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Genetic, environmental and biomarker considerations delineating the regulatory effects of vitamin D on central nervous system function

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Abstract

Studies show that vitamin D (vit-D) (25(OH)D), the bioactive metabolite ($1,25(OH)_2D_3$) and vit-D receptors (vit-D receptor; protein disulphide isomerase, family A member 3) are expressed throughout the brain, particularly in regions pivotal to learning and memory. This has led to the paradigm that avoiding vit-D deficiency is important to preserve cognitive function. However, presently, it is not clear if the common clinical measure of serum 25(OH)D serves as a robust surrogate marker for central nervous system (CNS) homeostasis or function. Indeed, recent studies report CNS biosynthesis of endogenous 25(OH)D, the CNS expression of the CYP group of enzymes which catalyse conversion to $1,25(OH)_2D_3$ and thereafter, deactivation. Moreover, in the periphery, there is significant ethnic/genetic heterogeneity in vit-D conversion to $1,25(OH)_2D_3$ and there is a paucity of studies which have actually investigated vit-D kinetics across the cerebrovasculature. Compared with peripheral organs, the CNS also has differential expression of receptors that trigger cellular response to $1,25(OH)_2D_3$ metabolites. To holistically consider the putative association of peripheral (blood) abundance of 25(OH)D on cognitive function, herein, we have reviewed population and genetic studies, pre-clinical and clinical intervention studies and moreover have considered potential confounders of vit-D analysis.

Key words: Vitamin D: Brain: Cognition: Cerebral vitamin D metabolism: Ageing: Neurodegenerative disorders: Vitamin D measurement

Vitamin D (vit-D) is a lipid-soluble vitamin well-documented for its role in the development and maintenance of skeletal health, as well as maintenance of Ca and P homeostasis[1–5]. Advances in molecular and cellular biology now show that vit-D has pleiotropic effects and perhaps direct effects within the central nervous system (CNS)[6]. Proposed mechanisms include maintaining neuronal Ca regulation and signalling, regulation of neurotrophic factors, enhanced neurotransmission, synaptogenesis, neurogenesis, neuroprotection and inhibition of degenerative processes, including apoptosis[7–10].

Presently, the recommended serum concentration range for vit-D is primarily based on historical studies in the context of skeletal health; however, it is unknown if this is appropriate when considering CNS integrity and cognitive function[11,12]. There is a substantive body of evidence that collectively demonstrates health risks associated with vit-D deficiency, including endocrine, autoimmune, metabolic, bone and cardiovascular disorders[13–23]. However, the putative effects of vit-D deficiency and by extension vit-D supplementation on CNS function are contradictory. An emerging body of pre-clinical and clinical studies suggests an inverse U-shaped association with low and greater abundance of serum vit-D both correlated with neurocognitive deficits[14,24,26]. Whilst the mechanisms for the latter are presently not established, preclinical studies suggest that chronically heightened levels of serum vit-D may compromise cognitive performance through a cerebrovascular axis. In two rodent species, dietary-induced hypervitaminosis-D increased cerebral capillary permeability, resulting in inappropriate kinetics of plasma proteins from blood into brain parenchyme and neurovascular inflammation[24], whilst genetically engineered mice with hypervitaminosis-D show an accelerated ageing phenotype[26,27]. Whilst the physiological sequelae associated with vit-D deficiency are reasonably well established, the putative detrimental effects of exaggerated vit-D are noteworthy in the context of public health

Abbreviations: BBB, blood–brain barrier; CNS, central nervous system; FGF-23, fibroblast-growth-factor-23; HPLC, high-pressure liquid chromatography; PDIA3, protein disulphide isomerase, family A member 3; RCT, randomised controlled trial; VDBP, vit-D binding protein; VDR, vitamin D receptor; vit-D, vitamin D.

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trends showing increased self-prescribed use of supplementary vit-D nutraceuticals and clinical trials with interventions of up to 7500 μg of vit-D_3 per dose[28–32].

Collectively, at present, there is no substantiated serum reference range for vit-D that can reliably be indicated to support optimal CNS function[14]. Moreover, adding to the complexity of interpreting putative vit-D effects on cognitive performance is that clinically, blood vit-D status is ordinarily indicated by considering the concentration of the inactive metabolite of vit-D, serum 25-hydroxyvitamin-D (25(OH)D), a biomarker of storage rather than biological function *per se*[33].

Serum vitamin D synthesis and homeostasis

Serum abundance of vit-D is partially indicative of the endogenous biosynthesis of cholecalciferol (vit-D_3) which occurs within the epidermis of mammalian skin[7,34] during exposure to UV-B radiation (290–315 nm range). However, in addition, serum vit-D is influenced through exogenous sources provided through the ingestion of either plant-derived food commodities enriched in ergocalciferol (vit-D_2); animal meats and dairy containing both cholecalciferol and ergocalciferol or through nutraceutical supplements (principally provided as cholecalciferol)[34] (Fig. 1).

Peripheral synthesis and metabolism of vitamin D

Significant hepatic uptake of both skin and dietary-derived vit-D_3 and vit-D_2 occurs when they are hydroxylated by the microsomal cytochrome P450 25-hydroxylase enzyme, CYP2R1, to 25-hydroxyvit-D (calcidiol (25(OH)D)). In blood, the 25(OH)D is thereafter chaperoned associated with the vit-D binding protein (VDBP), which can subsequently be internalised by the proximal tubule within the kidney via megalin, a transmembrane receptor protein with high affinity for VDBP (Fig. 1)[35]. Principally within the kidney, CYP27B1 converts 25(OH)D to the biologically active metabolite 1,25-dihydroxyvit-D (calcitriol ($1,25(OH)_2D_3$)), where it is released into circulation as an endocrine modulator. In blood, the biologically active $1,25(OH)_2D_3$ acts on target cells including cells of the parathyroid glands, osteoblasts, dendritic cells, T cells and keratinocytes. Ordinarily, homeostasis of the active metabolite is normally strictly controlled by highly regulated co-expression within kidney by CYP27A1, which deactivates $1,25(OH)_2D_3$ as a consequence of conversion to 24,25-dihydroxyvitamin-D ($24,25(OH)_2D_3$)[36–39].

The catalytic conversion of 25(OH)D to $1,25(OH)_2D_3$ is thought to be principally regulated via ionised Ca homeostasis[40,41]. When blood Ca is low, parathyroid hormone is released, stimulating expression of CYP27B1 and decreasing CYP24A1, resulting in an increase of $1,25(OH)_2D_3$ synthesis. Increased levels of $1,25(OH)_2D_3$ subsequently inhibit parathyroid hormone, thus avoiding potential risk for hypercalcaemia[35,42]. Moreover, a negative feedback loop occurs as a consequence of $1,25(OH)_2D_3$ to the vit-D receptor (VDR), with down-regulation of transcription for CYP27B1[39] and an increased expression of inactivating enzyme, CYP24A1[43,44]. Similarly, blood phosphate is a potent regulator of CYP-mediated conversion of vit-D metabolites. Dietary phosphates and heightened blood abundance stimulate fibroblast growth factor-23 (FGF-23) secretion[45–47] by osteocytes in the bone matrix of skeletal tissue. The FGF-23 inhibits kidney CYP27B1 and increases CYP24A1 expression, collectively suppressing $1,25(OH)_2D_3$[48]. Conversely, circulating low levels of phosphate increases the production of CYP27B1 and thus increases $1,25(OH)_2D_3$ concentrations in the blood; the latter would then stimulate FGF-23 production, which would complete the feedback loop and down-regulate CYP27B1 expression[41,48].

In 1987, Stumpf & O'Brien demonstrated that the bioactive metabolite of vit-D, $1,25(OH)_2D_3$, could diffuse across the blood–brain barrier (BBB) into brain parenchyme and accumulate in nuclei of the amygdala, hippocampus, hypothalamus, thalamus, pallium, pons, midbrain and cerebellum[49]. Stumpf and colleagues observed that the distribution of cerebral $1,25(OH)_2D_3$ was non-random and in tight regulation with endocrine, autonomic, sensory and motor systems[49]. Indeed, present clinical studies have substantiated that cerebrospinal fluid homeostasis of vit-D is associated with serum abundance of vit-D, supporting current dogma that modulating peripheral metabolism may have CNS modulatory effects[50].

The vitamin D receptor

The VDR is a member of the nuclear receptor superfamily and acts as a $1,25(OH)_2D_3$-inducible transcription factor[51–53]. In most cell types including neuronal cells, VDR are predominantly (58 %) found in the nucleus[54]; however, observational studies have also shown VDR on the plasma membranes of cells[55] and within the cytosol[54]. Genomic effects mediated by VDR consist of the activation and repression of gene transcription. First, the internalisation of $1,25(OH)_2D_3$ forms a complex with a retinoid X receptor (either RXRα, RXRβ or RXRγ) and VDR within the cell[52,56]. The $1,25(OH)_2D_3$-VDR-RXR complex then binds to specific enhancer elements, referred to as vit-D response elements (VDRE)[52], within the regulatory regions of target genes in the DNA. In a $1,25(OH)_2D_3$-dependent manner, the complex recruits various chromatin-active coregulatory complexes, thereby facilitating gene-selective transcription[56–59] (Fig. 1).

Vitamin D receptors and the cytochrome P450 enzymes in brain

It was not until commercial antibodies became available that research groups such as Veenstra *et al.*[60] and Prufer *et al.*[61] were able to map VDR expression throughout the rodent brain. Many studies have demonstrated the presence of VDR in the brain via Western blot, immuno-fluorescent and quantitative RNA techniques[9,55,62–65]; however, some researchers disputed these findings and suggested relatively low VDR abundance or exaggerated measures of VDR[66,67]. The uncertainty with respect to CNS VDR abundance is partly based on the work by Wang *et al.*, who did not detect VDR in the rat or human brain using ELISA and immunohistochemical methodologies[68]. However, in 2014, Eyles and colleagues provided further

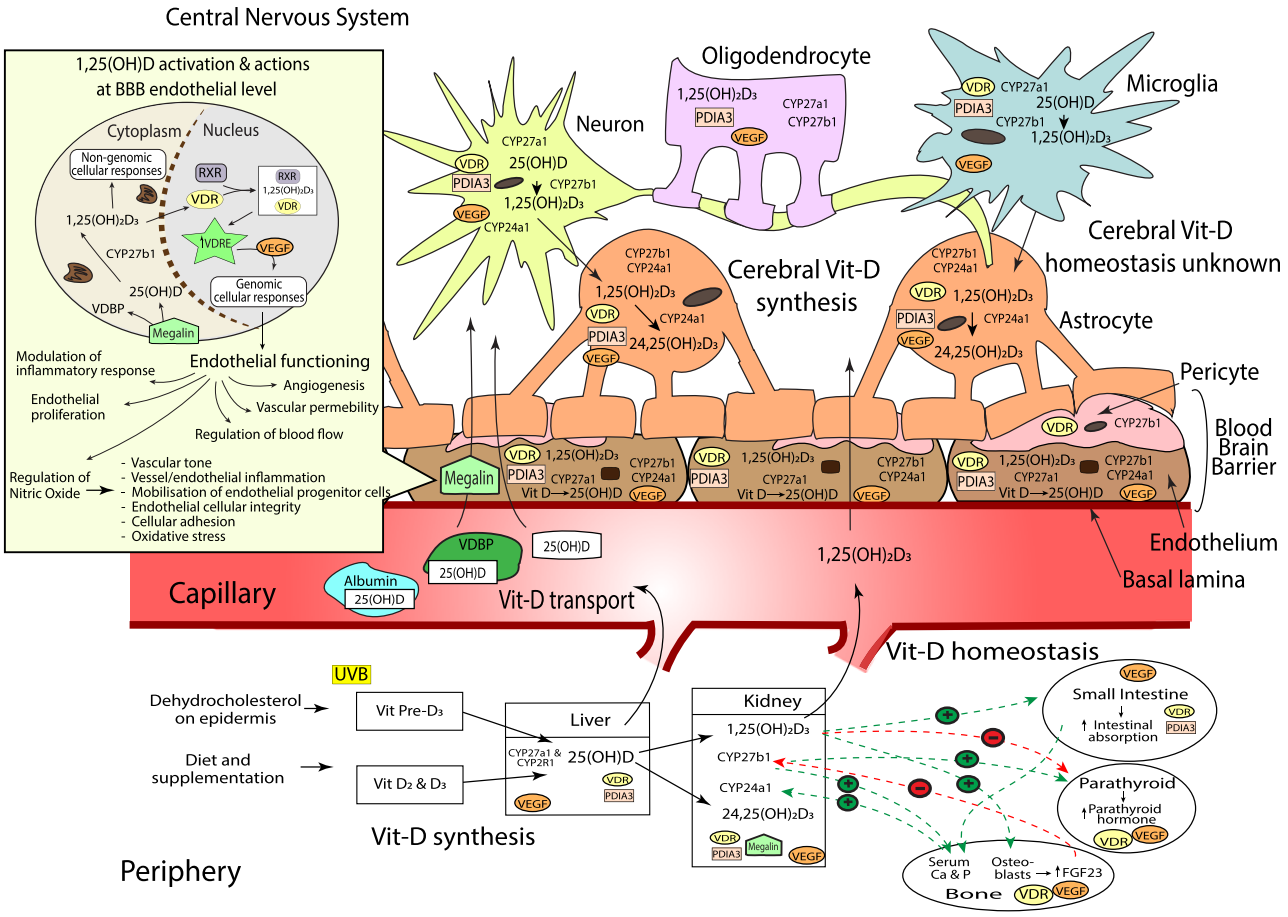

Fig. 1. (Colour online) Sources of vitamin D (vit-D), peripheral and cerebral synthesis and homeostasis, blood and blood–brain barrier (BBB) transport. Vit-D is obtained by the conversion of dehydrocholesterol on the epidermis when exposed to UVB (vit-D_3), diet and supplementation (vit-D_2 and D_3). Vit-D_2 and D_3 metabolites are converted to 25(OH)D (25-hydroxycholecalciferol) by CYP2R1 and CYP27A1 hydroxylase enzymes in the liver, before conversion to active $1,25(OH)_2D_3$ (calcitriol, by CYP27B1, or inactive $24,25(OH)2D_3$ (24,25-Dihydroxycholecalciferol) by CYP24A1 in the kidneys. Hydroxylation enzymes CYP27A1, CYP27B1 and CYP24A1, and vit-D receptors, VDR and PDIA, have been shown in varying concentrations throughout the brain. Neurons, endothelial cells and microglial express CYP27A1, CYP27B1, CYP24A1, VDR and PDIA. Astrocytes express CYP27A1, CYP24A1, VDR and PDIA. Oligodendrocytes express CYP27A1 and PDIA3; and pericytes express CYP27B1 and VDR. Vit-D synthesis from vit-D to 25(OH)D takes place within endothelial cells and neurons, before further hydroxylation to $1,25(OH)_2D_3$ in the neurons and microglia. $1,25(OH)_2D_3$ activates either genomic actions via VDR or non-genomic actions via PDIA3. As VDR is expressed in lower quantities in brain compared with kidney, PDIA3 may act as the main cerebral vit-D receptor. Peripheral vit-D homeostasis is tightly regulated by hormones such as calcium, parathyroid hormone (PTH), fibroblast growth factors (FGF-23) and abundance of $1,25(OH)_2D_3$; however, cerebral vitamin D homeostasis, along with the relationship between peripheral and cerebral homeostasis, remains unknown. Vit-D is predominantly transported in peripheral blood bound to VDBP, but can also be bound to albumin, or free (<1 %). Albumin can traverse the endothelium via transcytotic mechanisms, whilst free 25(OH)D and active $1,25(OH)_2D_3$ enter the endothelial cell and move across the BBB into the central nervous system (CNS) via passive diffusion. The BBB consists of the basal lamina, endothelial cells, pericytes and astrocytes. 25(OH)D bound to VDBP is dependent on internalisation by megalin transport protein. Once internalised, 25(OH)D unbinds from VDBP before conversion to $1,25(OH)_2D_3$ within the cytoplasm. $1,25(OH)_2D_3$ then translocates into the nucleus where it binds to VDR and retinoid X receptor (RXR). The $1,25(OH)_2D_3$, VDR, RXR complex then binds to target genes that contain a vitamin D response element (VDRE)[62,70,73,75,130]. Activation of VDRE promotes expression of vascular endothelial growth factor (VEGF)[234] which is located throughout the brain including astrocytes[235], neurons[236], oligodendrocytes[237], microglial cells[238] and the endothelium at the BBB[239]. VEGF binds to VEGF-specific receptors like those in the endothelium, controlling a wide range of endothelial cellular activities[234]. This includes regulation of vascular permeability, endothelial proliferation, activation of angiogenesis, regulation of blood flow via vasodilation/vasoconstriction and modulation of inflammatory response including platelet activation, migration and cell survival[234,240]. VEGF also induces the release of nitric oxide (NO) from endothelial cells[241]. NO is an important biomolecule which mediates various metabolic pathways[242] including vessel inflammation and integrity, regulation of vascular tone, cellular adhesion and oxidative stress. Additionally, NO is partly responsible for the mobilisation of endothelial progenitor cells essential for vessel maintenance and repair[243]. Vit-D and VEGF dysregulation can therefore substantially modulate endothelial cell function[234].

evidence of cerebral abundance of VDR using proteomic techniques[62].

Recently, another CNS-rich protein has been implicated in rapid intracellular signalling induced by $1,25(OH)_2D_3$, namely, protein disulphide isomerase, family A member 3 (PDIA3), a membrane-bound VDR[69]. Notably, the PDIA3 expression in brain is orders of magnitude greater than hepatic and renal PDIA3 expression, particularly in the cerebral cortex and hippocampus, regional areas critical to cognitive function (Fig. 1)[69]. Consistent with an autocrine pathway, Eyles *et al.*[70] also described the coexpression of CYP27B1 with VDR in human cerebral cortex, hippocampal formation and hypothalamus[9]. CYP27B1 and VDR were immunodetected in both neuronal and glial cells, with the expression of CYP27B1 restricted to the cytoplasm, and VDR within the nucleus, respectively (Fig. 1). VDR immunoreactivity was indicated within

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hippocampal formation regions, CA1 and CA2, with less prominence in CA3. Consistent with the regional distribution of VDR, CYP27B1 was clearly indicated throughout the entirety of the CA1, CA2 and CA3-hippocampal formation regions, brain regions pivotal for spatial learning and memory formation and considered one of the most vulnerable regions in Alzheimer's disease pathology[62,70–72] (Fig. 1). The substantive findings by Eyles *et al.* demonstrating a *de novo* CNS pathway for $1,25(OH)_2D_3$ biosynthesis and cellular activation in brain raises uncertainty as to the relative physiological significance of peripheral (serum) $1,25(OH)_2D_3$ homeostasis in the context of CNS function.

Cellular distribution of vitamin D receptor and associated enzymes in the central nervous system

At a cellular level, Smolders *et al.*[73] reported immunostaining for VDR in microglia and astrocytes; however, the CYP24A1 enzyme was restricted primarily to astrocytes (Fig. 1). In alignment with these findings, El-Atifi *et al.*[74] demonstrated CYP2R1 and VDR expression in human pericytes. As major structural and functional cells comprise the neurovascular unit, the aforementioned evidence supports the autocrine/paracrine modes of action of vit-D on neurovascular function (Fig. 1).

Within the neurovascular unit, CYP27A1, CYP27B1 and CYP24A1 expressions have been confirmed in astrocytes, endothelial cells, microglia, oligodendrocytes and notably, with significant expression of CYP27B1 particularly indicated in neurons[75]. However, differential expression of the VDR binding proteins has been described. The VDR is expressed principally in astrocytes, whereas PDIA3 mRNA is ubiquitously indicated. For the latter, there was striking abundance found in endothelial cells consistent with a functional role of peripheral (blood) 1,25(OH)D on vascular function[75]. Figure 1 summarises all present evidence for vit-D associated metabolites and receptors that have been characterised in the brain.

Cerebral vitamin D homeostasis relative to peripheral vitamin D homeostasis. Vit-D metabolites are able to traverse the BBB from blood into the brain paranchyme through mechanism which have not been equivocally delineated[76]. Unfortunately, there is a paucity of literature considering putative associations between serum and cerebral vit-D homeostasis. Xue *et al.*[76] showed that rats fed a vit-D_3-deficient diet had markedly lower cerebral $25(OH)D_3$ metabolite concentration in comparison with rats maintained on a vit-D_3 supplemented diet. Consistent with the possibility of diffusion or facilitated transport process across the BBB, Xue and colleagues showed that the serum vit-D_3 metabolite concentrations correlated with brain vit-D_3 metabolite levels. However, the relative significance of peripheral vit-D homeostasis in association with CNS vit-D homeostasis remains largely unexplored.

Vitamin D and cognitive performance

There is public popularity with the notion that higher vit-D status, indicated by increased serum vit-D (25(OH)D as the universal marker of vit-D status), is associated with better cognitive performance and reduced risk of neurodegenerative disorders. The public perception has resulted in the promotion of self-prescribed vit-D supplementation without clinical indication[77].

A number of randomised controlled trials (RCT) and meta-analyses suggest a positive association of serum vit-D with cognition[78], at a threshold serum concentration of 25(OH)D level >25 nmol/l. Benefits are indicated on global cognitive performance[79] and in specific cognitive domains including visuospatial skills, language, working memory, memory recall, concentration and attention[80]. However, other studies indicate that exaggerated levels of vit-D are also associated with poorer cognitive function[25,81–83]. Moreover, adding to the complexity of interpretating vit-D-associated CNS effects is the significant potential confounder of reverse causation and in lack of standardisation and heterogeneity of blood vit-D analytics[82,84].

Hypovitaminosis D and cognitive performance

Numerous studies report associations between vit-D deficiency and an increased risk of cognitive decline in older adults[14,19,20,85]; however, paradoxical findings suggest no such association[81] (See summary of studies to date in Table 1). An international task force considering vit-D and cognition in elderly individuals concluded that hypovitaminosis-D increases the risk of cognitive decline and dementia[14]. In Alzheimer's disease, hypovitaminosis-D is associated with a 2·4-fold increased risk for cognitive impairment[14]. In the large community-based Framingham Heart Study, there was an association between lower vit-D status and reduced hippocampal volume, poorer measures of neuropsychological function and a greater risk of dementia[86]. In agreement with these large cohort studies, Hooshmand *et al.*[87] reported positive associations between vit-D (25(OH)D) status with cognitive function and a reduction in CSF amyloid-β and brain volume. Moreover, regional cerebral blood flow and brain function were found to be positively associated with serum vit-D concentration in Alzheimer's disease patients. A lower concentration of serum vit-D was found to correlate with poorer executive functioning (heterogeneous set of complex processes that controls and regulates other abilities and behaviour); however, episodic memory was generally found to be unaffected[87]. Furthermore, Chaves *et al.*[88] recently concluded vit-D insufficiency as an independent risk factor for Alzheimer's disease.

In contrast to studies which indicate a positive association between serum vit-D and cognitive performance, an increasing number of prospective cohort studies, including in early childhood to individuals of advanced age, have failed to show a correlation between serum vit-D status and cognitive outcomes. Schneider *et al.*[89,90] found no association between vit-D deficiency with lower cognitive test scores in a 20 year (median) longitudinal study in late to middle aged adults. Graf *et al.*[91] reported no association between vit-D and increased risk for mild cognitive impairment, nor did serum vit-D predict the conversion from cognitively normal or mild cognitive impairment to dementia. Other studies have not found any association between low 25(OH)D and cognitive performance[92,93]. Using a Mendelian randomisation study design in a recent study, Maddock *et al.*[94] explored the relationship between serum 25(OH)D concentration and cognitive function. Data collected

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Table 1. Summary of randomised controlled trials and observational studies exploring vitamin D (vit-D) and cognitive outcomes

Reference/author/year	Study design	Sample size/participants	Primary cognitive measures	Intervention/follow-up time (if applicable)	Method of vit-D status measurement	Outcome
Randomised controlled trials						
Rossom *et al.* (2012)[92]	RCT	4143 elderly women (>65 years of age) without probable dementia	Global cognitive function; attention; working memory; verbal knowledge; memory, spatial ability	10 μg vit-D_3 combined with calcium carbonate/d; mean follow-up of 7·8 years	Serum 25(OH)D not measured	No associations were reported between vit-D supplementation and cognitive outcomes
Dean *et al.* (2011)[93]	RCT	128 healthy young adults (mean age of 21·45 years)	Working, response inhibition; cognitive flexibility	125 μg of vit-D_3/d for 6 weeks	25(OH)D (dried whole blood spot samples) via tandem mass spectroscopy	Vit-D supplementation did not influence cognitive or emotional functioning
Stein *et al.* (2011)[97]	RCT	Sixty-three community dwelling participants aged ≥60 years with mild-moderate AD	Visuospatial; language; concentration; attention; working memory; executive function and orientation	25 μg and 150 μg of vit-D_2/d for 8 weeks	Serum 25(OH)D via RIA	High-dose vit-D supplementation conferred no benefit for cognition in individuals with mild-moderate AD
Castle *et al.* (2019)[83]	RCT	Healthy, postmenopausal women (50–70 years of age; BMI 25–40 kg/m^2)	Executive functioning; attention and flexibility of attention; reaction time working memory capacity and spatial planning; motor control	15, 50 or 100 μg vit-D_3/d for 12 months	Serum 25(OH)D via RIA	Differential dosage effects of vit-D3 on specific cognitive domains were reported. Participants from the 50 μg/d group performed better in learning and memory measures in comparison with those taking 15 and 100 μg vit-D3/d. Slower reaction time was observed in participants on daily 100 μg vit-D3
Observational studies						
Lam *et al.* (2015)[100]	Cross-sectional	179 healthy, older aged individuals (47–84 years of age)	Verbal episodic memory and attention	N/A	Serum 25(OH)D enzyme immunoassay	Higher vit-D status was associated with poorer performance on verbal episodic memory in middle-aged and older individuals with normal vit-D-Ca-PTH homeostasis
Granic *et al.* (2015)[25]	Cross-sectional	775 older participants over 85 years	Global functioning and attention	N/A	Serum 25(OH)D via RIA	Low and high season-specific 25(OH)D quartiles were associated with cognitive deterioration and poorer overall performance in attention-specific tasks. Participants in the highest quartile with increased risk of worsened global cognition and attention were users of vit-D supplementation; this was not observed in non-users of vit-D supplements
McGrath *et al.* (2007)[81]	Cross-sectional	Adolescent group (*n* 1676, 12–17 years), adult group (*n* 4747, 20–60 years), elderly group (*n* 4809, 60–90 years)	Verbal and visuospatial learning and memory; visual attention; coding speed; concentration; psychomotor speed	N/A	Serum 25(OH)D via RIA	No association was observed between 25(OH)D levels and psychometric measures in both adolescent and adult groups. In the elderly group however, those within the highest 25(OH)-quintile showed learning and memory impairments
Maddock *et al.* (2014)[82]	Cohort	6496 participants from the 1958 British Cohort	Verbal memory and fluency; processing speed	5-year follow-up (Baseline: 45 years of age)	Serum 25(OH)D via automated enzyme immunoassay (OCTEIA)	Participants with both low and high serum 25(OH)D concentrations performed significantly worse on specific cognitive domains; immediate word recall
Schneider *et al.* (2018)[90]	Cohort	13 044 participants from the Atherosclerosis Risk in Communities Brain MRI Study (baseline age 45–65 years)	Recent memory and verbal learning; processing speed; executive function	20-year follow-up (mean age of 57 years at baseline)	Serum 25(OH)D by liquid chromatography-tandem MS	Over a 20-year follow-up period, there were no significant associations between lower serum 25(OH)D concentrations at midlife with greater rate of cognitive deterioration

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Table 1. (*Continued*)

Reference/author/year	Study design	Sample size/participants	Primary cognitive measures	Intervention/follow-up time (if applicable)	Method of vit-D status measurement	Outcome
Schneider *et al.* (2014)[89]	Cohort	1652 participants (52 % white; 48 % black) aged 45–65 years at baseline	Verbal and recent memory; executive function and processing speed; executive function and language	Median of 3·0, 10·6 and 16·6 years follow-up (mean age of 57 years at baseline)	Serum 25(OH)D by liquid chromatography-tandem MS	No significant associations were observed between lower levels of 25(OH)D measured in late-middle age and lower cognitive test scores at baseline nor change in scores over time or dementia risk
Graf *et al.* (2014)[91]	Cohort	428 elderly participants (mean age 85·2 years); cognitively normal (*n* 200); mild cognitive impairment (*n* 46); dementia (*n* 182)	Global functioning; episodic and verbal memory; attention	2-year follow-up	Plasma 25(OH)D electrochemi-luminesence immunoassay	Plasma 25(OH)D concentrations were not associated with cognitive status and did not predict conversion to dementia
Overmann *et al.* (2017)[95]	Cohort	2430 middle-aged and elderly men aged between 40 and 79 years	Visuo-constructional ability; visual memory; processing speed	4·4-year follow-up	Serum 25(OH)D via RIA and 1,25(OH)2D via liquid chromatography-tandem MS	No association reported between serum 25(OH)D or 1,25(OH)$_2$D concentrations and each of the cognition subdomains measured

RCT, randomised controlled trial; 25(OH)D, 25-hydroxyvitamin D; AD, Alzheimer's disease; N/A, not applicable.

from seventeen observational cohort studies, including over 170 000 participants, concluded no evidence of an association between serum 25(OH)D concentration as a causal factor for cognitive performance in later life. Similarly, a 4·5-year longitudinal male ageing study by Overman *et al.*[95] found no association between serum 25(OH)D and 1,25(OH)$_2$D$_3$ concentrations and multiple cognitive subdomains in 3369 men aged between 40 and 79 years of age.

Vitamin D supplementation and cognitive function. Results from RCT linking vit-D deficiency and cognitive decline have not been promising[89,96,97] (Table 1). There was no benefit on cognition or memory realised in vit-D-deficient older participants supplemented with vit-D[92,97,98]. Results from the Women's Health Initiative study found that vit-D and Ca supplementation over a period of 8 years had no beneficial effects on cognition[99]. Studies by Stein *et al.*[97] show that supraphysiological levels of vit-D did not confer beneficial effects in cognitive performance in individuals with mild-moderate Alzheimer's disease. A study by Rossom *et al.*[92] failed to demonstrate positive effects of vit-D supplementation in attenuating cognitive dysfunction, or on lowering the risk of dementia when compared with placebo. Moreover, Castle and colleagues recently reported on the differential dosage effects of vit-D on specific cognitive domains in healthy, post-menopausal women whereby higher doses were found associated with poorer reaction time and measures of learning and memory[83]. Furthermore, a recent literature review by Landel *et al.*[72] determined that there was no solid evidence to suggest that exogenous supplementation of vit-D improves cognition in those who already have sufficient serum levels of vit-D. Collectively, results of RCT do not consistently suggest postive effects of exogenous vit-D on cognitive performance.

Hypervitaminosis-D and cognitive performance

Recent studies suggest cognitive impairment may indeed also be indicated in individuals with greater levels of serum vit-D, especially those taking vit-D supplements. Granic *et al.*[25] reported that both the lowest and highest season-specific serum 25(OH)D quartiles had increased risk of cognitive impairment compared with those in the middle quartiles adjusted for socio-demographic, health and lifestyle confounders. An increased risk of poorer global cognition and attention amongst those in the highest quartile was observed, specifically in users of vit-D supplements.

Earlier reports by McGrath *et al.*[81] did not find significant associations between lower vit-D status and neurocognitive performance in adolescent and adult groups. However, there was a significant association between vit-D levels and cognitive performance in the elderly group, demonstrating unexpected results that those with increased levels of vit-D, particularly those taking vit-D supplements performing worse in learning and memory measures. Consistent with these findings, Lam *et al.*[100] found an association between higher serum vit-D status and poorer verbal episodic memory performance in those with normal Ca-parathyroid hormone homeostasis in middle-aged and older individuals.

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Whilst a significant number of cross-sectional, epidemiological and population studies have explored the association of serum vit-D status and cognition, mechanistic studies are lacking. Cell studies by Brown *et al.*[101] demonstrated that vit-D treatment of cultured hippocampal cells can initiate cellular apoptosis and similar findings have been indicated in cancerous cell lines[102–104]. Excessive activation of the VDR causes gene transcription associated with mitochondrial export of cytochrome C and subsequent cleavage of caspase-9, which consequently promotes DNA fragmentation and thereby apoptosis[105].

Vitamin D homeostasis and ageing. Population studies exploring the association between vit-D homeostasis and cognitive capacity in late-aged individuals may be confounded by 'reverse causation' due to lifestyle changes and immobility[106–109]. Moreover, the ability to synthesise endogenous vit-D within the epidermis or to activate 25(OH)D may be age-dependent[110]. Paradoxical reports have been published with some studies suggesting increased, decreased or unchanged vit-D metabolite concentrations with ageing in both preclinical and clinical studies[35,110,111]. In addition to putative age-associated changes in peripheral biosynthetic and conversion of vit-D metabolites, pre-clinical and clinical data indicate that there is a marked reduction in intestinal, kidney and skeletal responsiveness to $1,25(OH)_2D$ with age through mechanisms which are presently unclear[35]. Several studies have demonstrated a reduction in intestinal VDR expression[112–114] with ageing in both animal and human studies, although others have reported no change[115,116]. A putative age-related diminished intestinal responsiveness to $1,25(OH)_2D_3$ may be associated with modified recruitment of VDR, VDR co-activators or epigenetic changes[35]. Presently, no studies have investigated cerebral vit-D homeostasis, receptors or 'responsiveness' in the context of ageing, an important consideration in the context of CNS function and cognitive performance.

Hypervitaminosis-D and accelerated ageing. The global prevalence of hypovitaminosis-D during ageing is well documented[117]. However, possible effects of hypervitaminosis-D on the ageing process are not yet clear[27]. Based on the strict regulation of vit-D activation, hypervitaminosis-D is rare in humans based on dietary sources. Nonetheless, vit-D supplementation has been associated with early ageing, hypercalcaemia, cardiovascular complications (vascular-related) and early death, supporting the association between hypervitaminosis-D and accelerated ageing[118]. Two genes of interest in this area of study are FGF-23, involved in suppression of renal expression of $1,25(OH)_2D_3$, resulting in decreased production of calcitriol, and KLOTHO; a membrane protein involved with cellular functions and cell–matrix interactions[119]. Recent *in vivo* genetic-manipulation studies have shown increased serum levels of vit-D and altered mineral-ion homeostasis in mice that lack either FGF-23 or KLOTHO genes[27]. Furthermore, hypervitaminosis-D in these mutant mice exhibits an accelerated ageing phenotype[26]. Genetic ablation of FGF-23 or KLOTHO genes in other rodent models results in hypervitaminosis-D, hypercalcaemia and hyperphosphataemia, corresponding with a phenotype consistent with premature ageing[26]. Remarkably, Tsujikawa *et al.*[120] reported that dietary restriction of vit-D reverses the premature-ageing phenotypes and indeed prolongs life in the KLOTHO knockout mouse model, an observation now reported in other studies. Polymorphisms in the human KLOTHO gene have also been associated with the occurrence of a number of age-related pathologies such as CVD[121]. These findings provide strong evidence that hypervitaminosis-D may be causally associated with the ageing process.

Vitamin D receptor genetic polymorphisms and cognitive function

Genetic variability for vit-D metabolism may be associated for an individual's susceptibility to cognitive decline. Polymorphisms of the VDR gene have been shown to influence the susceptibility to age-related changes in cognitive functioning and progression of neurodegenerative diseases such as Alzheimer's disease, mild cognitive impairment, Parkinson's disease and implicated with cognitive function[122,123]. However, literature contributing to the delineation of the putative metabolic pathways that result in cognitive dysfunction is varied and inconclusive.

The VDR is synthesised by the *VDR* gene, located on chromosome 12 and composed of nine exons[124], of which several genetic variations have been recognised. Approximately 1 % of the population has a DNA gene variation of the VDR[125]. These genetic alterations can lead to significant defects on gene activation, affecting cell proliferation, mineral and hormonal metabolism and immune function. The most investigated polymorphisms of the VDR gene include Cdx-2, FokI, BsmI, ApaI and Taq1 and are often associated with phenotypes involving bone mineral density, osteoporotic fracture risk and cancer[122,123].

Kuningas *et al.*[123] observed a significant decline in cognitive performance in those carrying the BsmI and Taq1 polymorphisms; however, no cognitive deficit was observed in participants carrying the Cdx-2 or FokI polymorphisms (Table 2 for summary of studies to date). Interestingly, individuals with the ApaI polymorphisms performed better on tests measuring processing speed, attention and memory. These findings complemented those reported by Uitterlinden and colleagues in 2004[126]. In contrast, Leymann *et al.*[127] found the ApaI VDR polymorphisms to be significantly associated with cognitive decline and increased risk of AD, particularly in people under 75 years. Similarly, Keyimu *et al.*[128] found both BsmI and ApaI polymorphisms significantly associated with an increased risk of mild cognitive impairment in a cohort of elderly Uygur people. A 2015 meta-analysis, totalling seven studies with 2034 Parkinson's disease cases and 2432 healthy controls, found polymorphisms of ApaI, Bsm1 and Taq1 were not associated with the susceptibility to Parkinson's disease, while the FokI (C and T allele) polymorphisms were associated with an increased risk in Parkinson's disease[129].

In a recent study, Beydoun *et al.*[130] evaluated associations of VDR polymorphisms (Cdx, BsmI, ApaI and Taq1) and LDL receptor, megalin, with a decline in longitudinal cognitive performance in 1024 healthy African American adults. Megalin is expressed in endothelial cells of the BBB and potentially mediates vit-D transport from the blood to brain parenchyma (Fig. 1). Beydoun and others have reported that VDR (BsmI/ApaI/Taq1)

Table 2. Summary of cross-sectional and cohort studies exploring the link between vitamin D receptor (VDR) gene polymorphisms and cognitive osutcomes

Reference/author/year	Sample participants/size	Study design	VDR gene polymorphisms investigated	Study focus	Outcomes
Pettersen *et al.* (2015)[122]	Healthy adults (*n* 78)	Cross-sectional	FokI	The relationship between the VDR FokI genotype, serum 25(OH)D levels and cognitive functioning was investigated	Polymorphisms in the FokI VDR gene were associated with non-verbal executive task and global cognitive performance, independent of serum 25(OH)D levels
Kuningas *et al.* (2009)[123]	563 participants > 85-years of age from the Leiden 85-plus study	Cohort	Cdx-2; FokI; BsmI; ApaI; Taq1	This study investigated whether genetic variance in the VDR gene is associated with cognitive functioning and depressive symptoms	Genetic variance in the VDR gene influences the susceptibility to age-related changes in cognitive performance. Carriers of BsmI and Taq1 polymorphisms exhibited poorer cognitive functioning
Gatto *et al.* (2016)[137]	Non-Hispanic Caucasian PD participants (*n* 190) from the Parkinson Environment Gene study (average age of diagnosis 67·4 years)	Cohort	FokI	Explored the relationship of VDR polymorphisms and cognitive decline in individuals with PD (average follow-up of 7·1 years)	FokI, a functional VDR polymorphism, was associated with cognitive decline in PD individuals. Each additional copy of the FokI A allele was associated with a significant decrease in the cognitive test score per year of follow-up
Lehmann *et al.* (2011)[127]	Caucasian participants with Alzheimer's disease (*n* 255) and healthy elderly controls (*n* 260) (mean age 78·4 years)	Cohort	ApaI and Taq1	This study used DNA from Alzheimer's disease cases and cognitively screened elderly controls from the longitudinal cohort of the Oxford Project to investigate the relationship between two VDR polymorphisms and memory and ageing	ApaI T and Taq1 VDR polymorphisms were significantly associated with cognitive decline and increased risk of Alzheimer's disease
Keyimu *et al.* (2014)[128]	124 mildly cognitive impaired participants and 124 healthy controls	Cohort	ApaI and BsmI	This study explores the relationship between VDR gene polymorphisms, ApaI, BsmI and risk of mild cognitive impairment	Alleles associated with both BsmI and ApaI polymorphisms, T allele and A allele, respectively, were significantly associated with an increased risk of mild cognitive impairment
Beydoun *et al.* (2017)[121]	1024 healthy African American adults (mean age 52 years)	Cohort	VDR polymorphisms; BsmI; ApaI; Taq1; and megalin	This study explored the putative associations of VDR and megalin polymorphisms with longitudinal changes in cognitive function (follow-up ranged from 1 to 8 years; mean of approx. 4 years)	BsmI/ApaI/Taq1 and megalin gene polymorphisms altered age-related cognitive pathways, specifically in global mental status, verbal fluency, visual and working memory and executive function
Beydoun *et al.* (2012)[131]	Cohort study of 702 non-Hispanic white participants at risk of mild cognitive impairment or dementia (mean age of 52·3 years)	Cohort	VDR polymorphisms; BsmI; ApaI; Taq1; and megalin	Associations between single nucleotide polymorphisms of VDR and megalin were explored in conjunction with longitudinal cognitive performance	VDR and megalin gene polymorphisms were associated with longitudinal changes in cognitive decline, in a sex-specific manner

25(OH)D, 25-hydroxyvitamin D; PD, Parkinson's disease.

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and megalin gene polymorphisms to correlate with age-related cognitive decline, specifically in performance tasks assessing global mental status, verbal fluency, visual/working memory and executive function[123,131–134]. Studies investigating VDR polymorphisms and cognitive indices are heterogeneic, inconclusive and exacerbated by the confounder of uncertainy in plasma and cerebral homeostasis of vit-D[135].

Genetic and environmental interactions regulating vitamin-D homeostasis, vitamin D receptor polymorphisms and cognitive performance. The relationship between VDR polymorphisms, vit-D metabolite concentrations and CNS function remains unclear[136]. It has been demonstrated by multiple researchers that expression and functionality of VDR polymorphisms to transactivate specific DNA gene sequences are regulated by both genetics, environment and abundance of bioactive vit-D[128,134,137]. For example, Wilkinson *et al.*[138] observed the TT/Tt VDR genotype of Taq1 polymorphism was associated with tuberculosis in a UK Indian population, but only in a vit-D-deficient state. Wong *et al.*[139] found an individual's susceptibility to colon cancer doubles when genotyped with ff FokI polymorphisms when consuming a low Ca (increased $1,25(OH)_2D_3$ synthesis) in comparison with FF genotypes. Collectively, it is apparent the impact of VDR polymorphisms on the function of the VDR may be strongly influenced by an individual's vit-D status and thereby associated disease risk[135]. By extension, similar effects may be indicated with respect to CNS function and cognition.

Whilst plasma insufficiency of vit-D concomitant with expression of selected VDR isoforms has been associated with Alzheimer's disease and cognitive decline[122], the possibility of strong associations between vit-D concentrations above normal physiological levels and VDR polymorphisms remains to be investigated. Interestingly, some studies have shown that FokI polymorphisms change the VDR translation initiation site and alter its functional properties, producing multiple isoforms of the receptor which influence transcription factor expression[135,140]. The f allele (T nucleotide) on the FokI gene produces a longer VDR protein which is proposed to be less active in influencing transcription factor and thus affecting downstream effects[135]. Conversely, the F allele (C nucleotide) on the FokI gene results in a VDR protein truncated by three amino acids, which is more effective in activating transcription factor[135,141,142]. Orton *et al.*[143] found lower concentrations of 25(OH)D (25·8 ± 2·2 ng/ml) when coding for homozygous genotypes for the shorter VDR isoform, compared with greater 25(OH)D concentrations (33·3 ± 1·6 ng/ml) when coding for the longer VDR isoform in heterozygous and homozygous genotypes.

Vitamin D and neurovascular inflammation

The BBB is a semipermeable membrane comprising the cerebrovascular wall which separates the blood serum from the brain parenchyma[144]. The unique endothelial junctions of the BBB ensure the tight regulation of substances entering the CNS[145–147]. BBB dysfunction is characterised by compromised cerebrovascular integrity leading to unregulated extravasation of serum constituents into the brain parenchyma. An increasing number of studies suggest that impairment of cerebral capillaries at the endothelial cell may be a major risk factor prior to the progression of clinical cognitive dysfunction[24,144,148]. Recent experimental and clinical studies have shown therapeutic benefit in attenuating progression of neurodegeneration/cognitive decline if cerebrovascular disturbances are attenuated[149,150].

Lam *et al.*[24] demonstrated that vit-D-enriched diets resulted in increased brain capillary permeability and neuroinflammation in a dose-dependent manner and independent of serum Ca homeostasis, or suppression of parathyroid hormone. Lam's study is the first to demonstrate that provision of exogenous vit-D supplementation above ordinary physiological levels has significant cerebrovascular-regulating properties. Furthermore, Durk *et al.*[151] recently investigated VDR expression on cerebral endothelial cells, which when activated by its bioactive ligand, $1,25(OH)_2D_3$ was found to alter the kinetics of endothelial p-glycoprotein expression and its substrates. As p-glycoprotein is an ATP-driven efflux pump and a major blockade in the prevention of small-molecule delivery across the BBB and into the brain[152], Durk's findings, whilst not directly implicating vit-D and the regulation of cerebral capillary function, certainly support this notion that vit-D can influence BBB permeability.

High-dose vit-D supplementation has been associated with an increased systemic inflammatory phenotype in concert with increased colitis susceptibility in animal studies[153]. In alignment with the pro-inflammatory phenotype exacerbated by high-dose vit-D intake, a recent study by Krementsov *et al.*[154] reported intriguing results in a pre-clinical model of autoimmune neuroinflammation (multiple sclerosis) whereby high-dose vit-D supplementation unexpectedly exacerbated disease susceptibility, in a sex- and genotype-specific manner. It is apparent that present evidence suggests restoration of vit-D homeostasis to sufficient levels from a vit-D-deficient state has been shown to ameliorate CNS oxidative stress, mitochondrial dysfunction, neuroinflammation and apoptosis that culminate in neurodegeneration[155–159]. However to date, no studies have reported the effects of exaggerated vit-D metabolism on the aforementioned mechanisms on the CNS, and indeed, warrants further investigation.

Clinical considerations: reference serum vitamin D concentration

The definition for appropriate serum vit-D ranges remains controversial[160]. The U.S. Institute of Medicine states insufficient vit-D levels are defined by 25(OH)D levels below 50 nmol/l[161], the Endocrine Society whom reports insufficiency of 25(OH)D below 72·5 nmol/l[162], whilst the Vitamin D Council refers to insufficiency at a concentration below 97·5 nmol/l of circulating 25(OH)D[29]. There are also meta-analyses, RCT and observational studies that suggest sufficient vit-D levels are approximately 100 nmol/l[163–166], a concentration that some organisations would dispute and suggest may be potentially reaching toxic levels[162].

Ross *et al.*[167] and a more recent study by Manson *et al.*[11] state vit-D recommendations are based on bone health, while benefits for other non-skeletal systems, such as the brain, remain

unclear. A diagnosis of vit-D deficiency is generally based on the findings that supplementing with 15–20 μg of vit-D/d (Institute of Medicine RDA for adults) fails to increase vit-D serum levels above 50 nmol/l in a population of North America. Manson *et al.* suggests, however, that a vit-D level of 40 nmol/l would serve the requirement of half of the North American population, whilst vit-D levels of 50 nmol/l are considered adequate to the majority of the indicated population. Presently, it is difficult to robustly define a physiological reference range, particularly in context of optimal CNS function, given the numerous factors that may influence vit-D status and response. Moreover, there is a paucity of studies which consider potential adverse effects associated with persistently higher blood or tissue concentration of bioactive vit-D metabolites. The importance of defining a suitable range and appropriate biomarker to accurately reflect vit-D levels is urgently needed.

Measurement of vitamin D homeostasis

Blood vit-D homestasis is indicative of 25(OH)D bound to VDBP (85 %), 25(OH)D bound to albumin (15 %) and unbound (free) 25(OH)D (Fig. 1)[168].

Serum 25(OH)D is ordinarily used as the surrogate biomarker of homeostasis based on its long half-life, a critical intermediary in the utilisation of 1,25(OH)D by the body, and because tissue level hydroxylase enzymes, such as CYP27B1, function below their K_m values and are below detectable limits. However, recent studies have challenged the validity of utilising 25(OH)D as a surrogate marker of active biological effects illicited as a consequence of 1,25OHD binding to high-affinity receptors[169,170]. The recent updated international recommendations by a panel of 12 vit-D experts concluded that 25(OH)D was not an appropriate marker of vit-D physiological homeostasis[14].

Hilger *et al.*[169] recently conducted a systematic review of vit-D status in global populations in which 195 studies were analysed from forty-four countries, involving over 168 000 participants. The study reported substantial variability in mean serum 25(OH)D concentrations (range 4·9–136·2 nmol/l) within the same geographical regions around the world. Clearly on a global scale, there are huge discrepancies in using 25(OH)D metabolite alone as a reflection of total vit-D status[162,167].

Common methods to assess vit-D metabolites and homeostasis include MS and high-pressure liquid chromatography (HPLC-MS), enzyme immunoassays, competitive protein binding assays, RIA, chemiluminescent immunoassays and automated chemiluminescence protein-binding assays[171,172]. Clinical measures are not harmonised internationally making appropriate reference ranges difficult to compare (Table 1). Due to the significant assay variation in 25(OH)D measurement and substantial inter-assay and laboratory variability[173–175], unsubstantiated assumptions for 25(OH)D as a robust marker of vit-D status have been realised[176].

The Vitamin D External Quality Assessment Scheme is the world's largest specialist scheme for assessing and evaluating the reliability of 25(OH)D assays and has been in operation since 1989. As of January 2017, the data collected by Vitamin D External Quality Assessment Scheme was a contribution of fifty-six countries and covered analysis from approximately thirty different assay methods[176]. Indeed, multiple studies, including reports from Vitamin D External Quality Assessment Scheme, have indicated a great deal of variability between different 25(OH)D metabolite assays as well as inter-laboratory disagreement[173,177–182].

Snellman *et al.*[171] investigated the precision and accuracy of three common commercially available assays (HPLC-MS, RIA and chemiluminescent immunoassays) with diverse results. Researchers accounted for a multitude of confounders such as age, sex, ethnicity, season, altitude, geography, as well as limiting genetic variability, in their cohort of Swedish twins (*n* 204). Mean 25(OH)D concentrations between assays showed up to 30 % (25 nmol/l) variability (HPLC-MS 85 nmol/l; RIA 70 nmol/l; chemiluminescent immunoassays 60 nmol/l). Moreover, Black *et al.*[183] investigated inter-laboratory 25(OH)D concentrations using Australian participants (*n* 840) from three different laboratories. The three laboratories used DiaSorin Liaison and HPLC-MS-based 25(OH)D detection assays, which were analysed against a certified laboratory using a standardised HPLC-MS-based assay. Results from all four laboratories were wide-ranging. Researchers are now engaged in an international effort to suspend meta-analyses publications on whose methodologies are based on unstandardised 25(OH)D data[160]. According to the National Institute of Health, Office of Dietary Supplements USA (2017), an international effort to standardise the measurement of 25(OH)D and its metabolites is currently being led by the Vitamin D Standardization Program[184].

Contemporary liquid chromatography-MS (LC-MS/MS) analytical methods have been developed and validated to simultaneously quantify a comprehensive panel of vit-D compounds in human serum. Historically, the extraction and chromatographing of vit-D compounds have been particulary challenging due to the range of polarities and different molecular moieties. However, in the recent years, optimised precipitation and separation techniques have produced high-sensitivity, recovery and resolution results which can quantitate up to fifteen vit-D compounds (bioactive, inactive, catabolites) and indeed, an appropriate alternative to standardise analysis of vit-D status[185,186].

In cases of suspected clinical vit-D deficiency, physicians generally recommend either ergocalciferol (vit-D_2) or cholecalciferol (vit-D_3) supplementation[187]. Measurement of total vit-D status includes circulating serum calcifediol (25(OH)D) and metabolites 25(OH)D_2 and 25(OH)D_3; however, not all the immunoassays are able to detect 25(OH)D_2 in clinical practice[33]. Nonetheless, Tripkovic *et al.*[188] conducted a meta-analysis and systematic review of RCT from 1966 to 2011 that directly compared the effect both vit-D_2 and vit-D_3 on raising circulating serum 25(OH)D_2 or 25(OH)D_3 levels, respectively. Significantly higher 25(OH)D_3 levels than 25(OH)D_2 were achieved when participants were given 20 μg of vit-D_3, compared with those given 20 μg of vit-D_2, findings replicated in subsequent studies[189,190]. Swanson *et al.*[191] sought to quantify and examine the associations between 25(OH)D_2, and 25(OH)D_3, with their bioactive forms 1,25(OH)$_2D_2$, and 1,25(OH)$_2D_3$, respectively, in a large cohort of older men (*n* 679) to better understand how 25(OH)D_2 relates to the other vit-D metabolites. Interestingly, greater levels of 25(OH)D_2 were associated with

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lower levels of both $25(OH)D_3$ and biologically active $1,25(OH)_2D_3$. Furthermore, $25(OH)D_2$ was not found to be associated with higher total levels of 25(OH)D or physiologically relevant $1,25(OH)_2D$. Collectively, the findings suggest that differences between $25(OH)D_2$ and $25(OH)D_3$ are due to dissimilar affinities for the VDR, which appears to be linked to an extra step of 24-hydroxylation that inactivates $1,25(OH)_2D$[192]. Additionally, it is thought that $25(OH)D_3$ is the preferred substrate for hepatic 25-hydroxylase enzyme, CY2R1, which in combination may alter the rate of 24-hydroxylation[188].

Interestingly, Jones *et al.*[193] compared the serum half-lives of $25(OH)D_2$ and $25(OH)D_3$ in two separate populations from the UK and Gambia, Africa (*n* 36), with differing 25(OH)D status. Results showed that not only was the half-life of $25(OH)D_2$ shorter than $25(OH)D_3$ but also the half-lives were affected by VDBP concentration and genotype.

Vitamin D binding protein measurement and polymorphisms

Numerous tissues express the VDBP with liver as the major source for plasma abundance. However, VDBP expression has also been demonstrated in the brain, spinal cord, kidney, skeletal muscle, heart, lung, intestine and bone[194,195]. The VDBP can be determined in blood serum, cerebrospinal fluid, saliva, seminal fluid and breast milk[196]. VDBP is the primary chaperone protein for 25(OH)D due to higher affinity compared with albumin[197]. The free hormone hypothesis postulates that protein-bound hormones are biologically inactive, while unbound hormones are biologically free to exert their physiological activity[198].

Notionally, only 1 % of total 25(OH)D concentration is available for conversion to the bioactive $1,25(OH)D$[168]. VDBP serves as a sink for 25(OHD) and may be critical in the context of conversion to $1,25(OH)D$[197]. There are three forms of VDBP polymorphisms that exist, originally referred to as GC1F, GC1S and GC2. These allow for six allelic phenotypes (1s/1s, 1s/ 1f, 1s/2, 1f/1f, 1f/2 and 2/2)[199]. These phenotypes can be identified by genotyping for the two SNP, rs7041 and rs4588, in the GC gene[200]. These polymorphisms occur at a diverse range of frequencies among different races and ethnicities[197]. Allelic variants of VDBP are at varied concentrations within blood[201] and different binding affinities for 25(OH)D and $1,25(OH)D$[202,203]. Engelman *et al.*[204] showed that homozygosity for the CG1F allele occurred in 53 % of African Americans but only 6 % of Caucasians and 13 % of Hispanics. Interestingly, over 50 % of African Americans has a vit-D deficiency diagnosis, yet their low 25(OH)D concentrations do not appear to be linked with a higher risk of bone fractures, as they do in white Americans[205]. Consistent with the latter, Powe *et al.*[206] found racial, ethnic variation in VDBP polymorphism concentrations in black and white Americans and showed difference in total 25(OH)D, but comparable abundance of bioavailable 25(OH)D. Harris[207] previously compared vit-D metabolites in American Caucasians and African Americans, indicating African Americans have higher circulating concentrations of the bioactive vit-D at a given level of 25(OH)D compared with Caucasians. In studies by Lutsey *et al.*[208], they reported lower VDBP levels in African Americans in comparison with Caucasians were associated with a higher concentration of 25(OH)D. Furthermore, Sinottee *et al.*[209] evaluated the association between VDBP polymorphisms and 25(OH)D concentrations in white premenopausal Caucasian French women (*n* 741) and concluded that circulating 25(OH)D is highly correlated with VDBP polymorphisms. Other studies investigating VDBP polymorphisms have also found varied vit-D metabolite concentrations amongst race and ethnic groups[204,210–212].

In Tromsø, Northern Norway, longitudinal population-based health surveys covering general, medical and pathological information have been conducted at 6–7 years intervals since 1974[200]. Thus far, blood samples have totalled 27 000 participants and genotyping for VDBP polymorphisms has been undertaken in 11 704 participants. The prevalence in VDBP polymorphisms ranged between 4·4 and 30·9 % within population. Findings clearly demonstrate that 25(OH)D levels were not only dependent on VDBP and albumin concentrations but also significantly associated with VDBP polymorphisms. Additionally, factors influencing VDBP concentration and ultimately, VDBP polymorphisms and vit-D status, include age and sex[213,214], diurnal rhythms[215] and obesity[216,217]. Recent studies have further demonstrated that serum VDBP concentration is decreased in pathological states including, type 1 diabetes mellitus[218], chronic liver disease[168,219] and renal disease[220].

Clinical indications and health promotion

Vit-D insufficiency as currently determined is suggested in approximately 14 % of the world's population[221]. This has resulted to public vit-D recommendations from Government institutions and agencies between 25 and 250 μg/d[29–31]. In response to public health promotion of vit-D deficiency to resolve what has now been classed as a 'major global epidemic'[222], a significant number of middle-aged and elderly individuals are supplementing their vit-D intake in an attempt to reduce the putative health risks associated with vit-D deficiency, including cognitive decline and osteoporosis[223].

It is estimated that 86 % of the global population is reporting as having sufficient vit-D levels[221]. By extension, it is possible that recommendation for exogenous intake through fortified foods/supplements at 15 to 20 μg may be potentially harmful in some individuals, particularly if endogenous levels of $1,25(OH)_2D_3$ are already heightened[223]. Researchers in the USA analysed data from a national survey over 15 years (1999–2014) and found an 18 % increase in the people taking vit-D over 25 μg/d and a 2·8 % increase in the amount of people taking of over 100 μg of vit-D/d[223]. Unfortunately, this trend is not isolated and can be seen on a global scale. In Australia, the majority (77 %) of Australian citizens have supposedly sufficient vit-D levels, yet remarkably, one in twenty adults were reported to be taking vit-D supplements. Among those with high serum vit-D ((25(OH)D) >100 nmol/l), one in ten reported regular intake of exogenous vit-D[224]. Similarly, despite more than two-thirds (68 %) of Canadians reported having sufficient vit-D serum levels ((25(OH)D); >50 nmol/l), 34 % still report taking

a regular vit-D supplements[225]. Furthermore, several large national and international clinical trials have confirmed participant numbers between 5000 and 30 000 per trial for a variety of indications. These trials are currently administering from 50 μg vit-D/d[226] or 1500–2500 μg vit-D/month[227,228], exploring therapeutic rates of 250–7500 μg vit-D/dose[28,229–233] without adequate evidence such levels are safe in the context of cognitive function. Clearly, serum measures of vit-D must be considered carefully as a putative surrogate marker of CNS vit-D homeostasis.

Conclusion

There is substantial scientific, clinical and public health interest in how vit-D modulates CNS function. On the basis of purported benefits in cognitive performance, supplementary use of vit-D has increased markedly in developed countries, often without clinical indication. The latter may be of some concern given an emerging body of evidence which suggests either no benefit or possibly even harm in subjects taking exogenous vit-D supplementation who have otherwise adequate levels of vit-D as currently assessed. Significant limitations in our contemporary understanding of vit-D effects on the CNS include the relevance of serum measures to CNS homeostasis, regulation of conversion and deactivation of bioactive metabolites with studies suggesting significant subject variability. Other challenges include understanding longitudinal/life-long and possibly epigenetic effects when considering causal association realised over decades of life. Greater insight into fundamental physiological processes realised through robust pre-clinical models would be informative in supporting clinical conisiderations of vit-D homeostasis in the context of CNS health.

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