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Review

Vitamin D deficiency and Alzheimer disease: Common links

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ABSTRACT

Vitamin D (VitD) deficiency is a worldwide epidemic with estimates of 1 billion affected. In addition to the classically known roles of VitD in calcium regulation and bone health, recent studies demonstrated VitD to be an essential/vital neurosteroid hormone playing a wide variety of essential protective and regulatory roles in the brain. This paper reviews much of the mounting evidence of the detrimental effects of VitD deficiency on the brain and the association of many of these common links with Alzheimer's disease (AD). We also discuss the beneficial effects seen from VitD supplementation. Based on this accumulation of studies, we propose that VitD screening should be performed at least in those individuals at risk for VitD deficiency and AD. With appropriate medical counsel, those found to be VitD deficient should be considered for appropriate supplementation.

Contents

Abbreviations: ROS/RNS, reactive oxygen and reactive nitrogen species; VitD, vitamin D; Ca^{2+}, calcium; P_i, phosphate; AD, Alzheimer's disease; PCAD, preclinical Alzheimer's disease; MCI, mild cognitive impairment; Aβ, amyloid beta; NFTs, neurofibrillary tangles; APP, amyloid precursor protein; NE, norepinephrine; ApoE, apolipoprotein E; ApoA1, apolipoprotein A1; TNF-α, tumor necrosis factor-α; BBB, blood–brain barrier; UVB, sunlight; $VitD_3$, cholecalciferol; $VitD_2$, ergocalciferol; DBP, VitD-binding protein; 25-$(OH)D_3$, 25-hydroxyvitamin D_3; 1α,25-$(OH)_2D_3$, calcitriol; VDR, vitamin D receptor; RXR, retinoid X receptor; VDRE, vitamin D response element; CDC, Centers for Disease Control and Prevention; TLR, Toll-like receptor; PTM, post-translational modification; ACh, acetylcholine; •NO, nitric oxide; 24(OH)ase, 24-hydroxylase; mRNA, messenger ribonucleic acid; LVSCC-A1C, L-type voltage-sensitive calcium channel A1C; LTP, long-term potentiation; PUFA, polyunsaturated fatty acid; PET, positron emission tomography; PP2A, protein phosphatase 2A; MnSOD, manganese superoxide dismutase; NF-κB, nuclear factor κ-light chain enhancer of activated B cell; iNOS, inducible nitric oxide synthase; ONOO–, peroxynitrite; IκB-α, inhibitor of κB-α; GSH, reduced glutathione; GSSG, oxidized glutathione; VDUP1, vitamin D upregulating protein 1.

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1. Introduction

The natural course of brain aging as well as age-related neurodegenerative disorders are thought to be a contributing cause as well as a result of increases in reactive oxygen and reactive nitrogen species (ROS/RNS) in the brain (Butterfield and Lauderback, 2002; Calabrese et al., 2004, 2006b; Poon et al., 2004). These increases in ROS/RNS production occur through growing metabolic inefficiency, breakdown of antioxidant defenses, and free radical damage to metabolic and protective machinery (Anantharaman et al., 2006; Tangpong et al., 2008). Dietary deficiencies can lead to or, at least exacerbate, age-related failure of protective mechanisms (Bourre, 2006; Watson and Preedy, 2013).

Long-known historical functions of Vitamin D (VitD) center on the uptake and use of calcium (Ca^{2+}) and phosphate (P_i) primarily for the purposes of bone mineralization, bone resorption, and bone remodeling (DeLuca, 1986). However, it is now recognized that there are many other VitD functions. Mounting evidence supports significant roles for VitD in the brain, and VitD deficiency has been shown to have a long list of neurochemical consequences. This review provides an overview of the functions of VitD that affect the brain and, more specifically the effects of VitD insufficiency that may contribute to Alzheimer's disease (AD).

2. Alzheimer's disease

2.1. Incidence and overview

AD is a progressive neurodegenerative disorder characterized by a long preclinical stage (PCAD), often progressing into mild cognitive impairment (MCI) and eventual AD (Aluise et al., 2010a; Mosconi, 2005; Sperling et al., 2011). Currently more than 5 million Americans are estimated to have AD, with 35 million persons worldwide, numbers that are ever-increasing (2014a). Of these, approximately two-thirds are women (2014a). Gender differences were also reported for expression of antioxidant enzymes (Sullivan et al., 2012). Major pathological hallmarks of AD include amyloid beta-peptide (Aβ)-rich plaques, neurofibrillary tangles (NFTs), synapse loss, and brain atrophy especially in those areas of the brain associated with memory and higher executive function (Jack et al., 2005; Mosconi, 2005; Selkoe, 2001; Sperling et al., 2011). Major clinical signs and symptoms of AD include, among others, memory loss, cognitive impairment, glucose hypometabolism, and oxidative stress (Butterfield et al., 2001; Hensley et al., 1995a,1995b). Aging is the primary risk factor for AD (Perluigi et al., 2014; Sultana and Butterfield, 2013). Other major risk factors for AD include gender, and family history including genetics and lifestyle. Many of these cannot be changed, but steps can be taken to reduce risks once they are identified.

2.2. AD pathology

The classical primary pathological hallmarks of AD frequently used for diagnosis confirmation are amyloid plaques and NFTs. Amyloid plaques that accumulate outside neurons are made primarily of amyloid beta (Aβ) peptide, a product of improper cleavage of amyloid precursor protein (APP). NFTs, whose principal component is hyperphosphorylated tau, a microtubule associated protein with a growing number of identified potential functions, build up within neurons. The Alzheimer's Association also lists oxidative stress, brain inflammation, and cardiovascular disease comorbidity as pathological characteristics of AD. Other pathological substrates include brain atrophy and neurochemical changes (Yaari and Corey-Bloom, 2007). Brain atrophy includes widening sulci, shrinkage of gyri, and loss of synapses, though the specificity of the latter to AD has been questioned (Scheff et al., 2014). Neurochemical changes include well documented decreases in cholinergic activity, as well as changes including glutamate, norepinephrine (NE), and serotonin as well as biomarkers (Rapoport and Nelson, 2011).

Increased Aβ production and deficits in phagocytosis of Aβ by the immune system combined with decreased Aβ degradation and clearance contribute to Aβ accumulation and soluble Aβ-induced inflammation in AD (Chagas et al., 2012; Mizwicki et al., 2012, 2013). Aβ clearance from the brain by innate immune cells helps maintain normal brain function. In addition to Aβ increases, decreased glucose utilization in the brain is among the earliest known measurable changes that occur during the initiation and progression of AD perhaps even occurring prior to amyloidogenesis possibly decades before onset of clinical symptoms (Pedros et al., 2014; Sperling et al., 2011; Yao et al., 2009). This has led researchers to explore possible connections between AD and diabetes (de la Monte et al., 2006; Steen et al., 2005; Butterfield et al., 2014a).

The Ca^{2+} hypothesis of AD suggests that Aβ detrimentally alters neuronal Ca^{2+} signaling pathways that are involved in cognition (Berridge, 2010; Mattson, 1994). Neurotoxic Aβ oligomers increase Ca^{2+} entry into cells and may contribute to the release of Ca^{2+} from internal endoplasmic reticulum stores (Berridge, 2010, 2011). This dysregulation of Ca^{2+} is associated with learning and memory deficits like those that occur early in the progression of AD potentially preventing long-term storage of newly acquired memories (Berridge, 2010). As Aβ accumulates, progressive increases in resting Ca^{2+} occur, which may mimic a small global elevation of Ca^{2+} that occurs in slow oscillations in the brain during a phase of memory processing in which memories are erased during sleep and not retained for memory consolidation and storage (Berridge, 2014). Aβ deregulates this Ca^{2+} signaling, interfering with memory retention and inducing inflammatory responses in the brain (Berridge, 2014). Long-term dysregulation of Ca^{2+} levels and signaling can contribute to neurodegeneration seen in later stages of AD (Berridge, 2010, 2011; Camandola and Mattson, 2011; Hensley et al., 1995a; Mark et al., 1995; Mattson, 1994).

Though new evidence exists to suggest other potential functions of tau protein, it is long established that tau within neurons serves to stabilize microtubules for anterograde and retrograde transport along axons providing needed organelles and other factors, including mitochondria to the synapse (Brandt et al., 2005; Ko et al., 2005). Tau function in this capacity is regulated by phosphorylation/dephosphorylation by kinases and phosphatases. Hyperphosphorylation of tau results in the disconnection of tau from microtubules resulting in microtubule disassembly. If sustained, the synapse does not receive needed materials potentially leading to neuronal death. Hyperphosphorylated tau aggregates within the neuron to form NFTs, one of the major pathological hallmarks of AD mentioned (Braak and Braak, 1995).

2.3. Clinical presentation

The primary and often first-presenting complaint from a patient with an imminent diagnosis of AD is memory decline. This starts with

the impairment of short-term, and progressing to the loss of remote memory. As the disease progresses, memory issues worsen and are accompanied by impairments in executive function, logical reasoning, language, visuospatial function, motor function, behavioral, personality, and psychiatric changes (Yaari and Corey-Bloom, 2007). Less prevalent symptoms include anosmia, sleep disturbances, and seizures. Ability to perform activities of daily living gradually and progressively declines. Depression occurs in up to 50% of individuals with AD (Yaari and Corey-Bloom, 2007; Vos et al., 2015).

2.4. Risk factors

Risk factors for AD include age, gender, family history, genetics, and head trauma, and may include low educational attainment and environmental factors. Advancing age continues to be the biggest single known risk factor for AD with prevalence doubling approximately every five years after age 65 (Yaari and Corey-Bloom, 2007). Gender also contributes to AD risk as approximately two-thirds of diagnosed AD sufferers are women, even after correcting for small differences in overall lifespan (Selkoe, 2001). A family history of AD greatly increases one's risk of AD especially with diagnosed AD in first-degree relatives (van Duijn et al., 1991), possibly as a combination of genetic and environmental factors. Early onset AD generally occurs during the fifth decade of life, and accounts for fewer than 5% of AD cases. Inheritance of early-onset AD is attributed to mutated genes for presenilin-1 (chromosome 14), presenilin-2 (chromosome 1), and APP (chromosome 21). Down syndrome subjects have a third copy of the gene for APP (chromosome 21) likely accounting for the early appearance of AD pathology in these individuals. Late onset AD accounts for the vast majority of AD cases.

Though no definitive genetic link has been found to cause late-onset AD, susceptibility genes increase the risk of developing AD. For example, an individual's *apolipoprotein E* (*ApoE*) allele status has been shown to greatly affect the risk for AD. The primary role of ApoE is cholesterol transport, regulating lipid transport and injury repair in the brain. Additionally, ApoE plays roles in glucose metabolism, mitochondrial function, neuroinflammation, neuronal signaling, and Aβ processing (Dorey et al., 2014; Liu et al., 2013). Three different alleles of human *ApoE* exist, *ApoE2*, *ApoE3*, and *ApoE4*, conferring differential risk for AD. *ApoE4* is the highest genetic risk factor for late-onset AD. *ApoE3* allele confers an intermediate risk, while the *ApoE2* allele confers a low risk and is potentially neuroprotective (Corder et al., 1993; Liu et al., 2013; Seripa et al., 2011). These altered risks seem to hold across both genders and all races (Farrer et al., 1997), though the high risk for AD conferred by *ApoE4* seems to be even greater in women (Anttila et al., 2002; Mortensen and Hogh, 2001; Payami et al., 1996; Qiu et al., 2004), and evidence exists that ApoE2 may be more neuroprotective in men than in women (Johnson et al., 1998; Qiu et al., 2004).

2.5. Inflammation, apolipoprotein A1, and AD

Apolipoproteins have been shown to be integrally related to Alzheimer's disease pathology (Liu et al., 2013). In addition to the differential risk for AD afforded by ApoE status, changes to apolipoprotein A1 (ApoA1) in AD contributes to AD progression. Many Aβ-binding proteins including ApoE, ApoJ, and ApoA1 are known to accumulate in amyloid deposits (Moon et al., 2013). Like ApoE, ApoA1 plays major roles in cholesterol transport as well as regulation of inflammation (Aluise et al., 2011; Hyka et al., 2001; Keeney et al., 2013b; Quazi and Molday, 2011; Rader and Daugherty, 2008). Studies have shown decreased levels of ApoA1 in the plasma of AD subjects compared to healthy controls (Kawano et al., 1995; Liu et al., 2006; Merched et al., 2000). Decreased risk of dementia was associated with higher ApoA1 concentrations while lower ApoA1 levels in AD patients correlated with more severe cognitive impairment (Merched et al., 2000; Saczynski et al., 2007). In a mouse model of AD, ApoA1 overexpression protected learning and memory function, while ApoA1 deficiency increased memory deficits (Lewis et al., 2010). Studies have shown that ApoA1 binds to Aβ (Golabek et al., 1995), is present in amyloid plaques (Wisniewski et al., 1995), and prevents Aβ-induced toxicity in vitro (Koldamova et al., 2001) supporting a protective role of ApoA1 in AD pathology. Carriers of the high AD risk *ApoE4* allele have lower levels of ApoA1 (Raygani et al., 2006). ApoA1 oxidation leads to elevation of peripheral levels of the inflammatory cytokine tumor necrosis factor-α (TNF-α) that can cross the blood–brain barrier (BBB) activating microglia and contributing to further inflammatory and oxidative cascades known to lead to neuronal death (Aluise et al., 2011; Aluise et al., 2010b; Keeney et al., 2013b; Tangpong et al., 2008; Butterfield, 2014). Decreased plasma levels of ApoA1 are associated with late-onset AD and highly correlated with the severity of disease (Kawano et al., 1995; Merched et al., 2000).

2.6. Oxidative stress in disease progression

As aging progresses, an inevitable general decline in overall health occurs (Harman, 2001; Perluigi et al., 2014; Singh and Newman, 2011). Combinations of the aforementioned AD risk factors and pathological hallmarks prime the brain for the cascade of events providing risks to develop AD. One such neurodegenerative path is oxidative stress (Butterfield et al., 2001; Butterfield and Lauderback, 2002; Butterfield and Stadtman, 1997; Hensley et al., 1995b; Nunomura et al., 2001). Production of dangerous ROS/RNS is a natural and sometimes beneficial outcome of aerobic processes *in vivo* (Butterfield and Dalle-Donne, 2014; Dasuri et al., 2013; Halliwell, 2011; Sies, 2015; Valko et al., 2007). Metabolic processes in the mitochondria are one source of ROS (Adam-Vizi, 2005; Halliwell and Gutteridge, 1984; Scialo et al., 2013). Antioxidant defenses help to keep ROS/RNS in check under normal physiologic conditions (Halliwell, 2011). Oxidative stress occurs when increases in oxidants and/or decreases in antioxidant defenses create an oxidative imbalance (Butterfield and Lauderback, 2002; Butterfield and Stadtman, 1997; Sies, 1997). Oxidative stress plays a major role in the pathogenesis of AD (Butterfield et al., 2001; Dasuri et al., 2013; Sultana and Butterfield, 2010; Sultana et al., 2013; Swomley et al., 2014).

Mitochondria process oxygen to produce energy at potential self-risk (Adam-Vizi, 2005; Scialo et al., 2013). During aging, progressive oxidative and nitrosative damage to biomolecules in mitochondria lead to increased ROS/RNS production, further compromising antioxidant defenses. Metabolic processes become progressively less efficient posing severe challenges to neuronal survival (Klamt et al., 2002; Schulz et al., 2014; Stadtman and Berlett, 1998; Sultana and Butterfield, 2013). Major forms of oxidative and nitrosative stress include protein oxidation, protein nitration, lipid peroxidation, and oxidative damage to DNA and RNA (Halliwell, 2011; Sanders, 2014; Butterfield et al., 2007, 2010; Butterfield and Lauderback, 2002; Butterfield and Stadtman, 1997; Castegna et al., 2004; Fedirko et al., 2010; Bonda, 2014). Aβ peptide has also been shown to be associated with free radicals capable of beginning a chain reaction of oxidative damage to biomolecules (Hensley et al., 1994; Butterfield et al., 2001; Lauderback et al., 2001; Swomley et al., 2014). Due to factors including, among others, low anti-oxidant defenses, high oxygen consumption, and an abundance of lipid-resident unsaturated acyl chains containing labile allylic hydrogen atoms, the brain is particularly susceptible to oxidative damage (Butterfield and Stadtman, 1997). Aging brain is even more so (Butterfield and Dalle-Donne, 2014; Butterfield and Dalle-Donne, 2014, 2014a, 2014b; Calabrese et al., 2006a; Perluigi et al., 2010).

3. VitD overview

VitD, a steroid hormone (Fig. 1), has long been known to play roles in calcium homeostasis, bone mineralization, immune cell differentiation, and tumor inhibition (Brewer et al., 2006; Deeb et al., 2007; Holick,

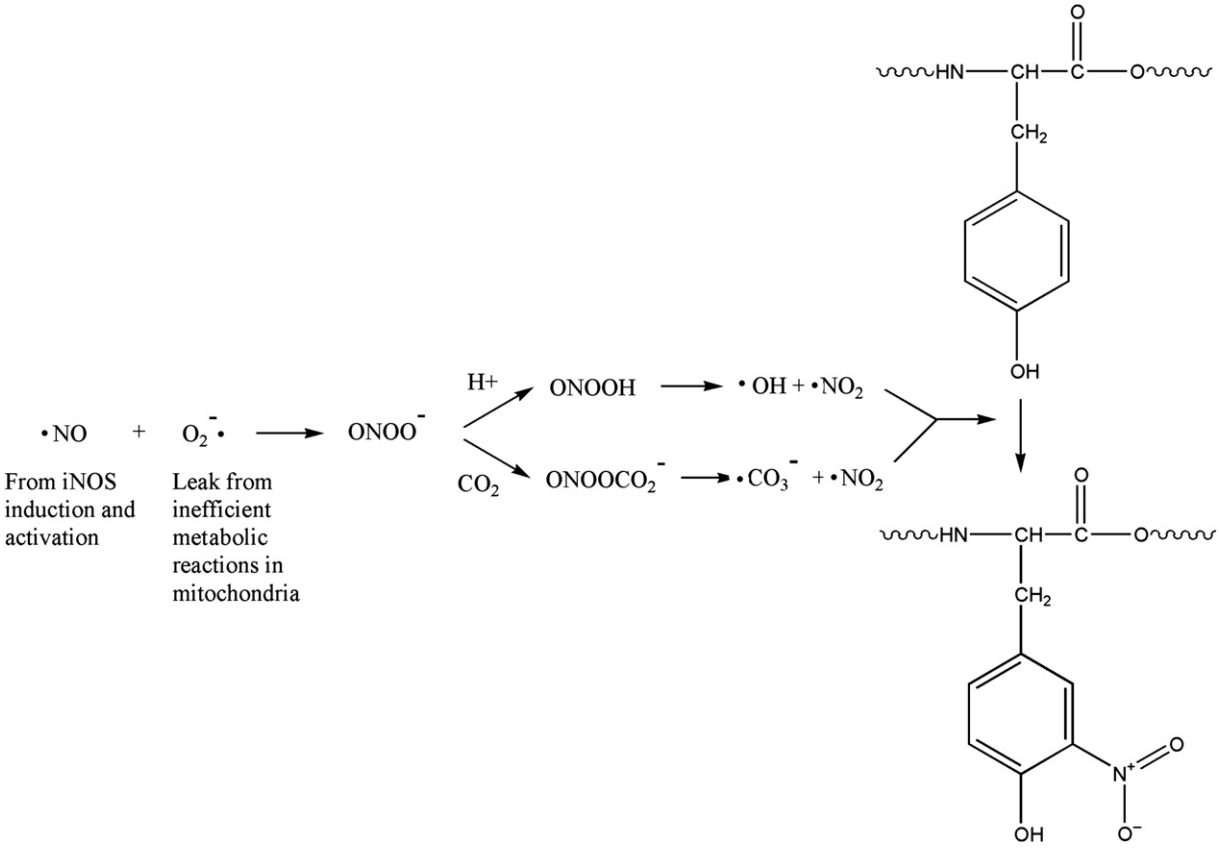

Fig. 1. General mechanism of synthesis of VitD. VitD synthesis from 7-dehydrocholesterol through a series of conversions to the longer-lived circulating 25(OH)D_3, the active 1α,25$(OH)_2D_3$, and 24,25$(OH)_2D_3$ for elimination.

2004; Nezbedova and Brtko, 2004). Recent studies indicate that long-term VitD deficiency may play an aggressive role in neurodegeneration including that associated with AD (Annweiler et al., 2009; Brewer et al., 2001; Carlberg, 2014b; Dusso and Brown, 1998; Farid et al., 2012; Keeney et al., 2013a). Genome-wide association studies suggest VitD as an important micro-nutrient with a wide variety of functions (Carlberg, 2014a; Carlberg and Molnar, 2012). Gene variants that involve hydroxylation (needed for VitD conversion to the active form) or VitD transport were found to affect VitD status; however, variants involved in cholesterol biosynthesis were also found to substantially increase risk of VitD insufficiency (Wang et al., 2010).

Upon exposure to sunlight (UVB), cholecalciferol ($VitD_3$) is synthesized in the skin from 7-dehydrocholesterol, the immediate biochemical precursor to cholesterol (Deeb et al., 2007). VitD can also be obtained from the diet. Few foods naturally contain VitD. Fatty fish, fish liver oil, beef liver, and egg yolk are among those that do (Holick, 2007). In many developed countries, foods including milk and breakfast cereals are fortified with VitD (Deeb et al., 2007). $VitD_3$, as synthesized, is inactive. Transported through the bloodstream bound to VitD-binding protein (DBP), $VitD_3$ is hydroxylated in the liver to 25-hydroxyvitamin D_3 (25-$(OH)D_3$) and further in the kidney to the biologically active calcitriol (1α,25-$(OH)_2D_3$) (Fig. 1) (Deeb et al., 2007). Active 1α,25$(OH)_2D_3$ is translocated to the nucleus. Most VitD-related signaling occurs *via* binding of 1α,25$(OH)_2D_3$ to its nuclear receptor, the vitamin D receptor (VDR). VDR forms a heterodimer with the retinoid X receptor (RXR), and the VDR–RXR complex binds with the VitD response element (VDRE), thereby modulating transcription (Ramagopalan et al., 2010). VDR–RXR interacts within the promoter regions of target genes on VDRE to regulate transcription (Bosse et al., 2009). 24-Hydroxylation leads to VitD degradation and excretion. Additionally, because cholesterol and VitD are synthesized from the same immediate precursor, statins, which inhibit cholesterol biosynthesis would thereby inhibit VitD biosynthesis, providing another route of VitD deficiency. In the USA, over 30 million individuals are on statin therapy, raising concerns about VitD insufficiency, even in economically developed countries (Robinson and Booth, 2010).

3.1. Incidence of VitD deficiency: age, gender, causes

VitD deficiency is widespread with approximately one billion people affected worldwide (Dickens et al., 2011). VitD levels decrease with age: even with regular sun exposure, elderly skin produces on average only 25% of the VitD that young skin produces (Kennel et al., 2010). About 50% of the general adult population is estimated to be VitD deficient across all age groups and ethnicities, and, as noted, a higher percentage of VitD-deficient individuals exists in the elderly.

Approximately 70–90% of AD patients have less than sufficient serum VitD levels (Annweiler and Beauchet, 2011; Bischoff-Ferrari, 2012; Durk et al., 2014; Holick, 2007; Nair and Maseeh, 2012). VitD deficiency is even more common in women (Calabrese et al., 2006b; Looker et al., 2011). In a 7 year follow-up study of elderly women, a higher incidence of AD was found in women with low VitD intake (Annweiler et al., 2012). In addition to being at higher risk of AD, women with low blood levels of VitD have been found to have increased risk of depression (Kerr et al., 2015). The percentage of adults achieving sufficient VitD levels has declined dramatically in recent years from 60% in whites and 10% in African Americans in the early 1990s to half these percentages in the early 2000s (Kennel et al., 2010). Reasons for low VitD in much of the population, especially the elderly, include low sun exposure (indoor, sedentary lifestyle), poor nutrition (lack of access/financial or physical or lack of desire), and genetics (discussed further below) (Binkley et al., 2012). VitD deficiency has been linked to increased overall mortality (Zittermann et al., 2009, 2012); conversely,

VitD supplementation is associated with significantly reduced mortality (Autier and Gandini, 2007).

3.2. VitD binding and transport: VDR and DBP

Most of the biological actions of VitD are mediated by the VDR, its nuclear ligand-induced transcription factor (Carlberg and Molnar, 2006; Dusso and Brown, 1998; Nezbedova and Brtko, 2004). In the absence of vitamin D, only 10–15% of dietary calcium is absorbed. That absorption increases to 30–40% upon interaction of VitD with VDR (Holick, 2007). VDR are present in many areas of the brain, particularly in the hippocampus (2014b; Annweiler et al., 2009). Expression of VDR in the hippocampus and cortex, key areas for cognition, suggest important functions for VitD in the brain. Additionally, Toll-like receptor (TLR) activated macrophages have been found to express both VDR and mitochondrial CP27B, that converts 25(OH)D to the active $1\alpha,25(OH)_2D_3$. The latter can then bind VDR to function as a transcription factor involved in immunity (Adams and Hewison, 2008; Fernandes de Abreu et al., 2009). VDR polymorphisms are known to be associated with altered AD susceptibility (Lee et al., 2014). These polymorphisms may alter the affinity of VDR for VitD, thereby increasing the risk for AD (Gezen-Ak et al., 2007).

By proteomics techniques, DBP, a protein involved in transport of VitD in the bloodstream, was identified as having decreased levels following plasma protein profiling of MCI subjects compared to healthy controls (Muenchhoff et al., 2015). Plasma VitD levels were also reported to be lower in MCI subjects (Olde Rikkert et al., 2014). These results suggest that impairment in VitD transport is present even in the early stages of AD, *i.e.*, prior to development of dementia. A study by Moon et al. found that DBP inhibited Aβ aggregation and prevented Aβ-mediated cell death in cultured hippocampal cells (Moon et al., 2013). Both monomeric and oligomeric Aβ bound to DBP in a dose-dependent manner. Further, following addition of Aβ, DBP treatment resulted in reduced synapse loss in mouse hippocampus and rescued Aβ-induced memory deficits (Moon et al., 2013).

3.3. Effects of VitD on brain structure/function

Young people with poor nutritional status reportedly experienced changes in cognition, behavior, and CNS physiology (Millet et al., 2014). During brain aging, poor nutrition could lead to deleterious consequences or exacerbate existing disease states (Millet et al., 2014). In recent years, VitD has emerged as an important neurosteroid with a wide variety of important functions affecting both the developing and adult brain (Table 1) (Annweiler et al., 2014d; Cui et al., 2015; Eyles et al., 2013; Groves et al., 2014; Kesby et al., 2011; Millet et al., 2014). Cui et al. suggest a two-hit hypothesis for the effects of VitD deficiency on the brain whereby deficient VitD may adversely affect the brain by both genomic and non-genomic means (Cui et al., 2015). VitD has been shown to play key roles in the brain including cell proliferation, cell differentiation, calcium signaling, neurotrophic factor regulation, neurotransmission, and synaptic plasticity (Annweiler, 2014; Brouwer-Brolsma and de Groot, 2015; Groves et al., 2014). Demonstrated physiological benefits of VitD include neurogenesis and synaptogenesis, improved synaptic transmission, improved cognitive performance, elevated Aβ clearance, and neuronal protection (Brouwer-Brolsma and de Groot, 2015). Consequently, low VitD levels are associated with adverse outcomes in the brain as outlined above (Cui et al., 2015).

In a 2012 study, VitD deficiency was found to interrupt neurogenesis and increase apoptotic cells in the dentate gyrus of adult mice. These consequences were ablated by replenishment of VitD (Zhu et al., 2012). A study by Eyles and colleagues showed that the VDR is concentrated in the subventricular zone of the dentate gyrus, and that maternal VitD deficiency alters neurogenesis in the brain of developing rat pups suggesting VitD to be a regulator of cell proliferation in the developing brain (Cui et al., 2007). A more recent study by Keilhoff et al. found that maternal VitD deficiency led to decreased neurogenesis in offspring (Keilhoff et al., 2010). Further, this prenatal restriction of VitD resulted in decreased neurogenesis in the hippocampal dentate gyrus of the offspring even in adulthood (Keilhoff et al., 2010), and the late gestation period may be the key window where maternal VitD deficiency results in disrupted brain function in adult rat offspring (O'Loan et al., 2007). VitD deficiency during brain development has been shown to result in alterations to brain structure, gene expression, and neurochemistry (Cui et al., 2015; Eyles et al., 2013). In addition, several biological pathways important to the brain are adversely and permanently affected by VitD deficiency during brain development (Becker et al., 2005; Burne et al., 2004). These include mitochondrial energy metabolism, redox balance, calcium homeostasis, synaptic plasticity and neurotransmission, cytoskeletal structure and maintenance, and protein post-translational modification (PTM) (Burne et al., 2004).

In addition to neonatal brain changes associated with VitD deficiency as discussed above, adult brain also reportedly adversely responds to low VitD levels, including a variety of neurochemical, cognitive, and structural consequences (Cui et al., 2015). In a 2014 meta-analysis, Annweiler and colleagues concluded that VitD deficiency was associated with decreased brain volume and larger lateral ventricles (Annweiler et al., 2014b). The greater brain atrophy present in subjects with deficient VitD levels was supported by other studies (Hooshmand et al., 2014). Conversely, higher plasma levels of VitD correlate with larger brain volumes (Hooshmand et al., 2014). The Annweiler group and others further demonstrated that low serum VitD levels are associated with increased white matter abnormalities in older adults similar to that seen in AD (Annweiler et al., 2014a, 2015a) especially in elderly women with amnestic MCI or AD (Sakurai et al., 2014).

3.4. VitD treatment and cognition/AD

AD brain exhibits decreased cholinergic activity, increased calcium-related neurotoxicity, increased glutamate excitotoxicity, and resultant learning and memory deficits (Annweiler and Beauchet, 2012). Although a few inconclusive or conflicting studies exist (Anastasiou et al., 2014; Schneider et al., 2014), mounting evidence suggests a direct correlation of serum VitD levels with cognitive ability (Annweiler and Beauchet, 2011; Brouwer-Brolsma et al., 2013; Schlogl and Holick, 2014). Abnormalities in cholinergic, dopaminergic, and noradrenergic neurotransmitter systems have been implicated in a wide variety of brain disorders. Manipulation of VitD levels through diet or supplementation has been shown to have positive effects on each of these neurotransmitter systems (Eyles et al., 2013). Studies have shown a variety of interactions between VitD and catecholamines including dopamine-induced VDR-mediated signaling as well as $VitD_3$ association with tyrosine hydroxylase (Sanchez et al., 2009; Wang et al., 2001), the rate-limiting enzyme in catecholamine biosynthesis (Daubner et al., 2011). Tyrosine hydroxylase has been shown to be inhibited by nitric oxide (•NO) (Abreu et al., 2000). •NO-related protein nitration in brain is a known downstream consequence of VitD deficiency as well as being present in MCI and AD brain, discussed in a later section (Butterfield et al., 2007; Calabrese et al., 2006c; Keeney et al., 2013a; Sultana et al., 2006). VitD supplementation in rats results in increased choline acetyltransferase activity and the resultant increase in available acetylcholine in brain areas relevant to AD (Sonnenberg et al., 1986). Further, VitD has been shown to play a beneficial regulatory role in cholinergic and dopaminergic receptor gene expression in diabetic rats (Peeyush et al., 2010). VitD administered to newborn rats resulted in increases in both dopamine and norepinephrine in the brainstem in adulthood (Tekes et al., 2009). Administration of VitD to adult rats increases dopamine release and storage in the striatum and protects against dopamine and serotonin depletion in response to normally depleting stressors (Cass et al., 2006, 2012).

VitD deficiency may exacerbate existing brain disorders and impair the ability to respond to stressors in the brain (Cui et al., 2015; Groves et al., 2014). Annweiler and coworkers demonstrated a potential

Table 1
AD associated pathological hallmarks, clinical presentations, and risk factors that are influenced by VitD status: selected references.

Key AD-related topics affected by VitD status	Reference	Title of study	Primary conclusions of study, impact of VitD	Category
Aβ-production	Wang et al. (2012)	Vitamin D receptor and Alzheimer's disease: a genetic and functional study	VitD treatment and VDR overexpression suppress APP transcription in cell culture	Pathology
Aβ-aggregation	Moon et al. (2013)	Vitamin D-binding protein interacts with Abeta and suppresses Abeta-mediated pathology	DBP decreased Aβ aggregation and prevented Aβ-mediated cell death in cultured hippocampal cells	Pathology
Aβ-levels	Durk et al. (2014)	1alpha,25-Dihydroxyvitamin D_3 reduces cerebral amyloid-beta accumulation and improves cognition in mouse models of Alzheimer's disease	In mice expressing human APP as a model of AD, acute treatment with VitD reduced soluble Aβ. Long-term VitD treatment resulted in decreased levels of both soluble and insoluble Aβ in the brain, particularly in the hippocampus, and improved performance on memory-related tasks	Pathology
Aβ-clearance	Hooshmand et al. (2014)	Vitamin D in relation to cognitive impairment, cerebrospinal fluid biomarkers, and brain volumes	Higher serum VitD levels correlate with increased Aβ in the CSF indicating increased clearance	Pathology
Aβ-clearance and load	Briones and Darwish (2012)	Vitamin D mitigates age-related cognitive decline through the modulation of pro-inflammatory state and decrease in amyloid burden	VitD supplementation in a rat model of brain aging not only improved learning and memory but increased Aβ clearance and decreased Aβ load in the brain of aged rats	Pathology
Aβ-degradation	Mizwicki et al. (2012)	Genomic and nongenomic signaling induced by 1alpha,25(OH)2-vitamin D_3 promotes the recovery of amyloid-beta phagocytosis by Alzheimer's disease macrophages	VitD activation of VDR-dependent signaling leads to the recovery of phagocytosis needed to protect the brain from these AD-inducing effects of Aβ	Pathology
Neurofibrillary tangles-Tau	Briones and Darwish, 2014	Decrease in age-related tau hyperphosphorylation and cognitive improvement following vitamin D supplementation are associated with modulation of brain energy metabolism and redox state	VitD supplementation restored activity PP2A and decreased Tau hyperphosphorylation to levels seen in younger control animals	Pathology
Neurochemical changes—catecholamine biosynthesis	Sanchez et al. (2009)	1,25-Dihydroxyvitamin D_3 administration to 6-hydroxydopamine-lesioned rats increases glial cell line-derived neurotrophic factor and partially restores tyrosine hydroxylase expression in the substantia nigra and striatum	VitD administration partially restores tyrosine hydroxylase expression, the rate limiting step in catecholamine biosynthesis	Pathology
Neurochemical changes—acetylcholine	Sonnenberg et al. (1986)	1,25-Dihydroxyvitamin D_3 treatment results in increased choline acetyltransferase activity in specific brain nuclei	VitD supplementation in rats results in increased choline acetyltransferase activity and the resultant increase in available acetylcholine in brain areas relevant to AD	Pathology
Neurochemical changes—dopamine	Wang et al. (2001)	Vitamin D(3) attenuates 6-hydroxydopamine-induced neurotoxicity in rats	VitD pretreatment attenuates dopamine neuronal toxicity induced by 6-hydroxydopamine	Pathology
Neurochemical changes—dopamine and norepinephrine	Tekes et al. (2009)	Influence of neonatal vitamin A or vitamin D treatment on the concentration of biogenic amines and their metabolites in the adult rat brain	VitD administered to newborn rats resulted in increases in both dopamine and norepinephrine in the brainstem in adulthood	Pathology
Glucose utilization	Chagas et al. (2012)	Focus on vitamin D, inflammation and type 2 diabetes	VitD positively influences glucose homeostasis *via* altering insulin secretion and sensitivity secondary to VitD's modulation	Pathology

(continued on next page)

Table 1 (*continued*)

Key AD-related topics affected by VitD status	Reference	Title of study	Primary conclusions of study, impact of VitD	Category
			of the inflammatory response	
Brain atrophy—gray matter, glucose utilization	Berti et al. (2015)	Nutrient patterns and brain biomarkers of Alzheimer's disease in cognitively normal individuals	VitD was among the nutrients that were positively associated with cerebral glucose metabolism and gray matter volume and negatively associated with markers of fibrillary Aβ	Pathology
Brain atrophy—white matter	Annweiler et al., 2014a	Vitamin D and white matter abnormalities in older adults: a cross-sectional neuroimaging study	Low serum 25(OH)D was associated with higher grade white matter abnormalities	Pathology
Brain atrophy—total brain volume	Annweiler et al. (2014b)	Vitamin D and brain volumetric changes: systematic review and meta-analysis	VitD deficiency was associated with decreased brain volume and larger lateral ventricles	Pathology
Brain atrophy—total brain volume	Hooshmand et al. (2014)	Vitamin D in relation to cognitive impairment, cerebrospinal fluid biomarkers, and brain volumes	Greater brain atrophy present in subjects with deficient VitD. Higher plasma levels of VitD correlate with larger brain volumes	Pathology
Synapse loss	Moon et al. (2013)	Vitamin D-binding protein interacts with Abeta and suppresses Abeta-mediated pathology	Following treatment with Aβ, DBP treatment resulted in reduced synapse loss in mouse hippocampus and rescued Aβ-induced memory deficits	Pathology
Brain inflammation	Fedirko et al. (2009)	Effects of vitamin D and calcium supplementation on markers of apoptosis in normal colon mucosa: a randomized, double-blind, placebo-controlled clinical trial	Upregulation of inhibitors of apoptosis as well as decreases in several pro-inflammatory cytokines including TNF-α in response to VitD treatment	Pathology
Brain inflammation—human females	Cavalcante et al. (2015)	Effect of vitamin D_3 supplementation and influence of BsmI polymorphism of the VDR gene of the inflammatory profile and oxidative stress in elderly women with vitamin D insufficiency: vitamin D_3 megadose reduces inflammatory markers	VitD insufficient elderly women given high dose VitD supplementation for four weeks showed reduced inflammatory markers	Pathology
Oxidative stress/redox status	Alvarez et al. (2014)	Vitamin D status is independently associated with plasma glutathione and cysteine thiol/disulfide redox status in adults	Higher serum VitD levels are associated with higher plasma GSH and lower GSSG	Pathology
Nitrosative stress	Keeney et al. (2013a)	Dietary vitamin D deficiency in rats from middle to old age leads to elevated tyrosine nitration and proteomics changes in levels of key proteins in the brain: implications for low vitamin D-dependent age-related cognitive decline	Long term VitD deficiency in the aging brain results in increased nitration of brain proteins through a free radical mechanism that occurs *via* the NF-κB pathway	Pathology
Cognition—memory, executive function	Annweiler et al. (2013)	Meta-analysis of memory and executive dysfunctions in relation to vitamin D	Low serum 25(OH)D levels are associated with executive dysfunction, VitD supplementation improved executive function, episodic memory disorders are modestly associated with low VitD status	Clinical presentation
Cognition—attention, working memory	Brouwer-Brolsma and de Groot (2015)	Cognitive performance: a cross-sectional study on serum vitamin D and its interplay with glucose homeostasis in Dutch older adults	Higher serum 25(OH)D concentrations are associated with better attention and working memory	Clinical presentation
Cognition—cognitive impairment, concentration	Llewellyn et al. (2011)	Vitamin D and cognitive impairment in the elderly U.S. population	VitD deficiency increases odds of cognitive impairment in the elderly (U.S.)	Clinical presentation
Behavior—depression	Kerr et al. (2015)	Associations between vitamin D levels and depressive symptoms in healthy young adult women	In addition to being at higher risk of AD, women with low blood levels of VitD have been found to have increased risk of depression	Clinical presentation
Advancing age	Annweiler et al. (2012)	Higher vitamin D dietary intake is associated with lower risk of Alzheimer's disease: a 7-year follow-up	In a 7 year follow-up study of elderly women, a higher incidence of AD was found in women with low VitD intake	Risk factors
Gender	Looker et al. (2011)	Vitamin D status: United States, 2001–2006	Like AD, VitD deficiency is more common in women	Risk factors

synergistic effect of memantine (an NMDA receptor antagonist acting on the glutamatergic system currently used as a treatment for AD). Memantine used in combination with VitD decreased cortical axon degeneration in neuronal culture during exposure to lysed blood-induced neurotoxicity (Annweiler et al., 2014c; Charier et al., 2015). The combination provided a greater protective effect than treatment with either component alone. This combination shows potential promise in protecting neurons during times of stress.

In a rat model of brain aging, older rats exhibited significant decline in learning and memory compared to young rats. VitD treatment ameliorated age-related decline in learning and memory in the older group, while no similar effect of VitD was seen in younger rats (Briones and Darwish, 2012). In a 6-year follow-up study on human subjects, low initial serum VitD levels were associated with substantial cognitive decline (Floyd, 1999). A 2011 Australian study found that when patients with mild to moderate AD were treated with low dose VitD (1000 IU/day for 8 weeks), cognition and memory improved. No further cognitive or memory improvements were seen following high dose VitD treatment (Stein et al., 2011). Latimer et al. found that middle-aged rats fed a high VitD diet for 5–6 months demonstrated enhanced basal synaptic transmission and significantly outperformed their normal and low VitD-diet counterparts on hippocampal-dependent learning and memory tasks (Latimer et al., 2014). Genes associated with synaptic transmission were upregulated in response to a high VitD diet.

A meta-analysis by Etgen et al. that included nearly 8000 human participants found a strongly significant increased risk of cognitive impairment associated with low VitD status (Etgen et al., 2012). In other studies, lower serum VitD levels also are reported in human subjects with AD *versus* age-matched controls (Annweiler et al., 2013). These results support the notion of a controlled clinical trial supplementing VitD in MCI patients.

3.5. VitD and inflammation

Serum VitD levels are inversely associated with systemic inflammation (Bellia et al., 2013).

Low serum VitD is associated with decreased ApoA1 levels (John et al., 2005) that have been associated with an increased inflammatory response mentioned previously. Serum VitD levels are reportedly inversely related to TNF-α levels, and, conversely, levels of TNF-α and other inflammatory markers are reduced upon VitD supplementation (Peterson and Heffernan, 2008; Stubbs et al., 2010). TNF-α is normally found in low levels in healthy brain (Keeney et al., 2013b) having both neuroprotective and neurotoxic roles, the latter involving both necrosis and apoptosis (Badiola et al., 2009; Schneider-Brachert et al., 2004). In other studies, upregulation of inhibitors of apoptosis as well as decreased pro-inflammatory cytokines, including TNF-α, in response to VitD treatment were reported (Fedirko et al., 2009; Hopkins et al., 2011). Calcium and VitD treatment each showed these protective effects; however, no synergistic effect was found. A recent study by Cavalcante et al. demonstrated that VitD insufficient elderly women given high dose VitD supplementation for four weeks showed reduced inflammatory markers (Cavalcante et al., 2015).

Cultured human brain pericytes, cells involved in control and maintenance of the BBB, treated with VitD demonstrated regulatory control of the expression of genes involved in neuroinflammation (Nissou et al., 2014). These cells also responded to exposure to inflammatory cytokines, including TNF-α, by upregulating genes (CYP27B1) involved in VitD metabolism to the active $1\alpha,25(OH)_2D$ form (Nissou et al., 2014). An implication of this study is that VitD conceivably may protect the BBB from inflammatory stress.

3.6. VitD and glucose utilization

Type 2 diabetes mellitus (T2DM) is a significant risk factor for AD (Butterfield et al., 2014a; Verdile et al., 2015; Barone and Butterfield, 2015). Though conflicting evidence exists, VitD has been shown to play a regulatory role in some pathways related to T2DM that may also have relevance to AD. VitD is reported to exert a positive influence on glucose homeostasis, a key process that shows detrimental changes early in AD brain, *via* altering insulin secretion and sensitivity secondary to VitD's modulation of the inflammatory response (Chagas et al., 2012). In 2015, Berti et al. measured the association of nutrient intake and brain biomarkers of AD and found that VitD was among the nutrients that were positively associated with cerebral glucose metabolism and gray matter volume (less brain atrophy) and negatively associated with markers of fibrillary Aβ (Berti et al., 2015).

3.7. VitD and Aβ

Insoluble Aβ plaques are a pathological hallmark of AD. Together with metabolic deficits, amyloid buildup and deposition is thought to be among the earliest changes in the brain in the progression of AD (Masters and Selkoe, 2011; Aluise et al., 2010a; Butterfield et al., 2001; Sperling et al., 2011; Swomley et al., 2014). However, soluble Aβ oligomers may be the more dangerous form of this damaging peptide (Masters and Selkoe, 2011; Butterfield et al., 2013). Both increased production of Aβ from APP and decreased elimination of Aβ due to defective Aβ phagocytosis, insufficient Aβ transport from brain to blood, and decreased degradation contribute to Aβ accumulation in the brain (Erickson et al., 2012; Mohmmad Abdul et al., 2006; Owen et al., 2010; Zhao et al., 2004). Recent evidence demonstrates that low VitD status results in increased Aβ and increased Aβ-related toxicities in the brain, whereas sufficient VitD improves Aβ clearance, decreases Aβ load, and provides protection against Aβ-related cognitive decline (Fig. 2). VitD treatment as well as VDR overexpression have each been shown to suppress APP transcription in cell culture (Wang et al., 2012).

Exposure of hippocampal neurons in culture to Aβ suppressed expression of VDR messenger ribonucleic acid (mRNA) and induced mRNAs for expression of 24-hydroxylase (24(OH)ase) that tags VitD for elimination as well as inducing L-type voltage-sensitive calcium channel A1C (LVSCC-A1C) mRNA (Dursun et al., 2013). Taken together these results suggest that Aβ may disrupt VitD/VDR signaling as well as contribute to the well-known calcium dysregulation and consequent neurodegeneration in AD brain (Garwood et al., 2013).

In a study of mice expressing human APP as a model of AD, acute treatment with VitD reduced soluble Aβ. Long-term VitD treatment of these mice resulted in decreased levels of both soluble and insoluble Aβ in brain, particularly in the hippocampus, leading to improved performance on memory-related tasks (Durk et al., 2014). Higher serum VitD levels correlate with increased Aβ in the CSF suggesting increased clearance from the brain (Hooshmand et al., 2014). VitD supplementation in a rat model of brain aging not only improved learning and memory but increased Aβ clearance and decreased Aβ load in the brain of aged rats (Briones and Darwish, 2014). Aβ treatment suppressed synaptic plasticity in the rat hippocampus and impaired the storage of memories *via* long-term potentiation (LTP) (Taghizadeh et al., 2014). Dietary VitD supplementation in these animals protected basic synaptic transmission and restored synaptic plasticity in the face of Aβ treatment (Taghizadeh et al., 2014). The effect of VitD on Aβ levels and clearance coupled with the Ca^{2+} regulatory effects of VitD are consistent with the notion that VitD supplementation may reduce Aβ-induced memory deficits.

In a cross-sectional neuroimaging human pilot study in cognitively normal individuals with known risk factors for AD, higher dietary levels of VitD as well as higher VitB12 and ω-3 polyunsaturated fatty acids, as part of a nutritious diet, were associated with decreased Aβ load assessed by positron emission tomography (PET) in AD relevant brain regions independent of gender, ApoE status, or family history and controlled for age and differences in total caloric intake (Mosconi et al., 2014).

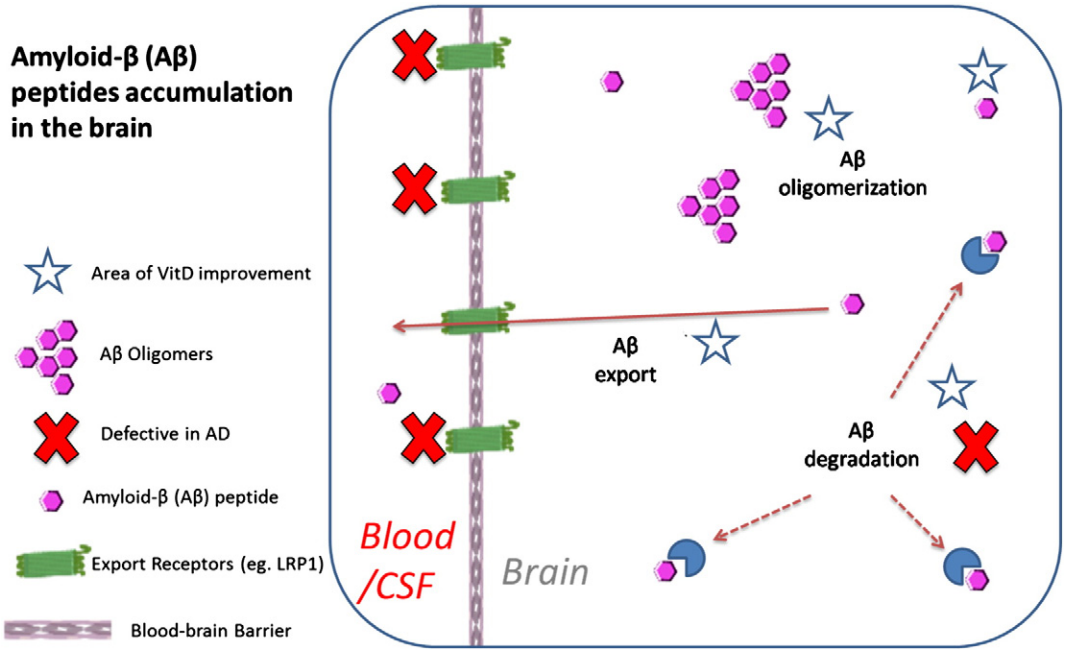

Fig. 2. Beneficial effects of VitD on amyloid accumulation in brain. Aβ accumulates in the brain due to increased production, decreased chemical and phagocytic degradation, and decreased clearance. Stars indicate areas involved in amyloid homeostasis where VitD has been shown to have beneficial effects.

As noted above, Aβ repressed VDR mRNA. VitD activation of VDR-dependent signaling led to recovery of phagocytosis needed to protect the brain from AD-inducing effects of Aβ (Mizwicki et al., 2012). Two types of macrophages are present in AD brain, Type I and Type II. In multiple studies, treatment with VitD strongly stimulated Aβ phagocytosis and clearance, rebalanced inflammation, and protected against apoptosis in both types of macrophages (Fiala, 2010; Masoumi et al., 2009). Some of these effects may be synergistic with curcuminoids (Masoumi et al., 2009). Additionally, VitD supplementation in combination with the anti-inflammatory agent, Resolvin D1, promoted Aβ phagocytosis and reversed Aβ-induced elevation of inflammatory cytokines and chemokines (Mizwicki et al., 2013). The study further concluded that low intake of both VitD and docosahexanoic acid may contribute to AD pathology (Mizwicki et al., 2013).

3.8. VitD and Tau

Studies by Hooshmand et al. and others found no significant differences in levels of Tau protein or Tau phosphorylation based on VitD status (Hooshmand et al., 2014). However, decreased age-related Tau hyperphosphorylation were reported following VitD supplementation (Briones and Darwish, 2014). In this latter study, previous findings of age-related decreases in brain energy metabolism, deleterious changes to the redox state in brain (assessed by levels of ROS, levels of glutathione, and activity of superoxide dismutase), increased Tau hyperphosphorylation, and learning and memory decline were validated (Briones and Darwish, 2014). All of these age-related, detrimental changes to the brain were attenuated by VitD supplementation. Further, the authors showed that VitD restored the activity of protein phosphatase 2A (PP2A), an enzyme that dephosphorylates Tau, and decreased Tau hyperphosphorylation to levels observed in younger control animals (Briones and Darwish, 2014). Consequently, taken together, VitD apparently does not affect levels of Tau, but beneficial effects of VitD appear to be the case with respect to Tau phosphorylation.

3.9. VitD and nitrosative stress

Keeney et al. demonstrated that long term VitD deficiency in aging rats resulted in increased nitration of brain proteins through a free radical mechanism (Keeney et al., 2013a). The highly reactive superoxide radical anion is produced due to inefficient mitochondrial respiration (Deby and Goutier, 1990; Fridovich, 1986; Halliwell and Gutteridge, 1984). Manganese superoxide dismutase (MnSOD), the primary mitochondria resident superoxide scavenger, has itself been shown to be a target of tyrosine nitration, rendering this enzyme less able to perform its antioxidant duties and exposing other biomolecules to the damaging effects of superoxide (Anantharaman et al., 2006; MacMillan-Crow et al., 1998; Sompol et al., 2008; Tangpong et al., 2008).

We demonstrated that VitD deficiency in aging brain leads to increased TNF-α-induced activation of nuclear factor κ-light chain enhancer of activated B cells (NF-κB) (Keeney et al., 2013a), increased translocation of NF-κB to the nucleus, and consequent increased levels of inducible nitric oxide synthase (iNOS), a downstream product of the NF-κB transcription pathway, resulting in increased nitration of cortical proteins (Keeney et al., 2013a) *via* a free radical pathway. •NO, a natural free radical, competes with MnSOD to react with superoxide (Amirmansour et al., 1999; Ischiropoulos, 2009). Normally, MnSOD efficiently reacts with superoxide converting this free radical to hydrogen peroxide and molecular oxygen. As MnSOD becomes damaged, the resulting elevated superoxide free radical is able to react with •NO to form the damaging peroxynitrite (ONOO−) (Surmeli et al., 2010). Protein nitration occurs at the 3-position of tyrosine as a result of $•NO_2$, a downstream product of ONOO− (Fig. 3), leading to changes in protein activation, deactivation, and function (Butterfield et al., 2007; Feeney and Schoneich, 2012; Ischiropoulos, 2009). The Butterfield laboratory previously showed that protein tyrosine nitration occurs early in the neurodegenerative process that leads to AD and continues to damage key proteins in AD brain (Castegna et al., 2003; Butterfield et al., 2007).

As discussed above, VitD decreased TNF-α levels (Diaz et al., 2009; Furman et al., 1996; Giulietti et al., 2007; Keeney et al., 2013a) and downregulated TLR (Sadeghi et al., 2006). VitD suppressed TNF-α-induced activation of NF-κB (Keeney et al., 2013a; Kwon et al., 2010), upregulated the inhibitor of κB-α (IκB-α), decreased IκB-α phosphorylation, and decreased translocation of NF-κB to the nucleus (Chagas et al., 2012; Keeney et al., 2013a). The VDRE may also inhibit NF-κB-directed expression (Haussler et al., 2008). Taken together, VitD inhibits NF-κB-related protein nitration and production of the inflammatory

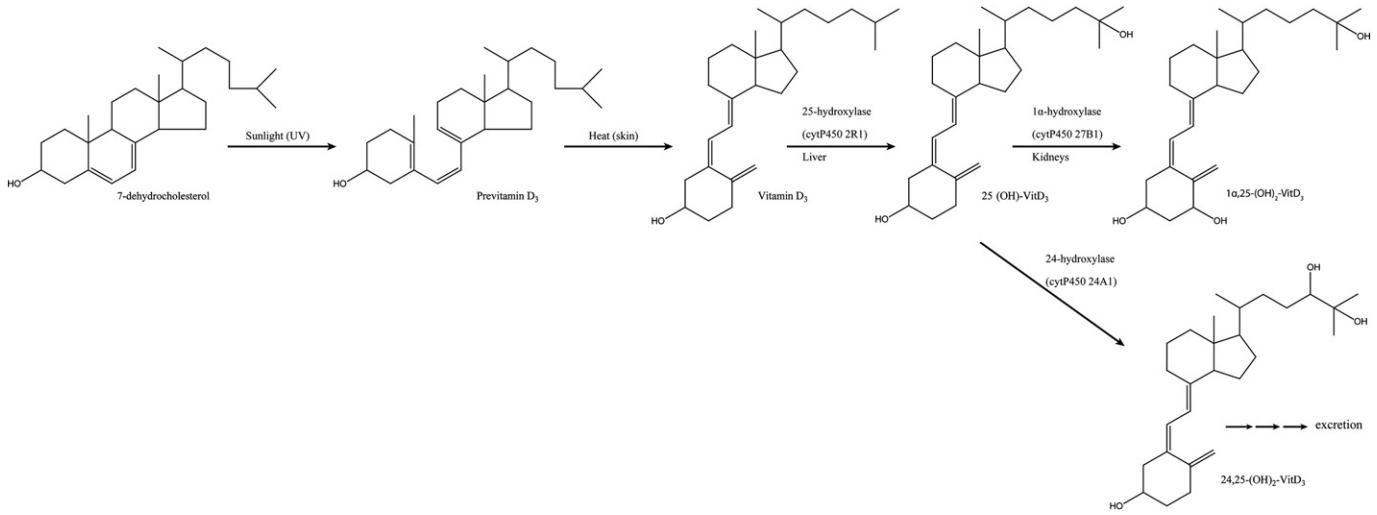

Fig. 3. Mechanism of tyrosine nitration and steric hindrance of a phosphorylation site. Nitric oxide reacts with superoxide by radical-radical recombination to form peroxynitrite. In the presence of hydrogen ion or carbon dioxide, peroxynitrite is converted to nitrogen dioxide which nitrates tyrosine in the 3-position hindering phosphorylation of the 4-position.

cytokine, TNF-α, thereby decreasing many downstream consequences of each (Fig. 4).

3.10. Regulation of redox status and redox signaling

Arguably the primary antioxidant in the brain is glutathione. The thiol group of GSH can react with a variety of ROS to protect the brain and maintain redox balance (Sies, 1997; Halliwell, 2011; Pocernich and Butterfield, 2012). The ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) gives a measure of the redox status of the brain. A report suggests that higher serum VitD levels are associated with higher plasma GSH and lower GSSG (Alvarez et al., 2014), indicating a favorable redox status to respond to and protect the brain from oxidative stress. Early trials in animal models by Fedirko et al. (2010) suggested that concurrent calcium and VitD supplementation decreased oxidative damage to DNA. Cavalcante et al. (2015) demonstrated that four weeks of high dose VitD supplementation in VitD-insufficient, elderly women resulted in increased antioxidant capacity in these subjects.

VitD may also indirectly regulate cellular redox signaling. Expression of vitamin D upregulating protein 1 (VDUP1), as the name implies, is regulated by VitD. VDUP1 was first known to be a regulator of thioredoxin expression and activity involved in redox regulation, but further studies showed that VDUP1 also plays roles in many cellular processes including cell proliferation, apoptosis, immune regulation, and fatty acid utilization (Chung et al., 2006). In 2006, Nemere et al.

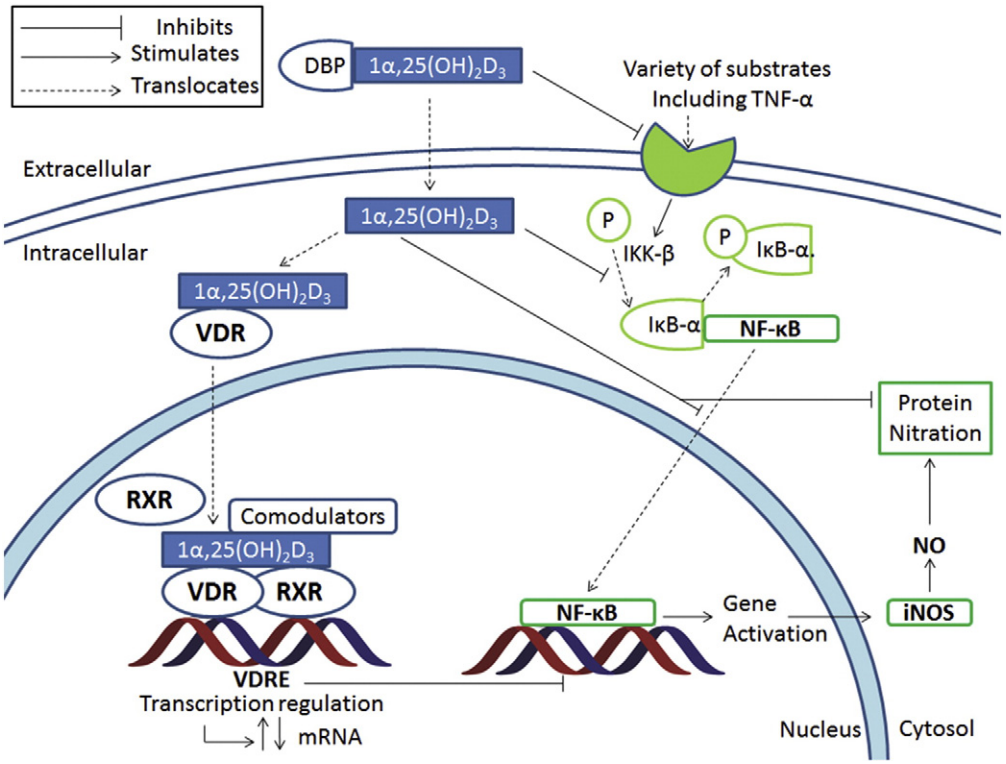

Fig. 4. VitD signaling and nitrosative stress. VitD inhibits TLR activation, IκB-α phosphorylation, and NF-κB translocation to the nucleus where VDRE inhibits NF-κB-induced transcription. Deficient VitD levels result in the loss of this control and consequent iNOS and TNF-α elevation, protein nitration, inflammatory processes, and oxidative stress-induced metabolic changes.

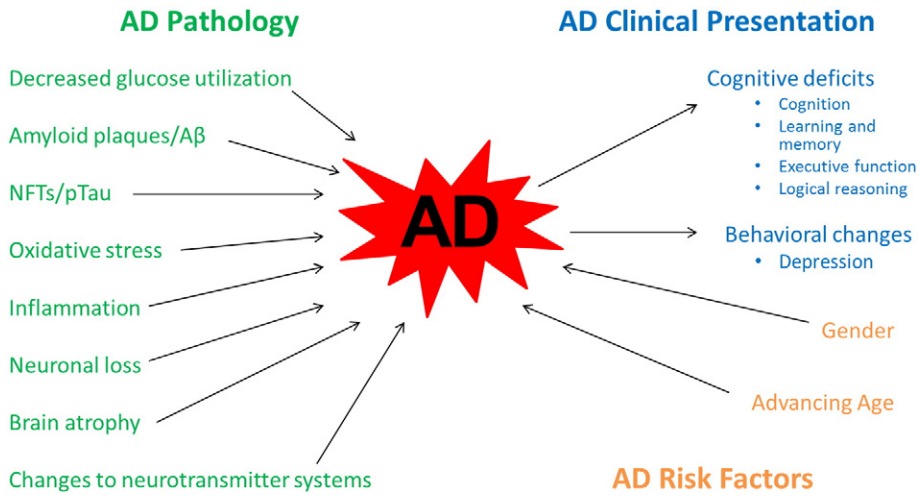

Fig. 5. Summary of the pathology and clinical presentation of AD where VitD has been shown to play potential beneficial roles. A pictorial representation of identified areas associated with AD where VitD has been shown to play beneficial roles.

demonstrated that $24,25(OH)_2D_3$, the elimination form of VitD, inhibited the stimulatory actions of the active $1\alpha,25(OH)_2D_3$ on Ca^{2+} and phosphate absorption through a mechanism that involved increased production of hydrogen peroxide by binding to and inactivating a key peroxidase, catalase (Nemere et al., 2006). Further studies of animal models revealed that diets high in antioxidants resulted in increased VitD binding to its corresponding receptors involved in both membrane-bound and nuclear transcription initiated pathways (Nemere et al., 2006; Richard et al., 2010).

3.11. Potential for VitD in human therapeutic intervention

A growing agreement exists among researchers that low VitD levels are associated with higher risk of cognitive decline and dementia, especially in the elderly segments of the population. However, currently, due to the association of VitD deficiency with many neurological, neurodegenerative, and non-CNS-related diseases and many areas of uncertainty, VitD status is a crucial but non-specific risk factor for AD (Annweiler et al., 2015b). Cui et al. suggested that specific 'critical windows' may exist during which VitD deficiency might result in the most detrimental brain outcomes, and during these times, VitD supplementation might be the most beneficial to prevent long-term damage to the brain (Cui et al., 2015). VitD level appears to be a modifiable risk factor for AD. A potential therapeutic window during which VitD might provide benefit to reduce the risk or delay the onset of AD may be during the pre-clinical and MCI stages in which measurable changes in glucose utilization and Aβ accumulation already occur (Etgen et al., 2011). The window of opportunity for the benefit of VitD would gradually close as neuronal loss increases. Early in disease progression, changes could still be made to potentially improve outcomes. Accordingly, we suggest that long-term, randomized clinical trials of VitD supplements among large populations of middle-aged adults in several countries should be considered with the end point being the conversion rate to MCI or AD.

4. Conclusions and future directions

More research is needed to deal with existing uncertainties in the roles of VitD in cognition and neurodegenerative diseases, including AD. The consensus of the literature is that VitD has a number of important beneficial effects on the brain, and that VitD deficiency contributes to and has common links with several deleterious consequences to the brain, many of which are strongly associated with AD (Fig. 5). It is our opinion that the current recommendations of daily intake of VitD are too conservative. Higher VitD levels may be essential for healthy brain aging. Further, based on a large number of AD-related issues that have common links with deficient VitD levels as outlined in this review, we opine that VitD should be considered as an adjunct therapy in conjunction with any AD treatment. VitD appears to play both acute and chronic roles in the brain. Critical periods may exist during which VitD deficiency may be the most harmful to the brain during which VitD supplementation might provide the most protective benefit.

As people age, they tend to become more sedentary, often due to physical limitations. As this occurs, older individuals tend to stay inside more, decreasing exposure to the sun. In combination with the declining ability of aging skin to synthesize VitD and the poor dietary status sometimes experienced in the elderly, VitD levels are often critically low. The widespread use of cholesterol-lowering drugs may also inhibit the ability to synthesize VitD. In our opinion, evidence of the potential contribution of VitD deficiency to neurodegeneration makes testing VitD levels and appropriate VitD supplementation as needed of utmost importance. We strongly urge the development of programs and awareness messages to produce more proactive relationships of patients and their physicians to address the widespread VitD deficiency and the value of supplementation as a part of quality of care/quality of life issues that potentially have a significant possibility to modulate the onset and progression of AD.

Acknowledgements

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References

Abreu, P., et al., 2000. Nitric oxide inhibits tyrosine hydroxylase of rat median eminence. Life Sci. 67, 1941–1946.
Adams, J.S., Hewison, M., 2008. Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. Nat. Clin. Pract. Endocrinol. Metab. 4, 80–90.
Adam-Vizi, V., 2005. Production of reactive oxygen species in brain mitochondria: contribution by electron transport chain and non-electron transport chain sources. Antioxid. Redox Signal. 7, 1140–1149.
Aluise, C.D., et al., 2010a. Preclinical Alzheimer disease: brain oxidative stress, Abeta peptide and proteomics. Neurobiol. Dis. 39, 221–228.
Aluise, C.D., et al., 2010b. Chemo brain (chemo fog) as a potential side effect of doxorubicin administration: role of cytokine-induced, oxidative/nitrosative stress in cognitive dysfunction. Adv. Exp. Med. Biol. 678, 147–156.
Aluise, C.D., et al., 2011. 2-Mercaptoethane sulfonate prevents doxorubicin-induced plasma protein oxidation and TNF-alpha release: implications for the reactive oxygen species-mediated mechanisms of chemobrain. Free Radic. Biol. Med. 50, 1630–1638.
Alvarez, J.A., et al., 2014. Vitamin D status is independently associated with plasma glutathione and cysteine thiol/disulphide redox status in adults. Clin. Endocrinol. 81, 458–466.

Amirmansour, C., et al., 1999. Tyrosine nitration in blood vessels occurs with increasing nitric oxide concentration. Br. J. Pharmacol. 127, 788–794.

Anantharaman, M., et al., 2006. Beta-amyloid mediated nitration of manganese superoxide dismutase: implication for oxidative stress in a APPNLH/NLH X PS-1P264L/P264L double knock-in mouse model of Alzheimer's disease. Am. J. Pathol. 168, 1608–1618.

Anastasiou, C.A., et al., 2014. Vitamin D and cognition: an update of the current evidence. J. Alzheimers Dis. 42 (Suppl. 3), S71–S80.

Annweiler, C., 2014. Vitamin D and Alzheimer's disease: from an intriguing idea to a therapeutic option. Biol Aujourdhui 208, 89–95.

Annweiler, C., Beauchet, O., 2011. Vitamin D-mentia: randomized clinical trials should be the next step. Neuroepidemiology 37, 249–258.

Annweiler, C., Beauchet, O., 2012. Possibility of a new anti-Alzheimer's disease pharmaceutical composition combining memantine and vitamin D. Drugs Aging 29, 81–91.

Annweiler, C., et al., 2009. Vitamin D and cognitive performance in adults: a systematic review. Eur. J. Neurol. 16, 1083–1089.

Annweiler, C., et al., 2012. Higher vitamin D dietary intake is associated with lower risk of Alzheimer's disease: a 7-year follow-up. J. Gerontol. A Biol. Sci. Med. Sci. 67, 1205–1211.

Annweiler, C., et al., 2013. Low serum vitamin D concentrations in Alzheimer's disease: a systematic review and meta-analysis. J. Alzheimers Dis. 33, 659–674.

Annweiler, C., et al., 2014a. Vitamin D and white matter abnormalities in older adults: a cross-sectional neuroimaging study. Eur. J. Neurol. 21 1436-e95.

Annweiler, C., et al., 2014b. Vitamin D and brain volumetric changes: systematic review and meta-analysis. Maturitas 78, 30–39.

Annweiler, C., et al., 2014c. Combination of memantine and vitamin D prevents axon degeneration induced by amyloid-beta and glutamate. Neurobiol. Aging 35, 331–335.

Annweiler, C., et al., 2014d. Vitamin D supplements: a novel therapeutic approach for Alzheimer patients. Front. Pharmacol. 5, 6.

Annweiler, C., et al., 2015a. Vitamin D and white matter abnormalities in older adults: a quantitative volumetric analysis of brain MRI. Exp. Gerontol. 63C, 41–47.

Annweiler, C., et al., 2015b. 'Vitamin D and cognition in older adults': updated international recommendations. J. Intern. Med. 277, 45–57.

Anttila, T., et al., 2002. Midlife income, occupation, APOE status, and dementia: a population-based study. Neurology 59, 887–893.

Autier, P., Gandini, S., 2007. Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. Arch. Intern. Med. 167, 1730–1737.

Badiola, N., et al., 2009. Activation of caspase-8 by tumour necrosis factor receptor 1 is necessary for caspase-3 activation and apoptosis in oxygen-glucose deprived cultured cortical cells. Neurobiol. Dis. 35, 438–447.

Barone, E., Butterfield, D.A., 2015. Insulin resistance in Alzheimer disease: is heme oxygenase-1 an Achilles' heel? Neurobiol. Dis. 84, 69–77.

Becker, A., et al., 2005. Transient prenatal vitamin D deficiency is associated with subtle alterations in learning and memory functions in adult rats. Behav. Brain Res. 161, 306–312.

Bellia, A., et al., 2013. Serum 25-hydroxyvitamin D levels are inversely associated with systemic inflammation in severe obese subjects. Intern. Emerg. Med. 8, 33–40.

Berridge, M.J., 2010. Calcium hypothesis of Alzheimer's disease. Pflugers Arch. 459, 441–449.

Berridge, M.J., 2011. Calcium signalling and Alzheimer's disease. Neurochem. Res. 36, 1149–1156.

Berridge, M.J., 2014. Calcium regulation of neural rhythms, memory and Alzheimer's disease. J. Physiol. 592, 281–293.

Berti, V., et al., 2015. Nutrient patterns and brain biomarkers of Alzheimer's disease in cognitively normal individuals. J. Nutr. Health Aging 19, 413–423.

Binkley, N., et al., 2012. Low vitamin D status: definition, prevalence, consequences, and correction. Rheum. Dis. Clin. N. Am. 38, 45–59.

Bischoff-Ferrari, H.A., 2012. "Vitamin D — why does it matter?" — defining vitamin D deficiency and its prevalence. Scand. J. Clin. Lab. Invest. Suppl. 243, 3–6.

Bonda, D.J., 2014. Neuronal failure in Alzheimer disease: a view through the oxidative stress looking-glass. Neurosci. Bull. 30, 243–252.

Bosse, Y., et al., 2009. Asthma and genes encoding components of the vitamin D pathway. Respir. Res. 10, 98.

Bourre, J.M., 2006. Effects of nutrients (in food) on the structure and function of the nervous system: update on dietary requirements for brain. Part 1: micronutrients. J. Nutr. Health Aging 10, 377–385.

Braak, H., Braak, E., 1995. Staging of Alzheimer's disease-related neurofibrillary changes. Neurobiol. Aging 16, 271–278 (discussion 278–84).

Brandt, R., et al., 2005. Tau alteration and neuronal degeneration in tauopathies: mechanisms and models. Biochim. Biophys. Acta 1739, 331–354.

Brewer, L.D., et al., 2001. Vitamin D hormone confers neuroprotection in parallel with downregulation of L-type calcium channel expression in hippocampal neurons. J. Neurosci. 21, 98–108.

Brewer, L.D., et al., 2006. Chronic 1alpha,25-(OH)2 vitamin D_3 treatment reduces Ca^{2+}—mediated hippocampal biomarkers of aging. Cell Calcium 40, 277–286.

Briones, T.L., Darwish, H., 2012. Vitamin D mitigates age-related cognitive decline through the modulation of pro-inflammatory state and decrease in amyloid burden. J. Neuroinflammation 9, 244.

Briones, T.L., Darwish, H., 2014. Decrease in age-related tau hyperphosphorylation and cognitive improvement following vitamin D supplementation are associated with modulation of brain energy metabolism and redox state. Neuroscience 262, 143–155.

Brouwer-Brolsma, E.M., de Groot, L.C., 2015. Vitamin D and cognition in older adults: an update of recent findings. Curr. Opin. Clin. Nutr. Metab. Care 18, 11–16.

Brouwer-Brolsma, E.M., et al., 2013. Serum 25-hydroxyvitamin D is associated with cognitive executive function in Dutch prefrail and frail elderly: a cross-sectional study exploring the associations of 25-hydroxyvitamin D with glucose metabolism, cognitive performance and depression. J. Am. Med. Dir. Assoc. 14 (852), e9–e17.

Burne, T.H., et al., 2004. Transient prenatal vitamin D deficiency is associated with hyperlocomotion in adult rats. Behav. Brain Res. 154, 549–555.

Butterfield, D.A., 2014. The 2013 SFRBM Discovery Award: selected discoveries from the Butterfield Laboratory of Oxidative Stress and its sequela in brain in cognitive disorders exemplified by Alzheimer disease and chemotherapy induced cognitive impairment. Free Radic. Biol. Med. 74, 157–174.

Butterfield, D.A., Dalle-Donne, I., 2014. Redox proteomics: from protein modifications to cellular dysfunction and disease. Mass Spectrom. Rev. 33, 1–6.

Butterfield, D.A., Lauderback, C.M., 2002. Lipid peroxidation and protein oxidation in Alzheimer's disease brain: potential causes and consequences involving amyloid beta-peptide-associated free radical oxidative stress. Free Radic. Biol. Med. 32, 1050–1060.

Butterfield, D.A., Stadtman, E.R., 1997. Protein oxidation processes in aging brain. Adv. Cell Aging Gerontol. 2, 161–191.

Butterfield, D.A., et al., 2001. Evidence of oxidative damage in Alzheimer's disease brain: central role for amyloid beta-peptide. Trends Mol. Med. 7, 548–554.

Butterfield, D.A., et al., 2007. Elevated levels of 3-nitrotyrosine in brain from subjects with amnestic mild cognitive impairment: implications for the role of nitration in the progression of Alzheimer's disease. Brain Res. 1148, 243–248.

Butterfield, D.A., et al., 2010. Involvements of the lipid peroxidation product, HNE, in the pathogenesis and progression of Alzheimer's disease. Biochim. Biophys. Acta 1801, 924–929.

Butterfield, D.A., et al., 2013. Amyloid beta-peptide (1–42)-induced oxidative stress in Alzheimer disease: importance in disease pathogenesis and progression. Antioxid. Redox Signal. 19, 823–835.

Butterfield, D.A., et al., 2014a. Elevated risk of type 2 diabetes for development of Alzheimer disease: a key role for oxidative stress in brain. Biochim. Biophys. Acta 1842, 1693–1706.

Butterfield, D.A., et al., 2014b. Mass spectrometry and redox proteomics: applications in disease. Mass Spectrom. Rev. 33, 277–301.

Calabrese, V., et al., 2004. Nitric oxide and cellular stress response in brain aging and neurodegenerative disorders: the role of vitagenes. In Vivo 18, 245–267.

Calabrese, V., et al., 2006a. Redox modulation of heat shock protein expression by acetylcarnitine in aging brain: relationship to antioxidant status and mitochondrial function. Antioxid. Redox Signal. 8, 404–416.

Calabrese, V., et al., 2006b. Redox regulation of cellular stress response in neurodegenerative disorders. Ital. J. Biochem. 55, 263–282.

Calabrese, V., et al., 2006c. Nitrosative stress, cellular stress response, and thiol homeostasis in patients with Alzheimer's disease. Antioxid. Redox Signal. 8, 1975–1986.

Camandola, S., Mattson, M.P., 2011. Aberrant subcellular neuronal calcium regulation in aging and Alzheimer's disease. Biochim. Biophys. Acta 1813, 965–973.

Carlberg, C., 2014a. Genome-wide (over)view on the actions of vitamin D. Front. Physiol. 5, 167.

Carlberg, C., 2014b. The physiology of vitamin D—far more than calcium and bone. Front. Physiol. 5, 335.

Carlberg, C., Molnar, F., 2006. Detailed molecular understanding of agonistic and antagonistic vitamin D receptor ligands. Curr. Top. Med. Chem. 6, 1243–1253.

Carlberg, C., Molnar, F., 2012. Current status of vitamin D signaling and its therapeutic applications. Curr. Top. Med. Chem. 12, 528–547.

Cass, W.A., et al., 2006. Calcitriol protects against the dopamine- and serotonin-depleting effects of neurotoxic doses of methamphetamine. Ann. N. Y. Acad. Sci. 1074, 261–271.

Cass, W.A., et al., 2012. Evoked dopamine overflow is augmented in the striatum of calcitriol treated rats. Neurochem. Int. 60, 186–191.

Castegna, A., et al., 2003. Proteomic identification of nitrated proteins in Alzheimer's disease brain. J. Neurochem. 85, 1394–1401.

Castegna, A., et al., 2004. Modulation of phospholipid asymmetry in synaptosomal membranes by the lipid peroxidation products, 4-hydroxynonenal and acrolein: implications for Alzheimer's disease. Brain Res. 1004, 193–197.

Cavalcante, I.G., et al., 2015. Effect of vitamin D_3 supplementation and influence of BsmI polymorphism of the VDR gene of the inflammatory profile and oxidative stress in elderly women with vitamin D insufficiency: vitamin D_3 megadose reduces inflammatory markers. Exp. Gerontol. 66, 10–16.

Chagas, C.E., et al., 2012. Focus on vitamin D, inflammation and type 2 diabetes. Nutrients 4, 52–67.

Charier, D., et al., 2015. Memantine plus vitamin D prevents axonal degeneration caused by lysed blood. ACS Chem. Neurosci. 6, 393–397.

Chung, J.W., et al., 2006. Vitamin D_3 upregulated protein 1 (VDUP1) is a regulator for redox signaling and stress-mediated diseases. J. Dermatol. 33, 662–669.

Corder, E.H., et al., 1993. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261, 921–923.

Cui, X., et al., 2007. Maternal vitamin D depletion alters neurogenesis in the developing rat brain. Int. J. Dev. Neurosci. 25, 227–232.

Cui, X., et al., 2015. Vitamin D and the brain: key questions for future research. J. Steroid Biochem. Mol. Biol. 148, 305–309.

Dasuri, K., et al., 2013. Oxidative stress, neurodegeneration, and the balance of protein degradation and protein synthesis. Free Radic. Biol. Med. 62, 170–185.

Daubner, S.C., et al., 2011. Tyrosine hydroxylase and regulation of dopamine synthesis. Arch. Biochem. Biophys. 508, 1–12.

de la Monte, S.M., et al., 2006. Therapeutic rescue of neurodegeneration in experimental type 3 diabetes: relevance to Alzheimer's disease. J. Alzheimers Dis. 10, 89–109.

Deby, C., Goutier, R., 1990. New perspectives on the biochemistry of superoxide anion and the efficiency of superoxide dismutases. Biochem. Pharmacol. 39, 399–405.

Deeb, K.K., et al., 2007. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. Nat. Rev. Cancer 7, 684–700.

DeLuca, H.F., 1986. The metabolism and functions of vitamin D. Adv. Exp. Med. Biol. 196, 361–375.

Diaz, L., et al., 2009. Calcitriol inhibits TNF-alpha-induced inflammatory cytokines in human trophoblasts. J. Reprod. Immunol. 81, 17–24.
Dickens, A.P., et al., 2011. Vitamin D, cognitive dysfunction and dementia in older adults. CNS Drugs 25, 629–639.
Dorey, E., et al., 2014. Apolipoprotein E, amyloid-beta, and neuroinflammation in Alzheimer's disease. Neurosci. Bull. 30, 317–330.
Durk, M.R., et al., 2014. 1alpha,25-Dihydroxyvitamin D-3 reduces cerebral amyloid-beta accumulation and improves cognition in mouse models of Alzheimer's disease. J. Neurosci. 34, 7091–7101.
Dursun, E., et al., 2013. Beta amyloid suppresses the expression of the vitamin D receptor gene and induces the expression of the vitamin D catabolic enzyme gene in hippocampal neurons. Dement. Geriatr. Cogn. Disord. 36, 76–86.
Dusso, A.S., Brown, A.J., 1998. Mechanism of vitamin D action and its regulation. Am. J. Kidney Dis. 32, S13–S24.
Erickson, M.A., et al., 2012. Lipopolysaccharide impairs amyloid beta efflux from brain: altered vascular sequestration, cerebrospinal fluid reabsorption, peripheral clearance and transporter function at the blood–brain barrier. J. Neuroinflammation 9, 150.
Etgen, T., et al., 2011. Mild cognitive impairment and dementia: the importance of modifiable risk factors. Dtsch. Arztebl. Int. 108, 743–750.
Etgen, T., et al., 2012. Vitamin D deficiency, cognitive impairment and dementia: a systematic review and meta-analysis. Dement. Geriatr. Cogn. Disord. 33, 297–305.
Eyles, D.W., et al., 2013. Vitamin D, effects on brain development, adult brain function and the links between low levels of vitamin D and neuropsychiatric disease. Front. Neuroendocrinol. 34, 47–64.
Farid, K., et al., 2012. Correlation between serum 25-hydroxyvitamin D concentrations and regional cerebral blood flow in degenerative dementia. Nucl. Med. Commun. 33, 1048–1052.
Farrer, L.A., et al., 1997. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease, a meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. JAMA 278, 1349–1356.
Fedirko, V., et al., 2009. Effects of vitamin D and calcium supplementation on markers of apoptosis in normal colon mucosa: a randomized, double-blind, placebo-controlled clinical trial. Cancer Prev. Res. (Phila.) 2, 213–223.
Fedirko, V., et al., 2010. Effects of supplemental vitamin D and calcium on oxidative DNA damage marker in normal colorectal mucosa: a randomized clinical trial. Cancer Epidemiol. Biomarkers Prev. 19, 280–291.
Feeney, M.B., Schoneich, C., 2012. Tyrosine modifications in aging. Antioxid. Redox Signal. 17, 1571–1579.
Fernandes de Abreu, D.A., et al., 2009. Vitamin D, a neuro-immunomodulator: implications for neurodegenerative and autoimmune diseases. Psychoneuroendocrinology 34 (Suppl. 1), S265–S277.
Fiala, M., 2010. Re-balancing of inflammation and abeta immunity as a therapeutic for Alzheimer's disease—view from the bedside. CNS Neurol. Disord. Drug Targets 9, 192–196.
Floyd, R.A., 1999. Antioxidants, oxidative stress, and degenerative neurological disorders. Proc. Soc. Exp. Biol. Med. 222, 236–245.
Fridovich, I., 1986. Biological effects of the superoxide radical. Arch. Biochem. Biophys. 247, 1–11.
Furman, I., et al., 1996. Differential expression of M-CSF, LIF, and TNF-alpha genes in normal and malignant rat glial cells: regulation by lipopolysaccharide and vitamin D. J. Neurosci. Res. 46, 360–366.
Garwood, C., Faizullabhoy, A., Wharton, S.B., Ince, P.G., Heath, P.R., Shaw, P.J., Baxter, L., Gelsthorpe, C., Forster, G., Matthews, F.E., Brayne, C., Simpson, J.E., Cognitive Function, M.R.C., Ageing Neuropathology Study Group, 2013. Calcium dysregulation in relation to Alzheimer-type pathology in the ageing brain. Neuropathol. Appl. Neurobiol. 39, 788–799.
Gezen-Ak, D., et al., 2007. Association between vitamin D receptor gene polymorphism and Alzheimer's disease. Tohoku J. Exp. Med. 212, 275–282.
Giulietti, A., et al., 2007. Monocytes from type 2 diabetic patients have a pro-inflammatory profile. 1,25-Dihydroxyvitamin D(3) works as anti-inflammatory. Diabetes Res. Clin. Pract. 77, 47–57.
Golabek, A., et al., 1995. Amyloid beta binding proteins in vitro and in normal human cerebrospinal fluid. Neurosci. Lett. 191, 79–82.
Groves, N.J., et al., 2014. Vitamin D as a neurosteroid affecting the developing and adult brain. Annu. Rev. Nutr. 34, 117–141.
Halliwell, B., 2011. Free radicals and antioxidants — quo vadis? Trends Pharmacol. Sci. 32, 125–130.
Halliwell, B., Gutteridge, J.M., 1984. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem. J. 219, 1–14.
Harman, D., 2001. Aging: overview. Ann. N. Y. Acad. Sci. 928, 1–21.
Haussler, M.R., et al., 2008. Vitamin D receptor: molecular signaling and actions of nutritional ligands in disease prevention. Nutr. Rev. 66, S98–S112.
Hensley, K., et al., 1994. A model for beta-amyloid aggregation and neurotoxicity based on free radical generation by the peptide: relevance to Alzheimer disease. Proc. Natl. Acad. Sci. U. S. A. 91, 3270–3274.
Hensley, K., et al., 1995a. A model for beta-amyloid aggregation and neurotoxicity based on the free radical generating capacity of the peptide: implications of "molecular shrapnel" for Alzheimer's disease. Proc. West. Pharmacol. Soc. 38, 113–120.
Hensley, K., et al., 1995b. Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation. J. Neurochem. 65, 2146–2156.
Holick, M.F., 2004. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. Am. J. Clin. Nutr. 80, 1678S–1688S.
Holick, M.F., 2007. Vitamin D deficiency. N. Engl. J. Med. 357, 266–281.
Hooshmand, B., et al., 2014. Vitamin D in relation to cognitive impairment, cerebrospinal fluid biomarkers, and brain volumes. J. Gerontol. A Biol. Sci. Med. Sci. 69, 1132–1138.
Hopkins, M.H., et al., 2011. Effects of supplemental vitamin D and calcium on biomarkers of inflammation in colorectal adenoma patients: a randomized, controlled clinical trial. Cancer Prev. Res. (Phila.) 4, 1645–1654.
Hyka, N., et al., 2001. Apolipoprotein A-I inhibits the production of interleukin-1beta and tumor necrosis factor-alpha by blocking contact-mediated activation of monocytes by T lymphocytes. Blood 97, 2381–2389.
Ischiropoulos, H., 2009. Protein tyrosine nitration—an update. Arch. Biochem. Biophys. 484, 117–121.
Jack Jr., C.R., et al., 2005. Brain atrophy rates predict subsequent clinical conversion in normal elderly and amnestic MCI. Neurology 65, 1227–1231.
John, W.G., et al., 2005. Hypovitaminosis D is associated with reductions in serum apolipoprotein A-I but not with fasting lipids in British Bangladeshis. Am. J. Clin. Nutr. 82, 517–522.
Johnson, J.K., et al., 1998. Initiation and propagation stages of beta-amyloid are associated with distinctive apolipoprotein E, age, and gender profiles. Brain Res. 798, 18–24.
Kawano, M., et al., 1995. Marked decrease of plasma apolipoprotein AI and AII in Japanese patients with late-onset non-familial Alzheimer's disease. Clin. Chim. Acta 239, 209–211.
Keeney, J.T., et al., 2013a. Dietary vitamin D deficiency in rats from middle to old age leads to elevated tyrosine nitration and proteomics changes in levels of key proteins in brain: implications for low vitamin D-dependent age-related cognitive decline. Free Radic. Biol. Med. 65C, 324–334.
Keeney, J.T., et al., 2013b. Apolipoprotein A-I: insights from redox proteomics for its role in neurodegeneration. Proteomics Clin. Appl. 7, 109–122.
Keilhoff, G., et al., 2010. Haloperidol normalized prenatal vitamin D depletion-induced reduction of hippocampal cell proliferation in adult rats. Neurosci. Lett. 476, 94–98.
Kennel, K.A., et al., 2010. Vitamin D deficiency in adults: when to test and how to treat. Mayo Clin. Proc. 85, 752–757 (quiz 757–8).
Kerr, D.C., et al., 2015. Associations between vitamin D levels and depressive symptoms in healthy young adult women. Psychiatry Res. 227, 46–51.
Kesby, J.P., et al., 2011. The effects of vitamin D on brain development and adult brain function. Mol. Cell. Endocrinol. 347, 121–127.
Klamt, F., et al., 2002. Time-related increase in mitochondrial superoxide production, biomolecule damage and antioxidant enzyme activities in cortical astrocyte cultures. Neuroreport 13, 1515–1518.
Ko, L.W., et al., 2005. Recent advances in experimental modeling of the assembly of tau filaments. Biochim. Biophys. Acta 1739, 125–139.
Koldamova, R.P., et al., 2001. Apolipoprotein A-I directly interacts with amyloid precursor protein and inhibits A beta aggregation and toxicity. Biochemistry 40, 3553–3560.
Kwon, H.J., et al., 2010. Vitamin D_3 upregulated protein 1 suppresses TNF-alpha-induced NF-kappaB activation in hepatocarcinogenesis. J. Immunol. 185, 3980–3989.
Latimer, C.S., et al., 2014. Vitamin D prevents cognitive decline and enhances hippocampal synaptic function in aging rats. Proc. Natl. Acad. Sci. U. S. A. 111, E4359–E4366.
Lauderback, 2001. The glutamate transporter, Glt-1, is oxidatively modified by 4-hydroxy-2-nonenal in the Alzheimer's disease brain: the role of Abeta1–42. J. Neurochem. 78, 413–416.
Lee, Y.H., et al., 2014. Vitamin D receptor polymorphisms and susceptibility to Parkinson's disease and Alzheimer's disease: a meta-analysis. Neurol. Sci. 35, 1947–1953.
Lewis, T.L., et al., 2010. Overexpression of human apolipoprotein A-I preserves cognitive function and attenuates neuroinflammation and cerebral amyloid angiopathy in a mouse model of Alzheimer disease. J. Biol. Chem. 285, 36958–36968.
Liu, H.C., et al., 2006. Proteomic identification of lower apolipoprotein A-I in Alzheimer's disease. Dement. Geriatr. Cogn. Disord. 21, 155–161.
Liu, C.C., et al., 2013. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. Nat. Rev. Neurol. 9, 106–118.
Llewellyn, D.J., Lang, I.A., Langa, K.M., Melzer, D., 2011. Vitamin D and cognitive impairment in the elderly U.S. population. J Gerontol A Biol Sci Med Sci 66, 59–65.
Looker, A.C., et al., 2011. Vitamin D status: United States, 2001–2006. NCHS Data Briefpp. 1–8.
MacMillan-Crow, L.A., et al., 1998. Peroxynitrite-mediated inactivation of manganese superoxide dismutase involves nitration and oxidation of critical tyrosine residues. Biochemistry 37, 1613–1622.
Mark, R.J., et al., 1995. Amyloid beta-peptide impairs ion-motive ATPase activities: evidence for a role in loss of neuronal Ca^{2+} homeostasis and cell death. J. Neurosci. 15, 6239–6249.
Masoumi, A., et al., 2009. 1alpha,25-Dihydroxyvitamin D_3 interacts with curcuminoids to stimulate amyloid-beta clearance by macrophages of Alzheimer's disease patients. J. Alzheimers Dis. 17, 703–717.
Masters, C.L., Selkoe, D.J., 2011. Biochemistry of amyloid β-protein: synaptic and network dysfunction. Cold Spring Harb. Perspect. Med. 2, a006262.
Mattson, M.P., 1994. Calcium and neuronal injury in Alzheimer's disease. Contributions of beta-amyloid precursor protein mismetabolism, free radicals, and metabolic compromise. Ann. N. Y. Acad. Sci. 747, 50–76.
Merched, A., et al., 2000. Decreased high-density lipoprotein cholesterol and serum apolipoprotein AI concentrations are highly correlated with the severity of Alzheimer's disease. Neurobiol. Aging 21, 27–30.
Millet, P., et al., 2014. Role of vitamin D in the physiopathology of neurodegenerative diseases. Biol Aujourdhui 208, 77–88.
Mizwicki, M.T., et al., 2012. Genomic and nongenomic signaling induced by 1alpha,25(OH)2-vitamin D_3 promotes the recovery of amyloid-beta phagocytosis by Alzheimer's disease macrophages. J. Alzheimers Dis. 29, 51–62.
Mizwicki, M.T., et al., 2013. 1alpha,25-Dihydroxyvitamin D_3 and resolvin D_1 retune the balance between amyloid-beta phagocytosis and inflammation in Alzheimer's disease patients. J. Alzheimers Dis. 34, 155–170.
Mohmmad Abdul, H., et al., 2006. Mutations in amyloid precursor protein and presenilin-1 genes increase the basal oxidative stress in murine neuronal cells and lead to

increased sensitivity to oxidative stress mediated by amyloid beta-peptide (1–42), HO and kainic acid: implications for Alzheimer's disease. J. Neurochem. 96, 1322–1335.
Moon, M., et al., 2013. Vitamin D-binding protein interacts with Abeta and suppresses Abeta-mediated pathology. Cell Death Differ. 20, 630–638.
Mortensen, E.L., Hogh, P., 2001. A gender difference in the association between APOE genotype and age-related cognitive decline. Neurology 57, 89–95.
Mosconi, L., 2005. Brain glucose metabolism in the early and specific diagnosis of Alzheimer's disease. FDG-PET studies in MCI and AD. Eur. J. Nucl. Med. Mol. Imaging 32, 486–510.
Mosconi, L., et al., 2014. Nutrient intake and brain biomarkers of Alzheimer's disease in at-risk cognitively normal individuals: a cross-sectional neuroimaging pilot study. BMJ Open 4, e004850.
Muenchhoff, J., et al., 2015. Plasma protein profiling of mild cognitive impairment and Alzheimer's disease across two independent cohorts. J. Alzheimers Dis. 43, 1355–1373.
Nair, R., Maseeh, A., 2012. Vitamin D: the "sunshine" vitamin. J. Pharmacol. Pharmacother. 3, 118–126.
Nemere, I., et al., 2006. Mechanism of 24,25-dihydroxyvitamin D_3-mediated inhibition of rapid, 1,25-dihydroxyvitamin D_3-induced responses: role of reactive oxygen species. J. Cell. Biochem. 99, 1572–1581.
Nezbedova, P., Brtko, J., 2004. 1alpha,25-Dihydroxyvitamin D_3 inducible transcription factor and its role in the vitamin D action. Endocr. Regul. 38, 29–38.
Nissou, M.F., et al., 2014. Additional clues for a protective role of vitamin D in neurodegenerative diseases: 1,25-dihydroxyvitamin D_3 triggers an anti-inflammatory response in brain pericytes. J. Alzheimers Dis. 42, 789–799.
Nunomura, A., et al., 2001. Oxidative stress is the earliest event in Alzheimer disease. J. Neuropathol. Exp. Neurol. 60, 759–767.
Olde Rikkert, M.G., et al., 2014. Differences in nutritional status between very mild Alzheimer's disease patients and healthy controls. J. Alzheimers Dis. 41, 261–271.
O'Loan, J., et al., 2007. Vitamin D deficiency during various stages of pregnancy in the rat; its impact on development and behaviour in adult offspring. Psychoneuroendocrinology 32, 227–234.
Owen, J.B., et al., 2010. Oxidative modification to LDL receptor-related protein 1 in hippocampus from subjects with Alzheimer disease: implications for Abeta accumulation in AD brain. Free Radic. Biol. Med. 49, 1798–1803.
Payami, H., et al., 1996. Gender difference in apolipoprotein E-associated risk for familial Alzheimer disease: a possible clue to the higher incidence of Alzheimer disease in women. Am. J. Hum. Genet. 58, 803–811.
Pedros, I., et al., 2014. Early alterations in energy metabolism in the hippocampus of APPswe/PS1dE9 mouse model of Alzheimer's disease. Biochim. Biophys. Acta 1842, 1556–1566.
Peeyush, K.T., et al., 2010. Cholinergic, dopaminergic and insulin receptors gene expression in the cerebellum of streptozotocin-induced diabetic rats: functional regulation with vitamin D_3 supplementation. Pharmacol. Biochem. Behav. 95, 216–222.
Perluigi, M., et al., 2010. Redox proteomics in aging rat brain: involvement of mitochondrial reduced glutathione status and mitochondrial protein oxidation in the aging process. J. Neurosci. Res. 88, 3498–3507.
Perluigi, M., et al., 2014. Redox proteomics and the dynamic molecular landscape of the aging brain. Ageing Res. Rev. 13, 75–89.
Peterson, C.A., Heffernan, M.E., 2008. Serum tumor necrosis factor-alpha concentrations are negatively correlated with serum 25(OH)D concentrations in healthy women. J. Inflamm. 5, 10.
Pocernich, C.B., Butterfield, D.A., 2012. Elevation of glutathione as a therapeutic strategy in Alzheimer disease. Biochim. Biophys. Acta 1822, 625–630.
Poon, H.F., et al., 2004. Free radicals and brain aging. Clin. Geriatr. Med. 20, 329–359.
Qiu, C., et al., 2004. Risk and protective effects of the APOE gene towards Alzheimer's disease in the Kungsholmen project: variation by age and sex. J. Neurol. Neurosurg. Psychiatry 75, 828–833.
Quazi, F., Molday, R.S., 2011. Lipid transport by mammalian ABC proteins. Essays Biochem. 50, 265–290.
Rader, D.J., Daugherty, A., 2008. Translating molecular discoveries into new therapies for atherosclerosis. Nature 451, 904–913.
Ramagopalan, S.V., et al., 2010. A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution. Genome Res. 20, 1352–1360.
Rapoport, S.I., Nelson, P.T., 2011. Biomarkers and evolution in Alzheimer disease. Prog. Neurobiol. 95, 510–513.
Raygani, A.V., et al., 2006. Association between apolipoprotein E polymorphism and serum lipid and apolipoprotein levels with Alzheimer's disease. Neurosci. Lett. 408, 68–72.
Richard, C.L., et al., 2010. Involvement of 1,25D_3-MARRS (membrane associated, rapid response steroid-binding), a novel vitamin D receptor, in growth inhibition of breast cancer cells. Exp. Cell Res. 316, 695–703.
Robinson, J.G., Booth, B., 2010. Statin use and lipid levels in older adults: National Health and Nutrition Examination Survey, 2001 to 2006. J. Clin. Lipidol. 4, 483–490.
Saczynski, J.S., et al., 2007. The relation between apolipoprotein A-I and dementia: the Honolulu–Asia aging study. Am. J. Epidemiol. 165, 985–992.
Sadeghi, K., et al., 2006. Vitamin D_3 down-regulates monocyte TLR expression and triggers hyporesponsiveness to pathogen-associated molecular patterns. Eur. J. Immunol. 36, 361–370.
Sakurai, T., et al., 2014. Lower vitamin D is associated with white matter hyperintensity in elderly women with Alzheimer disease and amnestic mild cognitive impairment. J. Am. Geriatr. Soc. 62, 1993–1994.
Sanchez, B., et al., 2009. 1,25-Dihydroxyvitamin D_3 administration to 6-hydroxydopamine-lesioned rats increases glial cell line-derived neurotrophic factor and partially restores tyrosine hydroxylase expression in substantia nigra and striatum. J. Neurosci. Res. 87, 723–732.
Sanders, L.H., 2014. Mitochondrial DNA damage: molecular marker of vulnerable nigral neurons in Parkinson's disease. Neurobiol. Dis. 70, 214–223.
Scheff, S.W., et al., 2014. Is synaptic loss a unique hallmark of Alzheimer disease? Biochem. Pharmacol. 88, 517–528.
Schlogl, M., Holick, M.F., 2014. Vitamin D and neurocognitive function. Clin. Interv. Aging 9, 559–568.
Schneider, A.L., et al., 2014. Vitamin D and cognitive function and dementia risk in a biracial cohort: the ARIC Brain MRI Study. Eur. J. Neurol. 21 (1211–8), e69–e70.
Schneider-Brachert, W., et al., 2004. Compartmentalization of TNF receptor 1 signaling: internalized TNF receptosomes as death signaling vesicles. Immunity 21, 415–428.
Schulz, E., et al., 2014. Mitochondrial redox signaling: interaction of mitochondrial reactive oxygen species with other sources of oxidative stress. Antioxid. Redox Signal. 20, 308–324.
Scialo, F., et al., 2013. Regulation of lifespan by the mitochondrial electron transport chain: reactive oxygen species-dependent and reactive oxygen species-independent mechanisms. Antioxid. Redox Signal. 19, 1953–1969.
Selkoe, D.J., 2001. Alzheimer's disease: genes, proteins, and therapy. Physiol. Rev. 81, 741–766.
Seripa, D., et al., 2011. The genetics of the human APOE polymorphism. Rejuvenation Res. 14, 491–500.
Sies, H., 1997. Oxidative stress: oxidants and antioxidants. Exp. Physiol. 82, 291–295.
Sies, H., 2015. Oxidative stress: a concept in redox biology and medicine. Redox Biol. 4C, 180–183.
Singh, T., Newman, A.B., 2011. Inflammatory markers in population studies of aging. Ageing Res. Rev. 10, 319–329.
Sompol, P., et al., 2008. A neuronal model of Alzheimer's disease: an insight into the mechanisms of oxidative stress-mediated mitochondrial injury. Neuroscience 153, 120–130.
Sonnenberg, J., et al., 1986. 1,25-Dihydroxyvitamin D_3 treatment results in increased choline acetyltransferase activity in specific brain nuclei. Endocrinology 118, 1433–1439.
Sperling, R.A., et al., 2011. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging–Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 7, 280–292.
Stadtman, E.R., Berlett, B.S., 1998. Reactive oxygen-mediated protein oxidation in aging and disease. Drug Metab. Rev. 30, 225–243.
Steen, E., et al., 2005. Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease—is this type 3 diabetes? J. Alzheimers Dis. 7, 63–80.
Stein, M.S., et al., 2011. A randomized controlled trial of high-dose vitamin D_2 followed by intranasal insulin in Alzheimer's disease. J. Alzheimers Dis. 26, 477–484.
Stubbs, J.R., et al., 2010. Cholecalciferol supplementation alters calcitriol-responsive monocyte proteins and decreases inflammatory cytokines in ESRD. J. Am. Soc. Nephrol. 21, 353–361.
Sullivan, N.J., et al., 2012. UV light B-mediated inhibition of skin catalase activity promotes Gr-1+ CD11b+ myeloid cell expansion. J. Invest. Dermatol. 132, 695–702.
Sultana, R., Butterfield, D.A., 2010. Role of oxidative stress in the progression of Alzheimer's disease. J. Alzheimers Dis. 19, 341–353.
Sultana, R., Butterfield, D.A., 2013. Oxidative modification of brain proteins in Alzheimer's disease: perspective on future studies based on results of redox proteomics studies. J. Alzheimers Dis. 33 (Suppl. 1), S243–S251.
Sultana, R., et al., 2006. Identification of nitrated proteins in Alzheimer's disease brain using a redox proteomics approach. Neurobiol. Dis. 22, 76–87.
Sultana, R., et al., 2013. Lipid peroxidation triggers neurodegeneration: a redox proteomics view into the Alzheimer disease brain. Free Radic. Biol. Med. 62, 157–169.
Surmeli, N.B., et al., 2010. Peroxynitrite mediates active site tyrosine nitration in manganese superoxide dismutase. Evidence of a role for the carbonate radical anion. J. Am. Chem. Soc. 51, 622–629.
Swomley, A.M., Förster, S., Keeney, J.T., Triplett, J., Zhang, Z., Sultana, R., Butterfield, D.A., 2014. Abeta, oxidative stress in Alzheimer disease: evidence based on proteomic studies. Biochim Biophys Acta 1842, 1248–1257.
Taghizadeh, M., Talaei, S.A., Djazayeri, A., Salami, M., 2014. Vitamin D supplementation restores suppressed synaptic plasticity in Alzheimer's disease. Nutr Neurosci 17, 172–177.
Tangpong, J., Sompol, P., St Clair, W., Butterfield, D.A., St Clair, D.K., 2008. Tumor necrosis factor alpha-mediated nitriv oxide production enhances manganese superoxide dismutase nitration and mitochondrial dysfunction in primary neurons: an insight into the role of glial cells. Neuroscience 151, 622–629.
Tekes, K., et al., 2009. Influence of neonatal vitamin A or vitamin D treatment on the concentration of biogenic amines and their metabolites in the adult rat brain. Horm. Metab. Res. 41, 277–280.
Valko, M., et al., 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol. 39, 44–84.
van Duijn, C.M., et al., 1991. Familial aggregation of Alzheimer's disease and related disorders: a collaborative re-analysis of case–control studies. J. Epidemiol. 20 (Suppl. 2), S13–S20.
Verdile, G., et al., 2015. The role of type 2 diabetes in neurodegeneration. Neurobiol. Dis. 84, 22–38.
Vos, S.J., et al., 2015. Prevalence and prognosis of Alzheimer disease at the mild cognitive impairment stage. Brain 138 (Pt5), 1327–1338.
Wang, J.Y., et al., 2001. Vitamin D(3) attenuates 6-hydroxydopamine-induced neurotoxicity in rats. Brain Res. 904, 67–75.
Wang, T.J., et al., 2010. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet 376, 180–188.
Wang, L., et al., 2012. Vitamin D receptor and Alzheimer's disease: a genetic and functional study. Neurobiol. Aging 33 (1844), e1–e9.

Watson, R.R., Preedy, V.R., 2013. Bioactive Food as Dietary Interventions for the Aging Population. Elsevier/Academic Press, Boston.
Wisniewski, T., et al., 1995. Conformational mimicry in Alzheimer's disease. Role of apolipoproteins in amyloidogenesis. Am. J. Pathol. 147, 238–244.
Yaari, R., Corey-Bloom, J., 2007. Alzheimer's disease. Semin. Neurol. 27, 32–41.
Yao, J., et al., 2009. Mitochondrial bioenergetic deficit precedes Alzheimer's pathology in female mouse model of Alzheimer's disease. Proc. Natl. Acad. Sci. U. S. A. 106, 14670–14675.
Zhao, L., et al., 2004. Insulin-degrading enzyme as a downstream target of insulin receptor signaling cascade: implications for Alzheimer's disease intervention. J. Neurosci. 24, 11120–11126.
Zhu, Y., et al., 2012. Abnormal neurogenesis in the dentate gyrus of adult mice lacking 1,25-dihydroxy vitamin D_3 (1,25-$(OH)_2D_3$). Hippocampus 22, 421–433.
Zittermann, A., et al., 2009. Vitamin D deficiency and mortality. Curr. Opin. Clin. Nutr. Metab. Care 12, 634–639.
Zittermann, A., et al., 2012. Vitamin D deficiency and mortality risk in the general population: a meta-analysis of prospective cohort studies. Am. J. Clin. Nutr. 95, 91–100.