Vitamin D Clinical Relevance in the Recovery From Traumatic Brain Injury Among the Military Population

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VITAMIN D CLINICAL RELEVANCE IN THE RECOVERY FROM TRAUMATIC BRAIN INJURY AMONG THE MILITARY POPULATION

by

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ABSTRACT

Background: Traumatic brain injury (TBI) still remains a difficult disorder to treat. TBI has been associated to chronic neuroinflammation and a high risk for neurodegenerative disorders. Since 2001 between ten to twenty percent of all deployed military members have suffered a combat-related TBI. Nearly twenty to thirty percent of those will experience chronic cognitive, behavioral and somatic symptoms after suffering a TBI.

Methods: The objective of this review is to evaluate current literature examining vitamin D as a neurosteroid with protective properties and its clinical relevance after traumatic brain injury. Vitamin D is known to participate in neurobiological processes and genomic regulation in the brain. Clinical and laboratory findings support that vitamin D modulates the immune responses to trauma, diminishes oxidative and toxic damage, and inhibiting activation and progression of the neuroinflammation. Inadequate levels of vitamin D have been identified as a common risk factor for many neurological disorders and have been linked to poorer recovery.

Results: This review found compelling evidence to support that the pathology of TBI is closely associated with neuroprotective mechanisms of vitamin D. Low vitamin D levels are common among US active duty military and veterans. The findings strongly suggest that optimizing vitamin D prior to injury could improve the recovery for military members after experiencing a TBI. Vitamin D ameliorates brain damage by modulating neuroinflammation, improving cell survival and down-regulating mechanisms involved in the progression of cell damage following a TBI. However, further studies are needed to evaluate the effects of vitamin D optimization in TBI outcomes.

Keywords: vitamin D, low vitamin D, neuroprotection, traumatic brain injury, TBI, calcitriol

DEDICATION

I would like to dedicate this work to my partner David B., for his unconditional support and love. Venturing back to school to start a new career in nursing has been one of the most difficult decisions of my life and he did not hesitate for a second to back me up. I cannot thank him enough for believing in me when I doubted myself and for helping me find the confidence to complete this work. He has been my source of hope and strength thought the difficult experiences of the last few years. Beyond doubt, sharing my life with such a kind, adventurous and intelligent human being inspires me to become the best person I can be.

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CHAPTER 1: INTRODUCTION

Traumatic brain injury is characterized as a complex and multidimensional neurological disorder (Brain Trauma Foundation [BTF], 2007; Institute of Medicine [IOM], 2011a; Stein, 2015). The onset of tissue and cellular alterations of TBIs emerge over a short period of time, but the consequences of the acute tissue damage has been known to predispose patients to other chronic neurological disorders, and shortens their lifespan (Chauhan, 2014; Daneshvar, Goldstein, Kiernan, Stein, & McKee, 2015; Gardner & Yaffe, 2015).

Neurological disorders are complex. Uncertainty about the ideal management is related to unclear or poorly understood mechanisms and an assortment of environmental, genetic and physiological risk factors that amplify the uniqueness of the clinical presentations and progression. In addition to this complexity, the diversity of outcome measures further complicates the clinical picture (Maas et al., 2012; Groves, McGrath & Burne, 2014; Veterans Affairs and Department of Defense [VA/DoD], 2016). Interestingly, a newly identified common risk factor shared by many of the disorders is inadequate vitamin D levels. After correcting for confounding variants, researchers have found that the incidence and symptom severity of neuropsychiatric and neurodegenerative disorders remains higher for subjects with low levels of vitamin D (Groves, McGrath & Burne, 2014).

In the last two decades, mounting epidemiologic evidence suggests that inadequate levels of vitamin D may be associated with an elevated risk for brain disorders such as schizophrenia (Cui et el., 2014; Groves, McGrath & Burne, 2014; Wrzosek et al., 2013), depression (Groves, McGrath & Burne, 2014), autism, Parkinson's disease (Cui et el., 2014; Groves, McGrath & Burne, 2014), Alzheimer's disease (Daneshvar et al., 2015; Groves, McGrath & Burne, 2014),

epilepsy (Groves, McGrath & Burne, 2014; Wrzosek et al., 2013), and brain atrophy (Annweiler, Annweiler, Montero-Odasso, Bartha & Beauchet, 2014; Wrzosek et al., 2013). The range of issues related to vitamin D deficiency speaks to a basic cellular set of activities that affect the entire system.

Animal experiments and in-vitro studies have shown and described some of the underlying mechanisms that produce the neuroprotective activities of vitamin D on brain cells. Vitamin D is capable of modulating inflammatory cytokines (Groves, McGrath & Burne, 2014; Holick, 2007; Moromizato et al., 2013; Wrzosek et al., 2013), offers antioxidant benefits (Eyles, Burne & McGrath, 2013; Wrzosek et al., 2013), intracellular calcium regulation (Annweiler et al., 2014; Cui et el., 2014; Eyles, Burne & McGrath, 2013; Groves, McGrath & Burne, 2014; Wrzosek et al., 2013), neurotrophic enhancement (Annweiler et al., 2014; Eyles, Burne & McGrath, 2013; Groves, McGrath & Burne, 2014; Wrzosek et al., 2013), protection of the BBB integrity (Moromizato et al., 2013; Won et al., 2015), and may reduce the toxic damage from proteins exiting damaged cells (Atif et al., 2013; Huang, Ho, Lai, Chiu, & Wang, 2015; Kajta et al., 2009).

This review will evaluate the literature to determine whether or not there is compelling evidence to support that vitamin D exerts a neuroprotective action in the acute phase following a traumatic brain injury (TBI). There is preliminary evidence that low vitamin D is prominent among active duty military (Alazzeh et al., 2015; Andersen et al., 2010; Bailey, Manning & Peiris, 2012; Hiserote et al., 2016; Wentz, Eldred, Henry, & Berry-Caban, 2014), a population with high prevalence of combat injuries involving trauma to the head (Bryan, 2013; Chauhan, 2014; IOM, 2011a; McKee & Robinson, 2014; Twamley, Jak, Delis, Bondi, & Lohr, 2014;

Troyanskaya et al., 2015; Department of Veterans Affairs & Department of Defense [VA/DoD], 2016; Vakhtin et al., 2013). This paper develops an argument for further investigation regarding the potential protective effect of vitamin D optimization on recovery from TBI, particularly for military personnel.

CHAPTER 2: METHODOLOGY

Due to the lack of studies evaluating the existence of a relationship between vitamin D and TBI, this review focused on articles concerning the neuroprotective effect of vitamin D on brain tissue. Publications were identified via Ebsco on the following databases: Medline, Cinahl Plus, Academic Search Premier, Conchrane Controlled Trials and Conchrane Systematic Reviews. Terms used for the initial search parameters included: inflammatory cytokines, or neuroinflammat*, or neuron* disruption or blood-brain barrier or BBB or BBB leakage or membrane leakage or L-type calcium channels or calcium channel or brain intracellular calcium or reactive oxygen species or ROS or oxidative stress or neurodegene* or ion gradients or excitotoxic* or cerebral edema or neuroedema or apoptosis or neurotrophic or lipid peroxidation or hypoxi* or vitamin d receptor or VDR or antioxidant or neuroprotect* or regulat* or modulat* or cytotoxic* or neuroprotective agent. Additionally, two different groups of terms were used to narrow down the specificity of the results; one using multiple terms for vitamin D and the other for TBI (full list of terms is available on Appendix A). Results were limited to publications in English, between 2006 and 2016.

Titles and abstracts were screened for inclusion/exclusion criteria with 14 articles selected. Duplicates across databases were removed during initial screening. The exclusion criteria included: (1) studies related to science or medical specialties other than neurology; (2) sample size of 1 patient; (3) age group: neonates, child* or infant or embryonic or pediatric or adolesce* or 0-18 years or birth or pregnancy or aged or aging or elderly or over 65; (4) did not include at least one of the topics from the inclusion criteria.

The inclusion criteria encompassed the following: (1) vitamin D neuroprotective effects in brain/axonal/neuronal injuries through mechanisms involving (2) neuroinflammation, blood-brain barrier integrity, L-type calcium channel, oxidative stress/reactive oxygen species (ROS) and excitotoxicity. Of the articles that met the inclusion criteria included 11 were primary research articles/animal model experiments, 1 randomized control trials (RCT) and 2 literature reviews.

CHAPTER 3: TRAUMATIC BRAIN INJURY BACKGROUND

Traumatic Brain Injury (TBI) comes in many forms. The characterization and definition of TBI is still yet to reach consensus (Gardner & Yaffe, 2015; Stein, 2015; VA/DoD, 2016). This should not come as a surprise, considering that the multidimensionality of the disorder results in a substantial assortment of clinical signs and symptoms severity, structural and functional alterations and heterogeneity of outcomes (BTF, 2007; IOM, 2011a; Stein, 2015). The most recent VA/DoD practice guidelines state that regardless of a confirmed exposure to an external force, a positive TBI diagnosis requires the reporting of one or more of the TBI clinical criteria immediately after sustaining an injury (VA/DoD, 2016).

Traumatic brain injuries occur after a person suffers an external blow to the head and the severity can range from mild (concussion symptoms) to severe (coma) (World Health Organization [WHO], 2006). The U.S. Centers for Disease Control and Prevention (CDC) and the Department of Defense (DoD) defined TBI as:

> a traumatically induced structural injury and/or physiological disruption of brain function as a result of an external force and is indicated by new onset or worsening of at least one of the following clinical signs immediately following the event: (1) any period of loss of or a decreased level of consciousness; (2) any loss of memory for events immediately before or after the injury (posttraumatic amnesia); (3) any alteration in mental state at the time of the injury (e.g., confusion, disorientation, slowed thinking, alteration of consciousness/mental state); (4) neurological deficits (e.g., weakness, loss of balance, change in vision, praxis, paresis/plegia, sensory loss, aphasia) that may or may not be transient; or, (5) intracranial lesion. (VA/DoD, 2016, p. 6)

Prevalence of Traumatic Brain Injury

The World Health Organization (2006) classifies TBI as one of the leading causes of disease burden worldwide for individuals under 40 years of age. TBI has been associated with almost fifty percent of all trauma related fatalities and are two to three times more common in males than on females (WHO, 2006). If the forecasted twofold increase of TBI by 2020 is fulfilled, it will become the third leading source of fatality and disability globally (Fernández-Gajardo et al., 2014).

Each year around 42 million individuals worldwide suffer a mild TBI (mTBI) (Gardner & Yaffe, 2015). In the United States, the number of emergency visits linked to TBIs are estimated between 1.7 and 2.2 million per year (Curtis & Epstein, 2014; VA/DoD, 2016; Vakhtin et al., 2013), and about 30% of deaths resulting from trauma are connected to TBI (Vakhtin et al., 2013). Every year approximately 52,000 deaths are caused by severe TBIs and 80,000 Americans (Sheriff & Hinson, 2015) join the estimated five million that live with a disability after experiencing a TBI (Chauhan, 2014; WHO, 2006).

Among the civilian population, TBIs are most commonly caused by motor vehicle accidents, falls, violence or sport-related injuries (Gardner & Yaffe, 2015; WHO, 2006). Reports of TBI prevalence among the military vary, however there is consensus on improvised explosive devices (IEDs) as the leading mechanism of injury for head trauma among active service members of Operation Iraqi Freedom and Operation Enduring Freedom (IOM, 2011a; VA/DoD, 2016). Intracranial injuries from IEDs involve a series of mechanical forces (shear stress, projectile debris, compression, pressure and tension) starting from the high energy center of the

explosion and many variables can impact the severity of the damage (VA/DoD, 2016). Researchers are actively working in deciphering the exact mechanisms behind blast-related acute structural changes (Goldstein, McKee, & Stanton, 2014).

Between 2001 and 2011, fatalities of the Iraq/Afghanistan conflicts combined documented 42% of casualties that were related to motor-vehicle or air-craft crashes; between 11-18% were registered as penetrating head injuries from bullets or projectiles from explosive; and about 15-20% were blast-related TBI. Thus, 26-38% of all deaths were directly linked to brain trauma and the majority were blast-related. Moreover, during 2004-2008, penetrating TBIs exceeded the number of closed or blast-related TBIs in a 2:1 ratio, a drastic decline in the occurrence of penetrating brain trauma was noted during the following three years (2008-2010), with a new 1.3:1 ratio (Chauhan, 2014).

According to a 2014 CDC report to Congress, between 2000 and 2011 the DoD documented 235,046 active duty personnel had a TBI diagnosis among the approximately 5.5 million Army, Air Force, Navy and Marine Corps service members (VA/DoD, 2016). However, prevalence estimates vary, even between different military sources. The CDC and other military sources, estimate that between ten to twenty percent of all deployed military members (Daneshvar et al., 2015) have suffered a blast-related TBI caused by IEDs (Bryan, 2013; Chauhan, 2014; Daneshvar et al., 2015; Gardner & Yaffe, 2015; McKee & Robinson, 2014; Twamley et al., 2014; Vakhtin et al., 2013; VA/DoD, 2016), and the majority are classified within the range of mild to moderate severity (Daneshvar et al., 2015; Maas et al., 2012; McKee & Robinson, 2014; Twamley et al., 2014; VA/DoD, 2016). According to Troyanskaya and colleagues (2015), twenty-five percent of combat zone emergency evacuations report injuries to

the head and neck and about fifteen percent of those who served in Operation Iraqi Freedom recounted having experienced a mTBI (Troyanskaya et al., 2015).

In 1992, Congress created the Defense and Veterans Brain Injury Center (DVBIC) following the recommendations from the Department of Defense (DoD) and the Department of Veterans Affairs (VA) on the relevance of TBI as a medical priority for the military. The purpose behind the DVBIC was to have a multidisciplinary team serve as a hub for the advancement of education and research, and the delivery of patient centered clinical care (IOM, 2011a). Improving knowledge on functional impairments following TBIs, the influencing factors that may contribute to persistency of symptoms and short- and long-term prognosis are at the top of the priorities (VA/DoD, 2016).

A small recent retrospective study underlined that vitamin D levels lower than 16ng/mL were prevalent in patients with a history of TBI, and showed a substantial negative association with cognitive test results, regardless of the severity or time since the injury. Those same $25OHD_3$ levels were inversely proportionate to the scores of the depression inventory. Overall levels were significantly lower than the general population, with 80.2% testing <26ng/mL, of which 46.5% were at less than 16ng/mL. These suggest that lower levels are more prevalent among post-TBI patients (Jamall et al., 2016).

Blast-related TBI.

The role of blast exposure as an etiology for brain trauma (Goldstein, McKee, & Stanton, 2014) was first reported among World War I infantry soldiers. The force from an explosion can generate a wide diversity of injuries compounded together into one (Daneshvar et al., 2015). The severity of a blast-related TBI is determined in part by the distance from the initial blast and

environmental differences such as exposure while out in the open versus inside of a vehicle and whether or not the person had properly secured personal protective equipment. Even low intensity recurrent blasts have been documented to cause neuronal tissue alterations (Goldstein, McKee, & Stanton, 2014).

A distinct characteristic of blast injuries is the additive neuronal damage caused by multiple forces (VA/DoD, 2016). The strong winds from the blast are traveling at such extreme, sustained speeds that can generate a shockwave capable of producing multiple intervals of dangerous, drastic contraction and expansion of tissue in fractions of a second. The shockwave may cause diffused or focal bleeding and inflammation as the result of shearing and tearing of neurovascular and neuronal tissues (Daneshvar et al., 2015). The violent atmospheric pressure changes of the blast generate projectile debris elevating the risk of penetrating or blunt injury, or both (Chauhan, 2014; VA/DoD, 2016). Lastly, as the body is tossed by the explosion, the brain remains susceptible to harm from the displacement of tissue inside the skull from rapid acceleration/deceleration and the risk of withstanding additional blunt to the head at landing (VA/DoD, 2016). Blast-related TBIs present with a collection of heterogeneous lesions representative of the many mechanisms of injury involved (Daneshvar et al., 2015).

TBI Pathophysiology

Brain tissue is organized into gray matter and white matter. Gray matter functions as the central nervous system (CNS) data processor and it is primarily composed by cell bodies and unmyelinated axons. White matter composition is predominantly myelinated axons,

oligodendrocytes, and other types of glia cells. It is a crucial element of the brain communication network, carrying transmission of signals between brain regions (Shi et al., 2015).

The brain's proper functions are tied to the cellular and interstitial environments. Cytoplasmic and interstitial parameters are maintained by interactions among neuroglia (microglia, oligodendrocyte and astrocyte) cells, endothelial cells of the BBB and neurons. Membrane proteins and signaling between cells enable adequate supply of oxygen and glucose for energy production, support electrolyte transport from general circulation, and facilitate the removal of metabolic waste (Chauhan, 2014; Shi et al., 2015).

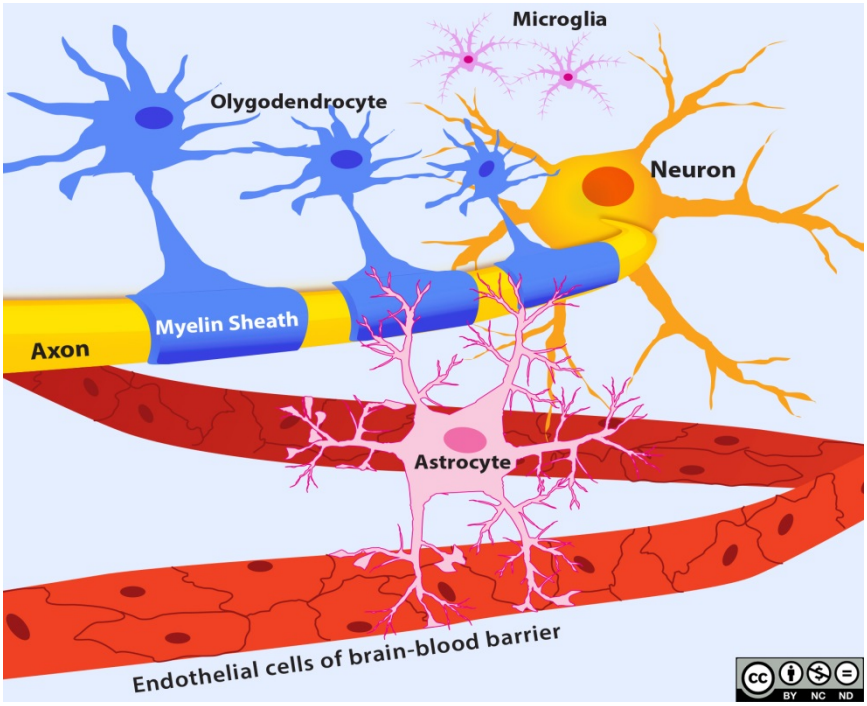


Figure 1: Endothelial Cells of the BBB, Neuroglia and Neuron.

Adapted from "Demyelination as a rational therapeutic target for ischemic or traumatic brain injury" by H. Shi, X. Hu, R. K. Leak, Y. Shi, C. An, J. Suenaga, J. Chen, & Y. Gao, 2015, *Experimental Neurology, 272*, p. 20.

The pathogenesis of TBI begins when an external force disrupts structures and neuronal homeostasis. Most TBIs present neurological deficits without or with minimally detectable anatomical changes, regardless of the severity. The tissue disruptions arising immediately after and as a direct consequence of biomechanical forces may include ischemia, neurovascular integrity alterations, and microscopic axonal shearing, and are commonly referred to as the primary injury or insult (Chauhan, 2014; Mayer, Huber & Peskind, 2013; VA/DoD, 2016; WHO, 2006).

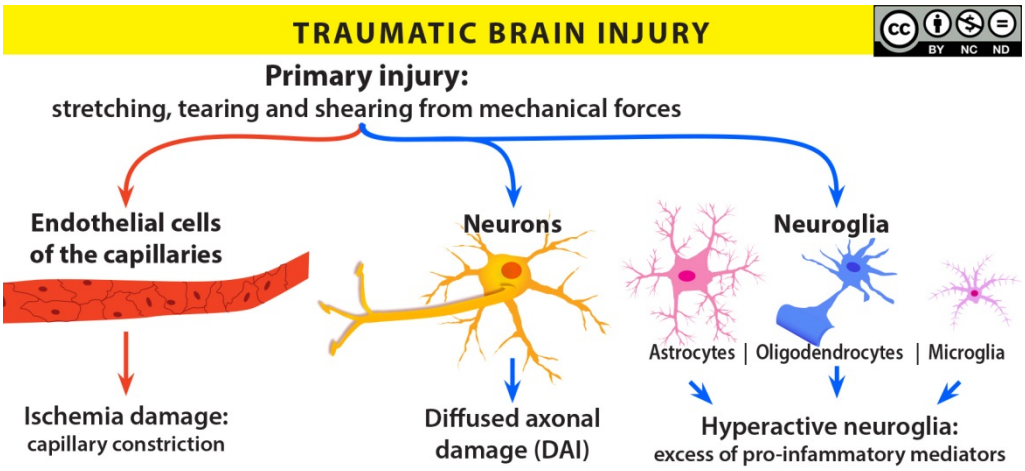


Figure 2: Primary injury from TBI.

(Chauhan, 2014; Daneshvar et al., 2015; Fernández-Gajardo et al., 2014; Mayer, Huber & Peskind, 2013; VA/DoD, 2016)

Co-occurrence of localized and widespread injuries from the same incident is quite typical (Chauhan, 2014). Energy transfer from rapid acceleration and deceleration to the skull causes brain tissue to shift, compromising cellular membrane and axonal cytoskeleton integrity. Mechanical forces cause microscopic neurovascular disruptions that have been linked to the clinical symptoms of TBI (Daneshvar et al., 2015; McKee & Robinson, 2014). Oftentimes, cellular damage occurs on the primary side of impact and to the opposite side as the brain and

skull come to sudden deceleration and the brain hits against surrounding intracranial structures, and it may rebound once more causing a secondary hit to the primary site of impact (VA/DoD, 2016).

Whether the damage is localized or widespread, the primary injury involves damage at the cellular level to neurons, neuroglial and neurovascular tissues (Chauhan, 2014; Fernández-Gajardo et al., 2014; VA/DoD, 2016), and subsequently initiates various neuropathological responses to the mechanical trauma that are classified as secondary injury or insult (Huang et al., 2015). The secondary multifaceted response includes a series of interrelated and complex molecular and cellular reactions involving macrophages, neuroglial cells, inflammatory messengers, systemic immune activation, excitotoxicity, disruptions of the blood-brain barrier, electrophysiological and ionic disturbances, and neurometabolic interruptions (Chauhan, 2014; Mayer, Huber & Peskind, 2013).

Common concerns during the secondary injury phase include neuroinflammatory activities, disruptions of the signal transmission, influx of calcium into cells, metabolic demands and abnormal electrophysiology, toxicity from blood-brain barrier leakage and buildup of neurotransmitters, intracranial edema and oxidative stress (Chauhan, 2014; Fernández-Gajardo et al., 2014; Mayer, Huber & Peskind, 2013; VA/DoD, 2016). Increased energy demands following trauma increases intracellular glucose requirements and releases catecholamines to stimulate glucose transport which in turn elevate serum glucose concentrations, producing acute hyperglycemia. After a TBI, high glucose attracts neutrophils to the wounded region intensifying the intracellular alterations, such as acidosis and production of reactive species, worsening of the extracellular detrimental environmental conditions including elevation of glutamate. Lastly, it

aggravates the inflammation by promoting glia secretion of pro-inflammatory mediators (Chauhan, 2014).

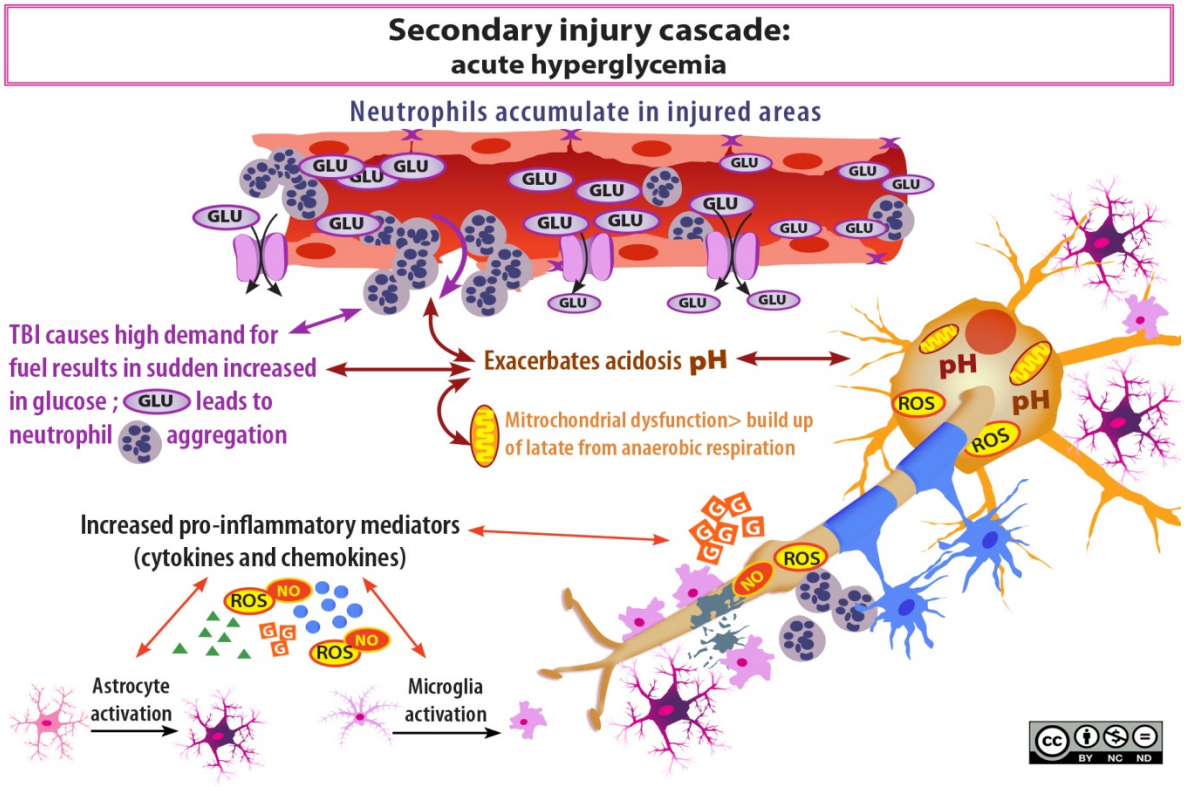


Figure 3: Acute hyperglycemia during TBI secondary injury cascade (Chauhan, 2014).

Adapted from "Traumatic brain injury," in Neurowiki, 2013, retrieved June 30, 2016, from http://neurowiki2013.wikidot.com/individual:traumatic-brain-injury.

The sums of the cytotoxic and neuroinflammatory processes collectively provoke cellular edema, oxidative stress, destruction of the mitochondria and eventually activate neuronal apoptosis (Chauhan, 2014). To appreciate the full profile of anatomical responses to the primary injury it is important to understand that multiple types of cells are involved in dynamics taking place simultaneously inside and outside of the affected cells. The responses are part of built-in mechanisms involving up- and down-regulations depending on the stimuli. The activation and

progression of inflammatory processes, even with the original purpose of tissue protection may exacerbate damage from trauma (Chauhan, 2014; Mayer, Huber & Peskind, 2013), and explains the neurological symptoms that develop following a TBI (Fernández-Gajardo et al., 2014). Even to this day, the multifaceted biological and structural alterations that occur as a result of a TBI are not fully understood (IOM, 2011a; Mayer, Huber & Peskind, 2013; VA/DoD, 2016). The following basic biologic mechanisms/steps are focused on processes where vitamin D is involved. Appendix B, contains a table of inflammatory mediators categorized by pro- and anti-inflammation with a brief description of their actions.

Neuroinflammation, excitotoxicity and oxidative stress.

Normally, the immune system inflammatory responses are aim at promotion of restorative and reparative actions (Fernández-Gajardo et al., 2014). In the central nervous system (CNS), inflammatory responses are controlled by CNS-devoted immune cells found in the interstitial space and are commonly referred to as neuroglia or glia. Activation of glia cells is generally inhibited by healthy neurons. Primary and secondary injuries stimulate concurrent release of proinflammatory mediators and elicit glia activation and proliferation (Mayer, Huber & Peskind, 2013).

Under typical conditions, the neuroglia or glia cells, comprised mostly of microglia and astrocytes, are immune cells dedicated to survey for pathogenic threats or injury (Mayer, Huber & Peskind, 2013). Clusters of activated microglia cells congregate at the site of injury immediately after trauma (Chauhan, 2014). Neurotoxic molecules released by the glia following activation from TBI are harmful to healthy cells, include reactive oxygen species (ROS), nitric oxide (NO), proinflammatory cytokines and chemokines (Huang et al., 2015; Mayer, Huber &

Peskind, 2013). Normally, the cell's built-in antioxidant system is able to keep ROS and NO from harmful high concentrations. However, under extraordinary circumstances, when cellular functions have been compromised, protective mechanisms struggle to safeguard the cell (Huang et al., 2015).

Neuroinflammation causes neuroglial to become hyperactive, which in turn instigate the phosphorylation of mitogen-activated protein kinase (MAPK) pathways. The ultimate goal of these pathways is to generate nuclear transcription factors (NTF) to regulate cellular inflammation, stress response, cell growth, differentiation and apoptosis (Hur et al., 2014). Initiation of the extracellular signal-regulated kinase (ERK), p38-MAPK and Jun-N-terminal (JNK) MAPK pathways elicited by activated microglia after brain trauma is responsible for the signaling actions to stimulate synthesis of proinflammatory mediators capable of furthering brain damage (Fu et al., 2013; Huang et al., 2015). Thus, the disproportionate or extended activation of microglia following traumatic injury may exacerbate neuronal damage by maintaining a cycle of glia activation and neuronal injury progression (Huang et al., 2015).

Table 1: Mitogen-activated protein kinase (MAPK) pathways

Extracellular stimuli	Cell membrane	Cytoplasm	MAPK NTF	Gene stimulation: protein synthesis affects	
1. ischemia, 2. toxicity, 3. inflammatory cytokines 4. hormones/ growth factors 5. mechanical trauma (TBI)	Activation of N-methyl D-aspartate (NMDA) glutamate receptor	Following NMDA receptor activation: • initiation of several phosphorylation steps produce a nuclear membrane transcriptor factor (NTF) • major extracellular glutamate efflux	**ERK MAPK**	Initiates apoptosis and shuts down the cell's survival mechanisms	Are involved in nitric oxide accumulation, reactive oxygen species production, and increases of pro-inflammatory cytokines/ chemokines.
			P38 MAPK	Promotes neuroinflamma-tion	
			JNK MAPK	Promotes neuroinflamma-tion	

⦚ cell membrane & nuclear membrane

Astrocytes are advantageously located between the neurovascular and neuronal cells with the purpose of regulating essential interactions concerning the BBB. Neurons rely on them for almost all of their cellular functions, counting on astrocytes to regulate concentrations of neurotransmitters, mediate synapsis and preserve ionic balances. It should not be a surprise that they outnumber any other type of cell in the brain. After head trauma, their activation can be triggered by damaged neurons or microglia activation. Activated astrocytes are beneficial in isolating injured neurons by substrate deposition after a traumatic event. However, this barrier also impedes axonal communication and restoration. They are also vulnerable to structural damage themselves, not only unable to support neuronal homeostasis, but releasing inflammatory mediators as well (Mayer, Huber & Peskind, 2013).

Oligodendrocytes' responsibilities include making, maintaining and regenerating the myelin sheath supporting the neuronal axons actions and integrity. However, they are particularly vulnerable due to a low capacity to fight the oxidative stress that follows head

trauma. The unmet demand of energy following acute trauma can reduce the cell's ability to perform its functions and lead to permanent loss of the myelin. Demyelination contributes to neuronal transmission alterations and cause neurological symptoms after TBI. Therapeutic approaches targeting oxidation and mitochondrial dysfunction could prevent or ameliorate axonal demyelination (Shi et al., 2015).

As neuroinflammation continues, Schwann cells become activated as well and contribute to the transmission dysfunctions of the wounded axons (Mayer, Huber & Peskind, 2013). Intracellular edema along with ionic and metabolic changes, eventually lead to further structural damage of the axons and infiltration of macrophages (Chauhan, 2014).

Excessive or persistent inflammatory response has been considered as a possible explanation for neurological deficits following a TBI (Mayer, Huber & Peskind, 2013). Multiple autopsy studies of individuals with mTBI found abnormal tissue structures correlated with neuroinflammation. Findings supporting inflammation included signs of axonal injury such as demyelination, varicosities, axonal bulbs at site of detachment and Wallerian degeneration, and clusters of activated microglia and macrophages containing degenerated myelin have also been found in the same area. All these alterations can be expected closely after trauma, except that these findings were observed up to 18 years post-TBI, in additions to thinning of neuronal tracts (Mayer, Huber & Peskind, 2013). Finding ways to mitigate, prevent or stop the overactive glia has been identified as a potential treatment target for TBI (Huang et al., 2015).

Neuroinflammation is intimately related to oxidative stress and excitotoxicity as originators and promoters of cellular damage. Their combined actions can consequently elicit cell necrosis, apoptosis and autophagy. Oxidation damage can initiate multiple pathways to

regulate cell death. Mitochondrial lysis is another known way to trigger apoptosis. Signs of apoptosis in autopsies of TBI patients have been observed up to a year after the original mechanical trauma. Autophagy initiated during ischemia/reperfusion, hypoxic stress or post-TBI is mostly driven by oxidative stress. Laboratory and clinical findings have identified a genetic pathway promoting autophagy after TBI (Fernández-Gajardo et al., 2014).

Oxidative stress from reactive oxygen and nitrogen species is known to occur in the brain after TBI and the antioxidant actions directly rely on the BBB to attain the best possible effects. Damage is subsequent to the discrepancies between high concentrations of reactive species and the cell's overwhelmed antioxidant mechanisms. Because of low antioxidant capacity, high demand for oxygen and high amounts of iron, neurons are particularly susceptible to elevation of reactive oxygen species (ROS) and reactive nitrogen species (NOS) following brain trauma (Fernández-Gajardo et al., 2014).

ROS contributions to intracellular damage are excitotoxicity, calcium influx, intracellular edema and metabolic collapse. ROS also facilitates structural weakening and disruption of the neurovascular vessel walls, thus increasing the permeability of the blood-brain barrier (BBB). Cell damage from oxidation causes declines in brain-derived neurotropic factors consequently worsening cognitive decline and neuronal communication (Fernández-Gajardo et al., 2014).

At the beginning of the secondary injury phase, a sudden neuronal membrane depolarization precedes the colossal release of the neurotransmitter glutamate. Glutamate efflux surpasses the reuptake ability of astrocytes (Charier et al., 2015). Excitotoxicity from glutamate triggers harmful influx of calcium ions, which precedes a series of neurotoxic events within the cell, augmenting cell edema and production of NOS. Glutamate toxicity harms the affected cells

as well as neighboring cells and creates a risk for apoptosis on all glutamate-stressed cells (Fernández-Gajardo et al., 2014).

Temporary increases of amyloid β and its precursor protein in the axons takes place following a TBI (Daneshvar et al., 2014). Studies showed that multiplied levels of amyloid β promote low-voltage calcium channel expression and suppressed the vitamin D receptor (Gezen-Ak, Dursun & Yilmazer, 2013).

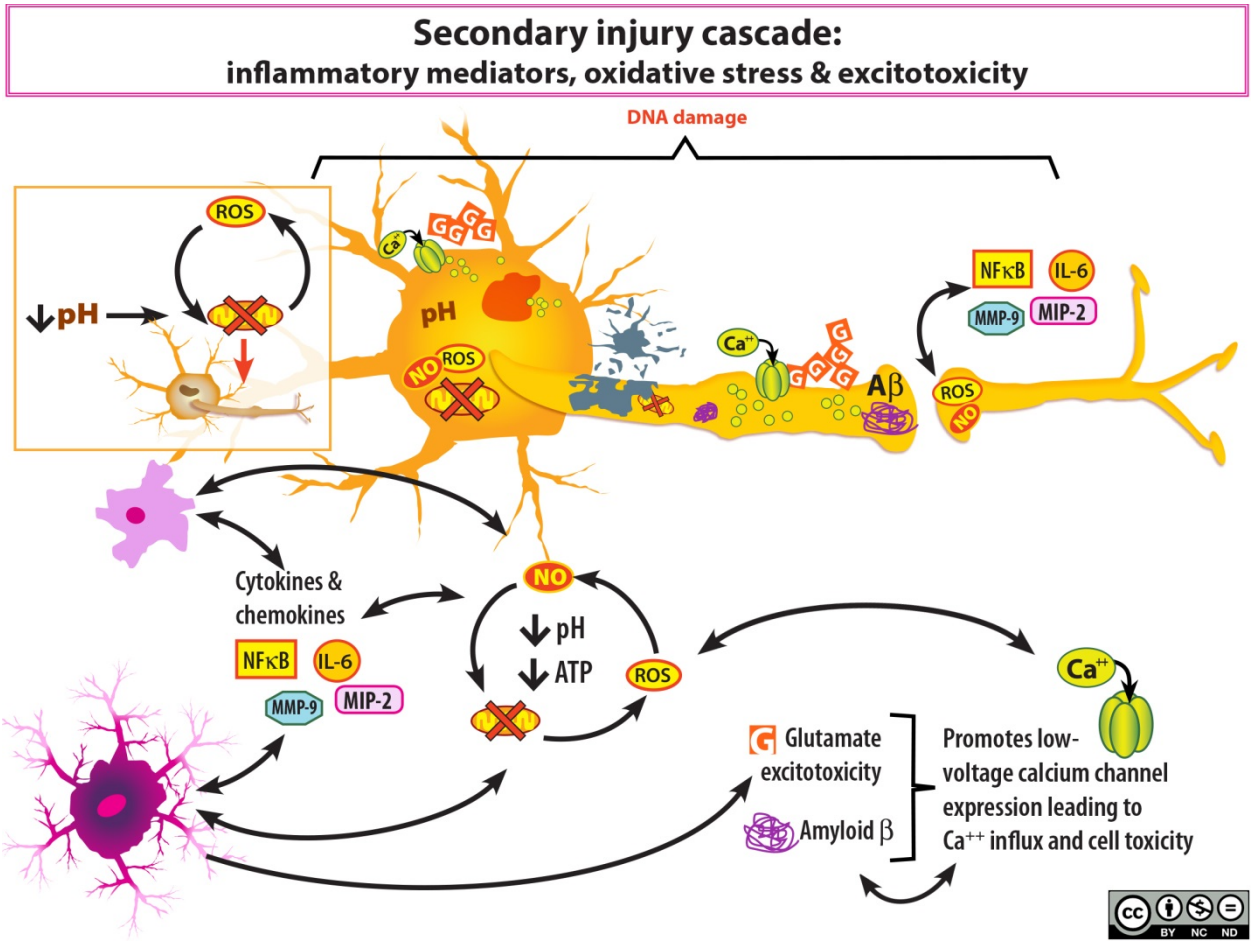


Figure 4: Inflammatory mediator, oxidative stress and excitotoxicity.

Adapted from "Traumatic brain injury," in Neurowiki, 2013, retrieved June 30, 2016, from http://neurowiki2013.wikidot.com/individual:traumatic-brain-injury. Copyright 2009 by E. Park, J. D. Bell, I. P. Siddiq & A. J. Baker.

Diffuse axonal injury.

Neurovascular vessels and axons are particularly susceptible to damage from rapid brain shifting because of their elongated structures (Daneshvar et al., 2015; McKee & Robinson, 2014). Diffuse axonal injury (DAI) or traumatic axonal injury (TAI) is seen in all types of TBIs, thus it is considered the signature injury of TBI (Daneshvar et al., 2015).

DAI is a dynamic process characterized by widespread microscopic injuries, that unfolds over time as a result of mechanical interruptions and membrane perforation to white matter tracts or axons. Compromised membrane integrity allows drastic influx of calcium while driving potassium out of the cell (Chauhan, 2014; Daneshvar et al., 2015; McKee & Robinson, 2014). Calcium influx stimulates sodium-calcium (Na^{+}/Ca^{++}) pumps and sets in motion regulatory actions of calcium-dependent enzymes to catabolized damaged proteins. The sudden increase of intracellular calcium leads to mitochondrial membrane leakage and axonal energy production collapses (Chauhan, 2014). Mitochondrial energy breakdown is a known predictor for poor outcomes (Fernández-Gajardo et al., 2014). Meanwhile, the Na^{+}/Ca^{++} pumps continue to exacerbate the burden on the axon cytoskeletal structures and the mitochondria by importing more calcium ions into the cell. Finally, the process of axonal degeneration culminates with necrosis and apoptosis (Chauhan, 2014).

Cellular changes that arise from the stretching and tearing of axons begin minutes to hours following the primary insult. Ramifications of the intracellular calcium overload include damage to the transmission structure on the cell and impaired transport. The large amount of ion transport proteins along the nodes of Ranvier makes this area particularly susceptible to

deterioration from calcium influx and membrane disturbances (Chauhan, 2014; Daneshvar et al., 2015).

Continuation of calcium import, with impaired ionic and neurotransmitters transport and intermittent areas of inflammation along the axon stimulate the formation of axonal varicosities and lead to further structural degeneration and deformations. Axonal varicosities are concentrated areas of inflammation considered weakened structural points along axonal cytoskeleton. Within minutes to hours after trauma, localized swelling and the buildup of un-transported molecules lead to severed axons from the body of the neuron and creates axonal bulbs at the detached ends of the axon, at the breaking point, usually at the level of the nodes of Ranvier (Chauhan, 2014).

As the axonal ends separate from the cell bodies, communication between different regions of the brain cease and Wallerian degeneration is initiated. Wallerian degeneration is a process in which the distal portion of a nerve fiber that has either been detached or damaged begins to degenerate. Using a DAI experiment model, detection of DAI occurred fifteen minutes after the injury. Axonal detachment was prevalent at thirty minutes and was followed by Wallerian degeneration beginning two to three hours after trauma (Chauhan, 2014).

Compromised myelin sheath renders transmission of action potential down the axon inoperable, impairs the cells energy sources and makes the unprotected axon more vulnerable to degradation (Shi et al., 2015). Acute deterioration of the myelin sheath exposes the axon facilitating infiltration of macrophages and Schwann cells. The progression of axonal demyelination is believed to continue years after the original trauma and may be the source for persistent symptoms and the effects of neuronal degeneration later in life (Chauhan, 2014).

Blood-brain barrier disruption.

The BBB can be described as an adaptable interface that is capable of restricting access to damaging substances from the circulatory system, while allowing crossing of necessary nutrients and substances to sustain the ideal conditions for proper neuronal function. Normally, neurovascular modifications that mediate these interactions occur from the influence of astrocytes on endothelial cells (Mayer, Huber & Peskind, 2013).

Regulation of the endothelial tight junctions is an extremely important protective mechanism for the brain since components of blood, plasma and peripheral leukocytes are toxic to brain cells. Structural alterations to microvascularity from mechanical forces, damage to astrocytes (Daneshvar et al., 2015; Mayer, Huber & Peskind, 2013), hypoxic death of endothelial cells, or oxidative damage to tight junction protein structures (Won et al., 2015) may lead to increased BBB permeability. Despite of the etiology, all TBIs whether from open or closed trauma, will compromise brain microvascular cells of the BBB. Consequently, the neurotoxicity caused by blood exposure exerting a pro-inflammatory action will increase activated glia and leakage of BBB (Chauhan, 2014; Mayer, Huber & Peskind, 2013).

Infiltration of peripheral leukocytes join in with other brain immune defenses, and lamentably, promote oxidative stress by increased production of nitric oxide (NO). The NO interactions on the microglia membranes foster additional ROS synthesis and suppress enzymatic activity necessary for the production of adenosine triphosphate (ATP) (Fernández-Gajardo et al., 2014).

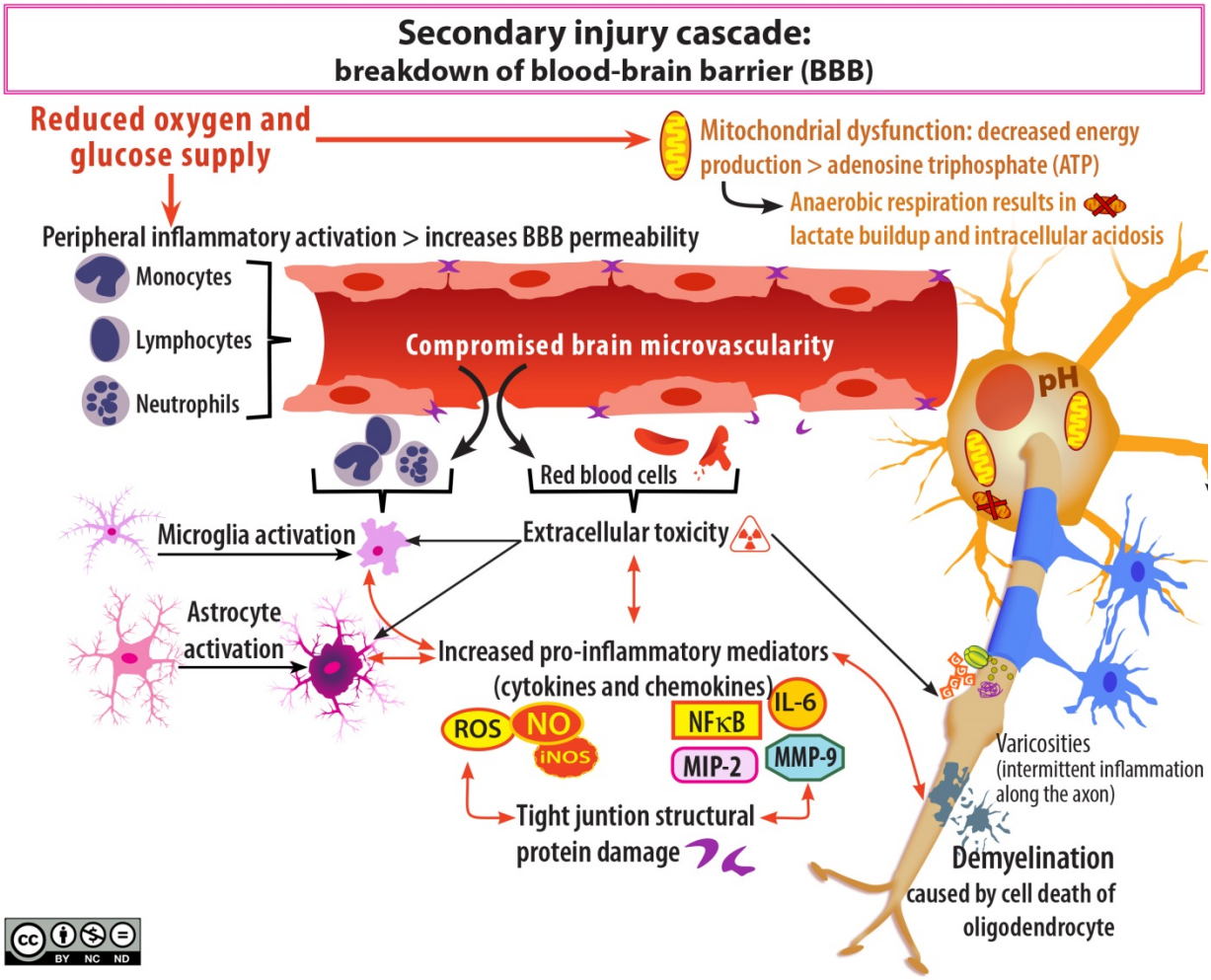


Figure 5: Secondary injury from BBB breakdown.

Adapted from "Disruption in the Blood-Brain Barrier: The Missing Link between Brain and Body Inflammation in Bipolar Disorder?," Scientific figure on ResearchGate, n.d., Retrieved June 30, 2016, from: https://www.researchgate.net/277352521_fig1_Proposed-model-of-blood-brain-barrier-BBB-disruption-in-bipolar-disorder-Increased-BBB.

Large amounts of uncoagulated blood coming into contact with brain cells, as seen with hematomas can cause severe brain edema and increased ICP. Edema and inflammation compromised brain functions even further and require immediate medical interventions to protect the brain cells (Chauhan, 2014). The infiltration of systemic leukocytes into the brain

interstitial space prompts additional recruitment of glia and peripheral immune cells resulting in a systemic immune reaction (Mayer, Huber & Peskind, 2013).

In summary, the pathological cascade triggered by different degrees of axonal and neurovascular structural alterations is not tied to a single, isolated timed-event. Evidence demonstrates that TBI damage is progressive in nature, with interlayering and complex anatomical responses and may persist over a span of months, years or even decades. Understanding the mechanisms triggering the secondary responses is crucial in order to develop therapies to prevent or attenuate their impact.

Overview of TBI Clinical Management

Clinical management of TBI can be cumbersome, in part because of the progressive nature of the disorder and complexities surrounding the wide variety of clinical signs. According to the IOM (2011a), approaches aiming at one specific aspect of the pathological alteration post-TBI have not yet succeeded and standardization of a particular consistent or effective protocol for TBI management has not been achieved (IOM, 2011a).

The use of imaging studies and laboratory results for conclusive diagnosis, guidance of clinical care or as prognosis predictors is not recommended (VA/DoD, 2016; WHO, 2006). Timely recognition of TBI symptoms enables early clinical management, improves outcomes and prevents undesirable consequences (WHO, 2006). It is important to underline that there is no definite correlation between the documented degree of severity of TBI and patient prognosis (Maas et al., 2012; Stein, 2015).

The unpredictability of physiological responses seen with TBI is related to the distinctive characteristics of the person at the time of the incident, amount and direction of the forces, and environmental factors. Other factors likely to modify the body's response may involve genetic makeup, a prior diagnosis of TBI, overall health, nutritional and hydration status preceding the incident, lack of sleep and high concentrations of stress hormones (VA/DoD, 2016).

Symptomology and Manifestations of Mild TBI

Given that the majority of reported TBIs are considered mild in severity (Gardner & Yaffe, 2015; Maas et al., 2012; Mayer, Huber & Peskind, 2013; McKee & Robinson, 2014; Stein, 2015; Twamley et al., 2014; VA/DoD, 2016), this section will focus on this data. It has been proposed that microscopic structural lesions correspond to milder post-injury symptoms (Daneshvar et al., 2015). However, the prevalence and severity of some symptoms have been found to be higher in mTBIs than in more severe cases, as it is the case with post-traumatic headaches (Mayer, Huber & Peskind, 2013). This seems to contradict the notion that the severity of structural damage usually corresponds to the severity of the TBI.

Although the magnitude of structural damage may be narrower for an mTBI, the metabolic and neuronal disruptions are thought to result from the aftermath of metabolic, ionic and physiologic alterations rather than the structural changes. The pathological profile created by the timing and magnitude of these anatomical responses has been known to progress into chronic neurodegeneration in a subgroup of the population (VA/DoD, 2016).

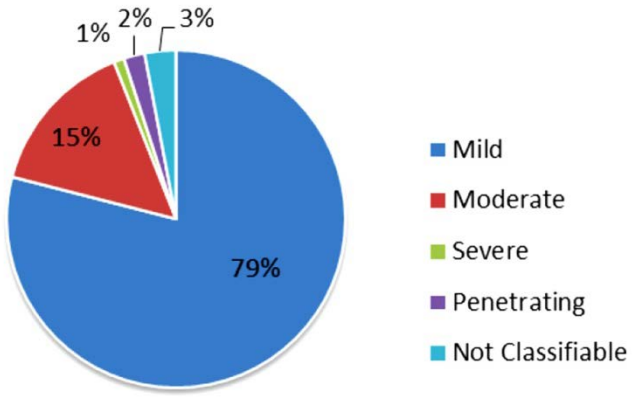


Figure 6: DoD TBI Diagnoses from 2002-2009.

Reprinted from "Clinical practice guideline for the management of concussion-mild traumatic brain injury (version 2.0)," by VA/DoD, 2016, p. 101, Retrieved June 19, 2016, from www.healthquality.va.gov/guidelines/Rehab/mtbi/.

Mild TBIs are characterized by lack of obvious anatomical trauma, the acute neurological deficits tend to be categorized as mild and frequently resolve on their own in a matter of few days or weeks (McKee & Robinson, 2014; WHO, 2006). However, about 10-15% of people will be diagnosed with postconcussive syndrome, a condition characterized by persistency of TBI-associated symptoms (McKee & Robinson, 2014). The invisible manifestations of a single or multiple close-head TBIs are often insidious and may take years to appear, though they may be traced back to the initial injury (Daneshvar et al., 2015; IOM, 2011a).

During the initial assessment of mTBI patients may present with a combination of physiological, cognitive and neuropsychological symptoms. Figure 7 contains the list of symptoms highlighted in the VA/DoD mTBI practice guidelines that may indicate the need for a neurological consult (VA/DoD, 2016).

1. Progressively declining level of consciousness 2. Progressively declining neurological exam 3. Pupillary asymmetry 4. Seizures 5. Repeated vomiting 6. Neurological deficit: motor or sensory	7. Double vision 8. Worsening headache 9. Cannot recognize people or disoriented to place 10. Slurred speech 11. Unusual behavior

Figure 7: Initial presentation: Indicators for immediate referral.

Reprinted from "Clinical practice guideline for the management of concussion-mild traumatic brain injury (version 2.0)," by VA/DoD, 2016, p. 17, Retrieved June 19, 2016, from www.healthquality.va.gov/guidelines/Rehab/mtbi/.

Some commonly reported physiological symptoms are headaches, dizziness, nausea, fatigue, sleep disturbances, and seizures (Chauhan, 2014). Roughly 30-90% of patients complain of post-traumatic headaches (VA/DoD, 2016) and around 24-74% of dizziness, both symptoms resolving within three months following an mTBI. Fatigue has been reported in about 73% of TBI patients and has been linked to lower levels of social interaction and isolation. Sleep disorders develop in about 30-73% of individuals diagnosed with TBI. Many of these symptoms exist side by side and may contribute to neuropsychological or cognitive deficits. Researchers found that improving sleep problems that developed after a TBI reduce the incidence of psychological impairments (Chauhan, 2014).

At least one psychosomatic behavioral alteration has been reported for nearly all TBI patients, involving the onset of depression, anxiety, or mood changes. Reports of post-TBI cognitive symptoms include difficulties with concentration, attention, decreased processing speed, impaired memory, judgement and problems with planning and multitasking (VA/DoD, 2016).

Sidebar 3: Possible Post-mTBI Related Symptoms***		
Physical Symptoms: Headache, dizziness, balance disorders, nausea, fatigue, sleep disturbance, blurred vision, sensitivity to light, hearing difficulties/loss, tinnitus, sensitivity to noise, seizure, transient neurological abnormalities, numbness, tingling	**Cognitive Symptoms:** Problems with attention, concentration, memory, speed of processing, judgment, executive control	**Behavior/Emotional Symptoms:** Depression, anxiety, agitation, irritability, impulsivity, aggression

*Alteration of mental status must be immediately related to the trauma to the head. Typical symptoms would be: looking and feeling dazed and uncertain of what is happening, confusion, difficulty thinking clearly or responding appropriately to mental status questions, and being unable to describe events immediately before or after the trauma event.
**In April 2015, the DoD released a memorandum recommending against the use of GCS scores to diagnose TBI. See the memorandum for additional information.
***Symptoms that may develop within 30 days post injury.

Figure 8: List of possible mTBI symptoms from the VA/DoD algorithm for the management of mTBI. Reprinted from "Clinical practice guideline for the management of concussion-mild traumatic brain injury (version 2.0)," by VA/DoD, 2016, p. 17, Retrieved June 19, 2016, from www.healthquality.va.gov/guidelines/Rehab/mtbi/.

Many of the symptoms reported by combat veterans recovering from TBI include cognitive dysfunctions such as problems with concentration, memory impairment and executive function irregularities (Daneshvar et al., 2015; Twamley et al., 2014). These deficiencies in executive functions diminish a person's ability to plan and organize their day, to manage their time, or even completely quench their inability to multitask. Studies have indicated that cognitive functionality can directly influence employability (Twamley et al., 2014). Thus, this shows that special attention should be paid at resources available to ensure combat veterans can return to their family and community with the highest level of reintegration and adaptability possible.

TBI Outcomes

TBI survival rates among combat-related wounded soldiers have increased significantly, in part due to modern advances in personal protective armour (Vakhtin et al., 2013) and

improved military medical interventions (Johannigman et al., 2015). Consequently, many veterans are coming back home or being returned to active duty after surviving their injuries.

Holistic, patient-oriented TBI management aims at improving symptoms, quality of life and functionality levels (VA/DoD, 2016). Most combat-related TBIs are considered mild in nature (Daneshvar et al., 2015; Maas et al., 2012; McKee & Robinson, 2014; Twamley et al., 2014; VA/DoD, 2016) and, although mortality rate is low, long-term disability is common (Curtis & Epstein, 2014; WHO, 2006). Curtis and Epstein (2014) state that as many as 20% of patients with a mild TBI will go on to experience chronic symptoms related to postconcussive syndrome (Curtis & Epstein, 2014), other estimates are as high as 30% (Vakhtin et al., 2013).

Disability levels vary among veterans who have suffered one or more TBI and rehabilitation outcome predictions are difficult. Severity of symptoms has been directly associated with the degree of disability, poorer degree of functionality, lower reintegration in the community and employability. Cognitive deficiencies contribute to learning difficulties that decrease their ability to learn new skills or earn a new degree (Twamley et al., 2014).

An estimated 7% of Iraq and Afghanistan combat veterans receiving health care at VA facilities continue to experience post-TBI symptoms related to cognitive deficits, sleep difficulties, chronic fatigue, emotional problems and post-concussive headaches (Twamley et al., 2014). However, it is unclear how the high frequency of neuropsychiatric comorbidities may contribute to cognitive impairments (Troyanskaya et al., 2015; Twamley et al., 2014).

A small sample, not-blinded pilot study, found that twelve-weeks of cognitive rehabilitation that included restorative and compensatory approaches could improve post-traumatic cognitive deficits and prospective memory in combat veterans with history of TBI,

even with PTSD as comorbidity (Twamley et al., 2014). Other studies have found a direct correlation between differences on the timing and quality of clinical care and rehabilitation programs with the overall outcomes (Maas et al., 2012; Stein, 2015). It is sensible to conclude that further examination of rehabilitation strategies may offer potential gains in service members' rehabilitation outcomes.

Classification disparities among facilities and practitioners (Gardner & Yaffe, 2015) and widespread treatment differences could explain some of the differences in outcomes (Maas et al., 2012). Additional challenges concerning therapeutic effectiveness are embedded in an unpredictable collection of physiological and environmental conditions. Genetic susceptibility, preinjury psychosocial wellbeing as well as post-injury factors influencing psychosocial health, pre-existing illness, the mechanism of trauma, as well as the quality and time of clinical care can arbitrarily and collectively affect therapeutic outcomes. The need to expand understanding of the multidimensionality of phenotypical expressions among comparable injuries is of utmost significance (Maas et al., 2012; VA/DoD, 2016). Thus far, thirty-two transcription factors have been related to genetic up-regulation following ischemic injury and are suspected to be involved in inflammatory responses in rat brain cells (Ridder et al., 2009).

TBI is known to present with acute and chronic alterations of neuronal functions (Daneshvar et al., 2015; Vakhtin et al., 2013). Multiple long-term complications that patients with a history of TBI may face include reduction of life expectancy (Chauhan, 2014) and an increased risk for developing neurodegenerative diseases later in life (Chauhan, 2014; Daneshvar et al., 2015; Gardner & Yaffe, 2015).

The body of evidence suggests that an isolated TBI may result in gray and white matter degeneration and can trigger or speed up age-associated neuronal deterioration. It also increases the likelihood of developing progressive neurodegenerative diseases such as Alzheimer's and Parkinson's. Postmortem brain examinations of combat veterans with a positive history of blast-related mTBI due to IEDs, showed evidence of tau neurofibrillary accumulation, chronic traumatic encephalopathy (CTE), axonal and microvascular tissue damage, signs of proliferation of astrocytes and microglial activation nearby lesions. The autopsies revealed initial signs of CTE in four out of five of the deceased combat veterans that overlapped with a diagnosis of post-traumatic stress disorder (PTSD) in life, implying a possible connection between the pathology of both conditions (Daneshvar et al., 2015).

A greater understanding of the immediate and lifelong consequences that mTBI has on functioning still remains a priority for research. Moving forward, it is important for researchers and clinicians to consider preventive, treatment and restorative strategies that can minimize the disease burden among past, current and future military service members. Unemployment and cognitive difficulties often co-exist. Reintegration programs for combat veterans dealing with persistent neurological deficits of mTBIs and their families could provide resources and cognitive training to support their transitions and improve their successful reintegration to the community.

CHAPTER 4: VITAMIN D BACKGROUND

Discovered in the early 20th century, vitamin D's clinical relevance did not become apparent until the connection of its extreme deficiency was identified as the leading cause of rickets. First described in the mid-1600s, the clinical signs of rickets are visibly detectable as bone deformities, growth delays and weakening of the muscles which were eventually seen as being due to extreme hypovitaminosis D (Holick, 2006). Early experiments provided the data needed to understand how rickets could be prevented by increasing sun exposure and vitamin D dietary intake. In the early 20th century scientists were finally able to appreciate the improvements on bone mineralization from this intervention, especially on the epiphyses of long bones, by examining children's x-rays (Holick, 2006; Eyles, Burne & McGrath, 2013).

The high prevalence of rickets, led the U.S. government to adopt the fortification of milk with the fat-soluble nutrient as part of public health policy program for the prevention of rickets. In 1928, the discovery of vitamin D's chemical structure by Adolf Windausin (Eyles, Burne & McGrath, 2013), facilitated new improvements for food fortification techniques (Holick, 2006). The success of the policy became evident by the marked reduction of rickets cases in the mid-1900s, almost eradicating the disease (Holick, 2006; Looker, Dawson-Hughes, Calvo, Gunter, & Sahyoun, 2002; Rovner & O'Brien, 2008).

Vitamin D Photosynthesis

Photosynthesis.

Vitamin D is a fat soluble molecule that undergoes a complex process from initial synthesis to activation before it can exert hormonal effects. Throughout a series of biochemical

reactions, vitamin D exists in several different forms and goes by many different names depending on the molecular structure. During the early stages of food fortification, the two different forms of vitamin D were identified and classified as D_2 or egocalciferol, which is found in plants, and D_3 or cholecalciferol, which is produced by animals. Fortification of food and oral supplements use either form (Eyles, Burne, & McGrath, 2013; Holick, 2007; Holick et al., 2011).

In animals, the metabolic process of vitamin D_3 begins when 7-dehydrocholesterol, commonly known as "cholesterol," an inert molecule on the epidermis layer of skin, is exposed to the solar ultraviolet-B (UVB) radiation giving rise to cholecalciferol, the inactive form of vitamin D_3. The inactive molecule of vitamin D_3 is transported in the circulatory system by transport proteins to the liver where several P450 enzymes attach the first hydroxyl group in a process known as hydroxylation. The newly formed 25-hydroxyvitamin D_3 ($25OHD_3$) or calcidiol has a lifespan between two to three weeks, thus making it the ideal biological marker to screen and monitor levels (Annweiler et al., 2014; Holick, 2007; Holick et al., 2011; Jamall et al., 2016; Wang, Ji, Tong & Zhang, 2014; Wentz et al., 2014). $25OHD_3$ undergoes a second hydroxylation in the kidneys from which the activated 1,25-dihydroxyvitamin D_3 or $1,25OH_2D_3$ emerges ready to function as a hormone (Eyles, Burne, & McGrath, 2013; Holick, 2006; Holick, 2007).

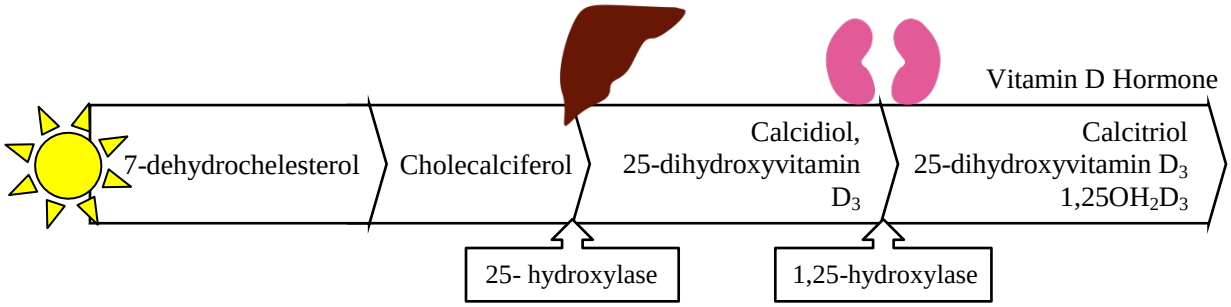


Figure 9: Photosynthesis steps of vitamin D.

Adapted from "Vitamin D deficiency," by M. F. Holick, 2007,

In *The New England Journal of Medicine, 357*(3), p. 269.

Vitamin D synthesized by sun exposure has been found to last twice as long in circulation when compared to the orally ingested form. The skin is an extremely efficient source of vitamin D. An estimated 1,000–1,500 hours out in the sun during the spring, summer and fall should provide enough vitamin D for the majority of humans to cover for the winter months. One dose of UVB irradiation, defined as the exposure needed by an adult dressed with a swimsuit to have superficial pink tone 24 hours later, can generate the equivalent of 10,000-25,000 IU of oral intake (Holick et al., 2011). Rovner & O'Brien (2008) stated that adequate synthesis of fair skin individuals would require UVB radiation exposure for about 5 to 15 minutes between the hours of 10 am and 3 pm during the abovementioned seasons. However, individuals with darker skin may need five to ten times longer UVB exposure length for an equivalent production (Rovner & O'Brien, 2008).

Factors influencing photosynthesis.

Vitamin D is a unique nutrient; although humans can ingest it from food sources, including oral supplements, human skin is perfectly capable of synthesizing this nutrient to adequate levels with appropriate exposure to sunlight. However, scientists have identified factors that influence the body's ability to produce vitamin D. Non-modifiable factors known to reduce a

person's ability to photosynthesize vitamin D include dark skin tone, age (decline of 7-dehydrocholesterol), weather (cloud coverage and smog), cold seasons (shorter duration of sun light) and latitude (higher than ~40°). On the other hand, adjustments to certain behaviors (length of time spent outside, frequency and duration of exposure to UVB), type of clothing (degree of skin coverage), dietary choices and supplementation that could potentially counteract the impact of non-modifiable elements (Groves, McGrath & Burne, 2014; Harms, Burne, Eyles, & McGrath, 2011; Holick, 2006; Holick, 2007; Looker et al., 2002; Rovner & O'Brien, 2008).

Individuals with dark skin tone require longer UVB exposure for adequate metabolism of vitamin D_3 due to higher concentration of melanin on their skin (Harms et al., 2011; Holick, 2007; Jamall et al., 2016). A decline in 7-dehydrocholesterol content in the skin is an expected change observed with aging, thus the decrease of the molecular precursor for cholecalciferol results in decreased synthesis (Holick, 2007). It has been reported that decreased intensity of UVB radiation during the winter months accounts for the seasonal differences of vitamin D photosynthesis, being especially significant in latitudes greater than ~40° (Holick, 2006; Holick, 2007; Looker et al., 2002). The photochemical smog of urban areas keeps sunlight from reaching past it and might explain the higher occurrence of hypovitaminosis D when compared to rural populations (Holick, 2007). Clothing and the application of sunscreen are known to decrease skin exposure to UVB radiation thus reducing production of vitamin D (Eyles, Burne & McGrath, 2013; Holick, 2006; Holick, 2007; Jamall et al., 2016).

Studies have found that certain medical conditions and medications increase the risk of hypovitaminosis D. Sequestration of vitamin D in excess fatty tissue reduces the vitamin D reaching circulation in obese individuals. In cases of liver and kidney insufficiency and failure,

organ damage results in metabolic disruption of active vitamin D. Skin grafts or extensive burn scarring deprive skin from its synthesis abilities (Holick, 2007). Medications for the treatment of epilepsy and HIV/AIDS promote destruction of $25OHD_3$ and $1,25OH_2D_3$ (Holick, 2007; Holick et al., 2011).

Testing for hypovitaminosis D.

The Institute of Medicine (IOM) and The Endocrine Society (TES) concluded that the evidence did not support regular screening of vitamin D levels except for individuals that are considered to be at risk for deficiency (IOM, 2011b; Holick et al., 2011). It is important during health assessments, to performed a thorough history intake that include questions on past medical diagnosis, current medications, family history and certain behaviors, as the answers could guide clinical judgement.

The Endocrine Society (2011) indicated screening when confirmed pathology of liver, kidney or gastric systems is present and with metabolic and endocrine conditions known to alter the metabolism of vitamin D (Holick et al., 2011). Clinicians should screen for deficiencies those (1) with a body mass index above 30, (2) taking antiepileptic, antiretroviral, glucocorticoids, and cholestyramine medications, (3) diagnosed with a malabsorption condition or (4) food allergies, (5) renal or hepatic insufficiencies, (6) advanced age, or (7) primary hyperparathyroidism (Holick, 2007; Holick et al., 2011). Screening is also important in persons with altered skin functions, as seen with extensive scaring (Holick, 2007; Holick et al., 2011).

Vitamin D Deficiency versus Insufficiency

At the time of this review, there is still no agreement concerning the assay criteria for deficiency and insufficiency. According to Looker and colleagues (2002), deficiency occurs when the levels of circulating $25OHD_3$ present with positive clinical symptoms of bone demineralization, secondary hyperparathyroidism, or other signs of poor bone health. Insufficiency, sometimes called subclinical deficiency occurs when levels are above deficiency but low enough to contribute to bone disease (Looker et al., 2002) and other pathologies (Holick, 2007; Holick et al., 2011).

The medical community has struggled to reach the consensus necessary to identify clinical guidelines for assessing, monitoring, and correcting vitamin D deficiencies (Eyles, Burne & McGrath, 2013; Looker et al., 2002; Rovner & O'Brien, 2008; Wentz et al., 2014). The IOM and TES do not concur on their clinical reference values or recommendations. In 2011, these two major organizations published their own set of clinical guidelines for the screening, classification and optimization practices (refer to table 2 for reference values) (Holick et al., 2011; IOM, 2011b).

Table 2: 2011 Vitamin D reference values from the IOM and TES.

	IOM, 2011b		Holick et al., 2011	
Sufficiency	**>20ng/mL**	(>50nmol/L)	**30 - 100 ng/mL**	(75 - 250nmol/L)
Insufficiency			**21 - 29 ng/mL**	(52 - 72nmol/L)
Deficiency	**<20ng/mL**	(<50nmol/L)	**<20 ng/mL**	(<50nmol/L)

The significant relationship between vitamin D and the musculoskeletal system has been established and accepted within the scientific community, and the mechanisms involved have been extensively studied. The IOM indicated that their committee of experts appraised the newer

scientific evidence available since publishing their previous clinical practice guidelines in 1997 and did not find the data to be supportive of any health benefits beyond bone health. Thus, the supplementation recommendation were designed to support optimal bone health based on the minimum $25OHD_3$ levels necessary to support the musculoskeletal system. The institute's 2011 update significantly increased the threshold for deficiency from 10ng/mL or 25nmol/L in 1997, to 20ng/mL or 50nmol/L. It is important to underline in both occasions the guidelines were based solely on bone homeostasis (IOM, 2011b).

The IOM set the adult vitamin D daily requirements to 400 IU (international units), with a dietary allowance of 600 IU and a total upper limit of 4,000 IU. Referencing the data from the NHANESIII survey, which showed daily dietary intake below standard requirements, the committee deduced that adequate solar exposure may explain why average levels were higher than 20ng/mL, which satisfies the cutoff proposed by the IOM. The committee raised concerns about possible public misperceptions regarding vitamin D, driving a trend for increased screenings and warns that inconsistencies on the cut-offs used between laboratories, which may mislabel a person as deficient (IOM, 2011b).

In summary, the IOM's dietary recommendations were established to keep a concentration of at least 20ng/mL. The committee counsels against surpassing the daily upper limit of 4,000 IU and warns that higher intake could come with detrimental risks to health and that the likelihood of excessive intake is heightened by the availability of fortified foods and an increased intake of supplements frequent in North America. The committee concludes that additional research is needed in order to expand the health benefits of vitamin D (IOM, 2011b).

In contrast with the IOM recommendations, after review of the available literature, The Endocrine Society (TES) set forth guidelines which suggest vitamin D levels should be higher in order to maintain health. The TES taskforce established the threshold for deficiency at 20ng/mL or 50nmol/L, aligning with the IOM parameter. However, they also defined the range between 21-29 ng/mL as insufficiency, and determined the sufficiency range to be at of 30-100 ng/mL. The insufficiency range was set just below the demonstrated ideal threshold in which parathyroid hormone levels and adequate intestinal calcium absorption are believed to be optimally balanced. When compared with the IOM report, the TES asserts that vitamin D deficiency is common in all age groups and that few foods contain vitamin D, and recommends supplementation based on age group and clinical presentation (Holick et al., 2011).

The TES taskforce indicated that optimization of vitamin D deficiency in adults can be achieved by administering either D_2 (egocalciferol) or D_3 (cholecalciferol). They suggest that healthy adults age 19 to 70 need at least 600 IU daily in order to maximize bone health and muscle function, but clarified that 600 IU may not be enough to increase vitamin D levels above 30ng/mL consistently or to support other non-skeletal benefits. The daily requirement was set between 1,500 and 2,000 IU for all adults in order to maintain $25OHD_3$ blood levels greater than 30ng/mL. In deficient adults older than 19 years of age initial supplementation involves 50,000 IU/week or 6,000 IU/day for two months to correct the deficiency, followed by the maintenance dose of 1,500 to 2,000 IU a day (Holick et al., 2011).

The TES guidelines set a tolerable upper limit (UL) at 4000 IU/day for children between 1 and 8 years of age and 10,000 IU/day for anyone older than 9 years old, except for pregnant or lactating teens, for which the 4,000 IU/day continues to apply. The committee decision to set the

UL to 10,000 IU/day was based on a study in which men were supplemented with this dose for 5 months without reports of increase blood calcium concentrations or renal calcium excretion (Holick et al., 2011).

Although the IOM committee warned that levels above 50ng/mL may be associated with higher mortality, the TES did not support this claim and stated the evidence does not support any disadvantages of increased intake, except for cases of lymphoma or chronic granuloma-forming disorders (such as tuberculosis and sarcoidosis). The TES stated that an upper threshold to prevent vitamin D toxicity has not been identified and the IOM UL recommendations were based on observations from the 1940s. TES referenced studies in adults and children that showed negative health concerns with $25OHD_3$ blood levels of 150ng/mL or above. For this reason they believed an UL of 100ng/mL offers a safe parameter to prevent the risk of developing hypercalcemia or hypercalciuria (increased calcium renal excretion) (Holick et al., 2011).

The TES does agree with the IOM in the desirability for better and more reliable assay to measure vitamin D serum levels. Their report concludes and suggests that additional, high-quality studies on vitamin D replacement and non-skeletal benefits are warranted in order to solidify the evidence and develop stronger recommendations (Holick et al., 2011).

Prevalence of Low Vitamin D

Cases of extreme, chronic hypovitaminosis D (the type that leads to rickets) are rare in the United States (Holick, 2006; Looker et al., 2002; Rovner & O'Brien, 2008). However, the findings published by Looker and colleagues in 2002, confirmed significant vitamin D deficiencies among noninstitutionalized adolescents and adults (Looker et al., 2002).

Looker and colleagues analyzed the data collected as part of the Third National Health and Nutrition Examination Survey (NHANES III 1988-1994), conducted by the U.S. National Center for Health Statistics with the purpose of focusing on the status of $25OHD_3$ levels in the population. The analysis accounted for differences in assessment methods by setting separate cutoffs for radioimmunoassay (RAI) and competitive protein binding (CPB) test, since CBP results tend to be 30% higher than RAI (Looker et al., 2002).

The cut-off point for vitamin D deficiency was set as a $25OHD_3$ blood level of <17.5 nmol/L (7 ng/mL) for the RAI, equating to <25 nmol/L (10ng/mL) when using the CPB, which followed the IOM guidelines from 1997. Based on the cutoff used, less than 1% of the participants tested were considered vitamin D deficient. Since there was no agreement for insufficiency cutoff at the time of their analysis, the authors used four different ranges for the calculations. The RAI values for insufficiency were set at (1) <25 nmol/L, (2) <37.5 nmol/L, (3) <50 nmol/L, and (4) 62.5 nmol/L with corresponding CPB values between <37.5 nmol/L and 87.5 nmol/L (Looker et al., 2002).

The NHANES III sample included 18,875 participants, 12-years or older, that were classified under three different ethnic/racial groups. Seasonal and latitudinal innate variances of vitamin D synthesis were identified as confounding factors and corrected early on in the analysis. In their analysis, the researchers concluded that regardless of seasonal and latitudinal differences, insufficient levels of $25OHD_3$, defined as <37.5-62.5 nmol/L, were prevalent across all ages, gender, race and ethnicity. The calculations found that for all surveyed participants of both genders the percentage of occurrence for $25OHD_3$ of <37.5nmol/L (15ng/mL) was 2-19%; for

the same sample the results showed 8-40% when using <50nmol/L (20ng/mL); and, when using <62.5nmol/L (25ng/mL), the prevalence was 21-58%.

The results from the NHANES III led the Center for Disease Control and Prevention to recognize that regardless of the food fortification efforts, varied degrees of subclinical deficiencies are prevalent among all ages, genders, and latitudes of the country (Looker et al., 2002). When using the 2011 recommendations for deficiency and insufficiency, up to 40% of the population sampled would fall below <50nmol/L or <20ng/mL to be considered deficient (IOM, 2011b), and up to 58% would meet insufficiency criteria (Holick et al., 2011).

Physiology of Vitamin D

Once the epidemic of rickets was under control, the interest surrounding vitamin D died down. Eventually it was discovered that biological interactions of vitamin D with body systems extend far beyond musculoskeletal functions. In the 1970s and 1980s, new discoveries prompted the reclassification of calcitriol ($1,25OH_2D_3$) as a hormone, launching a new area for scientific research (Eyles, Burne & McGrath, 2013; Harms et al., 2011; Holick, 2006; Kesby, Eyles, Burne, & McGrath, 2011; Rovner & O'Brien, 2008).

The abundance of the enzymes that activate and deactivate vitamin D and its receptors in the brain suggest that vitamin D is a neurosteroid that regulates brain bioprocesses. The expression of vitamin D receptors (VDRs) in the brain is capable of bioactivating $25OHD_3$ to $1,25OH_2D_3$ (Groves, McGrath & Burne, 2014; Harms et al., 2011). Expression and activation of the vitamin D receptors (VDR) during physiological activities can either exert genetic transcriptional modifications or simply act as a membrane-associated rapid response steroid by

influencing signaling mechanisms (MARRS) (Eyles, Burne & McGrath, 2013; Gezen-Ak, Dursun & Yilmazer, 2013; Kajta et al., 2009; Ridder et al., 2009). The ability to follow genomic or non-genomic paths is a commonality shared by other hormones categorized under the same family (Eyles, Burne & McGrath, 2013). Post-mortem brain examinations of stroke patients showed increased concentration of VDR in the region with infarction when compared to other unaffected areas or subjects without a stroke (Ridder et al., 2009).

The actions of vitamin D on the biological processes related to bone health have been studied extensively (Holick et al., 2011). When discussing dynamic biological processes, as with bone turnover, it is important to note that multiple body systems, organs, cells types and hormones collaborate simultaneously from different locations towards the completion of a common biological objective. A concise version of just the demineralization portion follows as a way to illustrate the parallel intricacies of biological processes.

The renal hydroxylation of $25OHD_3$ to produce $1,25OH_2D_3$ or calcitriol, is tightly controlled by the dynamics between serum concentrations of parathyroid hormone (PTH), calcium and phosphorous. Generally, when the hypothalamic feedback mechanism signals low calcium levels in the blood, it activates compensatory mechanisms which increase PTH secretion from the parathyroid glands. PTH works by translocating calcium ions from storage by dissolving bone tissue. In addition, when abnormal levels of serum calcium and phosphorous concentrations are detected, $1,25OH_2D_3$ interaction with VDRs in bone cells aid increase bone dissolution and promote calcium reabsorption in the kidneys (Holick, 2006; Holick, 2007; Holick et al., 2011).

Meanwhile, expression of VDR in the small intestines improves dietary calcium absorption from roughly 10-15% without $1,25OH_2D_3$, to about 30-40% and dietary phosphorous improves from 60% to 80%, in an attempt to balance their concentrations. Consequently, as phosphorous concentrations rise in the bloodstream, bone tissue secretes molecules to signal the kidneys to stop reabsorbing and start excreting excess phosphorous from the blood, and to reduce absorption of phosphorous from dietary sources by decreasing renal activation of $25OHD_3$ to $1,25OH_2D_3$. Thus, low vitamin D levels dampen the body's ability to absorb dietary calcium and phosphorous negatively impacting bone health and decreasing availability of these minerals (Holick, 2006; Holick, 2007; Holick et al., 2011).

In children, prolonged renal excretion of phosphorus due to secondary hyperparathyroidism causes bone deformities and it is known as rickets. Adults have fully developed bones, thus the mineralization defects from the inadequate ratio of calcium and phosphorus present as softening of the bones, a condition known as osteomalacia. Both conditions share symptomatic similarities such as widespread bone and muscle pain, fatigue and muscle weakness. Weakened muscles may contribute to difficulty standing, problems with gait, recurrent falls and increase of stress fractures. In adults, the direct, long term consequence of the bone demineralization is lower bone density, causing osteopenia and osteoporosis. Correction of serum vitamin D levels, improve calcium balance and can prevent or correct secondary hyperparathyroidism (Holick, 2006; Holick, 2007; Holick et al., 2011; Looker et al., 2002).

Since the recognition of vitamin D's hormonal properties, converging evidence from epidemiological, experimental and clinical studies has connected low vitamin D to negative health outcomes distinct from skeletal outcomes (Braun et al., 2011; Cui et al., 2015; Daubail et

al., 2013; McKinney et al., 2011; Moromizato et al., 2013; Turetsky et al., 2015; Wang et al., 2014) and opened the possibility of supplementation as preventive intervention or treatment (Amrein et al., 2014; Gominak & Stumpf, 2012). Retrospective and prospective observations support that low levels of vitamin D yielded worse outcomes on cerebrovascular attacks (Daubail et al., 2013; Turetsky et al., 2015; Wang et al., 2014), critically ill patients (Braun et al., 2011; McKinney et al., 2011; Moromizato et al., 2013), lower cognitive scores, higher score for depression inventory (Jamall et al., 2016), chronic fatigue in post-TBI (Schnieders, Willemsen, & de Boer, 2012), and increased risk for many neuropsychiatric or neurodegenerative conditions (Eyles, Burne & McGrath, 2013; Groves, McGrath & Burne, 2014; Harms et al., 2011; Holick, 2007; Kesby et al., 2011; Stein, 2015).

In a systematic review and meta-analysis concerning the association of vitamin D deficiency and brain volumetric variations, Annweiler and colleagues (2014), concluded that subjects with low vitamin D had lesser brain volume and larger ventricles when compared to control subjects. Taking into account that vitamin D is a relatively new research interest, only a few studies met inclusion criteria for the review, thus the connection of vitamin D to brain atrophy remains to be confirmed (Annweiler et al., 2014). Similar volumetric findings from animal studies have also been reported (Eyles, Burne & McGrath, 2013; Groves, McGrath & Burne, 2014; Kesby et al., 2011).

Scientists from the University of Queensland, Australia, have done extensive work exploring the neuronal implications of vitamin D in the brain, including distribution mapping of VDR and the enzymes for activation/deactivation (1,25-hydroxylase and 24- hydroxylase) in the human brain and the extraordinary similarities in the rat brains. Their collaborative work has

created a large databank supporting the neuroprotective qualities of vitamin D (Eyles, Burne & McGrath, 2013; Groves, McGrath & Burne, 2014; Harms et al., 2011; Kesby et al., 2011). Their experiments in rats has provided compelling evidence implicating low vitamin D level during gestational and early developmental age to structural alterations of the brain, genetic modifications linked to neuronal potentiation, increased cell proliferation, decreased apoptosis and cell differentiation, and deviations of dopamine (DA) signaling and breakdown (Eyles, Burne & McGrath, 2013; Groves, McGrath & Burne, 2014; Kesby et al., 2011). Epidemiological studies by the same group, demonstrated a significant risk for schizophrenia was inversely related to $25OHD_3$ levels, with levels $\leq$16ng/mL displaying a twofold increased (Eyles, Burne & McGrath, 2013).

Studies suggest an association between vitamin D and the incidence of neuropsychiatric conditions including depressive disorders, schizophrenia, Alzheimer disease, Parkinson's disease, cognitive decline, dementia and autism, among others. For some of these findings reverse-causation has not yet been discarded (Cui et al., 2015; Groves, McGrath & Burne, 2014; Kesby et al., 2011). Groves, McGrath and Burne (2014) organized this information (see figure 10 below) to illustrate the type of studies and the findings categorized by disease (Groves, McGrath & Burne, 2014).

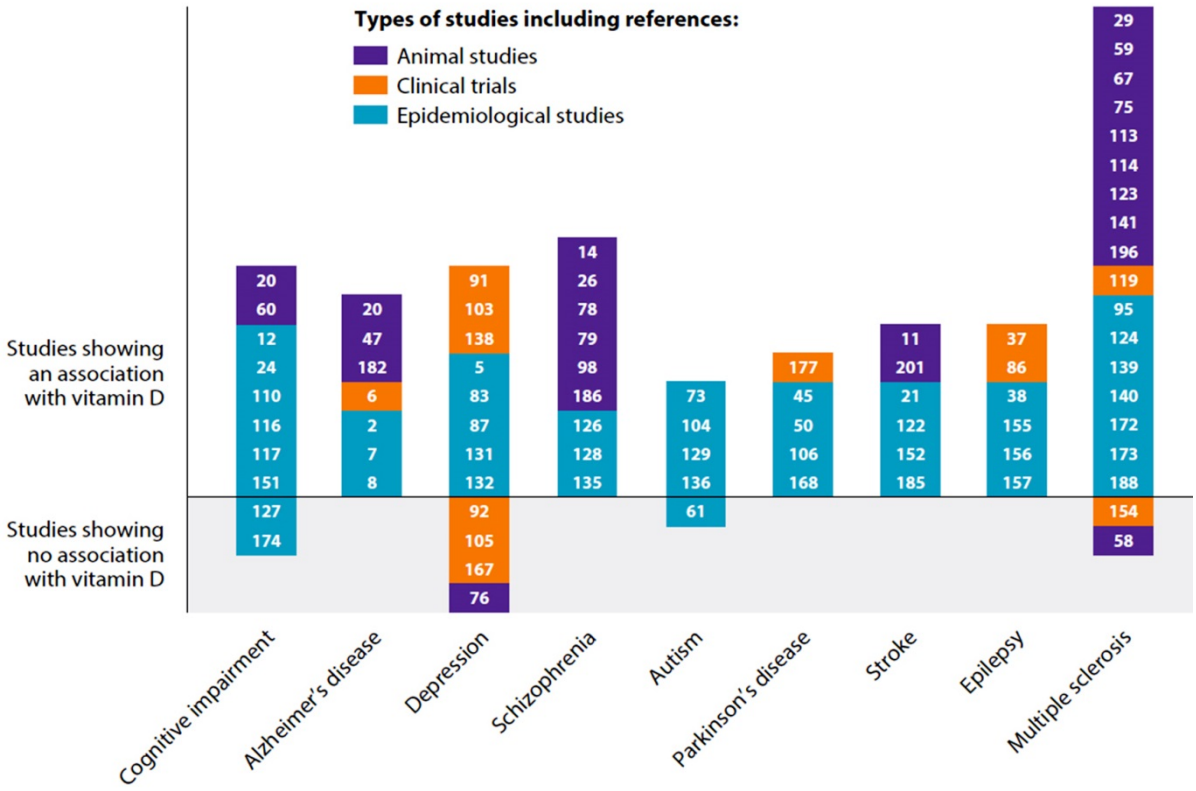


Figure 10: Strength of evidence based on animal studies, clinical trials, and epidemiological studies that show evidence of an association between vitamin D and various disorders.

Numbers in boxes correspond to reference numbers (Groves, McGrath & Burne, 2014, p. 128).

Based on the evidence from prospective cohort studies, there is reason to believe that vitamin D deficiency and insufficiency may contribute to cognitive decline in adults. Other prospective studies have found similar evidence, in addition to linking marked vitamin D deficiency to a significantly increase of the risk for Alzheimer disease and vascular dementia in the 30 year follow up. However, failure to collect baseline cognitive data or to use objective tools to test cognitive abilities limits the significance the findings (Toffanello et al., 2014).

Recent retrospective and observational studies found that prehospitalization low vitamin D level is a solid predictor of mortality for ICU patients during hospitalization (Braun et al.,

2011; McKinney et al., 2011; Moromizato et al., 2013), and at 30, 90 and 365 days from admission mortality remained high for $25OHD_3$ levels $\leq$29ng/mL prior to hospitalization (Braun et al., 2011). Another study revealed a strong connection between low $25OHD_3$ among critically ill and an elevated likelihood of developing acute kidney injury (Moromizato et al., 2013).

A randomized double-blind trial of 492 ICU patients with $25OHD_3 \leq 20$ng/mL were given either high doses of vitamin D or placebo for a period of five months. No statistical significance was found in the length of hospitalization or time in the ICU. However, the comparison of the fatality rates during hospital stay and at six months were lower for the group receiving high-dose calcitriol supplementation (28.6% and 34.7% respectively) versus the placebo group (46.1% and 50% respectively). After adjusting for covariate only the mortality rate during hospital stay remained significant (Amrein et al., 2014). The results from this clinical trial did not observe significant effect on the outcomes for patients with deficiency or insufficiency prior to admission in the ICU, or even after levels were improved by aggressive supplementation (Amrein et al., 2014). However, this peculiarity may be explained by results from laboratory experiments and clinical trials that suggested levels prior to cell trauma or stress offer the best neuroprotection, also the duration/timing of $25OHD_3$ exposure may have big implications in responses (Kajta et al., 2009; Obradovic et al., 2009), and lastly, the effectiveness of vitamin D may by directly related to a specific range of concentration (Atif et al., 2013; Gominak & Stumpf, 2012; Hur et al., 2014; Kajta et al., 2009).

Low levels of $25OHD_3$ in patients suffering from headaches who were also diagnosed with a sleep disorder and the discovery of high presence of VDR in regions of the hypothalamus and brainstem known to regulate sleep, led researchers to hypothesize that vitamin D could

possibly influence sleep patterns. A two year long, uncontrolled clinical trial of 1,500 patients with complaints of headache, poor sleep and fatigue found a marked sleep quality improvement following vitamin D supplementation regardless to the sleep disorder diagnosis. However, decreased fatigue and sleep improvements were best achieved when $25OHD_3$ concentration fluctuated between 60-80 ng/L (Gominak & Stumpf, 2012).

Studies have found that fatigue and sleep disorders are prevalent following a TBI (Chauhan, 2014; Jamall et al., 2016; VA/DoD, 2016). A recent cohort study of TBI patients with complaints of chronic fatigue, found that vitamin D levels <20ng/mL was the number one factor shared by 65% of the cohort, when compared to poor sleep (54%), anxiety (36%), inadequate levels of growth hormone (16%) and gonads hormonal deficiencies (9%). It is worth noting that 26.6% of the remaining was considered to have insufficiency with $25OHD_3$ levels <30ng/mL, thus nearly 92% was bellow 30ng/mL. The incidence and severity of chronic fatigue, sleep problems, attention and cognitive deficits after TBI are strongly and intricately related (Schnieders, Willemsen, & de Boer, 2012) and improvements in post-TBI sleep disturbances showed significant decrease of other neuropsychological impairments related to TBI (Chauhan, 2014).

It is worth noticing the work of researchers from Emory University, in Atlanta, Georgia, since they have made crucial contributions to the areas of stroke and TBI research over the last two decades. They focused on targeting multiple processes of acute head trauma by combining two neuroprotective agents with the idea that the combination would exert synergistic benefits. This concept was accepted by a panel of the National Health Institute with the idea of boosting favorable outcomes and functionality following brain injury. The preclinical results with

$1,25OH_2D_3$ administrated concomitantly with progesterone yielded significant improvements in TBI and stroke models (Atif et al., 2013). Unfortunately, this hormonal combination failed to translate with the same success as the clinical trials advanced (Stein, 2015). The Emory University laboratories have demonstrated that vitamin D attenuates the inflammatory response, protects neuronal and glia cells against apoptosis, exerts antioxidant protection, protects against excitotoxicity from glutamate, may impart a positive impact in certain functionality outcomes after ischemic damage (Atif et al., 2013), strengthen the integrity of the BBB and reduces damage by oxidative stress (Won et al., 2015).

Vitamin D neuroprotection.

Vitamin D deficiency has been implicated in defective actions of macrophages involving the secretion of pro-inflammatory cytokines and phagocytic performance. The connecting element between the innate immunity and stimulation of toll-like receptors is vitamin D. Moromizato and colleagues (2013), hypothesized that the elevated likelihood for sepsis or septic shock among critically ill patients is due to these the toll-like pathways interacting with vitamin D. VDR expression is induced by peripheral and neuronal immune cells activation. After multivariable adjustment and random selection validation sub-analysis, vitamin D levels $\leq$15ng/mL prior to admission were associated to a 2.5-fold increment on the risk for sepsis or septic shock (Moromizato et al., 2013).

Stressed brain cells increase VDR expression and $1,25OH_2D_3$ activation in cultured animal cells (Fu et al., 2013; Obradovic et al., 2009) and in the brain of deceased patients with a stroke. Similar findings concerning brain cells under ischemic stress were described by Ridder and colleagues (2009), showing VDRs translocation into the nucleus from vitamin D hormone

stimulation followed the ischemic event (Ridder et al., 2009). Vitamin D significantly decreased cell death from glutamate excitotoxicity (Kajta et al., 2009), from absence of oxygen-glucose (ischemia/reperfusion model) (Atif et al., 2013), from oxidative stress (Hur et al., 2014) and due to tumor necrosis factor apoptosis-inducing ligand (TRAIL) (Obradovic et al., 2009).

Preclinical experiments have shown that vitamin D neuroprotective gains may require preloading or pretreatment preceding exposure to harmful incident (Kajta et al., 2009; Obradovic et al., 2009), and may be dose- and time-dependent. For example neurons from different regions of the brain require higher or lower doses than others for the same benefit or in cases of delayed treatment, higher concentrations may be required to reach therapeutic benefits (Kajta et al., 2009).

Recent experiments confirm that activated microglia intensified the expression of 1,25-hydraxolase and VDR preceding depression of nitric oxide (NO) synthesis and a 90% improvement on cell viability, implying suppression of activated microglia by the active form of vitamin D (Hur et al., 2014). Activated microglia promotes neuroinflammation by stimulating mitogen-activated protein kinase (MAPK) pathways. However, vitamin D is capable of negative regulating the phosphorylation of p38 (Huang et al., 2015; Hur et al., 2014; Obradovic et al., 2009), extracellular signal-regulated kinase (ERK) (Fu et al., 2013; Huang et al., 2015) and Jun-N-terminal (JNK) (Huang et al., 2015) MAPK pathways. Increase in NR3 subunit by vitamin D, dampens phosphorylation of ERK pathway by mediation of a nuclear binding transcription factor (cAMP/Ca2+ response element binding protein or CREB) (Fu et al., 2013). Vitamin D's obstruction of p38 and ERK expression significantly hinders inducible nitric oxide synthase

(iNOS), reduces intracellular production of ROS (Huang et al., 2015) and buildup of NO (Huang et al., 2015; Hur et al., 2014).

$1,25OH_2D_3$ also prompt a marked decline in synthesis and signaling involving pro-inflammatory mediators such as interleukin-6 (IL-6) (Atif et al., 2013; Huang et al., 2015), macrophage-inflammatory protein-2 (MIP-2) (Huang et al., 2015), and nuclear factor kappa-B (NFκB) concentrations (Atif et al., 2013; Won et al., 2015). The significant declines of IL-6 and MIP-2 at protein and mRNA sites were largely mediated by the inhibitory effects on p38, ERK and JNK (Huang et al., 2015).

Hypoxic and mitochondrial injury stimulates activation of NFκB pathway. The activated NFκB translocates into the nucleus, where p65 subunits acts as a transcription factors by binding to a gene that upregulates MMP-9 expression (Won et al., 2015) and IL-6 (Fernández-Gajardo et al., 2014) promoting neuronal inflammation (Atif et al., 2013). In vitro experiments showed a marked decline in concentrations and expression of NFκB after vitamin D was added to brain cultured cells. Thus blocking phosphorylation of NFκB reduces inflammation and disruption of the BBB (Atif et al., 2013; Won et al., 2015).

Atif and colleagues (2013) from Emory University, found outstanding efficacy of vitamin D in elevating heme oxygenase-1 expression to attenuate oxidative damage after ischemia, as demonstrated by a twofold increment (Atif et al., 2013). Mitigation of free radical damage increasingly causing lipid peroxidation and degradation of DNA is ameliorated by vitamin D (Charier et al., 2015; Won et al., 2015). Vitamin D cause only a minor reduction of malondialdehyde (a biomarker present following membrane oxidative degradation) concentrations, however it produced a substantial boost in superoxide dismutase. Superoxide

dismutase is the potent antioxidant enzyme behind the protective catalysis of superoxide (Ekici, Ozyurt & Erdogan, 2009).

A combined in vitro/in vivo study showed that calcitriol, when administered within 30 minutes following a hypoxic-ischemic event, offered significant reduction of brain injury. The in vitro portion of the study showed that glutamate excitotoxicity damage is significantly weakened by administration of calcitriol to cultured cortical neuron and glia cells. Pretreatment and higher doses of calcitriol yielded superior protection. Interestingly, these positive benefits were not observed in cerebellar cells (Kajta et al., 2009). Vitamin D can ameliorate cell damage from glutamate toxicity by increasing NR3 expression, subsequently antagonizing proinflammatory extracellular signal-regulated kinase (ERK) MAPK pathway thought the activation of a nuclear transcription factor CREB. CREB expression has an anti-apoptotic effect, stimulates neurogenesis and may be helpful in protecting against glutamate excitotoxicity (Fu et al., 2013).

Extracellular glutamate in excess results in hyper-stimulation of low voltage calcium channel and generates calcium influx rising to toxic levels (Fu et al., 2013). Positive modulation of low voltage calcium channel subunit A1C (LVCC-A1C) has been observed in cultured neurons after suppression of VDR (Gezen-Ak, Dursun & Yilmazer, 2013). Downwards regulation of the LVCC-subunits is unleashed by vitamin D or VDR and thought to block calcium influx. Additionally, vitamin D enhanced expression of calcium binding proteins thus limiting free ions inside the cell (Groves, McGrath & Burne, 2014).

After exposure of cortical neurons to amyloid β, VDR expression was totally invalidated. Meanwhile, treatment with vitamin D protected cortical neurons against toxicity from amyloid β proteins through suppression of LVCC-A1C while stimulating the expression of VDR. Amyloid

β accumulation had an opposite effect by enhancing LVCC-A1C and suppressing VDR expression. This is interesting, since amyloid β accumulation has been proposed as a potential etiology for Alzheimer disease and other dementias (Gezen-Ak, Dursun & Yilmazer, 2013). Knowing that there is an upsurge of both amyloid β and its precursor protein after head trauma (Fernández-Gajardo et al., 2014), and that it can suppressed vitamin D activities during the secondary injury phase opens the possibility for therapeutic interventions. Studies on Alzheimer patients showed that vitamin D improves amyloid β elimination and phagocyte engulfment in the brain (Gezen-Ak, Dursun & Yilmazer, 2013).

Vitamin D considerably reduced permeability of viable brain endothelial cells under low oxygen conditions though dose-mediated protection on the integrity of proteins along the tight junctions, the space between endothelial cells that supports BBB integrity. Interruption of the BBB during hypoxia or ischemia is mostly driven by excessive generation of ROS and NO damaging cellular structures. VDR expression directed the antioxidant effects of vitamin D in the protection of the BBB permeability by hindering oxidative stress.

Thus $1,25OH_2D_3$ and VDR mediate the protection of tight junctions of endothelial cells in the BBB in several ways (Won et al., 2015) by negatively impacting the activity of matrix metalloproteinases (MMP), a group of enzymes that together with nuclear factor kappa-B (NF-κB), are known to disrupt cellular membrane permeability subsequential to ROS stimulation. Matrix metalloproteinase-9 (MMP-9) is an enzyme that sponsors mending of cell membranes. To achieve restoration the MMP-9 simultaneously disintegrates tight junction proteins to repair the membranes, thus worsening dysfunction of the BBB (Mayer et al., 2013; Won et al., 2015). As MMP-9 concentrations grow following brain injury, it paves the way for BBB disruption through

protein degradation (Won et al., 2015). MMP-9 is a proinflammatory mediator capable of activating neuroglia cells (Mayer et al., 2013). After a hypoxic event, vitamin D is able to promote the BBB impermeability by reducing MMP-9 expression (Won et al., 2015).

The abovementioned, are extremely important for brain cell protection after acute trauma, since disruption of the BBB precedes infiltration of peripheral and blood cells into the brain compartment that can result in neuronal and glia apoptosis (Fernández-Gajardo et al., 2014). Blood is so detrimental to the brain that hemorrhages are indicators for more advanced neurological impairment and higher mortality risk. When lysed blood comes into contact with neuronal cells it exacerbates the cellular stress from excess calcium. Recent laboratory data showed that axonal fragmentation began 12 hours after exposure of neuronal cultures to lysed blood and progressed until the entire axon was destroyed. Vitamin D application showed significant protection against blood-induced axonal fragmentation, but it did not offer a significant protection against substances emitted from blood clotting (Charier et al., 2015).

In vivo experiments indicated that $1,25OH_2D_3$ administration after an ischemic incident induces smaller infarction volume (Fu et al., 2013; Kajta et al., 2009) and increased cell survival (Atif et al., 2013), however, pretreatment exerted better results (Kajta et al., 2009). Data from studies on ischemic strokes are provided, since clinical data of TBI, vitamin D, and ischemic injury was not available at the time of this review. These findings may explain the observations from retrospective and prospective studies from China, France and the USA, in which an inverse association between vitamin D and infarction size was reported. Low vitamin D prior to admission showed larger infarct volume (Turetsky et al., 2015; Wang et al., 2014), increase severity on admission (assessed with the National Institutes of Health Stroke Scale) (Daubail et

al., 2013; Wang et al., 2014) and longer hospitalization length after an ischemic stroke (Wang et al., 2014), also served as an independent predictor for the magnitude of the outcomes. Two of the studies found that low $25OHD_3$ predicted worse functionality outcomes at hospital discharged (Wang et al., 2014) and at 90-days (Turetsky et al., 2015) and survivors were correlated with higher serum $25OHD_3$ (Wang et al., 2014). Turetsky and colleagues (2015) stated that for every 10ng/mL reduction in $25OHD_3$ levels, there was a twofold increase for inferior outcomes at 90 days post-ischemic brain attack, and proposed exploration of $25OHD_3$ as a biomarker to identify patients with heighten vulnerability for worse prognosis (Turetsky et al., 2015). All of the observational studies used difference vitamin D references for their analysis, as seen on table 3.

Table 3: Incidence of vitamin D deficiency as defined by the above referenced studies.

Stroke patients	Daubail et al., 2013	Turetsky et al., 2015	Wang et al., 2014
Country of origin	France	China	USA
Serum $25OHD_3$	<10ng/mL	≤29.9ng/mL	<20ng/mL $25OHD_3$
Percentage of participants	33%	64%	68%

Significance of Vitamin D on Brain Health

The neurological implications of vitamin D levels and the mechanisms involved with brain functions are still being assessed. Compelling evidence indicates that $1,25OH_2D_3$ may prevent brain cell damage by interacting with about 170 genes following ischemic injury and 32 transcription factors with gene upregulation potential (Ridder et al., 2009). An in vitro study using cultured neuronal stem cells from human origin, found more than 180 proteins involved in neurological processes and neurogenesis were expressed after the cells came into contact with $1,25OH_2D_3$ (Obradovic et al., 2009).

Noteworthy attenuation of the expression of iNOS, IL-6 and MIP-2 has been observed with calcitriol (Huang et al., 2015). Genomic regulations of ion voltage channels on cell membranes have been reported to aid in maintain ionic balance (Gezen-Ak, Dursun & Yilmazer, 2013; Groves et al., 2014). Thus, deficiency or insufficiency of vitamin D may enhance vulnerability when facing the pathological alterations of TBI.

In summary, available biological data supports that $1,25OH_2D_3$ and VDR expression have regulatory influences in known secondary responses that follow a TBI. Given the results, vitamin D promises to be a therapeutic agent to prevent the activation or slow down the progression of the TBI-related inflammatory responses and BBB disruption. The emerging literature associating vitamin D status with the prevention, prognosis, and progression of a large number of neurological deficits and long-term neurodegeneration supports the need to expand research.

Vitamin D Status Among the Military Population

New discoveries concerning vitamin D actions in multiple biological processes, as well as neuroprotective features, could be relevant to military personnel recovering from TBI (Cui et al., 2015; Wentz, Eldred, Henry, & Berry-Caban, 2014). Although only a few studies have evaluated vitamin D levels among active military and veterans, the available evidence suggests a high prevalence of deficiency and insufficiency (Alazzeh et al., 2015; Andersen et al., 2010; Bailey, Manning & Peiris, 2012; Hiserote et al., 2016; Wentz et al., 2014).

A retrospective study of active duty (1,928) and veteran (1,125) military personnel, examining medical records for 3,053 samples at the Womack Army Medical Center, Fort Bragg,

North Carolina, showed that 57.3% of participants had either vitamin D deficiency or insufficiency, regardless of the 35.1°N latitude of the sample. The findings (reference table 4 and 5 below) showed that 20.4% of the participants were deficient, 36.9% tested at insufficient levels and 42.7% were between 30-100ng/mL (Hiserote et al., 2016).

Table 4: Service members by $25OHD_3$ level (Hiserote et al., 2016).

	Deficiency <20ng/mL	**Insufficiency 21-29ng/mL**	**Sufficiency 30-100ng/mL**
All service members	n= 623	n= 1,127	n= 1,302
Mean $25OHD_3$	14.65 ± 3.44	24.77 ± 2.82	38.94 ± 9.83

Table 5: Vitamin D levels according to military status (Hiserote et al., 2016).

	Active duty (n= 1,350)	**Veterans (n= 1703)**
Mean $25OHD_3$	27.79 ± 11.28	30.47 ± 12.55

To this date, perhaps the most significant study evaluating vitamin D levels among military patients is the retrospective analysis of 15,340 individuals from six different facilities in the southeastern United States. Although the sample was primarily comprised of white males with an average age of 66.5 years, vitamin D deficiency (<20 ng/mL) was significant in all six sites, even after correcting for seasonal and latitudinal variances. In fact, the highest vitamin D levels were below 35 ng/mL at all locations, except for one site (Huntington) where it was between 38-39 ng/mL. Moreover, deficiency was independently related to higher cost of health care across the entire sample (as seen on figure 11). Health care cost was at least 70% higher for patients who were deficient and did not receive a vitamin D follow up test in comparison to who were either deficient or received a vitamin D follow up test. The deficient patients without a

follow up test had nearly 300% higher health care cost than the non-deficient who received follow up testing (Bailey, Manning & Peiris, 2012).

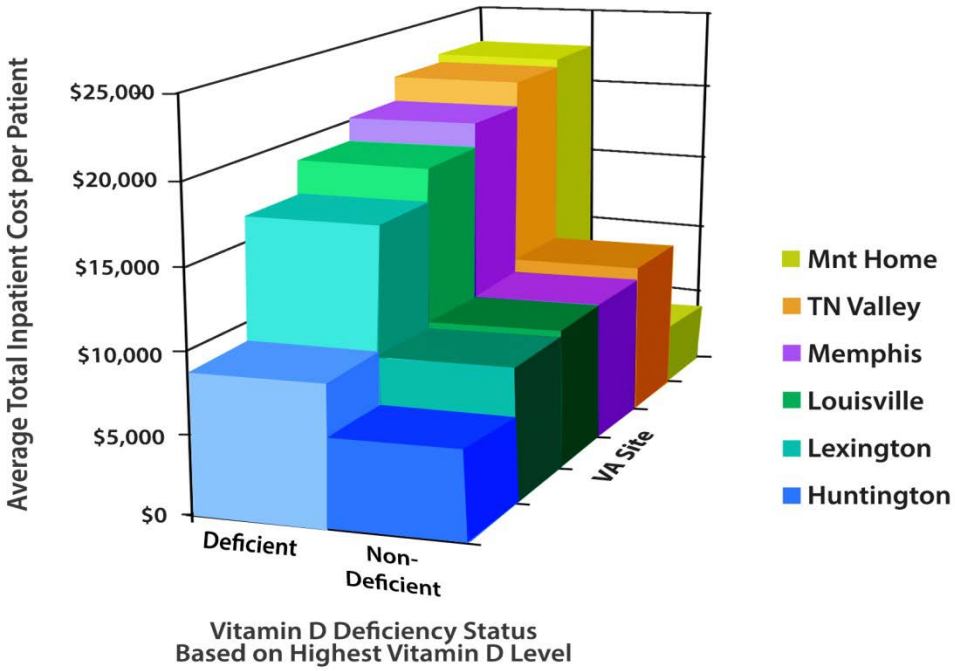


Figure 11: Total inpatient costs by VA site and vitamin D deficiency status

(Bailey, Manning & Peiris, 2012, p. 74).

A retrospective study analyzed of 3,608 American female veterans tested during a period of ten years (2001-2010), found a 44% deficiency of $25OHD_3$ (<20 ng/mL) with a higher incidence among minority veterans (Alazzeh et al., 2014). Another retrospective analysis of vitamin D status among critically ill veterans between 1999 and 2009, found that 38% tested deficient at <20ng/mL and 60% tested within the range for insufficiency (<30ng/mL) (McKinney et al., 2011).

A pilot study examined the impact of 8 weeks of basic combat training (BCT) on the vitamin D levels on new female recruits at the US Army BCT at For Jackson, South Carolina. The data from the randomized control trial was collected during summer/fall training and used to

evaluate vitamin D serum concentrations of the 74 trainees. The comparison of $25OHD_3$ levels from baseline and the end of the 8 weeks of basic combat training showed the mean levels declined from 72.9 $\pm$30.0nmol/L to 63.3 $\pm$19.8nmol/L, with lowest levels among non-Hispanic black. This study documented a decline in $25OHD_3$ levels among young female recruits during the summer-fall training, regardless of the time spent outdoors (Andersen et al., 2010).

The IOM and TES concluded that screening of vitamin D status for healthy, asymptomatic individuals is not necessary, unless there are identifiable risks suggesting otherwise (Holick et al., 2011; IOM, 2011b). Giving the significance of the evidence showing high health care cost with low vitamin D levels and progressive decreased during outdoor military training, it is appropriate to recommend evaluation of vitamin D optimization for active duty members and veterans.

Vitamin D: Advancing Future Research.

In their review of literature, Wentz and associates (2014) suggest that achieving optimal levels of vitamin D prior to injury may protect and improve rehabilitation efforts of wounded soldiers, while improving their outcomes (Wentz et al., 2014). There may also be the potential added benefits of improving survival rates for soldiers in critical condition, reducing disease progression and decreasing clinical symptoms (Alazzeh et al., 2015; Cui et al., 2014; Eyles, Burne & McGrath, 2013; McKinney et al., 2011; Moromizato et al., 2013; Toffanello et al., 2014; Wentz et al., 2014).

Advantages of working with military subjects include easy access to conduct health exams and laboratories prior to head trauma exposure. Monitoring and supplementation interventions can be performed within cohorts with shared characteristics. Additionally,

designing studies with subjects under a two or three year contract or with lifelong military careers may improve attrition rates.

In order to advance vitamin D research and practices it is necessary to address the lack of international reference values for screening, the need to standardize the assay methods, and reanalyzed the epidemiological and clinical findings.

CHAPTER 5: DISCUSSION

Active duty military and veterans share a history of exposure to TBI. TBI is responsible for inflammatory and metabolic processes with long-term progression. Screening of vitamin D status is a reasonable first step, given that adequate vitamin D levels prior to brain injury have been shown to exert neuroprotective actions and slow the pathological changes after head trauma.

The complexity of TBI management comes with acute and chronic pathologies. Clinical presentation is as individualized and unique as the injury itself. The progression, outcomes and response to current intervention protocols have made TBI a difficult disorder to treat.

To date, most research of TBI and vitamin D has been done in animal models. Thus far, translating these models into clinical trials has proven to be challenging. However, in order to improve knowledge, successes and failures are inherent, and arguably necessary in research.

The idea of having standard, acceptable measures to better guide future study of TBI has been a long standing goal of the scientific and clinical communities. The shortcomings of the methods used to measure outcomes contribute to the large number of clinical trial failures and continue to hold back prospects of fulfilling this goal.

Neuroinflammatory pathophysiological alterations of ischemic stroke parallel those of TBI. In reviewing human trials and observational studies of patients with hypoxic/reperfusion injuries, it was evident that vitamin D exerts a neuroprotective action. It reduces the volume of the injury, improves symptoms and prognosis, decreases mortality, lowers disability and is associated with better functionality. There is still much to answer about possible physiological

impact of low and insufficient vitamin D concentrations on health and thus far, causality is not even part of the conversation.

However, it is hard to ignore the inconsistencies in vitamin D classification and reference range used by many of the studies. Variations in optimization practice guidelines add to the challenges surrounding a complex field and compound to the difficulties of designing interventional trials. Consistency and validity of measuring methods are at the foundation of high quality research and should be prioritized. Only time will tell if the scientific and medical communities can indeed achieve consensus and provide the prevention and interventions of vitamin D. Collaboration to develop a common set of guidelines will be needed, if health care providers are to address clinical and subclinical deficiencies among military service personnel utilizing evidence-based practices.

The number of combat injured service personnel surviving serious injuries will continue to rise. Better, consistent diagnostics and treatments should be a priority for the nation. Accurate diagnosis and high quality TBI clinical management have the potential to improve the lives of veterans and improve their reintegration as civilians.

The scarcity of human studies on the neuroprotective effects of vitamin D on TBI-related outcomes, confirms that there is a need in this area. Future research of TBI management could include exploring the relationship of vitamin D screenings before combat exposure and post-TBI outcomes. It could also incorporate prognosis predictions based on $25OHD_3$ levels and investigating the possibility of long-term supplementation for the prevention and treatment of chronic neurodegeneration after combat-related head trauma.

CHAPTER 6: CONCLUSION

This review found compelling evidence supporting that the acute and chronic pathological changes following TBI are closely associated with the neuroprotective mechanisms of vitamin D. Calcitriol administration is considered safe, relatively low cost and screening risks are fairly low. The question of the potential benefit of a supplementation program for military personnel merits further inquiry. Furthermore, the evidence supports that an optimization program for active duty and veterans promises to lower cost of health care. Thus, the potential argument concerning the cost of vitamin D screening pales against the return on investment and the human capital gains.

Studies evaluating vitamin D status among military personnel shows that deficiency and insufficiency are prevalent, regardless of age, gender, ethnic background, or military status. Ongoing warfare will continue to expose military service personnel to the risk of TBI. Sufficient preclinical studies have shown that vitamin D neuroprotection is enhanced by preloading the cells before exposure to trauma and in some instances neuroprotection can only be achieved by meeting a specific range. Mounting evidence from clinical and epidemiological studies confirm that low vitamin D levels predispose patients to poorer health consequences.

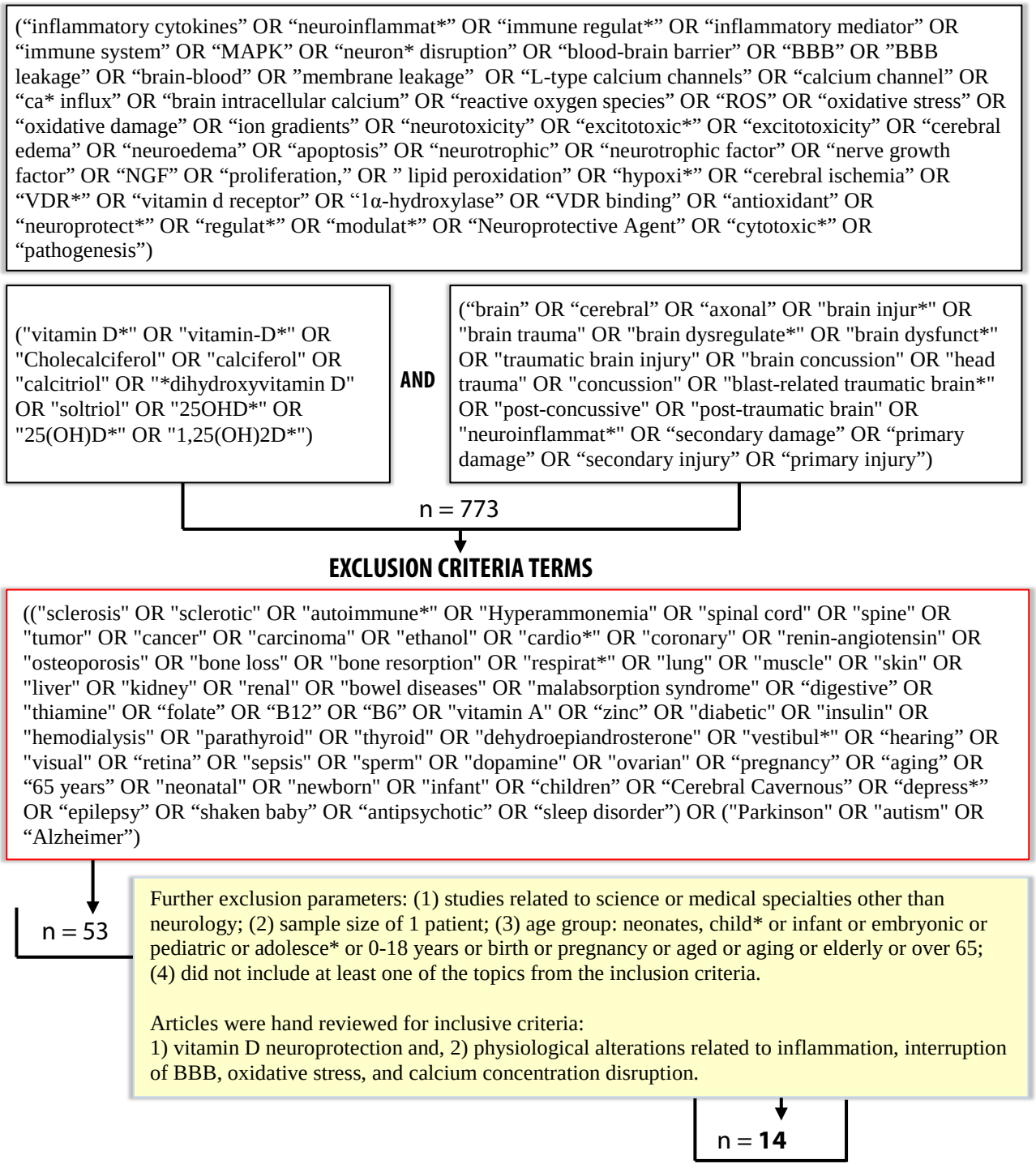
Soldiers, veterans and their families eagerly await evidence-based clinical practices that can reduce disease burden by improving outcomes, recovery and minimizing long-term sequelae. Based on the evidence reviewed, the medical community can no longer afford to ignore vitamin D.

APPENDIX A:

METHODOLOGY

Appendix A: Methodology

Searched subject headings, subjects and abstracts for:



APPENDIX B:

INFLAMMATORY MEDIATORS

Appendix B: Inflammatory Mediators

Pro-inflammatory mediators	Action	Vitamin D
Mitogen-activated protein kinase (MAPK) are initiated via expression of the NMDA receptor: 1. **p38** 2. **extracellular signal-regulated kinase (ERK)** 3. **Jun-N-terminal (JNK)**	• Initiate neuroinflammatory response by increasing synthesis of pro-inflammatory mediators (ROS, IL-6 and MIP-2) and NO accumulation (Huang et al., 2015). • ERK: signaling cascade to initiate apoptosis (Fu et al., 2013).	• Suppresses phosphorylation of p38, ERK and JNK MAPK pathways, reduces NO accumulation and ROS production and significantly decreases IL-6 and MIP-2 (Huang et al., 2015). • Restore concentration of the NR3A subunit by lessening its degradation, consequently triggering expression of the calcium-response element binding protein (p-CREB) (Fu et al., 2013). • Hinder the phosphorylation sequence of p38 MAPK and consequently inhibits expression of inducible nitric oxide synthase (iNOS), accumulation of nitric oxide (NO) and synthesis reactive oxygen species (ROS) (Hur et al., 2014).
Reactive oxygen species (ROS) synthesis	• Lipid peroxidation (Ekici et al., 2008), degradation of DNA (Fernández-Gajardo et al., 2014) and initiates endothelial and neuronal apoptosis (Won et al., 2015). • Activates NJK MAPK, triggers cellular edema, calcium influx and metabolic collapse (Fernández-Gajardo et al., 2014). • Decrease neurotrophic factors, worsen cognitive decline and neuronal communication, and increase BBB permeability (Fernández-Gajardo et al., 2014). • Promotes expression of NFκB and MMP-9 (Won et al., 2015).	• Reduces intracellular production of ROS by obstructing p38 and ERK expression in cultured brain cells (Huang et al., 2015). • Significant improves cell viability following exposure to ROS (Won et al., 2015). • Mitigates free radical damage increasingly causing lipid peroxidation and ameliorates degradation of DNA (Charier et al., 2015; Won et al., 2015). • Vitamin D significantly inhibited mitochondrial ROS production through VDR expression endothelial cells (Won et al., 2015).

Pro-inflammatory mediators	Action	Vitamin D
Nitric oxide (NO) accumulation	• Suppresses enzymatic activity in the mitochondria thus reducing adenosine triphosphate (ATP) production (Fernández-Gajardo et al., 2014; Huang et al., 2015; Hur et al., 2014). • Foster ROS synthesis (Huang et al., 2015).	• Obstruction of p38 and ERK expression significantly decreases buildup of NO (Huang et al., 2015; Hur et al., 2014). • Expression of 1,25-hydraxolase and VDR preceded decline of nitric oxide (NO) synthesis and a 90% improvement on cell viability (Hur et a., 2014).
Inducible nitric oxide synthase (iNOS)	• Linked to cognitive deficits in older adults (Huang et al., 2015).	• Significantly hinders iNOS expression by limiting activation of p38 and ERK (Huang et al., 2015).
Matrix metalloproteinase-9 (MMP-9) upregulation	• MMP-9 is capable of activating neuroglia cells (Mayer et al., 2013). • Sponsors mending of cell membranes, but at the same time it disintegrate proteins of the BBB tight junctions (Mayer et al., 2013; Won et al., 2015). • As concentration rises after injury, it produces protein degradation that leads to interruptions of BBB (Won et al., 2015).	• Protects BBB integrity after a hypoxic event, by reduced expression of transcriptor factor that promotes MMP-9 (Won et al., 2015).
Nuclear factor kappa-B (NFκB)	• Activation of NFκB pathway allows it to translocate into the nucleus, where p65 subunit acts as a transcription factor by binding to a gene that upregulates MMP-9 production (Won et al., 2015). • NFκB binding to genes increases IL-6 (Fernández-Gajardo et al., 2014) promoting neuronal inflammation (Atif et al., 2013).	• Marked decline in concentrations of NFκB (Atif et al., 2013), blocks phosphorylation and prevents translocation and gene expression. Thus, protects BBB integrity by reduction on MMP-9 concentrations (Won et al., 2015).
Toll-like receptors (TLR)	• Proteins capable of recognizing indicators of tissue damage and aid in communication of	• Vitamin D is the mediator between TLR and activation of innate immunity. Low levels had a 2.5-

Pro-inflammatory mediators	Action	Vitamin D
	neuroglia by releasing chemokines (Mayer et al., 2013), and may be involved in an elevated likelihood for sepsis or septic shock among critically ill patients (Moromizato et al., 2013).	fold increment on the risk for sepsis or septic shock in vulnerable patients. (Moromizato et al., 2013). • Vitamin D can down regulate the expression of TLR-8 and its direct gene transcription (Huang et al., 2015).
Glutamate excitotoxicity	• Overstimulates low voltage calcium channel (LVCC) and leads to deregulation of intracellular calcium by promoting massive influx, thus worsening of calcium influx and cellular edema (Fernández-Gajardo et al., 2014). • Causes necrosis and apoptosis of neuronal cells (Charier et al., 2015).	• Calcitriol significantly lessened damage by glutamate in cultured cortical neuron and glia cells. Pretreatment and higher doses of calcitriol yielded better protection (Kajta et al., 2009). • Ameliorate cell damage by increasing NR3 expression, subsequently antagonizing proinflammatory ERK MAPK pathway (Fu et al., 2013).
Low voltage calcium channel (LVCC) subunit A1C	• Up-regulation suppresses expression of vitamin D Receptor (VDR) by promoting amyloid- β accumulation. • Calcium is cytotoxic at high levels (Gezen-Ak, Dursun & Yilmazer, 2013). • Disrupts permeability of mitochondrial membrane, which compromises membrane action potential and uncouples ATP production. • Worsens oxidative stress (Fernández-Gajardo et al., 2014). • Low ATP production will lead to dysregulation of membrane transport proteins (Shi et al., 2015).	• Positive modulation of low voltage calcium channel subunit A1C (LVCC-A1C) has been observed in cultured neurons after suppression of VDR (Gezen-Ak, Dursun & Yilmazer, 2013). Downwards regulation of the LVCC-subunits is unleashed by vitamin D or VDR and thought to block calcium influx. • Additionally, vitamin D enhanced expression of calcium binding proteins thus limiting free ions inside the cell (Groves, McGrath & Burne, 2014).
amyloid β excitotoxicity	• Suppresses activation of vitamin D receptor while promoting the expression of LVCC-A1C.	• Protected cortical neurons against toxicity from amyloid β proteins through suppression of LVCC-

Pro-inflammatory mediators	Action	Vitamin D
	• Accumulation is strongly associated to the onset of Alzheimer disease (Gezen-Ak, Dursun & Yilmazer, 2013).	A1C while stimulating the expression of VDR. • Improves amyloid β elimination from the brain (Gezen-Ak, Dursun & Yilmazer, 2013).
Macrophage-inflammatory protein-2 (MIP-2) release	• A chemokine that promotes inflammation (Huang et al., 2015).	• Marked decline was mediated by inhibited expression of protein and gene transcription, and by blocking the phosphorylation of MAPK pathways (Huang et al., 2015).
Interleukin-6 (IL-6)	• Is a cytokine required to activate astrocytes (Mayer et al., 2013). • Following brain trauma, IL-6 appears to have positive effect on neuronal survival (Mayer et al., 2013), such as improving survival and differentiation of oligodendrocytes (Shi et al., 2015).	• Marked decline in synthesis and signaling at protein and mRNA sites (Atif et al., 2013), were largely mediated by vitamin D inhibitory effects on p38, ERK and JNK (Huang et al., 2015). • In animal experiments, deficiency was linked to elevated cytokines in the brain without a trauma, including IL-6 (Groves, McGrath & Burne, 2014).
Blood toxicity	• Blood-driven axonal fragmentation was noted to begin 12 hours after exposure and progressed to axonal death (Charier et al., 2015). • Initiates inflammation and toxicity (Chauhan, 2014).	• Vitamin D application protected brain cells against blood-induced toxicity and this effect was enhanced by concomitant use of an N-methyl D-aspartate (NMDA) glutamate receptor antagonist (Charier et al., 2015).
Caspase-3	• Activated by lysed mitochondria, this messenger is capable of triggering apoptosis (Atif et al., 2013).	• Inhibits signaling activity (Kajta et al., 2009).
Peripheral Leukocytes	• After infiltration, these cells assist neuroglia cells in the brain by promoting inflammation and produce toxic concentrations of nitic oxide (Fernández- Gajardo et al., 2014). • Promotes oxidative stress by	• VDR expression and $1,25OH_2D_3$ activation are induced by peripheral and neuronal immune cells activation, which in turn controls feedback mechanisms that diminish possible damage from overactive immune reactions

Pro-inflammatory mediators	Action	Vitamin D
	increasing production of nitric oxide (NO) (Fernández-Gajardo et al., 2014). • Prompts additional recruitment of glia and peripheral immune cells resulting in a systemic immune reaction (Mayer, Huber & Peskind, 2013).	(Groves, McGrath & Burne, 2014; Moromizato et al., 2013). • Deficiency is connected to defective actions of macrophages involving secretion of pro-inflammatory cytokines and phagocytic performance (Moromizato et al., 2013).

Anti-inflammatory mediator	Neuroprotective actions	Vitamin D
NR3A NMDA glutamate receptor subunit (found in brains of mammals)	• Antagonizes extracellular signal-regulated kinase (ERK) MAPK pathway by phosphorylation CREB, a protective nuclear signaling protein. • Reduces cell membrane permeability to calcium. • Co-expressed with NR3B, decreases calcium influx into mitochondria (Fu et al., 2013).	• Increases NR3A concentration by decreasing its degradation, consequently triggering expression of the calcium-response element binding protein (p-CREB), an anti-apoptotic mechanism (Fu et al., 2013).
cAMP/Ca^{2+}-response element binding protein (CREB), a nuclear transcriptor factor	• Stimulates neurogenesis, and may be effective in protection cells from glutamate excitotoxicity (Fu et al., 2013).	• Increases expression, thus promoting cell viability (Fu et al., 2013).
Superoxide dismutase	• Potent antioxidant enzyme behind the protective catalysis of superoxide (Ekici, Ozyurt & Erdogan, 2009).	• Produced a substantial boost in superoxide dismutase (Ekici, Ozyurt & Erdogan, 2009).
heme oxygenase-1 expression	• Produces potent antioxidant molecules in neuronal and neuroglia cells (Atif et al., 2013).	• Vitamin D produced a twofold increment, thus attenuating oxidative damage after ischemic event (Atif et al., 2013)

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