Is there an optimal vitamin D status for immunity in athletes and military personnel?

Cheng-Shiun He[1], Xin Hui Aw Yong[2], Neil P. Walsh[2] and Michael Gleeson[1]

[1] School of Sport, Exercise and Health Sciences, Loughborough University, United Kingdom.
[2] School of Sport, Health and Exercise Sciences, Bangor University, United Kingdom.

ABSTRACT

Vitamin D is mainly obtained through sunlight ultraviolet-B (UVB) exposure of the skin, with a small amount typically coming from the diet. It is now clear that vitamin D has important roles beyond its well-known effects on calcium and bone homeostasis. Immune cells express the vitamin D receptor, including antigen presenting cells, T cells and B cells, and these cells are all capable of synthesizing the biologically active vitamin D metabolite, 1, 25 dihydroxy vitamin D. There has been growing interest in the benefits of supplementing vitamin D as studies report vitamin D insufficiency (circulating 25(OH)D < 50 nmol/L) in more than half of all athletes and military personnel tested during the winter, when skin sunlight UVB is negligible. The overwhelming evidence supports avoiding vitamin D deficiency (25(OH)D < 30 nmol/L) to maintain immunity and prevent upper respiratory illness (URI) in athletes and military personnel. Recent evidence supports an optimal circulating 25(OH)D of 75 nmol/L to prevent URI and enhance innate immunity and mucosal immunity and bring about anti-inflammatory actions through the induction of regulatory T cells and the inhibition of pro-inflammatory cytokine production. We provide practical recommendations for how vitamin D sufficiency can be achieved in most individuals by safe sunlight exposure in the summer and daily 1, 000 IU vitamin D$_3$ supplementation in the winter. Studies are required in athletes and military personnel to determine the impact of these recommendations on immunity and URI; and, to demonstrate the purported benefit of achieving 25(OH)D > 75 nmol/L.

Keywords: Exercise; Immune; Infection; Cholecalciferol; Ergocalciferol

1. INTRODUCTION

Stress-induced immune dysregulation is widely acknowledged to have negative implications for health (48). Those working in the field of exercise immunology have shown us that individuals who undertake heavy physical exertion, particularly when combined with periods of psychological stress, nutritional inadequacy and sleep disruption (e.g. athletes and military personnel), risk compromising host defence and increasing their susceptibility to respiratory viral infections such as the common cold and possibly to other infectious microorganisms (58, 136, 137). In 1981, the British general practitioner and celebrated epidemiologist, R. Edgar Hope-Simpson was the first to hypothesise that respiratory viral infections (e.g. epidemic influenza) have a 'seasonal stimulus' intimately associated with solar radiation. He observed an increased incidence of respiratory viral infections during the winter that appeared to be more strongly related to the amount of solar radiation than the presence of anti-viral antibodies. The nature of this 'seasonal stimulus' remained undiscovered until the important immuno-modulatory effects of the sunlight-dependent secosteroid vitamin D were fully recognised (Figure 1) (24); indeed, vitamin D levels in the human body are known to fall to a nadir during the peak influenza season and peak when influenza is scarce (95).
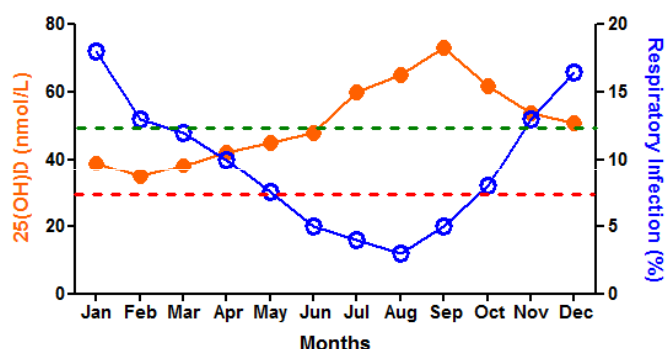

Figure 1. The association between circulating 25(OH)D (closed circles) and respiratory infection (open circles) in British adults. Adapted from Berry et al. (16). Upper dotted line indicates vitamin D sufficiency threshold (50 nmol/L) and lower dotted line indicates vitamin D deficiency threshold (30 nmol/L) as suggested by the Institute of Medicine (66).

Vitamin D refers to a group of fat-soluble secosteroids responsible for enhancing intestinal absorption of calcium, iron, magnesium, phosphate and zinc (63). In humans, vitamin D can be obtained either from sunlight exposure at the skin or in

Corresponding Author:
Prof. Neil P. Walsh FACSM, School of Sport, Health and Exercise Sciences, Bangor University, Bangor, LL57 2PZ, UK.
Email: n.walsh@bangor.ac.uk, Telephone: + 44 1248 383480

the foods we eat and by consuming dietary supplements. Vitamin D production as a result of sunlight ultraviolet (UV) B radiation penetrating the skin typically provides 80-100% of the body's vitamin D requirements. In humans, the most important compounds in the vitamin D group are vitamin D_3 (also known as cholecalciferol) and vitamin D_2 (ergocalciferol). Both cholecalciferol and ergocalciferol can be ingested from the daily diet and from supplements. Unlike other fat- and water-soluble vitamins, the body can also synthesise vitamin D (specifically cholecalciferol) in the skin, from cholesterol, when exposure from sunlight UVB is adequate. Evidence indicates the synthesis of vitamin D from sunlight UVB exposure is regulated by a negative feedback loop that prevents toxicity, but because of uncertainty about the cancer risk from overexposure to sunlight, currently no recommendations are issued by national bodies regarding the amount of sunlight exposure required to meet vitamin D requirements. Accordingly, the recommended daily dietary intake of vitamin D for adults (5 µg or 200 IU in the European Union and 15 µg or 600 IU in the USA) assumes that no synthesis occurs and all of a person's vitamin D is from food intake, although that will rarely occur in practice. As vitamin D can be synthesised in adequate amounts by humans and most other mammals exposed to sunlight, it is not strictly a vitamin (i.e. an organic compound and a vital nutrient that an organism requires in limited amounts), and following its hydroxylation in the body to 1, 25 dihydroxy vitamin D (1, 25(OH)$_2$D) it may be considered a hormone as its synthesis and biological activity occur in different locations. Its discovery in the 1930s can be attributed to key contributions by the chemist Adolf Windaus that included the elucidation of the chemical structures of vitamin D (144).

Inadequate nutrition in terms of dietary energy, macro- or micronutrients is a potential cause of depressed immune function in those engaging in heavy training regimens (137). While most individuals undergoing heavy training who consume a varied diet sufficient to meet their energy needs should meet their micronutrient requirements, one exception can be the failure to achieve adequate vitamin D status during the winter months due to limited vitamin D synthesis from reduced sunlight exposure (106). Therefore, dietary sources of vitamin D and oral vitamin D supplementation are of particular importance during the winter as will be discussed in this review.

The focus of this review is on the effects of vitamin D on immune function and susceptibility to infection and its poten-

tial importance for health maintenance in athletes and military personnel. After covering the structure, sources, metabolism and measurement of vitamin D we will present evidence showing that vitamin D deficiency (defined by the Institute of Medicine (IoM) as a circulating 25(OH)D concentration < 30 nmol/L and used hereafter) occurs commonly in athletes and military personnel. The influence of vitamin D status on innate and adaptive immunity, wound repair and respiratory infection with specific reference to those undergoing heavy training schedules will follow. Then we will discuss whether a circulating 25(OH)D level ≥ 75 nmol/L represents an optimal vitamin D status for immune function and host defence with some simple practical guidance on safe summer sunlight exposure and safe oral vitamin D supplementation during the autumn and winter. The reader is referred to other recent reviews for a consideration of the influence of vitamin D on bone health and risk of fractures, cancer prevention, hypertension and mortality (15, 20) and the emerging role of vitamin D in optimising muscle function and athletic performance (6, 80, 99, 105, 106, 123, 130).

1.1 Vitamin D structure and sources

All forms of vitamin D belong to a family of lipids called secosteroids which are very similar in structure to steroids except that two of the B-ring carbon atoms of the typical four steroid rings are not joined, whereas in steroids they are (Figure 2A). The biologically active metabolite, 1, 25(OH)$_2$D, acts very much like a steroid, binding to nuclear receptors and modulating gene expression and subsequently the synthesis of specific proteins.
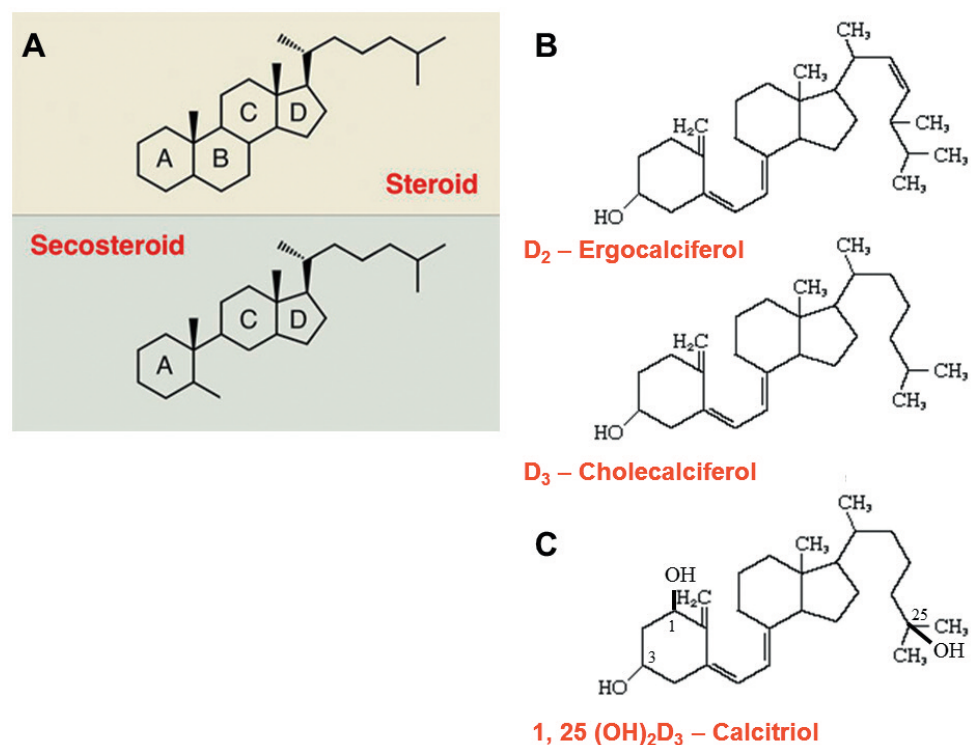

Figure 2. General structure of a secosteroid compared with that of a traditional steroid (A) and the structure of the vitamin D secosteroids (B): Ergocalciferol (D_2) is produced by UV irradiation of ergosterol, a membrane sterol which is produced by some kinds of plankton, invertebrates, yeasts and fungi. Cholecalciferol (D_3) is produced by ultraviolet B irradiation of 7-dehydrocholesterol in the skin which supplies 80-100% of the body's vitamin D requirements. Also shown (C) is the biologically active form of vitamin D, 1, 25-dihydroxy-vitamin D (1, 25(OH)$_2$D), known as calcitriol or calciferol.
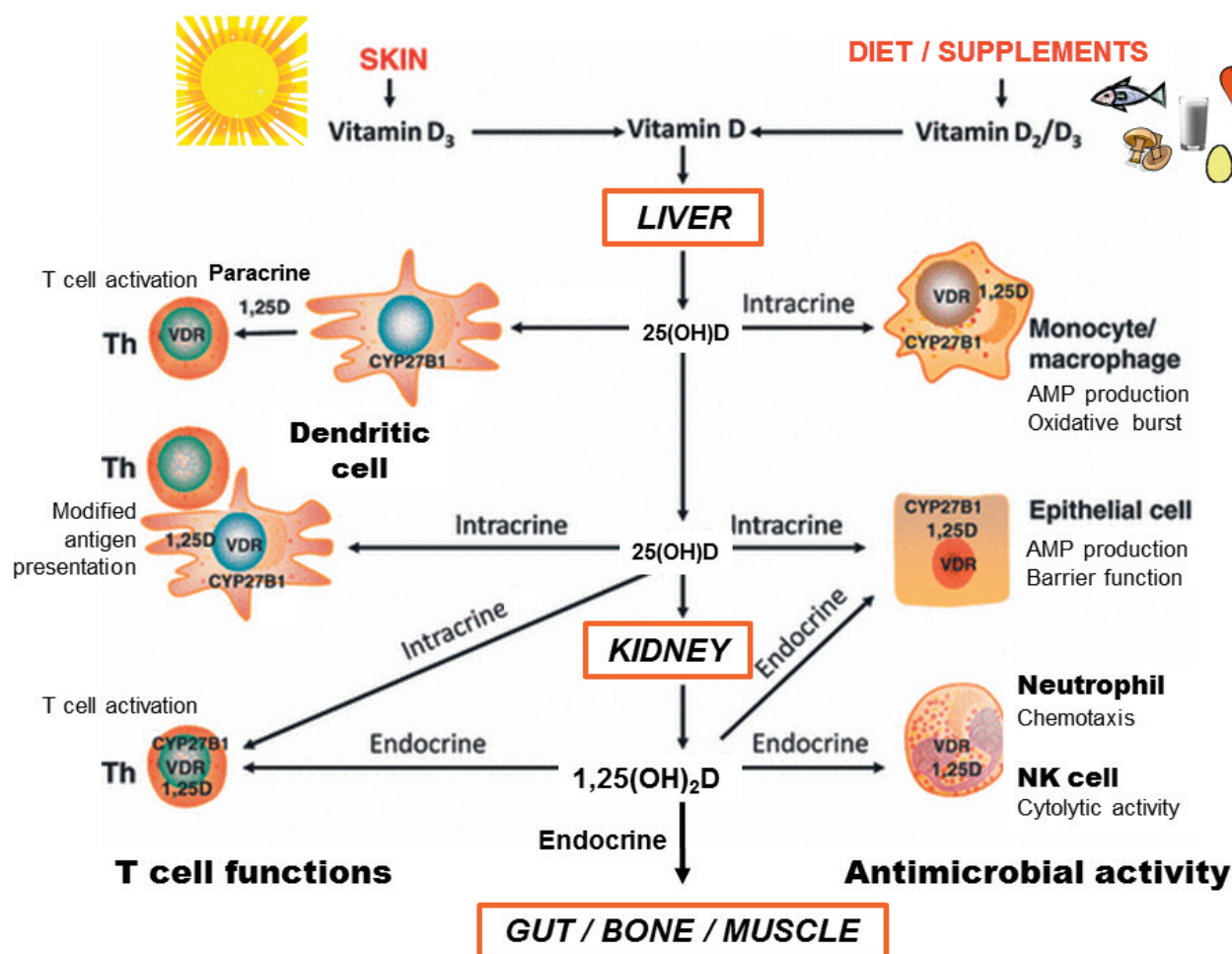
Figure 3. Mechanisms for innate and adaptive immune responses to vitamin D. Ergocalciferol (vitamin D_2) from the diet and cholecalciferol (vitamin D_3) from the diet or produced from the action of UVB on the skin are metabolised in the liver to form 25-hydroxyvitamin D (25(OH)D), the main circulating form of vitamin D. Target cells such as monocytes, macrophages and dendritic cells expressing the mitochondrial vitamin D-activating enzyme 1-α hydroxylase (CYP27B1) and the cytoplasmic vitamin D receptor (VDR) can then utilise 25(OH)D for intracrine responses via localised conversion to 1, 25-dihydroxy-vitamin D (1, 25(OH)$_2$D; calcitriol, shown in the Figure as 1,25D for intracellular locations). In monocytes and macrophages this promotes antibacterial responses to infection. In dendritic cells, intracrine synthesis of 1, 25(OH)$_2$D inhibits dendritic cell maturation, thereby modulating helper T-helper (Th) cell function. Th cell responses to 25(OH)D may also be mediated in a paracrine fashion, via the actions of dendritic cell-generated 1, 25(OH)$_2$D. Intracrine immune effects of 25(OH)D also occur in epithelial cells expressing the VDR and the 1-α hydroxylase (CYP27B1). However, other leukocytes such as neutrophils and natural killer (NK) cells do not appear to express CYP27B1 and are therefore likely to be directly affected by circulating levels of 1, 25(OH)$_2$D synthesised by the kidneys or locally produced in and secreted from tissue macrophages and dendritic cells. VDR-expressing Th cells are also potential targets for systemic 1, 25(OH)$_2$D, although intracrine mechanisms have also been proposed. In a similar fashion, epithelial cells can respond in an intracrine fashion to 25(OH)D, but may also respond to systemic 1, 25(OH)$_2$D to promote antibacterial responses.

Two forms of vitamin D can be obtained from dietary sources (Figure 2B): vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). While vitamin D_3 is found in food from animal origin, such as oily fish, egg yolk, liver and milk, vitamin D_2 is present in some plants and mushrooms (derived from UVB exposure of fungi and yeast ergosterols). Some foods including cereals, margarine and dairy products may be fortified, usually with vitamin D_3. The fractional absorption of both forms of vitamin D from lipid micelles (with the aid of bile salts) in the gut is about 50%. After uptake by intestinal mucosal cells they are incorporated into chylomicrons and enter the circulation via the lymphatic system.

Under optimal conditions of skin sunlight exposure, vitamin D_3 production from UVB-mediated conversion of 7-dehydrocholesterol in the plasma membrane of skin cells provides 80-100% of the body's vitamin D requirements (78). This process is rapid and the production of vitamin D_3 in the skin after only a few minutes of appropriate sunlight easily exceeds dietary sources. The UVB radiation (wavelength of 290-320 nm) promotes photolytic cleavage of 7-dehydrocholesterol into previtamin D in the epidermis, which is subsequently converted into vitamin D_3 by a spontaneous thermal isomerisation. Newly synthesised vitamin D_3 (and its metabolites) are bound to vitamin D-binding protein (VDBP) for systemic transport. Vitamin D_2 is more rapidly metabolised than vitamin D_3, is less well bound to VDBP and therefore has a shorter half-life.

1.2 Metabolism of vitamin D
Vitamin D needs to be hydroxylated twice to achieve the biologically active form, 1, 25(OH)$_2$D (Figure 2C). The endogenously synthesised vitamin D_3 and diet-derived D_2 and D_3

must first be hydroxylated in the liver into 25(OH)D (calcidiol or calcifediol) at the carbon 25-position by the enzyme, 25-hydroxylase. The main storage form of vitamin D, 25(OH)D is found in muscles and adipose tissue, and 25(OH)D is the major circulating metabolite of vitamin D, with a half-life of 2-3 weeks. Therefore, the total plasma concentration of 25(OH)D is considered to be the primary indicator of vitamin D status (11).

In the second hydroxylation, 25(OH)D is converted in the kidney to the biologically active form, 1, 25(OH)$_2$D (calcitriol or calciferol), by 1-α-hydroxylase, an enzyme which is stimulated by parathyroid hormone (PTH) when serum calcium and phosphate concentrations fall below their normal physiological range of 2.1–2.6 mmol/L and 1.0–1.5 mmol/L, respectively. 1, 25(OH)$_2$D, is released into the circulation from the kidney which is considered as a vital endocrine source of hormone (Figure 3). Normal concentrations of circulating 1, 25(OH)$_2$D are approximately 50-250 pmol/L, about 1000 times lower than its precursor, 25(OH)D; the plasma half-life of 1, 25(OH)$_2$D is 4-6 hours. Some cells other than kidney cells also express 1-α-hydroxylase and have the enzymatic machinery to convert 25(OH)D to 1, 25(OH)$_2$D in non-renal compartments including cells of the immune system as illustrated in Figure 3 (8). Importantly, 1, 25(OH)$_2$D limits its own activity in a negative feedback loop by inducing 24-hydroxylase, which converts 1, 25(OH)$_2$D into the biologically inactive metabolite, 1, 24, 25(OH)$_3$D. In addition, 1, 25(OH)$_2$D also inhibits the expression of renal 1-α-hydroxylase. This negative feedback loop reduces the likelihood of hypercalcaemia by preventing excessive vitamin D signalling, thus maintaining bone health.

1.3 Mode of action of 1, 25(OH)$_2$D

1, 25(OH)$_2$D exerts its functions by acting as a modulator of over 900 genes (73). Circulating 1, 25(OH)$_2$D passes through the plasma membrane of target cells and binds to the vitamin D receptor (VDR) in the cytoplasm. The VDR is a nuclear receptor and ligand-activated transcription factor. It is a member of the superfamily of nuclear hormone receptors and it is composed of an α-helical ligand-binding domain and a highly conserved DNA binding domain. High-affinity binding of 1, 25(OH)$_2$D to the α-helical ligand-binding domain of VDR activates transcription by heterodimerization with the retinoid X receptor (RXR), which is essential for the high-affinity DNA binding to cognate vitamin D response elements (VDRE). The 1, 25(OH)$_2$D-VDR-RXR heterodimer translocates to the nucleus where it binds to VDRE located in the regulatory regions of 1, 25(OH)$_2$D target genes and then induces expression of the vitamin D responsive genes (8).

1.4 Vitamin D measurement

Measurement of plasma or serum 25(OH)D concentration is widely used in clinical practice and research reports to assess vitamin D status as 25(OH)D is the major circulating metabolite of vitamin D in whole blood. It has been demonstrated that 25(OH)D in whole blood, serum or plasma is stable at room temperature or when stored at -20°C and is unaffected by multiple freeze-thaw cycles (2, 7, 143). For example, storage of serum samples for up to 3 years at -20°C does not affect serum 25(OH)D concentrations (2) and 25(OH)D con-

centrations in serum samples that have been thawed and refrozen up to four times are still reliable (7).

Plasma or serum 25(OH)D concentration can be measured by competitive protein binding assay, immunoassay, high pressure liquid chromatography (HPLC) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) (40). Current 25(OH)D ELISAs employ polyclonal or monoclonal antibodies that bind specifically to human 25(OH)D. Nevertheless, the competition between the 25(OH)D specific antibodies and VDBP in plasma samples makes these assays difficult to control (29, 38). The plasma 25(OH)D concentration cannot be measured accurately unless it is released from VDBP and the strong protein binding of 25(OH)D requires the employment of suitable conditions to release 25(OH)D from VDBP (40, 135). In addition, most commercial immunoassays cannot measure the concentration of 25(OH)D$_2$ and 25(OH)D$_3$ independently. It has been reported that there was an underestimation of plasma 25(OH)D$_2$ concentration in several commercial immunoassays which resulted in marked variations of the total plasma 25(OH)D levels (D$_2$ and D$_3$) (40, 135). The LC-MS/MS method is generally considered to be the gold standard method for the measurement of plasma or serum 25(OH)D levels because isotope dilution LC-MS/MS method can simultaneously and accurately quantitate both 25(OH)D$_2$ and 25(OH)D$_3$ (135, 146). Furthermore, both 25(OH)D$_2$ and 25(OH)D$_3$ can be extracted from plasma samples using isolute C18 solid phase extraction cartridges in the LC-MS/MS assay. Nonetheless, the use of LC-MS/MS is not without limitations. Significant inter-assay variability of 16.4% has been reported for 25(OH)D measurement using in-house standards and can only be avoided if laboratories use common standards (26) as well as adopt the similar preparation and calibration methods (39).

1.5 Classical biological role of vitamin D for bone health

The classic function of vitamin D is to maintain the health of bones and teeth by influencing calcium homeostasis. Vitamin D influences bone health by upregulating the expression of genes for several calcium transport proteins that enhance calcium absorption from the diet in the small intestine and increase calcium reabsorption in the renal tubules (in association with elevated PTH). Vitamin D also stimulates bone cell differentiation to promote calcium homeostasis and bone health (63). In the general population, individuals who maintain higher vitamin D status have higher bone mineral density in the hip and lumbar spine (63). In physically active populations, sufficient vitamin D is important for the prevention of stress fractures. For example, in Finnish military recruits stress fracture risk was 3.6 times higher in those with relatively low vitamin D status (25(OH)D concentration < 75 nmol/L) compared to those with higher status (118). A randomised, placebo-controlled, double-blind trial of vitamin D$_3$ supplementation (daily 800 IU with 2 g calcium) found a 20% reduction in stress fracture incidence in female US naval recruits compared with those taking a placebo (79).

1.6 Is there a consensus of opinion on vitamin D status classifications for immune health?

The simple answer to this question is 'no'. The IoM has recommended a circulating 25(OH)D level above 50 nmol/L to

Table 1. Classification of vitamin D status suggested by the Institute of Medicine[1].

Vitamin D status	Circulating 25(OH)D concentration (nmol/L)
Deficient	< 30
Inadequate	30 - 50
Sufficient	> 50

[1]Institute of Medicine (66).

achieve 'good bone health' in virtually all of the population but there are no such classifications for vitamin D status in relation to immunity and resistance to common infections (Table 1) (117). In fact, right now there is still no definitive consensus of opinion on the thresholds for vitamin D status and bone health. For example, the circulating 25(OH)D level below which represents deficiency for bone health has been proposed as 30 nmol/L by the IoM (117) but 50 nmol/L by the Endocrine Society and a number of world-leading researchers in the field (20, 64). Furthermore, based on the studies relating 25(OH)D with circulating PTH levels, as well as other evidence for reducing risk of fracture, improving muscle strength and preventing chronic diseases, the Endocrine Society recommends that vitamin D sufficiency should be defined as circulating 25(OH)D > 75 nmol/L (64). Also, a recent and comprehensive review that summarises the various studies that have attempted to evaluate threshold levels for circulating 25(OH)D levels in relation to bone mineral

Table 2. Vitamin D status in athletes and military personnel.

Season	Location (latitude)	Population	N	Age (years)	Circulating 25(OH)D concentration (nmol/L)			Ref
					% < 30 nmol/L[1] Deficient	% < 50 nmol/L[1] Insufficient	% < 75 nmol/L[2] Suboptimal	
Winter								
	Finland (60 - 70 ºN)	Finnish military recruits	196	18 - 28	19% < 25	78% < 40	-	Laaksi et al.(77)
	Liverpool, UK (53 ºN)	Elite soccer players	20	24	-	65%	-	Morton et al. (100)
	Liverpool, UK (53 ºN)	UK club athletes	30	20 - 24	20%	57%	-	Close et al. (34)
	Liverpool, UK (53 ºN)	Professional UK athletes	61	18 - 27	35%	64%	-	Close et al.(33)
	Loughborough, UK (53 ºN)	Recreational to elite athletes	225	21	8%	38%	-	He et al. (56)
	Barcelona (41 ºN)	Professional basketball players	21	25	10% < 25	57%	-	Garcia & Guisado(42)
Autumn								
	Washington, USA (47 °N)	Collegiate athletes	39	18 - 33	-	3%	26% < 80	Storlie et al. (127)
	Australia (35 °S)	Australian female gymnasts	18	10 - 17	-	33%	83%	Lovell (87)
Summer								
	Finland (60 - 70 ºN)	Finnish military recruits	756	18 - 29	-	4% < 40	-	Laaksi et al.(76)
	California, USA (34 °N)	Collegiate athletes	223	-	-	3%	34% < 80	Villacis et al.(133)
	Doha, Qatar (25 °N)	Middle-eastern sportsmen	93	13 - 45	59% < 25	91%	100%	Hamilton et al.(51)
	Doha, Qatar (25 °N)	Professional Qatar based footballers	342	16 - 33	12% < 25	56%	84%	Hamilton et al.(52)
All seasons								
	Carolina, USA (35 °N)	Young active military personnel	312	-	-	-	52%	Wentz et al.(142)
Not reported								
	East Germany (53 ºN)	Competitive gymnasts	85	8 - 27	37% < 25	-	-	Bannert et al.(12)
	Pittsburgh, USA (40 °N)	National football league players	80	22 - 37	-	26%	69%	Maroon et al.(92)
	Jerusalem, Israel (32 °N)	Athletes and dancers	98	10 - 30	-	-	73%	Constantini et al.(35)
	Texas, USA (31 °N)	Overweight and obese soldiers	314	31	-	21%	72% < 72	Funderburk et al.(41)

Age is presented as mean or range. Hyphen '-' indicates not reported.
[1]Values are based on current recommendations for bone health, where circulating 25(OH)D < 30 nmol/L is defined as deficient and < 50 nmol/L is defined as insufficient (66). Note: not all authors have used the IoM classification as reflected in the table.
[2]Evidence suggests that those with circulating 25(OH)D < 75 nmol/L have a higher adjusted odds of acute respiratory infections compared with individuals with 25(OH)D levels ≥ 75 nmol/L (97).

density, lower limb function, dental health, cancer prevention, risk of falls, fractures, incident hypertension and mortality concludes that for all endpoints, circulating levels of 25(OH)D < 50 nmol/L are associated with adverse effects or no benefit, while the most advantageous circulating levels for 25(OH)D appeared to be close to 75 nmol/L (20). Further research is clearly required to investigate whether a circulating 25(OH)D > 75 nmol/L is necessary to optimise immune function, as will be discussed in more detail in section 5.3. For the purposes of this review, vitamin D deficiency is denoted as circulating 25(OH)D < 30 nmol/L in line with the current IoM recommendations (117).

2. IS VITAMIN D DEFICIENCY A PROBLEM FOR ATHLETES AND MILITARY PERSONNEL?

The answer to this question appears to be 'yes' although to date we know of no evidence indicating that athletes are at greater risk of vitamin D deficiency than non-athletes. A summary of the current evidence on vitamin D status in athletes and military personnel is provided in Table 2. As logic dictates, vitamin D deficiency is more prevalent in the winter when skin sunlight UVB exposure and endogenous synthesis of vitamin D is low and in those who cover their skin whilst training outdoors in the summer (51) or who train predominantly indoors (12). In the winter months more than half of the athletes and military personnel studied could be considered to have insufficient vitamin D status (circulating 25(OH)D < 50 nmol/L) and as many as 35% could be considered vitamin D deficient. Important considerations when interpreting the data on the incidence of vitamin D deficiency in athletes and military personnel (Table 2) include: sunlight avoidance behaviour (fear of sunburn and skin cancer); season; latitude; skin type; clothing and sunscreen use, all of which will be discussed in section 7.

3. EMERGING BIOLOGICAL ACTIONS OF VITAMIN D

Many tissues other than kidney, including brain, lung, muscle, skin, adipose tissue and cells of the immune system possess both the 1-α-hydroxylase and VDR and are able to produce the biologically active 1, 25(OH)$_2$D from circulating 25(OH)D (11). It is important to note that extra-renal 1-α-hydroxylase differs from renal 1-α-hydroxylase in that it is not regulated by circulating PTH, calcium and phosphate concentrations (145). In recent years it has been established that vitamin D is not only important for calcium homeostasis and bone health but also for the optimal function of skeletal muscle and immune function.

3.1 Vitamin D and skeletal muscle function
Vitamin D can modulate skeletal muscle function by both genomic and nongenomic events. 1, 25(OH)$_2$D induces muscle gene transcription and protein synthesis to influence muscle cell proliferation and differentiation, calcium uptake and phosphate transport across the sarcolemma (50). The nongenomic responses include modulation of calcium uptake across

the sarcolemma and the activation of mitogen-activated protein kinase signalling pathways in muscle fibres (50). Vitamin D also up-regulates expression of insulin-like growth factor-1 (IGF-1) (5), which has a well-recognised role in muscle remodelling, hypertrophy and strength gains (74). IGF-1, which is mostly produced by the liver and bound by insulin-like growth factor binding protein 3 (IGFBP-3) in the serum, is a key component in muscle regeneration and could induce proliferation, differentiation and hypertrophy of skeletal muscle (5, 122). IGFBP-3 expression could be regulated by vitamin D as there are vitamin D response elements in the promoter region of the human IGFBP-3 gene which might lead to higher circulating amounts of IGFBP-3 and so delay the normally rapid clearance of IGF-1 in the bloodstream (50, 83). The obvious implication of these findings is that vitamin D status and vitamin D supplementation might affect muscle strength, endurance and athletic performance. This has received considerable attention over the past decade and the results of these studies have been the main focus of numerous recent reviews about vitamin D and the athlete (6, 80, 99, 105, 106, 123, 130). The general consensus at present is vitamin D deficiency could negatively impact athletic performance due to the influence of vitamin D on muscle function. However, there is insufficient evidence from a limited number of cross sectional vitamin D status studies and longitudinal, randomised, placebo-controlled vitamin D$_3$ supplementation studies in athletes to conclude that vitamin D is a direct performance enhancer (46).

4. VITAMIN D AND IMMUNE FUNCTION

Vitamin D is known to have important effects on both innate and adaptive immune function with implications for host defence. These issues are the main focus of the remainder of this review.

The discovery of VDR in almost all immune cells, including T lymphocytes, B lymphocytes, neutrophils and antigen presenting cells, such as monocytes, macrophages and dendritic cells prompted the idea that vitamin D could have a vital role in the regulation of immune responses (11). These immune cells also express the mitochondrial vitamin D-activating enzyme, 1-α-hydroxylase (CYP27B1) and thus possess the ability to convert 25(OH)D to 1, 25(OH)$_2$D. This conversion is regulated by circulating levels of 25(OH)D and can also be induced by activation of specific toll-like receptors (TLRs) (18) which act as pathogen detectors. Thus, 1, 25(OH)$_2$D could play important roles in both innate and adaptive immune responses (Figure 3). Four potential mechanisms by which vitamin D can influence immune function have been proposed: 1) direct endocrine actions on immune cells mediated by circulating 1, 25(OH)$_2$D formed in the kidney; 2) direct intracellular actions of 1, 25(OH)$_2$D following intracrine conversion of 25(OH)D to 1, 25(OH)$_2$D within immune cells; 3) paracrine actions of 1, 25(OH)$_2$D produced in and secreted from antigen presenting cells on local lymphocytes and neutrophils and 4) indirect effects on antigen presentation to T cells mediated by influence of circulating 1, 25(OH)$_2$D on antigen presenting cells (60, 112). The proposed actions of 1, 25(OH)$_2$D on the human immune system are summarised in

Table 3. The proposed effects of 1, 25 dihydroxy vitamin D on the immune system.

Target site	Actions of 1, 25 $(OH)_2D$
Antigen presenting cells	Upregulation of the production of antimicrobial proteins and peptides (AMPs) (e.g. cathelicidin, β-defensins)
	Increased generation of reactive oxygen species and the expression of inducible nitric oxide synthase
	Increased macrophage phagocytosis
	Upregulation of CD14 expression
	Downregulation of CD40 (required for B cell activation)
	Downregulation of CD80/86 (required for T cell activation)
	Downregulation of MHCII expression
	Elevation of IL-10 production
	Inhibition of production of pro-inflammatory cytokines
Saliva	Increased saliva flow and AMP secretion
Epithelial cells	Upregulation of genes for gap junction, adherens junction and tight junction proteins to strengthen barrier function
Natural Killer cells	Downregulation of production of IFN-γ
	Upregulation of expression of NK cytotoxicity receptors NKp30 and NKp44
	Augmentation of IL-2 activated cytolysis
T cells	Increased vitamin D receptor expression
	Suppression of T helper (Th) type 1 and induction of Th2
	Inhibition of production of pro-inflammatory cytokines IL-2 and IFN-γ by Th1 cells
	Elevation of IL-4 production by Th2 cells
	Suppression the development of Th17 cells and inhibition of the production of cytokines by Th17 cells
	Induction of Treg cells
	Increased IL-10 production by Treg cells
	Upregulation of phospholipase C-gamma 1 expression leading to increased antigen-specific T cell activation and proliferation
B cells	Increased vitamin D receptor expression
	Suppression of B cell proliferation and immunoglobulin production
	Inhibition of the differentiation of B cell precursors into plasma cells

Table 3. Although the actions of vitamin D do not alter numbers of circulating leukocytes, neutrophils, monocytes or lymphocytes, the proportions of lymphocyte subsets, particularly within the T cell compartment, can be modified as can the functions of various immune cells associated with both innate and acquired immunity.

4.1 Vitamin D, innate immunity and mucosal immunity

It has been demonstrated that 1, 25$(OH)_2D$ is a vital mediator of innate immune responses, enhancing the antimicrobial properties of immune cells such as monocytes and macrophages through the induction of antimicrobial proteins (AMPs) and stimulation of autophagy and autophagosome activity (18, 31). 1, 25$(OH)_2D$ is a key link between TLR activation and antimicrobial responses in innate immunity. Following activation of the TLR signalling cascade in the presence of microbes, 1, 25$(OH)_2D$ has a vital role in up-regulating the production of AMPs, such as cathelicidin and β-defensin (85, 138). The AMPs have a broad range of activities against microorganisms, particularly bacteria, and may also be involved in the direct inactivation of viruses through membrane destabilisation (68). They are produced by epithelial cells and macrophages and in the lungs are secreted into the biofilm covering the inner surface of the airways, thereby creating a barrier that is chemically lethal to microbes. Both macrophages and epithelial cells, possessing the 1-α-hydroxylase and VDR, are capable of responding to and producing 1, 25$(OH)_2D$. The biologically active form, 1, 25$(OH)_2D$, can induce expression of the vitamin D responsive genes to enhance the production of cathelicidin and β-defensin by binding to VDREs as described previously in section 1.3. The stimulation of TLRs by interaction with pathogen associated molecular patterns in macrophages or by wounding the epidermis in keratinocytes results in increased expression of both

the VDR and the 1-α-hydroxylase enzyme, which up-regulates the production of 1, 25$(OH)_2D$ to stimulate the expression of cathelicidin and β-defensins in the presence of adequate 25(OH)D as illustrated in Figure 4 (31, 85). 25(OH)D, the major circulating form used to determine vitamin D status, is an essential factor for the local production of 1, 25$(OH)_2D$ to up-regulate cathelicidin production in the skin and in macrophages. While 1, 25$(OH)_2D$ alone is sufficient for the strong induction of cathelicidin expression, the combination of IL-1β and 1, 25$(OH)_2D$ is required for the strong induction of β-defensin. 1, 25$(OH)_2D$ can double the induction of β-defensin production by IL-1β signalling which stimulates NF-κB transcription factor function (84).

In addition to its effects on AMPs, 1, 25$(OH)_2D$ strengthens epithelial barrier functions by up-regulating genes for the proteins required in tight junctions (e.g. occludin), gap juctions (e.g. connexin 43) and adherens junctions (e.g. E-cadherin) in epithelial cells, fibroblasts and keratinocytes (32, 47, 107). Furthermore, 1, 25$(OH)_2D$ enhances the effectiveness of monocytes and macrophages in killing microbes by enhancing the generation of reactive oxygen species and the expression of inducible nitric oxide synthase in these phagocytic cells (124) as well as augmenting IL-1β secretion and up-regulating the expression of CD14, the lipopolysaccharide (LPS) receptor.

Recent studies on natural killer (NK) cell function indicate that 1, 25$(OH)_2D$ upregulates the expression of NK cell surface cytotoxicity receptors NKp30, NKp44 and NKG2D, downregulates the expression of the killer inhibitory receptor CD158 and enhances NK cell cytolytic activity (3). Vitamin D appears to have rather limited effects on neutrophil function. Although neutrophils are recognised as an important source of

cathelicidin and do express VDRs, they seem to have no 1-α hydroxylase activity that would enable them to convert 25(OH)D into the biologically active 1, 25(OH)$_2$D necessary to initiate cathelicidin gene expression (59). However, neutrophils can be influenced directly by circulating 1, 25(OH)$_2$D and, as in monocytes, expression of CD14 on the cell surface is augmented by 1, 25(OH)$_2$D (129). Previous exposure of neutrophils to pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α) or granulocyte macrophage colony-stimulating factor or with the VDBP leads to alterations in complement activation peptide C5a-mediated neutrophil functions, including enhanced chemotaxis (19). A recent study on VDBP knockout mice reported that neutrophil recruitment to the lung in both C5a- and CXCL1-induced alveolitis was 50% lower than in the wild type controls (131) and that the reduced neutrophil response in VDBP knockout mice could be restored to wild-type levels by administering exogenous VDBP suggesting that VDBP may have a more significant role in neutrophil recruitment than previously recognised. These various effects of vitamin D can be suggested as improving innate immunity and could conceivably contribute to a reduced susceptibility to infections.

4.1.1 Vitamin D, innate immunity and mucosal immunity in athletes

A recent study in university athletes reported a higher level of plasma cathelicidin and salivary secretory immunoglobulin A (SIgA) secretion in those who had plasma 25(OH)D greater than 120 nmol/L compared with those who had lower vitamin D status (56) and a follow-up randomised, placebo controlled, double blind vitamin D$_3$ supplementation study (5, 000 IU/day for 14 weeks) by the same group (55) reported significant increases in salivary secretion rates of both SIgA and cathelicidin compared with no significant changes in the placebo group. This was due, at least in part to a significant increase in saliva flow rates over time in the vitamin D$_3$ group. Several animal studies have demonstrated that VDRs are present in the parotid, submandibular and sublingual salivary glands which points to a possible role for vitamin D in the regulation of salivary secretion. This is supported by the finding that salivary flow rates were stimulated after treatment with vitamin D$_3$ in vitamin D deficient rats (108, 128). The mechanism for how vitamin D affects salivary flow rates requires elucidation. But it was suggested that vitamin D might stimulate salivary secretion through the regulation of calcium as the rapid efflux of calcium plays a role in the stimulation of fluid secretion (108).

In summary, the discovery of increased VDR and 1α-hydroxylase (CYP27B1) expression in macrophages following a pathogen challenge, and the subsequent enhancement of AMP production, oxidative burst and autophagosome activity has underlined the importance of intracrine vitamin D as a key enhancer of innate immune function. It is now clear that both macrophages and dendritic cells are able to respond to 25(OH)D, the major circulating vitamin D metabolite, thereby providing a link between the function of these cells and the variations in vitamin D status among humans. Although the evidence is limited, recent studies in athletes show beneficial effects of high circulating vitamin D (> 120 nmol/L) on innate immunity and mucosal immunity.
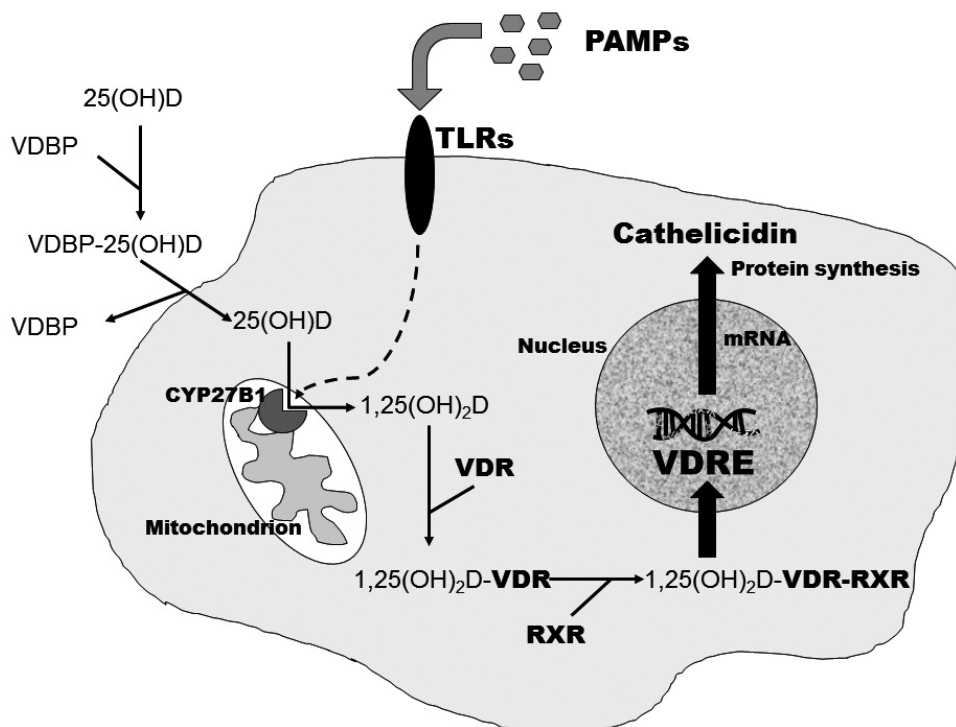

Figure 4. Cathelicidin induction via activation of TLRs and vitamin D. 25-hydroxy vitamin D (25(OH)D) is transported in the circulation bound to the vitamin D binding protein (VDBP). Pathogen associated molecular patterns (PAMPs) on invading microorganisms trigger toll-like receptors (TLR 1/2 and TLR4) and subsequent downstream signalling (dashed arrow) induces the mitochondrial 1-α hydroxylase (CYP27B1), increasing the intracellular conversion of 25(OH)D to 1, 25(OH)$_2$D which after binding to the vitamin D receptor (VDR) along with the retinoid X receptor (RXR) in the cytoplasm translocates to the nucleus where it binds to cognate vitamin D response elements (VDRE) located in the regulatory regions of 1, 25(OH)$_2$D target genes and then induces transcription of the vitamin D responsive genes leading to increased amounts of antimicrobial proteins (e.g. cathelicidin, β-defensin) being produced.

4.2 Vitamin D, adaptive immunity and inflammation

In contrast with the innate immune responses, many of the reported actions of vitamin D on adaptive immunity are indicative of anti-inflammatory and suppressive mechanisms, which could be beneficial for those with autoimmune disorders. The effect of 1, 25(OH)$_2$D on antigen presenting cells is to induce IL-10 and suppress IL-12 production, inhibit dendritic cell activation by down-regulating expression of costimulatory molecules CD40 and CD80/86 while up-regulating the production of AMPs and autophagosome activity (1). Furthermore, 1, 25(OH)$_2$D can inhibit T cell proliferation and also influence the phenotype of T cells, in particular through the suppression of Th1 cells which are associated with cellular immunity (81). Studies using human T cell cultures have shown that 1, 25(OH)$_2$D inhibits T cell proliferation and production of IL-2 and interferon gamma (IFN-γ) (102, 116, 132). In contrast, 1, 25(OH)$_2$D enhances cytokine production by Th2 cells (e.g. IL-4) that are associated with humoral immunity (21). Thus, vitamin D could help limit the inflammation and tissue damage associated with excessive Th1 cellular immunity by shifting the balance to a Th2 cell phenotype. 1, 25(OH)$_2$D also has an influence on the activity of Th17 cells, which are linked to inflammatory tissue damage. It appears that 1, 25(OH)$_2$D can suppress the development of Th17 cells and inhibit the production of cytokines by Th17 cells (27). In addition, it has been shown that treatment of naive CD4 T cells with 1, 25(OH)$_2$D potently induces the development of regulatory T cells (Treg) which are capable of producing cytokines that block Th1 development (49). Vitamin D also increases synthesis of the primary anti-inflammatory cytokine IL-10 by Treg cells and dendritic cells (45, 120). Overall, vitamin D is suggested to maintain a balance between inflammatory Th1/Th17 cells and immunosuppressive Th2/Treg cells to temper inflammation and tissue damage (59). It has also been demonstrated that 1, 25(OH)$_2$D can suppress B cell proliferation and immunoglobulin production and inhibit the differentiation of B cell precursors into plasma cells, which highlights a potential role for vitamin D in B cell related disorders (30).

The actions of vitamin D on adaptive immunity appear to be mostly suppressive or inhibitory, so why does this not impair immune responses to pathogens and increase susceptibility to infection? The answer to this paradox may be found in the recent studies indicating that vitamin D is essential in activating and controlling the T-cell antigen receptor and thus enhancing the recognition of antigens by T lymphocytes (73, 134) leading to an activation of the cellular immune response in response to pathogen exposure. Naive human T cells have very low expression of phospholipase C-gamma 1 (PLC-γ1), a key signalling protein downstream of many extracellular stimuli, and this is associated with low T cell antigen receptor (TCR) responsiveness in naive T cells. However, TCR triggering leads to a large up-regulation of PLC-γ1 expression, which correlates with greater TCR responsiveness. Induction of PLC-γ1 is dependent on vitamin D and expression of the VDR. Naive T cells do not express the VDR, but VDR expression is induced by TCR signalling via the alternative mitogen-activated protein kinase p38 pathway. Thus, initial TCR signalling via p38 leads to successive induction of VDR and PLC-γ1, which are required for subsequent classical TCR sig-

nalling and T cell activation. These findings indicate that vitamin D is crucial for the activation of the acquired immune system and therefore very important for the effective clearance of viral infections. The aforementioned suppressive actions of vitamin D on adaptive immunity may therefore be a reaction to prevent the development of an exaggerated immune response and excessive inflammation following T cell activation. This is, of course, important as the ideal immune response is rapid, proportionate, and effective but finite; an inflammatory response which is disproportionate or lasts too long risks injury to the host. The recognition that in adaptive immunity vitamin D is needed for its effective activation when challenged by pathogens is more in keeping with its role in promoting innate immunity and the reduction in respiratory infection incidence with improved vitamin D status which has been reported in several large scale studies in both the general population (44) and athletes (56) which are discussed in more detail in section 5 of this review.

It is also important to recognise that the primary influence of 1, 25(OH)$_2$D may vary with the tissue site. Systemic levels of 1, 25(OH)$_2$D may aid in maintaining tonic immunosuppression and thus prevent trivial antigenic stimuli from initiating an immune response. Upon initiation of an immune response to a significant antigenic challenge 1, 25(OH)$_2$D may, in concert with other suppressor mechanisms, limit the extent of the host response by inhibition of IL-2 and IFN-γ production. At local sites of chronic inflammation concentrations of 1, 25(OH)$_2$D may be elevated and may act in an autocrine or paracrine fashion to alter the immune response, for example, by increasing IL-1β production and antigen presentation by tissue macrophages. The activation of T cells is associated with increased expression of VDRs, thus potentially limiting T cell proliferation in the presence of the 1, 25(OH)$_2$D. Thus, the end result of the opposing effects of 1, 25(OH)$_2$D on immune cells and their secretory products may vary with the specific cells involved, their state of maturation and activation, and the local concentrations of 1, 25(OH)$_2$D.

The identification of hundreds of primary 1, 25(OH)$_2$D target genes in immune cells has provided new insight into the role of vitamin D in the adaptive immune system, such as the modulation of antigen-presentation and T cell proliferation and phenotype, with the over-arching effects being to suppress inflammation and promote immune tolerance, while also being able to activate the acquired immune response in the presence of pathogen challenge. Thus variations in 25(OH)D levels have the potential to influence both innate and adaptive immune responses.

4.3 Vitamin D and cytokine responses

The studies that have reported modulation of pro- and anti-inflammatory cytokine production by vitamin D have generally administered 1, 25(OH)$_2$D *in vivo* in animals (25, 147) or *in vitro* in human peripheral blood mononuclear cell cultures (70, 101, 115, 116) and observed increases in anti-inflammatory cytokines such as transforming growth factor-β, IL-4 and IL-10 and reductions in pro-inflammatory cytokines including IL-2, IL-6, IFN-γ and TNF-α. However, these studies have used supraphysiological (nanomolar) concentrations of 1, 25(OH)$_2$D$_3$ to determine mitogen- or bacteria-stimulated

cytokine production in human peripheral blood mononuclear cell cultures (the normal human plasma 1, 25(OH)$_2$D$_3$ concentration is 50-250 pmol/L). Furthermore, this experimental approach is not a true reflection of differences in vitamin D status, where marked differences in circulating 25(OH)D concentration may exist and might have more influence on immune cell functions than changes in levels of circulating 1, 25(OH)$_2$D. Using *in vitro* antigen-stimulated human whole blood culture, inhibition of IL-2, IL-6, IFN-γ and TNF-α production was only observed at 1, 25(OH)$_2$D$_3$ concentrations of 1, 000 or 10, 000 pmol/L and not within the more realistic range of 0 to 200 pmol/L (54). This suggests that antigen-stimulated cytokine production is unchanged within the normal reference range of 1, 25(OH)$_2$D$_3$ concentrations.

4.3.1 Vitamin D and cytokine responses in athletes

A recent study in athletes indicated that athletes deficient in 25(OH)D (circulating 25(OH)D < 30 nmol/L) had substantially lower *in vitro* antigen-stimulated production of the pro-inflammatory cytokines (IL-6, IFN-γ and TNF-α) by whole blood culture than athletes with high vitamin D status (circulating 25(OH)D > 90 nmol/L) (56). This is similar to a report of decreased macrophage IL-6, IL-1β and TNF-α production following *in vitro* LPS stimulation of peritoneal macrophages in vitamin D deficient mice (69). In that study the authors also reported that TNF-α and IL-6 concentrations in serum were ~50% lower following *in vivo* administration of LPS in vitamin-D deficient mice indicating that vitamin D deficiency does result in a defect of cytokine production. A higher pro-inflammatory cytokine production in response to an antigen challenge with better vitamin D status could be seen as being beneficial to host defence against pathogenic microorganisms. Indeed, athletes with high vitamin D status had fewer upper respiratory illness (URI) episodes during a 4-month winter period than those with vitamin D deficiency (56).

Further studies are warranted to understand the mechanisms by which vitamin D affects adaptive immunity and the implications for both infectious and autoimmune diseases. In particular, studies in athletes and military personnel are required to examine the influence of seasonal changes in vitamin D status and vitamin D supplementation (see section 6) on *in vivo* immune measures with known clinical endpoints such as the antibody response to vaccination (136).

It is also worth noting that some cytokines have an influence on vitamin D metabolism, For example IFN-γ is a Th1 pro-inflammatory cytokine that influences vitamin D metabolism in human monocytes (37, 126) and macrophages (72) by increasing 1α-hydroxylase activity which mediates the conversion of 25(OH)D to 1, 25(OH)$_2$D. In contrast to IFN-γ, IL-4 is a Th2 anti-inflammatory cytokine that initiates the catabolism of 25(OH)D to the biologically inactive 24, 25(OH)$_2$D (37). Furthermore, recent genome-wide analyses (31) have highlighted how cytokine signalling pathways can influence the intracrine vitamin D system and either enhance or abrogate responses to 25(OH)D.

4.4 Vitamin D, wound repair and rehabilitation from injury

The emerging evidence for an influence of vitamin D status during musculoskeletal rehabilitation following injury or surgery is of potential importance to athletes. One study reported that vitamin D status influenced strength and recovery in young, recreationally active individuals following anterior cruciate ligament repair (14). In this study, those with circulating 25(OH)D concentration below 75 nmol/L recovered more slowly and had significantly attenuated increases in peak isometric force compared to those with concentrations above 75 nmol/L. Another study by the same group reported that following an intense single limb exercise bout a faster recovery of muscle strength occurred with higher pre-exercise levels of circulating 25(OH)D (13). Studies in athlete populations are currently lacking but a few studies of patients in rehabilitation units support the idea that vitamin D may be important for rehabilitation (13, 71, 121). A study in a general rehabilitation unit found that vitamin D deficiency delayed rehabilitation and increased length of stay by 19% (71). Another randomised trial in female stroke patients found that supplementation with 1, 000 IU vitamin D/day improved muscle strength and increased the relative number and size of type II muscle fibres (121).

5. VITAMIN D STATUS AND RESPIRATORY INFECTION

5.1 Vitamin D status and respiratory infection in the general population

Several cross-sectional and cohort studies have reported a negative association between vitamin D status and respiratory infection incidence. In the National Health, Nutrition and Examination Survey involving 18, 883 participants above 12 years, those with circulating 25(OH)D < 25 nmol/L were 1.4 times more likely to report recent URI compared to those with 25(OH)D ≥ 75 nmol/L, even after adjusting for demographics and clinical data (season, body mass index, smoking history, asthma and chronic obstructive pulmonary disease) (44). The proportion of participants who had a self-reported URI was also significantly different between vitamin D groups (24% in those with circulating 25(OH)D levels < 25 nmol/L vs. 20% with levels of 25-75 nmol/L *vs.* 17% with levels of ≥ 75 nmol/L) (44). In a cohort study over 3.5 months in 198 healthy adults, there was a significant inverse association between circulating 25(OH)D concentration and risk of acute viral respiratory tract infection (45% in those with 25(OH)D < 95 nmol/L *vs.* 17% in those with circulating 25(OH)D ≥ 95 nmol/L). Circulating 25(OH)D > 95 nmol/L was also associated with a significant two-fold reduction in the risk of developing acute respiratory tract infections (119). The main strength of the study was that infection was confirmed by determination of pathogens in swabs collected from participants who exhibited symptoms of respiratory tract infection. Furthermore, in a nationwide study involving 6, 789 middle-aged British adults, 12% of those with circulating 25(OH)D < 25 nmol/L had a respiratory infection in the month prior to blood sampling compared to 6% in those with 25(OH)D > 100 nmol/L. Circulating 25(OH)D was inversely associated with risk of acute respiratory infection even after taking into account lifestyle and socio-economic factors. Each 10 nmol/L increase in circulating 25(OH)D significantly reduced the risk of self-reported acute respiratory infection by 7% (16). Hence, these population-wide studies indicate an inverse rela-

tionship between circulating 25(OH)D and the incidence of URI.

5.2 Vitamin D status and respiratory infection in military personnel and athletes

In 756 young Finnish conscripts who were starting military training during the summer time (July), 4% had low circulating 25(OH)D concentrations (stated by the authors as < 40 nmol/L). Although only a minority showed such low levels of circulating 25(OH)D, this group had significantly more duty days lost due to respiratory infection during the following 6 months of training to January (median: 4 *vs.* 2 days) than those with circulating 25(OH)D > 40 nmol/L. Those with low 25(OH)D were also 1.6 times more likely to miss duty due to respiratory infection (76). However, the study only measured circulating 25(OH)D at the start of military training and thus failed to account for any changes in 25(OH)D during the training period that might have influenced respiratory infection incidence.

Studies in athletic populations have yielded similar findings. Vitamin D status was assessed in a group of elite athletes who reported to a physician with URI symptoms. Athletes who had positive virology/bacteriology results (infectious group; mean ± SD circulating 25(OH)D 79 ± 164 nmol/L) or had mild to moderate leukocytosis (suggestive group; circulating 25(OH)D 77 ± 95 nmol/L) had significantly lower circulating 25(OH)D levels than those who had negative virology/bacteriology results and normal differential leukocyte counts (unknown group; 168 ± 251 nmol/L) (36). Irrespective of the high SDs reported, which suggest large between-participant variability in circulating vitamin D levels, the vitamin D level in the infectious and suggestive groups appear relatively high (means > 75 nmol/L): it's unclear if this finding can be explained by the assay used to determine circulating 25(OH)D as the assay method is not mentioned. In another group of endurance athletes, a significantly greater proportion of those with circulating 25(OH)D < 30 nmol/L presented with URI symptoms than those with 25(OH)D > 120 nmol/L (56). Furthermore, the total number of URI symptom days and the median symptom-severity score in athletes with circulating 25(OH)D < 30 nmol/L was significantly higher than those with 25(OH)D > 120 nmol/L (56).

In summary, though causality cannot be established from cross-sectional comparisons, studies in military personnel and athletes agree with the large general population studies (that used powerful logistical regression techniques to identify contributing factors to URI) by showing an inverse relationship between circulating 25(OH)D and the incidence of URI.

5.3 Is there an optimal vitamin D status to prevent respiratory infections?

As mentioned previously, circulating 25(OH)D is recommended to be > 50 nmol/L for optimum bone health as this represents the level that reduces circulating PTH to a minimum and allows maximum calcium absorption (66). Nonetheless, Chapuy et al. (28) reported an inverse relationship between circulating levels of 25(OH)D and PTH up to 75 nmol/L, at which point the decrease in PTH in response to increasing 25(OH)D levelled out. As such, many experts now

agree that a circulating 25(OH)D concentration of at least 75 nmol/L is desirable (4, 20, 64, 109). In accordance with this recommendation, one large scale study involving 14, 108 participants over 16 years of age (NHANES, 2001–2006) supports the proposed circulating 25(OH)D cut-off level of 75 nmol/L for the prevention of respiratory infection as there was a near linear inverse relationship between circulating 25(OH)D levels and the cumulative frequency of acute respiratory infection up to 25(OH)D levels ~75 nmol/L (97). Interestingly, in another study, a partition analysis determined that a circulating 25(OH)D cut-off level of 95 nmol/L best discriminated between groups that did or did not develop viral infections and it has been reported that adults with 25(OH)D status < 95 nmol/L had a significant two-fold increase in the risk of developing acute respiratory infection during winter months compared with those whose 25(OH)D status was > 95 nmol/L (119). Therefore, the optimal circulating 25(OH)D level required to prevent URI in athletes and military personnel has yet to be determined, but based on the limited evidence available, is likely to be 75 nmol/L or possibly higher (e.g. 95 nmol/L). Continued research using randomised-controlled trials of vitamin D supplementation (see the next section) is required to substantiate the purported 75 nmol/L cut-off for circulating 25(OH)D to prevent URI in athletes and military personnel.

6. THE EFFECTS OF ORAL VITAMIN D SUPPLEMENTATION AND UVB IRRADIATION ON VITAMIN D STATUS, IMMUNITY AND RESPIRATORY INFECTION

As described in section 5, a consistent observation in the extant literature is that vitamin D insufficiency is associated with increased URI incidence and symptom duration. Therefore, adopting strategies to avoid vitamin D insufficiency e.g. taking a daily oral vitamin D supplement during the winter and, where possible, practising safe summer sunlight exposure is important to optimise vitamin D status and defence against URI. The information covered in this section considered alongside the sections that follow on factors affecting vitamin D status (section 7) and toxicity (section 8) will form the backdrop for the closing section on simple recommendations to optimise vitamin D status and immune health for athletes and military personnel (section 10).

6.1 The effects of oral vitamin D supplementation on vitamin D status, immunity and respiratory infection

Although vitamin D_2 and D_3 are available as oral supplements, vitamin D_3 supplementation is more commonly used as it has a greater efficacy in raising circulating 25(OH)D compared to vitamin D_2 (65). Current evidence (Table 4) indicates that oral vitamin D supplementation enhances innate responses to mycobacterial infection (specifically, *Mycobacterium bovis* in the BCG-lux assay) (93) and increases circulating levels of the AMP cathelicidin (17, 55). A shift towards an anti-inflammatory cytokine profile (91, 125) and an increase in circulating regulatory T cells (111) has also been demonstrated with oral vitamin D supplementation. Nevertheless, there are weaknesses with some of these studies that limit the interpretation in terms of the

Table 4. Summary of evidence regarding the effects of oral vitamin D supplementation on immune function.

Study design	Population[1]	Season or month	Supplementation	Change in circulating 25(OH)D concentration[2] (pre to post, nmol/L)	Immune outcome	Ref
UT	25 healthy adults, 39 years	-	50,000 IU Vit D_2 every other day for 5 days	Vit D group: ↑ 62 from pre < 80	Group with largest increase in plasma 25(OH)D (80 - 160 nmol/L) showed increase in plasma cathelicidin	Bhan et al.(17)
RCT DB	39 athletes, 20 years	Winter	5,000 IU Vit D_3 or placebo daily for 14 weeks	Vit D group: ↑ 55 to 126 Placebo group: ↓ 57 to 33	Vit D group: Plasma cathelicidin ↑ 15% Placebo group: Plasma cathelicidin ↓ 9%	Heet al.(55)
RCT DB	131 healthy adults, 25 - 45 years	Winter and Spring	Single dose of 100,000 IU Vit D_2 or placebo at baseline	Vit D group: ↑ 35 to 67 Placebo group: -	Vit D group: Ability of whole blood to restrict BCG-lux luminescence ↑ 20% compared to placebo No difference in whole blood antigen-stimulated IFN-γ secretion	Martineau et al.(93)
RCT DB	39 MS patients	-	1,000 IU Vit D_3 and 800 mg calcium or placebo daily for 6 months	Vit D group: ↑ 42 to 70 Placebo group: -	Vit D group: TGF-ß1 levels ↑ 28% Placebo group: No effect	Mahon et al.(91)
UT	46 healthy adults, 31 years	Feb to Jun	140,000 IU of Vit D_3 at baseline and week 4	Vit D group: ↑ 60 to 145	Vit D group: % Tregs↑ 17%	Prietlet al.(111)
UT	15 MS patients	Oct to Dec	20,000 IU Vit D_3 daily for 3 months	Vit D group: ↑ 50 to 380 (median)	Vit D group: Proportion of IL-10^{+} CD4^{+} T cells ↑ 92% Ratio between IFN-γ^{+} and IL-4^{+} CD4^{+} T cells ↓ 19%	Smolders et al.(125)

[1]Mean, median or range is provided for age as reported.

[2]Mean values are reported unless stated otherwise.

Hyphen '-' indicates not reported. RCT = Randomised controlled trial. DB = Double-blinded. UT = Uncontrolled trial. BCG = Bacille Calmette Guérin, a vaccine against tuberculosis, prepared from a strain of *Mycobacterium bovis*. IFN-γ = Interferon gamma. MS = Multiple sclerosis.

Table 5. Summary of evidence regarding the effects of oral vitamin D supplementation on self-reported URI.

Study design	Population[1]	Season or month	Supplementation	Change in circulating 25(OH)D[2] (pre to post, nmol/L)	URI outcome	Sig.	Ref
RCT DB	162 healthy adults, 18 - 80 years	Winter	2,000 IU Vit D_3 or placebo daily for 3 months	Vit D group: ↑ 64 to 89 Placebo group: ↓ 63 to 61	<u>Episodes per group</u> Vit D group: 48 Placebo group: 50 <u>Symptom duration (days)</u> Vit D group: 5 Placebo group: 5	NS NS	Li-Ng et al.(82)
RCT DB	164 young Finnish conscripts, 18 - 28 years	Autumn and Winter	400 IU Vit D_3 or placebo daily for 6 months	Vit D group: ↓ 79 to 72 Placebo group: ↓ 74 to 51	<u>Symptom incidence (%, Vit D *vs.* Placebo)</u> Cough 65 *vs.* 57 Runny nose 74 *vs.* 75 Sore throat 48 *vs.* 45 Fever 31 *vs.* 38 <u>Absent from duty due to URI (days)</u> Vit D group: 2 Placebo group: 3	NS NS NS NS NS	Laaksi et al.(75)
RCT DB	322 healthy adults in New Zealand, 47 years	Feb to Nov	Initial dose of 200,000 IU Vit D_3, 200,000 IU a month later, subsequently 100,000 IU monthly or placebo for 18 months	Vit D group: ↑ 72 to > 120 Placebo group: ↓ 70 to < 50	<u>Episodes per person</u> Vit D group: 4 Placebo group: 4 <u>Missed work due to URI (days)</u> Vit D group: 1 Placebo group: 1 <u>Symptom duration (days)</u> Vit D group: 12 Placebo group: 12	NS NS NS	Murdoch et al.(103)

[1]Average age or age range provided where reported.

[2]Mean values are reported unless stated otherwise.

RCT = Randomised controlled trial. DB = Double-blinded. NS = Non-significant.

influence of vitamin D supplementation on immunity; including, the lack of experimental control (111, 125) and co-supplementation with calcium (91). In addition, a few of these studies were conducted using Multiple Sclerosis patients (91, 125), an autoimmune disease characterised by an inflammatory profile, and there is a large discrepancy amongst studies with regards the oral vitamin D dosing regimens (Table 4). As such, more randomised-controlled trials are needed in young, healthy athletic populations to confirm these findings.

Currently, there is little evidence to support vitamin D supplementation to reduce URI incidence and duration (Table 5). Three randomised-controlled trials showed no difference between oral vitamin D supplementation and placebo for URI incidence and duration (75, 82, 103). This is despite the fact that vitamin D supplementation increased circulating 25(OH)D compared to the placebo. The lack of an observed effect of oral vitamin D supplementation on URI in these studies may be due to participants having relatively high base-

ma (i.e. sunburn) for fairer skin types (140) and skin cancer (10). For example, a single minimum erythemal dose (MED: the minimum amount of sunlight that burns the skin) typically provides an oral equivalent vitamin D dose of 10, 000 to 25, 000 IU (140). As such, experts recommend short, frequent exposures to a standard erythemal dose (SED: equates to ~ ¼ to ½ MED for the white UK population) in shorts and t-shirt that does not burn the skin and provides the oral equivalent vitamin D dose of ~1, 000 IU (Figure 5). To ensure safety and
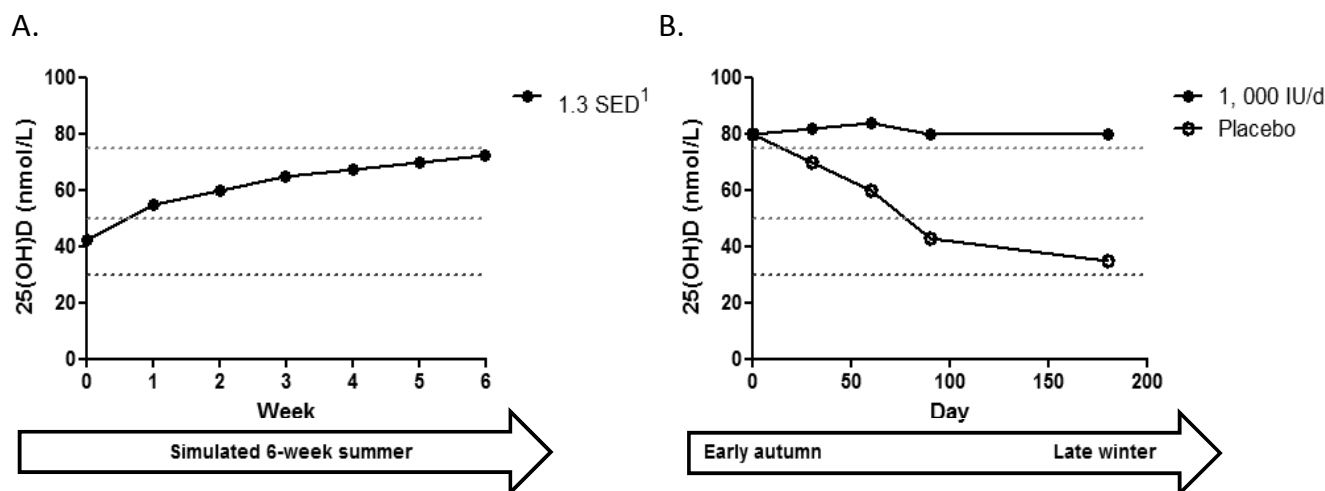
A.

B.

Figure 5. **(A)** Impact of simulated summer sunlight exposures on circulating 25(OH)D, adapted from Rhodes et al. (114). White adult Caucasians from Greater Manchester, UK (53.5 °N) received simulated summer sunlight exposures, specifically 1.3 standard erythemal dose (SED), three times weekly for 6 weeks, while wearing T-shirt and shorts. **(B)** Influence of daily 1, 000 IU oral vitamin D_3 supplementation (close circle) or placebo (open circle) for 6 months from early autumn to late winter in adults living in New Zealand (46 °S), adapted from Logan et al. (86). Dotted horizontal lines indicate Institute of Medicine thresholds for deficiency (30 nmol/L) and sufficiency (50 nmol/L) and the proposed optimal threshold (75 nmol/L) for circulating 25(OH)D to prevent URI (97). [1]To avoid the confounding influence of skin exposure to ambient UVB the study commenced in wintertime (53.5 °N) when sunlight is negligible and circulating 25(OH)D is at its nadir.

line vitamin D levels at the outset (baseline circulating 25(OH)D levels range from 64 to 79 nmol/L). Indeed, it has been suggested that boosting the 25(OH)D level in those with vitamin D deficiency (circulating 25(OH)D level < 30 nmol/L) activates various innate and adaptive immune responses that are critical in the control of some respiratory viral infections; however, boosting from a higher starting level of 25(OH)D probably provides no additional benefit (22). Randomised-control trials of oral vitamin D supplementation are sorely needed in athletes and military personnel around the winter-time nadir in circulating 25(OH)D level when evidence indicates vitamin D deficiency in up to 35% of individuals (Table 2). Moreover, for convenience, rather than confirming the presence of pathogens in oral/nasopharyngeal swabs, studies have tended to rely on self-report of URI using common-cold symptom questionnaires that have been criticised (36). Notwithstanding this limitation, symptoms of URI without a detectable pathogen appear to be common in those under heavy training stress (36) and likely have a negative impact upon training and performance (113).

6.2 The effects of UVB irradiation on vitamin D status and immunity
As introduced in section 1.1, for a range of skin colours and latitudes between 30 and 60 °N, the majority of vitamin D can be obtained through short-lasting skin exposures to natural UVB irradiation from summer sunlight (~15 min each day). Importantly, prolonged exposures give diminishing returns in terms of vitamin D formation (61) and raise the risk of erythe-

efficacy, exposing a large surface area over a shorter duration as opposed to a small surface area for prolonged periods is recommended to increase circulating levels of vitamin D. For example, full body exposure for 2 min is preferable to over-exposing a (bald) head and neck for 20 min (139).

It remains unknown if UVB exposure of the skin (either from sunlight or a sun cabinet) has additional benefits on immune function, health and performance independent of the synthesis of vitamin D. For example, UV radiation generates nitric oxide locally at the skin which has been associated with benefits to cardiovascular health via a decrease in systemic blood pressure (67). In addition to its effects on vasodilatation, nitric oxide may also influence neurotransmission, immune defence, regulation of cell death (apoptosis) and cell motility (67). The potential for mood enhancement (possibly mediated via increased β-endorphins) and stress reduction with skin sunlight or artificial UVB exposure to influence immune function, health and performance should not be overlooked. Indeed, it's possible, but remains unknown, that UVB exposure of the skin improves immune function and exercise performance to a greater extent than the equivalent oral vitamin D supplementation due to enhanced levels of nitric oxide, mood or some as yet unknown mechanism.

A method to replicate safe summer sunlight exposure using sub-erythemal solar simulated radiation in a laboratory-based irradiation cabinet has been developed by Rhodes and colleagues (Figure 5A) (114). During the winter months, when

vitamin D status was low, this method restored adequate circulating levels of vitamin D (25(OH)D level > 50 nmol/L) in the majority of volunteers. Importantly, in line with policy recommendations in the UK, this method simulates summer sunlight exposure (at latitudes between 30 and 60 °N for most skin types), on several occasions each week, for ~15 min wearing t-shirt and shorts without producing sunburn (140). Whether this method to restore adequate circulating vitamin D in the winter improves immunity and host defence remains largely unknown and requires investigation. Promising work shows a threefold increase in circulating Tregs (CD4+CD25^{hi}FoxP3$^+$) that correlated positively with the change in circulating 25(OH)D in patients with immune-mediated skin disease (e.g. psoriasis) after 4 weeks of phototherapy treatment (96).

7. FACTORS AFFECTING VITAMIN D STATUS IN ATHLETES AND MILITARY PERSONNEL

Vitamin D can be increased through natural food sources or the exposure of skin to UVB radiation. The vitamin D production in the skin from sufficient UVB exposure provides 80-100% of body requirements (78). In particular, several factors can affect the production of vitamin D via UVB exposure, these include season and latitude as well as age, skin colour, clothing and sunscreen use.

7.1 Season and latitude
The solar zenith angle (SZA) is the angle between the local vertical and the position of the sun in the sky. During the summer and at low latitudes the SZA is small. Conversely, the SZA is large during the winter and at high latitudes. At a large SZA, UVB radiation (290 – 320 nm) travels a longer path through the atmosphere and there is greater attenuation of the radiation compared to a small SZA (139). Consequently, the amount of UVB radiation reaching the Earth's surface is reduced and scattered over a larger area (139). This explains why vitamin D deficiency is more prevalent in countries at high latitudes and particularly during the winter months (43). Furthermore, it has been estimated that the contribution of sunlight to vitamin D status is only 20% during the winter (88) and 80% (89) during the spring and summer thus suggesting the importance of the increase in the contribution of diet during winter in order to prevent vitamin D deficiency.

7.2 Age
As one ages, cutaneous vitamin D production declines. This is due to the decrease in the amount of 7-dehydrocholesterol available in the epidermal layer of the skin, where the majority of vitamin D is formed following exposure to UVB radiation (90). Despite the decline in vitamin D$_3$ production with age, the elderly can still achieve adequate amounts of vitamin D in the summer through regular skin exposure to sunlight (141) (section 6.2). Nonetheless, older athletes living in the northern latitude may wear more clothing and train mostly indoors. As regular skin sunlight exposure is limited, this group of elderly people is at a greater risk of vitamin D deficiency. Therefore, a combination of regular sun exposure where possible and increased vitamin D intake from the daily diet and oral supplementation are important considerations to ensure that older athletes have adequate vitamin D.

7.3 Skin colour
The amount of melanin pigment in the skin can interfere with vitamin D synthesis by absorbing UVB radiation and blocking the wavelength of sunlight required to synthesise vitamin D (89), thus preventing the cutaneous production of pre-vitamin D$_3$. Melanin content is higher in dark-skinned individuals compared to fair-skinned individuals. Hence, for a given dose of UVB, a dark-skinned individual will produce less pre-vitamin D$_3$ than a fair-skinned individual (30). An analysis of the vitamin D status in 63 elite UK track and field athletes reported 7% of dark-skinned athletes with 25(OH)D < 50 nmol/L compared to only 1% of fair-skinned athletes during the summer (110). When individuals were given UVB doses adjusted for their skin colour, it was discovered that there was a tendency for dark-skinned individuals to show a smaller increase in 25(OH)D (9). Nonetheless, it should be noted that, dark-skinned individuals can produce equivalent amounts of vitamin D$_3$ as their fair-skinned counterparts when exposed to adequate amounts of UVB radiation (30).

7.4 Clothing
Clothing can act as a physical barrier preventing UVB radiation from reaching the skin. As the majority of vitamin D is synthesised in the skin, any area covered by clothing will reduce the exposed skin surface area to sunlight. Broadly speaking, in terms of vitamin D synthesis, there is an inverse relationship between the surface area of the skin exposed to sunlight and the duration of exposure. To illustrate this relationship, a fully clothed person with the head and neck exposed for 20 min would synthesise an equivalent amount of vitamin D to exposing the whole body for 2 min (139). In the summer, athletes who train and compete for prolonged periods in short-sleeved tops and shorts may not receive adequate sun protection. In contrast, military recruits who train in long-sleeved uniform and wear helmets (and those who train indoors or who cover their skin for religious reasons) are at risk of a lack of sun exposure and vitamin D deficiency. In these groups at risk of vitamin D deficiency, alternative methods to increase vitamin D levels e.g. solar-simulated radiation or oral supplementation warrant investigation (see recommendations in section 10).

7.5 Sunscreen use
The use of sunscreen interferes with vitamin D$_3$ formation by absorbing and reflecting UVB radiation, thus preventing UVB radiation from reaching the target skin cells. Topical application of a sunscreen of sun protection factor 8 was found to limit vitamin D$_3$ production in protected compared to unprotected participants (94). Although sunscreen use can be beneficial in preventing sunburn and skin cancer, it should be used appropriately. For example, in the summer, athletes exposed to UVB radiation for prolonged periods are at increased risk of sunburn and should be encouraged to apply a broad-spectrum water-resistant sunscreen of at least SPF 30-50 every 2 to 4 hours (53). On the other hand, the elderly have a reduced ability to synthesise vitamin D cutaneously and are at greater risk of vitamin D deficiency. It has been recommended that they expose their hands, face, arms and legs to summer sun-

Table 6. Dietary sources of vitamin D[1].

Sources	Vitamin D content
Natural foods	
Cod liver oil	~ 400 - 1000 IU/teaspoon vitamin D_3
Salmon (fresh, wild)	~ 600 - 1000 IU/100 g vitamin D_3
Salmon (fresh, farmed)	~ 100 - 250 IU/100 g vitamin D_3
Salmon (canned)	~ 300 - 600 IU/100 g vitamin D_3
Sardines (canned)	~ 300 IU/100 g vitamin D_3
Mackerel (canned)	~ 250 IU/100 g vitamin D_3
Tuna (canned)	~ 230 IU/100 g vitamin D_3
Herring in oil	~ 800 IU/100 g vitamin D_3
Pickled herring	~ 480 IU/100 g vitamin D_3
Shiitake mushrooms (fresh)	~ 100 IU/100 g vitamin D_2
Shiitake mushrooms (dried)	~ 1600 IU/100 g vitamin D_2
Egg yolk	~ 20 - 50 IU/yolk vitamin D_3
Cheese	~ 7 - 28 IU/100 g vitamin D_3
Cow's milk	~ 0.4 - 1.2 IU/100 ml vitamin D_3
Fortified foods[2]	
Fortified milk	~ 100 IU/237 ml vitamin D_3
Fortified orange juice	~ 100 IU/237 ml vitamin D_3
Fortified yoghurts	~ 100 IU/237 ml vitamin D_3
Fortified butter	~ 50 IU/100 g vitamin D_3
Fortified margarine	~ 430 IU/100 g vitamin D_3
Fortified cheeses	~ 100 IU/85 g vitamin D_3
Fortified breakfast cereals	~ 100 IU/30 g vitamin D_3

[1]Adapted from Holick et al. (63) and Pludowski et al. (109).
[2]Countries with fortification policies include Australia, Finland, UK and the USA (77, 89, 98, 104).
IU denotes international unit. 1 IU is equivalent to 0.025 μg. To convert μg to IU multiply by 40.

light two to three times a week for only ~¼ of the duration that will take for them to reach mid sunburn and apply SPF ≥ 15 on all exposed skin for any further time spent outdoors (61, 62). This will allow the elderly to obtain the beneficial effects of sunlight for vitamin D nutrition whilst avoiding the detrimental effects of overexposure.

7.6 Natural food sources
As mentioned earlier, vitamin D can be obtained from the diet (Table 6) by consuming foods such as oily fish (e.g. tuna, mackerel, salmon), shiitake mushrooms and egg yolks (63). Interestingly, an analysis of the vitamin D_3 content in a variety of oily fish showed that farmed salmon contains only 25% of the vitamin D_3 found in wild caught Alaskan salmon (30), suggesting that wild-type fish are a better source of vitamin D_3 than farmed varieties. In countries such as America and Canada, some foods such as milk, breakfast cereals and margarine are also fortified with vitamin D in order to increase vitamin D intake (23) (Table 6).

8. VITAMIN D TOXICITY

Excessive intake of vitamin D can result in vitamin D intoxication, which is characterised by hypercalcaemia (total serum calcium corrected for albumin > 2.6 mmol/L), renal stones and renal calcification, with kidney failure and death (57). Except for infrequent cases of accidental or intentional poisoning, this is extremely rare. Both the intoxication literature and several controlled dosing studies show no cases of confirmed intoxication at circulating 25(OH)D levels below 500 nmol/L. Correspondingly, the oral intakes needed to produce such levels are in excess of 20, 000 IU/day in otherwise healthy adults and 10, 000 IU/day (which is substantially

more than is apparently needed for any recognised efficacy endpoint) is considered as the tolerable upper intake level (57). Incidentally, it is worth noting that whole-body skin sun exposure, such as might be achieved in a few minutes on a summer day, produces an endogenous vitamin D production of 10, 000 to 20, 000 IU, depending upon skin type (9). Thus, frequent summer sun exposures produce inputs of the same magnitude as the proposed upper intake level (can be characterised as a "physiological") and there has never been a case of vitamin D intoxication reported as a result of sun exposure.

The toxicity of intakes of high oral doses of vitamin D has been established on the basis of relatively short-term studies and there has to be some concern about the longer-term implications for health of high vitamin D intakes over a lifetime. The IoM indicates that sparse data are available for upper circulating 25(OH)D levels in humans, and values above 125-150 nmol/L should raise concerns about potential adverse effects because of several large scale studies indicating an increased multivariable-adjusted risk of all-cause mortality not only for circulating 25(OH)D levels below 30 nmol/L, but also for levels above 125 nmol/L (66). The all-cause mortality data emerging from the examination of national survey data as well as observational studies suggest adverse effects at circulating 25(OH)D levels much lower than those associated with the toxicity demonstrated by short-term acute hypervitaminosis D. In general, these studies, as expected, indicated that low circulating 25(OH)D levels akin to < 30 nmol/L are associated with an increased risk of mortality. Furthermore, as circulating 25(OH)D levels increase up to a point mortality is lowered. However, some, but not all, of the studies have observed a troubling U-shaped relationship with a statistically significant trend between increasing circulating 25(OH)D levels and lower odds ratios for all-cause mortality. For these reasons, a circulating 25(OH)D of above 125-150 nmol/L is not recommended, corresponding to intakes of not more than 5, 000 IU/day in the absence of adequate sun exposure.

9. CONCLUSIONS

A multitude of studies have suggested that vitamin D deficiency (circulating 25(OH)D level < 30 nmol/L) not only has negative consequences on bone health but also increases the risk for many acute and chronic illnesses, including respiratory infections. Recent work in athletes shows beneficial effects of optimising vitamin D status on innate immunity and mucosal immunity and vitamin D exerts anti-inflammatory actions through the induction of regulatory T cells and the inhibition of pro-inflammatory cytokine production. Although the incidence of vitamin D insufficiency (circulating 25(OH)D level < 50 nmol/L) appears to be similar in athletic and non-athletic populations, studies show that more than half of all athletes and military personnel are vitamin D insufficient in the winter months and as many as 35% are vitamin D deficient. To date,

studies point to the benefits of avoiding vitamin D deficiency to maintain immunity and reduce the burden of URI in athletes and military personnel. In answer to the question posed in the title of this review, there is broad agreement that a circulating 25(OH)D level of 75 nmol/L represents an optimal vitamin D status for the prevention of URI (97). Fruitful future lines of enquiry include verification of this proposed optimal vitamin D status to maintain immunity and resistance against URI in athletes and military personnel. In addition, investigations should explore whether UVB exposure of the skin (either from sunlight or an irradiation cabinet) has additional benefits on immune function, health and exercise performance independent of the synthesis of vitamin D.

10. PRACTICAL RECOMMENDATIONS (FIGURE 6)

In Figure 6 we attempt to provide some simple practical recommendations for athletes and military personnel on how vitamin D sufficiency can be achieved in the summer and maintained during the winter. Mindful of key factors such as latitude and skin type, as little as 15 min of exposure to summer sunlight between 10am and 3pm wearing t-shirts and shorts on most days can achieve vitamin D sufficiency in most individuals and levels deemed optimal in some (Figure 5A and 6) (114, 140). Dietary sources of vitamin D and vitamin D supplements become important considerations during the winter months when skin sunlight as a source of vitamin D is absent or drastically reduced (Figure 6). Studies have shown that consuming a 1, 000 IU/day vitamin D_3 supplement during the winter can achieve vitamin D sufficiency in most individuals (86, 89) and maintain end-of-summer 25(OH)D levels throughout the autumn and winter (Figure 5B) (86). Finally, the recommendation to take a 1, 000 IU/day vitamin D_3 supplement in the autumn-winter may also be suitable for those who cannot achieve the safe summer sunlight guidance (Figure 6). For example, individuals training indoors in the summer or those required to wear clothing (for protective or religious reasons) that restricts skin sunlight exposure in the summer may benefit from 1, 000 IU/day vitamin D_3 supplementation year-round as there is evidence that these individuals can suffer vitamin D deficiency, even during the summer months (Table 2) (51, 52). Further research endeavours are required to determine whether following these recommendations for vitamin D benefit athletes and military personnel by maintaining immunity and increasing resistance against URI.
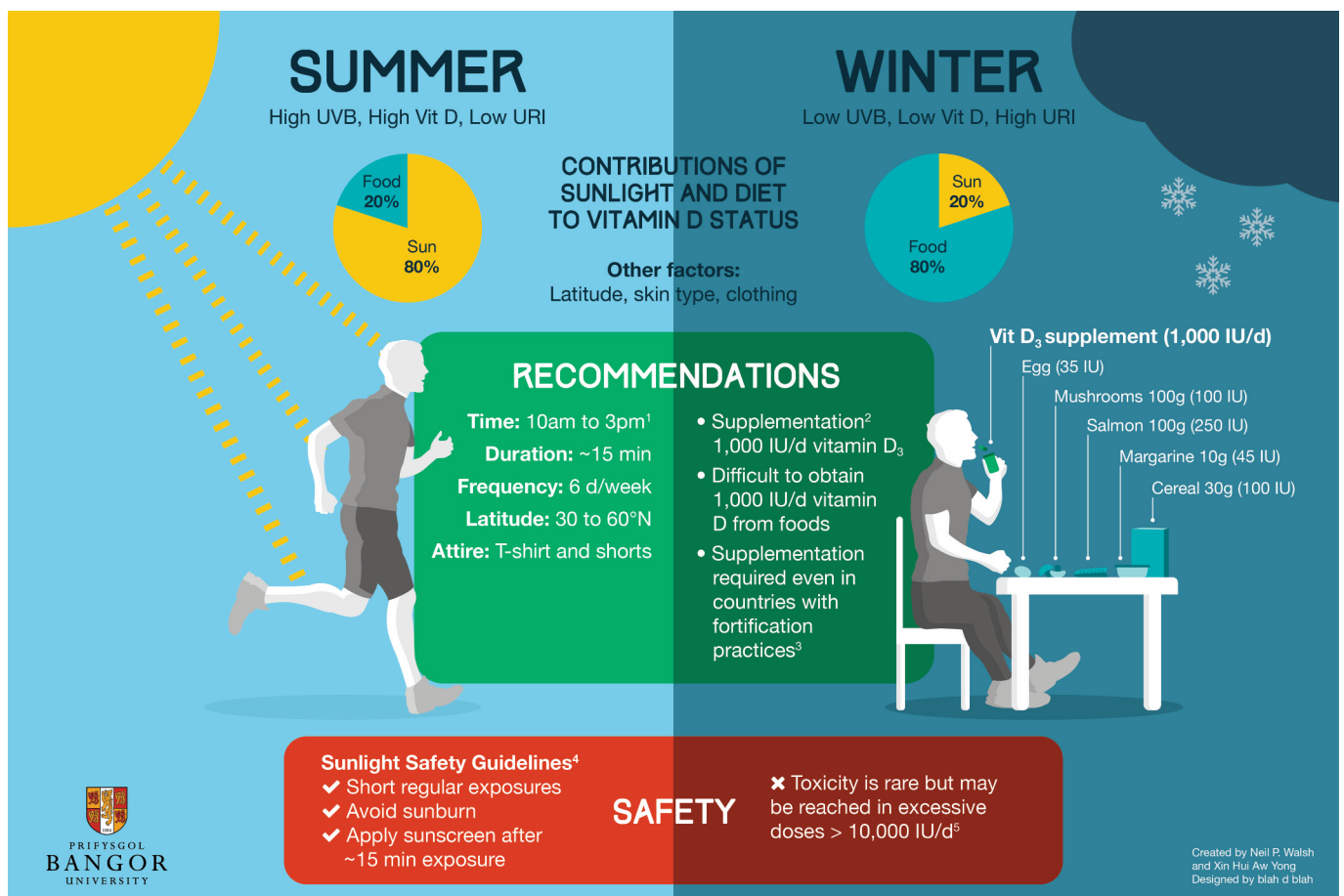

Figure 6. Practical recommendations for athletes and military personnel on how vitamin D sufficiency can be achieved in the summer and maintained during the winter. [1]Recommended summer sunlight exposure (114, 140). [2]Recommended oral vitamin D_3 supplementation (86, 89). [3]Countries with fortification policies include Australia, Finland, UK and the USA (77, 89, 98, 104) but average dietary vitamin D intake remains below 1, 000 IU/day. [4]Sunlight safety recommendations (139, 140). [5]Safety recommendations for oral vitamin D supplementation (57). UVB = ultraviolet-B. URI = upper respiratory illness.

REFERENCES

1. Adorini L and Penna G. Dendritic cell tolerogenicity: a key mechanism in immunomodulation by vitamin D receptor agonists. Hum Immunol 70: 345-352, 2009.

2. Agborsangaya CB, Surcel HM, Toriola AT, Pukkala E, Parkkila S, Tuohimaa P, Lukanova A and Lehtinen M. Serum 25-hydroxyvitamin D at pregnancy and risk of breast cancer in a prospective study. Eur J Cancer 46: 467-470, 2010.

3. Al-Jaderi Z and Maghazachi AA. Effects of vitamin D_3, calcipotriol and FTY720 on the expression of surface molecules and cytolytic activities of human natural killer cells and dendritic cells. Toxins 5: 1932-1947, 2013.

4. Alshahrani F and Aljohani N. Vitamin D: deficiency, sufficiency and toxicity. Nutrients 5: 3605-3616, 2013.

5. Ameri P, Giusti A, Boschetti M, Murialdo G, Minuto F and Ferone D. Interactions between vitamin D and IGF-1: from physiology to clinical practice. Clin Endocrinol 79: 457-463, 2013.

6. Angeline ME, Gee AO, Shindle M, Warren RF and Rodeo SA. The effects of vitamin D deficiency in athletes. Am J Sports Med 41: 461-464, 2013.

7. Antoniucci DM, Black DM and Sellmeyer DE. Serum 25-hydroxyvitamin D is unaffected by multiple freeze-thaw cycles. Clin Chem 51: 258-261, 2005.

8. Aranow C. Vitamin D and the immune system. J Invest Med 59: 881-886, 2011.

9. Armas LA, Dowell S, Akhter M, Duthuluru S, Huerter C, Hollis BW, Lund R and Heaney RP. Ultraviolet-B radiation increases serum 25-hydroxyvitamin D levels: the effect of UVB dose and skin color. J Am Acad Dermatol 57: 588-593, 2007.

10. Armstrong BK and Kricker A. The epidemiology of UV induced skin cancer. J Photochem Photobiol B 63: 8-18, 2001.

11. Baeke F, Takiishi T, Korf H, Gysemans C and Mathieu C. Vitamin D: modulator of the immune system. Curr Opin Pharmacol 10: 482-496, 2010.

12. Bannert N, Starke I, Mohnike K and Frohner G. Parameters of mineral metabolism in children and adolescents in athletic training. Kinderarztl Prax 59: 153-156, 1991.

13. Barker T, Henriksen VT, Martins TB, Hill HR, Kjeldsberg CR, Schneider ED, Dixon BM and Weaver LK. Higher serum 25-hydroxyvitamin D concentrations associate with a faster recovery of skeletal muscle strength after muscular injury. Nutrients 5: 1253-1275, 2013.

14. Barker T, Martins TB, Hill HR, Kjeldsberg CR, Trawick RH, Weaver LK and Traber MG. Low vitamin D impairs strength recovery after anterior cruciate ligament surgery. J Evid Based Complementary Altern Med 16: 201-209, 2011.

15. Bendik I, Friedel A, Roos FF, Weber P and Eggersdorfer M. Vitamin D: a critical and essential micronutrient for human health. Front Physiol 5: 248, 2014.

16. Berry DJ, Hesketh K, Power C and Hyppönen E. Vitamin D status has a linear association with seasonal infections and lung function in British adults. Br J Nutr 106: 1433-1440, 2011.

17. Bhan I, Camargo CA, Wenger J, Ricciardi C, Ye J, Borregaard N and Thadhani R. Circulating levels of 25-hydroxyvitamin D and human cathelicidin in healthy adults. J Allergy Clin Immunol 127: 1302-1304, 2011.

18. Bikle D. Nonclassic actions of vitamin D. J Clin Endocrinol Metab 94: 26-34, 2009.

19. Binder R, Kress A, Kan G, Herrmann K and Kirschfink M. Neutrophil priming by cytokines and vitamin D binding protein (Gc-globulin): impact on C5a-mediated chemotaxis, degranulation and respiratory burst. Mol Immunol 36: 885-892, 1999.

20. Bischoff-Ferrari HA. Optimal serum 25-hydroxyvitamin D levels for multiple health outcomes. Adv Exp Med Biol 810: 500-525, 2014.

21. Boonstra A, Barrat FJ, Crain C, Heath VL, Savelkoul HF and O'Garra A. 1, 25-Dihydroxyvitamin D3 has a direct effect on naive CD4+ T cells to enhance the development of Th2 cells. J Immunol 167: 4974-4980, 2001.

22. Bryson KJ, Nash AA and Norval M. Does vitamin D protect against respiratory viral infections? Epidemiol Infect 142: 1789-1801, 2014.

23. Calvo MS, Whiting SJ and Barton CN. Vitamin D fortification in the United States and Canada: current status and data needs. Am J Clin Nutr 80: 1710S-1716S, 2004.

24. Cannell JJ, Vieth R, Umhau JC, Holick MF, Grant WB, Madronich S, Garland CF and Giovannucci E. Epidemic influenza and vitamin D. Epidemiol Infect 134: 1129-1140, 2006.

25. Cantorna MT, Woodward WD, Hayes CE and DeLuca HF. 1, 25-Dihydroxyvitamin D3 is a positive regulator for the two anti-encephalitogenic cytokines TGF-ß and IL-4. J Immunol 160: 5314-5319, 1998.

26. Carter GD and Jones JC. Use of a common standard improves the performance of liquid chromatography-tandem mass spectrometry methods for serum 25-hydroxyvitamin-D. Ann Clin Biochem 46: 79-81, 2009.

27. Chang SH, Chung Y and Dong C. Vitamin D suppresses Th17 cytokine production by inducing C/EBP homologous protein (CHOP) expression. J Biol Chem 285: 38751-38755, 2010.

28. Chapuy MC, Preziosi P, Maamer M, Arnaud S, Galan P, Hercberg S and Meunier PJ. Prevalence of vitamin D insufficiency in an adult normal population. Osteoporosis Int 7: 439-443, 1997.

29. Chen J, Olivares-Navarrete R, Wang Y, Herman TR, Boyan BD and Schwartz Z. Protein-disulfide isomerase-associated 3 (Pdia3) mediates the membrane response to 1, 25-dihydroxyvitamin D3 in osteoblasts. J Biol Chem 285: 37041-37050, 2010.

30. Chen TC, Chimeh F, Lu Z, Mathieu J, Person KS, Zhang A, Kohn N, Martinello S, Berkowitz R and Holick MF. Factors that influence the cutaneous synthesis and dietary sources of vitamin D. Arch Biochem Biophys 460: 213-217, 2007.

31. Chun RF, Liu PT, Modlin RL, Adams JS and Hewison M. Impact of vitamin D on immune function: lessons learned from genome-wide analysis. Front Physiol 5: 151, 2014.

32. Clairmont A, Tessmann D, Stock A, Nicolai S, Stahi W and Sies H. Induction of gap junctional intercellular communication by vitamin D in human skin fibroblasts is dependent on the nuclear vitamin D receptor. Carcinogenesis 17: 1389-1391, 1996.

33. Close GL, Russell J, Cobley JN, Owens DJ, Wilson G, Gregson W, Fraser WD and Morton JP. Assessment of vitamin D concentration in non-supplemented professional athletes and healthy adults during the winter months in the UK: implications for skeletal muscle function. J Sports Sci 31: 344-353, 2013.

34. Close GL, Leckey J, Patterson M, Bradley W, Owens DJ, Fraser WD and Morton JP. The effects of vitamin D3 supplementation on serum total 25 [OH] D concentration and physical performance: a randomised dose-response study. Br J Sports Med 47: 692-696, 2013.

35. Constantini NW, Arieli R, Chodick G and Dubnov-Raz G. High prevalence of vitamin D insufficiency in athletes and dancers. Clin J Sport Med 20: 368-371, 2010.

36. Cox AJ, Gleeson M, Pyne DB, Callister R, Hopkins WG and Fricker PA. Clinical and laboratory evaluation of upper respiratory symptoms in elite athletes. Clin J Sport Med 18: 438-445, 2008.

37. Edfeldt K, Liu PT, Chun R, Fabri M, Schenk M, Wheelwright M, Keegan C, Krutzik SR, Adams JS, Hewison M and Modlin RL. T-cell cytokines differentially control human monocyte antimicrobial responses by regulating vitamin D metabolism. Proc Natl Acad Sci U S A 107: 22593-22598, 2010.

38. Farrell CJ, Martin S, McWhinney B, Straub I, Williams P and Herrmann M. State-of-the-art vitamin D assays: a comparison of automated immunoassays with liquid chromatography-tandem mass spectrometry methods. Clin Chem 58: 531-542, 2012.

39. Fraser WD. Standardization of vitamin D assays: art or science? Ann Clin Biochem 46: 3-4, 2009.

40. Fraser WD and Milan AM. Vitamin D assays: past and present debates, difficulties, and developments. Calcif Tissue Int 92: 118-127, 2013.

41. Funderburk LK, Daigle K and Arsenault JE. Vitamin D status among overweight and obese soldiers. Mil Med 180: 237-240, 2015.

42. Garcia RB and Guisado FR. Low levels of vitamin D in professional basketball players after wintertime: relationship with dietary intake of vitamin D and calcium. Nutr Hosp 26: 945-951, 2011.

43. Genuis SJ, Schwalfenberg GK, Hiltz MN and Vaselenak SA. Vitamin D status of clinical practice populations at higher latitudes: analysis and applications. Int J Environ Res Public Health 6: 151-173, 2009.

44. Ginde AA, Mansbach JM and Camargo CA, Jr. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. Arch Intern Med 169: 384-390, 2009.

45. Ginde AA, Mansbach JM and Camargo CA, Jr. Vitamin D, respiratory infections, and asthma. Curr Allergy Asthma Rep 9: 81-87, 2009.

46. Girgis CM, Clifton-Bligh RJ, Turner N, Lau SL and Gunton JE. Effects of vitamin D in skeletal muscle: falls, strength, athletic performance and insulin sensitivity. Clin Endocrinol 80: 169-181, 2014.

47. Gniadecki R, Gajkowska B and Hansen M. 1, 25-dihydroxyvitamin D3 stimulates the assembly of adherens junctions in keratinocytes: involvement of protein kinase C. Endocrinology 138: 2241-2248, 1997.

48. Godbout JP and Glaser R. Stress-induced immune dysregulation: implications for wound healing, infectious disease and cancer. J Neuroimmune Pharm 1: 421-427, 2006.

49. Gorman S, Kuritzky LA, Judge MA, Dixon KM, McGlade JP, Mason RS, Finlay-Jones JJ and Hart PH. Topically applied 1, 25-dihydroxyvitamin D3 enhances the suppressive activity of CD4+ CD25+ cells in the draining lymph nodes. J Immunol 179: 6273-6283, 2007.

50. Hamilton B. Vitamin D and human skeletal muscle. Scand J Med Sci Sports 20: 182-190, 2010.

51. Hamilton B, Grantham J, Racinais S and Chalabi H. Vitamin D deficiency is endemic in middle eastern sportsmen. Public Health Nutr 13: 1528-1534, 2010.

52. Hamilton B, Whiteley R, Farooq A and Chalabi H. Vitamin D concentration in 342 professional football players and association with lower limb isokinetic function. J Sci Med Sport 17: 139-143, 2014.

53. Harrison SC and Bergfeld WF. Ultraviolet light and skin cancer in athletes. Sports Health 1: 335-340, 2009.

54. He CS, Fraser WD and Gleeson M. Influence of vitamin d metabolites on plasma cytokine concentrations in endurance sport athletes and on multiantigen stimulated cytokine production by whole blood and peripheral blood mononuclear cell cultures. ISRN Nutr: 820524, 2014.

55. He CS, Gleeson M, Tanqueray E, Rudland-Thomas J, Brown K and Renwick S. The effect of chronic vitamin D3 supplementation on antimicrobial peptides and proteins in athletes. J Sports Sci 32, S6-S7, 2014.

56. He CS, Handzlik M, Fraser WD, Muhamad A, Preston H, Richardson A and Gleeson M. Influence of vitamin D status on respiratory infection incidence and immune function during 4 months of winter training in endurance sport athletes. Exerc Immunol Rev 19: 86-101, 2013.

57. Heaney RP. Vitamin D: criteria for safety and efficacy. Nutr Rev 66: S178-S181, 2008.

58. Hellard P, Avalos M, Guimaraes F, Toussaint JF and Pyne DB. Training-related risk of common illnesses in elite swimmers over a 4-yr period. Med Sci Sports Exerc 47: 698-707, 2015.

59. Hewison M. Vitamin D and immune function: an overview. P Nutr Soc 71: 50-61, 2012.

60. Hewison M. Vitamin D and immune function: autocrine, paracrine or endocrine? Scand J Clin Lab Invest Suppl 72: 92-102, 2012.

61. Holick MF. Environmental factors that influence the cutaneous production of vitamin D. Am J Clin Nutr 61: 638S-645S, 1995.

62. Holick MF. Vitamin D: A millenium perspective. J Cell Biochem 88: 296-307, 2003.

63. Holick MF. Vitamin D deficiency. New Engl J Med 357: 266-281, 2007.

64. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH and Weaver CM. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 96: 1911-1930, 2011.

65. Houghton LA and Vieth R. The case against ergocalciferol (vitamin D2) as a vitamin supplement. Am J Clin Nutr 84: 694-697, 2006.

66. Institute of Medicine (IOM). Dietary reference intakes for calcium and vitamin D. Washington DC: National Academies Press, 2011.

67. Juzeniene A and Moan J. Beneficial effects of UV radiation other than via vitamin D production. Dermatoendocrinol 4: 109-117, 2012.

68. Kamen DL and Tangpricha V. Vitamin D and molecular actions on the immune system: modulation of innate and autoimmunity. J Mol Med 88: 441-450, 2010.

69. Kankova M, Luini W, Pedrazzoni M, Riganti F, Sironi M, Bottazzi B, Mantovani A and Vecchi A. Impairment of cytokine production in mice fed a vitamin D3-deficient diet. Immunology 73: 466-471, 1991.

70. Khoo AL, Chai LYA, Koenen HJPM, Kullberg BJ, Joosten I, van der Ven AJ and Netea MG. 1, 25-dihydroxyvitamin D3 modulates cytokine production induced by Candida albicans: impact of seasonal variation of immune responses. J Infect Dis 203: 122-130, 2011.

71. Kiebzak GM, Moore NL, Margolis S, Hollis B and Kevorkian CG. Vitamin D status of patients admitted to a hospital rehabilitation unit: relationship to function and progress. Am J Phys Med Rehabil 86: 435-445, 2007.

72. Koeffler HP, Reichel H, Bishop JE and Norman AW. Gamma-interferon stimulates production of 1, 25-dihydroxyvitamin D 3 by normal human macrophages. Biochem Bioph Res Commun 127: 596-603, 1985.

73. Kongsbak M, Levring TB, Geisler C and Von Essen MR. The vitamin D receptor and T cell function. Front Immunol 4: 148, 2013.

74. Kraemer WJ and Ratamess NA. Hormonal responses and adaptations to resistance exercise and training. Sports Med 35: 339-361, 2005.

75. Laaksi I, Ruohola JP, Mattila V, Auvinen A, Ylikomi T and Pihlajamäki H. Vitamin D supplementation for the prevention of acute respiratory tract infection: a randomized, double-blinded trial among young Finnish men. J Infect Dis 202: 809-814, 2010.

76. Laaksi I, Ruohola JP, Tuohimaa P, Auvinen A, Haataja R, Pihlajamäki H and Ylikomi T. An association of serum vitamin D concentrations < 40 nmol/L with acute respiratory tract infection in young Finnish men. Am J Clin Nutr 86: 714-717, 2007.

77. Laaksi IT, Ruohola JS, Ylikomi TJ, Auvinen A, Haataja RI, Pihlajamäki HK and Tuohimaa PJ. Vitamin D fortification as public health policy: significant improvement in vitamin D status in young Finnish men. Eur J Clin Nutr 60: 1035-1038, 2006.

78. Lanteri P, Lombardi G, Colombini A and Banfi G. Vitamin D in exercise: physiologic and analytical concerns. Clin Chim Acta 415: 45-53, 2013.

79. Lappe J, Cullen D, Haynatzki G, Recker R, Ahlf R and Thompson K. Calcium and vitamin D supplementation decreases incidence of stress fractures in female navy recruits. J Bone Miner Res 23: 741-749, 2008.

80. Larson-Meyer DE and Willis KS. Vitamin D and athletes. Curr Sports Med Rep 9: 220-226, 2010.

81. Lemire JM. Immunomodulatory actions of 1, 25-dihydroxyvitamin D 3. J Steroid Biochem Mol Biol 53: 599-602, 1995.

82. Li-Ng M, Aloia JF, Pollack S, Cunha BA, Mikhail M, Yeh J and Berbari N. A randomized controlled trial of vitamin D3 supplementation for the prevention of symptomatic upper respiratory tract infections. Epidemiol Infect 137: 1396-1404, 2009.

83. Liao L, Chen X, Wang S, Parlow AF and Xu J. Steroid receptor coactivator 3 maintains circulating insulin-like growth factor I (IGF-I) by controlling IGF-binding protein 3 expression. Mol Cell Biol 28: 2460-2469, 2008.

84. Liu PT, Schenk M, Walker VP, Dempsey PW, Kanchanapoomi M, Wheelwright M, Vazirnia A, Zhang X, Steinmeyer A and Zugel U. Convergence of IL-1beta and VDR activation pathways in human TLR2/1-induced antimicrobial responses. PLoS One 4: e5810, 2009.

85. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schauber J, Wu K, Meinken C, Kamen DL, Wagner M, Bals R, Steinmeyer A, Zügel U, Gallo RL, Eisenberg D, Hewison M, Adams JS, Bloom BR and Modlin RL. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 311: 1770-1773, 2006.

86. Logan VF, Gray AR, Peddie MC, Harper MJ and Houghton LA. Long-term vitamin D3 supplementation is more effective than vitamin D2 in maintaining serum 25-hydroxyvitamin D status over the winter months. Br J Nutr 109: 1082-1088, 2013.

87. Lovell G. Vitamin D status of females in an elite gymnastics program. Clin J Sport Med 18: 159-161, 2008.

88. Macdonald HM, Mavroeidi A, Fraser WD, Darling AL, Black AJ, Aucott L, O'Neill F, Hart K, Berry JL and Lanham-New SA. Sunlight and dietary contributions to the seasonal vitamin D status of cohorts of healthy postmenopausal women living at northerly latitudes: a major cause for concern? Osteoporosis Int 22: 2461-2472, 2011.

89. Macdonald HM. Contributions of sunlight and diet to vitamin D status. Calcif Tissue Int 92: 163-176, 2013.

90. MacLaughlin J and Holick MF. Aging decreases the capacity of human skin to produce vitamin D3. J Clin Invest 76: 1536-1538, 1985.

91. Mahon BD, Gordon SA, Cruz J, Cosman F and Cantorna MT. Cytokine profile in patients with multiple sclerosis following vitamin D supplementation. J Neuroimmunol 134: 128-132, 2003.

92. Maroon JC, Mathyssek CM, Bost JW, Amos A, Winkelman R, Yates AP, Duca MA and Norwig JA. Vitamin D profile in national football league players. Am J Sports Med 43: 1241-1245, 2015.

93. Martineau AR, Wilkinson RJ, Wilkinson KA, Newton SM, Kampmann B, Hall BM, Packe GE, Davidson RN, Eldridge SM, Maunsell ZJ, Rainbow SJ, Berry JL and Griffiths CJ. A single dose of vitamin D enhances immunity to mycobacteria. Am J Resp Crit Care Med 176: 208-213, 2007.

94. Matsuoka LY, Ide L, Wortsman J, MacLaughlin J and Holick MF. Sunscreens suppress cutaneous vitamin D3 synthesis. J Clin Endocrinol Metab 64: 1165-1168, 1987.

95. Maxwell JD. Seasonal variation in Vitamin D. P Nutr Soc 53: 533-543, 1994.

96. Milliken SV, Wassall H, Lewis BJ, Logie J, Barker RN, Macdonald H, Vickers MA and Ormerod AD. Effects of ultraviolet light on human serum 25-hydroxyvitamin D and systemic immune function. J Allergy Clin Immunol 129: 1554-1561, 2012.

97. Monlezun DJ, Bittner EA, Christopher KB, Camargo CA and Quraishi SA. Vitamin D status and acute respiratory infection: Cross sectional results from the United States National Health and Nutrition Examination Survey, 2001-2006. Nutrients 7: 1933-1944, 2015.

98. Moore C, Murphy MM, Keast DR and Holick MF. Vitamin D intake in the United States. J Am Diet Assoc 104: 980-983, 2004.

99. Moran DS, McClung JP, Kohen T and Lieberman HR. Vitamin D and physical performance. Sports Med 43: 601-611, 2013.

100. Morton JP, Iqbal Z, Drust B, Burgess D, Close GL and Brukner PD. Seasonal variation in vitamin D status in professional soccer players of the English Premier League. Appl Physiol Nutr Metab 37: 798-802, 2012.

101. Muller K, Haahr PM, Diamant M, Rieneck K, Kharazmi A and Bendtzen K. 1, 25-dihydroxyvitamin D 3 inhibits cytokine production by human blood monocytes at the post-transcriptional level. Cytokine 4: 506-512, 1992.

102. Muller K, Ødum N and Bendtzen K. 1, 25-Dihydroxyvitamin D 3 selectively reduces interleukin-2 levels and proliferation of human T cell lines in vitro. Immunol Lett 35: 177-182, 1993.

103. Murdoch DR, Slow S, Chambers ST, Jennings LC, Stewart AW, Priest PC, Florkowski CM, Livesey JH, Camargo CA and Scragg R. Effect of vitamin D3 supplementation on upper respiratory tract infections in healthy adults: the VIDARIS randomized controlled trial. JAMA 308: 1333-1339, 2012.

104. Nowson CA and Margerison C. Vitamin D intake and vitamin D status of Australians. Med J Aust 177: 149-152, 2002.

105. Ogan D and Pritchett K. Vitamin D and the athlete: risks, recommendations, and benefits. Nutrients 5: 1856-1868, 2013.

106. Owens DJ, Fraser WD and Close GL. Vitamin D and the athlete: Emerging insights. Eur J Sport Sci 15: 73-84, 2015.

107. Palmer HG, Gonzalez-Sancho JM, Espada J, Berciano MT, Puig I, Baulida J, Quintanilla M, Cano A, de Herreros AG, Lafarga M and Munoz A. Vitamin D3 promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of ß-catenin signaling. J Cell Biol 154: 369-388, 2001.

108. Peterfy C, Tenenhouse A and Yu E. Vitamin D and parotid gland function in the rat. J Physiol 398: 1, 1988.

109. Pludowski P, Karczmarewicz E, Bayer M, Carter G, Chlebna-Sokól D, Czech-Kowalska J, Debski R, Decsi T, Dobrzanska A, Franek E, Gluszko P, Grant W, Holick M, Yankovskaya L, Konstantynowicz J, Ksiazyk J, Ksiezopolska-Orlowska K, Lewinski A, Litwin M, Lohner S, Lorenc R, Lukaszkiewicz J, Marcinowska-Suchowierska E, Milewicz A, Misiorowski W, Nowicki M, Povoroznyuk V, Rozentryt P, Rudenka E, Shoenfeld Y, Socha P, Solnica B, Szalecki M, Talalaj M, Varbiro S and Zmijewski M. Practical guidelines for the supplementation of vitamin D and the treatment of deficits in Central Europerecommended vitamin D intakes in the general population and groups at risk of vitamin D deficiency. Endokrynol Pol 64: 319-327, 2013.

110. Pollock N, Dijkstra P, Chakraverty R and Hamilton B. Low 25 (OH) vitamin D concentrations in international UK track and field athletes. S Afr J Sports Med 24: 55-59, 2012.

111. Prietl B, Pilz S, Wolf M, Tomaschitz A, Obermayer-Pietsch B, Graninger W and Pieber TR. Vitamin D supplementation and regulatory T cells in apparently healthy subjects: vitamin D treatment for autoimmune diseases? Isr Med Assoc J 12: 136-139, 2010.

112. Prietl B, Treiber G, Pieber TR and Amrein K. Vitamin D and immune function. Nutrients 5: 2502-2521, 2013.

113. Pyne DB, Hopkins WG, Batterham AM, Gleeson M and Fricker PA. Characterising the individual performance responses to mild illness in international swimmers. Br J Sports Med 39: 752-756, 2005.

114. Rhodes LE, Webb AR, Fraser HI, Kift R, Durkin MT, Allan D, O'Brien SJ, Vail A and Berry JL. Recommended summer sunlight exposure levels can produce sufficient (≥ 20 ng ml-1) but not the proposed optimal (≥ 32 ng ml-1) 25 (OH) D levels at UK latitudes. J Invest Dermatol 130: 1411-1418, 2010.

115. Rigby WF, Denome S and Fanger MW. Regulation of lymphokine production and human T lymphocyte activation by 1, 25-dihydroxyvitamin D3. Specific inhibition at the level of messenger RNA. J Clin Invest 79: 1659-1664, 1987.

116. Rigby WF, Stacy T and Fanger MW. Inhibition of T lymphocyte mitogenesis by 1, 25-dihydroxyvitamin D3 (calcitriol). J Clin Invest 74: 1451-1455, 1984.

117. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL and Jones G. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab 96: 53-58, 2011.

118. Ruohola JP, Laaksi I, Ylikomi T, Haataja R, Mattila VM, Sahi T, Tuohimaa P and Pihlajamäki H. Association between serum 25 (OH) D concentrations and bone stress fractures in Finnish young men. J Bone Miner Res 21: 1483-1488, 2006.

119. Sabetta JR, DePetrillo P, Cipriani RJ, Smardin J, Burns LA and Landry ML. Serum 25-hydroxyvitamin d and the incidence of acute viral respiratory tract infections in healthy adults. PLoS One 5: e11088, 2010.

120. Sandhu MS and Casale TB. The role of vitamin D in asthma. Ann Allerg Asthma Immunol 105: 191-199, 2010.

121. Sato Y, Iwamoto J, Kanoko T and Satoh K. Low-dose vitamin D prevents muscular atrophy and reduces falls and hip fractures in women after stroke: a randomized controlled trial. Cerebrovasc Dis 20: 187-192, 2005.

122. Schertzer JD, Gehrig SM, Ryall JG and Lynch GS. Modulation of insulin-like growth factor (IGF)-I and IGF-binding protein interactions enhances skeletal muscle regeneration and ameliorates the dystrophic pathology in mdx mice. Am J Pathol 171: 1180-1188, 2007.

123. Shuler FD, Wingate MK, Moore GH and Giangarra C. Sports health benefits of vitamin D. Sports Health 4: 496-501, 2012.

124. Sly LM, Lopez M, Nauseef WM and Reiner NE. 1, 25-Dihydroxyvitamin D3-induced monocyte antimycobacterial activity is regulated by phosphatidylinositol 3-kinase and mediated by the NADPH-dependent phagocyte oxidase. J Biol Chem 276: 35482-35493, 2001.

125. Smolders J, Peelen E, Thewissen Ml, Cohen Tervaert JW, Menheere P, Hupperts R and Damoiseaux J. Safety and T cell modulating effects of high dose vitamin D3 supplementation in multiple sclerosis. PLoS One 5: e15235, 2010.

126. Stoffels K, Overbergh L, Giulietti A, Verlinden L, Bouillon R and Mathieu C. Immune regulation of 25-hydroxyvitamin-D3-1alpha-hydroxylase in human monocytes. J Bone Miner Res 21: 37-47, 2006.

127. Storlie DM, Pritchett K, Pritchett R and Cashman L. 12-Week vitamin D supplementation trial does not significantly influence seasonal 25 (OH) D status in male collegiate athletes. Int J Health Nutr 2: 8-13, 2011.

128. Stumpf WE. Vitamin D and the digestive system. Eur J Drug Metab Pharmacokinet 33: 85-100, 2008.

129. Takahashi K, Nakayama Y, Horiuchi H, Ohta T, Komoriya K, Ohmori H and Kamimura T. Human neutrophils express messenger RNA of vitamin D receptor and respond to 1alpha,25-dihydroxyvitamin D3. Immunopharmacol Immunotoxicol 24: 335-347, 2002.

130. Todd JJ, Pourshahidi LK, McSorley EM, Madigan SM and Magee PJ. Vitamin D: Recent advances and implications for athletes. Sports Med 45: 213-229, 2015.

131. Trujillo G, Habiel DM, Ge L, Ramadass M, Cooke NE and Kew RR. Neutrophil recruitment to the lung in both C5a-and CXCL1-induced alveolitis is impaired in vitamin D-binding protein-deficient mice. J Immunol 191: 848-856, 2013.

132. Tsoukas C, Watry D, Escobar S, Provvedini D, Dinarello C, Hustmyer F and Manolagas S. Inhibition of interleukin-1 production by 1, 25-dihydroxyvitamin D3. J Clin Endocrinol Metab 69: 127-133, 1989.

133. Villacis D, Yi A, Jahn R, Kephart CJ, Charlton T, Gamradt SC, Romano R, Tibone JE and Hatch GFR. Prevalence of abnormal vitamin D levels among Division I NCAA athletes. Sports Health 6: 340-347, 2014.

134. Von Essen MR, Kongsbak M, Schjerling P, Olgaard K, Ødum N and Geisler C. Vitamin D controls T cell antigen receptor signaling and activation of human T cells. Nat Immunol 11: 344-349, 2010.

135. Wallace AM, Gibson S, De La Hunty A, Lamberg-Allardt C and Ashwell M. Measurement of 25-hydroxyvitamin D in the clinical laboratory: current procedures, performance characteristics and limitations. Steroids 75: 477-488, 2010.

136. Walsh NP, Gleeson M, Pyne DB, Nieman DC, Dhabhar FS, Shephard RJ, Oliver SJ, Bermon S and Kajeniene A. Position statement part two: maintaining immune health. Exerc Immunol Rev 17: 64-103, 2011.

137. Walsh NP, Gleeson M, Shephard RJ, Gleeson M, Woods JA, Bishop NC, Fleshner M, Green C, Pedersen BK, Hoffman-Goetz L, Rogers CJ, Northoff H, Abbasi A and Simon P. Position statement part one: immune function and exercise. Exerc Immunol Rev 17: 6-63, 2011.

138. Wang TT, Nestel FP, Bourdeau V, Nagai Y, Wang Q, Liao J, Tavera-Mendoza L, Lin R, Hanrahan JH, Mader S and White JH. Cutting edge: 1, 25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol 173: 2909-2912, 2004.

139. Webb AR. Who, what, where and when-influences on cutaneous vitamin D synthesis. Prog Biophys Mol Biol 92: 17-25, 2006.

140. Webb AR, Kift R, Berry JL and Rhodes LE. The vitamin D debate: translating controlled experiments into reality for human sun exposure times. Photochem Photobiol 87: 741-745, 2011.

141. Webb AR, Pilbeam C, Hanafin N and Holick MF. An evaluation of the relative contributions of exposure to sunlight and of diet to the circulating concentrations of 25-hydroxyvitamin D in an elderly nursing home population in Boston. Am J Clin Nutr 51: 1075-1081, 1990.

142. Wentz L, Berry-Cabán C, Eldred J and Wu Q. Vitamin D correlation with testosterone concentration in US army special operations personnel. The FASEB J 29: 733-735, 2015.

143. Wielders JP and Wijnberg FA. Preanalytical stability of 25(OH)-vitamin D3 in human blood or serum at room temperature: solid as a rock. Clin Chem 55: 1584-1585, 2009.

144. Wolf G. The discovery of vitamin D: the contribution of Adolf Windaus. J Nutr 134: 1299-1302, 2004.

145. Wu S, Ren S, Nguyen L, Adams JS and Hewison M. Splice variants of the CYP27b1 gene and the regulation of 1, 25-dihydroxyvitamin D3 production. Endocrinology 148: 3410-3418, 2007.

146. Zerwekh JE. Blood biomarkers of vitamin D status. Am J Clin Nutr 87: 1087S-1091S, 2008.

147. Zhu Y, Mahon BD, Froicu M and Cantorna MT. Calcium and 1, 25-dihydroxyvitamin D3 target the TNF-alpha pathway to suppress experimental inflammatory bowel disease. Eur J Immunol 35: 217-224, 2005.