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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 (VDR: vit-D receptor; PDIA3: 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 to 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.

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1.0. Introduction

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 calcium and phosphorous 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 calcium 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 demonstrate 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 suggest an inverse U-shaped association with low and greater abundance of serum vit-D both correlated with neurocognitive deficits [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[27; 28]. Whilst the physiological sequalae associated with vit-D deficiency are reasonably well established, the putative detrimental effects of exaggerated vit-D is noteworthy in the context of public health trends showing increased self-prescribed use of supplementary vit-D nutraceuticals and clinical trials with interventions of up to 300,000 international units (IU) of vit-D_3 per dose[29-33].

Collectively, at present there is no substantiated serum reference range for vit-D that can reliably be indicated to support optimal CNS function[25]. 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[34].

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2.0. 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; 35] during exposure to ultraviolet-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)[35] (Fig. 1).

2.1. 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[36]. Principally within the kidney, CYP27B1 converts 25(OH)D to the biologically active metabolite 1,25-dihydroxyvit-D (calcitriol; [1,25(OH)$_2$D$_3$]), where it is released into circulation as an endocrine modulator. In blood, the the biologically active 1,25(OH)$_2$D$_3$ 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)$_2$D$_3$ as a consequence of conversion to 24,25-dihydroxyvitamin-D (24,25(OH)$_2$D$_3$)[37-40].

The catalytic conversion of 25(OH)D to 1,25(OH)$_2$D$_3$ is thought to be principally regulated via ionised calcium homeostasis[41; 42]. When blood calcium is low, parathyroid hormone (PTH) is released, stimulating expression of CYP27B1 and decreasing CYP24A1, resulting in an increase of 1,25(OH)$_2$D$_3$ synthesis. Increased levels of 1,25(OH)$_2$D$_3$ subsequently inhibit PTH, thus avoiding potential risk for hypercalcemia[36; 43]. Moreover, a negative feedback loop occurs as a consequence of 1,25(OH)$_2$D$_3$ to the vitamin D receptor (VDR), with down-regulation of transcription for CYP27B1[40] and an increased expression of inactivating enzyme, CYP24A1[44; 45]. Similarly, blood phosphate is a potent regulator of CYP-mediated conversion of vit-D metabolites. Dietary phosphates and heightened blood abundance stimulates Fibroblast-Growth-Factor-23 (FGF-23) secretion [46-48] by osteocytes in the bone matrix of skeletal tissue. The FGF-23 inhibits kidney CYP27B1 and increases CYP24A1 expression, collectively suppressing 1,25(OH)$_2$D$_3$[49]. Conversely, circulating low levels of phosphate increases the production of CYP27B1, and thus increase 1,25(OH)$_2$D$_3$ concentrations in the blood; the latter would then stimulate FGF23 production, which would complete the feedback loop and downregulate CYP27B1 expression[42; 49].

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In 1987, Stumpf and 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[50]. 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[50]. Indeed, current 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[51].

2.2 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[52-54]. In most cell types including neuronal cells, VDRs are predominantly (58%) found in the nucleus[55], however, observational studies have also shown VDRs on the plasma membranes of cells[56] and within the cytosol[55]. 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[53; 57]. The $1,25(OH)_2D_3$-VDR-RXR complex then binds to specific enhancer elements, referred to as vit-D response elements (VDRE)[53], 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[57-60] (Fig. 1).

3.0. Vitamin D receptors and the CYP enzymes in brain

It was not until commercial antibodies became available that research groups such as Veenstra *et al.*[61] and Prufer *et al.*[62], 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; 56; 63-66], however, some researchers disputed these findings and suggested relatively low VDR abundance, or exaggerated measures of VDR[67; 68]. 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[69]. However, in 2014, Eyles and colleagues provided further evidence of cerebral abundance of VDR using proteomic techniques[63].

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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 vit-D receptor[70]. 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)[70]. Consistent with an autocrine pathway, Eyles *et al.*[71] also described the coexpression of CYP27B1 with VDR in human cerebral cortex, hippocampal formation (HPF) and hypothalamus[9]. CYP27B1 and VDR were immunodetected in both neuronal and glial cells, with expression of CYP27B1 restricted to the cytoplasm, and VDR within the nucleus, respectively (Fig. 1). VDR immunoreactivity was indicated within HPF 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- HPF regions, brain regions pivotal for spatial learning and memory formation and considered one of most vulnerable regions in Alzheimer's disease pathology [63; 71-73] (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.

3.1. Cellular distribution of vit-D receptor and associated enzymes in the CNS

At a cellular level, Smolders *et al.*[74] 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.*[75] demonstrated CYP2R1 and VDR expression in human pericytes. As major structural and functional cells comprising 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 expression has been confirmed in astrocytes, endothelial cells, microglia, oligodendrocytes and notably, with significant expression of CYP27B1 particularly indicated in neurons[76]. However, differential expression of the vit-D receptor 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 [77]. Figure 1 summarises all current evidence for vit-D associated metabolites and receptors that have been characterised in the brain.

3.1.1. Cerebral vitamin D homeostasis relative to peripheral vit-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[78]. Unfortunately, there is a paucity of literature considering putative associations between serum and cerebral vit-D homeostasis. Xue *et al.*[78] showed that rats fed a vit-D_3-deficient diet had markedly lower cerebral $25(OH)D_3$ metabolite concentration in comparison to rats maintained on a vit-D_3 supplemented diet. Consistent with the possibility of diffusion, or factilitated 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.

4.0. 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 pereption has resulted in the promotion of self-prescribed vit-D supplementation without clinical indication[79].

A number of randomised controlled trials (RCT) and meta-analyses suggest a positive association of serum vit-D with cognition[80], at a threshold serum concentration of $25(OH)D$ level >25 nmol/L. Benefits are indicated on global cognitive performance[81] and in specific cognitive domains including visuospatial skills, language, working memory, memory recall, concentration, and attention[82]. However, other studies indicate that exaggerated levels of vit-D are also associated with poorer cognitive function[26; 83-85]. 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[84; 86].

4.1. 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; 87], however, paradoxical findings suggest no such association[83] (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[25]. 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

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neuropsychological function and a greater risk of dementia[88]. In agreement with these large cohort studies, Hooshmand et al.[89] 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 was found to 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 were found to be unaffected[89]. Furthermore, Chaves et al.[90] 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 indivudals of advanced age, have failed to show a correlation between serum vit-D status and cognitive outcomes. Schneider et al.[91; 92] 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.[93] reported no association between vit-D and increased risk for mild cognitive impairment (MCI), nor did serum vit-D predict the conversion from cognitively normal or MCI to dementia. Other studies have not found any association between low 25(OH)D and cognitive performance[94; 95]. Using a Mendelian randomisation study design in a recent study, Maddock et al.[96] explored the relationship between serum 25(OH)D concentration and cognitive function. Data collected from 17 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.[97] found no association between serum 25(OH)D or 1,25(OH)$_2$D$_3$ concentrations and multiple cognitive subdomains in 3369 men aged between 40-79 years of age.

4.1.1. Vitamin D supplementation and cognitive function

Results from RCT's linking vit-D deficiency and cognitive decline have not been promising[91; 98; 99] (Table 1). There was no benefit on cognition or memory realised in vit-D deficient older participant's supplemented with vit-D [94; 99; 100]. Results from the Women's Health Initiative study found that vit-D and calcium supplementation over a period of 8 years had no beneficial effects on cognition[101]. Studies by Stein et al.[99] 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.[94] failed to demonstrate positive effects of vit-D supplementation in attenuating cognitive dysfunction, or on lowering the risk of dementia when compared to placebo. Moreover, Castle and colleagues recently reported on the differential dosage effects of vit-D on

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specific cognitive domains in healthy, post-menopausal women whereby higher doses were found associated with poorer reaction time and measures of learning and memory[85]. Furthermore, a recent literature review by Landel et al.[73] determined that there was no solid evidence to suggest that exogenous supplementation of vit-D improves cognition in those with already have sufficient serum levels of vit-D. Collectively, results of RCTS's do not consistently suggest postive effects of exogenous vit-D on cognitive performance.

4.2. Hypervitaminosis-D and cognitive performance

Recent studies suggests 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.[26] 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.[83] 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.[102] found an association between higher serum vit-D status and poorer verbal episodic memory performance in those with normal calcium-PTH homeostasis in middle-aged and older individuals.

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.[103] demonstrated that vit-D treatment of cultured hippocampal cells can initiate cellular apoptosis and similar findings have been indicated in cancerous cell lines[104-106]. 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[107].

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4.2.1. Vit-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[108-111]. Moreover, the ability to synthesize endogenous vit-D within the epidermis, or to activate 25(OH)D may be age-dependent[112]. 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[36; 112; 113]. In addition to putative age-associated changes in peripheral biosynthetic and conversion of vit-D metabolites, pre-clinical and clinical data indicates 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[36]. Several studies have demonstrated a reduction in intestinal VDR expression [114-116] with ageing in both animal and human studies, although others have reported no change[117; 118]. 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[36]. 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.

4.2.2. Hypervitaminosis D and accelerated ageing

The global prevalence of hypovitaminosis-D during ageing is well documented[119]. However, possible effects of hypervitaminosis-D on the ageing process are not yet clear[28]. 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, hypercalcemia, cardiovascular complications (vascular-related) and early death, supporting the association between hypervitaminosis-D and accelerated ageing[120]. Two genes of interest in this area of study is Fibroblast-Growth-Factor-23 (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[121]. 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[28]. Furthermore, hypervitaminosis-D in these mutant mice exhibit an accelerated ageing phenotype[27]. Genetic ablation of FGF-23 or KLOTHO genes in other rodent models results in hypervitaminosis-D, hypercalcemia and hyperphosphatemia; corresponding with a phenotype consistent with premature ageing[27]. Remarkably, Tsujikawa *et al.*[122] reported that dietary restriction of vit-D reverses the premature-ageing phenotypes and indeed, prolongs life in 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 cardiovascular disease [123]. These findings provide strong evidence that hypervitaminosis-D may be causally associated with the ageing process.

4.3. VDR 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 (MCI), Parkinson's disease (PD) and implicated with cognitive function[124; 125]. However, literature contributing to the delineation of the putative metabolic pathways that result in cognitive dysfunction are varied and inconclusive.

The VDR is synthesized by the *VDR* gene, located on chromosome 12 and composed of 9 exons[126], of which, several genetic variations have been recognized. Approximately 1% of the population has a DNA gene variation of the VDR[127]. 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 *TaqI*, and are often associated to phenotypes involving bone mineral density, osteoporotic fracture risk and cancer[124; 125].

Kuningas *et al.*[125] observed a significant decline in cognitive performance in those carrying the *BsmI* and *TaqI* 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[128]. In contrast, Leymann *et al.*[129] 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.*[130] found both *BsmI* and *ApaI* polymorphisms significantly associated with an increased risk of MCI in a cohort of elderly Uygur people. A 2015 meta-analysis, totalling seven studies with 2034 PD cases and 2432 healthy controls, found polymorphisms of *ApaI*, *BsmI*, and *TaqI* were not associated with the susceptibility to PD while the FokI (C and T allele) polymorphisms were associated with an increased risk in PD[131].

In a recent study, Beydoun *et al.*[132] evaluated associations of VDR polymorphisms (*Cdx, Bsml, Apal and Taql)* and low density lipoprotein receptor, megalin, with a decline in longitudinal cognitive performance in 1024 healthy African-American adults. Megalin is expressed in endothelial cells of the blood-brain barrier and potentially mediates vit-D transport from the blood to brain parenchyma (Fig. 1). Beydoun and others have reported that VDR (*BsmI/ApaI/TaqI)* and megalin gene

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polymorphisms to correlate with age-related cognitive decline, specifically in performance tasks assessing global mental status, verbal fluency, visual/working memory, and executive function [125; 133-136]. 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[137].

4.3.1. Genetic and environmental interactions regulating vit-D homeostasis, VDR polymorphisms and cognitive performance

The relationship between VDR polymorphisms, vit-D metabolite concentrations and CNS function remains unclear[138]. It has been demonstrated by multiple researchers that expression and functionality of VDR polymorphisms to transactivate specific DNA gene sequences is regulated by both genetics, environment and abundance of bioactive vit-D[130; 136; 139]. For example, Wilkinson *et al.*[140] observed the TT/Tt VDR genotype of *TaqI* polymorphism was associated with Tuberculosis in a UK Indian population, but only in a vit-D deficient state. Wong *et al.*[141] found an individual's susceptibility to colon cancer doubles when genotyped with ff *FokI* polymorphisms when consuming a low-calcium (increased $1,25(OH)_2D_3$ synthesis) in comparison to 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[137]. 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 have been associated with Alzheimer's disease and cognitive decline[124], the possibility of strong associations between vit-D concentrations above normal physiological levels and VDR polymorphisms remain to be investigated. Interestingly, some studies have shown that *FokI* polymorphisms changes the VDR translation initiation site and alters its functional properties, producing multiple isoforms of the receptor which influence transcription factor expression[137; 142]. 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[137]. Conversely, the F allele (C nucleotide) on the *FokI* gene results in a VDR protein truncated by 3 three amino acids, which is more effective in activating transcription factor[137; 143; 144]. Orton *et al.*[145] 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.

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4.4. Vitamin D and neurovascular inflammation

The blood-brain barrier (BBB) is a semipermeable membrane comprising the cerebrovascular wall which separates the blood serum from the brain parenchyma[146]. The unique endothelial junctions of the BBB ensure the tight regulation of substances entering the CNS[147-149]. 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; 146; 150]. Recent experimental and clinical studies have shown therapeutic benefit in attenuating progression of neurodegeneration / cognitive decline if cerebrovascular disturbances are attenuated[151; 152].

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 calcium 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*.[153] 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[154], Durks'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[155]. In alignment with the pro-inflammatory phenotype exacerbated by high dose vit-D intake, a recent study by Krementsov *et al*.[156] 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 gender and genotype specific manner. It is apparent that current 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[157-161]. 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.

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5.0. Clinical considerations: Reference serum vit-D concentration

The definition for appropriate serum vit-D ranges remains controversial[162]. The U.S. Institute of Medicine (IOM) states insufficient vit-D levels are defined by 25(OH)D levels below 50 nmol/L[163], the Endocrine Society whom report insufficiency of 25(OH)D below 72.5 nmol/L[164], whilst the Vitamin D Council refers to insufficiency at a concentration below 97.5 nmol/L of circulating 25(OH)D[30] . There are also meta-analyses, RCT's and observational studies, that suggest sufficient vit-D levels are approximately 100 nmol/L[165-168], a concentration that some organisations would dispute and suggest may be potentially reaching toxic levels[164].

Ross et al.[169] 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 600-800 international units (IU) of vit-D per/day (IOM Recommended Dietary Allowance (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 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.

6.0. Measurement of vitamin D homeostasis

Blood vit-D homestasis is indicative of 25(OH)D bound to vit-D binding protein (VDBP) (85%), 25(OH)D bound to albumin (15%), and unbound (free) 25(OH)D (Fig. 1)[170].

Serum 25(OH)D is ordinarily used as the surrogate biomarker of homeostasis based on its long half-life, a critical intermediary in the utilization 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[171; 172]. 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.[171] 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)

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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[164; 169].

Common methods to assess vit-D metabolites and homeostasis include mass spectrometry (MS) and high-pressure liquid chromatography (HPLC-MS), enzyme immunoassays (EIA), competitive protein binding assays (CPBA), radioimmunoassays (RIA), chemiluminescent immunoassays (CLIA), and automated chemiluminescence protein-binding assays (CLPBA)[173; 174]. 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 [175-177], unsubstantiated assumptions for 25(OH)D as a robust marker of vit-D status have been realised[178].

The Vitamin D External Quality Assessment Scheme (DEQAS) 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 DEQAS was a contribution of 56 countries and covered analysis from approximately 30 different assay methods[178]. Indeed, multiple studies, including reports from DEQAS have indicated a great deal of variability between different 25(OH)D metabolite assays as well as inter-laboratory disagreement[175; 179-184].

Snellman et al. [173] investigated the precision and accuracy of three common commercially available assays (HPLC-MS, RIA and CLIA) with diverse results. Researchers accounted for a multitude of confounders such as age, gender, 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; CLIA 60 nmol/L). Moreover, Black et al.[185] 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 unstandardized 25(OH)D data[162]. 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 (VDSP)[186].

Contemporary liquid chromatography-mass spectrometry (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

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the recent years, optimised precipitation and separation techniques have produced high-sensitivity, recovery and resolution, results which can quantitate up to 15 vit-D compounds (bioactive; inactive; catabolites) and indeed, an appropriate alternative to standardise analysis of vit-D status[187; 188].

In cases of suspected clinical vit-D deficiency, physicians generally recommend either ergocalciferol [vit-D_2] or cholecalciferol [vit-D_3] supplementation[189]. 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[34]. Nonetheless, Tripkovic et al.[190] conducted a meta-analysis and systematic review of RCT's 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 then $25(OH)D_2$ were achieved when participants were given 800 IU of vit-D_3, compared to those given 800 IU of vit-D_2, findings replicated in subsequent studies[191; 192]. Swanson et al.[193] 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 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 $1,25(OH)_2D$[194]. 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[190].

Interestingly, Jones et al.[195] compared the serum half-lives of $25(OH)D_2$ and $25(OH)D_3$ in two separate populations from the United Kingdom 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 that the half-lives were affected by VDBP concentration and genotype.

7.0. Vitamin D binding protein measurement and polymorphisms

Numerous tissues express the Vitamin-D-Binding-Protein (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[196; 197]. The VDBP can be determined in blood serum, cerebrospinal fluid, saliva, seminal fluid, and breast milk[198]. VDBP is the primary chaperone protein for 25(OH)D due to higher affinity compared to albumin[199]. The free hormone hypothesis postulates that protein bound hormones are biologically inactive, while unbound hormones are biologically free to exert their physiological activity[200].

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Notionally, only 1% of total 25(OH)D concentration is available for conversion to the bioactive 1,25(OH)D[170]. VDBP serves as a sink for 25(OHD) and may be critical in the context of conversion to 1,25(OH)D [199]. 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)[201]. These phenotypes can be identified by genotyping for the two single nucleotide polymorphisms, rs7041 and rs4588, in the GC gene[202]. These polymorphisms occur at a diverse range of frequencies among different race and ethnicities[199]. Allelic variants of VDBP are at varied concentrations within blood[203] and different binding affinities for 25(OH)D and 1,25(OH)D[204; 205]. Engelman *et al.*[206] 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 have 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[207]. Consistent with the latter, Powe *et al.*[208] 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[209] 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 to Caucasians. In studies by Lutsey *et al.*[210] they reported lower VDBP levels in African Americans in comparison to Caucasians was associated with a higher concentration of 25(OH)D. Furthermore, Sinottee *et al.*[211] 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 to VDBP polymorphisms. Other studies investigating VDBP polymorphisms have also found varied vit-D metabolite concentrations amongst race and ethnic groups[206; 212-214].

In Tromsø, Northern Norway, longitudinal population-based health surveys covering general, medical and pathological information has been conducted at 6–7 years intervals since 1974[202]. Thus far, blood samples have totalled 27,000 participants, and genotyping for VDBP polymorphisms have 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 gender[215; 216], diurnal rhythms[217] and obesity[218; 219]. Recent studies have further demonstrated that serum VDBP concentration is decreased in pathological states including, type 1 diabetes mellitus[220], chronic liver disease[170; 221], and renal disease[222].

8.0. Clinical indications and health promotion

Vit-D insufficiency as currently determined is suggested in approximately 14% of the world's population[223]. This has resulted to public vit-D recommendations from Government institutions and agencies from between 1000-10,000 IU/day[30-32]. In response to public health promotion of vit-D deficiency to resolve what has now been classed as a 'major global epidemic' [224], 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[225].

It is estimated that 86% of the global population are reporting as having sufficient vit-D levels[223]. By extension, it is possible that recommendation for exogenous intake through fortified foods/supplements at 600 IU to 800 IU may be potentially harmful in some individuals, particularly if endogenous levels of $1,25(OH)_2D_3$ are already heightened[225]. 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 1000 IU/day and a 2.8% increase in the amount of people taking of over 4000 IU of vit-D/day[225]. 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, 1 in 20 adults were reported to be taking vit-D supplements. Among those with high serum vit-D ([25(OH)D] >100 nmol/L), 1 in 10 reported regular intake of exogenous vit-D[226]. 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[227]. Furthermore, several large national and international clinical trials, have confirmed participant numbers between 5, 000-30, 000 per trial for a variety of indications. These trials are currently administering from 2000 IU vit-D/day[228], or 60,000-100,000 IU vit-D/month[229; 230], exploring therapeutic rates of 10, 000 - 300, 000 IU vit-D per dose[29; 231-235] 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.

9.0. 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 vitamin 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 vitamin 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 realized over decades of life. Greater insight into fundamental physiological processes realized through robust pre-clinical models would be informative in supporting clinical conisiderations of vit-D homeostasis in the context of CNS health.

Conflict of Interest

The authors declare no conflicts of interest.

Authorship

All authors contributed to the literature search, manuscript design, manuscript writing and revisions of the manuscript. All authors read and approved the final manuscript.

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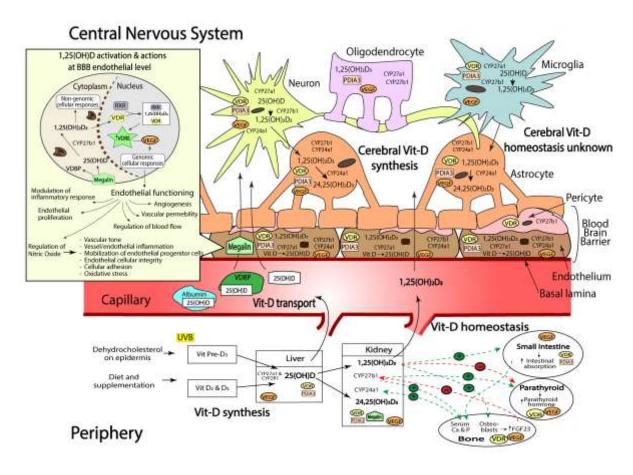
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Figure 1. Sources of vitamin D (vit-D), peripheral and cerebral synthesis and homeostasis, blood and blood brain barrier transport. Vit-D is obtained by the conversion of dehydocholesterol on the epidermis when exposed to UVB (vit-D$_3$), diet and supplementation (vit-D$_2$ & D$_3$). Vit-D$_2$ & 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)$_2$D$_3$ (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 take place within endothelial cells and neurons, before further hydroxylation to 1,25(OH)$_2$D$_3$ in the neurons and microglia. 1,25(OH)$_2$D$_3$ activates either genomic actions via VDR, or non-genomic actions via PDIA3. As VDR is expressed in lower quantities in brain compared to kidney, PDIA3 may act as the main cerebral vit-D receptor. Peripheral vit-D homeostasis is tightly regulated by hormones such as calcium (Ca), parathyroid

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hormone (PTH), fibroblast growth factors (FGF-23) and abundance of $1,25(OH)_2D_3$, however cerebral vitamin D homeostasis, along with relationship between peripheral and cerebral homeostasis, remain unknown. Vit-D is predominantly transported in peripheral blood bound to vit-D binding protein (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 via passive diffusion. The blood brain barrier consists of basal lamina, endothelial cells, pericytes and astrocytes. 25(OH)D bound to VDBP is dependent on internalisation by megalin transport protein. Once internalized, 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 responsive element (VDRE)[63; 71; 74; 76; 132]. Activation of VDRE promotes expression of vascular endothelial growth factor (VEGF)[236] which is located throughout the brain including astrocytes[237], neurons[238], oligodendrocytes[239], microglial cells[240] and the endothelium at the BBB [241]. VEGF binds to VEGF specific receptors like those in the endothelium, controlling a wide range of endothelial cellular activities[236]. 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[236; 242]. VEGF also induces the release of nitric oxide (NO) from endothelial cells[243]. NO is an important biomolecule which mediates various metabolic pathways[244] including vessel inflammation and integrity, regulation of vascular tone, cellular adhesion, and oxidative stress. Additionally, NO is partly responsible for the mobilization of endothelial progenitor cells essential for vessel maintenance and repair[245]. Vit-D and VEGF dysregulation can therefore substantially modulate endothelial cell function[236].

Table 1. Summary of randomised controlled trials and observational studies exploring 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[94]	RCT	4143 elderly women (> 65 years of age) without probable dementia.	Global cognitive function; attention; working memory;	400 IU vit-D₃ combined with calcium carbonate/day; mean follow-up of	Serum 25(OH)D not measured	No associations were reported between vit-D supplementation and

			verbal knowledge; memory, spatial ability	7.8 years		cognitive outcomes
Dean *et al.* 2011[95]	RCT	128 healthy young adults (mean age of 21.45 years)	Working, response inhibition; cognitive flexibility	5, 000 IU of vit-D_3/day 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[99]	RCT	63 community dwelling participants age ≥ 60 years with mild-moderate AD.	Visuospatial; language; concentration; attention; working memory; executive function and orientation	1, 000 IU & 6, 000 IU of vit-D_2/day for 8 weeks	Serum 25(OH)D via radioimmunoassay	High-dose vit-D supplementation conferred no benefit for cognition in individuals with mild-moderate AD.
Castle *et al* 2019[85]	RCT	Healthy, postmenopausal women (50–70 years of age; BMI: 25–40 kg/m²)	Executive functioning; attention and flexibility of attention; reaction time working memory capacity & spatial planning; motor control	600 IU, 2, 000 IU or 4, 000 IU vit-D_3/day for 12 months	Serum 25(OH)D via radioimmunoassay	Differential dosage effects of vit-D3 on specific cognitive domains were reported. Participants from the 2000 IU/day group performed better in learning and memory measures in comparison to those taking 600 IU and 4, 000 IU vit-D3 per day. Slower reaction time was observed

						in participants on daily 4, 000 IU vit-D3.
Observational studies						
Lam *et al* 2015[102]	Cross-section al	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-calcium-PTH homeostasis.
Granic *et al* 2015[26]	Cross-section al	775 older participants over 85yrs	Global functioning & attention	N/A	Serum 25(OH)D via radioimmunoas say	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

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						vit-D supplementation; this was not observed in non-users of vit-D supplements.
McGrath *et al* 2007[83]	Cross-sectional	Adolescent group (n= 1,676, 12-17 years), adult group (n= 4,747, 20-60 years), elderly group (n = 4,809, 60-90 years).	Verbal & visuospatial learning and memory; visual attention; coding speed; concentration; psychomotor speed	N/A	Serum 25(OH)D via radioimmunoassay	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[84]	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[92]	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 mass spectrometry	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.
Schneider *et al* 2014[91]	Cohort	1, 652 participants (52% white; 48% black) aged 45–65 years at baseline	Verbal & 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 mass spectrometry	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[93]	Cohort	428 elderly participants (mean age 85.2 years); cognitively normal (n = 200); mild cognitive impairment (n = 46); dementia (n = 182)	Global functioning; episodic & 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[97] | Cohort | 2,430 middle-aged and elderly men aged between 40–79 | Visuo-constructional ability; visual memory; processing speed | 4.4-year follow-up | Serum 25(OH)D via radioimmunoassay and 1,25(OH)2D via liquid chromatography-tandem mass spectrometry | No association reported between serum 25(OH)D or 1,25(OH)$_2$D concentrations and each of the cognition subdomains measured. |

Table 2. Summary of cross-sectional and cohort studies exploring the link between VDR gene polymorphisms and cognitive outcomes

Reference / Author/ Year	Sample participants / size	Study design	VDR Gene Polymorphisms Investigated	Study focus	Outcomes
Pettersen *et al.* 2015[124]	Healthy adults (n = 78)	Cross-sectional	Fokl	The relationship between the VDR Fokl genotype, serum 25(OH)D levels and cognitive functioning, was investigated.	Polymorphisms in the Fok1 VDR gene were associated with non-verbal executive task and global cognitive performance, independent of serum 25(OH)D levels.
Kuningas *et al.* 2009[125]	563 participants > 85-years of age from the Leiden 85-plus study	*Cohort*	*Cdx-2*; *FokI*; *BsmI*; *ApaI*; *TaqI*.	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 Bsml and Taql polymorphisms exhibited poorer cognitive functioning.
Gatto *et al.* 2016[139]	Non-Hispanic Caucasian Parkinson's disease (PD) participants (n = 190) from the Parkinson Environment Gene study (average age of diagnosis 67.4 years)	Cohort	Fokl	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 *Fok*I A allele was associated with a significant decrease in the cognitive test score per year of follow-up.
Lehmann *et al.* 2011[129]	Caucasian participants with	Cohort	Apa1 and Taq1	This study used DNA from Alzheimer's disease cases and	Apa1 T and Taq1 VDR polymorphisms were significantly associated

	Alzheimer's disease (n = 255) & healthy elderly controls (n = 260) (mean age 78.4 years)			cognitively screened elderly controls from the longitudinal cohort of the Oxford Project to investigate the relationship between two *VDR* polymorphisms and memory and ageing.	with cognitive decline and increased risk of Alzheimer's disease.
Keyimu *et al.* 2014[130]	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[123]	1024 healthy African American adults (mean age 52 years)	Cohort	VDR polymorphisms; BsmI; ApaI; TaqI; 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/TaqI and megalin gene polymorphisms altered age-related cognitive pathways, specifically in global mental status, verbal fluency, visual & working memory, and executive function.
Beydoun *et al.* 2012[133]	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; TaqI; 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 gender-specific manner.