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Vitamin B12 Deficiency Does Not Stimulate Amyloid-beta Toxicity in a Ceanorhabditis elegans Model of Alzheimer's Disease

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Ceanorhabditis elegans Model of Alzheimer's Disease

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by

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ABSTRACT

Vitamin B12 Deficiency Does Not Stimulate Amyloid-beta toxicity in a

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Alzheimer's disease (AD) is symptomized by amyloid-beta plaques in the brain and accounts for more than 65 percent of dementia cases. Vitamin B12 (cobalamin) deficiency can result in similar cognitive impairment and roughly 15% of the elderly are vitamin B12 deficient. Vitamin B12 deficiency results in the accumulation of toxic methylmalonic acid and homocysteine. Hyperhomocysteinemia is a strong risk factor for AD. To test if vitamin B12 deficiency stimulates amyloid-beta toxicity, *Caenorhabditis elegans* expressing amyloid-beta in muscle were fed either vitamin B12-deficient OP50-1 or vitamin B12-rich HT115(DE3) *E. coli* bacteria. Increased amyloid-beta toxicity was found in worms fed the 0P50-1 diet. Supplementation of the OP50-1 diet with vitamin B12 did not rescue the increased *C. elegans* toxicity. Knockdown of either of the only two *C. elegans* vitamin B12-dependent enzymes *metr-1 or mmcm-1* protected against toxicity. Therefore, vitamin B12 deficiency does not stimulate Alzheimer's amyloid-beta-mediated toxicity in *C. elegans*.

DEDICATION

To God: my heavenly Father, Jesus: my Lord, Savior and Friend. To the Holy Spirit:

my helper, counsellor, and comforter. I return all Glory to these three persons in Trinity.

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CHAPTER 1. INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder that is progressive in nature and in patients it features loss of memory, communication skills, and cognitive functions such as thinking, learning and problem-solving. AD causes brain cells (neurons) in certain areas of the brain such as the hippocampus to degenerate. Over time the neurodegeneration spreads to much of the frontal and temporal lobes of the cerebral cortex causing loss of synapses and eventually leading to brain atrophy (Morrison and Hof 2007; Alzheimer's Association 2019). AD is believed to begin two decades before clinical symptoms are seen in patients (Bennett et al. 2004). Although, AD accounts for more than 75% of the cases of dementia in individuals over 65 years of age (Erkkinen et al. 2018), it should not be viewed as a normal aspect of brain aging.

AD exists in two form. The Familial AD typically begins between the ages of 30 and 55 and is referred to as the early onset form accounting for less than 5% of AD cases. The late onset form (sporadic AD) is the most prevalent form of AD and begins after 65 years of age (Bird 2018). AD development is linked with multiple factors, and in the case of the early onset form, has been linked to mutation in three genes: amyloid precursor protein (APP), presenilin 1 (PS1) and presenilin 2 (PS2). The greatest risk factors for sporadic AD are aging, inheriting the apolipoprotein Ɛ4 allele, and family history (Bekris et al. 2010; Wallon et al. 2017; Giau et al. 2019). Epidemiological studies have also shown that subjects with cerebrovascular disorders, a high cholesterol level, a history of brain injury, diabetes, or mid-life hypertension are at higher risk of developing AD (Reitz and Mayeux 2014; Prince et al. 2016). Factors such as gender, level of education, race, and poor diet and lifestyle are associated with risk for AD development. (Scheltens et al. 2016).

Over 5 million Americans over 65 years of age are living with AD, and by 2050 a projected rise to 15.8 million is expected. AD is the 6th leading cause of death in the United States and a major cause of poor health and disability in individuals over 65 years of age (Alzheimer's Association 2019). The social, economic and financial burden to individuals living with AD, the family members who support them, and the nation is on the rise (Maresova et al. 2016; Prince et al. 2016).

Current pharmacological treatment for AD includes acetylcholinesterase inhibitors (ACHei) and N-methyl D-aspartate (NMDA) receptor antagonists (Aderinwale et al. 2010). However, these treatments provide only mild relief for cognitive function and are used to treat cognitive decline at the later stages of the disease. In addition, these drugs are not effective in all patients and have substantial side effects (Bianchetti et al. 2006; Small and Duff 2008). Non-pharmacological treatments such as exercise and music therapy have been found to be mildly effective in some cases. Both, like the pharmaceutical treatments, only provide symptomatic relief without stopping AD progression.

Despite the number of years dedicated towards research to understand the molecular and pathological basis of AD no drug has been developed to even slow down its progression and almost all drugs have failed clinical trials. AD represents a global challenge to public health with impacts on individuals, families, and society at large giving rise to economic and social hardship. Due to these devastating consequences there is an increasing need for the development of drugs that target novel molecular pathways to attempt to slow down AD disease progression.

Alzheimer's Disease Pathology

In the brain, pathophysiology of AD include extracellular deposits of amyloid plaques and intracellular aggregation of neurofibrillary tangles (NFTs), which were first described by

Alois Alzheimer in 1907 from studying the brain autopsy of AD patients (Murphy and Levine 2010; Serrano-Pozo et al. 2011). Amyloid plaques contain abnormally folded amyloid-beta (Aβ) peptides of 40/42 amino acids in length proteolytically formed from cleavage of amyloid precursor protein (APP) (Sheng et al. 2018). Aβ peptides form soluble oligomers and then aggregate into beta sheet structures in the neuronal extracellular space and form insoluble Aβ plaques. Recent studies posit that intracellular Aβ oligomers are more toxic than extracellular Aβ plaques as the oligomers induce neuronal damage and loss of synapses (Tu et al. 2014; Ferreira et al. 2015; Ono and Tsuji 2020). Intracellular neurofibrillary tangles are helical filaments composed of highly phosphorylated tau proteins, which prevent the movements of cargo up and down microtubules in the axons thereby disrupting neuronal function (Singh et al. 2016). Both Aβ plaques and tau NFTs inhibit neuronal communication via disrupting synapses and consequently lead to neurodegeneration and brain atrophy.

Hypotheses of AD Development and Progression

Since the first identification of AD, numerous hypotheses have been put forth to explain this multifaceted disorder. The first data-driven molecular hypothesis was postulated in 1976 by Peter Davies and A. J. F. Maloney (Davies and Maloney 1976). Behavioral changes such as irritability, agitation, depression, and psychosis are often observed at the later stage of AD and these are likely coupled with the loss of acetylcholine. These findings led to the cholinergic hypothesis, which posits that deficiency of acetylcholine, a neurotransmitter produced by the enzyme choline acetyltransferase at presynaptic clefts, causes the behavioral deficits seen in AD patients. Pharmacological therapies such as acetylcholinesterase inhibitors, which serve to decrease the rate of acetylcholine degradation, have been used to treat late onset AD (Francis et al. 1999; Yiannopoulou and Papageorgiou 2013; H. Ferreira-Vieira et al. 2016).

The amyloid-beta cascade hypothesis has been at the forefront of AD research for the past three decades. According to its founders (Hardy and Allsop 1991), the aggregation of Aβ peptide , which is a proteolytic product of the transmembrane protein amyloid precursor protein (APP), is the major culprit in AD. The downstream effects of Aβ plaque deposition result in intracellular neurofibrillary tangle deposition and neuronal and synaptic loss, ultimately leading to dementia. The Aβ cascade hypothesis was further corroborated when it was found that patients with the familial form of the disease have mutations in genes involved in the proteolytic processing of Aβ. The genes involved are APP, PS1 and PS2. Also, Down syndrome patients, who possess an extra copy of chromosome 21, have a higher risk for developing AD due to location of the APP gene on chromosome 21 (Ricciarelli and Fedele 2017).

As convincing as the amyloid-beta cascade hypothesis seems, many questions are yet to be resolved including how increased production of Aβ peptides lead to NFT formation, why anti-amyloid therapy has not been able to successfully treat the disease at the clinical level, why there are Aβ plaques in the aging brain of patients with no signs of dementia, why Aβ plaques and NFTs are seen in patients with late onset AD with no mutation in the APP, PS1,or PS2 genes, and how Aβ plaques and NFTs can develop independently of mutations in these genes (Swerdlow et al. 2010; Morris et al. 2014; Ricciarelli and Fedele 2017).

In a bid to explain the pathophysiology of the most common form of AD (sporadic late onset AD), the "mitochondrial cascade hypothesis" was put forth. The hypothesis posits that mitochondrial dysfunction, which increases with aging leads to decreased oxidative phosphorylation and increased reactive oxygen species (ROS) production and that this dysfunction drives Aβ deposition and NFT formation. Deposited amyloid-beta peptides are imported into mitochondria, where they further increase ROS production and decrease ATP

production leading to neuronal cell death (Butterfield 2002; Swerdlow and Khan 2004; Swerdlow et al. 2010).

Other hypotheses for development of AD include the tau hypothesis (Frost et al. 2009), the calcium hypothesis (Mattson et al. 1992), the metal ion hypothesis (Liu et al. 2018), the dual pathway hypothesis (Small and Duff 2008), and the oxidative stress hypothesis (Markesbery 1997). An alternative hypothesis recently put forth is the homocysteine hypothesis. Homocysteine is a non-protein sulfur containing amino acid, which is remethylated to form methionine in the methionine-homocysteine cycle. High serum level of homocysteine is an indicator of low B vitamin level (B12, B9, or B6) (Smith et al. 2018).

The homocysteine hypothesis was formulated to attempt to find a link between the cause for the early and late onset forms of AD. Familial AD has been linked with vitamin B12 deficiency as mutation in APP gene leads to endolysosomal protease inhibition preventing release of vitamin B12 bound to its carrier protein. This is further discussed later in the thesis. Likewise, mutation of the presenilin-1 (PS1) gene could disrupt lysosome function, preventing the release of cobalamin (vitamin B12) for use by the rest of the cell. Several studies have reported high serum homocysteine levels in older subjects before the onset of dementia and AD suggesting hyperhomocysteinemia as a risk factor for sporadic AD (Regland and McCaddon 2019).

Mitochondria and AD Development

Considering the importance of mitochondria as the main energy provider for the cell, they have been hypothesized to play a role in AD development and progression and are important targets for therapeutic purposes. Mitochondria produce adenosine triphosphate (ATP) via oxidative phosphorylation. The electron transport chain (complexes I, II, III, and IV) and ATP synthase (complex V) located in the mitochondrial inner membrane generate

energy from the NADH and FADH$_2$ produced from the mitochondrial citric acid cycle oxidation of respiratory substrates such as pyruvate, fatty acids, and amino acids. Cytoplasmic NADH derived from glucose oxidation is also shuttled into the mitochondrial matrix space for energy generation.

Genetic information is present in the mitochondrial matrix space in the form of mitochondrial DNA (mtDNA), which can replicate independently of the cell cycle. MtDNA is quite susceptible to mutations such as deletions and base substitution mutations. Mutations have been shown to accumulate during brain aging and in neurodegenerative disease, to a level which can compromise mitochondrial bioenergetics (Tillement et al. 2011). This was corroborated in studies that reported mtDNA mutations in neurons in patients with AD (Hirai et al. 2001; Wang et al. 2014).

Also, increased ROS level, reduced ATP production, reduced NADH dehydrogenase (complex I) activity, and reduced cytochrome c oxidase (complex IV) activity was reported in autopsied AD patient brain mitochondria and in studies that used AD cybrid cell lines, where mitochondria from patient blood cells were injected into cell lines depleted of mtDNA, and the cells were exposed to Aβ1-40 (Cardoso et al. 2004). β-amyloid oligomers have been implicated as the cause of several types of mitochondrial dysfunction. Several studies have noted that β-amyloid can be imported across the mitochondrial inner and outer membranes, and is able to interact with mitochondrial matrix space molecules (Lustbader et al. 2004; Singh et al. 2009).

A group that used human neuroblastoma cells reported that Aβ1-42 peptide crossed the mitochondrial outer membrane and interacted with complex II in the inner membrane (Tillement et al. 2006). This is not so surprising given that the mitochondrial outer membrane contains oligomeric VDAC proteins that form pores allowing the passage of solutes smaller

than 100 amino acids (~ 10 kD) in size. This study further showed that Aβ1-42 can also interact with complexes IV and V of the mitochondrial oxidative phosphorylation machinery.

Another study also noted a decrease in complex IV (cytochrome oxidase) activity in the brain of AD patients (Bosetti et al. 2002). Furthermore, examination of the hippocampus of patients with late onset AD revealed decreased cytochrome c oxidase activity (COX) compared to normal patients of the same age. Further analysis of the neuronal mtDNA-linked COX deficiency found that it was due to elevated mtDNA deletions, which increase with aging (Krishnan et al. 2012).

β-amyloid can further compromise membrane integrity and lead to cell membrane deformation, increased membrane permeability (Janson et al., 1999; Engel, 2009) and disrupted calcium homeostasis, which can interfere with normal mitochondrial function (Csordás et al. 1999). β-amyloid can also impede ATP production via inhibition of oxidative phosphorylation (Hauptmann et al. 2009), which consequently increases ROS production (Alexeyev et al. 2006). ROS overproduction can lead to DNA damage, lipid peroxidation, and cell apoptosis (Tillement et al. 2011). A plethora of evidence has shown a link between β-amyloid peptide deposits, mitochondrial dysfunction, and increased oxidative stress (Butterfield 2002; Chen and Zhong 2014; Wang et al. 2014; Huang et al. 2016).

Roles of B Vitamin in Brain Function

B Vitamins are a group of water-soluble vitamins, which play a role in normal cell function by serving as coenzymes/cofactors in enzymatic reactions (Mikkelsen, Stojanovska, and Apostolopoulos 2016). They play a role in maintaining mitochondrial energy production, the metabolism of protein, fats and carbohydrate, the synthesis of nucleic acids and amino acids, as well as play a role in DNA repair and immunity (Laquale 2006; Kennedy 2016).

The eight B vitamins with inter-related functions are thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), biotin (B7), folate (B9), and cobalamin (B12). Except for vitamin B12, which is synthesized only by some bacteria, all other B vitamins can be synthesized by plants. B vitamins are derived from dietary supplements of plant and animal origin such as meat, grains, fruits, fish, dairy, milk, and vegetables (Kennedy 2016).

Thiamine acts as a cofactor in pathways that are involved in carbohydrate metabolism. It plays a role in maintaining neuronal structure and function. Riboflavin and niacin function together in mitochondrial energy metabolism. Riboflavin is a precursor for two coenzymes, flavin mononucleotide and flavin adenine dinucleotide, while the active forms of niacin are nicotinamide adenine dinucleotide (NAD(H)) and NAD phosphate (NADP(H)). In addition, riboflavin regulates the recycling of niacin, folate, and vitamin B6 (pyridoxine). Pantothenic acid is a precursor for the synthesis of coenzyme A and is used in the metabolism of amino acids, fatty acids, cholesterols, and phospholipids. Biotin is used in carboxylation reactions and is also required for glucose and fatty acid metabolism (Mikkelsen, Stojanovska, and Apostolopoulos 2016).

Vitamin B6, B9, and B12 are involved in one-carbon metabolism of methyl groups, which are crucial for the methylation of proteins and nucleic acids. Pyridoxine (B6) serves as a cofactor in the folate cycle. Folate (B9) is also important for red blood cell maturation, synthesis of DNA, and metabolism of amino acids. Cobalamin (B12) is crucial for the synthesis of new red blood cells and for the conversion of folate into its active forms. These three vitamins are involved in the folate and methionine cycles, which produce the methyl groups required for DNA and RNA methylation. They are also important in lowering the plasma level of homocysteine, which is linked to heart disease, stroke, and AD (Kennedy 2016). Deficiency of B vitamins is common in the elderly and it is often linked with

cardiovascular disorders and neurological disorders such as AD (Mikkelsen, Stojanovska, and

Apostolopoulos 2016; Smith and Refsum 2016). Daily storage and uptake of up to 1-2 µg of

vitamin B12 is essential to maintain a healthy vitamin status (Kozyraki and Cases 2013). Ten

percent of adults over the age of 65 are vitamin B12-deficient due to the loss of intestinal

uptake with age (Mikkelsen, Stojanovska, Tangalakis, et al. 2016).

Vitamin B12 Deficiency and AD

  Vitamin B12 (cobalamin) is converted into its two active forms, which are

adenosylcobalamin in the mitochondrial matrix space and methylcobalamin in the cytoplasm.

Each is a cofactor for a single enzyme. Adenosylcobalamin is used by the mitochondrial

enzyme methylmalonyl-CoA mutase and methylcobalamin is used by the cytoplasmic

enzyme methionine synthase (Morris 2012). Methionine synthase, encoded by the human

MTR gene and the *C. elegans metr-1* gene, catalyzes the synthesis of methionine from

homocysteine by addition of a methyl group, which is donated by N5-methyl-tetrahydrofolate

to yield tetrahydrofolate (de Jager 2014). In the mitochondrial matrix, a series of enzymes

converts propionyl-CoA into D-methylmalonyl-CoA and L-methylmalonyl-CoA

intermediates. The methylmalonyl-CoA mutase enzyme, encoded by the human MMUT

(formerly MCM) gene and the *C. elegans mmcm-1* gene, with its adenosylcobalamin

coenzyme, converts L-methylmalonyl-CoA into succinyl-CoA, with the latter being part of

the citric acid cycle that supplies reducing equivalents for oxidative phosphorylation (Miller

2003; Birch et al. 2009). Vitamin B12 deficiency leads to the buildup of toxic compounds,

such as homocysteine and methylmalonic acid, directly upstream of the deficient enzymes

(Allen 1998; Morris 2012). The measurement of these compounds in the blood is used for the

diagnosis of vitamin B12 deficiency.

One mechanism through which vitamin B12 deficiency may cause dementia is through increasing homocysteine levels as decreased cytoplasmic methionine synthase activity leads to increased homocysteine levels and increased blood homocysteine is linked with AD dementia (Seshadri et al. 2002; Smith and Refsum 2016). A high homocysteine level has been suggested to cause increased oxidative stress (Birch et al. 2009), decreased DNA methylation, and increased DNA damage, subsequently leading to accumulation of amyloid-beta, neurofibrillary tangles, and neuronal cell death (Smith 2008; Smith and Refsum 2016).

Interestingly, experiments using a *C. elegans* AD model where the *metr-1* gene was knocked down showed an increased level of homocysteine and amyloid-beta toxicity (Leiteritz et al. 2018). This might be explained by the fact that hyperhomocysteinemia increases amyloid-beta toxicity through the homocysteinylation of peptide lysine residues, which leads to the increased formation and stabilization of toxic amyloid-beta oligomers (Khodadadi et al. 2012).

Several lines of study have established that B vitamin deficiency and increased plasma homocysteine levels are risk factors for cognitive decline, dementia, and AD. However, conflicting results have been reported within the last two decades when vitamins B6, B9, and B12, either alone or in combination, have been administered to AD patients. For example, a study that examined AD patients, non-demented hospital patients, and healthy controls living at home noted that the highest level of homocysteine and methylmalonic acid was present in the plasma of AD subjects compared to controls (Joosten et al. 1997). This was also confirmed by another group that noted that total serum homocysteine was significantly higher, while total serum folate and vitamin B12 were decreased in patients with AD dementia when compared to control subjects (Clarke et al. 1998).

Others performed an eight-year follow-up study of non-demented elderly who participated in the Framingham heart study and reported an increase in plasma homocysteine in some subjects with mild dementia, while patients with confirmed cases of AD showed higher levels of plasma homocysteine than controls (Seshadri et al. 2002). In contrast, a two-year study investigated if oral supplementation of folic acid and vitamin B12 could improve cognitive function in elderly subjects that had elevated plasma homocysteine and reported that there was no improvement in cognitive performance between the group that had been given the B vitamin supplement and the group that had received the placebo control. However, plasma homocysteine level was significantly reduced in the subjects given the B vitamin supplementation compared to the control (Van Der Zwaluw et al. 2014). A meta-analysis study of the effects of folate, cobalamin, and vitamin B6 (pyridoxine) supplementation on AD patients found that there was a decreased serum homocysteine level without an improvement in cognitive function (Zhang et al. 2017). A more recent publication that used the above-mentioned B vitamins supplemented to elderly with or without cognitive decline also saw no significant improvement in cognition between the treatment group and those that were given the placebo. They did however find a significant decrease in homocysteine levels in the plasma of subjects supplemented with the B vitamins (Ford and Almeida 2019). One possibility is that synapses are irreversibly lost or neurons die shortly after the onset of B vitamin deficiency and hyperhomocysteinemia in AD subjects and therefore, subsequent recovery of B vitamin levels and lowering of homocysteine levels are unable to recover the lost cognitive function.

Another mechanism through which vitamin B12 deficiency may cause dementia is through increasing the level of methylmalonic acid (MMA), which can cause mitochondrial electron transport chain dysfunction (Brusque et al. 2002; Chandler et al. 2009; Schuck et al. 2013). Elevated MMA can occur as a result of deficient vitamin B12-dependent

methylmalonyl-CoA mutase enzyme activity within the mitochondrial matrix (Grützner et al. 2013). Several reports have suggested that MMA causes oxidative stress in mitochondria through the accumulation of toxic metabolites, such as propionyl-CoA and propionic acid, although their roles in mitochondrial impairment have not been fully elucidated (Haijes et al. 2019).

Vitamin B12 Structure and Metabolism

The chemical structure of Vitamin B12 consists of a cobalt ion, which is the functional group, surrounded by a planar corrin ring. Various derivatives of vitamin B12 are formed by functional groups which are added to the corrin ring, such as cyanide forming cyanocobalamin, a methyl group forming methylcobalamin, or an adenosyl group forming adenosylcobalamin. Cyanocobalamin is a stable synthetic form of vitamin B12 used in dietary supplements (Kräutler 2012).

Many genes are involved in the absorption, transport, binding, and covalent modification of cobalamin. Absorption of vitamin B12 occurs in the stomach and small intestine. When a meal containing Vitamin B12 is ingested, vitamin B12 binds to haptocorrin (also known as transcobalamin 1 (TC1)) in the upper part of the stomach. In the duodenum, haptocorrin is degraded and vitamin B12 binds to the intrinsic factor glycoprotein in the ileum. In this area of the intestine, the enterocytes synthesize cubulin and amnionless, where they localize to the luminal membrane and function to internalize the intrinsic factor-vitamin B12 complex within an endosome. Degradation of intrinsic factor occurs within the endosome and vitamin B12 binds to another protein called transcobalamin 2 (TC2) and the complex is exocytosed into the systemic circulation. Some vitamin B12 in the circulation re-binds haptocorrin and is unable to be taken up by cells. For vitamin B12 to enter cells, the vitamin 12-TC2 complex binds a plasma membrane receptor called CD320 or TCb1R and the

entire complex is then endocytosed into the endolysosomal system. In the lysosome, proteolytic digestion of TC2 occurs. Mutation in TCN2 has been linked with AD and low vitamin B12 status in individuals with autoimmune gastritis. (Surendran et al. 2018).

Two membrane proteins LMBD1 and ABCD4 are required for the transport of cobalamin out of the lysosome. Defects in the genes encoding these proteins will cause vitamin B12 to be trapped within the lysosome and unable to be utilized as a coenzyme. Just as in these patients with an inborn error of metabolism, defects in lysosomal function in AD patients may prevent vitamin B12 from being processed properly into methylcobalamin or adenosylcobalamin in neurons leading to the local accumulation of toxic homocysteine and methylmalonic acid. But since glial cells may still be able to correctly process vitamin B12 and metabolize homocysteine and MMA, these toxic products may not increase in the bloodstream.

The MMACHC (methylmalonic aciduria type C and homocysteine) gene product is important in conversion of cobalamin into methylcobalamin. This gene encodes the cblC protein which is present on the outside of the lysosomal membrane and in the cytoplasm to catalyze the conversion of cyanocobalamin, methylcobalamin, or adenosylcobalamin into cob(II)alamin, a common intermediate that can be further converted into either methylcobalamin or adenosylcobalamin (Surendran et al. 2018). Reduced glutathione is a required cofactor for cblC. Glutathione levels are depleted in AD neurons that may perturb synthesis of the active forms of vitamin B12. The MMADHC gene locus encodes the cblD protein that functions in the partitioning of cob(II)alamin between the cytoplasmic and mitochondrial compartments (Gherasim, Hannibal, et al. 2013). Methionine synthase reductase also known as the cblE protein encoded by the MTRR gene catalyzes the methylation of inactive cob(II)alamin into the active form methylcob(I)alamin (Gherasim et al. 2013). The cblA protein encoded by the MMAA gene locus function in the transport of

cob(II)alamin into the mitochondrial matrix. The cblB (ATP-dependent cob(I)alamin adenosyltransferease) protein is encoded by the MMAB gene locus and functions in the synthesis of adenosylcob(I)alamin from cob(II)alamin and S-adenosylmethionine (Oussalah et al. 2017). Lastly, adenosylcob(I)alamin functions as a coenzyme for the methylmalonyl-CoA mutase (MMUT) enzyme, which catalyzes the conversion of methylmalonyl-CoA to succinyl-CoA that is used for the production of energy in the tricarboxylic acid cycle (Gherasim et al. 2013).

Since AD is widely understood to be a disease caused by gradual cytoplasmic and mitochondrial energy depletion and increased oxidative stress, it is important to discover specific molecular mechanisms through which vitamin B12 deficiency may sensitize mitochondria and cells to amyloid-beta toxicity. Once this is better understood, targeted therapies can be created to restore the levels of the active forms of vitamin B12 to decrease homocysteine and methylmalonic acid levels and improve neuronal function to delay the onset of the disease. As amyloid plaques can occur up to twenty years before cognitive dysfunction, it is important to maintain adequate B vitamin levels starting in middle adulthood.

Caenorhabditis elegans as a Model Organism

Use of mammalian models for neurodegenerative disease research has been plagued with setbacks ranging from high cost of maintenance to long experiment time. This has led to the use of simple and less expensive model organisms to understand the disease mechanism with the aim of developing potential drug therapies more rapidly (Chen et al., 2015)

Caenorhabditis elegans (*C. elegans*) is a non-parasitic, free-living nematode (roundworm) found in the soil that can consume bacteria and fungi as a source of food (Barrière and Félix 2014; Corsi et al. 2015). Since it was first introduced by Sydney Brenner

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in the 1960's*, C. elegans* has been shown to be a good model organism for investigating various biological studies ranging from host-microbiota interactions, aging and age-related diseases, development, and neurobiology (Kaletta and Hengartner 2006).

C. elegans is a microscopic organism with a maximum length of 1 mm and a simplified anatomy consisting of reproductive, digestive, muscular, and nervous systems, which are visible under the microscope due to its transparent body (Culetto and Satelle 2000). It has a short life cycle of 4-7 days depending upon the temperature at which it is cultured (between 15 and 25^0 C) and a mean lifespan of 2.5 weeks at 20^0C (Corsi et al. 2015). In the lab, it is typically grown on nematode growth media (NGM) agar in a Petri dish or in oxygenated liquid S-media. It is typically fed *E. coli* bacteria. It generates a large brood size and exists mostly as a hermaphrodite, but males are also found at a prevalence of approximately 0.1% in the population (Brenner 1974). It can be stored over long periods of time in liquid nitrogen. All these characteristics makes it a simple, inexpensive, and effective model organism for high throughput screening which has provided the means to elucidate molecular mechanisms of disease in a whole organism (Kaletta and Hengartner 2006; Sin et al. 2014).

C. elegans was the first multicellular organism to have its genome sequenced (Consortium 1998). About 40-60% of human genes have orthologs in the *C. elegans* genome (Kaletta and Hengartner 2006), while approximately 42% of genes known to cause human diseases such as APP, tau and parkin have orthologs in *C. elegans* (Culetto and Satelle 2000). *C. elegans* has been used to model neurodegenerative diseases such AD, Parkinson's disease (PD) and Huntington's disease (HD). This process usually involves gene knockout, knockdown, or the creation of a transgenic strain expressing a heterologous human protein involved in such diseases (Kaletta and Hengartner 2006; Ma et al. 2018), although

CRISPR/Cas9 can also be used to introduce a specific mutation in *C. elegans* genes to engineer a new disease model (Dickinson and Goldstein 2016).

The ease of genetic manipulation in *C. elegans* has proven useful in large scale genomic screening. RNA interference for gene knockdown is achieved by feeding *C. elegans* with *E. coli* expressing double stranded RNA (dsRNA), or soaking or injecting the worm with dsRNA (Kaletta and Hengartner 2006; Dimitriadi and Hart 2010). Due to the transparent body, fluorescent reporter strains of worms have been engineered. In these strains green fluorescent protein (GFP) is typically introduced downstream of an endogenous promoter, so the expression and tissue localization of that gene can be monitored by fluorescence measurements (Teschendorf and Link 2009). Numerous transgenic and knockout strains of *C. elegans* are also available from the University of Minnesota's *Caenorhabditis* Genome Center (CGC).

 C. elegans Model of Alzheimer's Disease

A strain of *C. elegans* has been engineered to express human Aβ peptide in the body wall muscle. The construct is made of a body wall muscle-specific promoter which drives expression of an Aβ$_{1-42}$ mini-gene fused with an artificial signal peptide from the *her-1* gene. The transgene plasmid was injected into the *C. elegans* gonad together with the *rol-6* plasmid, which expresses a mutated collagen gene with a roller marker phenotype causing worms to move in a distinctive C shape (Link 1995).

Aβ expression in *C. elegans* has been engineered to be inducible or constitutive. The former uses a *myo-3*-specific promoter and a temperature sensitive signal peptide, at which upshift of temperature induces Aβ expression causing a relatively quick (within 48 hours) paralysis of body wall muscle. In the constitutive expression model, the *unc-54* body wall muscle-specific promoter drives Aβ expression with a constitutive signal peptide causing

paralysis in aged worms (Teschendorf and Link 2009). The paralysis phenotype in both cases is easily scored by observing a lack of body movement in *C. elegans* or by the formation of a halo of cleared bacteria near the head region of the paralyzed worms, as the head, neck, and pharyngeal muscles do not become paralyzed.

A transgenic *C. elegans* strain expressing amyloid-beta from the *snb*-1 pan-neuronal promoter has also been created (Link 2006). In these worms, one group noted decreased chemotaxis and high sensitivity to serotonin with no apparent defect in body movement (Wu et al. 2006)

Some of the drawbacks as regards to the use of *C. elegans* as a model organism is the inability to investigate the molecular pathways that they lack but are present in mammals. Also, RNAi is not effective in neurons in most commonly used strains, and human disease models of *C. elegans* don't fully capture the entire pathophysiology of the disease seen in humans (Kaletta and Hengartner 2006; Teschendorf and Link 2009). Low uptake of drugs by *C. elegans* as a result of degradation by their *E. coli* food source and the impermeant external *C. elegans* cuticle which prevents drug uptake through the epidermis limits the use of *C. elegans* as a drug discovery model (O'Reilly et al. 2014), although strains with increased cuticle permeability have been identified (Xiong et al. 2017).

C. elegans and a Natural Vitamin B12 Deficient Diet

Like humans, *C. elegans* does not synthesize vitamin B12 but must obtain it from the diet. *E. coli*, unlike many other enteric bacteria, lacks the first of three portions of the cobalamin synthesis pathway and can only synthesize cobalamin if supplemented with the intermediate cobinamide. *E. coli* take up micronutrients from their LB growth media, a peptone and yeast extract-based media containing abundant peptides, fatty acids, simple sugars, and micronutrients (Maynard and Weinkove 2020). Two different strains of *E. coli*

are commonly fed to *C. elegans* in the laboratory. The most common *E. coli* strain used is OP50, while the next most common is HT115(DE3), which was used for the synthesis of the two global RNAi libraries.

It has been shown that the OP50 strain is moderately deficient in vitamin B12 synthesis due to the lack of the membrane transporter *tonB* for the uptake of cobalamin (MacNeil et al. 2013; Watson et al. 2014). So, when *C. elegans* feeds on OP50 bacteria, they become deficient in vitamin B12, which has been shown to just slightly reduce lifespan, fertility and retard growth in *C. elegans* (Watson et al. 2015). Also, an accumulation of toxic intermediates, such as methylmalonic acid and homocysteine, was seen in *C. elegans* grown on vitamin B12-deficient OP50 bacteria (Watanabe et al. 2013). A study by the Revtovich group also demonstrated an increased resistance to pathogens, heat, and oxidative stress and improved mitochondrial function in *C. elegans* fed the HT115(DE3) *E. coli* strain compared to worms fed the 0P50 *E. coli* strain. The increased resistance was eradicated when they knocked down the *mmcm-1* gene encoding mitochondrial methylmalonyl-CoA mutase, which uses vitamin B12 as a cofactor (Revtovich et al. 2019).

Furthermore, unpublished studies performed by another master's student from our laboratory showed that when *C. elegans* expressing amyloid-beta in body wall muscle was fed with vitamin a B12-deficient OP50 bacterial diet, they became paralyzed more quickly, roughly 48 hours after amyloid-beta expression was induced compared to when the *C. elegans* were fed a vitamin B12-rich HT115(DE3) bacterial diet, where they became paralyzed roughly 72 hours after amyloid-beta expression was induced.

Therefore, we hypothesized that vitamin B12-deficiency stimulates amyloid-beta toxicity. Consistent with this, a recent report showed that vitamin B12 prevented amyloid-beta monomer aggregation in a human neuronal cell line (Alam et al. 2017). Thus, following

the treatment of vitamin B12-deficient *C. elegans* expressing amyloid-beta with vitamin B12 we expected to find a decreased rate of amyloid-beta-induced body wall muscle paralysis. In addition, we hypothesized that vitamin B12-deficiency stimulates amyloid-beta toxicity through decreasing methylmalonyl-CoA mutase activity and that the addition of methylmalonic acid or knocking down the methylmalonyl-CoA mutase gene (*mmcm-1*) by RNAi to increase worm methylmalonic acid levels in vitamin B12-proficient worms will stimulate amyloid-beta-mediated body wall muscle paralysis to mimic the effects of vitamin B12 deficiency.

CHAPTER 2. MATERIALS AND METHODS

C. elegans AD Model

The *C. elegans* amyloid-beta expressing strain used for this study was CL4176 [*smg-1(cc546)]; dvIs27, (dvIs27 [myo-3p::A-Beta (1-42)::let-851 3'UTR) + rol-6(su1006)]* showing temperature-inducible expression of amyloid-beta in body wall muscle when upshifted to 25^0 C. The strain was obtained from University of Minnesota *Caenorhabditis* Genetics Center and reared at $15\,^0$C on Nematode Growth Media (NGM) agar plates and fed either the of *E. coli* OP50-1 strain or the HT115(DE3) strain of *E. coli*.

NGM Agar Plate Preparation

To prepare the NGM agar plates 1.5g NaCl, 8.5 g agar, and 1.25 g peptone were dissolved in 500 ml of double deionized water. The solution was autoclaved for 90 minutes and allowed to cool down in a 55^0C water bath for 15 minutes. After cooling, to the media was added 500 µl of 1 M $CaCl_2$, 1 M $MgSO_4$, Cholesterol in ethanol 5mg/ml and 12.5 ml of 1 M KH_2PO_4 followed by mixing thoroughly. 1 ml of 50 mg/ml ampicillin or streptomycin was added to the media based on the strain of *E. coli* that was grown on the plates. 1 ml of 10 mg/ml nystatin dissolved in ethanol was added to the media as an antifungal agent. 15 ml of the prepared NGM was dispensed to make one 90 mm by 15 mm NGM agar plate. After pouring, the plates were placed on a counter to dry and kept in a 15^0C incubator for future use.

E. coli Food Preparation

For this project two *E. coli* strains were used for feeding the worms, OP50 and HT115(DE3). Luria-Bertani (LB) broth media was prepared, autoclaved, and completely cooled down. The media was then inoculated aseptically with the appropriate strain of *E. coli* (OP50 or HT115(DE3)) and antibiotics, then placed in an incubator shaker and shaken at a

speed of 200-250 rpm at 37^0C for overnight growth (12-18 hrs). The overnight media was spun down at 5000g for 10 minutes to obtain the bacterial pellets. The pellets were washed three times to remove all traces of LB broth, then weighed and dissolved in sterile double deionized H_2O at 100 mg/ml concentration. The bacterial food was stored at 4^0C and used for up to 3 weeks. NGM agar plates used for the experiments were seeded with the appropriate *E. coli* food fro overnight growth.

B vitamins Preparation and Concentration

Methylcobalamin which is an active form of was used for this study. *C. elegans*, like mammals, can convert methylcobalamin to adenosylcobalamin. Methylcobalamin was diluted in deionized water at 200 ng/ml. Vitamin B9 (folic acid) and vitamin B6 (pyridoxal-5'-phosphate) were dissolved in double deionized water to a final concentration of 25 µM. 500 µl of the solution containing the compound was added to the surface of 15 ml NGM agar plates and allowed to dry in the absence of light before seeding with the appropriate strain of *E. coli* as a food source.

Amyloid-Beta Peptide Paralysis Assay

Before induction of amyloid-beta peptide expression in the CL4176 *C. elegans* AD strain, a two-generation age-synchronization was performed. First, an age synchronization of a worm population was obtained by picking 30 gravid worms onto a fresh NGM agar plate spread with a lawn of either the *E. coli* OP50-1 strain or the *E. coli* HT115(DE3) strain as a food source. The NGM agar plates were made with 50 mg/ml of the antibiotic streptomycin or ampicillin and 0.01 mg/ml of the antifungal nystatin from a 70% ethanol stock solution. The adult worms were left to lay eggs for 2 hours at 20^0C, after which the gravid worms were removed. The plates were then kept in a $15\ ^0$C incubator for one week. On day 7, 8-10 gravid worms were transferred using a worm pick onto a new NGM agar plate containing the

appropriate *E. coli* strain as food. After laying eggs for 2 hours, gravid worms were removed, and the eggs were kept at 15 ^{0}C for 48 hours until the nematodes developed into the third larval stage (L3). Plates were then transferred to a 25 ^{0}C incubator for another 28 hours to induce amyloid-beta expression. Worms were then checked every 2 hours for the next 20-24 hours for paralysis by prodding each one with a worm pick. Paralyzed worms were counted as those that ceased motion along at least half the length of their body. To test the protective effect of vitamin B12, the appropriate concentration of the drug was added to freshly prepared NGM agar plates and allowed to dry overnight, followed by adding 200 µl of 100 mg/ml 0P50-1 *E. coli* food, which was incubated for at least 8 hours at 37^{0}C. For each experiment, three plates were used, and each plate had at least 75 nematodes.

RNAi Interference Experiments

RNAi experiments were performed using the RNAi feeding method. The HT115(DE3) RNAi bacterial clones from the Ahringer and Vidal global RNAi libraries contain an L4440 plasmid that possesses a 0.5-1.5 kb insert corresponding to the gene of interest, which is flanked on each side by T7 RNA polymerase promoters. 1 mM isopropyl-D-thiogalactopyranoside (IPTG) was used to induce expression of the T7 RNA polymerase gene present in the bacterial genome leading to expression of both strands of the insert on the plasmid. For this method we incubated the HT115(DE3) *E. coli* RNAi-expressing clone with IPTG during the last 4 hours of their 16-hour culture in LB media at 37^{0}C to induce the expression of dsRNA. 50 µg/ml ampicillin added to the culture media. The overnight media was centrifuged for 10 minutes at 5000g. Bacteria was washed up to three times using double deionized water (ddw) and resuspended in ddw concentrated at 100 mg/ml and in the refrigerator until further use.

Knockdown of the vitamin B12-dependent mitochondrial methylmalonyl-CoA mutase *mmcm-1* gene or the vitamin B12-dependent methionine synthase *metr-1* gene was performed by using the RNAi feeding method. 25 µg/ml carbenicillin and 1 mM IPTG was added to the NGM agar plates. The *mmcm-1* and *metr-1 E. coli* RNAi clones are present in the Vidal RNAi library and were obtained from the Dharmacon (Horizon Discovery) company. The Aβ-mediated paralysis assays were performed on synchronized *C. elegans* feeding on the *metr-1*, *mmcm-1*, or empty vector *E. coli* RNAi clones.

Statistical Analysis

The paralysis assay data was analyzed using Sigma plot version 11.0 software using the Kaplan-Meier survival analysis and the Log-Rank test. For each survival curve, the experiment was done in triplicate. P-values < 0.05 were deemed statistically significant.

Effects of Different Diets on Amyloid-Beta Induced Paralysis

This research investigated a potential protective role of vitamin B12 on amyloid-beta induced toxicity in a *C. elegans* AD model. To induce cobalamin deficiency or proficiency, the worms were fed with two different *E. coli* strains, either the vitamin B12-deficient OP50 strain or the vitamin B12-proficient HT115(DE3) strain. Following age-synchronization of *C. elegans* feeding on the appropriate diet, a temperature upshift for 30 hours at the third larval stage was used to induce amyloid-beta peptide expression in the body wall muscle, which leads to paralysis within 48 hours after the temperature upshift.
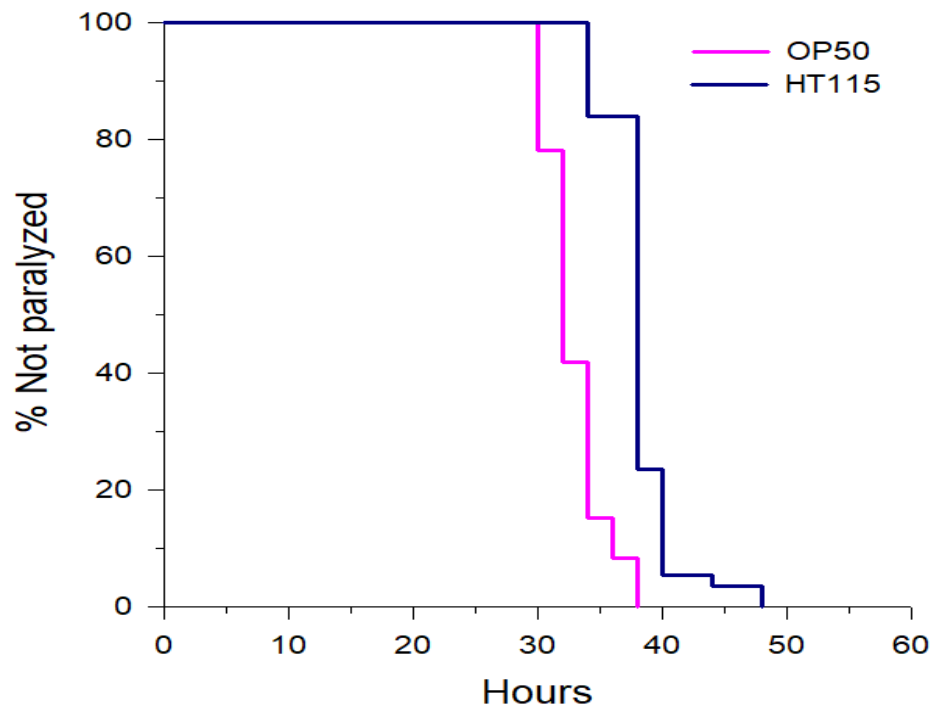

Figure 1: CL4176 Amyloid-Beta expressing Worms fed on the HT115 or the OP50

E. coli Diet

For this experiment, our hypothesis was that the OP50 *E. coli* diet, which is deficient in vitamin B12, will sensitize the worms to amyloid-beta toxicity and the worms would show an increased rate of paralysis, while worms fed the vitamin B12-replete HT115 *E. coli* diet will be protected showing a decreased rate of paralysis. The hypothesis was shown to be correct as the mean time until paralysis when feeding on HT115 was 16% greater than when feeding on OP50 ($p<0.001$) as shown in Figure 1.

Vitamin B12 Supplementation When Feeding the OP50 E. coli Diet

Following the protective effect of the HT115 *E. coli* diet for delaying paralysis in the *C. elegans* AD model compared to the accelerated paralysis in worms that were fed the OP50 diet, the OP50 diet was supplemented with 200 ng/ml of methylcobalamin. This was done to determine if restoration of vitamin B12 levels could mimic the effect of feeding HT115 *E. coli* to decrease the rate of paralysis and protect against amyloid-beta toxicity. However, methylcobalamin addition did not rescue the increased rate of paralysis when worms were fed the vitamin B12-deficient OP50 bacteria as shown in Figure 2 with ($p>0.05$).
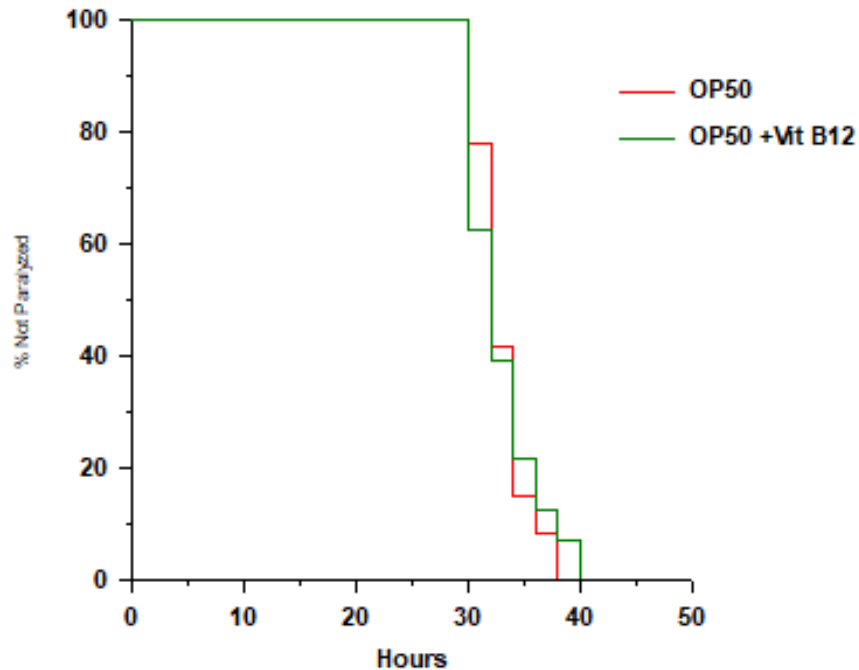
Figure 2: CL4176 *C. elegans* fed OP50 in the absence or presence of

Vitamin B12 (200 ng/ml)

Effects of metr-1 or mmcm-1 Knockdown on the C. elegans AD Model

It was possible in the previous experiment that the supplemented vitamin B12 was not
being efficiently taken up or metabolized by *C. elegans*, thus resulting in the negative result.
Therefore, further experiments were performed to test if decreased vitamin B12 levels could
be responsible for the increased toxicity of amyloid-beta peptide when worms were fed the
OP50 diet. As mentioned above, the only two enzymes in the *C. elegans* genome that use
vitamin B12 as a coenzyme are cytoplasmic methionine synthase (*metr-1*) and mitochondrial
methylmalonyl-CoA mutase (*mmcm-1*). To determine which of these genes may be important
in the protective effects conferred by the vitamin B12 replete HT115 *E. coli* diet, RNAi
knockdown targeting either *metr-1* or *mmcm-1* was performed. For these RNAi experiments
the *C. elegans* were fed on *E. coli* HT115 carrying either an empty vector, or a vector

expressing dsRNA targeted to *metr-1* or *mmcm-1*. If decreased vitamin B12 levels stimulate increased amyloid-beta toxicity, then knockdown of one of the two vitamin B12-dependent enzymes under vitamin B12 proficient conditions should mimic this effect and increase the rate of amyloid-beta-induced paralysis.
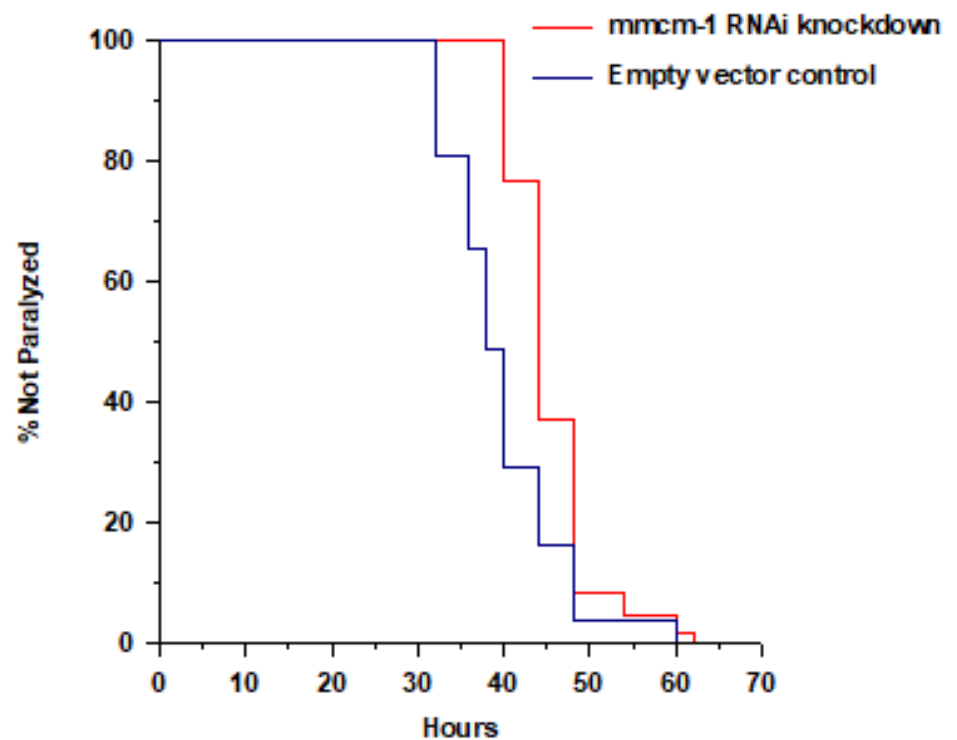
Figure 3: Effects of *mmcm-1* Knockdown on Amyloid-Beta Induced Paralysis in the
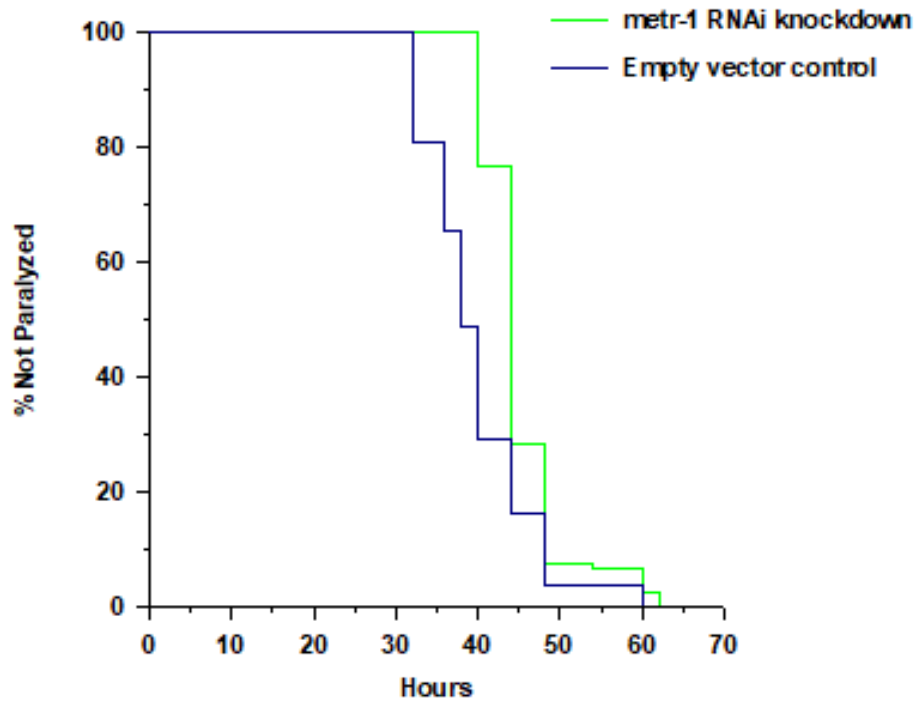
C. elegans AD Model

Figure 4: Effects of *metr-1* Knockdown on Amyloid-Beta Induced Paralysis in the

C. elegans AD Model

The mean time until paralysis when either *mmcm-1* or *metr-1* was knocked down was 20 % greater than the worms fed the HT115 empty vector control *E. coli* diet with (p<0.001) (Figure 3 and Figure 4). Therefore, knockdown of neither of these two vitamin B12-dependent enzymes increased the rate of amyloid-beta mediated paralysis as hypothesized, but instead decreased the rate of paralysis and were protective.

Vitamin B9 and B6 Supplementation When Feeding the OP50 E. coli Diet

To determine if other B vitamins of the folate cycle and one-carbon metabolism are protective against amyloid-beta toxicity in the *C. elegans* amyloid-beta toxicity model, 25 µM of vitamin B9 (folate) or vitamin B6 (pyridoxal-5'-phosphate) was added to the NGM agar plate. *C. elegans* was fed the OP50 *E. coli* diet, the temperature was upshifted to induce amyloid-beta expression, and the worms were scored for paralysis.

Figure 5: CL4176 *C. elegans* fed OP50 in the absence or presence of

Folate (25 µM)

Figure 6: CL4176 *C. elegans* fed OP50 in the absence or presence of

Pyridoxal-5-phosphate (25 µM)

Neither vitamin B9 (Figure 5) or vitamin B6 (Figure 6) was able to reduce the rate of

paralysis and protect against amyloid-beta toxicity in the CL4176 AD worm model.

CHAPTER 4. DISCUSSION

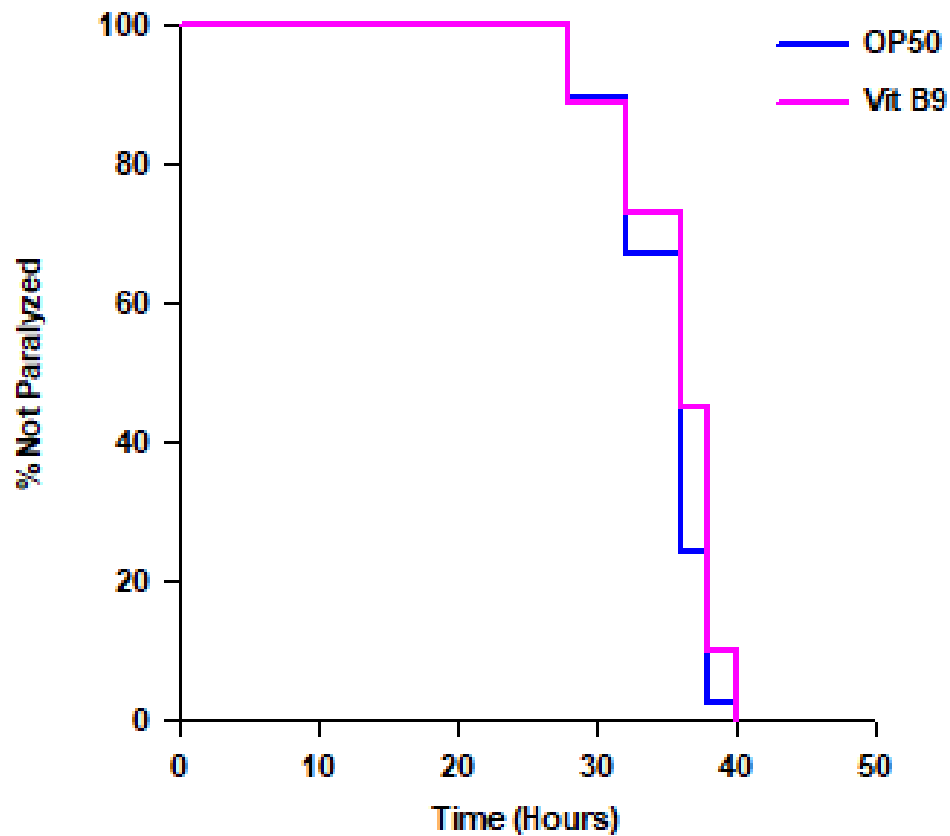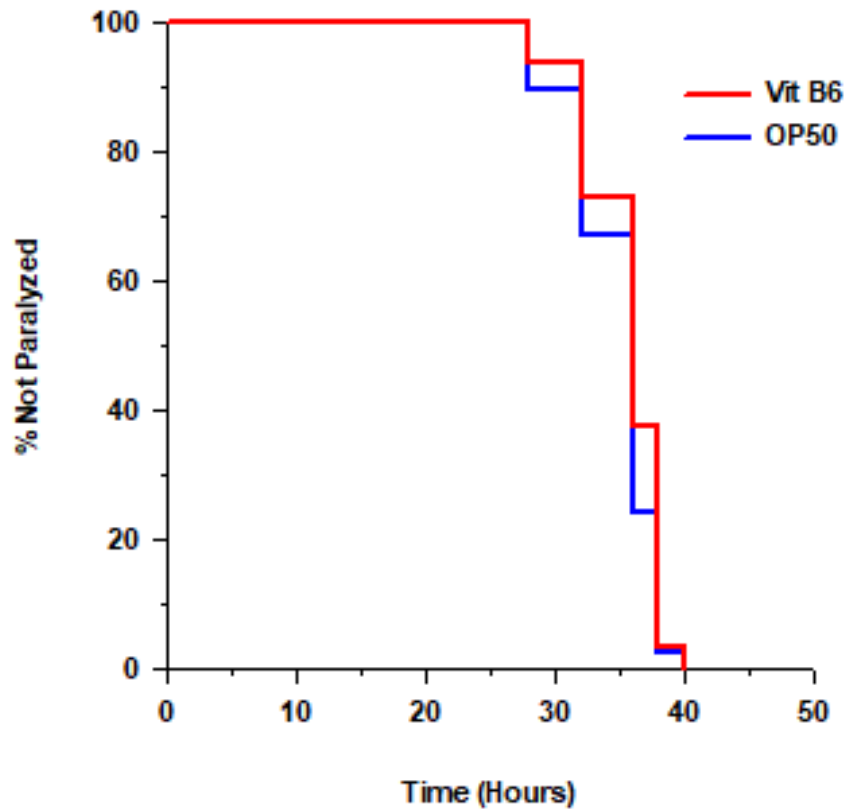Deficiency of the B vitamins is common in the elderly and is often linked with neurodegenerative disorders such as AD, and cardiovascular disease (Mikkelsen, Stojanovska, and Apostolopoulos 2016; Smith and Refsum 2016). Vitamin B6, B9, and B12 are involved in one carbon metabolism, which is crucial for protein and nucleic acid methylation. These three vitamins are also involved in the folate and methionine cycles, which are also important for lowering the level of toxic homocysteine in the blood (Kennedy 2016).

Over the past three decades, clinical trials administering these vitamins involved in one carbon metabolism individually or in combination to treat patients with cognitive decline, dementia or AD have reported conflicting results (Joosten et al. 1997; Clarke et al. 1998; Seshadri et al. 2002; Zhang et al. 2017; Ford and Almeida 2019). Most recently, the increased plasma homocysteine level seen in AD patients was proposed to be a strong, independent risk factor for AD (Smith and Refsum 2016). Hyperhomocysteinemia is associated with deficiencies of vitamins B6, B9, and B12. Discovering protective roles of vitamins functioning in one carbon-metabolism (vitamins B6, B9, and B12) has been a research interest to attempt to slow aging and the onset and progression of neurodegenerative diseases. This study therefore investigated the effects of vitamin B12 deficiency on amyloid-beta toxicity in a *C. elegans* model of AD.

Unpublished studies performed by another student from my lab showed that *C. elegans* fed with vitamin B12-deficient OP50 bacteria as their food became paralyzed more quickly, roughly 48 hours after amyloid-beta expression was induced, but *C. elegans* fed on vitamin B12-proficient HT115(DE3) bacteria became paralyzed roughly 72 hours after amyloid-beta expression was induced. Differences between the two *E. coli* diet were also

noted in the study performed by the Revtowich group in which there was an increased resistance to pathogens, heat, and oxidative stress and improved mitochondrial function in *C. elegans* fed HT115 *E. coli* compared to those fed 0P50 *E. coli* (Revtovich et al. 2019).

C. elegans feeds on different bacteria and fungi in the soil. However, in the laboratory their diet is mainly one of two different strains of *E. coli*. Their most common food source is the OP50 strain, which is a B strain of *E. coli*, and the next most common food source is the HT115 strain, which is a K-12 strain of *E. coli* possessing a mutant RNAse III gene that is mostly used for RNAi feeding experiments. The bacterial food source generally provides macronutrients such as carbohydrates, proteins, fats, lipids, and nucleic acids, but also provides vitamins and other cofactors (Zečić et al. 2019). Both *E. coli* strains have been reported to be composed of different compositions of macro and micronutrients, with HT115 having a higher carbohydrate composition compared to the OP50 strain (Brooks et al. 2009). Nutrition has a significant effect on cellular metabolism and animal health. *C. elegans* reared on these two different *E. coli* strains exhibited different physiological parameters such as lifespan, gene expression profile, fecundity, and growth rate (Stuhr and Curran 2020). The OP50 diet has been reported to contain low levels of vitamin B12, which leads to reduced fertility and a slow developmental rate without an effect on lifespan (Watson et al. 2014).

Like humans, but unlike many bacteria, *C. elegans* must obtain B vitamins from their diet. Therefore, to induce vitamin B12 deficiency the worms were first fed either a vitamin B12-deficient diet or a vitamin B12-replete diet. It was found that the vitamin B12-deficient diet induced an increased rate of amyloid-beta toxicity. Next, since there are several other differences in the nutritional makeup of the diets in addition to differences in vitamin B12 levels, vitamin B12 was added back to the vitamin B12-deficient diet. Surprisingly, adding back vitamin B12 did not rescue the increased rate of paralysis. The results therefore suggest that the protective effect against amyloid-beta-induced paralysis observed for the worms

reared on the HT115 diet is either not related to the vitamin B12 levels in the diet or that the supplemented vitamin B12 for some unknown reason was not restoring the activity of the two vitamin B12-dependent enzymes.

Our initial hypothesis was that vitamin B12-deficiency stimulates amyloid-beta toxicity and sensitizes mitochondria and cells to amyloid-beta toxicity. Therefore, we hypothesized that treatment with vitamin B12 would decrease the established pathological hallmarks of the disease. In addition, we hypothesized that vitamin B12-deficiency will stimulate amyloid-beta toxicity through decreasing methylmalonyl-CoA mutase activity and that knocking down the methylmalonyl-CoA mutase gene (*mmcm-1*) will increase worm methylmalonic acid levels in vitamin B12-proficient worms and stimulate amyloid-beta-mediated toxicity to mimic the effects of vitamin B12 deficiency. Contrary to our hypothesis, results from our knockdown of the *mmcm-1* gene showed a decreased paralysis rate demonstrating a protective effect. Likewise, knockdown of the *metr-1* gene decreased the paralysis rate demonstrating a protective effect. Our results are in contrast to reports from a group that noted an increased rate of paralysis after knockdown of *metr-1* in the *C. elegans* CL2006 AD model that constitutively expresses amyloid-beta in body wall muscle (Leiteritz et al. 2018).

It is likely that activation of the mitochondrial unfolded protein response (UPR[mt]), which can only be robustly induced during larval development, was responsible for the protective effects of *mmcm-1* and *metr-1* knockdown against amyloid-beta toxicity since UPR[mt] induction was shown to protect *C. elegans* from amyloid-beta toxicity (Sorrentino et al. 2018) and deficiency of either *mmcm-1* or *metr-1* was shown to induce UPR[mt] (Amin et al. 2020). It will be important to perform experiments knocking down the *mmcm-1* or *metr-1* genes starting from the first day of adulthood, to decrease the activation of the UPR[mt], using the CL2006 or the similar GMC101 AD strain of worms (Romani et al. 2021). We predict

that the knockdown of *metr-1* or *mmcm-1* would no longer be protective and may even lead to an increased rate of paralysis, as shown by others knocking down *metr-1* in the CL2006 strain (Fan and Chiu 2010; Leiteritz et al. 2018)

Our findings are different from those reported from Revtovich et. al., 2019 in which addition of exogenous methylcobalamin to the OP50 diet protected from pathogen, juglone (a superoxide generator), peroxide, or heat-induced worm death. In those experiments methylcobalamin addition significantly improved *C. elegans* mitochondrial health by increasing mitochondrial fusion and membrane potential. This group also found that *mmcm-1* knockdown in *C. elegans* prevented the protection conferred by methylcobalamin addition. However, they did not study amyloid-beta toxicity, which appears to induce toxicity by a different mechanism than the toxic treatments used in the prior study.

Our results, therefore, do not suggest that vitamin B12 deficiency stimulates amyloid-beta toxicity and suggest that vitamin B12 deficiency may even protect against amyloid-beta toxicity. The results of our study are in part supported by a report (Gagliano Taliun 2019), which used Mendelian randomized sampling of data from 6 different studies and found no link between low plasma vitamin B12 levels and an increased risk for late onset AD. In addition, the rate of AD is lower in India, which due to the high rate of vegetarians in this country, has a much higher rate of vitamin B12 deficiency than in the Unites States (Mathuranath et al. 2012). It should be determined if vitamin B12 deficiency activates the UPR^{mt} in human brain to delay the onset and progression of AD.

Homocysteine metabolism is dependent upon one-carbon metabolism, which requires vitaminsB6, B12, and B9 (folate). Vitamin B12 and vitamin B9 serve as cofactors in the remethylation cycle, which converts homocysteine to methionine, while vitamin B6 is a coenzyme in the transsulfuration pathway that converts homocysteine into cysteine, which is

not only important for protein synthesis, but is also used for the synthesis of the important antioxidant glutathione. (Nieraad et al. 2020). However, the addition of vitamin B9 or B6 did not rescue the increased amyloid-beta toxicity that occurs when worms were fed the OP50 *E. coli* diet in the AD worm model. It is likely that amyloid-beta does not affect vitamin B6 or B9 metabolism to deplete their levels. So, they are likely already present in sufficient amounts so that adding more to the culture media did not have any effect on the enzymes that use them as cofactors.

Conclusion

This study was not able to demonstrate a link between vitamin B12 deficiency and increased Alzheimer's amyloid-beta-mediated toxicity in *C. elegans*. In contrast, knockdown of either of the two enzymes that utilize vitamin B12 decreased the toxicity of amyloid-beta. So, the protection against amyloid-beta toxicity when *C. elegans* feeds on HT115 compared to OP50 *E. coli* is not due to the increased vitamin B12 levels in the HT115 *E. coli*, but must be due to other differences in the nutrient composition between these two strains. Recent studies have shown that the metabolites sucrose, maltose, lactic acid, aspartate, glutamate, lysine, GABA (a neurotransmitter), and betaine (involved in one-carbon metabolism) are present at higher levels in HT115 than OP50 *E. coli* and that oleic acid is present at lower levels in HT115 than OP50. So, one or more of these nutritional differences may explain the protection to *C. elegans* against amyloid-beta toxicity provided by the consumption of the HT115 *E. coli* diet. Consistent with this hypothesis, betaine was shown to protect *C. elegans* from amyloid-beta toxicity (Leiteritz et al. 2018). GABA was shown to improve cognitive function in the APP/PS1 mouse model of AD (Sun et al. 2012) and protect against neurodegeneration in *C. elegans* (Urrutia et al. 2020).

Future Directions

With three of the B vitamins playing essential roles in the one-carbon metabolism pathway, it is possible that a deficiency in any one of these three vitamins increases tau toxicity, but not amyloid-beta toxicity, to stimulate Alzheimer's disease. So similar experiments could be performed with a *C. elegans* strain that overexpresses tau protein and shows a decreased lifespan. Consistent with this idea, a link has been found between increased plasma homocysteine levels and neuronal tau pathology (Regland and McCaddon 2019). So, more research on B vitamins and AD is needed to determine if any important relationships exist that can be targeted therapeutically.

REFERENCES

Aderinwale OG, Ernst HW, Mousa SA. 2010. Current therapies and new strategies for the management of Alzheimers disease. Am J Alzheimers Dis Other Demen. 25:414–424.

Alam P, Siddiqi MK, Chaturvedi SK, Zaman M, Khan RH. 2017. Vitamin B12 offers neuronal cell protection by inhibiting Aβ-42 amyloid fibrillation. Int J Biol Macromol. 99:477–482.

Alexeyev MF, LeDoux SP, Wilson GL. 2006. Mitochondrial DNA and Aging. Handb Model Hum Aging. 364:507–519.

Allen RH. 1998. Relevance of vitamins, homocysteine and other metabolites in neuropsychiatric disorders. Eur J Pediatr Suppl. 157:122–127.

Amin MR, Mahmud SA, Dowgielewicz JL, Sapkota M, Pellegrino MW. 2020. A novel gene-diet interaction promotes organismal lifespan and host protection during infection via the mitochondrial UPR. PLoS Genet. 16:1–24.

Association A. 2019. 2019 Alzheimer's disease facts and figures. Alzheimer's Dement. 15:321–387.

Barrière A, Félix MA. 2014. Isolation of C. elegans and related nematodes. WormBook.:1–19.

Bekris LM, Yu CE, Bird TD, Tsuang DW. 2010. Review article: Genetics of Alzheimer disease. J Geriatr Psychiatry Neurol. 23:213–227.

Bennett DA, Schneider JA, Wilson RS, Bienias JL, Arnold SE. 2004. Neurofibrillary Tangles Mediate the Association of Amyloid Load with Clinical Alzheimer Disease and Level of Cognitive Function. Arch Neurol. 61:378–384.

Bianchetti A, Ranieri P, Margiotta A, Trabucchi M. 2006. Pharmacological treatment of Alzheimer's disease. Aging Clin Exp Res. 18:158–162.

Birch CS, Brasch NE, McCaddon A, Williams JHH. 2009. A novel role for vitamin B12: Cobalamins are intracellular antioxidants in vitro. Free Radic Biol Med. 47:184–188.

Bird TD. 2018. Alzheimer Disease Overview 1 . Clinical Characteristics of Alzheimer Disease. Gene Rev.:1–12.

Bosetti F, Brizzi F, Barogi S, Mancuso M, Siciliano G, Tendi EA, Murri L, Rapoport SI, Solaini G. 2002. Cytochrome c oxidase and mitochondrial F1F0-ATPase (ATP synthase) activities in platelets and brain from patients with Alzheimer's disease. Neurobiol Aging. 23:371–376.

Brenner S. 1974. The genetics of Caenorhabditis elegans. Genetics. 77:71–94.

Brooks KK, Liang B, Watts JL. 2009. The influence of bacterial diet on fat storage in C. elegans. PLoS One. 4

Brusque AM, Borba Rosa R, Schuck PF, Dalcin KB, Ribeiro CAJ, Silva CG, Wannmacher CMD, Dutra-Filho CS, Wyse ATS, Briones P, et al. 2002. Inhibition of the mitochondrial respiratory chain complex activities in rat cerebral cortex by methylmalonic acid. Neurochem Int. 40:593–601.

Butterfield DA. 2002. Amyloid β-peptide (1-42)-induced oxidative stress and neurotoxicity: Implications for neurodegeneration in Alzheimer's disease brain. A review. Free Radic Res. 36:1307–1313.

Cardoso SM, Santana I, Swerdlow RH, Oliveira CR. 2004. Mitochondria dysfunction of Alzheimer's disease cybrids enhances Aβ toxicity. J Neurochem. 89:1417–1426.

Chandler RJ, Zerfas PM, Shanske S, Sloan J, Hoffmann V, DiMauro S, Venditti CP. 2009. Mitochondrial dysfunction in mut methylmalonic acidemia . FASEB J. 23:1252–1261.

Chen X, Barclay JW, Burgoyne RD, Morgan A. 2015. Using C. elegans to discover therapeutic compounds for ageing-associated neurodegenerative diseases. Chem Cent J. 9:1–20.

Chen Z, Zhong C. 2014. Oxidative stress in Alzheimer's disease. Neurosci Bull. 30:271–281.

Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, Ueland PM. 1998. Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. Arch Neurol. 55:1449–1455.

Consortium TC elegans S. 1998. Genome sequence of the nematode C. elegans: A platform for investigating biology. Science. 282:2012–2018.

Corsi AK, Wightman B, Chalfie M. 2015. A Transparent window into biology: A primer on Caenorhabditis elegans. WormBook.:1–31.

Csordás G, Thomas AP, Hajnóczky G. 1999. Quasi-synaptic calcium signal transmission between endoplasmic reticulum and mitochondria. EMBO J. 18:96–108.

Culetto and Satelle. 2000. A role for Caenorhabditis elegans in understanding the function and interactions of human disease genes. Hum Mol Genet. 9:869–877.

Davies P, Maloney AJF. 1976. Selective loss of central cholinergic neurons in alzheimer's disease. Lancet. 308:1403.

de Jager, C. A. 2014. Critical levels of brain atrophy associated with homocysteine and cognitive decline. Neurobiology of Aging. 35:S35–S39.

Dickinson DJ, Goldstein B. 2016. CRISPR-based methods for caenorhabditis elegans genome engineering. Genetics. 202:885–901.

Dimitriadi M, Hart AC. 2010. Neurodegenerative disorders: Insights from the nematode Caenorhabditis elegans. Neurobiol Dis. 40:4–11.

Engel MFM. 2009. Membrane permeabilization by Islet Amyloid Polypeptide. Chem Phys Lipids. 160:1–10.

Erkkinen MG, Kim M, Geschwind MD. 2018. Major Neurodegenerative Diseases. 1–44.

Fan LY, Chiu MJ. 2010. Pharmacological treatment for Alzheimer's disease: Current approaches and future strategies. Acta Neurol Taiwan. 19:228–245.

Ferreira ST, Lourenco M V., Oliveira MM, De Felice FG. 2015. Soluble amyloid-β oligomers as synaptotoxins leading to cognitive impairment in Alzheimer's disease. Front Cell Neurosci. 9:1–17.

Ford AH, Almeida OP. 2019. Effect of Vitamin B Supplementation on Cognitive Function in the Elderly: A Systematic Review and Meta-Analysis. Drugs and Aging. 36:419–434.

Francis PT, Palmer AM, Snape M, Wilcock GK. 1999. The cholinergic hypothesis of Alzheimer's disease: A review of progress. J Neurol Neurosurg Psychiatry. 66:137–147.

Frost B, Jacks RL, Diamond MI. 2009. Propagation of Tau misfolding from the outside to the inside of a cell. J Biol Chem. 284:12845–12852.

Gagliano Taliun SA. 2019. Genetic determinants of low vitamin B12 levels in Alzheimer's disease risk. Alzheimer's Dement Diagnosis, Assess Dis Monit. 11:430–434.

Gherasim C, Hannibal L, Rajagopalan D, Jacobsen DW, Banerjee R. 2013. The C-terminal domain of CblD interacts with CblC and influences intracellular cobalamin partitioning. Biochimie. 95:1023–1032.

Gherasim C, Lofgren M, Banerjee R. 2013. Navigating the B12 road: Assimilation, delivery, and disorders of cobalamin. J Biol Chem. 288:13186–13193.

Giau V Van, Bagyinszky E, Yang YS, Youn YC, An SSA, Kim SY. 2019. Genetic analyses of early-onset Alzheimer's disease using next generation sequencing. Sci Rep. 9:1–10.

Grützner N, Heilmann RM, Stupka KC, Rangachari VR, Weber K, Holzenburg A, Suchodolski JS, Steiner JM. 2013. Serum homocysteine and methylmalonic acid concentrations in Chinese Shar-Pei dogs with cobalamin deficiency. Vet J. 197:420–426.

H. Ferreira-Vieira T, M. Guimaraes I, R. Silva F, M. Ribeiro F. 2016. Alzheimer's disease: Targeting the Cholinergic System. Curr Neuropharmacol. 14:101–115.

Haijes HA, Jans JJM, Tas SY, Verhoeven-Duif NM, van Hasselt PM. 2019. Pathophysiology of propionic and methylmalonic acidemias. Part 1: Complications. J Inherit Metab Dis. 42:730–744.

Hardy J, Allsop D. 1991. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. Trends Pharmacol Sci. 12:383–388.

Hauptmann S, Scherping I, Dröse S, Brandt U, Schulz KL, Jendrach M, Leuner K, Eckert A, Müller WE. 2009. Mitochondrial dysfunction: An early event in Alzheimer pathology accumulates with age in AD transgenic mice. Neurobiol Aging. 30(10):1574–1586.

Hirai K, Aliev G, Nunomura A, Fujioka H, Russell RL, Atwood CS, Johnson AB, Kress Y, Vinters H V, Tabaton M, et al. 2001. Mitochondrial Abnormalities in Alzheimer's Disease. 21:3017–3023.

Huang WJ, Zhang X, Chen WW. 2016. Role of oxidative stress in Alzheimer's disease (review). Biomed Reports. 4:519–522.

Janson J, Ashley RH, Harrison D, Mcintyre S, Butler PC. 1999 The Mechanism of Islet Amyloid Polypeptide. Diabetes. 48:491–498.

Joosten E, Lesaffre E, Riezler R, Ghekiere V, Dereymaeker L, Pelemans W, Dejaeger E. 1997. Is metabolic evidence for vitamin B-12 and folate deficiency more frequent in elderly patients with Alzheimer's disease? Journals Gerontol - Ser A Biol Sci Med Sci. 52:76–79.

Kaletta T, Hengartner MO. 2006. Finding function in novel targets: C. elegans as a model organism. Nat Rev Drug Discov. 5:387–399.

Kennedy DO. 2016. B vitamins and the brain: Mechanisms, dose and efficacy—A review. Nutrients. 8.

Khodadadi S, Riazi GH, Ahmadian S, Hoveizi E, Karima O, Aryapour H. 2012. Effect of N-homocysteinylation on physicochemical and cytotoxic properties of amyloid β-peptide. FEBS Lett. 586:127–131.

Kozyraki R, Cases O. 2013. Vitamin B12 absorption: Mammalian physiology and acquired and inherited disorders. Biochimie. 95:1002–1007.

Kräutler B. 2012. Biochemistry of B12-cofactors in human metabolism. Subcell Biochem. 56:323–46.

Krishnan KJ, Ratnaike TE, De Gruyter HLM, Jaros E, Turnbull DM. 2012. Mitochondrial DNA deletions cause the biochemical defect observed in Alzheimer's disease. Neurobiol Aging. 33:2210–2214.

Laquale K. 2006. B-complex vitamins' role in energy release. Athl Ther Today. 11:70–73.

Leiteritz A, Dilberger B, Wenzel U, Fitzenberger E. 2018. Betaine reduces β-amyloid-induced paralysis through activation of cystathionine-β-synthase in an Alzheimer model of Caenorhabditis elegans. Genes Nutr. 13:1–7.

Link CD. 1995. Expression of human β-amyloid peptide in transgenic Caenorhabditis elegans. Proc Natl Acad Sci U S A. 92:9368–9372.

Link CD. 2006. C. elegans models of age-associated neurodegenerative diseases: Lessons from transgenic worm models of Alzheimer's disease. Exp Gerontol. 41:1007–1013.

Liu JL, Fan YG, Yang ZS, Wang ZY, Guo C. 2018. Iron and Alzheimer's disease: From pathogenesis to therapeutic implications. Front Neurosci. 12:1–14.

Lustbader JW, Cirilli M, Lin C, Xu HW, Takuma K, Wang N, Caspersen C, Chen X, Pollak S, Chaney M, et al. 2004. ABAD Directly Links Aβ to Mitochondrial Toxicity in Alzheimer's Disease. Science. 304:448–452.

Ma L, Zhao Y, Chen Y, Cheng B, Peng A, Huang K. 2018. Caenorhabditis elegans as a model system for target identification and drug screening against neurodegenerative diseases. Eur J Pharmacol. 819:169–180.

MacNeil LT, Watson E, Arda HE, Zhu LJ, Walhout AJM. 2013. Diet-induced developmental acceleration independent of TOR and insulin in C. elegans. Cell. 153:240–252.

Maresova P, Klimova B, Novotny M, Kuca K. 2016. Alzheimer's and Parkinson's Diseases: Expected Economic Impact on Europe-A Call for a Uniform European Strategy. J Alzheimer's Dis. 54:1123–1133.

Markesbery WR. 1997. Oxidative stress hypothesis in Alzheimer's disease. Free Radic Biol Med. 23:134–147.

Mathuranath PS, George A, Ranjith N, Justus S, Kumar MS, Menon R, Sarma PS, Verghese J. 2012. Incidence of Alzheimer's disease in India: A 10 years follow-up study. Neurol India. 60:625–630.

Mattson MP, Cheng B, Davis D, Bryant K, Lieberburg I, Rydel RE. 1992. β-Amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicity. J Neurosci. 12:376–389.

Maynard C, Weinkove D. 2020. Bacteria increase host micronutrient availability: Mechanisms revealed by studies in C. elegans. Genes Nutr. 15:1–11.

Mikkelsen K, Stojanovska L, Apostolopoulos V. 2016. The Effects of Vitamin B in Depression. Curr Med Chem. 23:4317–4337.

Mikkelsen K, Stojanovska L, Tangalakis K, Bosevski M, Apostolopoulos V. 2016. Cognitive decline: A vitamin B perspective. Maturitas. 93:108–113.

Miller AL. 2003. The methionine-homocysteine cycle and its effects on cognitive diseases. Altern Med Rev. 8:7–19.

Morris GP, Clark IA, Vissel B. 2014. Inconsistencies and Controversies Surrounding the Amyloid Hypothesis of Alzheimer's Disease. Acta Neuropathol Commun. 2:1–21.

Morris MS. 2012. The Role of B Vitamins in Preventing and Treating. :801–812.

Morrison JH, Hof PR. 2007. Life and Death of Neurons in The Aging Cerebral Cortex. Int Rev Neurobiol. 81:41–57.

Murphy MP, Levine H. 2010. Alzheimer's Disease and the Beta-Amyloid Peptide. J Alzheimer's Dis. 19:1–17.

Nieraad H, de Bruin N, Arne O, Hofmann MCJ, Schmidt M, Saito T, Saido TC, Gurke R, Schmidt D, Till U, et al. 2020. Impact of hyperhomocysteinemia and different dietary interventions on cognitive performance in a knock-in mouse model for alzheimer's disease. Nutrients. 12:1–27.

O'Reilly LP, Luke CJ, Perlmutter DH, Silverman GA, Pak SC. 2014. C. elegans in high-throughput drug discovery. Adv Drug Deliv Rev. 69–70:247–253.

Ono K, Tsuji M. 2020. Protofibrils of amyloid-β are important targets of a disease-modifying approach for alzheimer's disease. Int J Mol Sci. 21:1–13.

Oussalah A, Levy J, Filhine-Trésarrieu P, Namour F, Guéant JL. 2017. Association of TCN2 rs1801198 c.776G>C polymorphism with markers of one-carbon metabolism and related diseases: A systematic review and meta-analysis of genetic association studies. Am J Clin Nutr. 106:1142–1156.

Prince M, Ali GC, Guerchet M, Prina AM, Albanese E, Wu YT. 2016. Recent global trends in the prevalence and incidence of dementia, and survival with dementia. Alzheimer's Res Ther. 8.

Regland B, McCaddon A. 2019. Alzheimer's Amyloidopathy: An Alternative Aspect. J Alzheimer's Dis. 68:483–488.

Reitz C, Mayeux R. 2014. Alzheimer disease: Epidemiology, Diagnostic Criteria, Risk Factors and Biomarkers. Biochem Pharmacol. 88:640–651.

Revtovich A V., Lee R, Kirienko N V. 2019. Interplay between mitochondria and diet mediates pathogen and stress resistance in caenorhabditis elegans. PLoS Genet. 15:1–27.

Ricciarelli R, Fedele E. 2017. The Amyloid Cascade Hypothesis in Alzheimer's Disease: It's Time to Change Our Mind. Curr Neuropharmacol. 15:926–935.

Romani M, Sorrentino V, Oh CM, Li H, de Lima TI, Zhang H, Shong M, Auwerx J. 2021. NAD+ boosting reduces age-associated amyloidosis and restores mitochondrial homeostasis in muscle. Cell Rep. 34:108660.

Scheltens P, Blennow K, Breteler MMB, de Strooper B, Frisoni GB, Salloway S, Van der Flier WM. 2016. Alzheimer's disease. Lancet. 388:505–517.

Schuck PF, Januário SB, Simon KR, Scaini G, Mafioleti RL, Malgarin F, Pettenuzzo LF, Streck EL, Ferreira GC. 2013. Acute renal failure potentiates brain energy dysfunction elicited by methylmalonic acid. Int J Dev Neurosci. 31:245–249.

Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT. 2011. Neuropathological alterations in Alzheimer disease. Cold Spring Harb Perspect Med. 1:1–23.

Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, Wilson PWF, Wolf PA. 2002. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med. 346:476–483.

Sheng M, Sabatini BL, Su TC. 2018. Synapses and Alzheimer's Disease. Cold Spring Harb Perspect Biol.:1–18.

Sin O, Michels H, Nollen EAA. 2014. Genetic screens in Caenorhabditis elegans models for neurodegenerative diseases. Biochim Biophys Acta - Mol Basis Dis. 1842:1951–1959.

Singh P, Suman S, Chandna S, Das TK. 2009. Possible role of amyloid-beta, adenine nucleotide translocase and cyclophilin-D interaction in mitochondrial dysfunction of Alzheimer's disease. Bioinformation. 3:440–445.

Singh SK, Srivastav S, Yadav AK, Srikrishna S, Perry G. 2016. Overview of Alzheimer's disease and some therapeutic approaches targeting A β by using several synthetic and herbal compounds. Oxid Med Cell Longev. 2016.

Small SA, Duff K. 2008. Linking Aβ and Tau in Late-Onset Alzheimer's Disease: A Dual Pathway Hypothesis. Neuron. 60:534–542.

Smith AD. 2008. The worldwide challenge of the dementias: A role for B vitamins and homocysteine? Food Nutr Bull. 29

Smith AD, Refsum H. 2016. Homocysteine, B Vitamins, and Cognitive Impairment. Annu Rev Nutr. 36:211–239.

Smith AD, Refsum H, Bottiglieri T, Fenech M, Hooshmand B, McCaddon A, Miller JW, Rosenberg IH, Obeid R. 2018. Homocysteine and Dementia: An International Consensus Statement. J Alzheimer's Dis. 62:561–570.

Sorrentino V, Romani M, Mouchiroud L, Beck JS, Amico DD, Moullan N, Potenza F, Schmid AW, Rietsch S, Counts SE, et al. 2018. HHS Public Access. 552:187–193.

Stuhr NL, Curran SP. 2020. Bacterial diets differentially alter lifespan and healthspan trajectories in C. elegans. Commun Biol. 3:653.

Sun X, Meng X, Zhang J, Li Y, Wang L, Qin X, Sui N, Zhang Y. 2012. GABA attenuates amyloid toxicity by downregulating its endocytosis and improves cognitive impairment. J Alzheimer's Dis. 31:635–649.

Surendran S, Adaikalakoteswari A, Saravanan P, Shatwaan IA, Lovegrove JA, Vimaleswaran KS. 2018. An update on vitamin B12-related gene polymorphisms and B12 status. Genes Nutr. 13:1–35.

Swerdlow RH, Burns JM, Khan SM. 2010. The Alzheimer's disease mitochondrial cascade hypothesis. J Alzheimer's Dis. 20

Swerdlow RH, Khan SM. 2004. A "mitochondrial cascade hypothesis" for sporadic Alzheimer's disease. Med Hypotheses. 63:8–20.

Teschendorf D, Link CD. 2009. What have worm models told us about the mechanisms of neuronal dysfunction in human neurodegenerative diseases? Mol Neurodegener. 4

Tillement L, Lecanu L, Papadopoulos V. 2011. Alzheimer's disease: Effects of β-amyloid on mitochondria. Mitochondrion. 11:13–21.

Tillement L, Lecanu L, Yao W, Greeson J, Papadopoulos V. 2006. The spirostenol (22R, 25R)-20α-spirost-5-en-3β-yl hexanoate blocks mitochondrial uptake of Aβ in neuronal cells and prevents Aβ-induced impairment of mitochondrial function. Steroids. 71:725–735.

Tu S, Okamoto S ichi, Lipton SA, Xu H. 2014. Oligomeric Aβ-induced synaptic dysfunction in Alzheimer's disease. Mol Neurodegener. 9:48.

Urrutia A, García-Angulo VA, Fuentes A, Caneo M, Legüe M, Urquiza S, Delgado SE, Ugalde J, Burdisso P, Calixto A. 2020. Bacterially produced metabolites protect C. elegans neurons from degeneration. 18.

Van Der Zwaluw, L. N, Dhonukshe-Rutten RAM, Wijngaarden JP van, Brouwer-Brolsma EM, Rest O van de, Veld PH In 't, Enneman AW, Dijk SC van, Ham AC, Swart KMA, et al. 2014. Results of 2-year vitamin B treatment on cognitive performance: Secondary data from an RCT. Neurology. 83:2158–2166.

Wallon D, Rovelet-lecrux A, Richard A, Pasquier F, Lacour M, Rollin-sillaire A, Martinaud O, Quillard-muraine M, De V, Boutoleau-bretonniere C, et al. 2017. APP , PSEN1 , and PSEN2 mutations in early- onset Alzheimer disease : A genetic screening study of familial and sporadic cases. PLoS Med. 1:1–16.

Wang X, Wang W, Li L, Perry G, Lee H, Zhu X. 2014. Oxidative Stress and Mitochondrial Dysfunction in Alzheimer's Disease. Biochim Biophys Acta. 1842:1240–1247.

Watanabe F, Bito T, Matsunaga Y, Yabuta Y, Kawano T. 2013. Vitamin B12 deficiency in Caenorhabditis elegans results in loss of fertility, extended life cycle, and reduced lifespan. FEBS Open Bio. 3:112–117.

Watson E, Macneil LT, Ritter AD, Yilmaz LS, Rosebrock AP, Caudy AA, Walhout AJM. 2014. Interspecies systems biology uncovers metabolites affecting C. elegans gene expression and life history traits. Cell. 156:759–770.

Watson E, Yilmaz LS, Walhout AJM. 2015. Understanding Metabolic Regulation at a Systems Level: Metabolite Sensing, Mathematical Predictions, and Model Organisms. Annu Rev Genet. 49:553–575.

Wu Y, Wu Z, Butko P, Christen Y, Lambert MP, Klein WL, Link CD, Luo Y. 2006. Amyloid-β-induced pathological behaviors are suppressed by Ginkgo biloba extract EGB 761 and ginkgolides in transgenic Caenorhabditis elegans. J Neurosci. 26:13102–13113.

Xiong H, Pears C, Woollard A. 2017. An enhanced C. elegans based platform for toxicity assessment. Sci Rep. 7:1–11.

Yiannopoulou KG, Papageorgiou SG. 2013. Current and future treatments for Alzheimer's disease. Ther Adv Neurol Disord. 6:19–33.

Zečić A, Dhondt I, Braeckman BP. 2019. The nutritional requirements of Caenorhabditis elegans. Genes Nutr. 14:1–13.

Zhang DM, Ye JX, Mu JS, Cui XP. 2017. Efficacy of Vitamin B Supplementation on

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and Meta-Analysis. J Geriatr Psychiatry Neurol. 30:50–59.

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