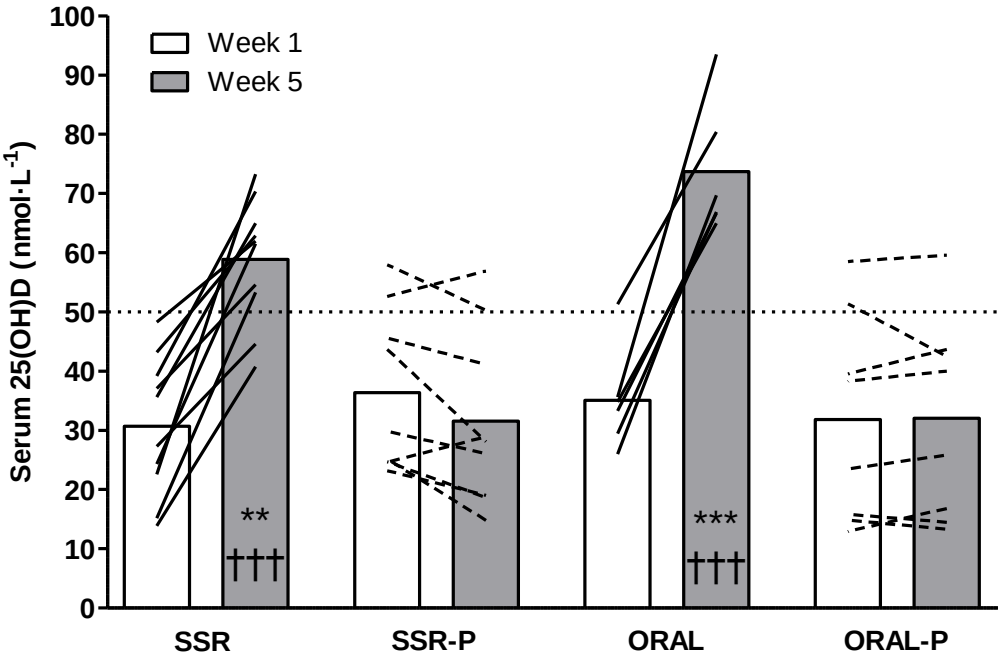

Figure 4.3. Restoration phase serum 25(OH)D response to 4-weeks simulated sunlight (SSR), placebo simulated sunlight (SSR-P), oral vitamin D_3 (ORAL) and oral placebo (ORAL-P). Group means are shown as vertical bars, and each line represents an individual participant (solid for active and dashed for placebo interventions). Horizontal dotted line indicates threshold for vitamin D sufficiency (25(OH)D ≥50 nmol·L^{-1}). ††† $P < 0.001$ *vs.* week 1; ** $P < 0.01$ and *** $P < 0.001$ *vs.* SSR-P and ORAL-P.

Maintenance phase: effect of simulated sunlight and oral vitamin D_3 on serum 25(OH)D

A group x time interaction was observed for serum 25(OH)D concentrations ($F(10,46) = 5.0$, $P < 0.001$; Figure 4.4). At week 5, serum 25(OH)D was higher among participants who had received simulated sunlight or oral vitamin D_3 during the restoration phase, compared with oral placebo (SSR, SSR→P, ORAL and ORAL→P *vs.* ORAL-P: $P < 0.05$); and was higher among participants who had received oral vitamin D_3 during the restoration phase, compared with placebo simulated sunlight (ORAL and ORAL→P *vs.* SSR-P: $P < 0.05$). Serum 25(OH)D remained higher at week 9 among ORAL compared with ORAL-P participants ($P < 0.05$). There were no other between group differences at weeks 5, 9 or 12 ($P > 0.05$). Serum 25(OH)D did not change during the maintenance phase among participants who received

simulated sunlight (SSR: $P = 0.05$); or participants who ceased active interventions (SSR→P: $P = 0.83$; ORAL→P: $P = 0.42$). Among participants who received oral vitamin D_3, serum 25(OH)D increased by +6.4 ± 0.4 nmol·L^{-1}, reaching 73.6 ± 2.0 nmol·L^{-1} at week 12 (ORAL: $P < 0.01$). Serum 25(OH)D also increased in participants who had received placebo interventions during both phases (SSR-P: +17.8 ± 6.3 nmol·L^{-1} and ORAL-P: +21.3 ± 6.9 nmol·L^{-1}; $P < 0.01$). On completion of the maintenance phase, all SSR and ORAL participants had sufficient vitamin D status ($n = 8$: 25(OH)D ≥50 nmol·L^{-1}). A quarter of participants who received active interventions during the restoration phase and placebo interventions during the maintenance phase became vitamin D insufficient at week 12 (2 out of 8 SSR→P and ORAL→P participants: 25(OH)D <50 nmol·L^{-1}). Sunlight exposure during the maintenance phase was not different between intervention groups (week 8: $P = 0.59$; week 12: $P = 0.89$; Table 4.2), indicating the vitamin D gained from ambient UVB was similar for all groups.

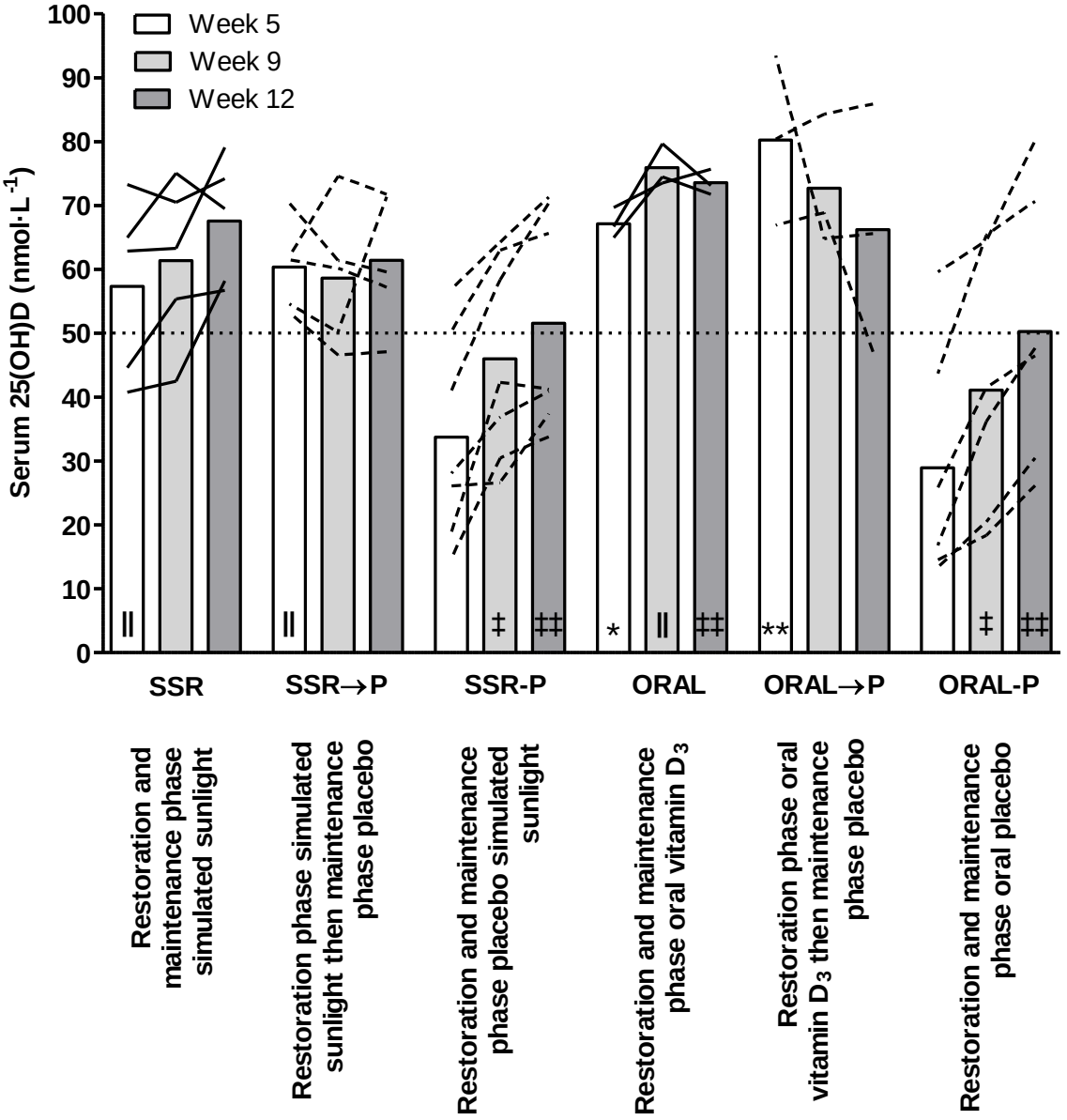

Figure 4.4. Serum 25(OH)D measured during the maintenance phase at week 5, 9 and 12. Group means are shown as vertical bars, and each line represents an individual participant (solid line for active and dashed line for placebo interventions). Horizontal dotted line indicates threshold for vitamin D sufficiency (25(OH)D ≥50 nmol·L^{-1}). ‡ $P < 0.05$ and ‡‡ $P < 0.01$ *vs.* week 5; * $P < 0.05$ and ** $P < 0.01$ *vs.* SSR-P and ORAL-P; ‖ $P < 0.05$ *vs.* ORAL-P.

Table 4.2. Sunlight exposure for the 6 intervention groups during the maintenance phase.

	Restoration and maintenance phase simulated sunlight	Restoration phase simulated sunlight then maintenance phase placebo	Restoration and maintenance phase placebo simulated sunlight	Restoration and maintenance phase oral vitamin D_3	Restoration phase oral vitamin D_3 then maintenance phase placebo	Restoration and maintenance phase oral placebo	
	SSR	SSR→P	SSR-P	ORAL	ORAL→P	ORAL-P	*P* value[a]
Week 8 sunlight exposure (SED·day^{-1})	0.12 *n* = 4	0.58 *n* = 3	0.35 *n* = 5	0.14 *n* = 3	0.68 *n* = 2	0.56 *n* = 5	0.59
Week 12 sunlight exposure (SED·day^{-1})	0.15 *n* = 3	0.13 *n* = 4	0.14 *n* = 5	0.17 *n* = 3	0.11 *n* = 3	0.22 *n* = 3	0.89

[a] Kruskal-Wallis one-way ANOVA between groups. Data are medians.

4.5 DISCUSSION

The purpose of this study was to investigate the effect of simulated sunlight and oral vitamin D_3 supplementation on serum 25(OH)D, using protocols designed to restore and subsequently maintain vitamin D sufficiency from its winter nadir, until the availability of ambient UVB increased during spring. It was intended for serum 25(OH)D to change by a similar magnitude using either simulated sunlight or oral supplementation. Aligned with the hypothesis, 4-weeks of simulated sunlight replicating safe, casual summer sun exposures (1.3 SED to 37% skin surface area, 3x·week^{-1}; Advisory group on Non-ionising Radiation, 2017) and oral vitamin D_3 supplementation (1,000 IU·day^{-1}) both successfully increased vitamin D status to sufficient concentrations in almost all young healthy adults (89%: 25(OH)D ≥50 nmol·L^{-1}; Figure 4.3). At the end of the subsequent 8-week maintenance phase, weekly simulated sunlight or oral vitamin D_3 (400 IU·day^{-1} as recommended by SACN, 2016) maintained vitamin D sufficiency in all; whereas, a quarter of participants who ceased active interventions became vitamin D insufficient (25(OH)D <50 nmol·L^{-1}; Figure 4.4). Therefore, as hypothesised, continued simulated sunlight or oral vitamin D_3 supplementation were necessary to maintain vitamin D sufficiency.

Restoration of vitamin D sufficiency

Serum 25(OH)D was not statistically different between active intervention groups and the effect sizes for restoration phase Δ25(OH)D by simulated sunlight and oral vitamin D_3 supplementation were both large (Cohen's *d* effect size >0.8). However, participants who received oral vitamin D_3 reached a non-significantly higher mean 25(OH)D concentration at week 5 compared with those who received simulated sunlight (73.7 ± 11.2 *vs.* 58.8 ± 10.5 nmol·L^{-1}). Furthermore, 2 participants in the simulated sunlight group failed to achieve vitamin D sufficiency by week 5 (Figure 4.3). A modest increase in the skin surface area

exposed to UVR could be used to better match the effect of oral vitamin D_3 and simulated sunlight on serum 25(OH)D (Matsuoka et al., 1992), and investigators should consider using such an approach in future studies.

The increase in serum 25(OH)D in participants who were exposed to simulated sunlight for 4-weeks (+28.2 nmol·L^{-1}; Figure 4.3) was marginally greater than the rise reported by Rhodes and colleagues (+25 nmol·L^{-1}; Rhodes et al., 2010). This small difference in 25(OH)D response can be explained by 3 differences between these studies. First, the larger skin surface area exposed to UVR in the present study (37% *vs.* 35%); with the potential for vitamin D synthesis enhanced by exposing more skin (Matsuoka et al., 1992). Second, the lower baseline 25(OH)D concentrations in the present study (30.7 *vs.* 44 nmol·L^{-1}); given the inverse relationship between baseline 25(OH)D and magnitude of increase with supplementation (Mazahery and von Hurst, 2015; Moan et al., 2009a; Trang et al., 1998). And third, the younger age of participants in the present study (18–29 years *vs.* 20–60 years); because young people have a greater availability of the epidermal 7-dehydrocholesterol and expression of vitamin D binding protein, thereby augmenting their potential for vitamin D_3 synthesis (MacLaughlin and Holick, 1985; Pop et al., 2015). The increase in 25(OH)D among participants who received 1,000 IU·day^{-1} oral vitamin D_3 for 4-weeks in the present study (+38.6 nmol·L^{-1}; Figure 4.3) was greater than the 25(OH)D increases reported by others who used the same oral dose for 4-weeks (+20 nmol·L^{-1}; Vieth et al. 2001), 8-weeks (+28.6 nmol·L^{-1}; Barger-Lux et al. 1998), ~10-weeks (+19 nmol·L^{-1}; Smith et al. 2009), or 11-weeks (+16 nmol·L^{-1}; Holick et al. 2008). Baseline 25(OH)D in the present study (35.1 nmol·L^{-1}) was lower than the four aforementioned investigations (mean min–max: 40–67 nmol·L^{-1}), and therefore accounts for the larger 25(OH)D response to vitamin D supplementation observed in the current study.

Maintenance of vitamin D sufficiency

Increases in serum 25(OH)D were observed during the maintenance phase among participants who received placebo interventions in both study phases (Figure 4.4). These increases were due to the increased availability of ambient UVB during spring and indicate ambient UVB likely contributed to the vitamin D status of all participants during the maintenance phase. A second caveat for the maintenance phase findings was the small sample size. The present study's required sample size was estimated and achieved for the restoration phase primary analysis; however, given the increase in the number of groups and subsequent smaller group sizes during the maintenance phase, statistical analysis may have been subject to type II error. Therefore, individual participants' changes in serum 25(OH)D will be discussed here, and are shown in Figure 4.4. Serum 25(OH)D decreased from week 5 to 12 among 2 out of the 3 participants who ceased oral vitamin D_3 supplementation after the restoration phase. Although continued oral vitamin D_3 supplementation increased serum 25(OH)D during the maintenance phase (3 out of 3 participants), this may have been due in part to exposure to ambient UVB. Hence, in the absence of ambient UVB, continued oral vitamin D_3 supplementation (400 $IU \cdot day^{-1}$) appears necessary to avoid a decrease in 25(OH)D. Serum 25(OH)D increased in all participants who received weekly simulated sunlight during the maintenance phase (5 out of 5 participants), whereas among participants who discontinued irradiation, 25(OH)D was similar at week 5 and 12 (Figure 4.4). It is anticipated that continued simulated sunlight would be required to maintain restored vitamin D status in the absence of ambient UVB.

To maintain vitamin D sufficiency, 400 $IU \cdot day^{-1}$ vitamin D_3 was effective and is the recommended daily intake in the UK (Scientific Advisory Committee on Nutrition, 2016). In agreement with the findings of the present study, daily supplementation with 400–500 IU

vitamin D_3 has previously been demonstrated to maintain 25(OH)D concentrations during winter (Macdonald et al., 2013; Meier et al., 2004). In the current study, withdrawal of oral vitamin D_3 supplementation following the restoration phase resulted in a decrease in 25(OH)D among 2 out of 3 participants; whereas among those who ceased simulated sunlight, 25(OH)D remained stable, on average (Figure 4.4). It has been suggested that orally administered vitamin D_3 is less effective at maintaining 25(OH)D than vitamin D_3 synthesised at the skin (Haddad et al., 1993). This may be because cutaneous synthesised vitamin D_3 is primarily associated with vitamin D binding protein, which slowly diffuses into the blood stream and is gradually delivered to the liver for hydroxylation (Fraser, 1983). In contrast, oral vitamin D_3 is associated with chylomicrons and lipoproteins which are readily taken up by the liver and hydroxylated more rapidly.

Strengths and limitations

The following strengths of the present study are notable. Firstly, the use of placebo interventions helped prevent control participants from commencing their own vitamin D supplementation. Placebo controls were effective with ~half of participants incorrectly guessing their allocated intervention; thus, the disguises used here could be used in future randomised controlled trials. Further strengths include demonstrating sunlight exposure and dietary vitamin D intake, as natural sources of vitamin D, were not different between groups. At week 4, ambient UVB was negligible, demonstrated by the low erythemal doses; similar to those reported in the UK during early spring (Macdonald, 2013). Higher erythemal doses were recorded at weeks 8 and 12, and were again similar to those expected during spring (Macdonald, 2013; Kift et al., 2013). The higher erythemal doses at week 8 *vs.* 12 were probably because week 8 was during the Easter vacation and included a period of sunny weather in the UK. Dietary vitamin D intake as a contributor to vitamin D status was low;

similar to that reported previously among UK adults; and would have contributed little to the vitamin D status of participants in all intervention groups (Macdonald, 2013). A limitation of the present study was the small sample size during the maintenance phase, which limited the statistical power of the maintenance phase analysis. Furthermore, only white-skinned individuals were studied, hence study findings cannot be applied to individuals with all sun-reactive skin types.

Conclusion

Four weeks of simulated sunlight (1.3 SED to 37% skin surface area, 3x·week^{-1}) or oral vitamin D_3 supplementation (1,000 IU·day^{-1}) increased vitamin D status to sufficient concentrations in 89% of young healthy adults; with no significant difference in serum 25(OH)D between the two supplementation routes. Hence, both methods represent effective strategies to correct wintertime vitamin D insufficiency. At the end of the subsequent 8-week maintenance phase, all simulated sunlight (1.3 SED to 37% skin surface area, once-a-week; n = 5) and oral vitamin D_3 (400 IU·day^{-1}; n = 3) participants had sufficient vitamin D status; whereas, a quarter of participants who switched to the placebo intervention were vitamin D insufficient (2 out of 8 participants). Therefore, continued simulated sunlight and oral vitamin D_3 supplementation appear necessary to maintain sufficient vitamin D status in the absence of ambient UVB, albeit in a small sample of participants. Future studies should monitor the maintenance of vitamin D sufficiency among a larger sample of participants; and investigate whether restoring and maintaining vitamin D sufficiency using simulated sunlight or oral vitamin D_3 is beneficial to meaningful outcomes, such as immune function or exercise performance.

CHAPTER FIVE

Influence of simulated sunlight and oral vitamin D_3 supplementation on exercise performance in white male military recruits

5.1 SUMMARY

Evidence to support a role of vitamin D in enhancing exercise performance is mainly cross-sectional; hence, randomised, placebo controlled trials are needed to enable cause and effect to be established. With vitamin D insufficiency (25(OH)D <50 nmol·L^{-1}) widespread among otherwise healthy adults during winter and spring, correcting vitamin D insufficiency at this time of year may benefit exercise performance. Therefore, the aim of this study was to investigate the influence of 12-weeks vitamin D supplementation on serum vitamin D metabolites, exercise performance and resting blood pressure, using simulated sunlight in accordance with recommendations on safe sunlight exposure, or oral vitamin D_3. Commencing in winter, 137 male military recruits (22 ± 3 years) received simulated sunlight (1.3 SED 3x·$week^{-1}$ for 4-weeks and then 1x·$week^{-1}$ for 8-weeks) or oral vitamin D_3 (1,000 IU·day^{-1} for 4-weeks and then 400 IU·day^{-1} for 8-weeks) in a randomised, double-blind placebo controlled trial. Serum 25(OH)D and 24,25$(OH)_2$D were assessed by LC-MS/MS and 1,25$(OH)_2$D by immunoassay. Endurance, strength and power exercise performance were assessed by 1.5-mile run, maximum dynamic lift and vertical jump, respectively. Vitamin D supplementation, by simulated sunlight or oral vitamin D_3, restored vitamin D sufficiency in almost all within 4-weeks (97%: 25(OH)D ≥50 nmol·L^{-1}) and maintained sufficiency for a further 8-weeks (96%: 25(OH)D ≥50 nmol·L^{-1}). Additionally, 59% of participants who received vitamin D had serum 25(OH)D ≥75 nmol·L^{-1} at week 5 which has been proposed to be optimal. Restoring vitamin D sufficiency (or even 'optimum' 25(OH)D ≥75 nmol·L^{-1}) did not improve exercise performance ($P \geq 0.06$) or reduce resting blood pressure ($P \geq 0.23$); suggesting vitamin D does not directly affect exercise performance in young healthy men.

5.2 INTRODUCTION

Correcting vitamin D deficiency (25(OH)D <30 nmol·L^{-1}) has been shown to increase strength in elderly adults (Stockton et al., 2011). Intriguingly, vitamin D_3 supplementation in a small cohort of athletes with low baseline serum 25(OH)D (~30 nmol·L^{-1}) has also been shown to improve musculoskeletal performance (Close et al., 2013b). The remaining evidence to support a positive role of vitamin D in enhancing exercise performance among healthy adults is cross-sectional (**Chapter 3**; Ardestani et al., 2011; Grimaldi et al., 2013), and therefore limited because cause and effect cannot be established. To investigate vitamin D's potentially important influence on exercise performance, and thereby determine optimal vitamin D thresholds and supplementation recommendations, randomised controlled trials are needed (Thomas et al., 2016). With vitamin D insufficiency (25(OH)D <50 nmol·L^{-1}) widespread among otherwise healthy adults during winter and spring (**Chapter 3; Figure 3.3**), vitamin D supplementation at this time of year, by either simulated sunlight (in accordance with recommendations on safe sunlight exposure; Advisory Group on Non-ionising Radiation, 2017), or oral vitamin D_3 may benefit exercise performance. If beneficial, simulated sunlight data could be used to make recommendations for natural sunlight exposures; or, in the absence of ambient UVB, simulated sunlight could be used as an alternative to oral supplementation.

During the last 5-years, randomised controlled trials have attempted to investigate the effect of oral vitamin D supplementation on exercise performance (**Chapter 1, section 1.7.2**). The findings of many of these studies may be limited because participants were on average vitamin D sufficient at baseline (Close et al., 2013a; Dubnov-Raz et al., 2015; Shanely et al., 2014; Nieman et al., 2014); or investigators used oral doses greater than the recommended tolerable upper intake of 4,000 IU·day^{-1} (Close et al., 2013a; Close et al., 2013b; Owens et

al., 2014; Jastrzebska et al., 2016). Claims have been made of benefits to exercise performance in early UVR studies using sun lamps (Cannell et al., 2009), including purported benefits of UVR for cardiovascular fitness and local muscular endurance (Allen and Cureton, 1945; Ronge, 1952; Hettinger and Seidl, 1956). Although intriguing, these claims should be interpreted with due caution as the studies involved were not placebo controlled and they made no assessment of 25(OH)D. As reviewed in **Chapter 1** (**section 1.8.2**), low serum 25(OH)D may be related to increased blood pressure and endothelial dysfunction (Gouni-Berthold et al., 2009). Therefore, vitamin D insufficiency may reduce cardiac output and increase peripheral vessel resistance, thereby impairing exercise performance. Whether restoring vitamin D sufficiency can reduce blood pressure, providing a mechanism for vitamin D to enhance endurance performance remains unclear (Lim et al., 1996). It is also unknown if sunlight exposure of the skin has additional benefits compared with vitamin D_3 obtained orally. The UVA component of sunlight has been shown to acutely elevate NO and reduce blood pressure (Feelisch et al., 2010) which could account for improved performance after UVR treatment rather than UVB stimulated changes in 25(OH)D.

Therefore, the aim of the present study was to investigate the effect of 12-weeks vitamin D supplementation, by either simulated sunlight (1.3 SED to 40% skin surface area, $3x \cdot week^{-1}$ for 4-weeks and then $1x \cdot week^{-1}$ for 8-weeks) or oral vitamin D_3 (1,000 $IU \cdot day^{-1}$ for 4-weeks and then 400 $IU \cdot day^{-1}$ for 8-weeks) on 3 major vitamin D metabolites, exercise performance, and resting blood pressure. This randomised placebo controlled trial commenced in winter, when vitamin D status is lowest and solar UVB is negligible, and continued until the availability of ambient UVB increased during spring (Webb et al., 1988). It was hypothesised that restoring serum 25(OH)D from winter to typical summer concentrations in 4-weeks and then maintaining EFSA and IOM defined vitamin D sufficiency for a further 8-weeks

(25(OH)D ≥50 nmol·L^{-1}) would improve endurance, strength and power exercise performance, and lower resting blood pressure among male military recruits.

5.3 METHODS

Participants. Male military recruits were eligible to participate if they had sun-reactive skin type I–IV (assessed as described in **Chapter 2, section 2.10**); were without a history of skin cancer, photosensitivity or systemic lupus erythematosus; were not currently consuming vitamin D supplements, fish oils or multivitamins containing vitamin D; and had not used a sun bed or travelled to a sunny climate in the 3-months before the study. The study took place in 2016 and 2017, with participants commencing in January or February. One-hundred and thirty-seven participants (100% white ethnicity) completed the 12-week intervention with a compliance ≥80% and having refrained from taking supplements containing vitamin D, using sun beds (other than their allocated intervention) or travelling outside of the British Isles (Table 5.1).

Table 5.1. Participant physical characteristics for the 4 intervention groups and combined data (all groups).

	SSR *n* = 37[a]	SSR-P *n* = 33[a]	ORAL *n* = 36	ORAL-P *n* = 31	All groups *n* = 137	*P* value[b]
Age (years)	21 ± 3	21 ± 3	22 ± 3	22 ± 3	22 ± 3	0.88
Body mass (kg)	76.0 ± 11.0	78.1 ± 11.9	76.6 ± 12.4	77.8 ± 11.0	77.0 ± 11.5	0.86
Height (cm)	177 ± 5	178 ± 7	177 ± 7	177 ± 6	177 ± 6	0.70
BMI ($kg \cdot m^{-2}$)	24.2 ± 3.3	24.5 ± 2.8	24.4 ± 3.1	24.8 ± 2.6	24.4 ± 3.0	0.86

[a] body mass, height and BMI data missing for 1 participant; [b] One-way ANOVA between groups. BMI, body mass index; SSR, simulated sunlight; SSR-P, placebo simulated sunlight; ORAL, oral vitamin D_3; ORAL-P, oral placebo. Data are mean ± SD.

Study design and experimental procedures. In a randomised double-blind placebo controlled trial, participants were block randomised within their platoons to one of four, 12-week intervention groups: 1) simulated sunlight (SSR); 2) placebo simulated sunlight (SSR-P); 3) oral vitamin D_3 (ORAL); or 4) oral placebo (ORAL-P). Block randomisation (using randomizer.org) resulted in an equal distribution of intervention groups within each platoon, and therefore ensured any differences in training conditions between platoons did not influence the study outcomes. Participants completed a 4-week restoration phase to restore vitamin D sufficiency, followed by an 8-week maintenance phase (Figure 5.1). Participants receiving the SSR or SSR-P intervention were exposed to simulated sunlight (1.3 SED to 40% skin surface area) or placebo, respectively, 3 times-a-week during the restoration phase, and once-a-week during the maintenance phase (as described in **Chapter 2, section 2.5**). Participants in the SSR group were exposed to a cumulative dose of 15.6 and 10.4 SED during the restoration and maintenance phases, respectively. Participants receiving the ORAL intervention consumed a vitamin D_3 capsule daily (1,000 and 400 $IU \cdot day^{-1}$ during the restoration and maintenance phases, respectively). For 12-weeks, ORAL-P participants consumed a placebo capsule daily (as described in **Chapter 2, section 2.6**). Exercise performance was assessed before commencing the intervention (baseline) and again at week 12, using a 1.5-mile run, maximum dynamic lift and maximum vertical jump (see **Chapter 2, section 2.4** for details). A 1.5-mile run was also completed at week 5. After ~10 minutes lying supine in an isolated room, resting blood pressure was measured in triplicate using an automated sphygmomanometer at baseline and week 12 (Omron M6 AC, Hoofddorp, The Netherlands), with the mean used for analyses. Mean arterial pressure was calculated using standard formula (Cywinski, 1980):

$$\text{Mean arterial pressure} = \text{diastolic blood pressure} + (1/3 \text{ x } (\text{systolic - diastolic blood pressure}))$$

Participants provided a blood sample before and after the restoration phase (at baseline and week 5, respectively) and at week 12, after the maintenance phase (as described in **Chapter 2, section 2.7**) for the analysis of vitamin D metabolites (serum 25(OH)D, 24,25$(OH)_2$D and 1,25$(OH)_2$D; see **Chapter 2, section 2.8** for details). Participants completed a FFQ at week 12, and wore PSF badges during weeks 4 and 11, preceding the collection of blood samples (as described in **Chapter 2, section 2.9**). All 137 participants gave a blood sample at baseline, week 5 and 12; and completed at least one exercise performance test. On completion of study procedures, participants were asked to guess which intervention they thought they had been receiving (i.e. active or control).

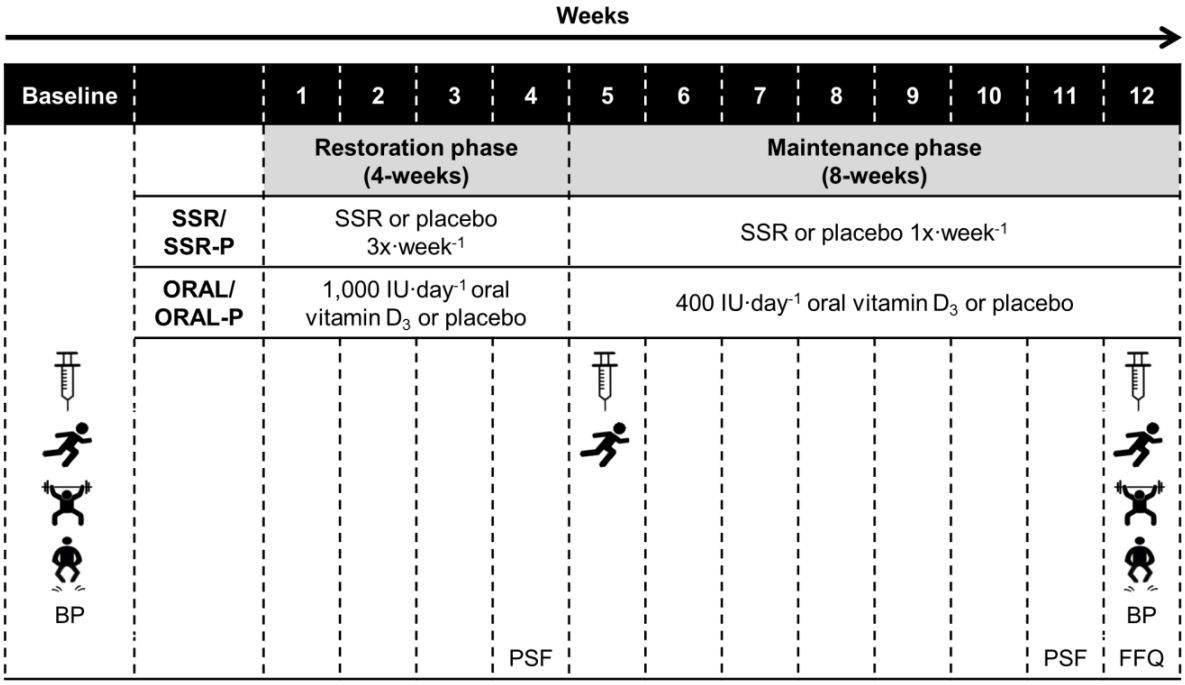

Figure 5.1. Schematic of study procedures, to investigate the effect of vitamin D supplementation by simulated sunlight (SSR), oral vitamin D_3 (ORAL), or placebo (SSR-P or ORAL-P) on vitamin D metabolites, exercise performance and resting blood pressure, using a 4-week restoration phase followed by an 8-week maintenance phase. Syringe icon represents blood sample; running icon represents 1.5-mile run; weightlifting icon represents maximum dynamic lift; jumping icon represents vertical jump; BP, resting blood pressure; PSF, polysulphone badge; FFQ, food frequency questionnaire.

Statistical analysis. The primary analyses were mixed model ANOVA to compare serum vitamin D metabolites, exercise performance and resting blood pressure between active vitamin D (SSR and ORAL combined together) and placebo groups. A sample size estimation for this analysis indicated that 19 participants per group were required to produce an 80% chance of obtaining statistical significance at the 0.05 level, based on the effect size (*f* = 0.175) and correlation between repeated measures (r = 0.67) determined from 1.5-mile run data (collected in **Chapter 3**; G*Power, version 3.1.9.2). In addition, individual active interventions were compared to their respective placebos (SSR *vs.* SSR-P; and ORAL *vs.* ORAL-P) by mixed model ANOVA for serum 25(OH)D, exercise performance and blood pressure. Exercise performance was also compared between SSR and ORAL participants who achieved proposed optimal vitamin D status (25(OH)D ≥75 nmol·L^{-1}; Thomas et al., 2016; Holick et al., 2011) *vs.* placebo participants who remained vitamin D insufficient (25(OH)D <50 nmol·L^{-1}) using independent Student's *t*-tests.

5.4 RESULTS

During the 12-week intervention, sunlight exposure (P = 0.31 and 0.73 at week 4 and 11, respectively; Table 5.2) and dietary vitamin D intake (P = 0.46) were not different between groups. Vitamin D gained from these natural sources was minor compared with the active interventions. Sunlight exposure of all groups pooled was not different between week 4 and 11 (0.17 ± 0.10 and 0.24 ± 0.40 SED·day^{-1}, respectively; P = 0.15). Participants were sufficiently blinded to the intervention since only 35% of participants correctly guessed their allocated group, 32% were incorrect, and 33% said they did not know whether they had received an active or placebo intervention.

Table 5.2. Sunlight exposure during the restoration and maintenance phases (week 4 and 11, respectively) and dietary vitamin D intake for the 4 intervention groups.

	SSR	SSR-P	ORAL	ORAL-P	P value[a]
Week 4 sunlight exposure (SED·day^{-1})	0.14 ± 0.08	0.17 ± 0.10	0.20 ± 0.10	0.17 ± 0.12	0.31
Week 11 sunlight exposure (SED·day^{-1})	0.24 ± 0.33	0.19 ± 0.34	0.33 ± 0.56	0.27 ± 0.50	0.73
Dietary vitamin D intake (IU·day^{-1})	120 ± 104	136 ± 100	100 ± 56	128 ± 88	0.46

[a] One-way ANOVA between groups. SSR, simulated sunlight; SSR-P, placebo simulated sunlight; ORAL, oral vitamin D_3; ORAL-P, oral placebo. Data are mean ± SD.
Week 4 sunlight exposure: SSR, n = 20; SSR-P, n = 16; ORAL, n = 21; ORAL-P, n = 16.
Week 11 sunlight exposure: SSR, n = 23; SSR-P, n = 27; ORAL, n = 25; ORAL-P, n = 18.
Dietary vitamin D intake: SSR, n = 36; SSR-P, n = 32; ORAL, n = 36; ORAL-P, n = 28.

Effect of simulated sunlight and oral vitamin D_3 on vitamin D metabolites

Serum 25(OH)D

Simulated sunlight and oral vitamin D_3 supplementation were effective strategies to increase serum 25(OH)D concentrations, with SSR and ORAL participants reaching typical summer vitamin D status at week 5 and 12 (see **Chapter 3, Figure 3.2A** for summer 25(OH)D concentrations). For serum 25(OH)D, a group x time interaction was observed for SSR and ORAL groups *vs.* placebo ($F(1.9,258.0) = 88.3$, $P < 0.001$; Figure 5.2A), SSR *vs.* SSR-P ($F(1.8,123.9) = 47.0$, $P < 0.001$; Figure 5.2C), and ORAL *vs.* ORAL-P ($F(2,130) = 41.5$, $P < 0.001$; Figure 5.2E). In all active interventions, serum 25(OH)D at week 5 and 12 was higher than baseline ($P < 0.001$); and higher than placebo groups at week 5 ($P < 0.001$) and 12 (SSR and ORAL *vs.* placebo, SSR *vs.* SSR-P: $P < 0.001$; ORAL *vs.* ORAL-P: $P < 0.05$). In all placebo groups, serum 25(OH)D at week 12 was higher than baseline and week 5 ($P < 0.001$).

At baseline, ~three-quarters (74%) of participants were vitamin D insufficient (25(OH)D <50 $nmol \cdot L^{-1}$; Figure 5.2B) and ~one-third (31%) of participants were vitamin D deficient (25(OH)D <30 $nmol \cdot L^{-1}$). After 4-weeks vitamin D supplementation almost all SSR and ORAL participants were vitamin D sufficient (97%: 25(OH)D ≥50 $nmol \cdot L^{-1}$) and none were vitamin D deficient; additionally, more than half (59%) had serum 25(OH)D ≥75 $nmol \cdot L^{-1}$. Vitamin D sufficiency was maintained at week 12, with 96% of SSR and ORAL participants having 25(OH)D ≥50 $nmol \cdot L^{-1}$. At week 5, only around a quarter (27%) of placebo participants were vitamin D sufficient, and 23% were vitamin D deficient. At week 12, ~two-thirds (64%) of placebo participants were vitamin D sufficient, and none were vitamin D deficient.

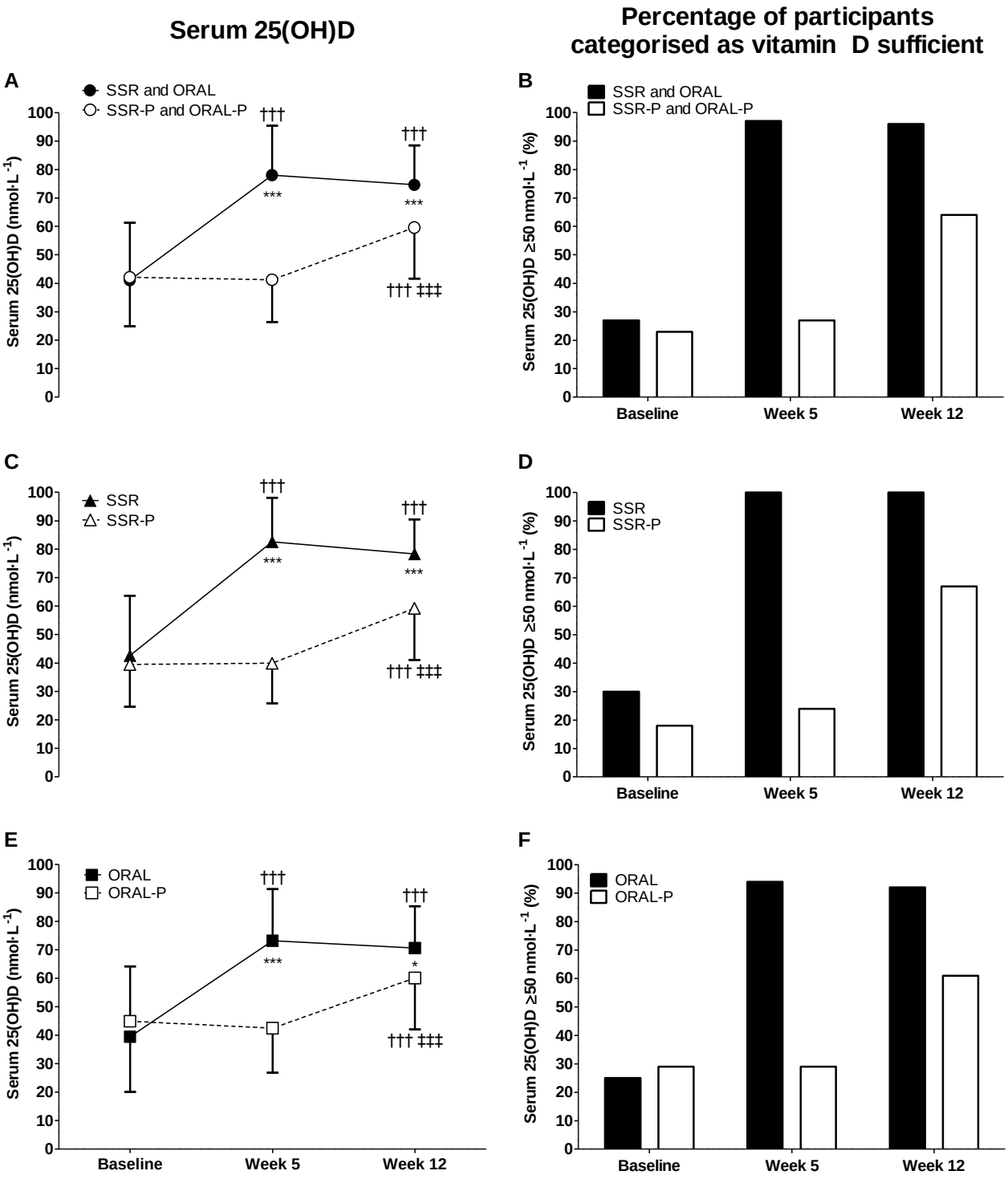

Figure 5.2. Serum 25(OH)D concentration and percentage of participants with 25(OH)D ≥50 nmol·L^{-1} in response to (A, B) simulated sunlight and oral vitamin D_3 (SSR and ORAL, *n* = 73) or placebo (SSR-P and ORAL-P, *n* = 64), (C, D) simulated sunlight (SSR, *n* = 37) or placebo (SSR-P, *n* = 33), and (E, F) oral vitamin D_3 (ORAL, *n* = 36) or placebo (ORAL-P, *n* = 31) measured at baseline, week 5 and 12. Panel A, C and E data are mean ± SD: ††† $P < 0.001$ *vs.* baseline; ‡‡‡ $P < 0.001$ *vs.* week 5; * $P < 0.05$ and *** $P < 0.001$ *vs.* placebo.

Serum 24,25(OH)$_2$D and 1,25(OH)$_2$D

A group x time interaction was observed for serum 24,25(OH)$_2$D (F(1.9,258.2) = 73.6, P < 0.001; Figure 5.3A) and 1,25(OH)$_2$D (F(2,270) = 3.8, P < 0.05; Figure 5.3B). In SSR and ORAL participants at week 5 and 12, serum 24,25(OH)$_2$D was higher than baseline, and higher than placebo participants (P < 0.001). Among placebo participants, serum 24,25(OH)$_2$D at week 5 was lower than baseline (P < 0.05); and higher at week 12 compared with baseline and week 5 (P < 0.001). In SSR and ORAL participants at week 5 and 12, serum 1,25(OH)$_2$D was higher than baseline (P < 0.05 and < 0.01, respectively), and at week 5 was higher than placebo (P < 0.05). Among placebo participants, serum 1,25(OH)$_2$D at week 12 was higher than week 5 (P < 0.01).

Serum vitamin D metabolites expressed as ratios

A group x time interaction was observed for the ratio of serum 25(OH)D to 1,25(OH)$_2$D (F(2,270) = 44.1, P < 0.001; Figure 5.4A); serum 25(OH)D to 24,25(OH)$_2$D (F(1.6,220.8) = 11.7, P < 0.001; Figure 5.4B); and serum 1,25(OH)$_2$D to 24,25(OH)$_2$D (F(1.3,170.5) = 20.9, P < 0.001; Figure 5.4C). In SSR and ORAL participants at week 5 and 12, the ratio of serum 25(OH)D to 1,25(OH)$_2$D was higher than baseline (P < 0.001) and higher than placebo participants (P < 0.001). Among placebo participants, the ratio of serum 25(OH)D to 1,25(OH)$_2$D at week 12 was higher than baseline and week 5 (P < 0.001). In SSR and ORAL participants, the ratio of serum 25(OH)D to 24,25(OH)$_2$D was lower at week 5 than baseline (P < 0.001); and at week 12 was lower than both baseline and week 5 (P < 0.001). Among SSR and ORAL participants the ratio of serum 25(OH)D to 24,25(OH)$_2$D at week 5 and 12 was lower than placebo (P < 0.001). In SSR and ORAL participants at week 5 and 12, the ratio of serum 1,25(OH)$_2$D to 24,25(OH)$_2$D was lower than baseline (P < 0.001), and lower

than placebo ($P < 0.001$). Among placebo participants, the ratio of serum $1,25(OH)_2D$ to $24,25(OH)_2D$ at week 12 was lower than baseline and week 5 ($P < 0.001$).

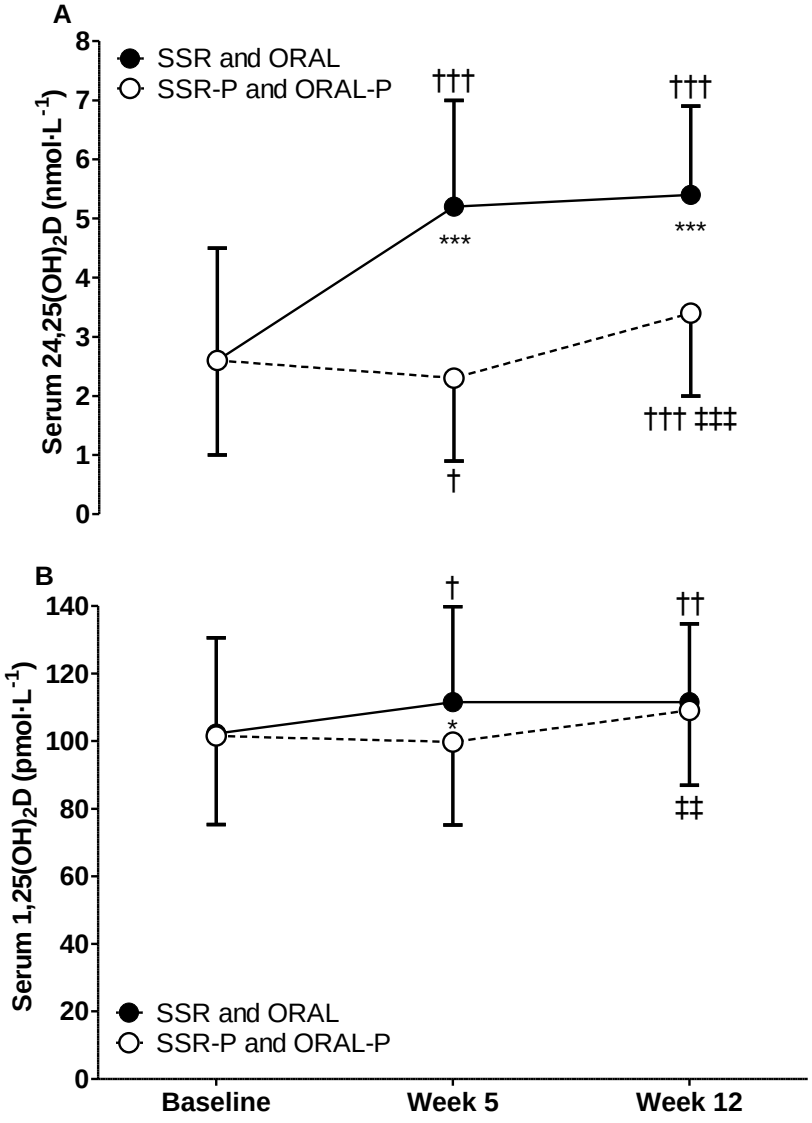

Figure 5.3. Serum (A) $24,25(OH)_2D$ and (B) $1,25(OH)_2D$ response to simulated sunlight and oral vitamin D_3 (SSR and ORAL, $n = 73$) or placebo (SSR-P and ORAL-P, $n = 64$) measured at baseline, week 5 and 12. Data are mean ± SD: † $P < 0.05$, †† $P < 0.01$ and ††† $P < 0.001$ *vs.* baseline; ‡‡ $P < 0.01$ and ‡‡‡ $P < 0.001$ *vs.* week 5; * $P < 0.05$ and *** $P < 0.001$ *vs.* placebo.

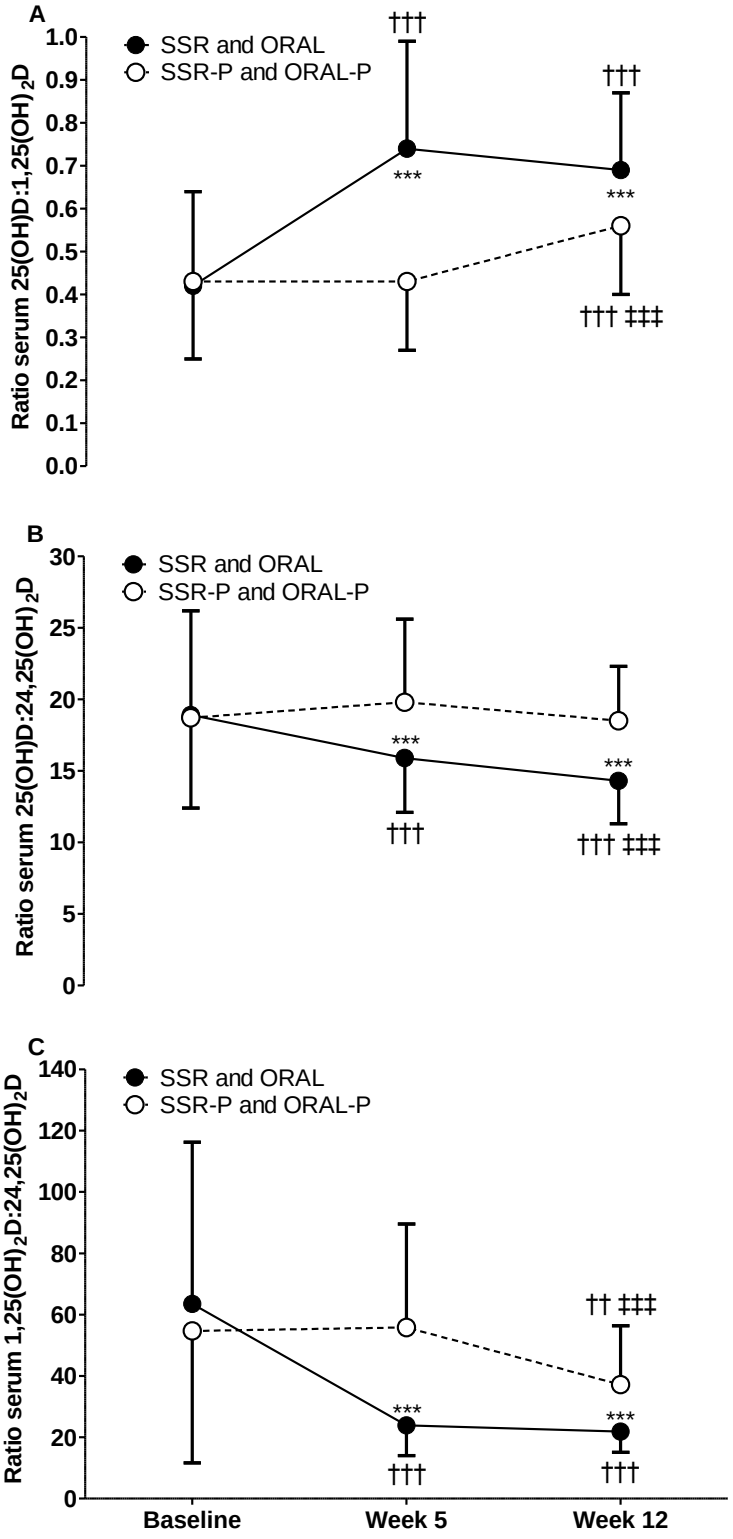

Figure 5.4. Relationship between serum vitamin D metabolites expressed as ratios: (A) ratio of 25(OH)D to $1,25(OH)_2D$, (B) ratio of 25(OH)D to $24,25(OH)_2D$, and (C) ratio of $1,25(OH)_2D$ to $24,25(OH)_2D$ in response to simulated sunlight and oral vitamin D_3 (SSR and ORAL, n = 73) or placebo (SSR-P and ORAL-P, n = 64) measured at baseline, week 5 and 12. Data are mean ± SD: ††† $P < 0.001$ and †† $P < 0.01$ *vs.* baseline; ‡‡‡ $P < 0.001$ *vs.* week 5; *** $P < 0.001$ *vs.* placebo.

Effect of simulated sunlight and oral vitamin D_3 on exercise performance

Endurance exercise performance

One-and-a-half mile run time was not affected by vitamin D supplementation, with no group x time interaction observed for SSR and ORAL *vs.* SSR-P and ORAL-P (F(1.5,83.8) = 0.3, P = 0.68; Figure 5.5A); SSR *vs.* SSR-P (F(2,54) = 0.2, P = 0.80; Figure 5.5B); or ORAL *vs.* ORAL-P (F(1.3,34.0) = 0.2, P = 0.69; Figure 5.5C). A main effect of time for 1.5-mile run time was observed for active intervention and placebo groups pooled, with faster run times at week 5 and 12 (all groups: F(1.5,83.8) = 54.0, P < 0.001; SSR and SSR-P: F(2,54) = 22.2, P < 0.001; ORAL and ORAL-P: F(1.3,34.0) = 32.3, P < 0.001). A main effect of group was observed for SSR *vs.* SSR-P, with SSR faster than placebo (F(1,27) = 4.3, P < 0.05). No main effect of group was observed for SSR and ORAL *vs.* SSR-P and ORAL-P (F(1,56) = 0.84, P = 0.37), or ORAL *vs.* ORAL-P (F(1,27) = 0.1, P = 0.71). Furthermore, SSR and ORAL participants who achieved 25(OH)D ≥75 nmol·L^{-1} by week 5 did not improve their 1.5-mile run time more than those who received placebo and remained vitamin D insufficient (1.5-mile run time improvement by week 5: participants ≥75 nmol·L^{-1} -28 ± 32 s (n = 22) *vs.* placebo participants <50 nmol·L^{-1} -24 ± 34 s (n = 20), P = 0.72; Cohen's d effect size = 0.1). At week 12, there was also no difference in 1.5-mile run time improvement for SSR and ORAL participants with 25(OH)D ≥75 nmol·L^{-1} *vs.* those who received placebo and were vitamin D insufficient (P = 0.61).

Muscular strength and power performance

There was no effect of vitamin D supplementation on maximum dynamic lift strength or muscular power output (Table 5.3). For maximum dynamic lift strength, no group x time interaction was observed for SSR and ORAL *vs.* SSR-P and ORAL-P (F(1,97) = 1.4, P = 0.23); SSR *vs.* SSR-P (F(1,49) = 0.01, P = 0.92); or ORAL *vs.* ORAL-P (F(1,46) = 3.7, P =

0.06); and no main effects of time or group were seen ($P \geq 0.07$). For muscular power output, no group x time interaction was observed for SSR and ORAL *vs.* SSR-P and ORAL-P ($F(1,125) = 0.1$, $P = 0.71$); SSR *vs.* SSR-P ($F(1,61) = 0.6$, $P = 0.43$); or ORAL *vs.* ORAL-P ($F(1,62) = 0.1$, $P = 0.82$). A main effect of time was observed for all groups pooled ($F(1,125) = 4.1$, $P = 0.045$), with muscular power output lower at week 12. No other main effects of time ($P \geq 0.11$), or any main effects of group were observed for muscular power ($P \geq 0.42$). Furthermore, there was no difference in maximum dynamic lift strength or muscular power for SSR and ORAL participants who achieved 25(OH)D ≥75 nmol·L^{-1} by week 12 ($n = 25$ and 35 for maximum dynamic lift and muscular power, respectively) *vs.* those who received placebo and remained vitamin D insufficient (25(OH)D <50 nmol·L^{-1}; $n = 15$ and 21 for maximum dynamic lift and muscular power, respectively; $P > 0.20$).

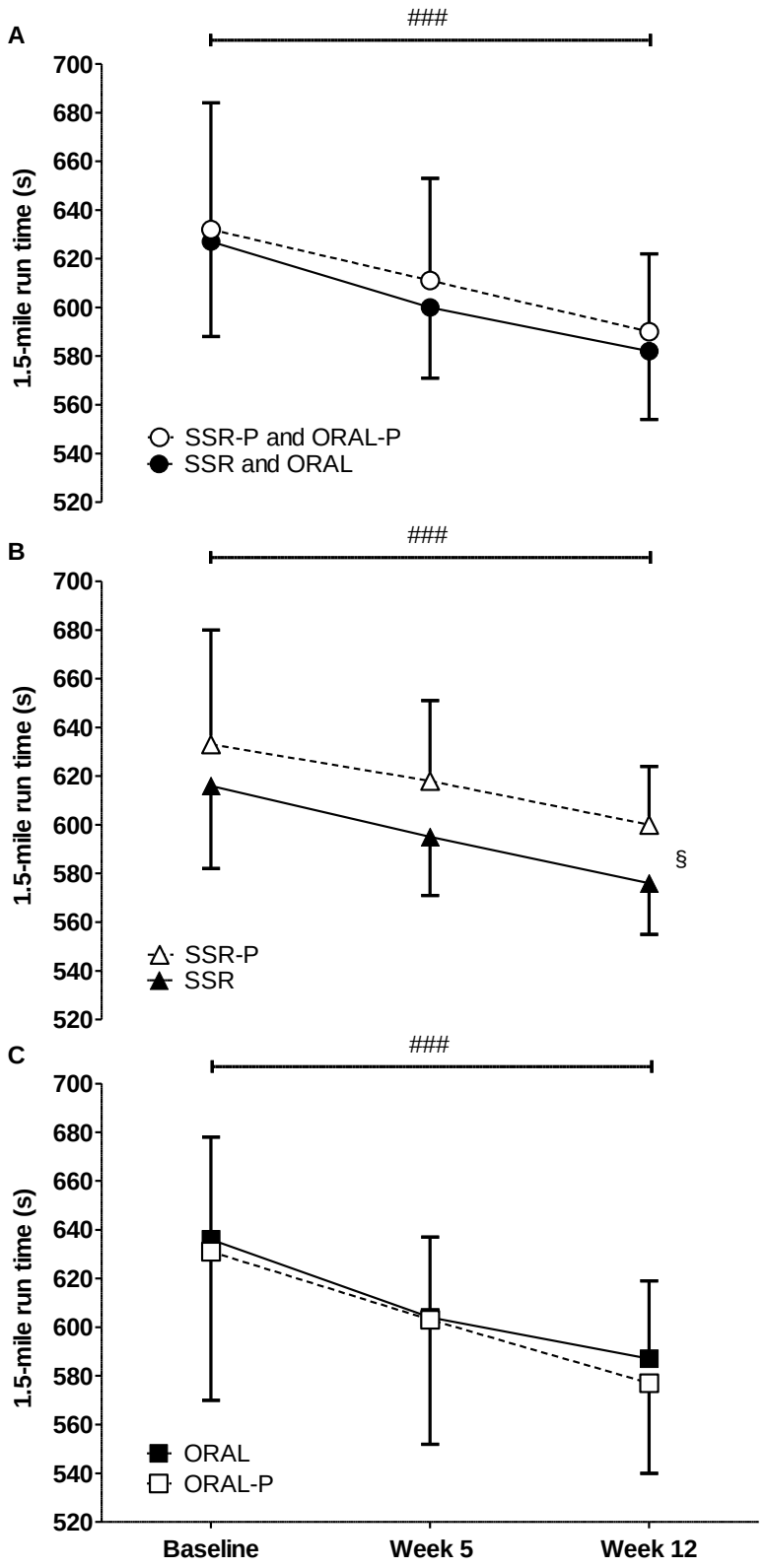

Figure 5.5. One-and-a-half-mile run time response to (A) simulated sunlight and oral vitamin D_3 (SSR and ORAL, *n* = 31) or placebo (SSR-P and ORAL-P, *n* = 27), (B) simulated sunlight (SSR, *n* = 14) or placebo (SSR-P, *n* = 15), and (C) oral vitamin D_3 (ORAL, *n* = 17) or placebo (ORAL-P, *n* = 12), measured at baseline, week 5 and 12. Data are mean ± SD: ### $P < 0.001$ main effect of time; § $P < 0.05$ main effect of group.

Table 5.3. Effect of simulated sunlight (SSR), placebo simulated sunlight (SSR-P), oral vitamin D_3 (ORAL) and oral placebo (ORAL-P) on maximum dynamic lift strength and muscular power output measured at baseline and week 12.

	Week	SSR	SSR-P	ORAL	ORAL-P	*P* value
Maximum dynamic lift strength (kg)	Baseline	70 ± 13	71 ± 12	68 ± 12	76 ± 13	0.23[a] 0.92[b]
	12	68 ± 13	70 ± 12	68 ± 11	73 ± 11	0.06[c]
Muscular power output (W)	Baseline	3888 ± 704	3930 ± 609	3808 ± 663	3911 ± 633	0.71[a] 0.44[b]
	12	3792 ± 565	3896 ± 575	3765 ± 589	3849 ± 482	0.82[c]

Group x time interaction: [a] SSR and ORAL *vs.* SSR-P and ORAL-P; [b] SSR *vs.* SSR-P; [c] ORAL *vs.* ORAL-P. Data are mean ± SD.
Maximum dynamic lift: SSR, $n = 27$; SSR-P, $n = 24$; ORAL, $n = 25$; ORAL-P, $n = 23$.
Muscular power output: SSR, $n = 33$; SSR-P, $n = 30$; ORAL, $n = 34$; ORAL-P, $n = 30$.

Effect of simulated sunlight and oral vitamin D_3 on blood pressure

There was no effect of vitamin D supplementation on resting blood pressure (Table 5.4). For mean arterial pressure, no group x time interaction was observed for SSR and ORAL *vs.* SSR-P and ORAL-P ($F(1,56) < 0.001$, $P = 0.99$); SSR *vs.* SSR-P ($F(1,32) = 1.1$, $P = 0.31$); or ORAL *vs.* ORAL-P ($F(1,22) = 1.4$, $P = 0.24$). Main effects of time were observed for active intervention and placebo groups pooled, with mean arterial pressure lower at week 12 (all groups, $F(1,56) = 53.0$, $P < 0.001$; SSR and SSR-P, $F(1,32) = 27.3$, $P < 0.001$; ORAL and ORAL-P, $F(1,22) = 24.5$, $P < 0.001$). No main effects of group were observed for mean arterial pressure ($P \geq 0.34$).

Table 5.4. Effect of simulated sunlight (SSR), placebo simulated sunlight (SSR-P), oral vitamin D_3 (ORAL) and oral placebo (ORAL-P) on mean arterial pressure measured at baseline and week 12.

	Week	SSR *n* = 17	SSR-P *n* = 17	ORAL *n* = 14	ORAL-P *n* = 10	*P* value
Mean arterial pressure (mmHg)	Baseline	84 ± 8	86 ± 4	89 ± 8	90 ± 6	0.99[a], 0.31[b], 0.24[c]
	12	79 ± 6	78 ± 6	80 ± 5	84 ± 5	

Group x time interaction: [a] SSR and ORAL *vs.* SSR-P and ORAL-P; [b] SSR *vs.* SSR-P; [c] ORAL *vs.* ORAL-P. Data are mean ± SD.

5.5 DISCUSSION

The effect of vitamin D supplementation using simulated sunlight in accordance with recommendations on safe sunlight exposure, or matched oral vitamin D_3 on 3 major vitamin D metabolites, exercise performance and blood pressure were investigated in a randomised placebo controlled trial. As anticipated, vitamin D supplementation via simulated sunlight or oral vitamin D_3 restored serum 25(OH)D from winter to typical summer concentrations in 4-weeks and then maintained EFSA and IOM defined vitamin D sufficiency for a further 8-weeks (Figure 5.2). Restoring vitamin D sufficiency also increased the concentration of serum 24,25$(OH)_2$D and 1,25$(OH)_2$D (Figure 5.3); the main metabolic products of 25(OH)D (**Chapter 1, Figure 1.1**). However, contrary to the hypotheses, vitamin D supplementation did not affect exercise performance (Figure 5.5 and Table 5.3) or resting blood pressure (Table 5.4) among young healthy male military recruits. These findings suggest vitamin D does not directly affect exercise performance.

Vitamin D metabolites

Prior to supplementation, ~three-quarters of young, otherwise healthy, UK-based male participants were vitamin D insufficient (serum 25(OH)D <50 nmol·L^{-1}). Typical summer serum 25(OH)D concentrations were reached using simulated sunlight or oral vitamin D_3 supplementation within 4-weeks and maintained during the subsequent 8-weeks, which may be beneficial for musculoskeletal health (Institute of Medicine, 2011; European Food Safety Authority, 2016). Dietary vitamin D intake as a contributor to vitamin D status was low, and similar to that reported previously in UK adults, and would have contributed little to the vitamin D status of participants in all intervention groups (Macdonald, 2013). Exposure to ambient UVB at week 11 was higher than week 4 (although not significantly different), with erythemal doses similar to those typical during spring in the UK (Table 5.2; Macdonald,

2013). This exposure to ambient UVB accounts for the increase in concentration of vitamin D metabolites detected among placebo group participants at week 12.

How serum 24,25$(OH)_2$D and 1,25$(OH)_2$D responded to safe simulated sunlight and oral vitamin D_3 supplementation intended to restore vitamin D sufficiency (serum 25(OH)D ≥50 nmol·L^{-1}) were measured for the first time. As the main metabolic products of 25(OH)D, the concentrations of serum 24,25$(OH)_2$D and 1,25$(OH)_2$D increased at week 5 and 12 (Figure 5.3). The larger relative increase in serum 25(OH)D compared with 1,25$(OH)_2$D meant the ratio of 25(OH)D to 1,25$(OH)_2$D was also increased at week 5 and 12, and was higher than placebo (Figure 5.4A). As expected (Owens et al., 2017; Wagner et al., 2011), the ratio of 25(OH)D to 24,25$(OH)_2$D was lower at week 5 than baseline, and lower still at week 12 suggesting a higher catabolic rate of 25(OH)D towards its 24,25$(OH)_2$D metabolite with vitamin D supplementation (Figure 5.4B). A lower ratio of 25(OH)D to 24,25$(OH)_2$D has been demonstrated to impair increases in 25(OH)D (Wagner et al., 2011); hence the scope for further increases in 25(OH)D was reduced as vitamin D sufficiency was restored. The ratio of 1,25$(OH)_2$D to 24,25$(OH)_2$D was lower at week 5 and 12 compared with baseline indicating 25(OH)D metabolism was driven to produce 24,25$(OH)_2$D rather than 1,25$(OH)_2$D (Figure 5.4C). A decrease in the ratio of 1,25$(OH)_2$D to 24,25$(OH)_2$D has been demonstrated previously, albeit using higher oral vitamin D_3 doses (5,000 and 10,000 IU·day^{-1}; Owens et al., 2017). Evidence is emerging that 24,25$(OH)_2$D might bind to the VDR and inhibit 1,25$(OH)_2$D activity (Curtis et al., 2014); therefore, the observed increase in 24,25$(OH)_2$D may have impaired VDR–1,25$(OH)_2$D mediated adaptations beneficial for exercise performance.

Exercise performance

In line with the study aim, supplementation via safe simulated sunlight or oral vitamin D_3 achieved EFSA and IOM defined vitamin D sufficiency in almost all participants (Figure 5.2). More than more than half of participants (59%) also achieved serum 25(OH)D ≥75 $nmol \cdot L^{-1}$, which has been proposed to be optimal for training induced adaptations and to maximise the effect of vitamin D on calcium, bone and muscle metabolism (Thomas et al., 2016; Holick et al., 2011). Contrary to the hypothesis, achieving and maintaining vitamin D sufficiency did not benefit endurance, strength or power exercise performance. In agreement with previous randomised, placebo controlled trials using oral vitamin D_3 supplementation (Todd et al., 2017; Owens et al., 2014; Close et al., 2013a), and here for the first time using simulated sunlight, no beneficial effect of achieving proposed optimal vitamin D status on exercise performance was demonstrated. Whether larger doses, achieving greater than normal seasonal changes in vitamin D (for example to >100 $nmol \cdot L^{-1}$) would have a beneficial effect on exercise performance remains unknown; however, higher doses of simulated sunlight, and oral vitamin D_3 in excess of 4,000 $IU \cdot day^{-1}$ risk skin damage and vitamin D toxicity, respectively (Institute of Medicine, 2011; Advisory Group on Non-ionising Radiation, 2017).

Blood pressure

Restoring vitamin D sufficiency via simulated sunlight or oral vitamin D_3 did not influence resting blood pressure (Table 5.4). This may be because participants' blood pressure was within the normal range at baseline; however, 12-weeks of military training did reduce blood pressure to within the optimal range, indicating there was scope for improvement (Burt et al., 1995). In agreement with the present study, several trials investigating the effect of oral vitamin D_3 supplementation on blood pressure reported no reduction in normo- or hyper-tensive patients (n = 4,541; Beveridge et al., 2015). With only one small, uncontrolled study

(n = 9) showing a blood pressure lowering effect of UVB to date (Krause et al., 1998), vitamin D supplementation is ineffective for decreasing resting blood pressure.

Unrelated to vitamin D mediated mechanisms, irradiation with UVA has been demonstrated to reduce blood pressure acutely in healthy individuals (Oplander et al., 2009; Liu et al., 2014). No blood pressure lowering effect of simulated sunlight (UVA and UVB) was observed in the present study; however, blood pressure was not measured immediately after irradiations. Speculatively, inverse relationships previously reported between vitamin D status and blood pressure (Forman et al., 2007) could be explained by vitamin D status serving as marker of sunlight exposure, with individuals with higher 25(OH)D exposed to more UVA.

Strengths and limitations

Strengths of the current study include the use of double-blind placebo control groups which enabled the effect of vitamin D on exercise performance to be measured with control for any placebo effect and without investigator bias (Beedie and Foad, 2009). Furthermore, functionally relevant and meaningful measures of exercise performance were used (Fortes et al., 2011; Friedl et al., 2015). Measuring sunlight exposure and dietary vitamin D intake demonstrated there were no differences between groups for these sources of vitamin D. The following limitations were present in the current study. Increases in serum 25(OH)D were observed in placebo group participants due to exposure to ambient UVB; thereby reducing active intervention *vs.* placebo group differences at week 12. However, no differences in exercise performance were observed between groups when vitamin D sufficient placebo participants were removed from analyses. Blood pressure was measured at rest so may not reflect any possible beneficial effect of vitamin D on cardiovascular function during exercise.

Finally, these findings cannot be conclusively applied to all because women were not included in the present study; and only white-skinned individuals were studied.

Conclusion

Vitamin D supplementation in white male military recruits using simulated sunlight in accordance with recommendations on safe sunlight exposure, or matched oral vitamin D_3 restored serum 25(OH)D from winter to typical UK summer concentrations in 4-weeks and then maintained EFSA and IOM defined vitamin D sufficiency for a further 8-weeks, but did not affect exercise performance or resting blood pressure. This lack of beneficial effect was observed despite the majority of supplemented participants also achieving possible optimal vitamin D status. These findings suggest vitamin D does not directly affect exercise performance.

CHAPTER SIX

General discussion

This thesis set out to investigate the role of vitamin D in optimising exercise performance among young healthy adults. In **Chapter 3**, it was demonstrated that vitamin D insufficiency was prevalent among otherwise healthy military recruits during winter and spring, and vitamin D status was positively associated with endurance exercise performance, but not muscular strength or power. To restore and then maintain vitamin D sufficiency from its winter nadir, vitamin D supplementation protocols using safe simulated sunlight and oral vitamin D_3 were demonstrated to be effective in **Chapter 4**. Then in **Chapter 5**, despite simulated sunlight and oral vitamin D_3 supplementation restoring and then maintaining vitamin D sufficiency in almost all male military recruits, there was no effect on exercise performance. Taken together, these findings suggest that vitamin D does not directly affect exercise performance.

6.1 Vitamin D metabolites

A seasonal variation in three major vitamin D metabolites (serum 25(OH)D, $24,25(OH)_2D$ and $1,25(OH)_2D$) was observed in **Chapter 3**, with concentrations lowest during winter and highest during summer. The nadir was a result of the reduced availability of solar UVB in the UK from late autumn to early spring (Webb et al., 1988). The observed seasonal variation in $1,25(OH)_2D$ was unexpected because circulating $1,25(OH)_2D$ concentrations are tightly regulated (Chesney et al., 1981). However, because vitamin D metabolites were measured in different participants in each season rather than the same participants throughout the year, whether a seasonal intra-individual variation in $1,25(OH)_2D$ would have occurred remains to be seen. The concentration of vitamin D metabolites changed minimally over 12-weeks (≈1

season) from baseline to follow-up in **Chapter 3**. For example, only 16% of males and 2% of females changed from being categorised as vitamin D deficient to sufficient (Δ25(OH)D <30 to ≥50 nmol·L^{-1}).

It was not an aim of this thesis to investigate between sex differences in vitamin D metabolites; however, the concentrations of vitamin D metabolites in **Chapter 3** were consistently higher in females than males. Sex differences in serum 25(OH)D have been observed previously, but with no sex consistently higher than the other (Hypponen and Power, 2007). Speculatively, the higher concentrations of vitamin D metabolites among women in **Chapter 3** may have been because female recruits training for a variety of Army professions might have been from higher socioeconomic backgrounds than males joining the infantry. Sun exposure and diet, and therefore vitamin D intake, may be affected by socioeconomic factors and lifestyle; for example lower concentrations of vitamin D metabolites have been observed among low income groups (Weaver et al., 2010). In addition, sun beds were used more regularly by women than men (9% of males and 21% of females were regular sun bed users). The higher latitude where males (Catterick, 54°N) *vs.* females (Pirbright, 51°N) were located for military training would not have affected baseline 25(OH)D concentration because serum was collected <1 week after participants had arrived from their hometowns (mean latitude: males 53°N and females 53°N).

6.2 Vitamin D insufficiency

In **Chapter 3**, 80% of male and female military recruits' vitamin D intake was inadequate to achieve EFSA (2016) and IOM (2011) recommended vitamin D sufficiency during winter (25(OH)D ≥50 nmol·L^{-1}). Similarly, and also during winter, 85% of young men and women were vitamin D insufficient in **Chapter 4**; and 74% of male military recruits were vitamin D

insufficient in **Chapter 5**. The prevalence of vitamin D insufficiency in this thesis was higher than previous wintertime observations, for example among adults living in the UK (69%) and Ireland (61%; Cashman et al., 2016).

6.3 Effect of simulated sunlight and oral vitamin D_3 on vitamin D metabolites

Avoiding vitamin D insufficiency is widely accepted as important for musculoskeletal health (Institute of Medicine, 2011; European Food Safety Authority, 2016); therefore, the protocols used in **Chapters 4 and 5** to safely restore and then maintain vitamin D sufficiency in almost all are valuable for most adults living in the UK or countries at similar latitudes. Cutaneous vitamin D synthesis is tightly regulated via a negative feedback loop (Holick, 1995), hence simulated sunlight can be used without risk of vitamin D overproduction and toxicity. By using a sub-erythemal dose of simulated sunlight, the risk of sun burn was minimised and did not occur, which is important given sunburn is a risk factor for skin cancer (Elwood and Jopson, 1997). Skin exposure to UVB is regarded as the safest means to increase vitamin D status (Webb et al., 2011), and can be used by individuals with intestinal fat malabsorption disorders for whom oral supplementation is unlikely to be effective. By using an oral vitamin D_3 dose below the tolerable upper intake recommended by EFSA and IOM (<4,000 $IU \cdot day^{-1}$), the risk of vitamin D intoxication or hypervitaminosis D was minimised in **Chapters 4 and 5**. However, markers of vitamin D intoxication such as hypercalcaemia and hypercalciuria were not measured to confirm this assumption.

Using a blanket dose of vitamin D_3 meant the oral dose given to participants in **Chapters 4 and 5** was not relativised to their body mass or composition. This, along with differences in baseline vitamin D status contributed to the inter-individual variability in serum Δ25(OH)D (Barger-Lux et al., 1998). In contrast, the standard erythemal dose of simulated sunlight

received by participants was relativised to their body size. As shown previously by Rhodes et al. (2010), serum 25(OH)D increases after exposure to simulated sunlight were still subject to some variation in **Chapters 4 and 5**. This may have been due to differences in baseline 25(OH)D concentrations and the exact area of skin exposed to UVR. For example, participants taller than the 180 cm irradiation tubes or with tattoos may have exposed less skin; whereas participants without hair exposed more skin than intended. In **Chapters 4 and 5**, a standard erythemal dose (1.3 SED) of simulated sunlight (95% UVA; 5% UVB) was given to a skin surface area likely to be exposed to summer sunlight whilst wearing casual summer clothing (37–40%). To their disadvantage, numerous intervention studies (**Table 1.3, Chapter 1**) have used fluorescent lamps with a UV emission removed from that of natural sunlight; exposed near whole-body surface areas; and sometimes used personalised (MED-related) UVR doses, limiting the practical applicability of their findings.

In **Chapter 4**, the increase in serum 25(OH)D using oral vitamin D_3 supplementation (1,000 $IU \cdot day^{-1}$: +39 $nmol \cdot L^{-1}$) was similar to that achieved using simulated sunlight (1.3 SED, $3x \cdot week^{-1}$: +28 $nmol \cdot L^{-1}$). However, to better match the two supplementation methods, the skin surface area exposed to UVR was increased from 37 to 40% in **Chapter 5.** This successfully resulted in a +40 $nmol \cdot L^{-1}$ increase in participants who received simulated sunlight *vs.* a +34 $nmol \cdot L^{-1}$ increase in participants who received oral vitamin D_3. The unintentional smaller increase in 25(OH)D achieved using oral vitamin D_3 in **Chapter 5** *vs.* **Chapter 4** probably occurred due to higher baseline 25(OH)D concentrations (40 *vs.* 35 $nmol \cdot L^{-1}$) and possible over reporting of compliance by military recruits in **Chapter 5**. The estimation of compliance to oral supplementation is recognised as a limitation of the studies in **Chapters 4 and 5**; although typical of large studies that have used daily supplementation

(Macdonald et al., 2013). Future investigations would benefit from supervising the daily consumption of oral vitamin D_3 to verify compliance.

For the first time, in **Chapter 4** serum 25(OH)D was measured in the 8-weeks after vitamin D sufficiency had been restored using simulated sunlight or oral vitamin D_3 in a small sample of participants. Among those who ceased receiving simulated sunlight, serum 25(OH)D, on average, remained relatively stable from week 5 to 12 (n = 5). In contrast, serum 25(OH)D decreased from week 5 to 12 among 2 out of the 3 participants who ceased consuming oral vitamin D_3. This may have been because participants who stopped oral vitamin D_3 supplementation had higher 25(OH)D concentrations than the simulated sunlight group, and thus had more to lose (80 *vs.* 60 $nmol \cdot L^{-1}$ at week 5). Alternatively, it may be that orally administered vitamin D_3 is less effective at maintaining 25(OH)D than vitamin D_3 synthesised at the skin (Haddad et al., 1993). This may be because cutaneous synthesised vitamin D_3 is primarily associated with vitamin D binding protein and is gradually delivered to the liver for hydroxylation (Fraser, 1983). In contrast, oral vitamin D_3 is associated with chylomicrons and lipoproteins which are readily taken up by the liver and hydroxylated more rapidly. Hence participants who consumed oral vitamin D_3 may have experienced a more rapid increase, but subsequent faster decline in 25(OH)D after ceasing supplementation.

6.4 Ambient ultraviolet B

Increases in serum 25(OH)D were observed in **Chapters 4 and 5** placebo groups due to exposure to ambient UVB. Thus active intervention *vs.* placebo group differences were narrowed at week 12, and likelihood of detecting a positive effect of vitamin D was reduced. It would have been desirable for all participants to commence supplementation in January thereby avoiding the maintenance phase concluding as late as May, when the availability of

ambient UVB is significant (Macdonald, 2013); but this was not logistically possible. PSF badges were used to quantify the erythemal dose participants received from ambient UVB, and demonstrated no between group differences (**Chapters 4 and 5**). Compliance was self-reported and badges may have been shaded by clothing or body position; hence UVB exposure may have been under-reported. Furthermore, the actual skin surface area exposed to sunlight whilst wearing badges was unknown. To overcome these limitations, future studies should closely monitor compliance and record the skin areas exposed when badges are worn.

6.5 Exercise performance

Chapter 3 sought to address some of the limitations of previous studies that have examined the relationship between vitamin D and exercise performance by: assessing meaningful measures of endurance, strength *and* power; controlling for covariates known to influence performance (body composition, smoking and season); studying only young healthy men *and* women; and collecting data throughout the year to give a seasonal range of 25(OH)D concentrations. The results indicated that serum 25(OH)D and 24,25$(OH)_2$D were positively associated with endurance exercise performance, but not muscular strength or power. As expected, the biologically active 1,25$(OH)_2$D was not associated with any measure of exercise performance. The latter finding was observed because even during vitamin D deficiency, 1,25$(OH)_2$D production is normal or even elevated; and exercise performance assessments were not made at exactly the same time as serum was collected, which is relevant since 1,25$(OH)_2$D has a half-life of only ~4 hours (Zerwekh, 2008; Holick, 2009). This thesis expands upon previous literature that has reported bivariate correlations between 25(OH)D and exercise performance (Fitzgerald et al., 2014; Forney et al., 2014; Koundourakis et al., 2014) because the results from **Chapter 3** were observed in both sexes, after controlling for body composition, smoking and season; and were seen again after 12-

weeks of military training, with control for incomplete training days due to illness or injury. Previous work by Ardestani et al. (2011) reported a positive association between 25(OH)D and $\dot{V}O_{2max}$ after controlling for BMI in a study that included elderly participants. Notably, BMI is a measure of relative body mass that does not distinguish between fat and lean mass, especially in active individuals (Flegal et al., 2009). The study in **Chapter 3** is the first to control for body fat when investigating the relationship between vitamin D status and endurance exercise performance. Adipose tissue sequesters vitamin D; therefore, individuals with high fat mass have lower 25(OH)D concentrations (Wortsman et al., 2000). High fat mass also impairs exercise performance in young adults (Mattila et al., 2007); consequently, high body fat and low availability of vitamin D may be responsible for poor performance in individuals with insufficient 25(OH)D concentrations. Therefore, the influence of vitamin D on performance may have been overestimated in studies that do not control for body composition (for example, Grimaldi et al. (2013) and Ardestani et al. (2011)). In agreement with previous observational studies (Hamilton et al., 2014; Marantes et al., 2011), there was no relationship between vitamin D metabolites and strength in **Chapter 3**. In contrast, 25(OH)D was positively associated with strength in a study by Grimaldi et al. (2013), but using isolated measures of muscle function rather than the functional assessments used in this thesis; and without controlling for body composition.

Vitamin D supplementation restored serum 25(OH)D from winter to typical summer concentrations in 4-weeks and then maintained EFSA and IOM defined vitamin D sufficiency for a further 8-weeks in **Chapter 5**. Most participants who received vitamin D (59%) achieved serum 25(OH)D $\geq$75 nmol·L^{-1}, which has been proposed to be optimal for training induced adaptations and to maximise the effect of vitamin D on calcium, bone and muscle metabolism (Thomas et al., 2016; Holick et al., 2011). However, this study's hypotheses

cannot be accepted because vitamin D supplementation did not affect exercise performance or resting blood pressure. Previous studies have also reported no effect on performance when proposed optimal vitamin D status was reached using oral vitamin D_3 (Todd et al., 2017; Owens et al., 2014; Close et al., 2013a). The following strengths make **Chapter 5** a valuable addition to the existing literature. Firstly, by using safe doses of vitamin D_3 (1,000 then 400 $IU \cdot day^{-1}$) below the IOM and EFSA recommended tolerable upper intake (4,000 $IU \cdot day^{-1}$); in contrast, Owens et al. (2014) and Close et al. (2013a) used doses >4,000 $IU \cdot day^{-1}$, where the risk for harm begins to increase (Institute of Medicine, 2011). Secondly, by studying individuals for whom vitamin D insufficiency was prevalent, thereby maximising the potential for supplementary vitamin D to be beneficial (mean 25(OH)D at baseline of 41 $nmol \cdot L^{-1}$; 74% <50 $nmol \cdot L^{-1}$); whereas participants studied by Close et al. (2013a) had mean 25(OH)D at baseline >50 $nmol \cdot L^{-1}$. And thirdly, by assessing functional measures of exercise performance; whereas Todd et al. (2017) measured $\dot{V}O_{2max}$, and Owens et al. (2014) used isokinetic dynamometry and electromyostimulation to assess lower limb muscle function.

To date, only one randomised placebo controlled trial has shown a beneficial effect of vitamin D supplementation on exercise performance in young adults (Close et al., 2013b). In a cohort of young males with a mean 25(OH)D of ~30 $nmol \cdot L^{-1}$, 8-weeks oral vitamin D_3 supplementation with 5,000 $IU \cdot day^{-1}$ improved vertical jump height and 10 m sprint times, with 25(OH)D increasing to a mean ~103 $nmol \cdot L^{-1}$. However, the placebo group also showed improvements in most of the performance measures, albeit not statistically significant changes. With only 5 participants in each group, whether a true physiological effect of vitamin D was observed by Close and colleagues (2013b) is unclear.

It was intended for endurance performance to be assessed using a multistage fitness test in **Chapter 5**. However, participants' effort in this test to exhaustion was not judged to be maximal (Leger et al., 1988); hence, endurance performance was assessed using a 1.5-mile run. Participants were highly motivated during this test because their best effort was required for progression in their military careers. **Chapter 5** findings cannot be conclusively applied to all because women were not studied. Men were studied in **Chapter 5** due to the higher prevalence of vitamin D insufficiency among males *vs.* females in **Chapter 3 (Figure 3.3)**. Participants' performance on the tests used in **Chapters 3 and 5** were typical of military recruits who had passed military entry standards, and directly relevant to young, physically active adults. Further research is recommended to confirm these findings in elite athletes.

The field-study nature of **Chapters 3 and 5** precluded absolute control of participants' behaviour before exercise tests, including diet, hydration, and nicotine intake, which as threats to internal validity may have confounded results (Halperin et al., 2015). The measures of exercise performance used in this thesis were chosen principally because they enabled a large number of participants to be tested in a timely manner. The chosen assessments also allowed functionally relevant and meaningful measures of exercise performance to be made. Maximum dynamic lift strength and vertical jump height were assessed because they utilise muscle groups often used by military personnel (Fortes et al., 2011). Maximum dynamic lift strength has been shown to correlate with military tasks such as load carriage and artillery ammunition loading (Nindl et al., 1997). Furthermore, maximum dynamic lift strength has been demonstrated to be superior to grip strength, isometric pull strength and $\dot{V}O_{2max}$ for predicting success in functional tasks (Friedl et al., 2015). Isokinetic or isometric dynamometry, as used by Grimaldi et al. (2013), Marantes et al. (2011), Hamilton et al. (2014) and Owens et al. (2014) enabled sensitive assessments of single joint muscle function

to be made in their respective studies. However, athletic or functional movements involve multiple joints working in synergy. Furthermore, relatively weak relationships have been observed between tests of muscle function and dynamic performance; and muscle function tests have been shown to lack the capability to track training induced changes in performance (Murphy et al., 1994; Murphy and Wilson, 1997). Therefore, the maximum dynamic lift and vertical jump tests used in **Chapters 3 and 5** were probably less sensitive than assessing muscle function using dynamometry, but were reflective of functional performance. Endurance exercise performance, assessed in this thesis using a 1.5-mile run, is important for young adults and military personnel because individuals with higher aerobic fitness are able to sustain physical tasks for longer and are at lower risk of injury (Friedl et al., 2015; Knapik et al., 2001; Jones et al., 1993). The 1.5-mile run is used widely among military personnel, with performance indicative of an individual's maximal aerobic capacity (Friedl et al., 2015). The improvements in endurance performance and absence of increases in strength or power in **Chapters 3 and 5** reflect the aerobic nature of Basic Military Training, and were aligned with existing literature (Williams, 2005; Williams et al., 1999; Legg and Duggan, 1996).

Maximum dynamic lift strength has a high test-retest reliability (r = 0.91; Nindl et al., 1997) and maximum vertical jump height has been shown to have a test-retest reliability of r = 0.90 (Moir et al., 2008). Running time trials of a similar distance to the 1.5-mile run (1,500–5,000 m) have also been demonstrated to have good reliability (coefficient of variation (CV) = 0.8–3.3%; Hodges et al., 2006; Laursen et al., 2007). Data to assess the reliability of the exercise performance assessments used in this thesis were not collected. Therefore, whether the tests used provide similar results from day-to-day, enabling small differences in performance to be detected, is unproven in this thesis and is recognised as a limitation (Currell and Jeukendrup, 2008).

6.6 Direct or indirect effects of vitamin D on exercise performance?

In **Chapter 5**, vitamin D supplementation achieved EFSA and IOM defined vitamin D sufficiency in almost all participants; and more than half also achieved proposed optimal vitamin D status (Thomas et al., 2016; Holick et al., 2011). Despite this, there was no beneficial effect on exercise performance, suggesting vitamin D does not directly affect performance. Notably, the positive relationships observed between 25(OH)D and endurance performance in **Chapter 3** remained after removing those with 25(OH)D ≥75, or <30 $nmol \cdot L^{-1}$; suggesting achieving high or avoiding low 25(OH)D is unnecessary for optimal performance. Neither strength nor power were influenced by vitamin D in **Chapter 3 or 5**, supporting an absence of a beneficial effect of vitamin D on exercise performance.

The positive association observed in this thesis between vitamin D status and endurance exercise performance may exist because 25(OH)D acts indirectly to benefit performance; however, the mechanism responsible for such an indirect effect is not clear. Vitamin D could be indirectly associated with endurance performance because physically active individuals with higher 25(OH)D have been shown to miss fewer training days due to injury or illness (Laaksi et al., 2007; He et al., 2013). However, this hypothesis was not supported by **Chapter 3** because the association between vitamin D and endurance performance remained at follow-up, after controlling for incomplete training days due to illness or injury. Unfortunately, the study in **Chapter 5** did not have an adequate sample size to investigate the effect of vitamin D supplementation on the incidence of illness or injury, and missed training. Alternatively, the positive association between vitamin D status and endurance performance in **Chapter 3** may be explained by reverse causation: individuals with greater long-term physical activity are more likely to have greater aerobic fitness, spend more time outdoors exposed to sunlight and, in-turn, have higher serum 25(OH)D. Therefore, vitamin D status

may simply represent a biomarker for general health and fitness. If 25(OH)D concentration in **Chapter 3** was reflective of an individual's long-term vitamin D status, it may be long-term vitamin D sufficiency is necessary for optimal endurance performance. Therefore, 12-weeks of supplementation in **Chapter 5** may have been an inadequate duration to benefit performance**.** In accordance with this notion, an extended period of vitamin D supplementation improved skeletal muscle remodelling in untrained, young males during a progressive resistance training programme (Agergaard et al., 2015). However, the reported benefits of longer-term vitamin D supplementation on skeletal muscle remodelling did not translate to improved muscular strength (Agergaard et al., 2015).

6.7 Perspectives, practical implications, and future directions

This thesis has demonstrated that vitamin D insufficiency (serum 25(OH)D <50 nmol·L^{-1}) is widespread among young otherwise healthy adults, principally during winter and spring. Therefore, the supplementation strategies used in **Chapters 4 and 5** may be beneficial for most white adults living in the UK. Only white-skinned individuals were studied in **Chapters 4 and 5**, hence the conclusions from these studies cannot be applied to individuals with darker sun-reactive skin types. Correcting vitamin D insufficiency could be useful for active young adults, including athletes and military personnel, by increasing their training availability: potentially via maintenance of immune function and increasing their resistance against respiratory tract infections (He et al., 2016); reducing risk of injury (Lappe et al., 2008); or enhancing recovery following muscle or ligament damage (Owens et al., 2015; Barker et al., 2011). Adequately powered, randomised controlled trials are required to definitively confirm or reject these possible benefits. Monitoring markers of vitamin D intoxication (hypercalcaemia and hypercalciuria) in future supplementation studies is recommended. A placebo version of simulated sunlight was successfully developed, piloted

and used for the first time in this thesis, and could be used in future investigations. Studies are also required to further our understanding of how genetic variation between individuals (for example in vitamin D binding protein; Owens et al., 2018) might affect health and exercise performance outcomes in response to vitamin D supplementation.

In **Chapter 3**, serum 25(OH)D and 24,25$(OH)_2$D were positively associated with endurance exercise performance, but not muscle strength or power. The magnitude of the associations between vitamin D and endurance performance were relatively small (Cohen, 1988). Restoring and then maintaining vitamin D sufficiency from its winter nadir did not improve exercise performance; suggesting vitamin D does not directly affect exercise performance. The relatively small increase in 1,25$(OH)_2$D **(Figure 5.3B)** could conceivably account for the absence of a beneficial effect on exercise performance. Whether larger vitamin D doses, achieving greater than normal seasonal changes in 25(OH)D (for example >100 nmol·L^{-1}), would benefit exercise performance remains unclear. Although this may appear to be the next logical step in supplementation studies, larger vitamin D doses may be ineffective because this has been shown to increase serum 24,25$(OH)_2$D, which may impair VDR–1,25$(OH)_2$D mediated adaptations beneficial for exercise performance (Owens et al., 2017; Owens et al., 2018). Moreover, higher doses of simulated sunlight, and oral vitamin D_3 in excess of tolerable upper intakes (4,000 IU·day^{-1}) risk skin damage and vitamin D toxicity, respectively (Advisory Group on Non-ionising Radiation, 2017; Institute of Medicine, 2011).

Rather than restoring vitamin D sufficiency from its winter nadir as in this thesis, studies should investigate the effect of preventing a decline in end of summer serum 25(OH)D by commencing vitamin D supplementation in late summer or early autumn and continuing until spring (~6-months), when ambient UVB returns as a source of vitamin D. The

supplementation protocols used in the maintenance phase of **Chapter 5** could be used for the purpose of maintaining end of summer vitamin D sufficiency. Studies should also aim to elucidate potential mechanisms whereby vitamin D may affect exercise performance, such as via improved endothelial function (reviewed in **Chapter 1; Figure 1.2**). Although notably, a recent meta-analysis found no evidence of benefit of vitamin D supplementation on a range of markers of vascular function (Beveridge et al., 2018). Exposure to simulated summer sunlight using an irradiation cabinet can be used to safely restore and then maintain vitamin D sufficiency. Whether UVR exposure of the skin has additional benefits independent of the synthesis of vitamin D remains to be seen, but no beneficial effects on exercise performance or resting blood pressure were observed in this thesis. Two advantages of using simulated sunlight *vs.* blanket oral vitamin D_3 supplementation are, first, the dose received is proportional to the participants' body size; and second vitamin D intoxication is not possible. However all things considered, oral vitamin D_3 supplementation presents a more practical means of increasing serum 25(OH)D, because there is no time burden for an individual; and bulky irradiation cabinets and regular calibrations are not required. Oral vitamin D_3 supplementation can also be used in all regardless of skin pigmentation.

Serum 25(OH)D concentration is widely accepted as the best available indicator of vitamin D status, reflecting contributions from cutaneous synthesis and diet (Institute of Medicine, 2011); yet the relationship between tissue and serum concentrations of vitamin D metabolites have not been evaluated. For example, it is uncertain how well the concentration of serum 25(OH)D represents tissue exposure to biologically active $1,25(OH)_2D$ (Davis, 2008). Future studies should assess the concentration of vitamin D metabolites in local tissues (for example skeletal muscle; Wagner et al., 2012); investigate their association with exercise performance; and study the effect of vitamin D supplementation.

Most circulating 25(OH)D and 1,25$(OH)_2$D are bound to vitamin D binding protein (80–90%) and albumin (10–20%); however a small fraction remains unbound (0.02–0.05% of 25(OH)D; 0.2–0.6% of 1,25$(OH)_2$D; Zerwekh, 2008). It has been suggested that differentiating between total and unbound vitamin D may be insightful because only unbound metabolites are available to act on target cells (Shieh et al., 2017; Owens et al., 2018). Health outcomes such as bone mineral density reportedly relate more closely to unbound vitamin D than total circulating 25(OH)D (Allison et al., 2018). Therefore, serum unbound or 'bioavailable' vitamin D metabolites may have a better association with exercise performance than total serum metabolites. Future investigations should investigate this hypothesis by quantifying the concentration of unbound vitamin D metabolites.

6.8 Conclusions

The major conclusions from this thesis are:

1. Vitamin D insufficiency was widespread among 967 military recruits, with 80% of these otherwise healthy men and women vitamin D insufficient during winter. A seasonal variation was observed in three major vitamin D metabolites, with 25(OH)D, 24,25$(OH)_2$D and 1,25$(OH)_2$D lowest during winter, and highest during summer.

2. In young, healthy male and female military recruits, both serum 25(OH)D and 24,25$(OH)_2$D were positively associated with endurance exercise performance, but not muscle strength or power, after controlling for confounding variables, including body composition, smoking and season. Serum 1,25$(OH)_2$D was not associated with exercise performance.

3. Four weeks of simulated sunlight (1.3 SED, 3x·week^{-1}) in accordance with recommendations on safe sunlight exposure, and oral vitamin D_3 supplementation (1,000 IU·day^{-1}) were both effective strategies to correct wintertime vitamin D insufficiency. Continued simulated sunlight (1.3 SED, 1x·week^{-1}) or oral vitamin D_3 supplementation (400 IU·day^{-1}) were necessary to maintain vitamin D sufficiency for a further 8-weeks, until the availability of ambient UVB increased during spring.

4. Vitamin D supplementation using safe simulated sunlight, or oral vitamin D_3 among white male military recruits restored serum 25(OH)D from winter to typical summer concentrations in 4-weeks and then maintained EFSA and IOM defined vitamin D sufficiency for a further 8-weeks, but did not affect exercise performance or resting blood pressure. These findings suggest vitamin D does not directly affect exercise performance.

5. The positive association between vitamin D status and endurance performance may exist because individuals with greater long-term physical activity are more likely to have higher aerobic fitness, spend more time outdoors exposed to sunlight and, in-turn, have higher 25(OH)D. Therefore, vitamin D status may simply represent a biomarker for general health and fitness.

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Appendix A: Medical questionnaire (Chapter 4)

Bangor University
SCHOOL OF SPORT, HEALTH AND EXERCISE SCIENCES

Name of Participant ……..……

Age ……

Email address ……..…..@bangor.ac.uk Mobile number ……...

Are you in good health? ☐ YES ☐ NO

If no, please explain

How would you describe your present level of activity?
Tick intensity level and indicate approximate duration.

Vigorous		Moderate		Low intensity	

Duration (minutes)…….

How often?

< Once per month		2-3 times per week	
Once per month		4-5 times per week	
Once per week		> 5 times per week	

Have you ever suffered from a serious illness or accident? ☐ YES ☐ NO

If yes, please give particulars:

Do you suffer from allergies? ☐ YES ☐ NO

If yes, please give particulars:

Do you suffer, or have you ever suffered from:

	YES	NO		YES	NO
Asthma			Epilepsy		
Diabetes			High blood pressure		
Bronchitis			Skin cancer		
Photosensitivity			Systemic lupus erythematosus		

Appendix A: Medical questionnaire (Chapter 4)

Are you currently taking medication? ☐ YES ☐ NO

If yes, please give particulars:

Are you currently taking a vitamin D supplement? ☐ YES ☐ NO

If yes, please give particulars:

Are you currently taking any multivitamins? ☐ YES ☐ NO

If yes, please give particulars:

Are you currently taking any fish oil supplements? ☐ YES ☐ NO

If yes, please give particulars:

Are you pregnant or currently breastfeeding? ☐ YES ☐ NO

If yes, please give particulars:

Have you used a sunbed or travelled to a sunny climate in the past 3 months? ☐ YES ☐ NO

If yes, please give particulars:

Do you plan to travel to a sunny climate in the next 3 months? ☐ YES ☐ NO

If yes, please give particulars:

Are you currently attending your GP for any condition or have you consulted your doctor in the last three months? ☐ YES ☐ NO
If yes, please give particulars:

Have you, or are you presently taking part in any other laboratory experiment?

Appendix A: Medical questionnaire (Chapter 4)

If yes, please give particulars: ☐ YES ☐ NO

PLEASE READ THE FOLLOWING CAREFULLY

Persons will be considered unfit to participate in experimental procedures if they:

- have a fever
- have chronic or acute symptoms of gastrointestinal bacterial infections (e.g. Dysentery, Salmonella);
- have a history of infectious diseases (e.g. HIV, Hepatitis B)

PLEASE COMPLETE AND SIGN THE DECLARATION BELOW

DECLARATION

I agree that I have none of the above conditions and I hereby volunteer to be a participant in experiments/investigations during the period of February to May 2015.

My replies to the above questions are correct to the best of my belief and I understand that they will be treated with the strictest confidence. The experimenter has explained to my satisfaction the purpose of the experiment and possible risks involved.

I understand that I may withdraw from the experiment at any time and that I am under no obligation to give reasons for withdrawal or to attend again for experimentation.

Furthermore, if I am a student, I am aware that taking part or not taking part in this experiment, will neither be detrimental to, or further, my position as a student.

I undertake to obey the laboratory/study regulations and the instructions of the experimenter regarding safety, subject only to my right to withdraw declared above.

Signature *(participant)* …….. Date ……..

Print name ……..

Signature *(experimenter)* ……. Date ……..

Print name ……..…...

Appendix B: Informed consent (Chapter 4)

Bangor University
SCHOOL OF SPORT, HEALTH AND EXERCISE SCIENCES

Title of Research Project: ……

Please tick boxes

1 I confirm that I have read and understand the Information Sheet dated …… for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2 (i) Patients: ☐

I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason, without my medical care or legal rights being affected.

(ii) Students: ☐

I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason. If I do decide to withdraw I understand that it will have no influence on the marks I receive, the outcome of my period of study, or my standing with my supervisor or with other staff members of the School.

(iii) General members of the public: ☐

I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason.

3 I understand that I may register any complaint I might have about this experiment with Professor Tim Woodman, Head of School of Sport, Health and Exercise Sciences, and that I will be offered the opportunity of providing feedback on the experiment using the standard report forms. ☐

4 I agree to take part in the above study. ☐

Name of Participant …….

Signature …… Date ……..

Name of Person taking consent…….

Signature …… Date ……..

WHEN COMPLETED – ONE COPY TO PARTICIPANT, ONE COPY TO RESEARCHER FILE

Appendix C: Informed consent (Chapters 3 and 5)

CONSENT FORM FOR PARTICIPANTS IN RESEARCH STUDIES

Title of Study:

Ministry of Defence Research Ethics Committee Reference:

Please initial each box

- **The nature, aims and risks of the research have been explained to me. I have read and understood the Information for Participants and understand what is expected of me. All my questions have been answered fully to my satisfaction.** ☐
- **I understand that if I decide at any time during the research that I no longer wish to participate in this project, I can notify the researchers involved and be withdrawn from it immediately without having to give a reason. I also understand that I may be withdrawn from it at any time, and that in neither case will this be held against me in subsequent dealings with the Ministry of Defence.** ☐
- **I understand that the lifestyle and dietary process to decide if I am suitable to be selected as a participant may include completing a medical lifestyle and dietary questionnaire and/or a physical examination by a medical officer and I consent to this.** ☐
- **I consent to the processing of my personal information for the purposes of this research study. I understand that such information will be treated as strictly confidential and handled in accordance with the provisions of the Data Protection Act 1998.** ☐
- **I agree to volunteer as a participant for the study described in the information sheet and give full consent.** ☐
- **This consent is specific to the particular study described in the Information for Participants attached and shall not be taken to imply my consent to participate in any subsequent study or deviation from that detailed here.** ☐
- **I understand that in the event of my sustaining injury, illness or death as a direct result of participating as a volunteer in Ministry of Defence research, I or my dependants may enter a claim with the Ministry of Defence for compensation under the provisions of the no-fault compensation scheme, details of which are attached.** ☐

Participant's Statement:

I _____

agree that the research project named above has been explained to me to my satisfaction and I agree to take part in the study. I have read both the notes written above and the Information for Participants about the project, and understand what the research study involves.

Signed **Date**

Witness **Name**

Signature

Appendix D: Food frequency questionnaire

Protect - Personal (when completed)

ARTD DIETARY QUESTIONNAIRE 410/MODREC/13

These questions are designed to estimate the amount of vitamin D, calcium, iron and vitamin C you get from the foods and drinks you usually consume. They are not to check if you have a healthy diet, or to criticise what you eat.

How do I fill in the questionnaire?

To indicate your response place a **CROSS** in the box e.g. [X]

To cancel a response fill in the box *e.g.* ■ and record the new response with a cross.

If you have any questions please ask.

Thank you.

Personal Details

Q1 **Today's Date:** D D / M M / Y Y Y Y

Q2 **UIN:**

Q3 **Surname**

Protect - Personal (when completed)

Protect - Personal (when completed)

Dietary Habits 410/MODREC/13

Indicate your response with a cross in the box *e.g.* [X]

Q4 **How often do you eat the following breakfast cereal?**

	Never	1 time / month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	1 time / day	2-3 times / day	4 times / day or more
Cornflakes	☐	☐	☐	☐	☐	☐	☐	☐	☐
Rice krispies	☐	☐	☐	☐	☐	☐	☐	☐	☐
All-Bran	☐	☐	☐	☐	☐	☐	☐	☐	☐
Weetabix	☐	☐	☐	☐	☐	☐	☐	☐	☐
Shredded Wheat	☐	☐	☐	☐	☐	☐	☐	☐	☐
Branflakes	☐	☐	☐	☐	☐	☐	☐	☐	☐
Special K	☐	☐	☐	☐	☐	☐	☐	☐	☐
Porridge made with water	☐	☐	☐	☐	☐	☐	☐	☐	☐
Porridge made with milk and water	☐	☐	☐	☐	☐	☐	☐	☐	☐
Porridge made with whole milk	☐	☐	☐	☐	☐	☐	☐	☐	☐
Muesli	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other cereals (specify in Q6)	☐	☐	☐	☐	☐	☐	☐	☐	☐

Q5 **Typically, how much breakfast cereal would you eat each time?**

Please use the food atlas to help you choose the portion size you usually eat

	A	B	C
Cornflakes	☐	☐	☐
Rice krispies	☐	☐	☐
All-Bran	☐	☐	☐
Weetabix	☐	☐	☐
Shredded Wheat	☐	☐	☐
Branflakes	☐	☐	☐
Special K	☐	☐	☐
Porridge made with water	☐	☐	☐
Porridge made with milk and water	☐	☐	☐
Porridge made with whole milk	☐	☐	☐
Muesli	☐	☐	☐

Q6 **Other cereals (please specify type and amount, *e.g.* Cheerios, same as photo B for rice krispies):**

Q7 **Which type of milk do you usually have with your cereal?**

Please tick only one box to indicate the milk you typically have.

None	☐
Whole (full fat)	☐
Semi-skimmed (half-fat)	☐
Skimmed (fat free)	☐
Fortified	☐
Soya (fortified/ non-fortified)	☐

Q8 **Typically, how much milk would you:**

	A	B	C
Have on your cereal each time?	☐	☐	☐

Protect - Personal (when completed)

Protect - Personal (when completed)

Dietary Habits 410/MODREC/13

Indicate your response with a cross in the box *e.g.* X

Q9 How often do you eat bread, wraps and/or rolls?

	Never	1 time / month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	1 time / day	2-3 times / day	4 times / day or more
White/ white softgrain bread	☐	☐	☐	☐	☐	☐	☐	☐	☐
Brown bread	☐	☐	☐	☐	☐	☐	☐	☐	☐
Wholemeal bread	☐	☐	☐	☐	☐	☐	☐	☐	☐
Granary bread	☐	☐	☐	☐	☐	☐	☐	☐	☐
Rye bread	☐	☐	☐	☐	☐	☐	☐	☐	☐
White roll	☐	☐	☐	☐	☐	☐	☐	☐	☐
Brown roll	☐	☐	☐	☐	☐	☐	☐	☐	☐
Wholemeal roll	☐	☐	☐	☐	☐	☐	☐	☐	☐
Granary roll	☐	☐	☐	☐	☐	☐	☐	☐	☐
Bagel	☐	☐	☐	☐	☐	☐	☐	☐	☐
White Pitta bread	☐	☐	☐	☐	☐	☐	☐	☐	☐
Wholemeal Pitta bread	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ciabatta	☐	☐	☐	☐	☐	☐	☐	☐	☐
French stick	☐	☐	☐	☐	☐	☐	☐	☐	☐
Garlic bread	☐	☐	☐	☐	☐	☐	☐	☐	☐
Tortilla	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chapatti	☐	☐	☐	☐	☐	☐	☐	☐	☐
Poppadum	☐	☐	☐	☐	☐	☐	☐	☐	☐

Q10 Typically, how much bread, wraps and/or rolls would you eat each time?

Please use the food atlas to help you choose the portion size you usually eat

	A	B	C
White/ white softgrain bread	☐	☐	☐
Brown bread	☐	☐	☐
Wholemeal bread	☐	☐	☐
Granary bread	☐	☐	☐
Rye bread	☐	☐	☐
White roll	☐	☐	☐
Brown roll	☐	☐	☐
Wholemeal roll	☐	☐	☐
Granary roll	☐	☐	☐
Bagel	☐	☐	☐
White Pitta bread	☐	☐	☐
Wholemeal Pitta bread	☐	☐	☐
Ciabatta	☐	☐	☐
French stick	☐	☐	■
Garlic bread	☐	☐	☐
Tortilla	☐	☐	☐
Chapatti	☐	☐	☐
Poppadum	☐	☐	☐

Protect - Personal (when completed)

Protect - Personal (when completed)

Dietary Habits 410/MODREC/13

Indicate your response with a cross in the box *e.g.* X

Q11 How often do you usually eat the following chocolate, biscuits and/or cakes?

	Never	1 time / month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	1 time / day	2-3 times / day	4 times / day or more
Chocolate covered bar with fruit/ nuts	☐	☐	☐	☐	☐	☐	☐	☐	☐
Milk chocolate	☐	☐	☐	☐	☐	☐	☐	☐	☐
Dark chocolate	☐	☐	☐	☐	☐	☐	☐	☐	☐
Plain digestive biscuits	☐	☐	☐	☐	☐	☐	☐	☐	☐
Shortbread biscuits	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chocolate covered biscuits	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chocolate cake	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sponge cake with/ without cream	☐	☐	☐	☐	☐	☐	☐	☐	☐
Madeira cake/ muffins	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fruit cake	☐	☐	☐	☐	☐	☐	☐	☐	☐
Malt loaf	☐	☐	☐	☐	☐	☐	☐	☐	☐
Carrot cake	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other biscuits/ cakes (Specify in Q13)	☐	☐	☐	☐	☐	☐	☐	☐	☐

Q12 Typically, how much chocolate and how many biscuits and/or cakes would you have each time?

Please use the food atlas to help you choose the portion size you usually eat

	A	B	C
Chocolate covered bar with fruit/ nuts	☐	☐	☐
Milk chocolate	☐	☐	☐
Dark chocolate	☐	☐	☐
Plain digestive biscuits	☐	☐	☐
Shortbread biscuits	☐	☐	☐
Chocolate covered biscuits	☐	☐	☐
Chocolate cake	☐	☐	☐
Sponge cake with/ without cream	☐	☐	☐
Madeira cake/ muffins	☐	☐	☐
Fruit cake	☐	☐	☐
Malt loaf	☐	☐	☐
Carrot cake	☐	☐	☐

Q13 Other biscuits/ cakes (please specify type and amount, *e.g.* Hobnobs x 2, one small slice of lemon drizzle cake):

Protect - Personal (when completed)

Protect - Personal (when completed)

Dietary Habits **410/MODREC/13**

Indicate your response with a cross in the box *e.g.* [X]

Q14 **How often do you eat the following meat and/or poultry?**

	Never	1 time / month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	1 time / day	2-3 times / day	4 times / day or more
Bacon	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ham	☐	☐	☐	☐	☐	☐	☐	☐	☐
Roast beef	☐	☐	☐	☐	☐	☐	☐	☐	☐
Roast lamb	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pork chop	☐	☐	☐	☐	☐	☐	☐	☐	☐
Roast chicken	☐	☐	☐	☐	☐	☐	☐	☐	☐
Liver/ kidney stew with gravy	☐	☐	☐	☐	☐	☐	☐	☐	☐
Beefburger	☐	☐	☐	☐	☐	☐	☐	☐	☐
Black pudding	☐	☐	☐	☐	☐	☐	☐	☐	☐
Corned beef	☐	☐	☐	☐	☐	☐	☐	☐	☐
Liver pate	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sausages	☐	☐	☐	☐	☐	☐	☐	☐	☐
Beef/ lamb stew with veg and gravy	☐	☐	☐	☐	☐	☐	☐	☐	☐
Beef/ lamb curry	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chicken curry/ casserole	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lasagne	☐	☐	☐	☐	☐	☐	☐	☐	☐
Bolognese sauce	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other meat (specify in Q16)	☐	☐	☐	☐	☐	☐	☐	☐	☐

Q15 **Typically, how much meat and/or poultry would you eat each time?**

Please use the food atlas to help you choose the portion size you usually eat

	A	B	C
Bacon	☐	☐	☐
Ham	☐	☐	☐
Roast beef	☐	☐	☐
Roast lamb	☐	☐	☐
Pork chop	☐	☐	☐
Roast chicken	☐	☐	☐
Liver/ kidney stew with gravy	☐	☐	☐
Beefburger	☐	☐	☐
Black pudding	☐	☐	☐
Corned beef	☐	☐	☐
Liver pate	☐	☐	☐
Sausages	☐	☐	☐
Beef/ lamb stew with veg and gravy	☐	☐	☐
Beef/ lamb curry	☐	☐	☐
Chicken curry/ casserole	☐	☐	☐
Lasagne	☐	☐	☐
Bolognese sauce	☐	☐	☐

Q16 **Other meat (please specify type and amount, *e.g.* chilli con carne, same as photo B for beef/lamb curry):**

Protect - Personal (when completed)

Protect - Personal (when completed)

Dietary Habits **410/MODREC/13**

Indicate your response with a cross in the box *e.g.* [X]

Q17 **How often do you eat the following fish and/ or shellfish?**

	Never	1 time / month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	1 time / day	2-3 times / day	4 times / day or more
Fish fingers/ deep-fried fish	☐	☐	☐	☐	☐	☐	☐	☐	☐
Plaice/ Cod/ Haddock	☐	☐	☐	☐	☐	☐	☐	☐	☐
Salmon/ Trout	☐	☐	☐	☐	☐	☐	☐	☐	☐
Tinned salmon	☐	☐	☐	☐	☐	☐	☐	☐	☐
Mackerel	☐	☐	☐	☐	☐	☐	☐	☐	☐
Tinned tuna in water/ oil	☐	☐	☐	☐	☐	☐	☐	☐	☐
Tinned sardine in water/ oil/ tomato	☐	☐	☐	☐	☐	☐	☐	☐	☐
Prawns	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other fish (specify in Q19)	☐	☐	☐	☐	☐	☐	☐	☐	☐

Q18 **Typically, how much fish and/ or shellfish would you eat each time?**

Please use the food atlas to help you choose the portion size you usually eat

	A	B	C
Fish fingers/ deep-fried fish	☐	☐	☐
Plaice/ Cod/ Haddock	☐	☐	☐
Salmon/ Trout	☐	☐	☐
Tinned salmon	☐	☐	☐
Mackerel	☐	☐	☐
Tinned tuna in water/ oil	☐	☐	☐
Tinned sardine in water/ oil/ tomato	☐	☐	☐
Prawns	☐	☐	☐

Q19 **Other fish (please specify type and amount, *e.g.* red snapper, same as photo B for salmon/trout):**

Q20 **How often do you usually eat pasta and/or rice?**

	Never	1 time / month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	1 time / day	2-3 times / day	4 times / day or more
White rice	☐	☐	☐	☐	☐	☐	☐	☐	☐
Wholegrain rice/ brown rice/ wild rice	☐	☐	☐	☐	☐	☐	☐	☐	☐
White pasta	☐	☐	☐	☐	☐	☐	☐	☐	☐
Wholewheat pasta	☐	☐	☐	☐	☐	☐	☐	☐	☐
White spaghetti	☐	☐	☐	☐	☐	☐	☐	☐	☐
Wholewheat spaghetti	☐	☐	☐	☐	☐	☐	☐	☐	☐

Q21 **Typically, how much pasta and/or rice would you eat each time?**

Please use the food atlas to help you choose the portion size you usually eat

	A	B	C
White rice	☐	☐	☐
Wholegrain rice/ brown rice/ wild rice	☐	☐	☐
White pasta	☐	☐	☐
Wholewheat pasta	☐	☐	☐
White spaghetti	☐	☐	☐
Wholewheat spaghetti	☐	☐	☐

Protect - Personal (when completed)

Protect - Personal (when completed)

Dietary Habits **410/MODREC/13**

Indicate your response with a cross in the box *e.g.* X

Q22 How often do you eat the following fruit and vegetables?

	Never	1 time / month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	1 time / day	2-3 times / day	4 times / day or more
Broccoli	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cabbage	☐	☐	☐	☐	☐	☐	☐	☐	☐
Spinach	☐	☐	☐	☐	☐	☐	☐	☐	☐
Peppers	☐	☐	☐	☐	☐	☐	☐	☐	☐
Watercress	☐	☐	☐	☐	☐	☐	☐	☐	☐
Tomatoes	☐	☐	☐	☐	☐	☐	☐	☐	☐
Oranges/ clementines/ satsumas/ mandarins	☐	☐	☐	☐	☐	☐	☐	☐	☐
Strawberries	☐	☐	☐	☐	☐	☐	☐	☐	☐
Kiwi fruit	☐	☐	☐	☐	☐	☐	☐	☐	☐
Dried apricots	☐	☐	☐	☐	☐	☐	☐	☐	☐
Raisins/ sultanas	☐	☐	☐	☐	☐	☐	☐	☐	☐
Dates	☐	☐	☐	☐	☐	☐	☐	☐	☐

Q23 Typically, how much fruit and vegetables would you eat each time?

Please use the food atlas to help you choose the portion size you usually eat

	A	B	C
Broccoli	☐	☐	☐
Cabbage	☐	☐	☐
Spinach	☐	☐	☐
Peppers	☐	☐	☐
Watercress	☐	☐	☐
Tomatoes	☐	☐	☐
Oranges/ clementines/ satsumas/ mandarins	☐	☐	☐
Strawberries	☐	☐	☐
Kiwi fruit	☐	☐	☐
Dried apricots	☐	☐	☐
Raisins/ sultanas	☐	☐	☐
Dates	☐	☐	☐

Q24 How often do you eat the following beans and nuts?

	Never	1 time / month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	1 time / day	2-3 times / day	4 times / day or more
Baked beans	☐	☐	☐	☐	☐	☐	☐	☐	☐
Kidney beans	☐	☐	☐	☐	☐	☐	☐	☐	☐
Almonds	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cashew nuts	☐	☐	☐	☐	☐	☐	☐	☐	☐
Peanuts	☐	☐	☐	☐	☐	☐	☐	☐	☐

Q25 Typically, how much beans and nuts would you eat each time?

Please use the food atlas to help you choose the portion size you usually eat

	A	B	C
Baked beans	☐	☐	☐
Kidney beans	☐	☐	☐
Almonds	☐	☐	☐
Cashew nuts	☐	☐	☐
Peanuts	☐	☐	☐

Protect - Personal (when completed)

Protect - Personal (when completed)

Dietary Habits **410/MODREC/13**

Indicate your response with a cross in the box *e.g.* X

Q26 How often do you eat eggs?

	Never	1 time / month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	1 time / day	2-3 times / day	4 times / day or more
Scrambled eggs	☐	☐	☐	☐	☐	☐	☐	☐	☐
Boiled eggs	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fried eggs	☐	☐	☐	☐	☐	☐	☐	☐	☐
Poached eggs	☐	☐	☐	☐	☐	☐	☐	☐	☐

Q27 Typically, how many eggs would you eat each time?

Please use the food atlas to help you choose the portion size you usually eat

	A	B	C
Scrambled eggs	☐	☐	☐
Boiled eggs	☐	☐	☐
Fried eggs	☐	☐	☐
Poached eggs	☐	☐	☐

Q28 How often do you eat cheese?

	Never	1 time / month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	1 time / day	2-3 times / day	4 times / day or more
Cheddar cheese	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cheddar type cheese, low fat	☐	☐	☐	☐	☐	☐	☐	☐	☐
Processed cheese, plain	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cheese spread, plain	☐	☐	☐	☐	☐	☐	☐	☐	☐
Full fat/ reduced fat soft cheeses	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cottage cheese	☐	☐	☐	☐	☐	☐	☐	☐	☐
Soft cheese *e.g.* Brie	☐	☐	☐	☐	☐	☐	☐	☐	☐
Parmesan	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other cheeses (specify in Q30)	☐	☐	☐	☐	☐	☐	☐	☐	☐

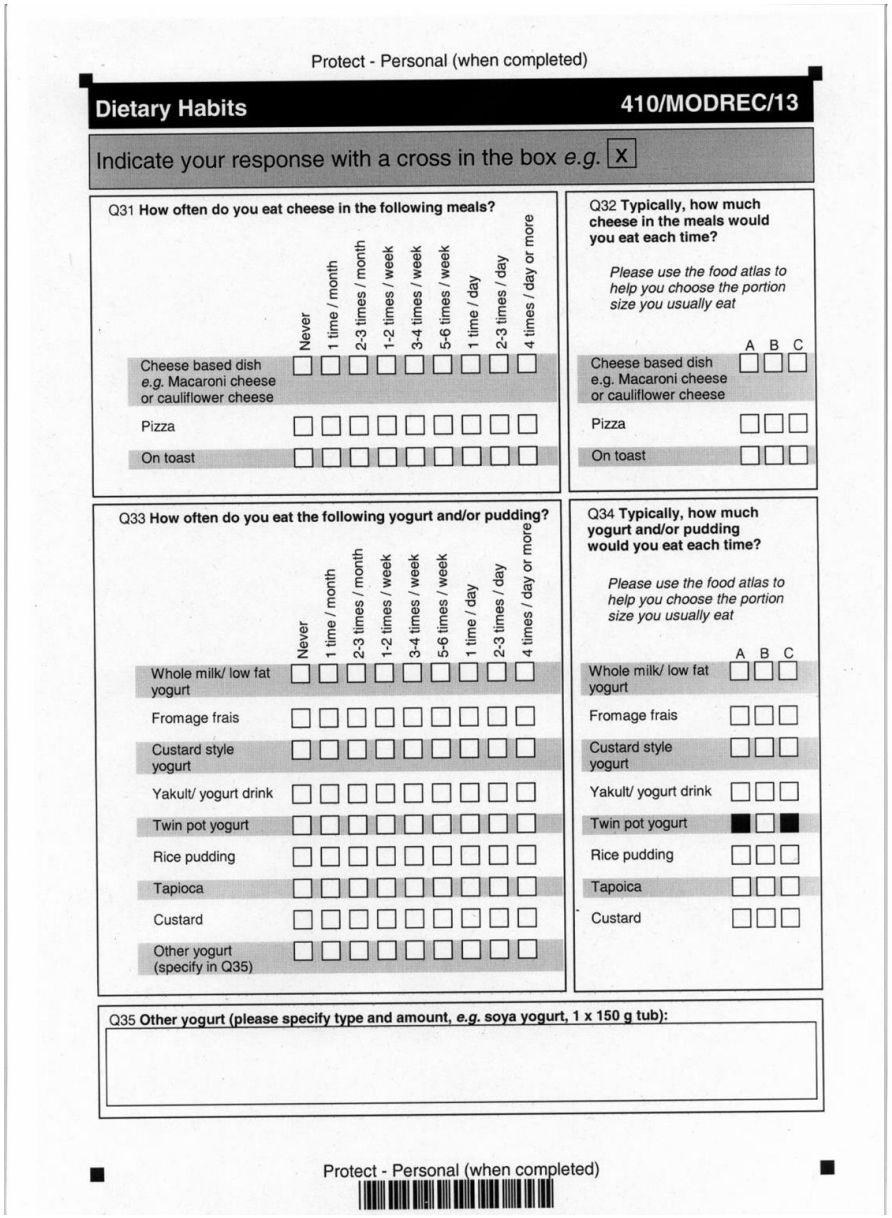
Q29 Typically, how much cheese would you eat each time?

Please use the food atlas to help you choose the portion size you usually eat

	A	B	C
Cheddar cheese	☐	☐	☐
Cheddar type cheese, low fat	☐	☐	☐
Processed cheese, plain	☐	☐	☐
Cheese spread, plain	☐	☐	☐
Full fat/ reduced fat soft cheeses	☐	☐	☐
Cottage cheese	☐	☐	☐
Soft cheese *e.g.* Brie	☐	☐	☐
Parmesan	☐	☐	☐

Q30 Other cheese (please specify type and amount, *e.g.* soya cheese, 2 slices):

Protect - Personal (when completed)



Protect - Personal (when completed)

Dietary Habits **410/MODREC/13**

Indicate your response with a cross in the box *e.g.* X

Q31 **How often do you eat cheese in the following meals?**

	Never	1 time / month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	1 time / day	2-3 times / day	4 times / day or more
Cheese based dish *e.g.* Macaroni cheese or cauliflower cheese	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pizza	☐	☐	☐	☐	☐	☐	☐	☐	☐
On toast	☐	☐	☐	☐	☐	☐	☐	☐	☐

Q32 **Typically, how much cheese in the meals would you eat each time?**

Please use the food atlas to help you choose the portion size you usually eat

	A	B	C
Cheese based dish e.g. Macaroni cheese or cauliflower cheese	☐	☐	☐
Pizza	☐	☐	☐
On toast	☐	☐	☐

Q33 **How often do you eat the following yogurt and/or pudding?**

	Never	1 time / month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	1 time / day	2-3 times / day	4 times / day or more
Whole milk/ low fat yogurt	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fromage frais	☐	☐	☐	☐	☐	☐	☐	☐	☐
Custard style yogurt	☐	☐	☐	☐	☐	☐	☐	☐	☐
Yakult/ yogurt drink	☐	☐	☐	☐	☐	☐	☐	☐	☐
Twin pot yogurt	☐	☐	☐	☐	☐	☐	☐	☐	☐
Rice pudding	☐	☐	☐	☐	☐	☐	☐	☐	☐
Tapioca	☐	☐	☐	☐	☐	☐	☐	☐	☐
Custard	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other yogurt (specify in Q35)	☐	☐	☐	☐	☐	☐	☐	☐	☐

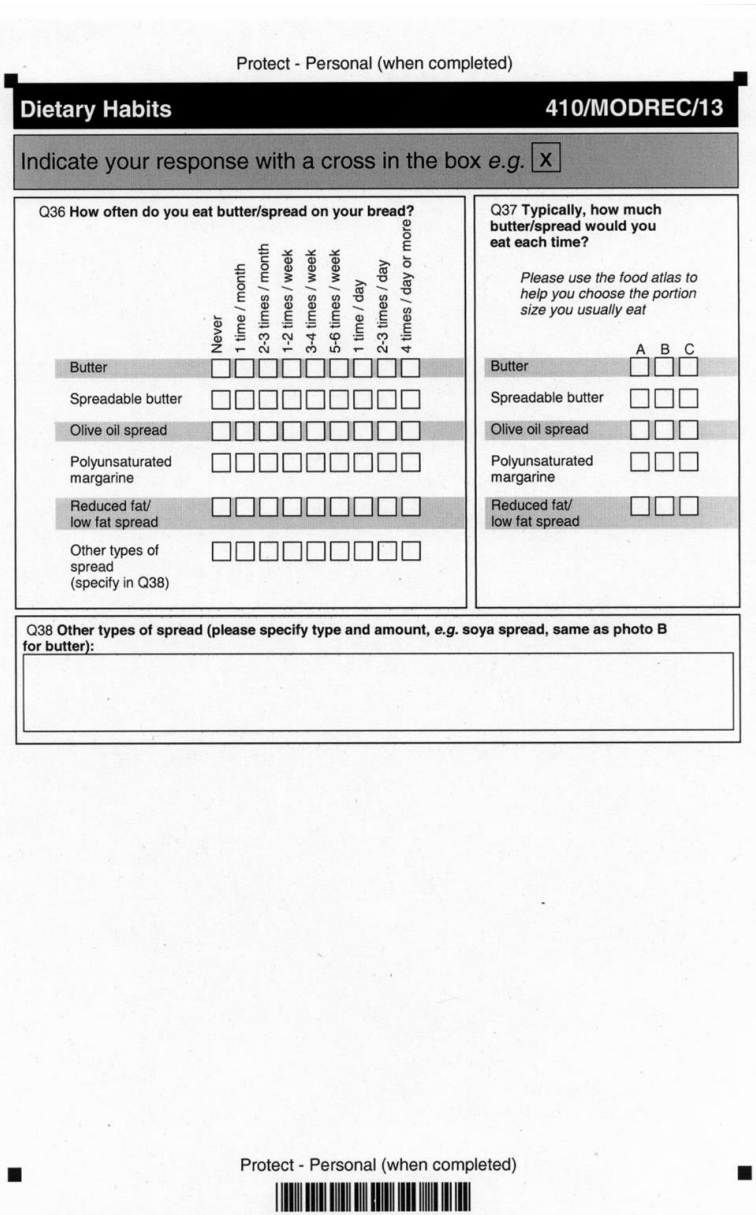
Q34 **Typically, how much yogurt and/or pudding would you eat each time?**

Please use the food atlas to help you choose the portion size you usually eat

	A	B	C
Whole milk/ low fat yogurt	☐	☐	☐
Fromage frais	☐	☐	☐
Custard style yogurt	☐	☐	☐
Yakult/ yogurt drink	☐	☐	☐
Twin pot yogurt	■	☐	■
Rice pudding	☐	☐	☐
Tapioca	☐	☐	☐
Custard	☐	☐	☐

Q35 **Other yogurt (please specify type and amount, *e.g.* soya yogurt, 1 x 150 g tub):**

Protect - Personal (when completed)



Protect - Personal (when completed)

Dietary Habits **410/MODREC/13**

Indicate your response with a cross in the box *e.g.* X

Q36 **How often do you eat butter/spread on your bread?**

	Never	1 time / month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	1 time / day	2-3 times / day	4 times / day or more
Butter	☐	☐	☐	☐	☐	☐	☐	☐	☐
Spreadable butter	☐	☐	☐	☐	☐	☐	☐	☐	☐
Olive oil spread	☐	☐	☐	☐	☐	☐	☐	☐	☐
Polyunsaturated margarine	☐	☐	☐	☐	☐	☐	☐	☐	☐
Reduced fat/ low fat spread	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other types of spread (specify in Q38)	☐	☐	☐	☐	☐	☐	☐	☐	☐

Q37 **Typically, how much butter/spread would you eat each time?**

Please use the food atlas to help you choose the portion size you usually eat

	A	B	C
Butter	☐	☐	☐
Spreadable butter	☐	☐	☐
Olive oil spread	☐	☐	☐
Polyunsaturated margarine	☐	☐	☐
Reduced fat/ low fat spread	☐	☐	☐

Q38 **Other types of spread (please specify type and amount, *e.g.* soya spread, same as photo B for butter):**

Protect - Personal (when completed)

Protect - Personal (when completed)

Dietary Habits — 410/MODREC/13

Indicate your response with a cross in the box *e.g.* X

Q39 How often do you:

	Never	1 time / month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	1 time / day	2-3 times / day	4 times / day or more
Drink tea:									
With whole milk	☐	☐	☐	☐	☐	☐	☐	☐	☐
With semi-skimmed milk	☐	☐	☐	☐	☐	☐	☐	☐	☐
With skimmed milk	☐	☐	☐	☐	☐	☐	☐	☐	☐
With fortified milk	☐	☐	☐	☐	☐	☐	☐	☐	☐
WIth soya milk	☐	☐	☐	☐	☐	☐	☐	☐	☐
Without milk	☐	☐	☐	☐	☐	☐	☐	☐	☐
Drink coffee:									
With whole milk	☐	☐	☐	☐	☐	☐	☐	☐	☐
With semi-skimmed milk	☐	☐	☐	☐	☐	☐	☐	☐	☐
With skimmed milk	☐	☐	☐	☐	☐	☐	☐	☐	☐
With fortified milk	☐	☐	☐	☐	☐	☐	☐	☐	☐
WIth soya milk	☐	☐	☐	☐	☐	☐	☐	☐	☐
Without milk	☐	☐	☐	☐	☐	☐	☐	☐	☐
Drink a glass (hot or cold) of:									
Whole milk	☐	☐	☐	☐	☐	☐	☐	☐	☐
Semi-skimmed milk	☐	☐	☐	☐	☐	☐	☐	☐	☐
Skimmed milk	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fortified milk	☐	☐	☐	☐	☐	☐	☐	☐	☐
Soya milk	☐	☐	☐	☐	☐	☐	☐	☐	☐
Drink a glass (hot or cold) of:									
Flavoured milk	☐	☐	☐	☐	☐	☐	☐	☐	☐
Have a milky coffee *e.g.* Latte, cappuccino made with:									
Whole milk	☐	☐	☐	☐	☐	☐	☐	☐	☐
Semi-skimmed milk	☐	☐	☐	☐	☐	☐	☐	☐	☐
Skimmed milk	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fortified milk	☐	☐	☐	☐	☐	☐	☐	☐	☐
Soya milk	☐	☐	☐	☐	☐	☐	☐	☐	☐

Q40 How much milk would you:

Please use the food atlas to help you choose the portion size you usually eat or drink

	A	B	C
Have in tea:			
With whole milk	☐	☐	☐
With semi-skimmed milk	☐	☐	☐
With skimmed milk	☐	☐	☐
With fortified milk	☐	☐	☐
With soya milk	☐	☐	☐
Have in coffee:			
With whole milk	☐	☐	☐
With semi-skimmed milk	☐	☐	☐
With skimmed milk	☐	☐	☐
With fortified milk	☐	☐	☐
With soya milk	☐	☐	☐
Drink as a glass (hot or cold) of:			
Whole milk	☐	☐	☐
Semi-skimmed milk	☐	☐	☐
Skimmed milk	☐	☐	☐
Fortified milk	☐	☐	☐
Soya milk	☐	☐	☐
Drink as a glass (hot or cold) of:			
Flavoured milk	☐	☐	■
Have in a milky coffee *e.g.* Latte, cappuccino:			
With whole milk	☐	☐	☐
With semi-skimmed milk	☐	☐	☐
With skimmed milk	☐	☐	☐
With fortified milk	☐	☐	☐
With soya milk	☐	☐	☐

Protect - Personal (when completed)

Protect - Personal (when completed)

Dietary Habits — 410/MODREC/13

Indicate your response with a cross in the box *e.g.* X

Q41 How often do you consume the following drinks?

	Never	1 time / month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	1 time / day	2-3 times / day	4 times / day or more
Orange juice	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fortified orange juice *e.g.* Tropicana Orange Juice with Calcium	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fizzy drinks *e.g.* cola, lemonade	☐	☐	☐	☐	☐	☐	☐	☐	☐
Energy drinks	☐	☐	☐	☐	☐	☐	☐	☐	☐
Wine	☐	☐	☐	☐	☐	☐	☐	☐	☐
Beer	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cider	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lager	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other drinks fortified with calcium and/or Vit D (specify in Q43)	☐	☐	☐	☐	☐	☐	☐	☐	☐

Q42 Typically, how much would you drink each time?

Please use the food atlas to help you choose the portion size you usually drink

	A	B	C
Orange juice	☐	☐	☐
Fortified orange juice *e.g.* Tropicana Orange Juice with Calcium	☐	☐	☐
Fizzy drinks *e.g.* cola, lemonade	☐	☐	☐
Energy drinks	☐	☐	☐
Wine	☐	☐	☐
Beer	☐	☐	☐
Cider	☐	☐	☐
Lager	☐	☐	☐

Q43 Other drinks/fortified drinks (please specify type and amount *e.g.* cranberry juice x 1, tumbler):

Protect - Personal (when completed)

Appendix D: Food frequency questionnaire

Protect - Personal (when completed)

Dietary Habits **410/MODREC/13**

Indicate your response with a cross in the box *e.g.* [X]

Q44 **If you take dietary supplements, please provide details below.**

	Brand name and strength	Amount usually taken per WEEK *e.g.* 7 tablets, 4 teaspoons
Multi-vitamin (*e.g.* Centrum, Solgar)		
Vitamin D and Calcium combined (*e.g.* Calcichew D3 Forte)		
Calcium alone (*e.g.* Os cal, Calcichew)		
Vitamin D alone		
Vitamin C alone		
Vitamin B12		
Iron		
Cod liver oil (either tablet or liquid)		

Thank you for completing the questionnaire.

Appendix E: Skin type questionnaire

SKIN TYPING QUESTIONNAIRE

1. If I was to go outside on a sunny June day in the UK and stay there for 40 minutes **without** sun cream, my skin will later:

   PLEASE CROSS ONE BOX ONLY

turn pink/red	
tan	
both of the above	
none of the above	

2. In general, when exposed to the sun in the UK:
   PLEASE CROSS ONE BOX ONLY

I virtually always **burn** and never/hardly ever **tan**	
I usually **burn** and **tan** lightly	
I sometimes **burn** and **tan** moderately	
I rarely **burn** and **tan** heavily	

3. I am:

   PLEASE CROSS ONE BOX ONLY

an easy burner	
an easy tanner	

4. Please include any additional information about your burning/tanning response below

   _____

   _____

PLEASE TURN OVER

INVESTIGATOR ONLY				
Fitzpatrick skin type:	**I**	**II**	**III**	**IV**

Appendix E: Skin type questionnaire

5. My natural hair colour is:

   PLEASE CROSS ONE BOX ONLY

red	
blond	
brown	
black	

6. My natural eye colour is:

   PLEASE CROSS ONE BOX ONLY

blue	
grey	
hazel	
brown	

7. i) I would describe my number of freckles as:
   PLEASE CROSS ONE BOX ONLY

none	
few	
moderate	
many	

   ii) My freckles only appear in the summer

agree	
disagree	