ROLE OF HYPOXIA INDUCIBLE FACTOR (HIF) 1 α IN THIAMINE INSUFFICIENCY MEDIATED ALZHEIMER DISEASE LIKE PATHOLOGY

by

MARIA LUISA VALLE

(Under the Direction of Jason Zastre)

ABSTRACT

Insufficiencies of the micronutrient thiamine (Vitamin B1) have been associated with inducing Alzheimer's Disease (AD)-like neuropathology. Thiamine is a critical enzyme cofactor within the glycolytic metabolic network that is fundamentally required to sustain the bioenergetic and anabolic needs of all cells. The hypometabolic state associated with chronic thiamine insufficiency (TI) has been demonstrated to be a contributor towards the development of amyloid plaque deposition and neurotoxicity within the thalamus, hippocampus, and cortex. However, the molecular mechanism underlying TI induced AD pathology is still unresolved. Previously, we have established that TI stabilizes the metabolic stress transcriptional factor, Hypoxia Inducible Factor-1α (HIF1α). Utilizing neuronal hippocampal cells, TI-induced HIF1α activation triggered the amyloidogenic cascade through transcriptional activation and increased activity of β-secretase (BACE1). Knockdown and pharmacological inhibition of HIF1α significantly reduced BACE1 and C99 formation. TI also increased the expression of the HIF1α regulated pro-apoptotic protein, BNIP3. Correspondingly, cell toxicity during TI conditions was significantly reduced with HIF1α

and BNIP3 knockdown. The role of BNIP3 in TI-mediated toxicity was further highlighted by localization of dimeric BNIP3 into the mitochondria and nuclear accumulation of Endonuclease G. Cell toxicity via the HIF1α/BNIP3 cascade required TI induced oxidative stress. TI decreased mitochondrial membrane potential and enhanced chromatin fragmentation. In addition, we observed that HIF1α, BACE1 and BNIP3 expression was induced in 3xTg AD mice after TI. Treatment with the HIF1α inhibitor YC1 significantly attenuated HIF1α and target genes levels. Overall, these findings demonstrate a critical stress response during TI involving the induction of HIF1α transcriptional activity that directly promotes neurotoxicity and AD-like pathology.

INDEX WORDS: Thiamine, HIF1α, Alzheimer disease, neurodegeneration, aging, hypoxia

ROLE OF HYPOXIA INDUCIBLE FACTOR (HIF) 1 α IN THIAMINE INSUFFICIENCY MEDIATED ALZHEIMER DISEASE LIKE PATHOLOGY

by

MARIA LUISA VALLE

BS, University of Bari (Italy), 2012

MS, University of Bari (Italy), 2014

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2021

ROLE OF HYPOXIA INDUCIBLE FACTOR (HIF) 1 α IN THIAMINE INSUFFICIENCY MEDIATED ALZHEIMER DISEASE LIKE PATHOLOGY

by

MARIA LUISA VALLE

Major Professor:	Jason Zastre
Committee:	Jesse Schank
	Neil Grimsey
	Phillip Greenspan

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
December 2021

DEDICATION

This thesis is dedicated to my parents, Mario and Leonarda.

They taught me the importance of education and encouraged me to follow my dreams. Thank you for your constant support and for believing in me! I hope this achievement makes you proud!

To all the teachers that guided me from kindergarten to Graduate school: thank you for helping me growing as a student and as a person.

And a special thanks to all the Angels that look after me- wherever they are.

ACKNOWLEDGEMENTS

Thanks to my parents for always being supportive and encouraging. Comin to the US meant new opportunities for me, but for you meant having your only daughter on the other side of the world! I am really lucky to have such great parents!

When I started to study Biotechnology at the University of Bari, I would have never imagined pursuing a PhD in the US. I need to thank my mentor at the university of Bari, Dr. Tortorella for having me in his lab as undergrad and master student. That's how my interest in pharmaceutical sciences started.

A special thanks to my Italian Biotech friends Ada, Valerio, and Gabriella for keeping up with me. Looking forward to celebrating with you guys in Italy!

During these six years in Georgia, I was really lucky to meet wonderful people that helped me during this journey.

Dr. Yeudall, thank you for taking me into your lab at Augusta and for your trust.

Linah, thank you for teaching me how to work in the lab. You are a great scientist and the bravest woman I know. Hope you will forgive me one day for having preferred UGA over Augusta!

Silvia, you are an amazing friend – thank you for taking care of me during these years! Thanks for all the homemade Italian lunches and dinners, for all the teas and coffees! Thank you for introducing me to your lab mates that are now my friends as well!

Dear Don, you are my guardian angel and my family here. Thank you for watching over me during those years. I would not have made it without your help. Thank you for listening to me almost every day – I am sure you are an expert on thiamine by now! It does not matter how far we are; you were and always will be my reference.

I was also lucky to have great roommates during these years!

Sydney, staying with you and the lovely Piper Louise really helped me managing the stress of classes and exams. Mona, thanks to you and to your wonderful family for taking care of me during the last two years. I really felt at home even if it was the first Christmas far from my family. Not everybody would have welcomed a stranger in their home during a pandemic!

Yasmin, the lab was pretty lonely for the majority of the time, and I was so happy that you came last year! Thank for helping me during the past months - I know that the lab is in good hands now.

To my committee members: thank you for your time and expertise.

Last but not least, thanks to my PI, Dr. Zastre.

Thank you for your support, patience, time, and guidance during these years- especially during the pandemic. Thank you for being a great and dedicate mentor and for helping me becoming a better scientist.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

ABBREVIATIONS

Aβ: Amyloid β peptides

AD: Alzheimer's Disease

AICD: Amyloid intracellular domain

ALD: Aldolase

ApoE: Apolipoprotein E

APP: Amyloid Precursor Protein

ATP: Adenosine Triphosphate

BACE: Beta secretase

bHLH: Basic Helix-Loop-Helix

BBB: Blood Brain Barrier

BCKDH: Branched Chain Ketoacid Dehydrogenase

BNIP3: BCL2/adenovirus E1B 19 kDa protein-interacting protein 3

BrdU: Bromodeoxyuridine

C83: C-terminal fragment of 83 amino acids

C99: C-terminal Fragment of 99 amino acids

CMR: Cerebral Metabolic Rate

CREB: cAMP Response Element-Binding Protein

CSF: Cerebrospinal Fluid

CTL: Control

DMOG: Dimethyloxallyl Glycine

DMSO: Dimethyl Sulfoxide

DFO: Deferoxamine Mesylate

EDTA: Ethylenediaminetetraacetic acid

EGTA: Ethylene glycol-bis (β-aminoethyl ether)-N, N, N’, N'-tetra acetic acid

ELISA: Enzyme-Linked Immunosorbent Assay

EP: Ethyl Pyruvate

EPO: Erythropoietin

FBS: Fetal Bovine Serum

FDG:(18) F-fluorodeoxyglucose

FIH: Factor Inhibiting HIF

Glu: Glutamate

GLUT: Glucose Transporter

HIF: Hypoxia Inducible Factor

HRE: Hypoxic Responsive Element

HRP: Horseradish Peroxidase

IL: Interleukin

IP: Intraperitoneal

KG: α-Ketoglutarate

KGDH: α-Ketoglutarate Dehydrogenase

KS: Korsakoff Syndrome

LDHA: Lactate Dehydrogenase

MCAO: middle cerebral artery occlusion

MQ: Mitoquinone

MMP: Mitochondrial Membrane Potential

MPTP: Mitochondrial Translocator Protein

NAC: N-acetyl Cysteine

NAD: Nicotinamide Adenine Dinucleotide

NADP: Nicotinamide Adenine Dinucleotide Phosphate

NFkB: Nuclear Factor kappa-light-chain-enhancer of activated B cells

NMR: Nuclear Magnetic Resonance

NOS: Nitric Oxide Synthase

NP40: Nonidet P40

OCT: Organic Cation Transporter

P3: 3kDa Protein fragments

PAS: Per-Arnt-Sim

PBS: Phosphate Buffered Saline

PCR: Polymerase Chain Reaction

PD: Parkinson's Disease

PET: Positron Emission Tomography

PHD: Prolyl Hydroxylase

PMSF: phenylmethane sulfonyl fluoride

PPP: Pentose Phosphate Pathway

PSEN: Presenilin

PT: Pyrithiamine Hydrobromide

RFC1: Reduced Folate Carrier 1

ROS: Reactive Oxygen Species

SDS: Sodium Dodecyl Sulfate

sGC: Soluble Guanyl Cyclase

TBS-T: Tris Buffered Saline-Tween 20

TCA: Tricarboxylic Acid

THTR: Thiamine Transporter

TI: Thiamine Insufficiency

TKT: Transketolase

TMP: Thiamine Monophosphate

TNFα: Tumor Necrosis Factor-α

TPC: Thiamine Pyrophosphate Carrier

TPK1: Thiamine Pyrophosphokinase 1

TPP: Thiamine Pyrophosphate

TPPT: Thiamine Pyrophosphate Transporter

VEGF: Vascular Endothelial Growth Factor

VHL: von Hippel Lindau Protein

WCL: Whole Cell Lysate

WE: Wernicke's Encephalopathy

WKS: Wernicke-Korsakoff Syndrome

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Alzheimer Disease

Dementia is a broad term used to describe diseases characterized by progressive deterioration in cognition, function, and behavior. The current prevalence of dementia is estimated to 24 million and predicted to quadruple by the year 2050 (Paroni, Bisceglia et al. 2019). The most frequent type of dementia is Alzheimer's Disease (AD). AD is the fifth leading cause of death in Americans aged 65 and older and it is associated with estimated health-care costs of $172 billion per year (Hendrie, Albert et al. 2006). The most common form of AD is late onset AD affecting subjects at an age of 65 years. About 5% of all AD cases have an early-onset reported in subjects aged from 30 years to 65 years (Mendez 2017) .

AD results in a progressive neurodegeneration and shrinkage of the brain. AD warning signs include memory loss, decreased problem-solving abilities, confusion, impairment in speech and writing, poor judgement and change in personality. Besides these signs, AD etiology is still under investigation. Recent studies were able to locate the first areas of damage within the medial temporal lobe (MTL) structures, in the hippocampus, responsible for the formation of new memories (Bonner-Jackson, Mahmoud et al. 2015). Therefore, MTL atrophy is associated with episodic memory impairment which progressively declines over the course of the disease. In the successive stages, the damage progresses to the occipital lobe involving the areas that control language, to the cortex and, in the worse scenario, to the cerebellum causing movement impairment. Rare AD forms may include specific lobes such as the Posterior cortical atrophy

(PCA) which affects the parietal and occipital lobes, frontotemporal dementia in the frontal area and the Lewy Bodies dementia within the cortex (Nelson, Sweeney et al. 2016).

Various factors such as stroke, high blood pressure and hypercholesterolemia, diabetes, oxidative stress and inflammation, as well as sedentary lifestyle have been associated with AD. Despite their heterogenicity, they all converge to molecular mechanisms centered on amyloid plaques and tau neurofibrillary tangles formation, both recognized as AD hallmarks (Figure 1.1).

1.2 Alzheimer Pathology

The major component of the amyloid plaques is the amyloid-β (Aβ) peptide which derives from the amyloid precursor protein (APP). The APP can be processed through two alternative pathways. In the nonamyloidogenic pathway APP is cleaved between amino acids 16 and 17 by α-secretase, which releases a soluble APP ectodomain and a membrane-bound carboxy-terminal fragment of 83 amino acids (C83). C83 can be degraded by lysosomes or further processed by γ-secretase to produce a short hydrophobic nonamyloidogenic peptides that are collectively called p3 fragments (p3) (Rodrigue, Kennedy et al. 2009).

In the amyloidogenic pathway the cleavage by β-site APP-cleaving enzyme (BACE1) forms a C-terminal fragment of 99 amino acids (C99) which is the direct precursor to Aβ after the cleavage of γ-secretase (O'Brien and Wong 2011). When Aβ production reaches a threshold, the peptide self-aggregates to form toxic oligomers that trigger degeneration of neuronal cells (Evin and Barakat 2014). Neurofibrillary tangles are composed of highly phosphorylated forms of the microtubule- associated protein tau. Tau is the major microtubule associated protein (MAP) of a normal mature neuron which interacts with tubulin and promotes its assembly into microtubules and stabilization of the microtubule network.

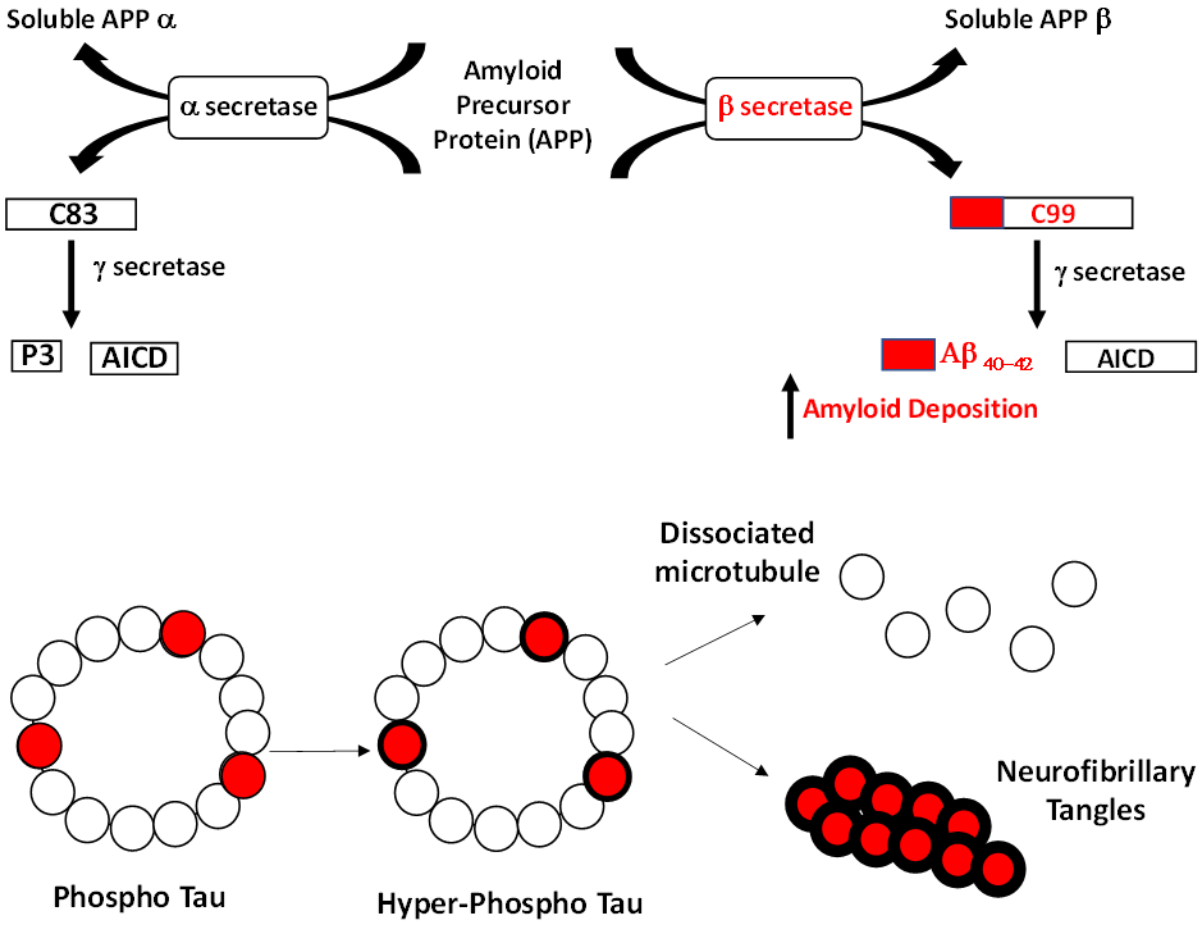


Figure 1. 1 Alzheimer pathogenesis.
APP can be cleaved by α-secretase, which releases soluble APP and C83 fragment. C83 can be processed by γ-secretase to produce p3 fragments and amyloid intracellular domain (AICD). On the contrary, the cleavage BACE1 leads to C99, the direct precursor to Aβ fragments. Hyper-phosphorylation of the microtubule associated protein Tau causes microtubular dissociation and tangles deposition leading to cell death.

Tau function is regulated by its grade of phosphorylation. Normal adult human brain tau contains 2–3 moles phosphate per mole of tau while, in AD, tau is three to four-fold more hyperphosphorylated (Iqbal, Liu et al. 2010). In hyperphosphorylated state, tau is polymerized into paired helical filaments (PHF) and mixed with straight filaments (SF) forming neurofibrillary tangles. Overall, the presence of neurofibrillary tangles within neurons and Aβ plaques outside the cells, causes shrinkage pressure, which leads to nerve cell apoptosis.

1.2.1 Familiar and sporadic Alzheimer

Genetically, AD is divided into Familial AD (FAD) and sporadic AD.

FAD cases are characterized by Mendelian inheritance and have mostly an early onset. In particular, mutations characterize three key genes involved in amyloid processing: APP, presenilin (PSEN) 1 and PSEN2 (Piaceri, Nacmias et al. 2013). Mutations in the APP gene account for about 9% of FAD and are mostly located in exons 16 and 17, in proximity of the α-secretase cleavage site (Goate, Chartier-Harlin et al. 1991). The most common APP mutation is known as the Swedish double mutation (K670N/M671L). Found in a Swedish family, it is located before the BACE1 cleavage site (Haass, Lemere et al. 1995). Other pathogenic APP mutations described include the Arctic (E693G), London (V717I), Indiana (V717F), Florida (I716V), French (V715M), German (V715A), and Austrian (T714I) mutations (Lanoiselée, Nicolas et al. 2017). The majority of them have been exploited to recapitulate AD pathogenesis in AD mice models (Esquerda-Canals, Montoliu-Gaya et al. 2017).

PSEN1 and 2 are among the components of the γ - secretase complex together with nicastrin (NCT), presenilin enhancer-2 (PEN-2) and anterior pharynx defective1 (APH1). Pathogenic PSEN1 mutations represent the most common FAD mutations with 185 mutations

identified in 405 AD families (Larner and Doran 2006). PSEN1 mutations are typically at early onset (average is 45 years) leading to a more severe AD form. Among the most common PSEN1 mutations there are M146V, M146L, L286V, ΔE9 while N141I mutation affects PSEN2 (Piaceri, Nacmias et al. 2013, Lanoiselée, Nicolas et al. 2017). They all alter γ-secretase activity and have been exploited in transgenic models.

The sporadic AD form affects more than 90% of AD patients and usually has a late onset. Sporadic AD is more complex and characterized by genetic and environmental influences. To date, the presence of the allele E4 on Apoliprotein E (ApoE) has been confirmed as AD risk factor (Corder, Saunders et al. 1993, Coon, Myers et al. 2007, Muñoz, Garner et al. 2019). Although ApoE regulates cerebral cholesterol homeostasis, its involvement in AD pathogenesis is still unclear. It has been suggested that ApoE may bind to the amyloid protein regulating its conversion from the monomeric non-toxic form to the oligomeric and fibrillary toxic amyloid (Castano, Prelli et al. 1995). In support of this hypothesis, ApoE has been detected in senile plaques and tangles (Roses 1997, Muñoz, Garner et al. 2019). According to other studies, ApoE isoforms would not interact with amyloid but would instead be involved in an alternative neurological signaling pathway affecting neuronal plasticity (Genin, Hannequin et al. 2011).

1.2.2 Experimental limitations in Alzheimer models

About a century after the discovery of AD, only five drugs have been approved by the US Food and Drug Administration (FDA): acetylcholinesterase inhibitors rivastigmine (Exelon), galantamine (Razadyne and Reminyl), tacrine (Cognex), donepezil (Aricept), and the N-methyl-d-aspartate (NMDA) receptor antagonist memantine (Namenda). The majority of the therapeutic compounds tested in clinical trials in the past two decades were designed to target αβ fragments

and tangles deposition. However, none of them were successful. The massive trials failure was caused by different limitations in vitro as well as in vivo models compared to actual AD patients (Doig, Del Castillo-Frias et al. 2017). Aβ and tau are difficult to reproduce experimentally, and the concentrations used were too high, causing a spontaneous aggregation which was unlikely to occur in humans. Also, none of the available animal models truly replicated the AD neuropathology spectrum. Despite the utility of transgenic models to better understand AD pathology, most of them are based on mutations from early-onset AD forms which represent only 5% of the AD total cases (Doig, Del Castillo-Frias et al. 2017).

Beside experimental limitations, one of the most evident limits of the amyloid hypothesis was its simplicity and divergency with aging. It could not explain the presence of amyloid deposition in elderly (90 years) subjects who were not diagnosed with AD or any other type of dementia (Dewachter, Van Dorpe et al. 2000, Rodrigue, Kennedy et al. 2009, Fjell, McEvoy et al. 2014). Moreover, deposited plaques seem to be less toxic compared to the soluble oligomers around them, since they are more biologically active (Makin 2018). Plaque and tangles formation alone do not imply any cellular and metabolic changes within the neurons. Several studies showed that plaques and tangles may be necessary but not sufficient to induce the pathological AD state (Provenzano, Muraskin et al. 2013, Fjell, McEvoy et al. 2014, Paroni, Bisceglia et al. 2019). On the contrary, energy hypometabolism is among the most recurrent and earliest abnormalities seen in AD and in dementia states (Hirai, Aliev et al. 2001, Rosenthal, Amiel et al. 2001).

1.3 Metabolic Alterations in AD

1.3.1 Hypometabolism

Glucose is the main source of energy exploited by the mammalian brain. It has been estimated that the human brain demands for 20% of the glucose-derived energy although it represent only 2% of the total body weight (Mergenthaler, Lindauer et al. 2013). Glucose metabolism is in fact essential to satisfy neuronal and non-neuronal cell maintenance, neurotransmitter synthesis and of course ATP generation. Due to the dependency for high glucose demand, brain metabolism is vulnerable to glucose utilization. Subjects experiencing insufficient glucose utilization had poor cognitive performances (Ferris, de Leon et al. 1980, Gold 2005, Euser, Sattar et al. 2010). However, when glucose availability was experimentally increased in selected brain areas, cognitive and memory tasks improved (Schroeder and Packard 2003, Schroeder and Packard 2004). Decrease glucose availability is a condition likely to happen gradually during aging. Average cerebral glucose metabolic rate showed a 26% decrease in 78 year old patients compared to 18 years old (Kuhl, Metter et al. 1982). However, beside aging, impairment in glucose metabolism has been recognized as invariant pathophysiological feature in AD.

A significant decline in cerebral metabolic rate (CMR) for glucose has been shown in several studies using Positron emission tomography (PET) imaging combined with 18F-2-deoxy-2-fluoro-D-glucose comparing AD patients with elderly controls (Ferris, de Leon et al. 1980, de Leon, Ferris et al. 1983, Nordberg, Rinne et al. 2010, Marcus, Mena et al. 2014). Declines were detected before AD symptomatology and memory impairment in specific brain areas that have been linked with dementia such as posterior cingulate, parietal, and temporal cortices (Reiman, Caselli et al. 1996, Rosenthal, Amiel et al. 2001). Glucose hypometabolism can be detected years before cognitive decline. Mosconi *et al.* performed an FDG-PET study to track the progression of

CMR decline from normal cognition to Mild Cognitive Impairment (MCI) and AD (Mosconi, Mistur et al. 2009). The FDG-PET study showed that reduction in glucose CMR was a reliable predictor of cognitive decline and the dementia severity years before the clinical symptomatology. Moreover, the glucose pattern from PET scans can even predict the progression from MCI to dementia (Mosconi, Tsui et al. 2008). Although hypometabolism has been recognized as AD hallmark, the causes behind impairment in glucose utilization in AD are still unclear. Diminished glucose availability due to diabetes, cerebral hypoperfusion, hypoxic conditions as well as defects in glucose transporters and glycolytic enzymes, are so far possible explanations behind the sequalae of events that lead to dementia onset.

1.3.2 Decrease in Glucose Utilization

The Solute Carrier (SLC) family of glucose transporters (GLUTs) have a fundamental role in maintaining glucose utilization within the brain and their alterations have been described in AD pathogenesis. Due to their inability to synthesize or store glucose, brain neurons rely on glucose transport across the Blood Brain Barrier (BBB), which is facilitated by GLUTs (Sweeney, Sagare et al. 2018). In particular, GLUT-1 and GLUT-3 are the predominant glucose transporters. GLUT-1 is specifically expressed on endothelia of the BBB and post-mortem analysis on AD subjects have confirmed the decrease in the expression together with a decreased GLUT-3, expressed on neurons. BBB alterations in early AD cases have been correlated to decrease in glucose uptake by the brain parenchyma due to the diminished activity of GLUT-1 (Nelson, Sweeney et al. 2016). Furthermore, the brain areas most vulnerable to glucose impairment are the most metabolically active and glucose-dependent, the cortex and hippocampus (Nelson, Sweeney et al. 2016). Liu *et al*. reported than decrease in GLUT-1 and GLUT-3 expression was correlated with the increase in

tau hyperphosphorylation as well as in the density of neurofibrillary tangles in human brains (Liu, Liu et al. 2008). Additionally, Winkler et al. investigated the contribution of GLUT-1 reductions in cerebrovascular impairment and on AD progression suggesting its role as early pathogenic AD marker (Winkler, Nishida et al. 2015). Lack of the transporter resulted in altered brain angioarchitecture, cerebral blood flow and BBB integrity.

Besides GLUTs, astrocytes have a critical role in brain energy homeostasis as main regulators of neuronal metabolism and glucose transport. Due to the high amount of glycogen stored, astrocytes may utilize anaerobic metabolism of glucose and glycogenolysis to produce lactate which will serve as alternative source of energy for neuronal metabolism during hypoglycemia (Verkhratsky, Olabarria et al. 2010, Stobart and Anderson 2013). Initial impairments of brain connectivity and synaptic transmission can result from generalized atrophy of astrocytes (Verkhratsky, Olabarria et al. 2010). The atrophy may alter synaptic transmission and reduce metabolic support to neurons leading to synaptic loss and early cognitive decline.

1.3.3 Hyperglycemia and Insulin Resistance

Metabolic alterations such as hyperglycemia and insulin resistance, typically observed in diabetes and obesity cases, are a common comorbidity in AD. It is well established that Alzheimer's pathology shares some degenerative pathways with diabetes and obesity, both considered AD risk factors. These common mechanisms are involved in oxidative stress, mitochondrial dysfunction, and inflammation (Pugazhenthi, Qin et al. 2017). Different clinical studies suggest that obese middle-aged subjects are more likely to develop cognitive impairment later in life (Anstey, Cherbuin et al. 2011, Xu, Atti et al. 2011, Nepal, Brown et al. 2014). Prolonged consumption of a high fat diet, a known causes of obesity, has also been shown to

promote dementia (Cole, Ma et al. 2010). Furthermore, carriers of the allele e4 in the lipid metabolism gene apolipoprotein E (ApoE) have an increased risk in developing cognitive disorders at an earlier age, especially if homozygote for the e4 allele (Corder, Saunders et al. 1993). This correlation was further established by imaging studies reporting that ApoE e4 subjects had higher levels of amyloid fragments compared to non- carriers (Sunderland, Mirza et al. 2004). On the other hand, hyperglycemia, hyperglycosylated hemoglobin and insulin resistance represented key factors in cognitive decline in diabetic patients. In particular, postmortem analyses on AD brains revealed evidence of insulin resistance implying that insulin positively influences cellular processes such as growth and survival (Gudala, Bansal et al. 2013). In particular, decreased expression of the insulin receptor and the insulin receptor substrate (IRS), phosphoinositide-3-kinase (PI3K), AKT and AKT phosphorylation within the insulin pathway have all been reported in the AD scenario (Steen, Terry et al. 2005, Lee, Kumar et al. 2009, Moloney, Griffin et al. 2010, Liu, Liu et al. 2011). Thus, it is not surprising that insulin is among the current treatments for AD.

The beneficial effect of insulin on memory has been showed in several studies which reported that mentally impaired individuals (young or elderly) receiving high insulin infusion rates exhibited improved memory performance and attention (Craft, Newcomer et al. 1996, Craft, Asthana et al. 1999, Kern, Peters et al. 2001). Besides IV administration, direct insulin delivery to the CNS has been investigated using intranasal administration via olfactory and trigeminal pathways (Born, Lange et al. 2002, Hanson and Frey 2008). When tested on cognitively impaired or AD subjects, intranasal insulin improved story recall, attention, and delayed memory (Reger, Watson et al. 2008, Craft, Baker et al. 2012). Beside insulin therapy, the insulin-sensitizer drug Metformin represents a standard type 2 diabetes therapy. Clinical studies revealed that metformin administration was effective in lowering dementia risk in diabetic patients, especially if taken for

a prolonged time (Chin-Hsiao 2019). Although the mechanism is still unclear, some studies proposed that metformin acts via activation of AMPK-dependent pathway which exerts neuroprotective effects in the brain (Markowicz-Piasecka, Sikora et al. 2017, Chin-Hsiao 2019).

1.3.4 Impact in glycolytic enzymes and cofactors

Due to hypoperfusion, AD patients experience a decrease in cofactors levels. Examples of enzyme cofactors are organic molecules such as vitamins (ascorbate, niacin, folic acid, pantothenic acid, biotin, riboflavin, and thiamine), or inorganic ions (magnesium, iron, selenium, zinc). Geriatric patients with dementia had borderline blood levels of the cofactors pantothenic acid, thiamine and ascorbate in a study conducted by Basu *et al*. (Basu, Jordan et al. 1976). Decreased levels of vitamin D (Llewellyn, Lang et al. 2010, Llewellyn, Lang et al. 2011, Toffanello, Coin et al. 2014), vitamin C (Dixit, Bernardo et al. 2015, Warner, Kang et al. 2015, Mi, Dixit et al. 2018), NAD+/NADH (Hou, Lautrup et al. 2018, Xie, Gao et al. 2019) have been reported in AD subjects and are likely to be a predictive indicator for cognitive impairment. A reduction in cofactors levels has a direct impact on enzymes functions. In particular, glycolytic enzymes are particularly affected during AD. A metabolomic study conducted on CSF of Alzheimer and non -dementia patients revealed a significant differences in the metabolites related to glycolysis. In particular, phosphoenolpyruvate, 2- and 3-phosphoglycerate, pyruvate and dihydroxyacetone phosphate were the Tricarboxylic acid cycle (TCA) metabolites showing a significant difference between the two groups (Bergau, Maul et al. 2019). The TCA and the pentose phosphate pathway (PPP) are two predominant pathways for glucose metabolism within the brain parenchyma. Decreases in TCA cycle enzyme α-ketoglutarate dehydrogenase (KGDH), Pyruvate Dehydrogenase complex (PDH) were reported in AD patients (Freeman, Nielsen et al. 1987, Gibson, Sheu et al. 1988,

Mastrogiacoma, Bettendorff et al. 1996, Bubber, Ke et al. 2004, Lu'o'ng and Nguyen 2011). Impairment in the PPP cytosolic enzyme Transketolase (TK) were also found in AD subject (Parker, Filley et al. 1990, Onyango, Dennis et al. 2016). TK is involved in the nonoxidative branch of the pentose phosphate pathway, which ultimately supplies NADPH, ATP and GTP for antioxidant defenses, fatty acid, and nucleic acid synthesis.

Interestingly, a diminished activity and/or expression of such enzymes was also described in aging and age-related neuropathology (Freeman, Nielsen et al. 1987, Jeyasingham, Pratt et al. 1987, Hoffman 2016, Yu and Zhong 2018). Beside playing a key role in glucose metabolism, KDGH, PDH and TKT require vitamin B1 or thiamine as a cofactor. Deficiency in thiamine represents a well-established AD comorbidity affecting glucose metabolism. Chronic thiamine insufficiency (TI) dramatically decreased cerebral oxidative metabolism promoting oxidative stress, mitochondrial damage, inflammation, and eventually neuronal loss (Gold, Chen et al. 1995, Calingasan, Chun et al. 1999, Sang, Pan et al. 2018). Inadequate levels of blood thiamine highly correlate with senile dementia prognosis and is a predictive peripheral biomarker for AD (Pan, Fei et al. 2015, Pan, Sang et al. 2017).

1.4 Role of Thiamine in cerebral metabolism

Thiamine (vitamin B1) is an essential water-soluble micronutrient; continuous dietary intake is needed to satisfy cellular metabolism. According to the National Institute of Health (NIH), the recommended diet allowance (RDA) for healthy adults' range between 1.1 to 1.4 mg per day with expected plasma level concentrations ranging between 10-20 nanomolar (Zastre, Sweet et al. 2013). Structurally thiamine is composed by a pyrimidine and a thiazole ring linked together by a methylene bridge. The thiazole ring contains a methyl and hydroxyethyl side chain

which is esterified with pyrophosphate to obtain the active cofactor Thiamine Pyrophosphate (TPP). The quaternary nitrogen makes thiamine a hydrophilic and a positive charged molecule at physiological pH with a consequent need of a carrier-mediated system to regulate its absorption.

The transporters that facilitate thiamine intake are THTR1 (SLC19A2) and THTR2 (SLC19A3) and belong to the solute carrier family. In particular, THTR1 is a low affinity – high-capacity transporter with an estimated Km of 2.5 μM while THTR2 has a higher affinity of 27 nanoM (Ganapathy, Smith et al. 2004, Zastre, Sweet et al. 2013). THTR1 and THTR2 regulate thiamine uptake through a passive antiport mechanism. Thiamine is exchanged with hydrogen ions in a 1:1 stoichiometry exploiting the transmembrane pH gradient (Ganapathy, Smith et al. 2004). Both carriers are expressed ubiquitously. In polarized cells, THTR1 has been located predominantly on basolateral membrane while THTR2 has been identified on the apical side. Furthermore, the organic cationic transport (OCT)1 (SLC22A1) functions as hepatic thiamine transporter. OCT1 modulates hepatic glucose and lipid metabolism mediating thiamine uptake in the liver (Liang, Yee et al. 2018). Recent studies have identified the thiamine pyrophosphate (TPP) transporter TPPT (SLC44A4) in the colon. TPPT regulates the absorption of microbiota generated TPP in the large intestine (Nabokina, Inoue et al. 2014).

Once in the cell, thiamine conversion to Thiamine Pyrophosphate (TPP), the active form, occurs through phosphorylation via thiamine pyrophosphokinase-1 (TPK1). TPK1 catalyzes the transfer of two phosphate groups onto thiamine in the presence of adenosine triphosphate (ATP) and Mg^{++} (Jonus, Hanberry et al. 2018). Transport of TPP across the mitochondrial is mediated by the thiamine pyrophosphate carrier (SLC25A19 gene) (Zastre, Sweet et al. 2013). Four enzymes necessitate thiamine as cofactor to function: the cytosolic enzymes transketolase (TKT) and the mitochondrial enzymatic complexes α-keto glutarate dehydrogenase (KGDH), pyruvate

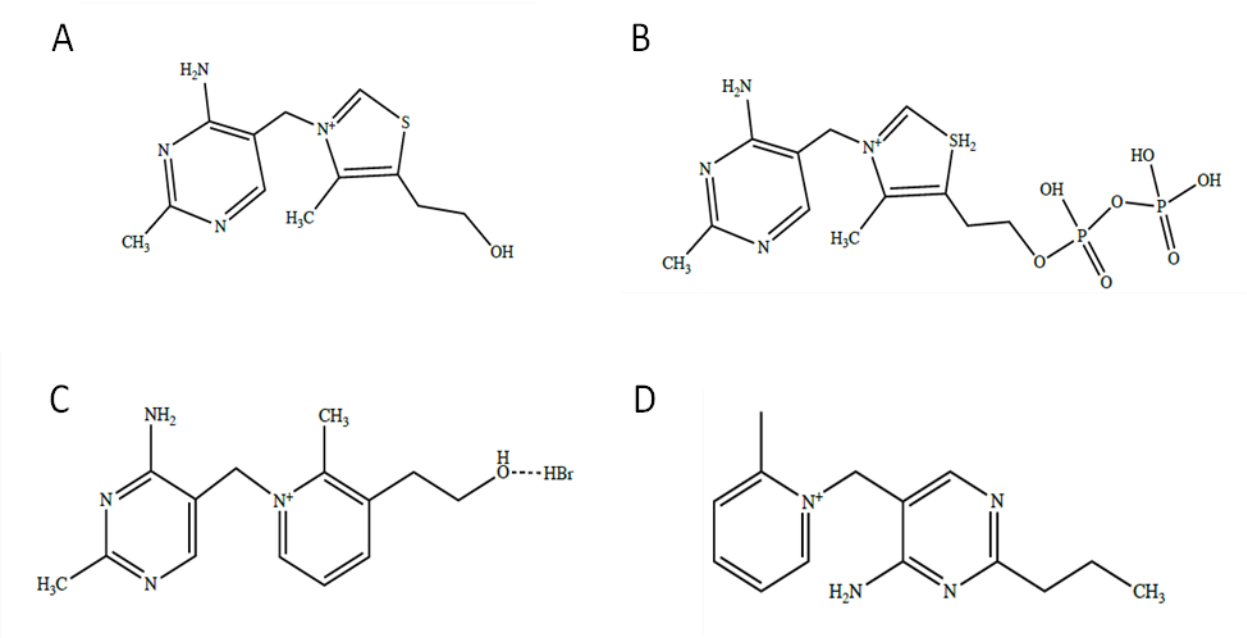


Figure 1.2. Structure of thiamine and thiamine analogues.
Thiamine or vitamin B1 (A) can be phosphorylated into the active cofactor Thiamine Pyrophosphate (B) by TPK1. The compound pyrithiamine (C) is an antivitamin that inhibits thiamine uptake and conversion into TPP. Amprolium (D) is an inhibitor of thiamine transporters.

dehydrogenase (PDH) and branched chain α-keto acid dehydrogenase complex (BCK).

The cytosolic thiamine dependent enzyme TKT connects the pentose phosphate pathway (PPP) to glycolysis. Within the non-oxidative portion of PPP, TKT catalyzes the conversion of ribose-5-phosphate into fructose-6-phosphate and glyceraldehyde-3-phosphate in a reversible reaction (Lonsdale 2007). Change in TKT activity is exploited to assess thiamine status in the TKT assay. In this biochemical assay the increase in the activity of TKT is measured after TPP supplementation in whole blood samples or erythrocytes. In case of thiamine deficiency, the exogenous TPP will stimulate TKT activity, determining the TPP effect (Basu, Dickerson et al. 1974, Boni, Kieckens et al. 1980).

The mitochondrial enzyme KGDH converts α-ketoglutarate (KG) to succinyl-coA and NADH providing electrons for the respiratory chain. Beside glycolysis, KG may be derived from glutamine deamination as anaplerotic substrate for the TCA cycle. For this reason, KGDH has a key role in mediating glutamine metabolism and the TCA cycle (DeBerardinis, Mancuso et al. 2007). KGDH activity is modulated by low concentrations of calcium and ADP and inhibited by high concentration of NADH, succinyl- CoA and reactive oxygen species (ROS) (Tretter and Adam-Vizi 2005).

The mitochondrial enzyme PDH is the rate-limiting enzyme of the pyruvate dehydrogenase complex. The PDH complex converts the pyruvate produced by glycolysis to Acetyl-coA for the entry into the TCA cycle. Due to its key role in cellular metabolism, PDH is tightly regulated by the NADH/NAD+ ratio, coA levels, ATP levels, and pyruvate levels (Bowker-Kinley, Davis et al. 1998) . PDH activity is regulated by 4 kinases known as pyruvate dehydrogenase kinases (PDKs). PDKs inactivate PHD by phosphorylation of three serine residues (Ser293, Ser300, and Ser232) (Kolobova, Tuganova et al. 2001).

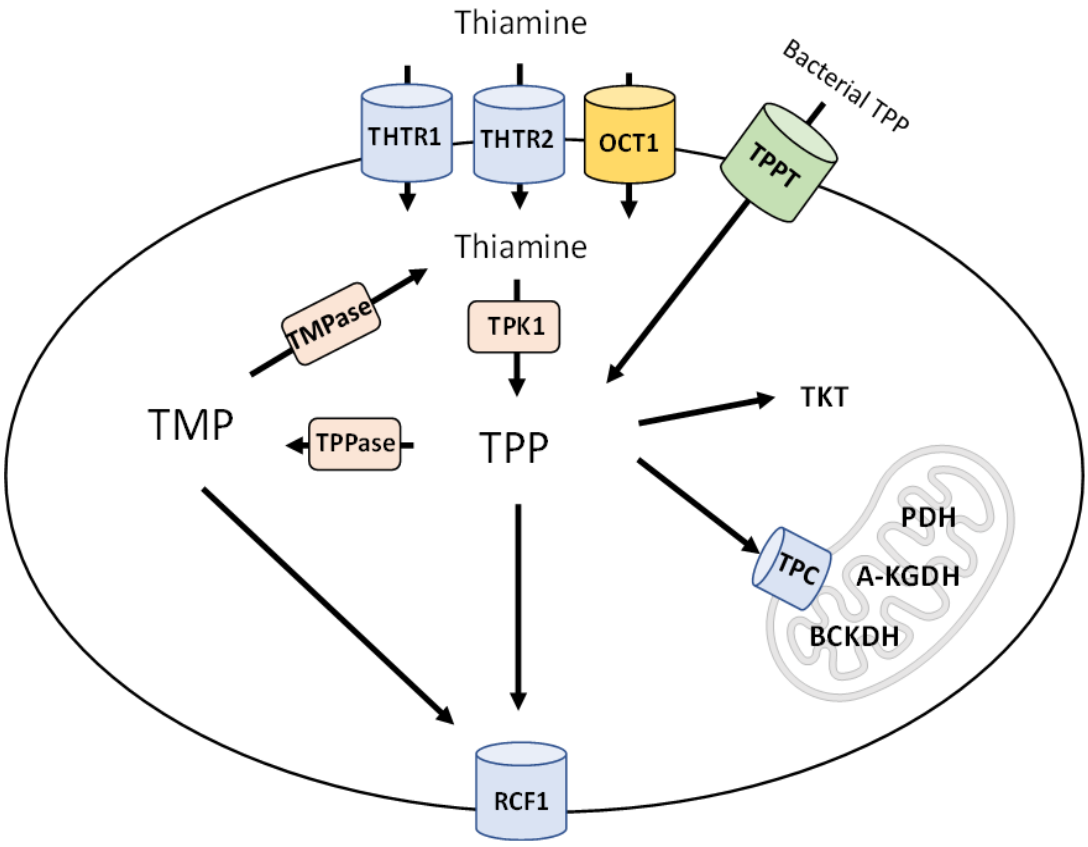


Figure 1.3 Thiamine Homeostasis.
Thiamine is transported inside the cells via THTR1 and THTR2. OCT1 is expressed in the liver while TPPT regulates the entrance of TPP produced by the microbiome in the intestine. Thiamine is then converted into thiamine pyrophosphate (TPP) by TPK1 phosphorylation. TPP is the active cofactor for transketolase (TKT) in the cytosol and pyruvate dehydrogenase (PDH), α-keto glutarate dehydrogenase (KGDH) and branched chain α-keto acid dehydrogenase complex (BCK) in the mitochondria. TPP transport into mitochondria is mediated by the thiamine pyrophosphate carrier (TPC). TPP can also be reconverted into thiamine monophosphate (TMP) by Thiamine Pyrophosphatase (TPPase) enzyme and back to thiamine by the enzyme Thiamine Monophosphatase (TMPase).

Among them, Ser293 is the predominant site, and its phosphorylation leads to inactivation. PDK1 was found to phosphorylate all three sites while the other isoforms primarily target Ser293 and Ser300 (Yeaman, Hutcheson et al. 1978).

BCK regulates the metabolism of essential branched chain amino acids (BCAAs) valine, isoleucine, and leucine. Like PDH, BCK is a mitochondria enzymatic complex composed by three suubnits. BCAAs undergo transamination to the α-keto acid followed by irreversible oxidative decarboxylation by BCKDC to form an acyl-CoA derivative (Zastre, Sweet et al. 2013). In particular, acetyl-coA is derived from leucine and succinyl-coA from valine and isoleucine (Harper, Miller et al. 1984). Another similarity with PDH is the regulation via reversible phosphorylation by branched-chain α-keto acid dehydrogenase kinase (BDK) and phosphatase (BDP) (Harper, Miller et al. 1984).

1.5 Thiamine Insufficiency

1.5.1 Role of Diet in Thiamine Insufficiency

A prolonged lack of thiamine within the diet may lead to thiamine insufficiency (TI). Although thiamine can be obtained from diverse sources (such as cereals, pork and beef meat, seeds and nuts) it can be easily denatured by high temperature and pH changes (Kimura and Itokawa 1990). In a physiological state, 25-30 mg of thiamine can be stored in the human body. High metabolic organs such as skeletal muscles, heart, brain liver and kidneys are mainly involved in thiamine storage (Vangeison, Carr et al. 2008). However, this amount can last approximately 2-3 weeks in case of prolonged deficiency (Sechi and Serra 2007). According to the World Health Organization (WHO), TI due to inadequate diet may happen in war refugees as described in Thailand (1980's and in the 1990's), Guinea (1990), Djibouti (1993) and in Nepal (1993-1995)

(Vangeison, Carr et al. 2008). Beside this scenario, populations with a poor varied diet may be more exposed to TI outbreaks. For example, the WHO estimated that the milled rice-based diet in South-East Asia was the cause for the TI outbreaks at the beginning of the century. The introduction of polished rice (in which vitamins had not been depleted through the milling process) in the diet alleviated the TI symptoms in the population (Vangeison, Carr et al. 2008).

Yet, a thiamine deficiency state due to inadequate diet is unlikely to happen in developed countries. In fact, several commonly consumed foods, such as cereals and breads, are supplemented with thiamine, increasing the overall daily intake. TI is still a recurrent comorbidity in Western world in cancer (Zastre, Sweet et al. 2013, Isenberg-Grzeda, Hsu et al. 2015, Lonsdale 2018), diabetes (Abdou and Hazell 2015, Groeneveld, Kappelle et al. 2016, Daulatzai 2017), alcoholism (de la Monte and Kril 2014, Ba 2017, Brinkman, Bekema et al. 2017), post-bariatric surgery (Goodman 2015, Kerns, Arundel et al. 2015, Maguire, Talwar et al. 2018), cardiac disfunction (Ahmed, Azizi-Namini et al. 2015, DiNicolantonio, Liu et al. 2018, Eshak and Arafa 2018), pregnancy (Butterworth 2001), AIDS-HIV (Butterworth, Gaudreau et al. 1991), aging and dementia (Gibson, Sheu et al. 1988, Abdul-Muneer, Alikunju et al. 2018, Whitfield, Bourassa et al. 2018).

1.5.2 Genetic Mutations in Thiamine homeostasis genes

Genetic alterations within thiamine homeostasis genes have been correlated with AD pathophysiology (Lu'o'ng and Nguyen 2011). TK structural abnormalities occur in AD patients and TK variants have been found in patients with thiamine-deficient encephalopathy (Lu'o'ng and Nguyen 2011). Also, a reduction in TK (45%) and KGDH (75%) levels has been described in AD patients derived fibroblasts and red blood cells. Mutations of the SLC19A3 gene are associated

with cognitive impairment and dementia. The most common SLC19A3- related genetic disorder is Biotin-thiamine-responsive basal ganglia disease (BTBGD) (also called Thiamine metabolism dysfunction syndrome 2) whose symptomatology includes encephalopathy characterized by confusion, seizures, ataxia, dystonia, ophthalmoplegia (Tabarki, Al-Hashem et al. 1993, Zastre, Sweet et al. 2013, Algahtani, Ghamdi et al. 2017). Basal ganglia nuclei, thalamus, infra-and supratentorial brain cortex, and brain stem are commonly affected by BTBGD (Brown 2014). SLC19A3 was listed among the putative genes responsible for spatial memory performance following chronic stress (Jung, Brownlow et al. 2017). SLC19A3 variants have been associated with AD and cognitive impairment in a genome wide association study conducted in families with multiple AD cases (Saad, Brkanac et al. 2015). Furthermore, SLC19A3 selective inhibition by the anticancer compound Fedratinib induced the TI-driven neuropathology Wernicke encephalopathy in treated patients, forcing the termination of the clinical trial (Zhang, Zhang et al. 2014, Pardanani, Harrison et al. 2015, Vora, Green et al. 2020). SLC19A2 mutations cause Thiamine-responsive megaloblastic anemia syndrome (TRMA) or Rogers syndrome. TRMA is an autosomal recessive disease characterized by megaloblastic anemia, diabetes mellitus, and sensorineural deafness (Labay, Raz et al. 1999). Thiamine supplementation has proven to be beneficial in ameliorating anemia and diabetes due to THTR2 functionality (Labay, Raz et al. 1999, Brown 2014, Habeb, Flanagan et al. 2018). Thiamine intake does not seem to be effective on deafness or on neuropathology associated with TRMA (Onal, Bariş et al. 2008, Brown 2014).

1.5.3 Thiamine Insufficiency as comorbidity in Neuropathology

Thiamine insufficiency has been linked to diverse neuropathology. One of the first neuropathology associated with TI was beriberi that can manifest as dry or wet. Dry beriberi affects

the peripheral nervous system causing peripheral neuropathy with sensory and motor impairment of distal and proximal limbs (Stroh, Meyer et al. 2014). If the pathology remains untreated, patients may experience loss of muscular strength and paralysis. Patients with wet beriberi show cardiomyopathy with heart failure, dyspnea, and peripheral edema (DiNicolantonio, Liu et al. 2018). Dry beriberi may also affect the Central Nervous System (CNS) with a congruent symptomatology of the Wernicke- Korsakoff Syndrome (WKS), the most recurrent yet life-threating comorbidity in chronic alcoholics affecting the CNS. The term WKS is commonly used to include a first reversible phase known as Wernicke Encephalopathy (WE). If untreated, WE will eventually lead to the irreversible Korsakoff psychosis (KS). First described in 1881 by Carl Wernicke, Wernicke Encephalopathy (WE) is an acute neurological disorder caused by thiamine insufficiency. Although it is mostly associated with alcoholism, non-alcoholic WE has been described in TI patients with malnutrition that was not caused by alcohol abuse (Butterworth 2007, Day and del Campo 2014). WE symptomatology is usually characterized by a triad of symptoms i.e., ophthalmoplegia, ataxia and mental confusion. Furthermore, post-mortem analysis of patients diagnosed with WKS revealed a selective damage within the thalamus and mammillary bodies with upregulated levels of pro-inflammatory cytokines and chemokines suggesting their contribution in neuronal cell death (Day and del Campo 2014). This neurodegenerative scenario was reported to occur with a regional selectivity for the thalamus and mammillary bodies. In contrast other regions like the cortex were unaffected (Fellgiebel, Scheurich et al. 2003).

Nevertheless, the rationale behind TI selective damage is still unknown. Furthermore, TI can lead to another neurodegenerative condition known as the Marchiafava-Bignami disease (MBD). Subjects diagnosed with MBD manifest dementia, depression, and an altered mental status as a consequence of poor nutrition and /or alcoholic syndrome (Hillbom, Saloheimo et al. 2014,

Fernandes, Bezerra et al. 2017). A significant association between Parkinson Disease (PD) and low levels of serum thiamine has also been demonstrated (Lương and Nguyê~n 2012, Luong and Nguyễn 2013). Diverse pathways are thought to regulate thiamine induced PD pathology; these include oxidative stress, inflammation, and cellular metabolism (Lương and Nguyê~n 2012). Genetic studies on PD have pointed out alterations in DJ-1 gene, excitatory amino acid transporters (EAATs), KGDH, coenzyme Q10, lipoamide dehydrogenase (LAD), transcription factor p53, the renin-angiotensin system (RAS), heme oxygenase-1 (HO-1), and poly(ADP-ribose) polymerase-1 gene (PARP-1) (Luong and Nguyễn 2013).

1.6 Similarities between Thiamine Insufficiency and Alzheimer Pathology

In congruency with AD, TI induces changes in oxidative metabolism promoting a sequalae of events that eventually lead to oxidative stress, mitochondrial damage, inflammation, and neuronal loss (Karuppagounder, Xu et al. 2009, Abdou and Hazell 2015). Post-mortem analysis of brains from WKS patients revealed a regional selective damage affecting bilaterally thalamic nuclei, inferior colliculi, inferior olivary nuclei, mammillary bodies, and lateral vestibular nuclei (Todd and Butterworth 1999). Microarray studies conducted on thalamus and inferior colliculus of TI models revealed a significant change in expression of genes involved in inflammation, oxidative stress, cell death and metabolism (Vemuganti, Kalluri et al. 2006). Reactive astrocytes and microglia were detected as well. On the contrary, cortex, caudate nuclei and hippocampi were not significantly affected by neuronal loss (Jhala and Hazell 2011, Stobart and Anderson 2013). Similar alterations were described in post-mortem analysis of WKS subjects including mitochondrial impairment, oxidative stress, inflammation, and excitotoxicity as potential players

behind the TI-mediated neuropathology (Todd and Butterworth 1999). In the next section the main pathological mechanisms that have been linked to TI-induced neuropathology will be described.

1.6.1 Mitochondrial dysfunction

Mitochondrial dysfunction is a well-characterized hallmark in neuropathology (Bubber, Haroutunian et al. 2005, Bubber, Hartounian et al. 2011). First mitochondrial alterations within the brain parenchyma were reported in the 1970s in electron microscopy studies (Johnson and Blum 1970, Wiśniewski, Terry et al. 1970). Key hallmarks of mitochondrial dysfunction include compromised glucose metabolism, imbalanced oxidative stress and hypometabolism. Interestingly, all alterations were described during TI-mediated brain damage. Loss of thiamine as cofactor results in a dramatical impairment within the TCA cycle enzymes and oxidative metabolism with decrease in ATP production and energy levels (Aikawa, Watanabe et al. 1984, Bettendorff, Sluse et al. 1995). Congruently, TK activity gradually decreases with prolonged TI (Trebukhina, Ostrovsky et al. 1983). In particular, decrease and loss in activity of the mitochondrial enzyme KGDH has been detected in early TI stages and seem to have a key role in initiating the cascade of biochemical lesions in selective brain regions (Butterworth 2007). KGDH dysfunction contributes to the changes in energy metabolism, synthesis of glucose-derived neurotransmitters, focal lactate accumulation as well as to cell death via cytochrome c release and caspase activation (Butterworth, Giguère et al. 1986, Héroux and Butterworth 1988, Butterworth 1989). Lactate accumulation is a result of the preferential pyruvate conversion into lactate due to the blockage of its entering to the TCA cycle (Huckabee 1958). This step is regulated by the mitochondrial thiamine-dependent enzyme PDH whose loss of activity is mainly caused by TI. Lactic acidosis is a major consequence of TI and it can lead to cytotoxic edema (Goldman,

Pulsinelli et al. 1989). Glial cells and astrocytes in particular are the main cells susceptible to lactate-induced swelling that will lead to brain edema and excitotoxicity (Kimelberg, Rutledge et al. 1995, Abdou and Hazell 2015).

1.6.2 Oxidative Stress

One of the most devastating consequence of TI-driven mitochondrial dysfunction is the increase in Reactive Oxygen Species (ROS). Thiamine has antioxidant properties that are lost in case of inadequate levels resulting in loss of balance in ROS regulation (Lukienko, Mel'nichenko et al. 2000, Sharma, Bist et al. 2013, Jonus, Hanberry et al. 2018).

Imbalanced production of ROS such as superoxide anion, hydroxyl radical, and hydrogen peroxide contributes to the early phases of AD pathology (Li, Calingasan et al. 2004, Swerdlow and Khan 2004, Ham and Raju 2017, Tönnies and Trushina 2017). Although they may have a physiological role at small concentrations, high ROS concentrations lead to oxidative stress with direct damage to nuclear and mitochondrial DNA, increased lipid and protein peroxidation, and decreased brain glucose metabolism (Bardaweel, Gul et al. 2018). Oxidative stress has been associated to aging (Harman 1956, Harman 1972, Hekimi, Lapointe et al. 2011) as well as with Aβ fragments and tangles production highlighting a possible involvement in the clinical aspects of AD (Westermann 2009). Amyloid fragments in turn accelerate ROS production by binding to mitochondrial membranes, leading to a disruption in cell metabolism and to synapsis loss (Beal 2005, Calkins, Manczak et al. 2011). In a similar trend, WKS patients exhibit elevated oxidative stress markers and lipid peroxidation. TI models exhibited elevated levels of oxidative stress markers such as ICAM-1, hemeoxygenase, endothelial NOS, iNOS, heme oxygenase-1, superoxide dismutase, ferritin, and malondialdehyde together with lipid peroxidation (Gibson and

Zhang 2002, Karuppagounder, Xu et al. 2009, Abdou and Hazell 2015). On the contrary, levels of antioxidative enzymes such as superoxide dismutase, catalase, and glutathione peroxidase were significantly reduced in TI brain (Langlais, Anderson et al. 1997, Gibson and Zhang 2002, Karuppagounder, Xu et al. 2009). Thiamine dependent enzymes showed a very low tolerance to oxidative damage with KGDH being the most sensitive enzyme (Schoonen, Wanamarta et al. 1990). Another deleterious effect of free radicals' accumulation is the impairment of BBB, which has been reported in TI cases (Watanabe and Kanabe 1978, Watanabe, Tomita et al. 1981, Abdou and Hazell 2015). Impairment in endothelial cells transport and integrity have been described as free radicals' mediated damages contributing to BBB breakdown. Furthermore, cerebral endothelial cells forming brain capillaries exhibited a decrease in GLUT-1 expression (Sarkar, Liachenko et al. 2016). Due to the key role of capillaries in oxygen diffusion, TI lesions may be a consequence of hypoperfusion and changes in metabolism (Binnewijzend, Benedictus et al. 2016, Wolters, Zonneveld et al. 2017).

The rise in oxidative stress occurred in late TI stages in selective brain regions preceding brain damage and cell death (Langlais, Anderson et al. 1997). The sub medial thalamus nuclei have been suggested as initial site of TI- mediated damage since elevated levels of iNOS, the oxidative stress marker heme oxygenase-1 (HO-1) together with the lipid peroxidation product, 4-hydroxynonenal (HNE) are observed (Calingasan, Chun et al. 1999).

1.6.3 Alzheimer Pathogenesis

The most interesting but yet unresolved connection between TI and AD is the fact that TI can recapitulate the pathological AD hallmarks. In congruency with AD, TI is characterized by selective neuronal loss, decreased activities of TPP-dependent enzymes, cholinergic deficits and

memory loss. Early studies with rats and mice fed with a TI diet, showed that TI accelerated neurodegeneration (Freeman, Nielsen et al. 1987, Freeman and Gibson 1988). Neuronal loss was achieved after 9-11 days of TI in mice and its pattern corresponded to elevation in iNOS and heme oxygenase (OH)-1 production in microglia (Calingasan, Chun et al. 1999).

Furthermore, TI induced plaques resembled the AD amyloid plaques. Rats fed with a TI diet and injected with the thiamine antagonist pyrithiamine (PT) showed pathological lesions accompanied with APP positive plaques and abnormal neurites within the thalamus, mammillary bodies and medial geniculate nucleus after 13 days (Calingasan, Gandy et al. 1995). However, TI did not affect presenilin 1 (Calingasan, Gandy et al. 1995). In mouse brain, TI produces region-specific alterations of the BBB that preceded APP alterations and neuroroxicity (Calingasan, Gandy et al. 1996). Another study using TI mice reported that TI facilitated the nuclear traslocation of the carboxy-terminal fragments (C99) of APP at day 3 of TI (Karuppagounder, Xu et al. 2008). This event precedes neuronal loss within the sub-medial thalamic nucleus (SmTN) at day 9. No CTF accumulation was detecetd within the cortex. This finding suggest that APP metabolism is an early TI event that may play a key role in TI-slective neuronal loss. Further studies tested the effect of TI on transgenic Tg19959 AD mice (overexpressing a doubly mutated human APP). TI exacerbated AD pathology by increasing plaque deposition, astroglia activation and neuritic cluster formation. In particular plaques were detected within cortex, hippocampus, and thalamus together with an increase in C99 levels by 33% and BACE1 protein levels by 43% (Karuppagounder, Xu et al. 2009). The detection of Aβ fragment within the cortex was of particular interest due to the highly selective TI-damage within the thalamic area. The same study also reported an increase in tau phosphorylation in TI mice although the mechanism was not clear.

1.6.4 Inflammation

Microglia-driven inflammatory response is a key player in TI-induced regional selective neuropathology. Microglia, together with astrocytes, are a key component in the spatial and temporal events of inflammation. Further studies confirmed that microglia activation and the following inflammatory response, contributes to the initial phase of the TI neurological dysfunction (Calingasan, Park et al. 1998, Todd and Butterworth 1999). Recently, Bowyer et al. showed that microglial activation occurs 24-48 hours prior to neuronal and astrocyte degeneration and vascular leakage in a TI model. In particular, the microglial response within the thalamus seems to impact the vasculature leading to vascular dysfunction (Bowyer, Tranter et al. 2018).

Lack of thiamine has an effect in changing inflammatory markers, lipid peroxidation and leukocytes recruitment profile during sepsis (de Andrade, Gayer et al. 2014) leading to increase in peroxynitrite, ROS and pro-inflammatory cytokines production (Butterworth and Heroux 1989, Butterworth 2007). In particular, up-regulation of inflammatory genes was reported within the TI susceptible areas of thalamus and inferior colliculus. Pro-inflammatory cytokines such as IL-6, IL-18, tumor necrosis factor (TNF)-α, chemokines such as Monocyte Chemoattractant Protein (MCP)-1, interferons and innate immune genes together with transcription factors involved in inflammatory response were reported to be increased (Vemuganti, Kalluri et al. 2006). Furthermore, the close relationship between the inflammatory response and TI was further validated in sepsis patients who exhibited TI as a comorbidity. Experimental sepsis conditions showed a greater pro-inflammatory and oxidative stress when conducted in TI conditions compared to regular thiamine concentration (Moskowitz and Donnino 2020). Recent clinical studies tested whether intravenous infusion of vitamin B1 had any beneficial effect on subject with sepsis or sepsis shock (Donnino, Andersen Lw Fau - Chase et al. , Moskowitz, Andersen et al.

2017, Mitchell, Ryan et al. 2019). Although none of the studies show any significant benefit after thiamine IV, septic patients with acute kidney injury (AKI) experienced an improvement in renal function suggesting a potential role for thiamine in sepsis-related renal injuries (Moskowitz, Andersen et al. 2017).

1.6.5 Excitotoxicity

Glutamate injections caused TI-like lesions (Todd and Butterworth 1999).This finding prompted the investigation toward glutamate-mediated excitotoxicity as a TI mediated mechanism. Further research conducted on WKS cases found that glutamate concentration was selectively elevated within the thalamic nuclei and that use of a selective antagonist of the N-methyl-D-aspartate (NMDA) receptor had neuroprotective effects (Todd and Butterworth 1998) . Thalamic lesion in TI patients resemble excitotoxic mediated events such as the downregulation of astrocytic glutamate transporters GLT-1 (Glutamate Transporter 1) and GLAST (Glutamate/Aspartate Transporter) (Hazell, Rao et al. 2001). Furthermore, glutamate transporters seem vulnerable to oxidative damage resulting in reduction in their function and build-up of neurotoxic glutamate. This mechanism has been described in TI as well as in Alzheimer's Disease due to the key role of glutamate receptors in cognitive process and memory (Trotti, Danbolt et al. 1998, Hynd, Scott et al. 2004, Esposito, Belli et al. 2013).

1.6.6 Thiamine Insufficiency as predictive peripheral Alzheimer's biomarker

Due to the correlation between low thiamine levels and dementia, blood thiamine derivatives levels were recently considered as a predictive peripheral AD biomarker. Current approaches for AD diagnosis do not include a routine diagnostic test for AD due to their high cost

and difficulty in performance (Molina, Jiménez-Jiménez et al. 2002, Mosconi 2005, Mosconi, Pupi et al. 2008, Anoop, Singh et al. 2010, Nordberg, Rinne et al. 2010). On the other way, either amyloid and tau fragments (Mattsson, Zetterberg et al. 2016) or Aβ40-Aβ42 (Lövheim, Elgh et al. 2017) failed as reliable predictive markers. Thus, more attention was given to brain glucose hypometabolism and associated factors, as invariant feature of AD pathophysiology. FDG-PET studies showed that glucose metabolism and blood TPP levels were both significantly reduced in patients with AD. Interestingly, no significant correlations between blood TMP or TPP levels and amyloid deposition were found in AD within the frontal, parietal, or temporal cortex. These results suggested that brain amyloid deposition did not significantly affect TPP levels in AD patients (Sang, Pan et al. 2018). Furthermore, thiamine levels in plasma and red blood cells were significantly decreased in senile dementia of the Alzheimer's type (SDAT) but not in non-dementia subjects (Gold, Chen et al. 1995) or idiopathic Parkinson patients (Chiu, Tsai et al. 2016). These studies suggested that thiamine may be considered a biomarker specific for AD.

This hypothesis was further strengthened by HPLC fluoroscopic studies conducted by Pan et al. who measured blood thiamine metabolites in AD patients compared to controls, vascular dementia (VD) and fronto-temporal dementia (FTD) subjects, respectively (Pan, Sang et al. 2017). Blood TPP levels in all dementia patients were significantly reduced as compared with control subjects. However, the AD group retained the lowest TPP values thus demonstrating that thiamine metabolites can differentiate AD from non-AD dementia cases (Chen, Pan et al. 2017). To clarify the mechanics behind TPP decrease, the activities of three main thiamine metabolizing enzymes thiamine diphosphatase (TDPase), thiamine monophosphatase (TMPase), and thiamine pyrophosphokinase (TPK) were studied in AD patients and control subjects (Pan, Sang et al. 2017). The study showed enhanced TDPase and TMPase activities, which leads to elevated

dephosphorylation in thiamine which cannot act as active cofactor. On the contrary, no significant change in TPK1 activity was detected. The authors concluded that this imbalance between phosphorylation and dephosphorylation in thiamine metabolism may contribute to brain glucose hypometabolism in AD, representing a potential target for AD prevention and treatment. Interestingly, blood TPP levels were significantly decreased in female AD patients compared to AD male patients but no sex-related difference was detected in controls (Wang, Fei et al. 2018). Thus, TPP levels may be another factor contributing to the greater AD prevalence rate in females.

1.6.7 Thiamine analogues in Alzheimer

One major limitation of thiamine is its low bioavailability, estimated around 5%, which affects its clinical effectiveness (Tallaksen, Sande et al. 1993). To overcome this problem, lipid-soluble thiamine derivatives benfotiamine, solbuthiamine and fursultiamine have been developed with an advanced pharmacokinetic profile. To date, only one clinical trial (Mimori, Katsuoka et al. 1996) and one study (Ollat, Laurent et al. 2007) focused on fursultiamine effects on dementia. Although the compound showed mild beneficial effect on cognitive abilities in AD patients without adverse reactions, its neuroprotective effect has not been fully clarified yet. On the contrary**,** benfotiamine has shown beneficial effects in diabetes, inflammation, vascular endothelial dysfunction, and Alzheimer's disease (Fraser, Diep et al. 2012, Raj, Ojha et al. 2018, Safavi, Hosseini-Sharifabad et al. 2020). Benfotiamine administration improved mice survival rate and behavioral deficits (Pan, Gong et al. 2010, Tapias, Jainuddin et al. 2018), reduced stress-induced suppression of hippocampal neurogenesis (Vignisse, Sambon et al. 2017), and ameliorated the cognitive ability of patients with mild to moderate AD, without reducing amyloid deposition (Pan, Chen et al. 2016). These findings further support the theory that alteration in cognitive capacities

do not depend on amyloid deposition. A recent study among MCI and mild AD patients showed that oral benfotiamine was able to minimize the decline in glucose utilization and slow the cognitive decline associated with the progression of dementia (Gibson, Luchsinger et al. 2020).

1.7 Rational and Goal

Several preclinical and clinical studies have highlighted the similarities in pathophysiology and in memory deficit between TI and Alzheimer's patients suggesting the possible role of thiamine as predictive biomarker for AD (Gibson, Sheu et al. 1988, Agbayewa, Bruce et al. 1992, Gibson and Zhang 2002, Karuppagounder, Xu et al. 2009, Gibson, Hirsch et al. 2016, Sang, Pan et al. 2018, Tapias, Jainuddin et al. 2018, Gibson, Luchsinger et al. 2020). In congruency with AD, TI state triggers the amyloidogenic cascade, oxidative stress, inflammation, excitotoxicity leading to neuronal death. Nevertheless, the molecular mechanism behind TI-induced neurotoxicity and AD-like pathophysiology has not been elucidated yet.

Our previous work has shown that thiamine insufficiency stabilizes Hypoxia Inducible Factor (HIF) 1α, the main transcription factor involved in hypoxic stress, through an oxygen independent mechanism (Sweet and Zastre 2013, Zera and Zastre 2017). Our lab was the first to link thiamine and thiamine homeostasis genes with the hypoxic transcription factor in breast cancer. We report that the high affinity thiamine transporter THTR2 (SLC19A3) was a hypoxia responsive gene in breast cancer (Sweet, Paul et al. 2010). Further studies confirmed that HIF1α directly binds to the SLC19A3 promoter to adaptively induce its expression during hypoxic stress (Zera, Sweet et al. 2016).

During normal oxygen levels, HIF1α is rapidly hydroxylated by Prolyl Hydroxylases (PHDs) and directed towards proteasome-mediated degradation. In hypoxia state, PHDs activity

is altered due to the lack of oxygen. This results in HIF1α stabilization and migration in the nucleus where it can function as transcription factor. HIF1α can be stabilized even in the presence of normal oxygen conditions known as pseudo-hypoxia. We previously showed that in TI conditions a buildup of the metabolic intermediate pyruvate can create a pseudo-hypoxic state in which HIF1α is stabilized (Zera and Zastre 2018). This mechanism is driven by a PDH complex which needs TPP as cofactor. This complex utilizes pyruvate formed as the product of glycolysis to generate acetyl coA for entry into the TCA. Because PDH activity is essential to the maintenance of cellular energy status, it is tightly regulated in response to various metabolic changes such as the NADH/NAD+ ratio, CoA levels, ATP levels, and pyruvate levels. In case of thiamine deprivation and TPP lacking, pyruvate cannot be converted and accumulates within the cells. This pyruvate surplus results in the inhibition of PHDs due to its structural similarity with their cofactor 2-oxoglutarate. Subsequently, HIF1α is stabilized and can migrate in the nucleus acting as transcription factor even in the absence of hypoxia.

HIF1α target genes may induce a protective effect or a pro-apoptotic and pro-inflammatory response (Semenza 2002, Semenza 2012). Although the exact mechanism has not been fully elucidated, an acute activation of HIF1α is likely to regulate the transcription of genes involved in oxygen utilization, angiogenesis, and metabolism. For example, erythropoietin (EPO) controls red blood cells production and Vascular endothelial growth factor (VEGF) regulates the formation of new vessels and promotes angiogenesis (Gleadle and Ratcliffe 1997). HIF1α also mediates the switch from oxidative to glycolytic metabolic metabolism via activation of metabolic enzymes such as lactate dehydrogenase A (LDHA), Glucose transporter (GLUT)1 and Pyruvate Dehydrogenase Kinase (PDK)-1. In this way, HIF1α maintains glucose metabolism while reducing TCA cycle, oxidative phosphorylation, and consequently oxidative stress induction. In

particular, upregulation of PDK1, activation of the hypoxia-sensitive subunit 2 in cytochrome c oxidase and induction of autophagy are among the mechanisms proposed to explain the HIF1α protective response (Brunelle, Bell et al. 2005, Fukuda, Zhang et al. 2007, Zhang, Bosch-Marce et al. 2008).

Chronic HIF1α activation resulted in a pro-apoptotic response. Prolonged hypoxia potentiated cell death in dendritic cells and endothelial cells together with transplanted stem cells in ischemic myocardium (Hu, Wei et al. 2011, Filippi, Morena et al. 2014, Jian, Shi et al. 2015). Upregulation of proapoptotic proteins such as BNIP3, Nix and Noxa after a chronic hypoxia condition has been described (Halterman and Federoff 1999, Bruick 2000, Kothari, Cizeau et al. 2003, Mellor and Harris 2007). In particular, activation of BNIP3 in presence of oxidative stress was shown to trigger cell death by membrane depolarization and mitochondrial pore opening (Kubli, Quinsay et al. 2008). Other studies have highlighted the role of HIF1α in the regulation of p53-mdm2 signaling during hypoxia (An, Kanekal et al. 1998, Koumenis, Alarcon et al. 2001, Chen, Li et al. 2003). Caspase 3 induction by HF1α was also described after photothrombotic cerebral ischemia (Van Hoecke, Prigent-Tessier et al. 2007). This mechanism would contribute to promote cytochrome c release and inhibition of electron transport chain (Greijer and van der Wall 2004).

Thus, HIF1α seems a promising candidate to bridge TI neuropathogenesis with cognitive decline. Therefore, the purpose of this work was to investigate if HIF1α may be the critical upstream mediator between TI and AD pathophysiology.

We also hypothesize that the HIF-BNIP3 signaling may mediate the TI-induced neurotoxicity in accordance with our previous work in astrocytes where TI induced a time-dependent increase in the pro-apoptotic and pro-inflammatory HIF1α target genes MCP1, BNIP3,

Nix and Noxa (Zera and Zastre 2017). Understanding the role of HIF1α may help clarify the pathophysiology of TI. This will give a better comprehension of AD as well as other TI- mediated neurological disorders such as Wernicke-Korsakoff syndrome and Parkinson's. Thus, targeting the HIF1α signaling may provide potential therapeutic benefits to AD and dementia patients.

In summary, the objectives for this work were:

Object 1: Investigate if TI-induced neurotoxicity is mediated by the HIF-BNIP3 signaling

Object 2: Elucidate if HIF1α mediates AD-pathogenesis via BACE1 activation during TI

Object 3: Determine the role of TI-induced HIF1α in AD-pathogenesis and neurotoxicity in an in vivo AD model

CHAPTER 2

THIAMINE INSUFFICIENCY- INDUCED HIF1α TRIGGERS NEUROTOXICITY VIA BNIP3 [1]

[1] Valle M.L, Anderson Y., Grimsey N., and Zastre J.
To be submitted to *Journal of Neurochemistry.*

2.1 Introduction

Neurotoxicity is a well-established consequence of thiamine insufficiency (TI) and related neuropathology (Freeman, Nielsen et al. 1987, Freeman and Gibson 1988, Hazell, McGahan et al. 1998). The impaired oxidative metabolism caused by decreased activity of thiamine-dependent enzymes leads to a multifactorial cascade of pro-apoptotic events including focal lactic acidosis, glutamate excitotoxicity, production of free radicals and BBB alterations (Hazell, McGahan et al. 1998, Hazell, Todd et al. 1998, Todd, Hazell et al. 1999).

TI neurotoxicity was shown to be regional selective within the thalamus and inferior colliculus. In these regions a significant increase in genes involved in inflammation, oxidative stress, cell death and metabolic perturbation was reported during TI (Vemuganti, Kalluri et al. 2006). NMR studies in rats injected with PT reported an increased synthesis of lactate within the medial thalamus to 148% and 226% compared to controls (Pannunzio, Hazell et al. 2000). WE/TI mice exhibited damaged BBB permeability with loss in protein expression of occludin, ZO-1 and ZO-2 (Beauchesne, Desjardins et al. 2009). Furthermore, rat astrocytes within the TI-vulnerable thalamic region showed a significant decrease in protein levels of the astrocyte glutamate transporters GLT-1 and GLAST as well as in aquaporin-4 (Hazell, Rao et al. 2001, Hazell, Pannunzio et al. 2003, Chan, Butterworth et al. 2004, Hazell and Wang 2005).

Despite the numerous studies trying to elucidate TI neuropathology, the molecular mechanism of TI induced neurotoxicity is less clear. In our previous study on primary murine astrocytes, TI induced a time-dependent increase in proapoptotic HIF1α target genes and induced apoptotic death in congruency with ischemic injury (Zera and Zastre 2017). In particular, the proapoptotic HIF1α target gene BNIP3 was significant upregulated during TI.

In congruency with TI, upregulation of proapoptotic protein BNIP3 has been described after chronic hypoxia and early AD stage models (Halterman and Federoff 1999, Bruick 2000, Kothari, Cizeau et al. 2003, Mellor and Harris 2007, Zhang, Yang et al. 2007, Ułamek-Kozioł, Czuczwar et al. 2019). Thus, these findings suggest that the HIF-BNIP3 signaling may be involved in TI-induced neurotoxicity. For the purposes of this dissertation, additional background information on hypoxia, HIF1α and BNIP3 has been included in the introduction.

2.1.1 Role of hypoxia in neurodegeneration

Cerebral hypoperfusion is a well-established comorbidity in patients with mild cognitive impairment (MCI) or dementia, mostly caused by pre-existent vascular damage. Significant differences in cerebral blood flow (CBF) rates were detected via MRI in controls compared to Alzheimer's Disease (AD) subjects, with MCI patients scoring intermediate values (Binnewijzend, Benedictus et al. 2016). Furthermore, subjects in advanced AD states had lower CBF rates compared to patients with less severe grades of AD (Binnewijzend, Kuijer et al. 2013, Binnewijzend, Benedictus et al. 2016). Reduced CBF leads to a diminished delivery of oxygen and metabolites that will eventually result in neuronal energy crisis and neurodegeneration (Wolters, Zonneveld et al. 2017). Hypoxia is a natural consequence of inadequate cerebral oxygen perfusion, likely to eventually trigger neuronal death. Chronic hypoxia is one of the most critical risk factors in AD pathogenesis (Sapin, Peyron et al. 2015, Macheda, Roberts et al. 2019, Zhang, Niu et al. 2019). Elderly patients with a history of a chronic hypoxia conditions are prone to develop AD symptomatology due to the repeated exposure to low oxygen levels followed by re-oxygenation (Daulatzai 2013). Prolonged exposure to hypoxic conditions impacted amyloid and tau metabolism, autophagy, neuroinflammation, oxidative stress, and mitochondrial and synaptic

dysfunction (Iyalomhe, Swierczek et al. 2017). Examples of chronic hypoxia scenarios reported in elderly are stroke, traumatic brain injury (TBI), chronic respiratory conditions, or sleep disorders like obstructive sleep apnea syndrome (OSAS) and chronic obstructive pulmonary disease (COPD). About 30% of ischemic strokes are followed by an immediate or delayed vascular cognitive impairment. Microinfarction, microvascular changes, and focal neuronal atrophy are among the critical post-ischemia alterations which lead to dementia (Kalaria 2016). Furthermore, early midlife TBI patients have a risk two to four-fold higher to develop dementia during aging compared to the general population. Multiple cerebral traumas seem to increase the risk as well (Shively, Scher et al. 2012, Nordström and Nordström 2018). Interestingly, alteration in glucose utilization has been reported in those hypoxia-driven conditions. Glucose intolerance has been diagnosed as comorbidity in cases of chronic pulmonary disease and obstructive sleep apnea (Oltmanns, Gehring et al. 2004), TBI, (Young, Ott et al. 1989, Jauch-Chara and Oltmanns 2014) and stroke (Hewitt, Castilla Guerra et al. 2012). At a molecular level, hypoxic response is controlled by the hypoxia inducible transcription factor, HIF1α.

2.1.2 HIF1α regulation

HIF1 is a heterodimeric transcription factor composed by an oxygen-dependent α subunit and a β subunit (also known as ARNT, Aryl hydrocarbon nuclear translocator) constitutively expressed. The 826 aa-long alpha subunit contains the domain required for the oxygen-dependent degradation (ODD), a basic helix-loop-helix (bHLH) domain, a PAS (Per Arnt Sim) homology domain and an amino and carboxy terminal transactivation domains (N-TAD and C-TAD) involved in the heterodimer formation (Wang and Semenza 1993, Semenza 2003, Semenza 2012). Beside HIF1α, the isoforms HIF2α and HIF3α have been identified (Semenza 2002). HIF2α

regulates cellular response to hypoxia similarly to HIF1α. The protein shares the same consensus sequence and domain structure with HIF1α a part for the N-TAD domain, responsible for gene specificity (Moniz, Biddlestone et al. 2014). Nevertheless, HIF2α has limited expression and activity compared to HIF1α (Raval, Lau et al. 2005). HIF2α has been implicated in mediating tumor growth, cell cycle progression and maintaining stem cell pluripotency (Covello, Kehler et al. 2006, Toledo, Qin et al. 2013). Less clear is the role of HIF3α that has been suggested to act as a dominant negative isoform of HIF1α (Duan 2016).

The oxygen dependent regulation occurs via post-translational modifications on proline residues 402 and 564 by prolyl hydroxylase isoforms (PHDs) that hydroxylate HIF1α using molecular oxygen as cofactor. PHDs belong to the α-ketoglutarate dependent dioxygenase family together with collagenase hydroxylases (Semenza 2012). These enzymes rely on iron, ascorbate and α-ketoglutarate as cofactors and use oxygen to hydroxylate their substrates on the LXXLAP target motif. In particular, in the hydroxylation reaction one oxygen atom is added to a proline residue to form hydroxyproline, whereas the other is used in a coupled decarboxylation reaction that converts α-ketoglutarate into succinate (Berra, Ginouvès et al. 2006). Oxygen activation is mediated by iron which is in turn maintained in a reduced state by ascorbate. Iron binds to the active site of the enzyme after the decarboxylation reaction and is therefore necessary for its re-activation (Smirnova, Hushpulian et al. 2012). The hydroxylation on Proline 402 and Proline 564 allows the binding of the von Hippel-Lindau protein factor (VHL) that, once bound, recruits the Elongin C ubiquitin-ligase complex. Then, the enzyme SSAT2 (spermidine/spermine N-acetyltransferase-2) binds to the HIF1-VHL-Elongin C complex leading to HIF1α polyubiquitination and degradation via the 26s proteasome (Baek, Liu et al. 2007). In contrast, hypoxia state causes the loss of oxygen inactivating PHDs. This results in HIF1α stabilization with

consequent binding with HIF1β and translocation to the nucleus. Within the nucleus, HIF1α binds to the hypoxic responsive elements (HREs) containing the sequence 5'-RCGTG-3' in the promoter region of various target genes involved in a wide array of processes including angiogenesis, proliferation, glucose metabolism, apoptosis, tumor invasion and metastasis (Semenza, Nejfelt et al. 1991, Wang and Semenza 1993). An alternative regulation of HIF1α involves the Factor Inhibiting HIF-1 (FIH-1). FIH-1 acts via hydroxylation of asparagine 803 on C-TAD but this modification prevents the interaction with co-activators p300 and CREB Binding Protein (CBP) resulting in an inhibition of the transcriptional activation of HIF1α (Semenza 2002, Semenza 2003). Other post-translation modifications involving HIF1α include phosphorylation and acetylation that may accelerate or inhibit transcriptional activity according to the targeted residue (Semenza 2002).

2.1.3 Prolyl Hydroxylases (PHDs)

In mammalian cells, three Prolyl Hydroxylases (PHDs) isoforms known as PHD1, PHD2 and PHD3, have been described. PHD2 is the most abundant isoform, and it can be found in almost every tissue (Freeman, Hasbani et al. 2003). PHD1 and PHD3 are highly localized within the heart and testis, but cerebral expression has also been reported (Freeman, Hasbani et al. 2003).PHDs are considered as oxygen sensors due to their low oxygen affinity and a Km of 230-250 μM (Lu, Dalgard et al. 2005). Beside HIF1α, PHDs recognize other substrates such as p53 (Deschoemaeker, Di Conza et al. 2015), the transcription factors ATF4 and FOXO34 (Hiwatashi, Kanno et al. 2011, Zheng, Zhai et al. 2014) as well as the NFkB modulator IKKβ (Cummins, Berra et al. 2006). Due to their dependency on oxygen as cofactor, hypoxia/ischemia led to PHDs

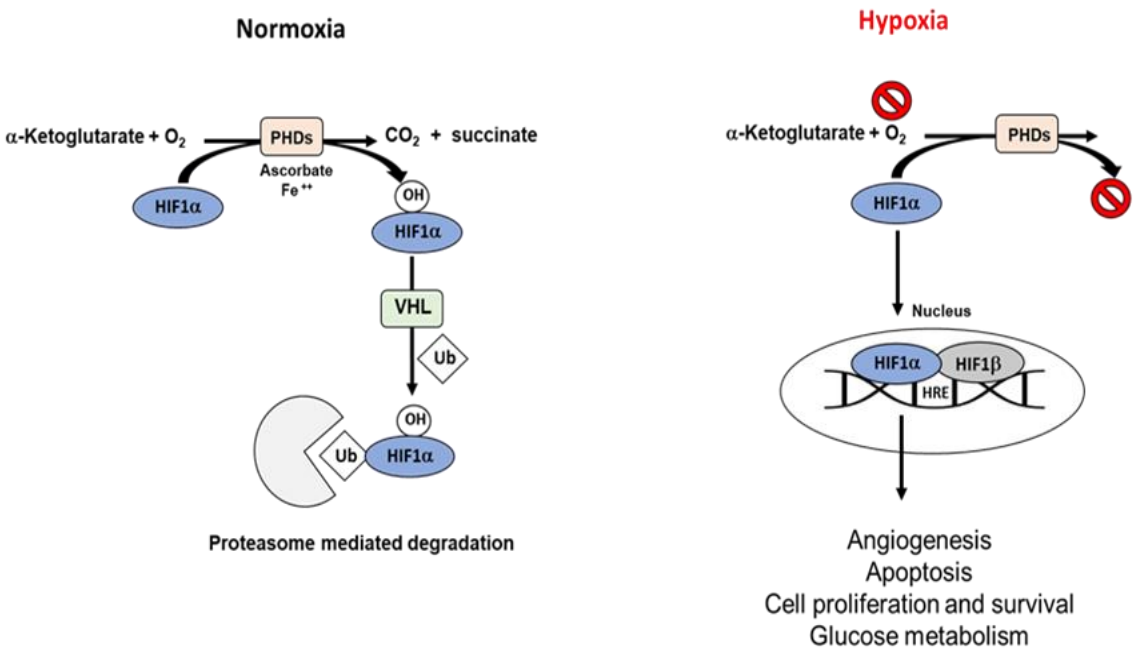


Figure 2.1 Canonical HIF1α Regulation.
During normoxia, HIF1α is rapidly hydroxylated by Prolyl Hydroxylases (PHDs) and directed to proteasome mediated degradation via VHL (von Hippel-Lindau Factor). During hypoxia, PHDs do not function properly leading to HIF1α stabilization and activation. Once stabilized, HIF1α migrates to the nucleus where it binds to HIF1β and transactivate target genes involved in angiogenesis, survival, apoptosis, or metabolism.

inactivation. However, PHDs inhibition can be oxygen independent. In cancer cells with deficiency or mutation of the mitochondrial enzymes' fumarate hydratase and succinate dehydrogenase the build-up of succinate and fumarate were shown to competitively inhibit PHDs activity even in the presence of oxygen. The same effect was also reported with isocitrate, pyruvate and lactate accumulation, respectively (Kaule, Timpl et al. 1998, Dalgard, Lu et al. 2004). Interestingly, PHDs expression has been associated with different oxygen vulnerability in different brain regions (Freeman, Hasbani et al. 2003).

Lamanna *et al.* showed an age-dependent correlation between PHDs expression and activity and oxygen sensitivity in senescent brain (Ndubuizu, Chavez et al. 2009). Based on the decreased HIF1α expression within the cortex, PHD1 and PHD3 mRNA and expression were found increased in 24-month rats exposed to hypoxia while PHD2 were found elevated even in 6 month rats. Interestingly, pharmacological inhibition of PHDs by iron chelators or 2-oxoglutarate analogues was associated to neurodegeneration. For example, in vivo PHDs inhibition by DMOG in hippocampus showed an impairment in long-term potentiation within the CA1 region (Wall, Corcoran et al. 2014). Also, PHDs inhibition by iron chelators was associated to neurodegeneration in Alzheimer's and Parkinson's (Speer, Karuppagounder et al. 2013, Lane, Ayton et al. 2018, Ratan 2019) suggesting the potential of PHDs as therapeutic target not limited to hypoxic conditions.

2.1.4 Dual role of HIF1α during hypoxia/ischemia

2.1.4.1 HIF1a protective response

It is well established that HIF1α mediated pathway may either induce a protective effect or a pro-apoptotic and pro-inflammatory response (Semenza 2002, Semenza 2012).

Table 2.1. Main characteristics of Prolyl Hydroxylases (PHDs).

	PHD1	**PHD2**	**PHD3**
Alternative Names	EGLN-2, HPH-3	EGLN-1, HPH-2	EGLN-3, HPH-1
Protein length	407 aa	426 aa	239 aa
Cellular Location	Nucleus	Cytoplasm	Nucleus + Cytoplasm
Tissue location	Testis > Brain	Ubiquitous	Heart = Liver > Brain
Oxygen Km	230-250 μM	230-250 μM	230-250 μM
Homozygous loss in vivo	Tolerated	Not compatible with life	Tolerated
Substrates	HIF1α, IKK β, FOXO3a, Cep192, MAPK6, p53, ATF4		
Inhibitors	Deferoxamine (DFO), Dimethyloxalylglycine (DMOG), Roxadustat, Ethyl 3,4-dihydroxybenzoate (EDHB)		

Although the exact mechanism behind the nature HIF1α response has not been fully elucidated, an acute activation of HIF1α is likely to regulate the transcription of genes involved in oxygen utilization (EPO), angiogenesis (VEGF), glucose metabolism (GLUT1) and metabolic adaptation to glycolytic metabolism (LDHA, PKM2 and PDK1) (Gleadle and Ratcliffe 1997). A non-lethal acute insult seems to protect the brain from a prolonged and more severe ischemic episode in animal models. In particular, a mild hypoxic episode may induce tolerance to a more severe insult allowing for an adaptative modification mediated by HIF1α that will prepare for an earlier recovery of the tissue. This scenario is known as 'hypoxic preconditioning' (HP) and it is particularly relevant in ischemic studies.

Studies conducted in newborn rats subjected to hypoxia preconditioning (8% O_2 for 3 hours) reported significant reduced brain damage after cerebral hypoxia compared with controls (Gidday, Fitzgibbons et al. 1994, Vannucci, Towfighi et al. 1998). Using the same model,

Bergeron and Chen groups highlighted the key role of HIF1α signaling in mediating an adaptative response elicited by the hypoxic precondition (Bergeron, Gidday et al. 2000, Chen, Jadhav et al. 2008). High levels of HIF1α and HIF1β within the more susceptible brain areas to hypoxia (cortex, hippocampus, thalamus, and striatum) were detected. The same result was achieved with the use of the divalent metal cobalt chloride and the iron chelating agent desferrioxamine (DFO), as chemical inducer of HIF1α stabilization (Guo, Song et al. 2006). Additional studies showed that hypoxic preconditioning led to changes in protein expression of GLUT-1, VEGF, ALD, and LDH, which are genes involved in the maintenance of cellular energy supplies (Jones and Bergeron 2001, Miller, Perez et al. 2001, Wick, Wick et al. 2002, Wacker, Perfater et al. 2012). Furthermore, after inducing mild ischemia (30 min ischemia with unilateral common carotid artery occlusion) to a neuron-specific knockdown of HIF1α murine model, Baranova et al. observed a detrimental effect in tissue damage and reduced survival rate (Baranova, Miranda et al. 2007). With a similar trend, a short-term hypoxic preconditioning (up to 24 hours) led to a HIF1α-driven protective response in dendritic cells and transplanted stem cells in ischemic myocardium (Hu, Wei et al. 2011, Filippi, Morena et al. 2014). Using wild type and HIF heterozygote (HET) mice, Cai et al. found that HET mice lost the acute cardio protection after HP and exhibited more caspase 3 activation and cell loss (Cai, Zhong et al. 2007). A reduced level of EPO was also found in HET mice.

Different mechanisms have been postulated in order to clarify the protective effect and metabolic reprogramming mediated by HIF1α activation. Induction of the HIF1α target gene Pyruvate Dehydrogenase kinase (PDK) 1 was considered as protective mechanism to prevent excessive ROS production. PDK1 limits Pyruvate Dehydrogenase (PDH) activity, resulting in a decreased flux towards the TCA cycle, oxidative phosphorylation but also in ROS generation. Directing the pyruvate towards reduction to lactate would also allow NAD+ regeneration to permit

glycolysis and ATP production during hypoxia. This model could also justify the role of hypoxia-induced mitochondrial ROS in the stabilization of HIF1α (Brunelle, Bell et al. 2005, Kaelin 2005). In a feedback mechanism, this response would lead to PDK1 activation that will in turn attenuate any potential lethal ROS.

Another proposed mechanism focused on the role of mitochondria during hypoxia. HIF1α activation would trigger the switching from subunit 1 to the hypoxia-sensitive subunit 2 of cytochrome c oxidase (COX). This adaptative response may optimize COX activity during hypoxia due to the greater oxygen affinity of subunit 2 (Fukuda, Zhang et al. 2007). Furthermore, using renal carcinoma cells lacking VHL, Zhang et al. showed that HIF1α negatively regulates mitochondrial metabolism and biogenesis by inhibition of c-Myc (Zhang, Gao et al. 2007). Induction of mitochondrial autophagy has also been taken into consideration as component of the HIF1α mediated metabolic adaptation (Zhang, Bosch-Marce et al. 2008). Studying the response of HIF1α knock out mouse embryonic fibroblasts (MEFs) in hypoxia, Zhang et al. showed that hypoxia induced autophagy via BNIP3. In particular, the HIF1α target gene BNIP3 would disrupt the interaction between Beclin1 and Bcl2 leading to Atg5 dependent autophagy. As adaptative metabolic cell response, the induction of autophagy would prevent increase in ROS level and cell death.

2.1.4.2 HIF1a pro-apoptotic and pro-inflammatory response

Chronic hypoxia stimuli resulted in a pro-apoptotic and pro-inflammatory responses and cell death. Neuronal primary culture exposed to glucose and oxygen deprivation showed reduced neuronal damage with a HIF dominant negative construct (Carmeliet, Dor et al. 1998). Prolonged hypoxia state (48-72 hours) triggered cell death in dendritic cells, endothelial cells together with

transplanted stem cells in ischemic myocardium (Hu, Wei et al. 2011, Filippi, Morena et al. 2014, Jian, Shi et al. 2015). In primary cortical neurons exposed to hypoxia, HIF1α induced delayed cell death via p53 activation (Halterman and Federoff 1999). In particular, HIF1α would lead to transcriptional upregulation of p53-dependent BH3-only family members, such as NOXA or PUMA (Cregan, Arbour et al. 2004, Aminova, Siddiq et al. 2008). HIF1α mediated growth arrest and apoptosis via p21 and p27 was also reported in embryonic stem cells exposed to prolonged hypoxia or hypoglycemia (Koshiji, Kageyama et al. 2004).

As possible mechanism to explain HIF1α induced cell death, direct binding between HIF1α and p53 to the ODD domain has been proposed (Hansson, Friedler et al. 2002). Hansson et al. localized two homologous p53 binding motifs separated by 120 residues within the ODD domain with a binding affinity independent or the proline 564 hydroxylation state. The authors speculated that each sequence would bind to different p53 subunits in order to strengthen the binding. Interestingly, HIF1α interacts with wild type p53 but not to the tumor derived mutant p53 (An, Kanekal et al. 1998). This finding seems to suggest that the HIF1α- p53 interaction may be mediated by the p53 ubiquitin ligase mdm2 (Chen, Li et al. 2003). Mdm2 is a E3 ubiquitin ligase that regulates both p53 degradation and nuclear export of p53 through ubiquitination (Haupt, Maya et al. 1997). Wildtype p53, but not the tumor-derived p53, is capable of induction of endogenous mdmd2. Thus, because of the lack of function of tumor p53, HIF1α would fail to interact with p53 due to the low levels of mdm2 in cells. Although the exact mechanism has not been elucidated yet, several studies have highlighted the key role of HIF1α in the regulation of p53-mdm2 signaling during hypoxia (An, Kanekal et al. 1998, Koumenis, Alarcon et al. 2001, Chen, Li et al. 2003) . Another mechanism to induce cell death involves the upregulation of proapoptotic proteins such as BNIP3, Nix and Noxa after a chronic hypoxia condition (Halterman and Federoff 1999, Bruick

2000, Kothari, Cizeau et al. 2003, Mellor and Harris 2007). The most investigated apoptotic effector in hypoxia/ischemia studies is BNIP3. BNIP3 may activate apoptotic, autophagic, or necrotic pathways (Kubli, Ycaza et al. 2007, Burton, Eisenstat et al. 2009). However, their overexpression has been shown to be necessary but not sufficient to mediate death. The addition of a secondary event such oxidative stress or acidosis, would potentiate HIF1α-induced oxidative death (Aminova, Siddiq et al. 2008, Chen, Ostrowski et al. 2010). In the case of BNIP3, an acidic pH seems to protect the protein from protease degradation (Lee, Kubli et al. 2015). Oxidative stress instead, seems to favor the dimerization of BNIP3 allowing its migration to the mitochondria where it triggers cell death by membrane depolarization and mitochondrial pore opening (Kubli, Quinsay et al. 2008).

Cerebral ischemia initiates inflammation which contributes to aggravate the pathogenesis. HIF1α target gene inducible Nitric Oxide Synthase (iNOS) showed a key role in mediating the inflammatory events during cerebral ischemia (Jung, Palmer et al. 2000). Expression of iNOS was increased in post-ischemic brain after transient focal ischemia contributing to the ischemic brain damage (Iadecola, Zhang et al. 1996). Treatment with isoflavonoid shortly before induction of middle cerebral artery occlusion (MCAO) in rats, attenuated focal cerebral ischemia and reduced infarct size. Interestingly, the compounds significantly decrease the expression of HIF1α, iNOS, caspase 3 and necrosis factor-α (TNF-α) in ischemic regions (Chang, Hsieh et al. 2009). In addition, HIF1α regulates several pro-inflammatory cytokines during ischemic stroke. Among them, human monocyte chemoattractant protein 1 (MCP1/CCL2) and murine monocyte chemoattractant protein 5 (MCP5/Ccl12) are potent chemokines produced by astrocytes during hypoxia. In human and murine astrocytes, Petrovic *et al*. showed the presence of HIF1-binding sites on MCP1 and MCP5 promoter regions adding them to the HIF1α target genes (Mojsilovic-

Petrovic, Callaghan et al. 2007). MCP1 and MCP5 induce transmigration of monocytes/macrophages across the BBB to reach the inflammatory sites within the brain (Feuerstein, Wang et al. 1998).

2.1.5 BNIP3 regulation

BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3) is a 194 amino acid protein constituted by four domains. The N- terminal proline (P), glutamic acid (E), serine (S), threonine (T), aspartic acid (D) domain known as PEST involved in BNIP3 degradation, the BH3 domain, a conserved domain and the carboxy-terminal transmembrane (TM) domain that allows BNIP3 migration to the mitochondria (Vasagiri and Kutala 2014). Unlike the other members, BNIP3 does not require its BH3 domain for dimerization or the involvement of Bcl-2 proteins for its pro-death activity (Ray, Chen et al. 2000). The TM domain is of particular importance for BNIP3 pro-apoptotic function since it allows BNIP3 binding to mitochondrial membrane. The GXXXG motif (a tandem AXXXG ‘‘glycine zipper’’ motif Ala 176, Gly 180, and Gly 184) within TM domain is essential for BNIP3 dimerization (Sulistijo and MacKenzie 2006). Sulistijo *et al.* showed that residues Ser 172 and His 173 are essential for the electrostatic interactions that regulate dimer formation and its stability (Sulistijo, Jaszewski et al. 2003, Sulistijo and MacKenzie 2006). Since its overexpression induces cell death, BNIP3 expression is tightly regulated. BNIP3 is mostly activated by HIF1α due to the presence of a HRE motif on its promoter (Kothari, Cizeau et al. 2003). Glucocorticoids and nitric oxide have also been described as BNIP3 induced stimuli in neurons and macrophages, respectively (Yook, Kang et al. 2004, Sandau and Handa 2007). On the other hand, excessive hypoxia-induced cell death by BNIP3 may be attenuated by Retinoblastoma protein (Rb) or Nuclear Factor kappa-light-chain-enhancer of activated B cells

(NFkB) signaling (Baetz, Regula et al. 2005, Tracy, Dibling et al. 2007). In normal tissues and non-hypoxic cells BNIP3 is detectable in the cytosol. However, its cellular localization may change with the effect of preventing or inducing cell death. Nuclear localization of BNIP3 correlates with a pro-survival response probably via transcriptional repression of the apoptosis inducing factor (AIF) gene (Burton, Eisenstat et al. 2009, Burton and Gibson 2009, Burton, Henson et al. 2013). This scenario has been reported in solid tumors, in particular glioblastoma, where high levels of BNIP3 have been detected in viable cells within hypoxic region suggesting its contribution to cell survival (Giatromanolaki, Koukourakis et al. 2004, Burton, Eisenstat et al. 2009). On the contrary, apoptosis is triggered by BNIP3 localization to the mitochondria. Here, by opening the permeability transition pore, the protein in its dimeric form triggers loss of mitochondrial membrane potential (MMP) as well as increase reactive oxygen species production, eventually leading to apoptosis (Zhang, Yang et al. 2007, Burton and Gibson 2009).

Previous studies analyzing the HIF1α-BNIP3 signaling, reported that the BNIP3 pro-death function could be achieved only with a secondary event identified with oxidative stress (Aminova, Siddiq et al. 2008). Lee *et al.* examined the difference sensibility to BNIP3 mediated death between of young and adult cardiomyocytes (Lee, Kubli et al. 2015). After hypoxia, adult myocytes were more protected from BNIP3 toxicity due to a higher presence of antioxidant enzyme manganese superoxide dismutase. Overexpression of BNIP3 alone was not sufficient to induce toxicity but addition of oxidants stimuli caused MMP disruption and apoptosis. Kubli *et al*. suggested the role of BNIP3 as oxidative stress sensor during myocardial ischemia and reperfusion (I/R) (Kubli, Quinsay et al. 2008). The group showed that BNIP3 exist in an inactive state in adult myocyte and increase in ROS during I/R would promote its activation by targeting the serine 64 residue on the

N-terminal domain. This conserved residue is a known activation switch for BCL2 family proteins and would promote dimerization by disulfide bonding.

In alternative, BNIP3 localization to mitochondria has also been correlated with autophagy and mitochondrial degradation (Tracy, Dibling et al. 2007). Depending on the cell type, BNIP3 induced cell death may involve or not caspase activation. In particular, a caspase-independent mechanism has been proposed in neurons after hypoxia (Zhang, Yang et al. 2007). According to the study, during neuronal apoptosis BNIP3 would trigger the release of endonuclease G (EndoG) from the mitochondria but not cytochrome c. Once released, EndoG migrates to the nucleus allowing the caspase-independent cleavage of chromatin into nucleosomal fragment.

2.1.6 Rationale and Goal

The purpose of the work presented in this chapter is to elucidate the molecular mechanism regulating TI - induced neurotoxicity. Our hypothesis is that TI-induced HIF1α stabilization will activate BNIP3 expression leading to apoptosis.

Upregulation of proapoptotic protein BNIP3 has been described in our previous study with astrocytes exposed to TI (Zera and Zastre 2017) together with hypoxia and early AD stage models (Halterman and Federoff 1999, Bruick 2000, Kothari, Cizeau et al. 2003, Mellor and Harris 2007, Zhang, Yang et al. 2007, Ułamek-Kozioł, Czuczwar et al. 2019). In rat cortical neurons exposed to amyloid protein fragments, Zhang *et al*. showed that BNIP3 had a key role in mediating the amyloid- induced oxidative stress (Zhang, Zhang et al. 2007). Also, in an early AD stage model, death of pyramidal neurons in the hippocampal region of CA3 was dependent on BNIP3, Beclin and Caspase-3 overexpression (Ułamek-Kozioł, Czuczwar et al. 2019). BNIP3 pro-apoptotic

activation can be achieved only with a secondary event, identified with oxidative stress (Kubli, Ycaza et al. 2007, Aminova, Siddiq et al. 2008).

The importance of oxidative stress in BNIP3 induced cell death represents an interesting connection with TI mediated toxicity. Increase in oxidative stress is a well-established TI consequence due to the mitochondrial dysfunction (Calingasan, Chun et al. 1999, Sharma, Bist et al. 2013, Liu, Ke et al. 2017). In primary cultures of rat cerebellar granule cells exposed to TI, supplementation of vitamin E as antioxidants resulted in neuroprotection (Todd and Butterworth 1998, Pannunzio, Hazell et al. 2000). Treatment of TD rats with the antioxidant N-acetylcysteine resulted in a decreased EAAT2 downregulation in the medial thalamus, and reduction in astrocytes and neuronal loss (Beauchesne, Desjardins et al. 2010, Hazell, Sheedy et al. 2010). These findings suggest a key role for ROS in induced neuronal death during thiamine depletion.

Therefore, we hypothesize that neurotoxicity during TI is mediated by HIF-BNIP3 activation in presence of oxidative stress.

2.2 Materials and Methods

2.2.1 Reagents

Cell culture reagents including RPMI 1640 media, penicillin/streptomycin, and trypsin/EDTA were purchased from Corning (Manassas, VA). Fetal bovine serum (FBS) was obtained from Sigma Aldrich (St. Louis, MO). Thiamine deficient RPMI was custom formulated by United States Biological (Salem, MA). Cell culture treated flasks and dishes were purchased from Greiner Bio-One (Monroe, NC). Chemicals including Dimethyloxallyl Glycine (DMOG), pyrithiamine hydrobromide (PT) and ethyl pyruvate (EP) were purchased from Sigma Aldrich (St. Louis, MO). YC1 (3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole) was purchased from Tocris (Minneapolis, MN) and Octyl-α-ketoglutarate (Octyl-KG) from Cayman Chemical (Ann Arbor, MI).

2.2.2 Cell culture treatments

HT22 murine hippocampal cells were a kind gift from Dr. William Hill (Augusta University, GA). Cells were cultured in RPMI 1640 supplemented with 1% penicillin/streptomycin and 10% fetal bovine serum at 37°C with 5% CO_2. HT22 were plated on 100-mm culture dishes at a density of 10,000 cell/cm^2 and let to attach overnight. The next day medium was removed, and treatments were started. Control cells were maintained in regular RPMI 1640 media containing 3 μM thiamine for the duration of all treatments. For all treatments, media was changed every 24 h. To induce thiamine insufficiency, custom formulated thiamine deficient RPMI 1640 (TD-RPMI) containing 10 nanoM thiamine was utilized. TD-RPMI was supplemented with 10% FBS and 1% penicillin/streptomycin with the addition of 10 μM of the thiamine antagonist Pyrithiamine hydrobromide (PT) for 72 h. This protocol was previously used to induce

TI in primary astrocytes (Zera and Zastre 2017). In addition, other PT concentrations were also tested (Supplemental Figure 2.S.1). To confirm that the HIF1α activation was due to the induction of TI and not to a non-specific PT effect, cells were also treated with the thiamine transporter inhibitor amprolium (Moraes, Rodrigues et al. 2018). Pharmacological inhibition of HIFα was achieved by supplementation of the HIF1α inhibitor YC1 (Kim, Yeo et al. 2006) . After a 24 h pre-treatment with a 20 μM dose, 10 μM of YC1 was added for 3 days with or without PT. This protocol was previously described to attenuate HIF1α signaling in primary astrocytes (Zera and Zastre 2017). To restore Prolyl Hydroxylase (PHDs) activity, HT22 were treated with 2 mM Octyl-KG (MacKenzie, Selak et al. 2007). 10 μM PT was added after 24 h of pretreatment with Octyl-KG. To investigate the role of pyruvate in PHDs inhibition, 1 mM of EP was added to HT22 as previously described (Kim, Choi et al. 2010). To limit pyruvate accumulation during PT treatment, an inhibitor of pyruvate kinase (NCGC00188636) was used as previously described (Zera and Zastre 2018). Cells were treated with 50 μM of pyruvate kinase inhibitor (PKI) for 3 days with or without 10 μM PT (Supplemental Figure 2.S2). As positive control for HIFα stabilization, HT22 were treated with 150 μM of Dimethyloxallyl Glycine (DMOG), a known PHD pan inhibitor (Wall, Corcoran et al. 2014) .

2.2.3 qRT-PCR

Gene expression was assessed using quantitative real time PCR analysis. RNA was extracted using the E.Z.N.A. Total RNA Kit I (Omega Bio-Tek, Norcross, GA) following the manufacturer's instructions. RNA was quantified using a Nanodrop 2000c Spectrophotometer (Thermo Scientific), and 1 μg was reverse transcribed to cDNA with the qScript cDNA Synthesis Kit (Quanta BioSciences) following the manufacturer's instructions. Changes in gene expression

of HIF1α, BACE1, LDHA and BNIP3 were evaluated using a LightCycler 480 II (Roche Applied Science, Indianapolis, IN) and calculated by the $2^{-\Delta\Delta Ct}$ method. Gene specific primers were designed through the Roche Universal Probe Library website to correspond with a specific fluorescein-labeled hydrolysis probe. The actin reference assay kit supplied by Roche Applied Science was used as housekeeping gene.

Table 2.2. Mouse RT-PCR Primers. Primer sequences and probes from Roche Universal Probe Library used for RT-PCR analysis in HT22 cells.

Gene	Primer sequence	Probe
HIF1α	F: 5'-gcactagacaaagttcacctgaga-3' R: 5'-cgctatccacatcaaagcaa-3'	# 95
LDHA	F: 5'-ggcactgacgcagacaag-3' R: 5' -tgatcacctcgtaggcactg-3'	# 12
BNIP3	F: 5'-cctgtcgcagttgggttc-3' R: 5'-gaagtgcagttctacccaggag-3'	# 52

2.2.4 Assessment of protein expression

After treatments, HT22 were harvested as whole cell lysates (WCL) or nuclear extracts for Western blot analysis as previously described (Sweet, Paul et al. 2010) . Briefly, 50 μM of WCL or 25 μM of nuclear lysate was resolved via electrophoresis using an 8% SDS-PAGE for APP detection and 10% SDS-PAGE gels for all other proteins. After transfer, polyvinylidene difluoride membranes were blocked in 3% non-fat milk in tris buffered saline-tween 20 (TBS-T) for 1 h at room temperature (RT). Membranes were immunoblotted for HIF1α (GTX127309, Genetex), LDHA (GTX101416, Genetex), BNIP3 (ab38621, Abcam), EndoG (#22148-1-AP, Proteintech), β-actin (#A2228, Sigma), Beclin (#3495S, Cell Signaling), LC3BI (#AB48394, Abcam), p84

(GTX70220, Genetex), TPK1 (GTX103943, Genetex), PDH (GTX104015, Genetex) and Hydroxy-HIF1α Pro564 (#3434, Cell Signaling) at 4°C for 15 h. Subsequently, blots were washed 3 times each for 10 min in TBS-T, and then immunoblotted with 1:20,000 goat anti-mouse or goat anti-rabbit horseradish peroxidase (HRP)-conjugated secondary antibody (Millipore, Billerica, MA) for 1 h at RT. Blots were visualized using Supersignal West Pico (Thermo Scientific, Rockford, IL) and captured with a Fluorchem HD2 digital imager (Alpha Innotech, San Leandro, CA). Densitometry relative to actin was performed using Image J software on at least n=3 independent experiments.

2.2.5 shRNA Transduction

HT22 cells were seeded on a 96-well plate at 20,000 cells/mL and cultured in regular RPMI until 70% of confluency was reached. At this point 8 μg /ml of Hexadimethrine bromide (H9268-5G) and MISSION lentiviral particles targeting HIF1α (CSTVRS, NM_01043.1, 1864s21c1, TRCN0000232222) or BNIP3 (CSTVRS, NM_009760.2, 524s1c1, TRCN0000009691) were added at a multiplicity of infection (MOI) of 2. As scramble control, non-Mammalian shRNA Control Transduction Particles (SHC002V-1EA, #090919303MN) were added at the same MOI. After 24 h of incubation, media was switched to RPMI containing 1.5 μg/ml puromycin for selection. Transfection with shRNA vector to knockdown HIF1α (shHIF1α) or BNIP3 (shBNIP3) resulted in approximately 90% decrease in HIF1α and BNIP3 mRNA (Figure 2.4).

2.2.6 MTT assay

Changes in cell viability were determined via MTT assay. HT22 were seeded at a density of 1,000 cells/cm2 in 96-well plates and left 24 h in regular RPMI 1640 to attach. Cells were then

treated with PT (0.1 μM to 100 μM) or EP (0.1 mM to 100 mM). In addition, the effect of YC1, octyl-KG, glutamate, Mitoquinol (MQ) and N-acetyl cysteine (NAC) on cell viability was also evaluated. After 72 h, 0.5 mg/ml of 3-(4,5-dimethythiazol2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reagent (5 mg/ml, # 475989, Calbiochem) were added to each well and incubated for 3.5 h at 37˚C. Then the solution was aspirated, and the adherent cells with formazan crystals were dissolved with 150 μl dimethyl sulfoxide for 15 min on a shaker. The absorbance was read at 590 nm using a SpectraMax M2 spectrophotometer (Molecular Devices; Sunnyvale, CA). Values were normalized to the untreated control.

2.2.7 Mitochondrial isolation

Mitochondrial fraction was obtained by differential centrifugation. HT22 were grown in 125-mm dishes with 10 μM PT, 1 mM EP or regular RPMI for 72 h. Cells were then harvested with trypsin/EDTA. After centrifugation, each pellet was resuspended in 1 ml of mitochondria isolation buffer (200 mM mannitol, 100 mM sucrose, 10 mM HEPES, 1 mM EGTA, 5 mM MgCl2, pH 7.4) containing protease and phosphatase inhibitors as described (Pfeiffer, Jaeckel et al. 2014). Cell pellets were disrupted with Dounce homogenizer and centrifuged at 1,000 x g for 10 min. Mitochondria pellets were isolated from the post-nuclear supernatants via centrifugation at 16,000 x g for 30 min and resuspended in mitochondrial isolation buffer. All steps were performed at 4˚C.

2.2.8 Changes in Mitochondrial Membrane Potential

The potentiometric dye tetramethylrhodamine methyl ester (TMRM) (Sigma Aldrich, St. Louis, MO) was used to assess changes in mitochondrial membrane potential (MMP) as previously described (Hanberry, Berger et al. 2014) . HT22 were seeded at a density of 1,000 cells/cm^2 in 96-

well plates and left 24 h in regular RPMI 1640 to attach. Cells were then treated with increasing PT doses from 0.1 μM to 100 μM. After 72 h, 50 nanoM of TMRM was added to each well and incubated at 37°C for 30 min. The media was then aspirated, and wells washed twice with 37°C PBS to remove any residual dye. Fluorescence was measured at Ex/Em=550/575 nm using a SpectraMax M2 spectrophotometer (Molecular Devices; Sunnyvale, CA). Data was normalized to untreated control.

2.2.9 Cellular DNA Fragmentation

Cell apoptosis was measured using the cellular DNA fragmentation ELISA kit (#11585045001, Roche Applied Sciences) according to the manufacturer's protocol. HT22 were grown in regular 1640 RPMI or treated with 10 μM PT or 1 mM EP. After 72 h, 10^5 cells/mL were metabolically prelabeled with 10 μM BrdU for 16 h. After which, BrdU-labeled cells were lysed and incubated on a 96-well plate coated with anti-DNA solution for 90 min at RT. The DNA was then fixed and denatured by microwave irradiation for 5 min. After washes, samples were incubated with an anti-BrdU-Peroxidase conjugate solution at RT for 90 min. The substrate solution was then added, and the plate was incubated for 5 min in the dark with gentle shaking. Following color development, stop solution was added and the absorbance was measured at 450 nm using a SpectraMax M2 spectrophotometer (Molecular Devices; Sunnyvale, CA). Cellular DNA fragmentation was calculated as the fold change compared to untreated control.

2.2.10 Oxidative Stress Detection

Intracellular levels of reactive oxygen species (ROS) were determined by the free radical sensor CM-H2DCFDA according to the manufacturer’s protocol (Invitrogen, Eugene, OR). HT22

cells were seeded at 20,000 cells/cm2 into a 6 well plate and let attach for 15 h. Once attached, cells were treated with the antioxidants 100 μM NAC and 1 μM MitoQ for 72 h. 25 μM Antimycin A (AA) was used as oxidant. Cells were harvested with trypsin/EDTA, briefly centrifuged, and resuspended in 37°C PBS containing 5 μM CM-H2DCFDA for 30 min at 37° C. Subsequently, the probe was removed, cells were washed with PBS and fluorescence was read at Ex/Em= 495/520 nm using a SpectraMax M2 spectrophotometer (Molecular Devices; Sunnyvale, CA). RFU values were further normalized by total protein.

2.2.11 Statistical Analysis

All experiments were performed with a minimum of three independent experiments unless otherwise stated. Statistical significance was evaluated between groups using either Student's t-test or one-way ANOVA analysis of variance with Tukey's post hoc test with a significance level of $p<0.05$ using GraphPad Prism 9 ® (GraphPad Software; La Jolla, CA).

2.3 Results

2.3.1 Thiamine insufficiency stabilizes and activates HIF1a in HT22.

Treatment with 10 μM PT for 3 days resulted in HIF1α stabilization and increase in HIF1α and LDHA expression that was significantly attenuated by shHIF1α knockdown (Figure 2.2A). HIF1α nuclear migration with PT treatment was also significantly reduced by shHIF1α knockdown (Figures 2.2C). Additionally, the HIF1α inhibitor YC1 also attenuated HIF1α stabilization and LDHA expression with PT (Figures 2.2B) and significantly reduced HIF1α nuclear migration (Figures 2.2D). No change in HIF2α stabilization was observed with PT treatment (Figure 2.2E). HIF1α stabilization was also achieved with amprolium supplementation (Figure 2.2F).

2.3.2 Role of Prolyl Hydroxylases (PHDs) in HIF1α stabilization during TI.

PHDs are the main enzymes regulating HIF1α. HIF1α hydroxylation on proline 564 by PHDs directs HIF1α to proteasome-mediated degradation. Supplementation of the PHDs cofactors octyl-KG and ascorbate significantly reduced HIF1α stabilization with PT treatment and limited the expression of LDHA (Figures 2.3A and 2.3B). Octyl-KG and ascorbate treatments also attenuated PT induced HIF1α nuclear migration (Figures 2.3C and 2.3D). No change in the PHDs substrate ATF4 expression during TI was detected (Figure 2.3E). Supplementation of Octyl-KG significantly increased HIF1α hydroxylation on Proline 564 compared to PT (Figure 2.3F).

2.3.3 TI-induced toxicity is triggered by the HIF1a-BNIP3 signaling.

To establish the involvement of BNIP3 in TI-mediated neurotoxicity, we employed BNIP3 knockdown. PT supplementation stabilized HIF1α increasing expression of BNIP3 that was significantly attenuated with shHIF1α (Figures 2.4A+B). The BNIP3 knock out group showed a

significantly less BNIP3 expression after PT treatment compared to control (Figures 2.4C+D). In addition, a significant decrease in protein levels and mRNA of BNIP3 was observed when Octyl-KG (Figures 2.4E) or YC1 were supplemented with PT (Figures 2.4F). MTT assay was used to assess changes in HT22 viability after 72 hours of PT treatment. Cell viability was significantly reduced after treatment with PT but was ameliorated in shHIF1α and shBNIP3 groups (Figures 2.4G+H). TI-induced cell toxicity was also significantly attenuated after Octyl-KG and YC1 treatment (Figures 2.4I+J).

2.3.4 TI induced HIF-BNIP3 toxicity is triggered by oxidative stress.

Increase in HIF1α, LDHA, BACE1 and BNIP3 expression after treatment with 1 mM ethyl pyruvate (EP) was attenuated by supplementation of Octyl-KG (Figure 2.5A) and YC1 (Figure 2.5B). However, 1mM EP did not induced significant toxicity in HT22 and Octyl-KG or YC1 supplementation did not recover cell viability (Figures 2.5C+D). The increase in cell death induced by EP at higher concentration was not mediated by HIF1α or BNIP3 (Figure 2.5E and supplemental figure 2S.3). A significant increase in cell toxicity was detected when an oxidant stimulus (glutamate) was supplemented to EP (Figure 2.5F). In addition, we found that EP treatment did not lead to increase in ROS compared to PT (Figure 2.5G). Supplementation of antioxidants Mitoquinol (MQ) or N-acetyl cysteine (NAC) to PT significantly recovered HT22 viability (Figure 2.5H) without altering HIF1α expression (Figure 2.5I).

2.3.5 BNIP3 potentiates neurotoxicity via MMP impairment and EndoG release.

By mitochondria lysates, we observed the mitochondrial localization of BNIP3 dimer after PT treatment in control but not in shHIF1α or shBNIP3 groups (Figure 2.6A). Supplementation of

glutamate to EP significantly induced BNIP3 mitochondrial localization at comparable levels of PT (Figure 2.6B). Reduction of oxidative stress by antioxidants supplementation to PT, significantly decreased BNIP3 expression in mitochondria (Figure 2.6C). Endo G was detected in nuclear lysates after PT treatment suggesting its implication in BNIP3 mediated toxicity (Figure 2.6D). Impairment of mitochondrial membrane potential (MMP) was not altered in absence of HIF1α or BNIP3 while a significant loss of potential was detected during TI in control group (Figure 2.6E). Increase in chromatin fragmentation was detected after PT treatment but not EP or when HIF1α or BNIP3 were silenced (Figure 2.6F).

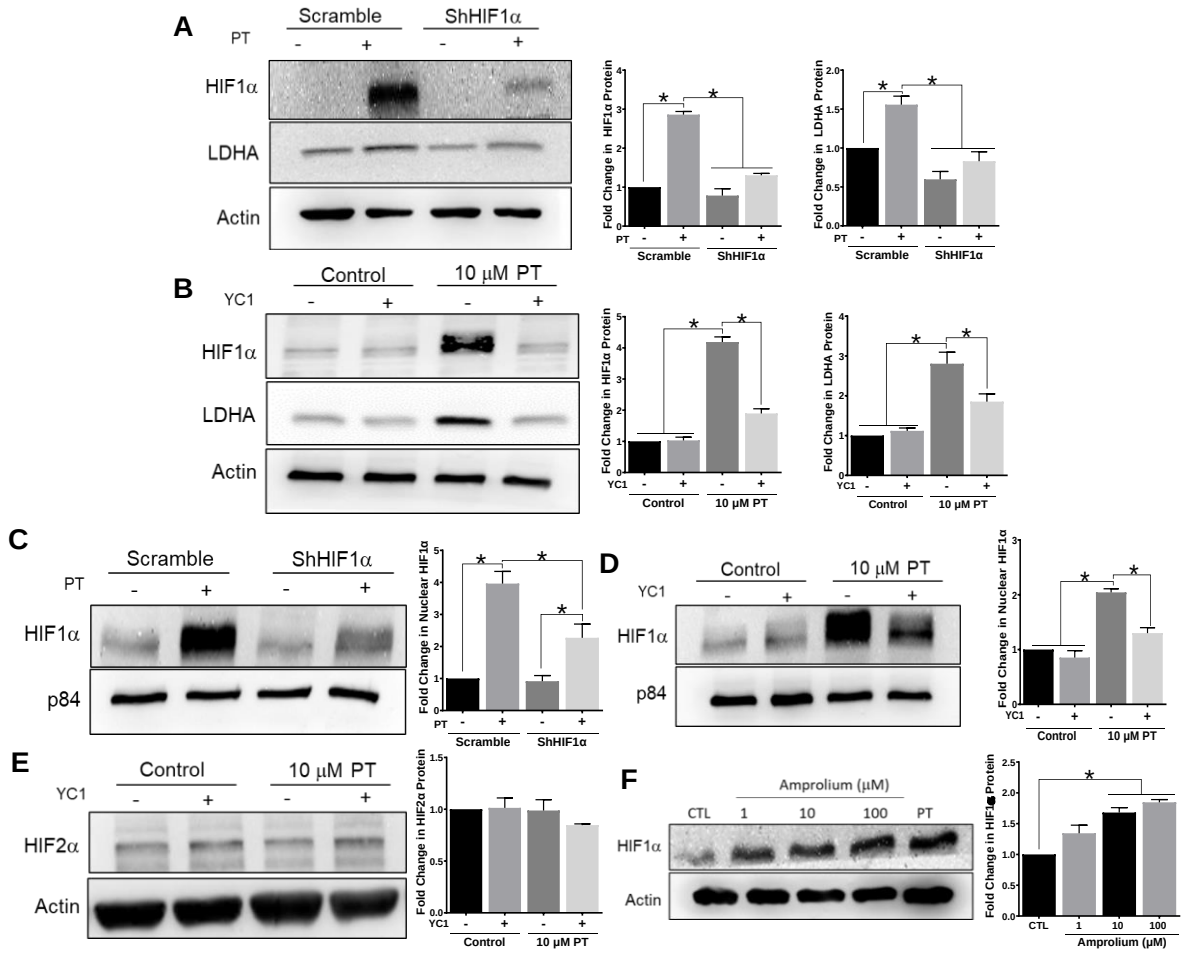


Figure 2.2. HIF1α activation in HT22 after thiamine insufficiency. (A) Representative WB of HIF1α and LDHA protein expression in whole cell lysate (WCL) with relative densitometry after HIF1α silencing and (B) after YC1 treatment. (C) Representative WB of HIF1α protein expression in nuclear lysate after HIF1α silencing with relative densitometry and (D) after YC1 supplementation. (E) Representative WB of HIF2α in WCL after YC1 supplementation with densitometry. (F) Representative WB of HIF1α with densitometry after amprolium treatment (1-100 μM) for 72 hours. HT22 were treated with 10 μM of pyrithiamine (PT) for 3 days. Control group was grown in regular RPMI containing 3 μM thiamine for 3 days. Actin and p84 were used as loading control for WCL and nuclear lysate, respectively. Scramble control was treated with a non-mammalian shRNA. (*****) Represents a statistically significant difference with $p < 0.05$ based on the results of a one-way ANOVA with Tukey's Post-hoc test of at least n=3 independent replicates.

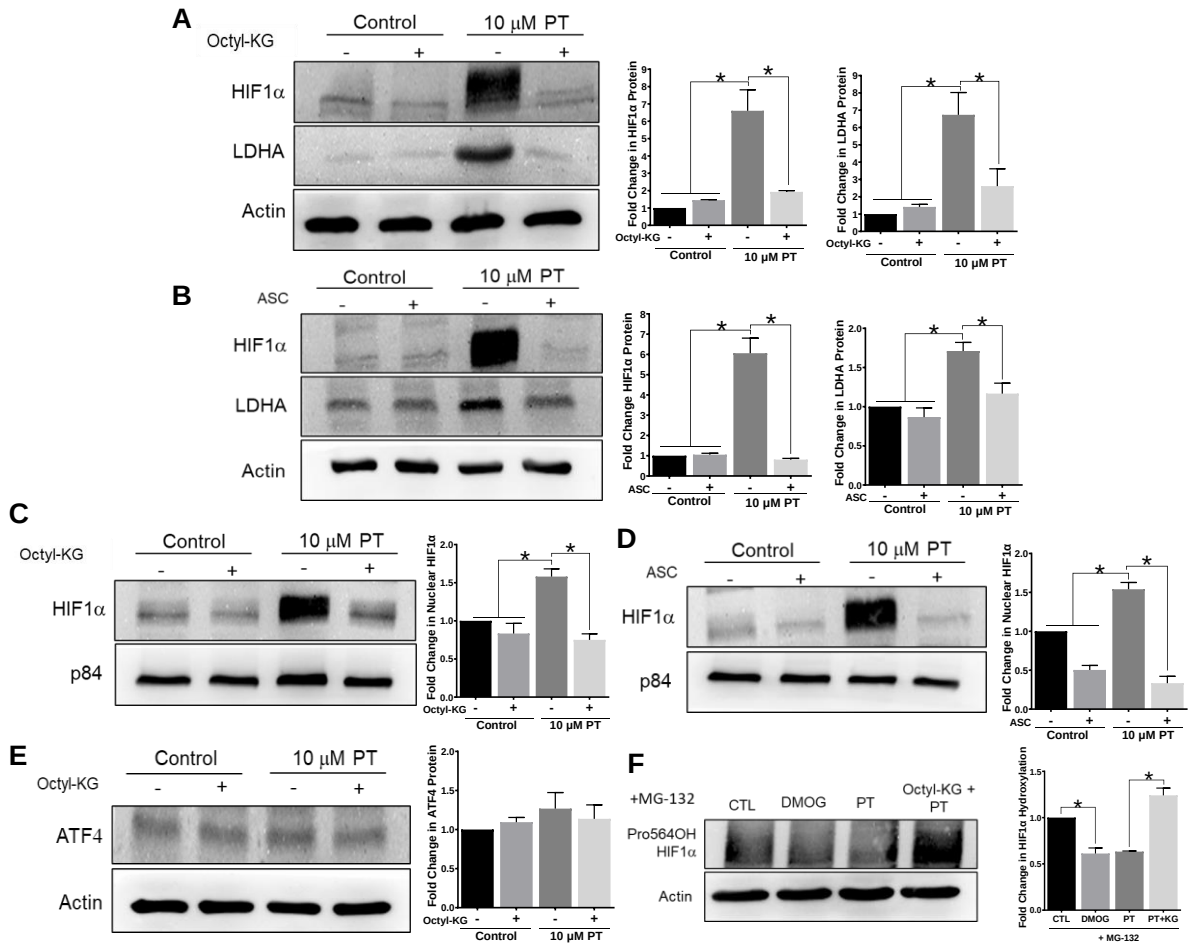


Figure 2.3. Prolyl Hydroxylases regulate HIF1α during TI. (A) Representative WB of HIF1α and LDHA protein expression in whole cell lysate (WCL) with relative densitometry after 2 mM Octyl-KG supplementation and (B) 500 μM of ascorbate supplementation. (C) Representative WB of HIF1α protein expression in nuclear lysate after Octyl-KG supplementation with relative densitometry and after ascorbate (D). (E)Representative WB of ATF4 protein expression in WCL with densitometry after Octyl-KG supplementation. (F) Representative WB and densitometry of changes in Proline 564 hydroxylation in HIF1α after treatment with DMOG, PT +/- Octyl-KG. 10 μM of MG-132 was used to inhibit proteasome degradation. HT22 were treated with 10 μM of pyrithiamine (PT) for 3 days. Control group was grown in regular RPMI containing 3 μM thiamine for 3 days. Actin and p84 were used as loading control for WCL and nuclear lysate, respectively. (*****) Represents a statistically significant difference with $p < 0.05$ based on the results of a one-way ANOVA with Tukey's Post-hoc test of at least n=3 independent replicates.

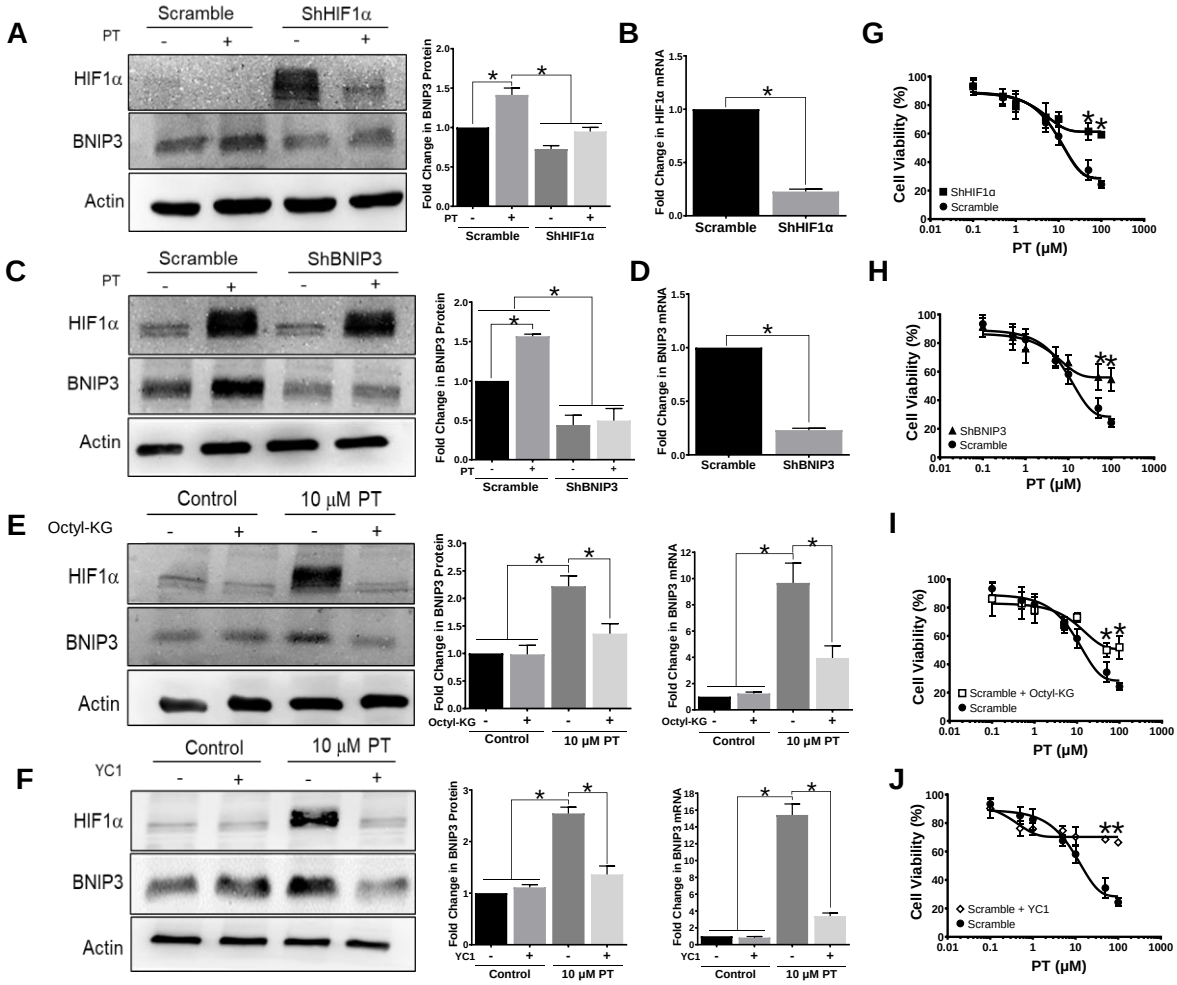


Figure 2.4 Role of HIF1α and BNIP3 in TI-induced toxicity. (A) Representative WB of HIF1α and BNIP3 protein expression in whole cell lysate (WCL) with relative densitometry after shHIF1α transfection and after BNIP3 transfection (C). Knock down validation via Real-time PCR of (B) HIF1α and (D) BNIP3 shRNAs. (E) Representative WB of HIF1α and BNIP3 protein expression in WCL with relative densitometry and fold change in BNIP3 mRNA after 2 mM Octyl-KG treatment and 10 μM YC1 supplementation (F). Change in cells viability via MTT assay after (G) shHIF1α or (H) shBNIP3 transfection, treatment with (I) Octyl-KG or with (J) YC1. HT22 were treated with 10 μM of pyrithiamine (PT) or with different PT doses (0.1 to 100 μM) for 3 days. Control group was grown in regular RPMI containing 3 μM thiamine for 3 days. Actin was used as loading control for WCL and PCR. Scramble control was treated with a non-mammalian shRNA. (*****) Represents a statistically significant difference with $p < 0.05$ based on the results of a one-way ANOVA with Tukey's Post-hoc test of at least n=3 independent replicates.

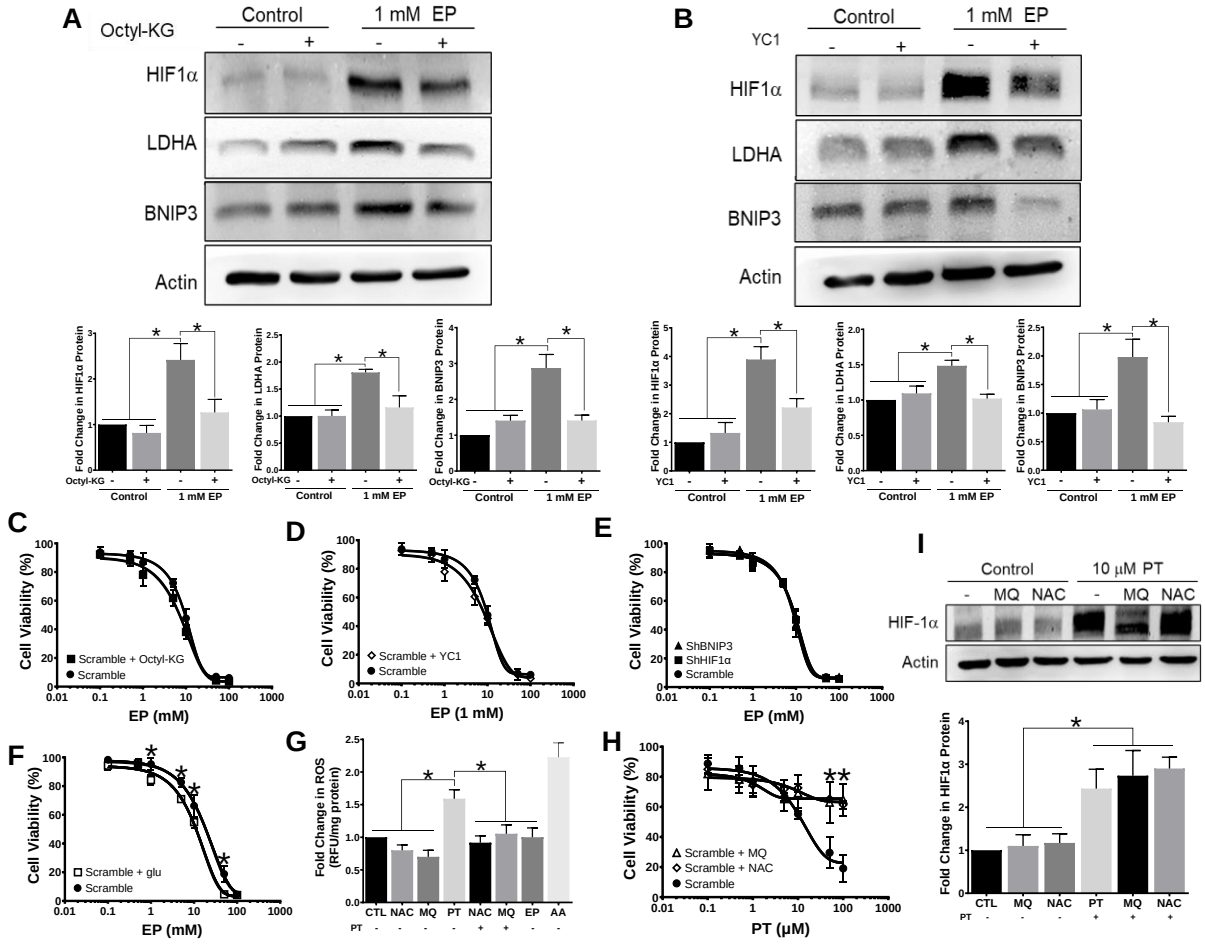


Figure 2.5. BNIP3 toxicity is induced by oxidative stress during TI. (A) Representative WB of HIF1α, LDHA and BNIP3 protein expression in whole cell lysate (WCL) with relative densitometry after 2 mM Octyl-KG treatment and after 10 μM YC1 treatment (B). Change in cells viability via MTT assay after (C) Octyl-KG, (D) YC1 treatment, (E) shBNIP3 transfection or (F) 0.5 mM glutamate supplementation to EP. (G) Fold change in ROS production via CM-H2DCFDA dye. Fluorescence values were normalized to mg of protein. (H) Change in cell viability via MTT assay after treatment with 1 μM Mitoquinone (MQ) and 100 μM N-acetyl cysteine (NAC). (I) representative WB of HIF1α protein expression in WCL with densitometry after MQ or NAC treatment +/- PT. HT22 were treated with 1 mM of EP or 10 μM PT or with different EP (0.1 to 100 mM) or PT doses (0.1 to 100 μM) for 3 days. Actin was used as loading control for WCL and PCR. Scramble control was treated with a non-mammalian shRNA (*****) Represents a statistically significant difference with $p < 0.05$ based on the results of a one-way ANOVA with Tukey's Post-hoc test of at least n=3 independent replicates.

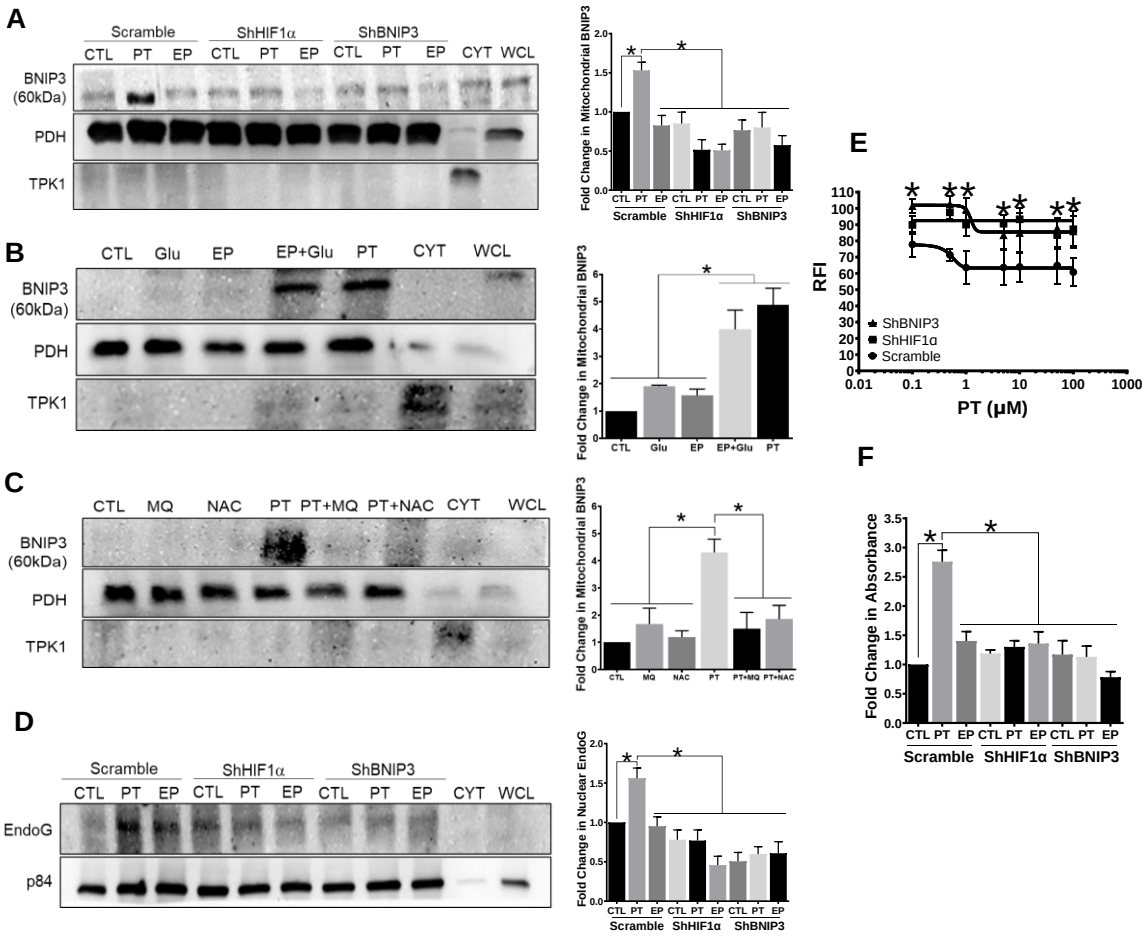


Figure 2.6. BNIP3 potentiates neurotoxicity during TI. (A) Representative WB of dimeric (60 kDa) BNIP3 protein expression in mitochondrial lysate with relative densitometry in scramble, shHIF1α and shBNIP3 groups after PT or EP treatment. (B) Representative WB of dimeric BNIP3 protein expression in mitochondrial lysate with relative densitometry in HT22 treated with 1 μM Mitoquinone (MQ) and 100 μM N-acetyl cysteine (NAC) +/- PT. (C) Representative WB of dimeric BNIP3 protein expression in mitochondrial lysate with relative densitometry in HT22 treated with 0.5 mM glutamate (Glu) +/- EP. (D) Representative WB of Endonuclease G (EndoG) protein expression in nuclear lysate with relative densitometry in scramble, shHIF1a and shBNIP3 groups after PT or EP treatment. (E) Change in mitochondrial membrane potential was assessed via TMRM dye shHIF1a and shBNIP3 groups after PT treatment. (F) A BrdU based ELISA assay was used for chromatin fragmentation detection in shHIF1α and shBNIP3 groups after PT or EP treatment. Actin and p84 were used as loading control for WCL and nuclear lysate, respectively. TPK1 was used as cytosolic control while PDH was used as mitochondrial control. A WCL from control group was used as WCL control for mitochondrial lysates. Scramble control was treated with a non-mammalian shRNA. (*****) Represents a statistically significant difference with $p < 0.05$ based on the results of a one-way ANOVA with Tukey's Post-hoc test of at least n=3 independent replicates.

2.4 Discussion

Mitochondrial dysfunction is a common feature of TI and neurodegeneration onset resulting in impairment in cerebral energy metabolism (Karuppagounder, Xu et al. 2009, Abdou and Hazell 2015). During chronic TI, prolonged reduction in the activity of the mitochondrial thiamine dependent enzyme KGDH leads to TCA cycle impairment, increase in lactic acidosis and excitotoxicity. Diminished thiamine-dependent enzymes' activity mainly impairs glucose metabolism and mitochondrial function leading eventually to neuronal death (Calingasan, Gandy et al. 1996, Karuppagounder, Xu et al. 2009). Compromised mitochondrial activity increases ROS production and oxidative stress that contribute to inflammation and neuronal death. Induction of TI in adult male C57BL/6N mice resulted in severe neuronal loss (90%) after 10-11 days accompanied by microglia activation. Thiamine supplementation seems to attenuate the neurotoxicity at early time points (until day 8) but was ineffective in the last part of the study (day 10-11) (Ke, Degiorgio et al. 2003). In order to explain the TI-induced neurotoxicity, changes in oxidative metabolism due to thiamine depletion, increase in oxidative stress and activation of caspase 3 or Jnk Kinase were considered (Wang, Hua et al. 2000, Gibson and Zhang 2002, Ke, Degiorgio et al. 2003, Karuppagounder, Xu et al. 2009). However, the molecular mechanism has not been described yet.

Here, we showed for the first time that the HIF-BNIP3 signaling may regulate TI-induced neurotoxicity. Our group has previously reported that TI is able to stabilize HIF1α expression and activity in different cell lines (Sweet, Paul et al. 2010, Zastre, Hanberry et al. 2013, Zera and Zastre 2017, Zera and Zastre 2018). Here, we observed that the stabilization and activity of HIF1α was increased after TI in neuronal hippocampal cells. In support of our findings, HIF1α inhibition via knock down and YC1 supplementation decreased HIF1α expression and nuclear migration (Figure

2.2). We did not detect any change in expression in HIF2α. In addition, we treated HT22 with another chemical known to induce TI, amprolium. Amprolium inhibits thiamine transport within the cell but does not affect TPK1 activity. Treatment with amprolium resulted in HIF1α stabilization in HT22 thus confirming that HIF activation was mediated by TI and not due to any PT off-target effects.

The decrease in HIF1α expression after octyl-KG and ascorbate supplementation suggested the involvement of Prolyl Hydroxylases (PHDs) in HIF1α regulation during TI (Figure 2.3). This hypothesis was further supported by the increase in HIF1α hydroxylation in Proline 564 after octyl-KG supplementation to PT. The hydroxylation was at a comparable level to the pan-PHDs inhibitor DMOG. Although further work will be required to clarify PHDs regulation during TI, their involvement may suggest a connection with the Nuclear Factor-kB (NFkB) pathway. The NFkB regulatory kinases IKKα and IKKβ contain the LXXLAP motif recognized by PHDs, adjacent to key phosphorylation sites in the activation loop (Strowitzki, Cummins et al. 2019) . Thus, TI may modulate a pro-inflammatory NFkB mediated response via PHDs.

Activating transcription factor 4 (ATF4) represents another transcription factor that is among the PHDs target genes. ATF4 is a transcription factor induced under severe hypoxia and mitochondrial stress state in cancer and mammalian cell lines (Ye and Koumenis 2009, Rzymski, Milani et al. 2010, Quirós, Prado et al. 2017). ATF4 expression did not change during TI suggesting that ATF4 is not involved in TI response.

Previous hypoxia studies have highlighted the central role of BNIP3 as pro-apoptotic inducer among BH3 -only proteins within the Bcl2 family (Mellor and Harris 2007, Burton and Gibson 2009). Here, we found that silencing of BNIP3 had beneficial effects on neuronal viability at comparable levels of HIF1α silencing (Figure 2.4). This suggests the involvement of BNIP3 as

pro-apoptotic downstream mediator of the TI-induced HIF1α signaling. To exclude the possibility of off-target PT effects, we decided to recreate the metabolic change mediated by TI by supplementing cells with pyruvate. Findings from our and other groups, suggest the role of the intermediate pyruvate in TI driven metabolic HIF1α stabilization (Lu, Dalgard et al. 2005, Zera and Zastre 2018) (Supplemental figure 2.S2) Addition of the more permeable pyruvate analogue, ethyl pyruvate resulted in HIF1α stabilization and activation of BNIP3, comparable to TI. Interestingly, ethyl pyruvate alone did not cause any significant change in cell viability independently from Octyl-KG and YC1 supplementation. Concentrations of ethyl pyruvate higher than 1 mM caused cell death due to the high toxicity of the chemical that was not correlated with HIF1α (Figure 2.5).

These results suggested that pyruvate was only able to increase BNIP3 expression without triggering its activation. Previous studies analyzing the HIF-BNIP3 signaling, reported that the BNIP3 pro-death function could be achieved only with a secondary event identified with oxidative stress (Kubli, Quinsay et al. 2008, Lee, Kubli et al. 2015). Hypoxia studies on cardiomyocytes have established that BNIP3 exists in an inactive state within the cytoplasm until an increase in ROS promote its activation by targeting the serine 64 residue on the N-terminal domain. This conserved residue is a known activation switch for BCL2 family proteins and would promote dimerization by disulfide bonding (Kubli, Quinsay et al. 2008). We observed an increased ROS levels in our neuronal model after PT but not pyruvate supplementation. Antioxidants were beneficial for neuronal viability but did not affect HIF1α expression during TI excluding their involvement in HIF1α stabilization (Figure 2.6). In congruency with previous BNIP3 studies (Zhang, Yang et al. 2007, Burton and Gibson 2009), we detected dimeric BNIP3 within the mitochondria, nuclear EndoG, loss of MPP and chromatin fragmentation only during PT treatment.

No significant change was seen when HIF1α or BNIP3 were silenced or when pyruvate was supplemented. Addition of glutamate as oxidant stimulus to ethyl pyruvate induced BNIP3 mitochondrial localization. Antioxidants addition to PT decreased mitochondrial BNIP3 levels. These findings further highlighted the involvement of a secondary event to trigger BNIP3 induced cell death during TI.

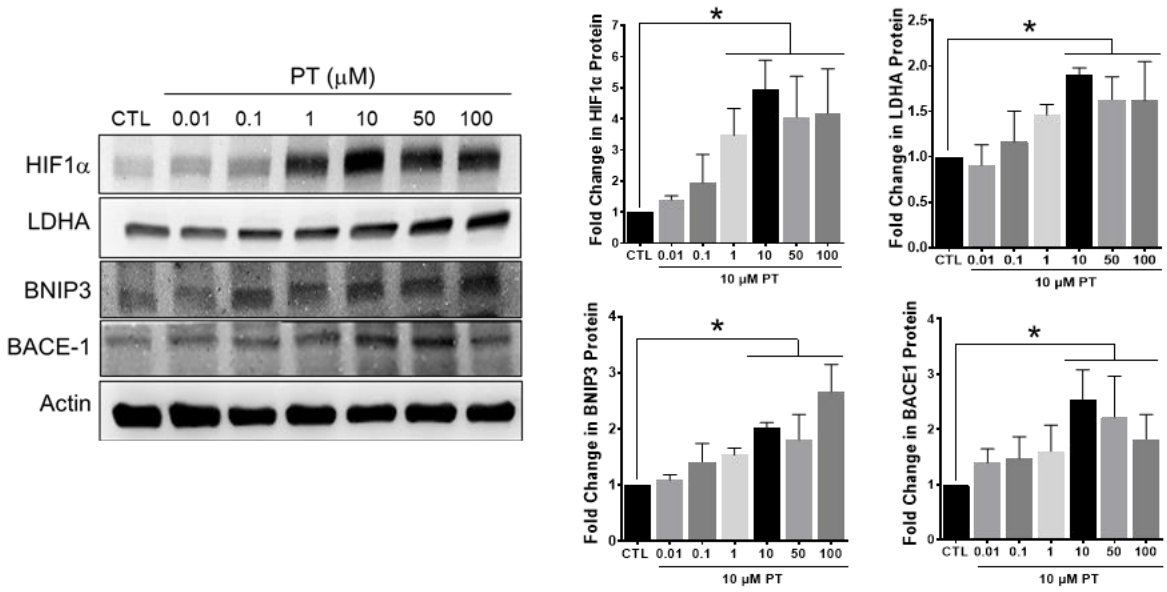
The pro-apoptotic nature of HIF1α response after TI in neurons correlates with our previous work conducted in astrocytes and to other chronic hypoxia/ischemia studies (Helton, Cui et al. 2005, Baranova, Miranda et al. 2007, Hu, Wei et al. 2011, Filippi, Morena et al. 2014, Jian, Shi et al. 2015, Zera and Zastre 2017, Zhang, Yao et al. 2018). As described at the beginning of this chapter, HIF1α mediated apoptosis may be mediated by p53-dependent signaling. Although not described in this study, we have previously investigated changes in p53 expression in astrocytes and neuroblastoma cells during TI. In both cases, p53 seems not to be involved in TI-induced apoptosis (unpublished data). Studies from other groups have reported that thiamine and TPP inhibit p53 DNA binding and reduce p53 expression and p53 intracellular activity in breast cancer cells and in diabetic retinopathy. (McLure, Takagi et al. 2004, Yang, Ge et al. 2004). Thus, more work needs to be conducted to clarify if p53 signaling is involved in TI mediated toxicity.

BNIP3 localization in the mitochondrial compartment has been associated with autophagy. Depending on the circumstances, autophagy can be protective or detrimental for cells (Maiuri, Zalckvar et al. 2007). BNIP3 would induce an autophagic response via liberation of the autophagy effector protein, Beclin1, from an inhibitory complex with Bcl-2 or via inhibition of the mTOR-activating GTPase (Dorn 2010, Quinsay, Thomas et al. 2010). To investigate if autophagy was induced during TI, we looked at change in expression of Beclin and the autophagy marker LC3BI proteins in presence of PT. TI or silencing of HIF1a and BNIP3 did not induce any significant

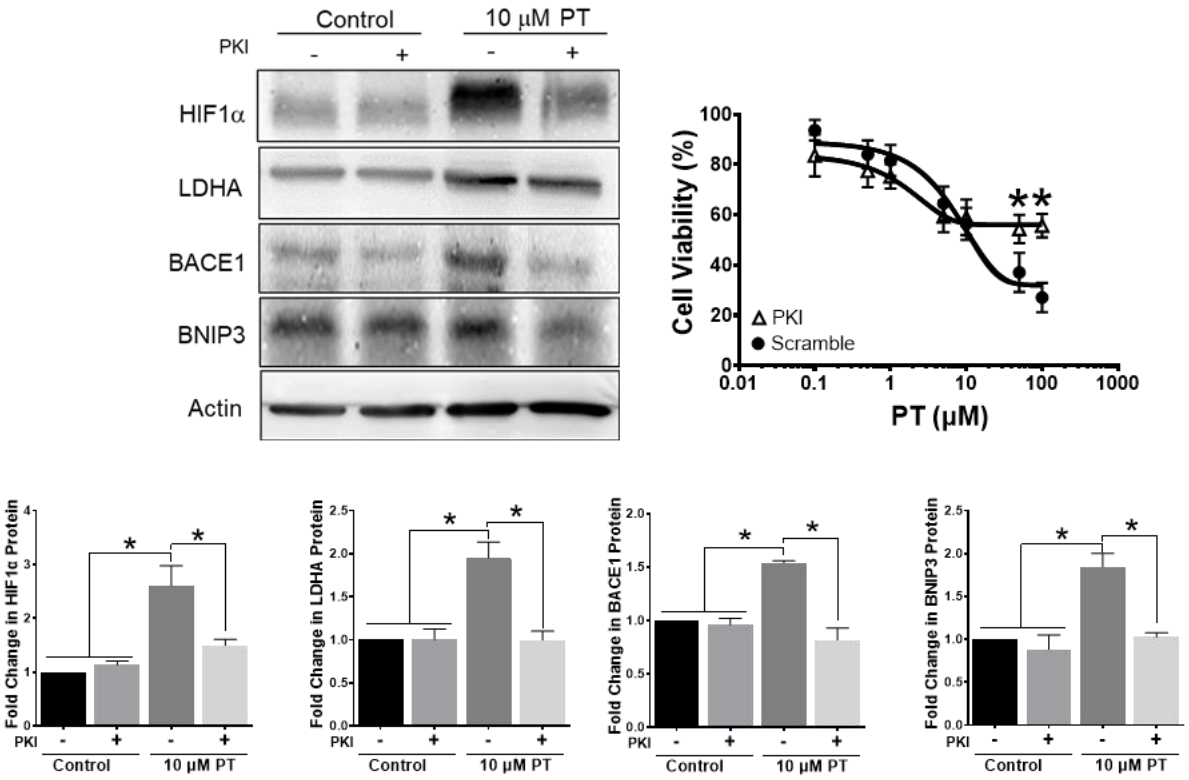
change in Beclin or LC3BI levels in HT22 (Supplemental figure 2.S4). However, TI studies conducted in vitro and in vivo showed that a protective autophagic response occurs during TI induced neurodegeneration (Meng, Yong et al. 2013). The authors suggest that TI may activate autophagy via oxidative stress, ER stress or via mTOR and AMPK pathways (Meng, Yong et al. 2013, Liu, Ke et al. 2017). Therefore, further studies are needed to clarify the involvement and consequences of autophagy during TI.

In conclusion, this work demonstrates that the HIF-BNIP3 signaling is involved in TI-mediated toxicity. Furthermore, oxidative stress has a key role for BNIP3 activation during TI. Overall, these findings suggest an important function of TI-induced HIF1α as mediator of a pro-apoptotic response in neuropathology.

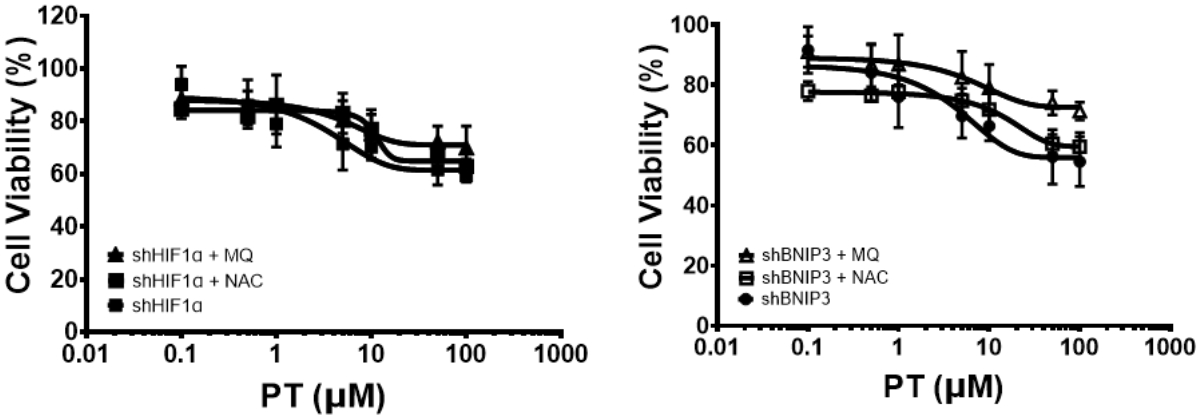
2.5 Supplementary Figures



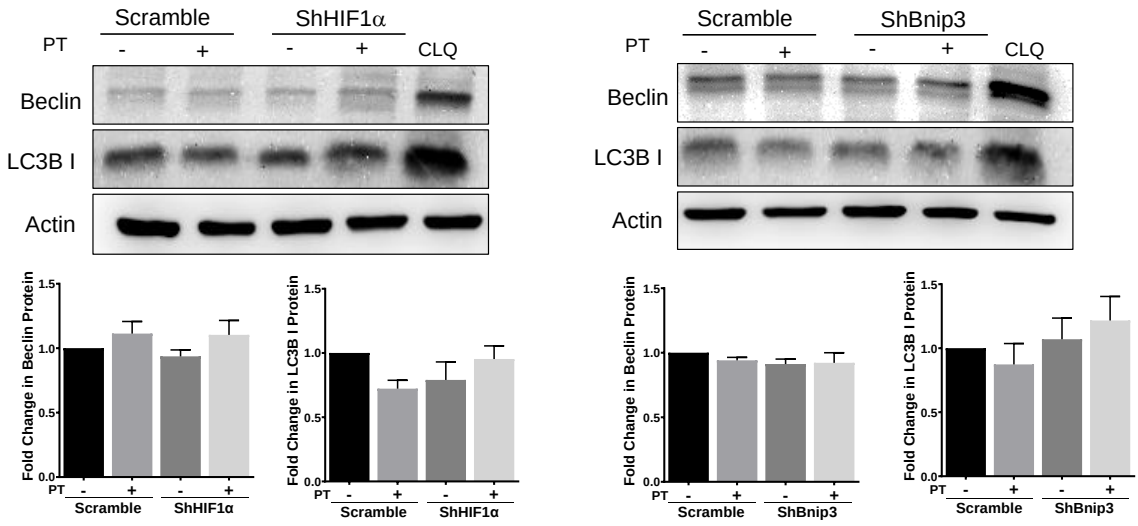
Supplemental Figure 2S.1 PT dose-dependent changes in HIF1α expression. Representative WB of HIF1α, LDHA, BNIP3 and BACE1 and densitometry of HT22 treated with 1, 10, 50 and 100 μM of PT supplementation for 72 hours. Actin was used as loading control. (*****) Represents a statistically significant difference with $p < 0.05$ based on the results of a one-way ANOVA with Tukey's Post-hoc test of at least n=3 independent replicates.



Supplemental Figure 2S.2. Pyruvate accumulation stabilizes HIF1α in HT22. MTT assay and representative WB of HIF1α, LDHA, BNIP3 and BACE1 and densitometry of HT22 treated with PT +/- 50 μM Pyruvate Kinase Inhibitor (PKI) for 72 hours. Actin was used as loading control. (*****) Represents a statistically significant difference with $p < 0.05$ based on the results of a one-way ANOVA with Tukey's Post-hoc test of at least n=3 independent replicates.



Supplemental Figure 2S.3. Antioxidant treatment is independent from HIF1α signaling. Change in cell viability via MTT assay after treatment with 1 μM Mitoquinone (MQ) and 100 μM N-acetyl cysteine (NAC) in ShHIF1α and shBNIP3 groups. (*****) Represents a statistically significant difference with $p < 0.05$ based on the results of a one-way ANOVA with Tukey's Post-hoc test of at least n=3 independent replicates.



Supplemental Figure 2S.4. BNIP3 does not induce autophagy during TI. Representative WB of Beclin and LC3BI with densitometry of scramble, shHIF1α and shBNIP3 groups treated with PT for 72 hours. Actin was used as loading control. 500 μM of chloroquine (CLQ) for 24 hours was used as positive control. (*****) Represents a statistically significant difference with $p < 0.05$ based on the results of a one-way ANOVA with Tukey's Post-hoc test of at least n=3 independent replicates.

CHAPTER 3

THIAMINE- INDUCED HIF1α MEDIATES ALZHEIMER PATHOGENESIS IN VITRO AND IN VIVO [2]

[2] Valle M.L, Anderson Y., Grimsey N., and Zastre J.
To be submitted to *Journal of Neurochemistry.*

3.1 Introduction

Age-dependent decline in thiamine levels has a devastating impact on the brain parenchyma. The neurotoxicity promoted by chronic thiamine insufficiency (TI) is a well-established comorbidity of AD, the most prevalent age-related metabolic neurodegenerative disease. Inadequate levels of blood thiamine and derivates highly correlate with senile dementia prognosis as predictive peripheral AD biomarker. Impaired activity of thiamine-dependent enzymes due to TI, dramatically decreased cerebral metabolism promoting oxidative stress, mitochondrial damage, inflammation, and eventually neuronal loss.

Recently, TI was shown to stabilize HIF1α, the major hypoxia inducible transcription factor (Sweet and Zastre 2013) . HIF1α mediated pathway may either induce a protective effect or a pro-apoptotic response according to the cell type and severity of the insult (Bergeron, Gidday et al. 2000, Semenza 2012). In several ischemic/hypoxic models, chronic HIF1α activation was associated with neurodegeneration and AD related gene expression (Gibson 1989, de la Monte, Neely et al. 2000, Kalaria, Akinyemi et al. 2016, Daulatzai 2017) . HIF1α signaling activates a neuropathological cascade involving free radical production, microglial activation, tau hyperphosphorylation, plaques deposition with subsequent neuronal loss and memory impairment (Aminova, Siddiq et al. 2008, Varela-Nallar, Rojas-Abalos et al. 2014, Zhang, Yang et al. 2014). In particular, HIF1α potentiates the amyloidogenic cascade via direct transcriptional activation of β-secretase (BACE)1 (Li, Zhou et al. 2006, Xue, Jia et al. 2006).

Thus, HIF1a may be the upstream mediator between TI and AD-like pathology. For the purposes of this dissertation, additional background information on HIF1α role in AD and AD models has been included in the introduction.

3.1.1 Dual HIF1α response in AD

3.1.1.1 HIF1a protective response

In congruency with the hypoxia studies, HIF1α can mediate a protective or pro-death signaling during AD. A beneficial HIF1α response was detected in primary murine astrocytes after amyloid ($A\beta_{1-42}$) activation (Schubert, Soucek et al. 2009). Astrocytes can be activated by stress or pathological conditions leading to a morphological (reactive gliosis) and a functional change in which the cells release oxidants and cytokines (Pekny and Nilsson 2005). Treatment with $A\beta_{1-42}$ fragments induces astrocytes activation with increase in ROS production. In the model described by Schubert *et al*., the authors speculate that Aβ fragments would impair proteasome activity affecting HIF1α stability. The degradation of HIF1α would dramatically impact glycolysis and ATP production with a consequent increase in cellular glutamate and oxidative stress within the cerebral parenchyma. The supplementation of the iron chelator and PHDs inhibitor DFO induced HIF1α stabilization, resulting in cellular protection and reactivation of glycolysis. Interestingly, other in vitro and in vivo studies highlighted the beneficial effect of DFO in dementia (Weinreb, Amit et al. 2010, Guo, Wang et al. 2013, Guo, Hao et al. 2016). DFO nasal administration to APP mice improved cognitive abilities and inhibited amyloid and tangles deposition. The protective effect of HIF1α was related to the upregulation of transferrin receptor (TFR), divalent metal transporter 1 (DMT1), and brain-derived neurotrophic factor (BDNF) resulting in a reduction in iron-overload and cell toxicity (Guo, Zhang et al. 2015).

Using a gene therapy approach, Chai *et al.* investigated the HIF1α protective role in vitro and in vivo (Chai, Kong et al. 2014). The group chose a recombinant adeno-associated virus (rAAV), reconstructed from a non-pathogenic wild-type adeno-associated virus (AAV) carrying the human HIF1α gene (rAAV-HIF1α). HT22 transduced with the construct showed a significant

reduction in amyloid induced death. In addition, direct intracerebral administration of rAAV-HIF1α induced prolonged HIF1α expression and decreased neuronal apoptosis in rat hippocampus. Although the authors were unsure about the mechanism behind HIF1α induced neuroprotection, they considered activation of HIF1α target antiapoptotic Bcl2 proteins or reduction in calcium levels.

A lot of attention was given to M30, another iron chelator that has shown to activate HIF1α inducing neuroprotection. Systemic M30 administration in an APP/PSEN1 mice model, reduced plaque formation. Furthermore, the compound upregulated HIF1α in various brain regions (cortex, striatum, and hippocampus) and spinal cord of adult mice. Increased levels of HIF1α target genes VEGF, EPO, enolase, transferrin, iNOS and GLUT1 were also detected (Youdim 2013). The drug has shown protective effects in Parkinson's and Amyotrophic lateral sclerosis (ALS) models as well (Youdim 2013). As explanation for the M30 protective mechanism induced by HIF1α activation, mitochondrial autophagy, a selective protective mechanism to removed damaged mitochondria, was suggested (Youdim 2013, Wu and Chen 2015).

To elucidate the molecular mechanism behind neurotoxicity, Soucek *et al.* established an amyloid resistant model exposing PC12 and B12 lines to amyloid fragments for four months (Soucek, Cumming et al. 2003). The Aβ resistant cells did not die after amyloid treatment and were more resistant to oxidative stress suggesting a higher glycolysis. To elucidate the nature of this metabolic advantage, the authors investigated if amyloid was able to stabilize HIF1α. Using western blot and electrophoretic mobility shift assays (EMSA), the authors were able to detect HIF1α expression not only in their in vitro model but also in cortical extracts from 22- and 23-month-old Tg2576 mice. These findings suggest that amyloid directly induced HIF1α expression and activity, leading to a HIF1α induced protective effect.

3.1.1.2 HIF1a pro-amyloidogenic response

It is well-established that hypoxia and cerebral hypoperfusion play a key role in AD pathophysiology. As further evidence of the involvement of HIF in AD neuropathogenesis, severe cerebral hypoxia may trigger apoptosis and mitochondrial dysfunction with consequent neuronal loss and memory impairment. Decreasing hypoxia state by enhancing oxygen supply or via the cerebral vasodilator Hydergine proved to be effective in ameliorating AD progression in early stages (Olin, Schneider et al. 2001, Sun, He et al. 2006).

Activation of HIF1α regulate plaque deposition by modulation of BACE1. BACE1 cleaves the APP into 99-residue membrane-associated fragments (C99) involved in the generation of β-amyloid peptides. Chronic hypoxia can alter APP production via BACE1 upregulation through HIF1α pathway (Wen, Onyewuchi et al. 2004, Sun, He et al. 2006). BACE1 is one of the downstream target genes of the HIF1α pathway having an HRE in its promoter region (Sun, He et al. 2006). In HIF1α conditional knock-out mice, BACE1 protein expression was significantly reduced in the hippocampus and cortex regions (Sun, He et al. 2006). Interestingly, even a slight increase in BACE1 expression could lead to a dramatic increase in amyloid production (Li, Zhou et al. 2006). Besides AD, increase in BACE1 levels have been reported after other causes of hypometabolism such as traumatic brain injury, ischemia, and oxidative stress (Tamagno, Bardini et al. 2002, Wen, Onyewuchi et al. 2004, Velliquette, O'Connor et al. 2005, Vassar, Kovacs et al. 2009).

Wang *et al*. also reported that hypoxia can upregulate γ-secretase via upregulation of anterior pharynx defective1 (APH1). The authors detected a HRE within the promoter of APH1, component of the γ - secretase complex together with presenilin (PS), nicastrin (NCT) and presenilin enhancer-2 (PEN-2) (Li, Zhang et al. 2009). Furthermore, in neuroblastoma cell SH-

SY5Y, chromic hypoxia inhibited the expression but not the mRNA of α-secretase within the non-amyloidogenic pathway (Marshall, Rattray et al. 2006). This inhibition would result in the reduction of C83 and soluble APP deposition in favor to C99 accumulation. Hypoxia state also downregulated protein and mRNA levels of neprylisin (NEP), critical neuropeptidase for Aß degradation (Kerridge, Kozlova et al. 2015). Additionally, amyloid deposition increases expression and activity of BNIP3, well known HIF1α proapoptotic target gene, via oxidative stress (Zhang, Zhang et al. 2007). Neurons treated with amyloid fragments induce ROS accumulation that in turn stabilized HIF1a via PHDs inhibition. When BNIP3 was silenced, neuronal death was inhibited suggesting its involvement in the neurotoxicity. Increased levels of BNIP3 were also reported in post-ischemic neurodegeneration of the hippocampus with subsequent development of dementia. The in vivo study found a significant increase in BNIP3 and caspase 3 genes up to 48 hours after ischemia (Pluta, Barcikowska et al. 1998, Pluta, Ouyang et al. 2021) .

3.1.2 Mice Models in AD

Considering the complexity of AD, the establishment of AD animal models has been challenging and led to failure in clinical studies. Transgenic mice are the most common model used to study AD pathology. In this section, the most common AD models will be discussed.

3.1.2.1 APP mutations

67 APP Mutations, among which 51 pathogenic, have been reported so far (Esquerda-Canals, Montoliu-Gaya et al. 2017). These mutations have been named according to the geographic origins of the first identified carrier family. The most common is the Swedish double mutation (K670N/M671L) located on the BACE1 cleavage site that increase amyloid production.

The transgenic strains Tg2576 and APP23 carry the Swedish mutation resulting in 5 to 7-fold increase in amyloid production., senile plaques, gliosis and learning impairment by 9-10 months (Hsiao, Chapman et al. 1996).

Development of angiopathy is a common feature of the Flemish (A692G), Dutch (E693Q), and Italian (E693K) mutations used to recapitulate cerebrovascular amyloid angiopathy (CAA). An increase Aβ toxicity has been linked to the Arctic (E693G), London (V717I), Indiana (V717F), Florida (I716V), French (V715M), German (V715A), and Austrian (T714I) mutations. Transgenic models known as TgCRND8 and J20 carry the Swedish and the Indiana mutations, while the TASD-41 strain carries the Swedish and London mutation. These models showed plaque deposit and cognitive impairment around 3-5 months (Chishti, Yang et al. 2001).

3.1.2.2 APP and presenilin 1 mutations

The combination of amyloid and presenilin mutation was another strategy employed for more effective AD models. Presenilin 1 and 2 are component of the γ-secretase catalytic together with the anterior pharynx-defective 1 protein (APH-1), nicastrin, and presenilin enhance protein 2 (PEN2). PSEN1 mutation is very common in FAD with 219 out of 230 mutations described being pathogenic (Shen and Kelleher 2007). PSEN1 mutations M146V, M146L, L286V, and ΔE9 alter γ-secretase activity and have been exploited in transgenic models. The most common Tg models bearing APP and PSEN1 mutations are the 3xTg and 5xTg models.

As suggested by its name, the 5xTg model combines three APP mutations (Swedish, Florida and London) with two PSEN1 mutations (M146V and L286V) under the control of the murine Thy-1 promoter. The severe AD pathology of this model includes amyloid genesis, gliosis at month 2, neuronal loss and cognitive impairment at month 4 (Oakley, Cole et al. 2006).

Engineered by the LaFerla group, the 3xTg model carries the Swedish APP, tau (P301L) and PS1 M146V mutations allowing the study of tauopathies in addition to amyloid pathology. The model is characterized by synaptic dysfunction, cognitive impairment at 4 months, amyloid deposition at 6 months and tau pathology at 12 months (Oddo, Caccamo et al. 2003).

3.1.2.3 APP and ApoE

Apolipoprotein E (ApoE) e4 allele is among the risks factor inducing sporadic AD patients. ApoE is a 35 kDa glycoprotein highly expressed in the brain whose contribution to AD pathology is still unclear. In particular, ApoE isoform E4 has been associated with increased risk in sporadic AD. Isoform E3 is commonly present in the population and isoform E2 seems to decrease the risk in dementia developing (Muñoz, Garner et al. 2019). A transgenic mouse model containing the E4 isoform instead of the endogenous gene was successfully crossed with TgCRND8 mice carries the Swedish and Indican APP mutations. The resulting strain is known as TgCRND8x (Graybeal, Bozzelli et al. 2015).

3.1.3 Rationale and goal

The purpose of the work presented in this chapter was to elucidate the molecular mechanism behind TI-induced AD like pathology. Our hypothesis was that HIF1α may mediate Alzheimer's Disease-like symptomatology during thiamine insufficiency.

Chronic hypoxia is one of the most critical risk factors in AD pathogenesis (Sapin, Peyron et al. 2015, Macheda, Roberts et al. 2019, Zhang, Niu et al. 2019). APP production can be altered via upregulation of BACE1 or γ-secretase or by inhibition of α-secretase and neprylisin (Wen, Onyewuchi et al. 2004, Marshall, Rattray et al. 2006, Sun, He et al. 2006, Li, Zhang et al. 2009,

Kerridge, Kozlova et al. 2015). BACE1 is one of the downstream target genes of the HIF1α pathway having an HRE in its promoter region (Sun, He et al. 2006). In HIF1α conditional knock-out mice, BACE1 protein expression was significantly reduced in the hippocampus and cortex regions (Sun, He et al. 2006). Furthermore, our previous work on astrocytes has shown that HIF1α is stabilized during TI leading to a pro-apoptotic response (Zera and Zastre 2018).

Therefore, we investigated if HIF1α mediated AD-like pathology during TI via BACE1 activation in an in vitro and in vivo AD model.

3.2 Materials and methods

3.2.1 Reagents

Cell culture reagents including RPMI 1640 media, penicillin/streptomycin, and trypsin/EDTA were purchased from Corning (Manassas, VA). Fetal bovine serum (FBS) was obtained from Sigma Aldrich (St. Louis, MO). Thiamine deficient RPMI was custom formulated by United States Biological (Salem, MA). Cell culture treated flasks and dishes were purchased from Greiner Bio-One (Monroe, NC). Chemicals including Dimethyloxallyl Glycine (DMOG), pyrithiamine hydrobromide (PT) and ethyl pyruvate (EP) were purchased from Sigma Aldrich (St. Louis, MO). YC1 (3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole) was purchased from Tocris (Minneapolis, MN) and Octyl-α-ketoglutarate (Octyl-KG) from Cayman Chemical (Ann Arbor, MI).

3.2.2 Cell culture treatments

HT22 murine hippocampal cells were a kind gift from Dr. William Hill (Augusta University, GA). Cells were cultured in RPMI 1640 supplemented with 1% penicillin/streptomycin and 10% fetal bovine serum at 37°C with 5% CO_2. HT22 were plated on 100-mm culture dishes at a density of 10,000 cell/cm^2 and let to attach overnight. The next day medium was removed, and treatments were started. Control cells were maintained in regular RPMI 1640 media containing 3 μM thiamine for the duration of all treatments. For all treatments, media was changed every 24 h. To induce thiamine insufficiency, custom formulated thiamine deficient RPMI 1640 (TD-RPMI) containing 10 nanoM thiamine was utilized. TD-RPMI was supplemented with 10% FBS and 1% penicillin/streptomycin with the addition of 10 μM of the thiamine antagonist Pyrithiamine hydrobromide (PT) for 72 h. This protocol was previously used to induce

TI in primary astrocytes (Zera and Zastre 2017). Pharmacological inhibition of HIFα was achieved by supplementation of the HIF1α inhibitor YC1 (Kim, Yeo et al. 2006) . After a 24 h pre-treatment with a 20 μM dose, 10 μM of YC1 was added for 3 days with or without PT. This protocol was previously described to attenuate HIF1α signaling in primary astrocytes (Zera and Zastre 2017). To restore Prolyl Hydroxylase (PHDs) activity, HT22 were treated with 2 mM Octyl-KG (MacKenzie, Selak et al. 2007). 10 μM PT was added after 24 h of pretreatment with Octyl-KG. As positive control for HIFα stabilization, HT22 were treated with 150 μM of Dimethyloxallyl Glycine (DMOG), a known PHD pan inhibitor (Wall, Corcoran et al. 2014) .

3.2.3 qRT-PCR

Gene expression was assessed using quantitative real time PCR analysis. RNA was extracted using the E.Z.N.A. Total RNA Kit I (Omega Bio-Tek, Norcross, GA) following the manufacturer's instructions. RNA was quantified using a Nanodrop 2000c Spectrophotometer (Thermo Scientific), and 1 μg was reverse transcribed to cDNA with the qScript cDNA Synthesis Kit (Quanta BioSciences) following the manufacturer's instructions. Changes in gene expression of HIF1α, BACE1, LDHA and BNIP3 were evaluated using a LightCycler 480 II (Roche Applied Science, Indianapolis, IN) and calculated by the $2^{-\Delta\Delta Ct}$ method. Gene specific primers were designed through the Roche Universal Probe Library website to correspond with a specific fluorescein-labeled hydrolysis probe. The actin reference assay kit supplied by Roche Applied Science was used as housekeeping gene.

Table 3.1. Mouse RT-PCR Primers. Primer sequences and probes from Roche Universal Probe Library used for RT-PCR analysis in HT22 cells and mouse brains.

Gene	Primer sequence	Probe
HIF1α	F: 5'-gcactagacaaagttcacctgaga-3' R: 5'-cgctatccacatcaaagcaa-3'	# 95
LDHA	F: 5'-ggcactgacgcagacaag-3' R: 5' -tgatcacctcgtaggcactg-3'	# 12
BNIP3	F: 5'-cctgtcgcagttgggttc-3' R: 5'-gaagtgcagttctacccaggag-3'	# 52
BACE1	F:5'-ccctttcctgcatcgctac-3' R: 5'-tacacacccttttcggaggtc-3'	# 34

3.2.4 Assessment of protein expression

After treatments, HT22 were harvested as whole cell lysates (WCL) or nuclear extracts for Western blot analysis as previously described (Sweet, Paul et al. 2010) . Briefly, 50 μM of WCL or 25 μM of nuclear lysate was resolved via electrophoresis using an 8% SDS-PAGE for APP detection and 10% SDS-PAGE gels for all other proteins. After transfer, polyvinylidene difluoride membranes were blocked in 3% non-fat milk in tris buffered saline-tween 20 (TBS-T) for 1 h at room temperature (RT). Membranes were immunoblotted for HIF1α (10006421, Cayman), LDHA (GTX101416, Genetex), BACE1 (GTX113319, Genetex), BNIP3 (ab38621, Abcam), Anti-Amyloid Precursor Protein, C-terminal (#A8717, Sigma), β-actin (#A2228, Sigma) at 4°C for 15 h. Subsequently, blots were washed 3 times each for 10 min in TBS-T, and then immunoblotted with 1:20,000 goat anti-mouse or goat anti-rabbit horseradish peroxidase (HRP)-conjugated

secondary antibody (Millipore, Billerica, MA) for 1 h at RT. Blots were visualized using Supersignal West Pico (Thermo Scientific, Rockford, IL) and captured with a Fluorchem HD2 digital imager (Alpha Innotech, San Leandro, CA). Densitometry relative to actin was performed using Image J software on at least n=3 independent experiments.

3.2.5 BACE1 activity assay

Changes in BACE1 activity was measured using the β-Secretase (BACE1) Activity Assay Kit (#K388, Biovision, Milpitas CA) following the manufacturer's instructions. Briefly, HT22 cells were lysed with ice-cold BACE1 Extraction Buffer and placed on ice for 10 min. Lysates were then centrifuged at 10,000 x g for 5 min at 4°C. Supernatants were collected and treated with saturated ammonium sulfate (#7096, Biovision, 1:2 ratio) in order to precipitate proteins. After 30 min of incubation, samples were spun at 10,000 x g at 4°C for 10 min and pellets were resuspended with BACE1 Assay Buffer. Samples were then aliquoted to a 96-well plate and a reaction mix containing the BACE1 substrate was added to each well. Fluorescence was measured at Ex/Em=345/500 nm after 2 min and 15 min of incubation at 37°C using a SpectraMax M2 spectrophotometer (Molecular Devices; Sunnyvale, CA). BACE1 activity was proportional to the fluorescence intensity and values were normalized by the total amount of protein.

3.2.6 In vivo model

The animal protocol was approved by the University of Georgia Institutional Animal Care and Use Committee and was compliant with Guidelines for the Use and Care of Laboratory Animals from the National Institutes of Health. Thirty-two female 3xTg-AD mice (Jackson Laboratories) (strain name B6;129 ^{Psen1tm1Mpm} Tg (APPSwe, tauP301L)1Lfa/Mmjax) were used for

the study. These mice contain three mutations associated with familial Alzheimer's disease: Swedish APP mutation KM670/671NL, presenilin (PSEN)1 M146V mutation together with microtubule associated protein tau (MAPT) P301L mutation. 3xTg-AD mice develop progressive AD neuropathology showing cognitive impairment at 4 months followed by plaques detectable after 6 months and tangles around 12 months (Oddo, Caccamo et al. 2003). In addition, gliosis and changes in long term potentiation have been detected at 6-7 months of age (Stover, Campbell et al. 2015). Based on the symptomatology of the strain, the study started when mice were 3 months old to avoid the development of the AD symptomatology in the control group. Mice were fed with a control chow (BioServ #F7745; Flemington, NJ) or thiamine deficient chow (BioServ #F7744; Flemington, NJ) for the duration of the study. In addition to thiamine deficient diet, PT was used to recapitulate TI pathogenesis in 9-10 days as established model (Watanabe and Kanabe 1978, Resende, Ribeiro et al. 2012) . YC1 was used to pharmacologically inhibit HIF1α (Yeh, Lu et al. 2007, Lee, Tai et al. 2018). The study involved four groups: control group (mice fed with control chow and injected an equivalent volume of 1% DMSO in bacteriostatic normal saline USP, n=8), PT group (mice fed with TI diet plus 2 mg/Kg PT IP daily injection, n=8), YC1 control group (mice fed with control diet and injected IP with YC1 at a dose of 5 mg/Kg once per day, n=8) and YC1 and PT group (mice fed with TI diet receiving 5 mg/Kg YC1 and 2 mg/Kg PT IP daily injection, n=8). YC1 injections began 2 days prior to initiating TI diet and PT injections. All animals were group housed during the duration of testing and were provided food and water ad libitum. They were maintained in a 12:12 light cycle. Food consumption per cage and weight loss was monitored daily. Diet was restricted in the saline and YC1 control group to remain isocaloric with the treatment groups.

3.2.7 Ledge test

Mice were evaluated daily for TI symptomatology. The ledge test was used to assess ataxia and motor impairment as previously described (Resende, Ribeiro et al. 2012). Mice were individually placed on the ledge of a cage and allowed to walk until they lowered themselves back down into the cage. For each mouse, the time taken to lower themselves back in the cage was measured. Furthermore, balance, the ability to grip the ledge with their hind paws together with their ability to gracefully lower themselves back down into the cage were also observed. Each measurement was performed daily in a blinded manner.

3.2.8 Statistical Analysis

All experiments were performed with a minimum of three independent experiments unless otherwise stated. Statistical significance was evaluated between groups using either Student's t-test or one-way ANOVA analysis of variance with Tukey's post hoc test with a significance level of $p<0.05$ using GraphPad Prism 9 ® (GraphPad Software; La Jolla, CA).

3.3 Results

3.3.1 TI-induced HIF1α mediates AD-like pathogenesis via BACE1.

To evaluate if HIF1α mediated AD-like pathology during TI, changes in BACE1 expression, activity, and C99 deposition were investigated. PT treatment resulted in an increase in mRNA, mature BACE1, and C99 that was significantly decreased with HIF1α knockdown (Figures 3.1A+B). Activation of PHDs via Octyl-KG supplementation (Figures 3.1C+D) and pharmacological inhibition of HIF1α via YC1 (Figure 3.1E+F) reduced BACE1 mRNA as well as BACE1 and C99 expression. Assessment of BACE1 activity after PT resulted in a significant increase that was attenuated by shHIF1α (Figure 3.1G), Octyl-KG (Figure 3.1H) or YC1 supplementation (Figure 3.1I).

3.3.2 TI-induced HIF1α mediates AD-like pathogenesis in vivo.

To determine if TI activated the HIF1α signaling in vivo 3xTg-AD mice were administered 2mg/kg PT alone or with 5mg/kg YC1 through IP injection every day for 9 days. Analysis of the murine brain via WB showed that the increase in expression of in HIF1α, LDHA, BNIP3, BACE1 and C99 induced by PT was significantly reduced by YC1 administration (Figure 3.2A). In accordance with the PT model, by the last day of treatment mice treated with PT had a significant loss in their weight (Figure 3.2B) and in food consumption (Figure 3.2C) compared to the saline control, YC1 control and YC1+PT groups. Ledge test scores confirmed the progressive aggravation of TI symptomatology in PT group compared to the saline and YC1 controls although no significant difference was detected between PT and PT+YC1 groups (Figure 3.2D).

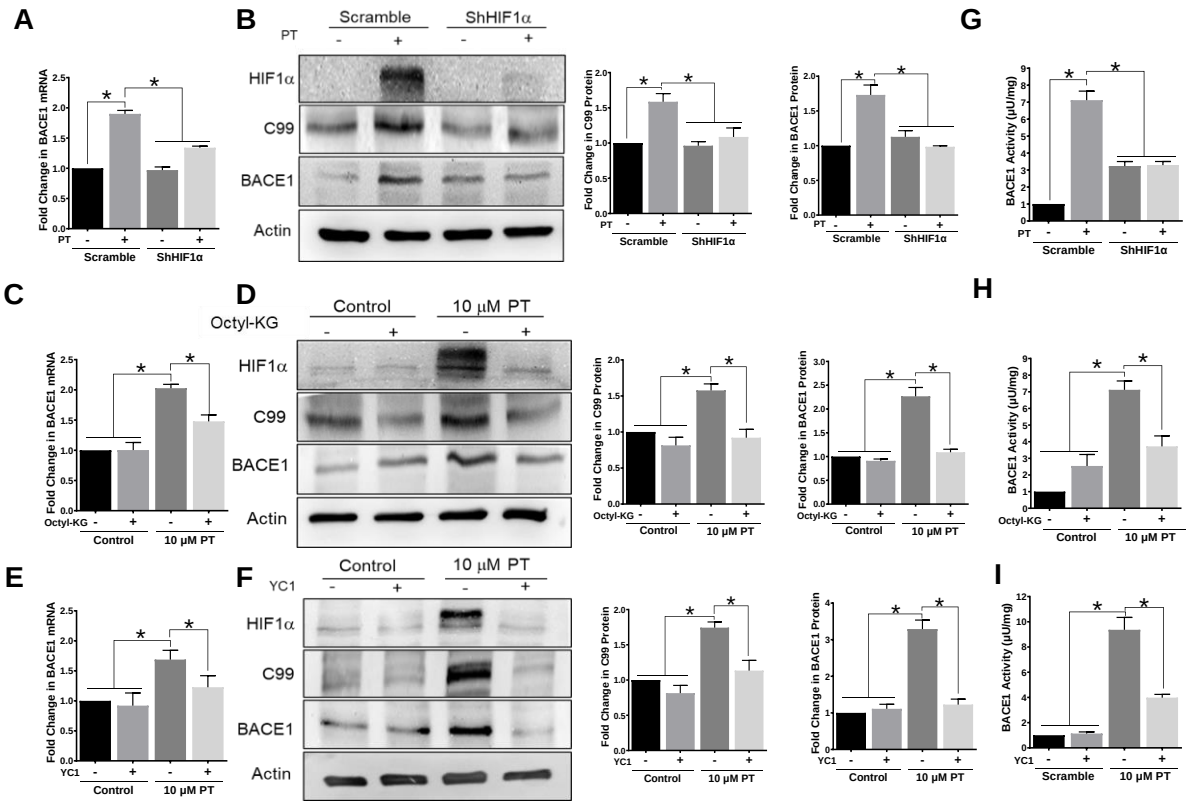


Figure 3.1. TI-induced HIF1α activates BACE1 and amyloid production. (A) Fold change in BACE1 mRNA after PT treatment in +/- shHIF1α group, (C) Octyl-KG or (E) YC1 supplementation. (B) Representative WB of HIF1α, C99 and BACE1 protein expression with relative densitometry after HIF1α silencing, (D) treatment with 2 mM Octyl-KG and (F) treatment with 10 μM YC1. Fold change in BACE1 activity after (G) HIF1α silencing, (H) treatment with Octyl-KG and (I) YC1. HT22 were treated with 10 μM PT for 3 days. Control group was grown in regular RPMI containing 3 μM thiamine for 3 days. Actin was used as a loading control for WCL and RT-PCR. Scramble control was treated with a non-mammalian shRNA. (*) Represents a statistically significant difference with $p < 0.05$ based on the results of a one-way ANOVA with Tukey's Post-hoc test of at least n=3 independent replicates

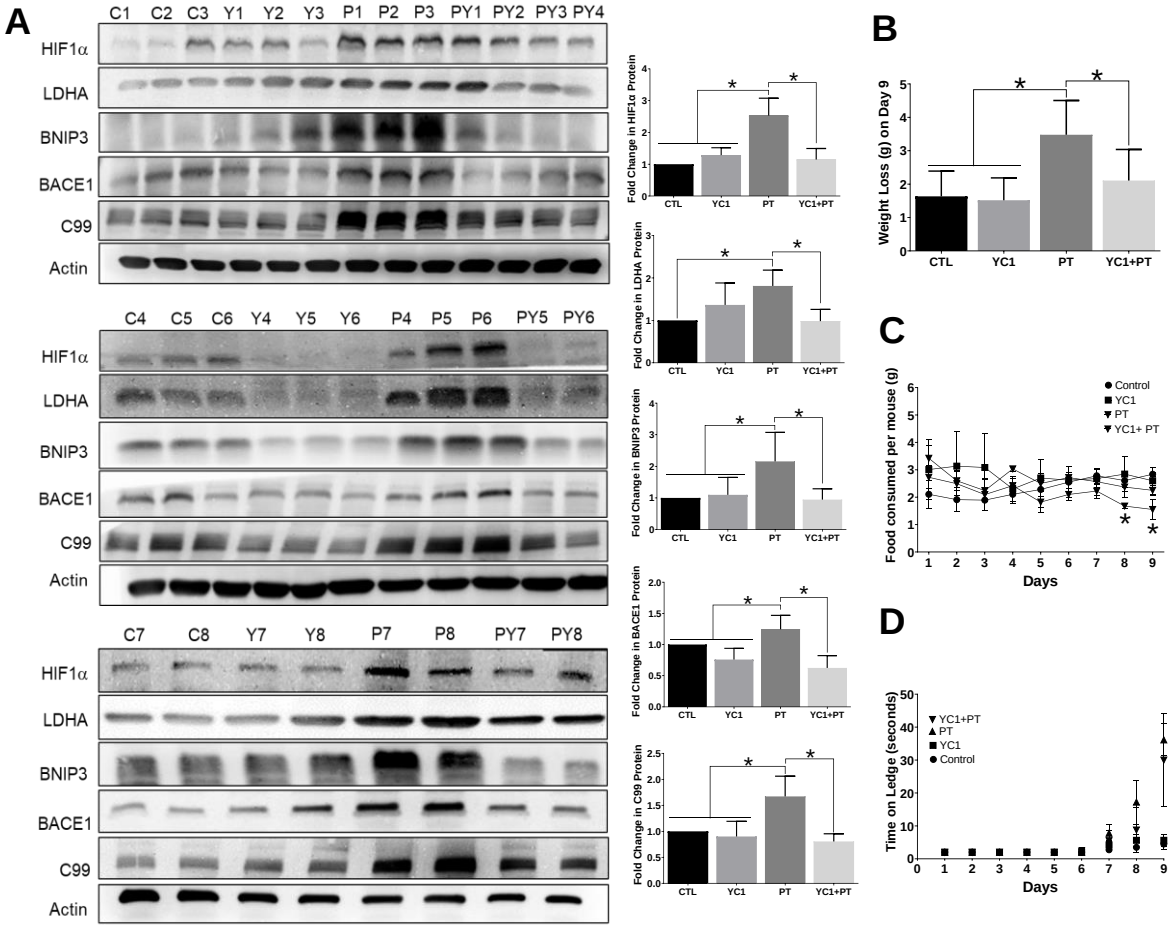


Figure 3.2. TI-induced HIF1α mediates AD-like pathogenesis in vivo. (A) Total WB from whole brain lysates of HIF1α, LDHA, BNIP3, BACE1 and C99 protein expression with relative densitometry in saline control (C1-8), YC1 control (Y1-8), PT (P1-8) and PT+YC1 (PY1-8) groups. (B) Mice weight loss at day 9 of treatment. (C) Daily changes in food consumption and (D) ledge test performance per mouse from day 1 to day 9. (*) Represents a statistically significant difference with $p < 0.05$ based on the results of a one-way ANOVA with Tukey's Post-hoc test of at least n=3 independent replicates.

3.4 Discussion

TI has been linked to dementia for decades (Basu, Jordan et al. 1976, Aikawa, Watanabe et al. 1984, Gibson, Sheu et al. 1988, Butterworth 1989, Meador 1993, Gold, Hauser et al. 1998, Gibson, Hirsch et al. 2016). In congruency with AD, TI induces changes in oxidative metabolism promoting a sequalae of events that eventually lead to oxidative stress, mitochondrial damage, inflammation, and neuronal loss (Karuppagounder, Xu et al. 2009, Abdou and Hazell 2015). Using APP Tg199959 plaque competent mice fed with TI diet and injected with the thiamine antagonist Pyrithiamine, Gibson et al. showed that TI exacerbated AD pathology (Karuppagounder, Xu et al. 2009). In particular, TI reduced KGDH activity by 42%, caused neuronal loss, increased plaque deposition, astroglia activation, oxidative stress and neuritic cluster formation (Calingasan, Gandy et al. 1995, Calingasan, Gandy et al. 1996, Calingasan, Gandy et al. 1997, Karuppagounder, Xu et al. 2009). Plaques were detected within the cortex, hippocampus, and thalamus together with an increase in C99 levels by 33% and BACE1 protein levels by 43% (Karuppagounder, Xu et al. 2009).

In order to explain the TI-induced AD pathology and neurotoxicity, changes in oxidative metabolism due to thiamine depletion, increase in oxidative stress and activation of caspase 3 or Jnk Kinase were considered (Wang, Hua et al. 2000, Gibson and Zhang 2002, Ke, Degiorgio et al. 2003, Karuppagounder, Xu et al. 2009). However, the molecular mechanism elucidating the AD-like pathology induced by TI has not been described yet. For the first time to our knowledge, we showed that HIF1α may be the critical upstream mediator between TI and AD pathophysiology

When HIF1α was silenced genetically or pharmacologically, BACE1 levels decreased further highlighting the key role of HIF1α in mediating TI induced AD-like pathogenesis. Interestingly, neuronal cells stably treated with amyloid fragment showed high levels of HIF1α

(Soucek, Cumming et al. 2003). The same study also reported the detection of HIF1α in cortical extracts from six 22- and 23-month-old Tg2576 mice. Although the mechanism on how amyloid stabilizes HIF1α is still not clear, these findings further strengthen the key role of HIF1α in AD pathogenesis.

The potential role of the HIF1α signaling in AD pathology translated in vivo where its inhibition by YC1 decreased expression of BACE1 and APP in transgenic AD mice. To induce a chronic TI state in the mice, we coupled TI diet with IP injection of the antivitamin pyrithiamine (PT). Thiamine depletion diet alone may take up to 3 months to show symptomatology. In contrast, PT rapidly crossed the BBB leading to neurological symptoms within 10-15 days in mice or rats (Savage, Hall et al. 2012). Therefore, the use of PT and TI diet is a well-established predictable and reproducible methodology.

As previously reported, 5mg/kg of YC1 treatment was not toxic for the duration of the study (Yan, Zhou et al. 2011, Yuan, Dong et al. 2011). Although recognized as an effective HIF1α inhibitor, YC1 was designed as soluble guanylate cyclase (sGC) inhibitor (Friebe and Koesling 1998). However, in vivo studies conducted with low YC1 doses (1-30 mg/kg) administered for a short period of time did not report any off-target YC1 effect (Shin, Kim et al. 2007, Yan, Zhou et al. 2011, Lee, Tai et al. 2018).

In this work YC1 administration significantly reduced HIF1α signaling suggesting its key role in AD pathogenesis. In physiologic conditions HIF1α is rapidly degraded and its expression is almost undetectable. Although not as high as PT, basal expression in HIF1α protein can be detected among control and YC1 control groups. Probably mice may have briefly experience hypoxia during the euthanasia procedures (isoflurane treatment and cervical dislocation) causing

HIF1α stabilization. More research will be needed to clarify if TI-induced HIF1α activation is regionally selective or affects the whole brain parenchyma.

Due to the acute TI symptomatology caused by PT and the short time point of the study we did not perform a behavioral test. Nevertheless, PT effectiveness was monitored via ledge test and food consumption daily. Interestingly, YC1 supplementation to PT significantly ameliorated weight loss and had some beneficial effect on ataxia. More research is needed to clarify the pharmacokinetic of YC1 and investigate how the drug may affect weight loss and motor coordination.

Overall, our results demonstrated a central role for HIF1α within the molecular mechanism underlying TI- induced AD pathology. Clarifying the pathophysiology of TI will give a better comprehension of AD as well as other TI- mediated neurological disorders such as Wernicke-Korsakoff syndrome and Parkinson's. Thus, targeting the HIF1α signaling may provide potential therapeutic benefits to AD and dementia patients.

CHAPTER 4

SUMMARY AND LIMITATIONS

4.1 Summary

Hypoxia inducible factor (HIF) 1α plays a key role in hypoxia and Alzheimer's Disease (AD). During hypoxia, HIF1α is stabilized and promotes activation of genes involved in cellular metabolism, glycolysis, glucose metabolism, angiogenesis (Semenza, Nejfelt et al. 1991). This metabolic adaptation allows cells to survive and adapt to hypoxic stress since it leads to reduction in oxidative stress and maintenance of energy levels via glycolysis. A protective HIF1α response was detected after acute hypoxia events, pre-treatment before a more prolonged insult (hypoxic preconditioning), or treatment with cobalt chloride and deferoxamine (DFO) (Vannucci, Towfighi et al. 1998, Bergeron, Gidday et al. 2000, Guo, Song et al. 2006, Baranova, Miranda et al. 2007). In contrast, chronic hypoxic stimuli have been associated with a pro-apoptotic and pro-inflammatory response and cell death (Carmeliet, Dor et al. 1998, Mojsilovic-Petrovic, Callaghan et al. 2007, Aminova, Siddiq et al. 2008, Kubli, Quinsay et al. 2008).

A similar dual response has been reported during AD where HIF1α signaling has proven to be beneficial or detrimental for the brain parenchyma. Activation of HIF1α signaling after amyloid treatment in vitro reduced neurotoxicity and oxidative stress (Soucek, Cumming et al. 2003, Schubert, Soucek et al. 2009). In addition, HIF1α stabilization via treatment with DFO and M30 resulted in neuroprotection and less plaque formation in vivo (Weinreb, Amit et al. 2010,

Guo, Wang et al. 2013, Youdim 2013). In contrast, other studies associated HIF1α response with an increased amyloidogenesis via β- and γ-secretase activation as well as neurotoxicity via BNIP3 and caspase 3 pathways (Pluta, Barcikowska et al. 1998, Sun, He et al. 2006, Zhang, Gao et al. 2007, Li, Zhang et al. 2009). Thus, despite the amount of research dedicated to HIF1α, HIF1α mediated response is still complex and influenced by different variables.

Our group was the first to show that HIF1α stabilization occurred during thiamine insufficiency (TI) via an oxygen independent mechanism (Sweet, Paul et al. 2010, Sweet and Zastre 2013, Zera and Zastre 2017). In particular, we determined that Prolyl Hydroxylases (PHDs), the main enzymes regulating HIF1α stability, are impaired by surplus of pyruvate that competes with their substrate α-ketoglutarate. Pyruvate displaces α-ketoglutarate from the active site of PHDs limiting HIF1α hydroxylation and degradation. The pyruvate accumulation is a direct consequence of TI that impairs the activity of the thiamine-dependent enzyme Pyruvate Dehydrogenase (PDH) that convers pyruvate into acetyl-coenzyme A (Zera and Zastre 2018).

The correlation between TI and HIF1α is of particular importance since it may clarify the molecular mechanism behind the pathophysiology induced by TI in neurological disorders. TI is a well-established comorbidity of dementia and AD. A significant decrease in thiamine levels in plasma and red blood cells was detected in Alzheimer patients but not in non-dementia subjects (Gold, Chen et al. 1995, Pan, Fei et al. 2015, Sang, Pan et al. 2018). Moreover, TI impairs the TCA cycle and oxidative metabolism, contributing to a decrease in ATP production, reduced glucose-derived neurotransmitters, and ultimately cell death (Trebukhina, Ostrovsky et al. 1983, Butterworth, Giguère et al. 1986, Butterworth 1989). In congruency with established AD pathology, TI also promotes inflammation, oxidative stress, plaque formation tau phosphorylation, and neuronal loss (Todd and Butterworth 1999, Karuppagounder, Xu et al. 2009). Despite progress

into the relationship between reduced thiamine levels and AD neuropathology, mechanistic insight into how thiamine insufficiency promotes AD hallmarks is lacking. Therefore, the purpose for this work was to investigate whether HIF1α was an upstream mediator between TI and AD.

Using murine hippocampal cells (HT22) as in vitro model, we found that HIF1α stabilization during TI led to transcriptional activation of pro-apoptotic target gene BNIP3. BNIP3 activation occurred via pyruvate accumulation and oxidative stress leading to loss of mitochondrial potential, chromatin fragmentation via endonuclease G and apoptosis. BNIP3 has been shown to induce neuronal apoptosis in hypoxia and early AD stage models (Zhang, Yang et al. 2007, Ułamek-Kozioł, Czuczwar et al. 2019). In addition, activation of the HIF-BNIP3 response was reported in our previous study on primary murine astrocytes (Zera and Zastre 2017). These results suggested that TI-induced toxicity may be mediated by HIF-BNIP3 signaling in presence of oxidative stress.

Oxidative stress is a major player in TI-induced toxicity due to mitochondrial dysfunction. Due to the disruption of oxidative metabolism in TI, mitochondria produce excessive ROS (Calingasan, Chun et al. 1999, Sharma, Bist et al. 2013, Liu, Ke et al. 2017). The loss in activity of KGDH and subsequent decreased TCA cycle was associated with the induction of eNOS and iNOS (Pulsinelli, Waldman et al. 1982). The increase in NOS activity and NO production is associated with toxicity since NO can react with superoxide radicals to form peroxynitrite, that is toxic to neurons (Calingasan, Park et al. 1998). Another deleterious effect of free radicals' accumulation is the impairment of BBB, which has been reported in TI cases (Watanabe and Kanabe 1978, Watanabe, Tomita et al. 1981, Abdou and Hazell 2015). Supplementation of antioxidants in TI models in vitro and in vivo ameliorated TI symptomatology and resulted in neuroprotection (Todd and Butterworth 1998, Pannunzio, Hazell et al. 2000, Beauchesne,

Desjardins et al. 2010, Hazell, Sheedy et al. 2010). These findings suggest a key role for ROS in induced neuronal death during thiamine depletion.

To elucidate if HIF1α may mediate AD-like symptomatology during thiamine insufficiency, we used 3xTg AD mice as in vivo model together with HT22. In both models, chronic TI state induced increase expression of HIF1α and its pro-amyloidogenic target gene BACE1. We also detected an increased in BACE1 activity and production of C99, precursor of Aβ fragments. Supplementation of the pharmacological HIF1α inhibitor YC1 attenuated the expression of HIF1α, BACE1 and C99 supporting the role of HIF1α as mediator between TI and AD.

To date, the mechanism regulating HIF1α switch to a pro-survival or pro-apoptotic response is still unclear. The literature so far suggests that type and duration of stimuli triggering HIF1α seems to be involved. In our approach, we induced a chronic thiamine depletion state in cells and mice that led to neurotoxicity. This result is consistent with chronic hypoxia/ischemia studies that led to a pro-apoptotic response. Less clear is the mechanism behind HIF1α response in AD and additional work is needed to give a better understanding of HIF1α regulation. The contribution of HIF1α to TI-mediated neuropathology represents a novel finding to better understand the molecular mechanism regulating TI. Previous studies have hypothesized the involvement of oxidative stress, activation of caspase 3 or Jnk Kinase in TI-mediated toxicity (Wang, Hua et al. 2000, Gibson and Zhang 2002, Ke, Degiorgio et al. 2003, Karuppagounder, Xu et al. 2009). However, none of them described a molecular mechanism elucidating the AD-like pathology induced by TI. Here, we showed that HIF1α contributes to pro-apoptotic and pro-amyloidogenic response via BACE1 and BNIP3 transcriptional activation.

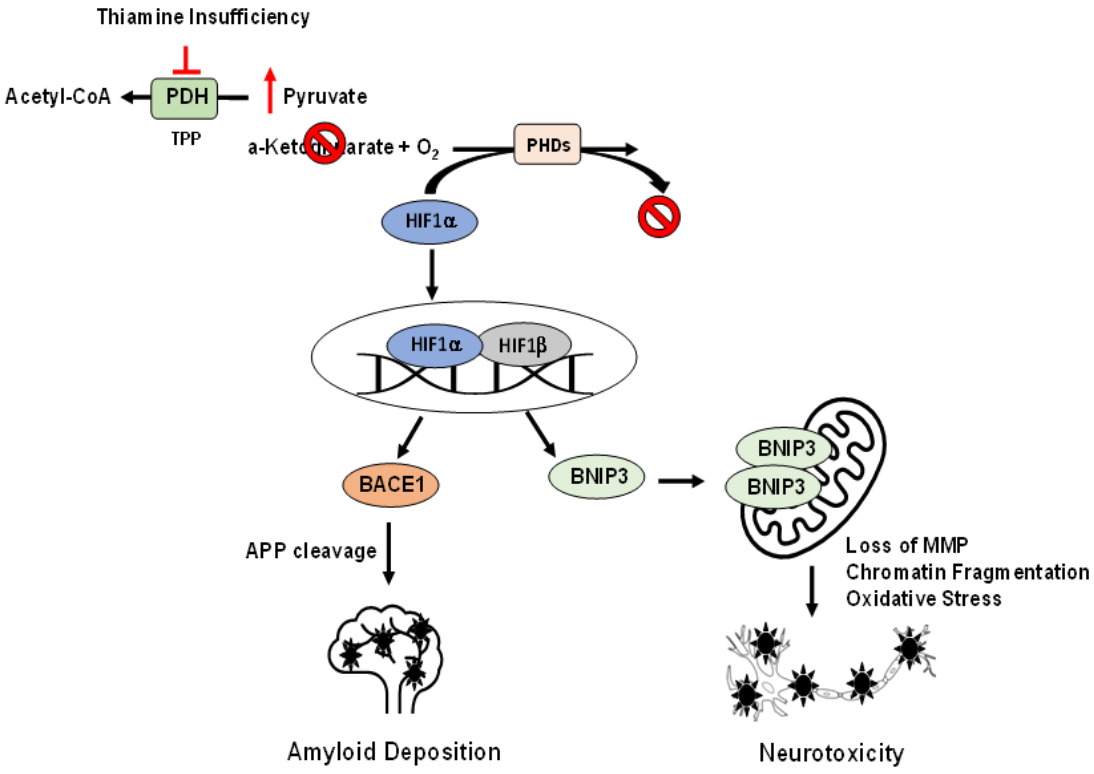


Figure 4.1. Molecular mechanism of TI-induced AD-pathogenesis and neurotoxicity. Thiamine insufficiency (TI) inhibits Prolyl Hydroxylases (PHDs) activity via pyruvate accumulation leading to HIF1α stabilization. Once stabilized, HIF1α migrates into the nucleaus where it binds to HIF1β. We found that HIF1α trascriptionally activates β-secretase (BACE) 1 triggering amyloid precursor protein (APP) cleavage and amyloid deposition. TI-inducd HIF1α also activates the pro-apoptotci proein BNIP3. BNIP3 migrates to the mitochodnria as a dimer and triggers loss of mitochondrial membrane potential (MMP), chromatine fragmentation and neurotoxicity. Oxidative stress is needed for BNIP3 activation.

Overall, this study suggests the HIF1α signaling during chronic TI contributes to cell death and to AD-like pathogenesis. Due to the involvement of TI in different neurodegenerative disorders, targeting HIF1α may have the potential to elucidate the pathophysiology of TI, AD and TI-induced neuropathies.

4.2 Limitations of the study

4.2.1 Use of HT22 cell line

Our in vitro experiments were conducted in HT22, a mouse hippocampal cell line. HT22 is an immortalized cell line subcloned from the HT-4 cell line. HT-4 derived from immortalization of mouse neuronal tissues with a temperature sensitive SV40 T-antigen (Davis and Maher 1994). The derivation from the hippocampus makes HT22 a valuable model due to the crucial role that hippocampus has in memory and in AD pathogenesis. In addition, HT22 are sensitive to glutamate excitotoxicity, a recurrent event in neurodegenerative disorders (Morimoto and Koshland 1990). Although HT22 are not a cancerous cell line, its immortalization may represent a limitation. For future studies, we plan to isolate murine primary neuronal cells to mimic the neuronal response to TI more closely. In addition, we aim to incorporate human cell lines in our future in vitro studies.

4.2.3 Induction of thiamine insufficiency in vitro

The purpose for our in vitro model was to recapitulate the metabolic changes in neuronal cells during thiamine insufficiency. To reduce thiamine levels in the culture medium, we used a customized thiamine deficient RPMI containing about 10 nanoM thiamine. This concentration is similar to the physiological thiamine concentration in blood (Zastre, Sweet et al. 2013). Despite the use of the thiamine depleted medium, thiamine may still be present in the FBS. Although the

use of dialyzed FBS or reduced FBS amount in the medium may further reduce thiamine levels, it may halt cell growth. For this reason, depletion of thiamine within the medium is not a reliable approach to induce TI. Addition of the antivitamin pyrithiamine (PT) induces a more rapid TI symptomatology blocking thiamine uptake and conversion to TPP within the cell. The rapid and severe action of PT does not reflect the gradual metabolic changes that happen during TI, representing a limitation of the treatment. Nevertheless, PT represents to date the standard methodology for TI treatments.

Due to the structural similarity to thiamine, it is unclear whether PT may bind to other thiamine -dependent enzymes. Knocking down TPK1 may represent an alternative way to induce TI limiting off-target effects. Although this approach will not affect thiamine uptake, it will block its conversion to the active form.

4.2.4 In vivo limitations

Our in vivo study aimed at elucidating the role of HIF1α in AD-pathogenesis during TI. Due to the limited number of mice, we only focused on protein change in whole brain without performing immunohistochemistry or tissue staining.

We also acknowledge the lack of a behavioral test for the study. This choice was based on the short duration of the TI model whose symptomatology is severe only on the last day of treatment. The ledge test does not account as behavioral test since it only shows the severity of TI symptomatology. In future studies, we plan to perform short-term behavioral test such as the novel object recognition test. This test is commonly used for testing different phases of learning and memory in mice and it can be performed within three days (Lueptow 2017).

4.3 Future Directions

4.3.1 Investigating TI-mediated HIF1a response in microglia

TI-induced HIF1α response in astrocytes (Zera and Zastre 2017) and neuronal cells led to cell death in both cases. This finding seems to suggest that HIF1α response during TI may not be cell specific as described during hypoxia. When astrocytes and neurons were exposed to hypoxia in a co-culture model, HIF1α induced a pro-death response in astrocytes while neurons were driven toward survival (Vangeison, Carr et al. 2008). Investigating the HIF1α mediated response in other brain cell types, such as microglia, would further clarify the nature of TI-driven HIF1α response.

Microglia, together with astrocytes, are a key component in the spatial and temporal events of inflammation. Microglia activation and the following inflammatory response contributes to the initial phase of the TI neurological dysfunction (Calingasan, Park et al. 1998, Todd and Butterworth 1999). Studies in TI animal models reported that microglial changes precede neuronal death (Ke, Degiorgio et al. 2003). In particular, microglia activation includes a series of biochemical and morphological changes affecting migration, proliferation, and mediators release as well as expression of specific markers. High levels of the activated microglia markers OX-42, CD11b, ED-1 have been localized in early TI stages prior to focal neuronal loss (Todd and Butterworth 1999, Sarkar, Liachenko et al. 2016) indicating that microglial response in TI-vulnerable brain regions may trigger the later neuropathology and cell death. Additionally, benfotiamine supplementation modulates oxidative activity of microglia, decreases CD40 expression and NFkB signaling (Bozic, Savic et al. 2015).

Another interesting aspect would be to investigate NFkB signaling in microglia exposed to TI. During normoxia, NFkB is inhibited by IkB. Activated IKKβ phosphorylates IkB, causing its degradation. This event allows NFkB translocation into the nucleus where it can start a pro-

inflammatory response. IKKβ is negatively regulated by PHDs, key enzyme sin HIF1α regulation (Kiernan, Smith et al. 2016). Based on our data, TI inhibits PHDs activity and HIF1α degradation. We have not investigated other PHDs targets yet. Therefore, microglia may be representing an interesting model to clarify TI effect on NFkB pathway.

4.3.2 Clarify HIF1a role in AD pathogenesis during TI

TI has a devastating impact in the brain. TI impairs mitochondrial metabolism, TCA cycle, promotes oxidative stress, inflammation, and excitotoxicity (Butterworth, Giguère et al. 1986, Todd and Butterworth 1999, Butterworth 2007). These events are manifested in focal brain regions identified with the thalamus and mammillary bodies (Todd and Butterworth 1999). Although their similarity with ischemia lesions, the underlying mechanism behind TI selective damage is still not known.

Unpublished in vivo studies from our groups in 11-months old C57BL/6 mice, showed HIF1α activation within the thalamus during day 9 of TI treatment. However, TI-induced HIF1α cerebral localization has to be elucidated in AD models. In AD mice TI exacerbated plaques formation within the cortex and other regions not limited to thalamus and mammillary bodies (Karuppagounder, Xu et al. 2009).

Future work will also clarify if TI-induction of HIF1α plays any role in tau phosphorylation and tangles formation. TI was shown to increase tauopathy in Tg19959 AD mice, but the mechanism was not elucidated (Karuppagounder, Xu et al. 2009). To date, few studies focused on the relationship between HIF1α and tangles provoding controversial results (Shin, Kim et al. 2007, Ashok, Ajith et al. 2017).

4.3.2 Determine the involvement of PHDs in TI

Prolyl Hydroxylases (PHDs) are the main enzymes regulating HIF1α. Target PHDs is a well-established strategy to prevent HIF1α degradation. Compounds like DMOG, DFO and cobalt chloride target PHDs substrates or cofactors leading to HIF1α stabilization. Furthermore, PHDs represent an interesting target to study aging and neurodegeneration (Ndubuizu, Chavez et al. 2009, Speer, Karuppagounder et al. 2013, Wall, Corcoran et al. 2014). The knowledge of TI effect on PHDs is still limited. We described that HIF1α stabilization during TI is mediated by PHDs inactivation. However, we did not focus on a particular isoform or investigate if all of them are affected by TI. Isoforms PHD1, PHD2 and PHD3 have been identified so far. PHD2 is the most studied while less clear are the roles of PHD1 and PHD3 (Ndubuizu, Chavez et al. 2009). Neuronal activation of PHD2 resulted in reduced infarct size and neuronal apoptosis after ischemia and post-stroke recovery (Kunze, Zhou et al. 2012, Li, Saliba et al. 2016). In another PHD2 neuronal knock down model, neuronal cell death was delayed and an increased protection against ischemic injury in the hippocampus was described (Corcoran, Kunze et al. 2013). Thus, PHD2 seems the isoform that plays a major role within the brain. Future research will clarify if TI has an isoform specific of pan -inhibition on PHDs.

REFERENCES

Abdou, E. and A. S. Hazell (2015). "Thiamine deficiency: an update of pathophysiologic mechanisms and future therapeutic considerations." Neurochem Res **40**(2): 353-361.

Abdul-Muneer, P. M., S. Alikunju, H. Schuetz, A. M. Szlachetka, X. Ma and J. Haorah (2018). "Impairment of Thiamine Transport at the GUT-BBB-AXIS Contributes to Wernicke's Encephalopathy." Molecular Neurobiology **55**(7): 5937-5950.

Agbayewa, M. O., V. M. Bruce and V. Siemens (1992). "Pyridoxine, ascorbic acid and thiamine in Alzheimer and comparison subjects." Can J Psychiatry **37**(9): 661-662.

Ahmed, M., P. Azizi-Namini, A. T. Yan and M. Keith (2015). "Thiamin deficiency and heart failure: the current knowledge and gaps in literature." Heart Fail Rev **20**(1): 1-11.

Aikawa, H., I. S. Watanabe, T. Furuse, Y. Iwasaki, E. Satoyoshi, T. Sumi and T. Moroji (1984). "Low energy levels in thiamine-deficient encephalopathy." J Neuropathol Exp Neurol **43**(3): 276-287.

Algahtani, H., S. Ghamdi, B. Shirah, B. Alharbi, R. Algahtani and A. Bazaid (2017). "Biotin-thiamine-responsive basal ganglia disease: catastrophic consequences of delay in diagnosis and treatment." Neurol Res **39**(2): 117-125.

Aminova, L. R., A. Siddiq and R. R. Ratan (2008). "Antioxidants, HIF prolyl hydroxylase inhibitors or short interfering RNAs to BNIP3 or PUMA, can prevent prodeath effects of the transcriptional activator, HIF-1alpha, in a mouse hippocampal neuronal line." Antioxidants & redox signaling **10**(12): 1989-1998.

An, W. G., M. Kanekal, M. C. Simon, E. Maltepe, M. V. Blagosklonny and L. M. Neckers (1998). "Stabilization of wild-type p53 by hypoxia-inducible factor 1alpha." Nature **392**(6674): 405-408.

Anoop, A., P. K. Singh, R. S. Jacob and S. K. Maji (2010). "CSF Biomarkers for Alzheimer's Disease Diagnosis." International journal of Alzheimer's disease **2010**: 606802.

Anstey, K. J., N. Cherbuin, M. Budge and J. Young (2011). "Body mass index in midlife and late-life as a risk factor for dementia: a meta-analysis of prospective studies." Obesity Reviews **12**(5): e426-e437.

Ashok, B. S., T. A. Ajith and S. Sivanesan (2017). "Hypoxia-inducible factors as neuroprotective agent in Alzheimer's disease." Clin Exp Pharmacol Physiol **44**(3): 327-334.

Ba, A. (2017). "Alcohol and thiamine deficiency trigger differential mitochondrial transition pore opening mediating cellular death." Apoptosis **22**(6): 741-752.

Baek, J. H., Y. V. Liu, K. R. McDonald, J. B. Wesley, H. Zhang and G. L. Semenza (2007). "Spermidine/spermine N(1)-acetyltransferase-1 binds to hypoxia-inducible factor-1alpha (HIF-1alpha) and RACK1 and promotes ubiquitination and degradation of HIF-1alpha." J Biol Chem **282**(46): 33358-33366.

Baetz, D., K. M. Regula, K. Ens, J. Shaw, S. Kothari, N. Yurkova and L. A. Kirshenbaum (2005). "Nuclear factor-kappaB-mediated cell survival involves transcriptional silencing of the mitochondrial death gene BNIP3 in ventricular myocytes." Circulation **112**(24): 3777-3785.

Baranova, O., L. F. Miranda, P. Pichiule, I. Dragatsis, R. S. Johnson and J. C. Chavez (2007). "Neuron-Specific Inactivation of the Hypoxia Inducible Factor 1α Increases Brain Injury in a Mouse Model of Transient Focal Cerebral Ischemia." The Journal of Neuroscience **27**(23): 6320-6332.

Bardaweel, S. K., M. Gul, M. Alzweiri, A. Ishaqat, H. A. Alsalamat and R. M. Bashatwah (2018). "Reactive Oxygen Species: the Dual Role in Physiological and Pathological Conditions of the Human Body." The Eurasian journal of medicine **50**(3): 193-201.

Basu, T. K., J. W. Dickerson, R. W. Raven and D. C. Williams (1974). "The thiamine status of patients with cancer as determined by the red cell transketolase activity." Int J Vitam Nutr Res **44**(1): 53-58.

Basu, T. K., S. J. Jordan, M. Jenner and D. C. Williams (1976). "Blood values of some vitamins in long-stay psycho-geriatric patients." Int J Vitam Nutr Res **46**(1): 61-65.

Beal, M. F. (2005). "Oxidative damage as an early marker of Alzheimer's disease and mild cognitive impairment." Neurobiol Aging **26**(5): 585-586.

Beauchesne, E., P. Desjardins, R. F. Butterworth and A. S. Hazell (2010). "Up-regulation of caveolin-1 and blood-brain barrier breakdown are attenuated by N-acetylcysteine in thiamine deficiency." Neurochem Int **57**(7): 830-837.

Beauchesne, E., P. Desjardins, A. S. Hazell and R. F. Butterworth (2009). "Altered expression of tight junction proteins and matrix metalloproteinases in thiamine-deficient mouse brain." Neurochem Int **55**(5): 275-281.

Bergau, N., S. Maul, D. Rujescu, A. Simm and A. Navarrete Santos (2019). "Reduction of Glycolysis Intermediate Concentrations in the Cerebrospinal Fluid of Alzheimer's Disease Patients." Frontiers in neuroscience **13**: 871-871.

Bergeron, M., J. M. Gidday, A. Y. Yu, G. L. Semenza, D. M. Ferriero and F. R. Sharp (2000). "Role of hypoxia-inducible factor-1 in hypoxia-induced ischemic tolerance in neonatal rat brain." Ann Neurol **48**(3): 285-296.

Berra, E., A. Ginouvès and J. Pouysségur (2006). "The hypoxia-inducible-factor hydroxylases bring fresh air into hypoxia signalling." EMBO reports **7**(1): 41-45.

Bettendorff, L., F. Sluse, G. Goessens, P. Wins and T. Grisar (1995). "Thiamine deficiency--induced partial necrosis and mitochondrial uncoupling in neuroblastoma cells are rapidly reversed by addition of thiamine." Journal of neurochemistry **65**(5): 2178-2184.

Binnewijzend, M. A., J. P. Kuijer, M. R. Benedictus, W. M. van der Flier, A. M. Wink, M. P. Wattjes, B. N. van Berckel, P. Scheltens and F. Barkhof (2013). "Cerebral blood flow measured with 3D pseudocontinuous arterial spin-labeling MR imaging in Alzheimer disease and mild cognitive impairment: a marker for disease severity." Radiology **267**(1): 221-230.

Binnewijzend, M. A. A., M. R. Benedictus, J. P. A. Kuijer, W. M. van der Flier, C. E. Teunissen, N. D. Prins, M. P. Wattjes, B. N. M. van Berckel, P. Scheltens and F. Barkhof (2016). "Cerebral perfusion in the predementia stages of Alzheimer's disease." European radiology **26**(2): 506-514.

Boni, L., L. Kieckens and A. Hendrikx (1980). "An evaluation of a modified erythrocyte transketolase assay for assessing thiamine nutritional adequacy." J Nutr Sci Vitaminol (Tokyo) **26**(5): 507-514.

Bonner-Jackson, A., S. Mahmoud, J. Miller and S. J. Banks (2015). "Verbal and non-verbal memory and hippocampal volumes in a memory clinic population." Alzheimer's Research & Therapy **7**(1): 61.

Born, J., T. Lange, W. Kern, G. McGregor, U. Bickel and H. L Fehm (2002). Sniffing neuropeptides: a transnasal approach to the human brain.

Bowker-Kinley, M. M., I. W. Davis, P. WU, A. R. HARRIS and M. K. POPOV (1998). "Evidence for existence of tissue-specific regulation of the mammalian pyruvate dehydrogenase complex." Biochemical Journal **329**(1): 191-196.

Bowyer, J. F., K. M. Tranter, S. Sarkar and J. P. Hanig (2018). "Microglial activation and vascular responses that are associated with early thalamic neurodegeneration resulting from thiamine deficiency." NeuroToxicology **65**: 98-110.

Bozic, I., D. Savic, I. Stevanovic, S. Pekovic, N. Nedeljkovic and I. Lavrnja (2015). "Benfotiamine upregulates antioxidative system in activated BV-2 microglia cells." Frontiers in Cellular Neuroscience **9**(351).

Brinkman, D. J., J. K. Bekema, M. A. Kuijenhoven, J. W. Wijnia, M. J. Dekker and M. A. van Agtmael (2017). "[Thiamine in patients with alcohol use disorder and Wernicke's encephalopathy]." Ned Tijdschr Geneeskd **161**: D931.

Brown, G. (2014). "Defects of thiamine transport and metabolism." J Inherit Metab Dis **37**(4): 577-585.

Bruick, R. K. (2000). "Expression of the gene encoding the proapoptotic Nip3 protein is induced by hypoxia." Proceedings of the National Academy of Sciences of the United States of America **97**(16): 9082-9087.

Brunelle, J. K., E. L. Bell, N. M. Quesada, K. Vercauteren, V. Tiranti, M. Zeviani, R. C. Scarpulla and N. S. Chandel (2005). "Oxygen sensing requires mitochondrial ROS but not oxidative phosphorylation." Cell Metabolism **1**(6): 409-414.

Bubber, P., V. Haroutunian, G. Fisch, J. P. Blass and G. E. Gibson (2005). "Mitochondrial abnormalities in Alzheimer brain: Mechanistic implications." Annals of Neurology **57**(5): 695-703.

Bubber, P., V. Hartounian, G. E. Gibson and J. P. Blass (2011). "Abnormalities in the tricarboxylic acid (TCA) cycle in the brains of schizophrenia patients." Eur Neuropsychopharmacol **21**(3): 254-260.

Bubber, P., Z. J. Ke and G. E. Gibson (2004). "Tricarboxylic acid cycle enzymes following thiamine deficiency." Neurochem Int **45**(7): 1021-1028.

Burton, T. R., D. D. Eisenstat and S. B. Gibson (2009). "BNIP3 (Bcl-2 19 kDa interacting protein) acts as transcriptional repressor of apoptosis-inducing factor expression preventing cell death in human malignant gliomas." J Neurosci **29**(13): 4189-4199.

Burton, T. R. and S. B. Gibson (2009). "The role of Bcl-2 family member BNIP3 in cell death and disease: NIPping at the heels of cell death." Cell death and differentiation **16**(4): 515-523.

Burton, T. R., E. S. Henson, M. B. Azad, M. Brown, D. D. Eisenstat and S. B. Gibson (2013). "BNIP3 acts as transcriptional repressor of death receptor-5 expression and prevents TRAIL-induced cell death in gliomas." Cell Death Dis **4**(4): e587.

Butterworth, R. F. (1989). "Effects of thiamine deficiency on brain metabolism: implications for the pathogenesis of the Wernicke-Korsakoff syndrome." Alcohol Alcohol **24**(4): 271-279.

Butterworth, R. F. (2001). "Maternal thiamine deficiency: still a problem in some world communities." The American Journal of Clinical Nutrition **74**(6): 712-713.

Butterworth, R. F. (2007). "Thiamin deficiency and brain disorders." Nutrition Research Reviews **16**(2): 277-284.

Butterworth, R. F., C. Gaudreau, J. Vincelette, A. M. Bourgault, F. Lamothe and A. M. Nutini (1991). "Thiamine deficiency and Wernicke's encephalopathy in AIDS." Metab Brain Dis **6**(4): 207-212.

Butterworth, R. F., J. F. Giguère and A. M. Besnard (1986). "Activities of thiamine-dependent enzymes in two experimental models of thiamine-deficiency encephalopathy. 2. alpha-Ketoglutarate dehydrogenase." Neurochem Res **11**(4): 567-577.

Butterworth, R. F. and M. Heroux (1989). "Effect of pyrithiamine treatment and subsequent thiamine rehabilitation on regional cerebral amino acids and thiamine-dependent enzymes." J Neurochem **52**(4): 1079-1084.

Cai, Z., H. Zhong, M. Bosch-Marce, K. Fox-Talbot, L. Wang, C. Wei, M. A. Trush and G. L. Semenza (2007). "Complete loss of ischaemic preconditioning-induced cardioprotection in mice with partial deficiency of HIF-1α." Cardiovascular Research **77**(3): 463-470.

Calingasan, N. Y., W. J. Chun, L. C. Park, K. Uchida and G. E. Gibson (1999). "Oxidative stress is associated with region-specific neuronal death during thiamine deficiency." J Neuropathol Exp Neurol **58**(9): 946-958.

Calingasan, N. Y., S. E. Gandy, H. Baker, K. F. Sheu, K. S. Kim, H. M. Wisniewski and G. E. Gibson (1995). "Accumulation of amyloid precursor protein-like immunoreactivity in rat brain in response to thiamine deficiency." Brain Res **677**(1): 50-60.

Calingasan, N. Y., S. E. Gandy, H. Baker, K. F. Sheu, J. D. Smith, B. T. Lamb, J. D. Gearhart, J. D. Buxbaum, C. Harper, D. J. Selkoe, D. L. Price, S. S. Sisodia and G. E. Gibson (1996). "Novel neuritic clusters with accumulations of amyloid precursor protein and amyloid precursor-like protein 2 immunoreactivity in brain regions damaged by thiamine deficiency." The American journal of pathology **149**(3): 1063-1071.

Calingasan, N. Y., S. E. Gandy and G. E. Gibson (1997). "Thiamine deficiency alters APP but not presenilin-1 immunoreactivity in vulnerable brain regions." Neuroreport **8**(11): 2631-2634.

Calingasan, N. Y., L. C. Park, L. L. Calo, R. R. Trifiletti, S. E. Gandy and G. E. Gibson (1998). "Induction of nitric oxide synthase and microglial responses precede selective cell death induced by chronic impairment of oxidative metabolism." Am J Pathol **153**(2): 599-610.

Calkins, M. J., M. Manczak, P. Mao, U. Shirendeb and P. H. Reddy (2011). "Impaired mitochondrial biogenesis, defective axonal transport of mitochondria, abnormal mitochondrial dynamics and synaptic degeneration in a mouse model of Alzheimer's disease." Hum Mol Genet **20**(23): 4515-4529.

Carmeliet, P., Y. Dor, J. M. Herbert, D. Fukumura, K. Brusselmans, M. Dewerchin, M. Neeman, F. Bono, R. Abramovitch, P. Maxwell, C. J. Koch, P. Ratcliffe, L. Moons, R. K. Jain, D. Collen and E. Keshert (1998). "Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis." Nature **394**(6692): 485-490.

Castano, E. M., F. Prelli, T. Wisniewski, A. Golabek, R. A. Kumar, C. Soto and B. Frangione (1995). "Fibrillogenesis in Alzheimer's disease of amyloid beta peptides and apolipoprotein E." Biochem J **306 (Pt 2)**(Pt 2): 599-604.

Chai, X., W. Kong, L. Liu, W. Yu, Z. Zhang and Y. Sun (2014). "A viral vector expressing hypoxia-inducible factor 1 alpha inhibits hippocampal neuronal apoptosis." Neural Regen Res **9**(11): 1145-1153.

Chan, H., R. F. Butterworth and A. S. Hazell (2004). "Primary cultures of rat astrocytes respond to thiamine deficiency-induced swelling by downregulating aquaporin-4 levels." Neurosci Lett **366**(3): 231-234.

Chang, Y., C. Y. Hsieh, Z. A. Peng, T. L. Yen, G. Hsiao, D. S. Chou, C. M. Chen and J. R. Sheu (2009). "Neuroprotective mechanisms of puerarin in middle cerebral artery occlusion-induced brain infarction in rats." J Biomed Sci **16**(1): 9.

Chen, C., R. P. Ostrowski, C. Zhou, J. Tang and J. H. Zhang (2010). "Suppression of hypoxia-inducible factor-1alpha and its downstream genes reduces acute hyperglycemia-enhanced

hemorrhagic transformation in a rat model of cerebral ischemia." J Neurosci Res **88**(9): 2046-2055.

Chen, D., M. Li, J. Luo and W. Gu (2003). "Direct interactions between HIF-1 alpha and Mdm2 modulate p53 function." The Journal of biological chemistry **278**(16): 13595-13598.

Chen, W., V. Jadhav, J. Tang and J. H. Zhang (2008). "HIF-1alpha inhibition ameliorates neonatal brain injury in a rat pup hypoxic-ischemic model." Neurobiol Dis **31**(3): 433-441.

Chen, Z., X. Pan, G. Fei, S. Pan, W. Bao, S. Ren, Y. Guan and C. Zhong (2017). "Diagnostic value of blood thiamine metabolites in Alzheimer's disease examined by (11)C-PiB PET scanning." Future Sci OA **3**(2): FSO172.

Chin-Hsiao, T. (2019). "Metformin and the Risk of Dementia in Type 2 Diabetes Patients." Aging and disease **10**(1): 37-48.

Chishti, M. A., D.-S. Yang, C. Janus, A. L. Phinney, P. Horne, J. Pearson, R. Strome, N. Zuker, J. Loukides, J. French, S. Turner, G. Lozza, M. Grilli, S. Kunicki, C. Morissette, J. Paquette, F. Gervais, C. Bergeron, P. E. Fraser, G. A. Carlson, P. S. George-Hyslop and D. Westaway (2001). "Early-onset Amyloid Deposition and Cognitive Deficits in Transgenic Mice Expressing a Double Mutant Form of Amyloid Precursor Protein 695*." Journal of Biological Chemistry **276**(24): 21562-21570.

Chiu, P.-Y., C.-T. Tsai, P.-K. Chen, W.-J. Chen and T.-J. Lai (2016). "Neuropsychiatric Symptoms in Parkinson's Disease Dementia Are More Similar to Alzheimer's Disease than Dementia with Lewy Bodies: A Case-Control Study." PloS one **11**(4): e0153989-e0153989.

Cole, G. M., Q. L. Ma and S. A. Frautschy (2010). "Dietary fatty acids and the aging brain." Nutr Rev **68 Suppl 2**(0 2): S102-111.

Coon, K. D., A. J. Myers, D. W. Craig, J. A. Webster, J. V. Pearson, D. H. Lince, V. L. Zismann, T. G. Beach, D. Leung, L. Bryden, R. F. Halperin, L. Marlowe, M. Kaleem, D. G. Walker, R. Ravid, C. B. Heward, J. Rogers, A. Papassotiropoulos, E. M. Reiman, J. Hardy and D. A. Stephan (2007). "A high-density whole-genome association study reveals that APOE is the major susceptibility gene for sporadic late-onset Alzheimer's disease." J Clin Psychiatry **68**(4): 613-618.

Corcoran, A., R. Kunze, S. C. Harney, G. Breier, H. H. Marti and J. J. O'Connor (2013). "A role for prolyl hydroxylase domain proteins in hippocampal synaptic plasticity." Hippocampus **23**(10): 861-872.

Corder, E. H., A. M. Saunders, W. J. Strittmatter, D. E. Schmechel, P. C. Gaskell, G. W. Small, A. D. Roses, J. L. Haines and M. A. Pericak-Vance (1993). "Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families." Science **261**(5123): 921-923.

Covello, K., J. Kehler, H. Yu, J. Gordan, A. Arsham, C.-j. Hu, P. Labosky, M. Simon and B. Keith (2006). "Covello KL, Kehler J, Yu H, Gordan JD, Arsham AM, Hu CJ, Labosky PA, Simon MC, Keith BHIF-2alpha regulates Oct-4: effects of hypoxia on stem cell function, embryonic development, and tumor growth. Genes Dev 20:557-570." Genes & development **20**: 557-570.

Craft, S., S. Asthana, J. W. Newcomer and et al. (1999). "Enhancement of memory in alzheimer disease with insulin and somatostatin, but not glucose." Archives of General Psychiatry **56**(12): 1135-1140.

Craft, S., L. D. Baker, T. J. Montine and et al. (2012). "Intranasal insulin therapy for alzheimer disease and amnestic mild cognitive impairment: A pilot clinical trial." Archives of Neurology **69**(1): 29-38.

Craft, S., J. Newcomer, S. Kanne, S. Dagogo-Jack, P. Cryer, Y. Sheline, J. Luby, A. Dagogo-Jack and A. Alderson (1996). "Memory improvement following induced hyperinsulinemia in alzheimer's disease." Neurobiology of Aging **17**(1): 123-130.

Cregan, S. P., N. A. Arbour, J. G. Maclaurin, S. M. Callaghan, A. Fortin, E. C. Cheung, D. S. Guberman, D. S. Park and R. S. Slack (2004). "p53 activation domain 1 is essential for PUMA upregulation and p53-mediated neuronal cell death." J Neurosci **24**(44): 10003-10012.

Cummins, E. P., E. Berra, K. M. Comerford, A. Ginouves, K. T. Fitzgerald, F. Seeballuck, C. Godson, J. E. Nielsen, P. Moynagh, J. Pouyssegur and C. T. Taylor (2006). "Prolyl hydroxylase-1 negatively regulates IκB kinase-β, giving insight into hypoxia-induced NFκB activity." Proceedings of the National Academy of Sciences **103**(48): 18154-18159.

Dalgard, C. L., H. Lu, A. Mohyeldin and A. Verma (2004). "Endogenous 2-oxoacids differentially regulate expression of oxygen sensors." The Biochemical journal **380**(Pt 2): 419-424.

Daulatzai, M. A. (2013). "Death by a Thousand Cuts in Alzheimer's Disease: Hypoxia—The Prodrome." Neurotoxicity Research **24**(2): 216-243.

Daulatzai, M. A. (2017). "Cerebral hypoperfusion and glucose hypometabolism: Key pathophysiological modulators promote neurodegeneration, cognitive impairment, and Alzheimer's disease." J Neurosci Res **95**(4): 943-972.

Davis, J. B. and P. Maher (1994). "Protein kinase C activation inhibits glutamate-induced cytotoxicity in a neuronal cell line." Brain Res **652**(1): 169-173.

Day, G. S. and C. M. del Campo (2014). "Wernicke encephalopathy: a medical emergency." CMAJ **186**(8): E295.

de Andrade, J. A. A., C. R. M. Gayer, N. P. d. A. Nogueira, M. C. Paes, V. L. F. C. Bastos, J. d. C. B. Neto, S. C. Alves, Jr., R. M. Coelho, M. G. A. T. da Cunha, R. N. Gomes, M. B. Águila, C. A. Mandarim-de-Lacerda, P. T. Bozza and S. da Cunha (2014). "The effect of thiamine deficiency on inflammation, oxidative stress and cellular migration in an experimental model of sepsis." Journal of inflammation (London, England) **11**: 11-11.

de la Monte, S. M. and J. J. Kril (2014). "Human alcohol-related neuropathology." Acta Neuropathol **127**(1): 71-90.

de la Monte, S. M., T. R. Neely, J. Cannon and J. R. Wands (2000). "Oxidative stress and hypoxia-like injury cause Alzheimer-type molecular abnormalities in central nervous system neurons." Cell Mol Life Sci **57**(10): 1471-1481.

de Leon, M. J., S. H. Ferris, A. E. George, D. R. Christman, J. S. Fowler, C. Gentes, B. Reisberg, B. Gee, M. Emmerich, Y. Yonekura, J. Brodie, Kricheff, II and A. P. Wolf (1983). "Positron emission tomographic studies of aging and Alzheimer disease." AJNR Am J Neuroradiol **4**(3): 568-571.

DeBerardinis, R. J., A. Mancuso, E. Daikhin, I. Nissim, M. Yudkoff, S. Wehrli and C. B. Thompson (2007). "Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis." Proc Natl Acad Sci U S A **104**(49): 19345-19350.

Deschoemaeker, S., G. Di Conza, S. Lilla, R. Martín-Pérez, D. Mennerich, L. Boon, S. Hendrikx, O. D. Maddocks, C. Marx, P. Radhakrishnan, H. Prenen, M. Schneider, J. Myllyharju, T. Kietzmann, K. H. Vousden, S. Zanivan and M. Mazzone (2015). "PHD1 regulates p53-mediated colorectal cancer chemoresistance." EMBO Molecular Medicine **7**(10): 1350-1365.

Dewachter, I., J. Van Dorpe, L. Smeijers, M. Gilis, C. Kuipéri, I. Laenen, N. Caluwaerts, D. Moechars, F. Checler, H. Vanderstichele and F. Van Leuven (2000). "Aging Increased Amyloid Peptide and Caused Amyloid Plaques in Brain of Old APP/V717I Transgenic Mice by a Different Mechanism than Mutant Presenilin1." The Journal of Neuroscience **20**(17): 6452.

DiNicolantonio, J. J., J. Liu and J. H. O'Keefe (2018). "Thiamine and Cardiovascular Disease: A Literature Review." Prog Cardiovasc Dis **61**(1): 27-32.

Dixit, S., A. Bernardo, J. M. Walker, J. A. Kennard, G. Y. Kim, E. S. Kessler and F. E. Harrison (2015). "Vitamin C deficiency in the brain impairs cognition, increases amyloid accumulation and deposition, and oxidative stress in APP/PSEN1 and normally aging mice." ACS chemical neuroscience **6**(4): 570-581.

Doig, A. J., M. P. Del Castillo-Frias, O. Berthoumieu, B. Tarus, J. Nasica-Labouze, F. Sterpone, P. H. Nguyen, N. M. Hooper, P. Faller and P. Derreumaux (2017). "Why Is Research on Amyloid-β Failing to Give New Drugs for Alzheimer's Disease?" ACS Chem Neurosci **8**(7): 1435-1437.

Donnino, M. W., M. Andersen Lw Fau - Chase, K. M. Chase M Fau - Berg, M. Berg Km Fau - Tidswell, T. Tidswell M Fau - Giberson, R. Giberson T Fau - Wolfe, A. Wolfe R Fau - Moskowitz, H. Moskowitz A Fau - Smithline, L. Smithline H Fau - Ngo, M. N. Ngo L Fau - Cocchi and M. N. Cocchi "Randomized, Double-Blind, Placebo-Controlled Trial of Thiamine as a Metabolic Resuscitator in Septic Shock: A Pilot Study." (1530-0293 (Electronic)).

Dorn, G. W. (2010). "Mitochondrial Pruning by Nix and BNip3: An Essential Function for Cardiac-Expressed Death Factors." Journal of Cardiovascular Translational Research **3**(4): 374-383.

Duan, C. (2016). "Hypoxia-inducible factor 3 biology: complexities and emerging themes." American Journal of Physiology-Cell Physiology **310**(4): C260-C269.

Eshak, E. S. and A. E. Arafa (2018). "Thiamine deficiency and cardiovascular disorders." Nutr Metab Cardiovasc Dis **28**(10): 965-972.

Esposito, Z., L. Belli, S. Toniolo, G. Sancesario, C. Bianconi and A. Martorana (2013). "Amyloid β, Glutamate, Excitotoxicity in Alzheimer's Disease: Are We on the Right Track?" CNS Neuroscience & Therapeutics **19**(8): 549-555.

Esquerda-Canals, G., L. Montoliu-Gaya, J. Güell-Bosch and S. Villegas (2017). "Mouse Models of Alzheimer's Disease." Journal of Alzheimer's Disease **57**: 1171-1183.

Euser, S. M., N. Sattar, J. C. M. Witteman, E. L. E. M. Bollen, E. J. G. Sijbrands, A. Hofman, I. J. Perry, M. M. B. Breteler and R. G. J. Westendorp (2010). "A Prospective Analysis of Elevated Fasting Glucose Levels and Cognitive Function in Older People." Results From PROSPER and the Rotterdam Study **59**(7): 1601-1607.

Evin, G. and A. Barakat (2014). Critical analysis of the use of β-site amyloid precursor protein-cleaving enzyme 1 inhibitors in the treatment of Alzheimer's disease.

Fellgiebel, A., A. Scheurich, T. Siessmeier, L. G. Schmidt and P. Bartenstein (2003). "Persistence of disturbed thalamic glucose metabolism in a case of Wernicke-Korsakoff syndrome." Psychiatry Res **124**(2): 105-112.

Fernandes, L. M. P., F. R. Bezerra, M. C. Monteiro, M. L. Silva, F. R. de Oliveira, R. R. Lima, E. A. Fontes-Júnior and C. S. F. Maia (2017). "Thiamine deficiency, oxidative metabolic pathways and ethanol-induced neurotoxicity: how poor nutrition contributes to the alcoholic syndrome, as Marchiafava–Bignami disease." European Journal of Clinical Nutrition **71**(5): 580-586.

Ferris, S. H., M. J. de Leon, A. P. Wolf, T. Farkas, D. R. Christman, B. Reisberg, J. S. Fowler, R. Macgregor, A. Goldman, A. E. George and S. Rampal (1980). "Positron emission tomography in the study of aging and senile dementia." Neurobiol Aging **1**(2): 127-131.

Feuerstein, G. Z., X. Wang and F. C. Barone (1998). "The Role of Cytokines in the Neuropathology of Stroke and Neurotrauma." Neuroimmunomodulation **5**(3-4): 143-159.

Filippi, I., E. Morena, C. Aldinucci, F. Carraro, S. Sozzani and A. Naldini (2014). "Short-term hypoxia enhances the migratory capability of dendritic cell through HIF-1α and PI3K/Akt pathway." J Cell Physiol **229**(12): 2067-2076.

Fjell, A. M., L. McEvoy, D. Holland, A. M. Dale and K. B. Walhovd (2014). "What is normal in normal aging? Effects of aging, amyloid and Alzheimer's disease on the cerebral cortex and the hippocampus." Progress in Neurobiology **117**: 20-40.

Fraser, D. A., L. M. Diep, I. A. Hovden, K. B. Nilsen, K. A. Sveen, I. Seljeflot and K. F. Hanssen (2012). "The Effects of Long-Term Oral Benfotiamine Supplementation on Peripheral Nerve Function and Inflammatory Markers in Patients With Type 1 Diabetes." Diabetes Care **35**(5): 1095.

Freeman, G. B. and G. E. Gibson (1988). "Dopamine, acetylcholine, and glutamate interactions in aging. Behavioral and neurochemical correlates." Ann N Y Acad Sci **515**: 191-202.

Freeman, G. B., P. E. Nielsen and G. E. Gibson (1987). "Effect of age on behavioral and enzymatic changes during thiamin deficiency." Neurobiology of Aging **8**(5): 429-434.

Freeman, R. S., D. M. Hasbani, E. A. Lipscomb, J. A. Straub and L. Xie (2003). "SM-20, EGL-9, and the EGLN family of hypoxia-inducible factor prolyl hydroxylases." Mol Cells **16**(1): 1-12.

Friebe, A. and D. Koesling (1998). "Mechanism of YC-1-Induced Activation of Soluble Guanylyl Cyclase." Molecular Pharmacology **53**(1): 123.

Fukuda, R., H. Zhang, J. W. Kim, L. Shimoda, C. V. Dang and G. L. Semenza (2007). "HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells." Cell **129**(1): 111-122.

Ganapathy, V., S. B. Smith and P. D. Prasad (2004). "SLC19: the folate/thiamine transporter family." Pflügers Archiv **447**(5): 641-646.

Ganapathy, V., S. B. Smith and P. D. Prasad (2004). "SLC19: the folate/thiamine transporter family." Pflugers Arch **447**(5): 641-646.

Genin, E., D. Hannequin, D. Wallon, K. Sleegers, M. Hiltunen, O. Combarros, M. J. Bullido, S. Engelborghs, P. De Deyn, C. Berr, F. Pasquier, B. Dubois, G. Tognoni, N. Fiévet, N. Brouwers, K. Bettens, B. Arosio, E. Coto, M. Del Zompo, I. Mateo, J. Epelbaum, A. Frank-Garcia, S. Helisalmi, E. Porcellini, A. Pilotto, P. Forti, R. Ferri, E. Scarpini, G. Siciliano, V. Solfrizzi, S. Sorbi, G. Spalletta, F. Valdivieso, S. Vepsäläinen, V. Alvarez, P. Bosco, M. Mancuso, F. Panza, B. Nacmias, P. Bossù, O. Hanon, P. Piccardi, G. Annoni, D. Seripa, D. Galimberti, F. Licastro, H. Soininen, J. F. Dartigues, M. I. Kamboh, C. Van Broeckhoven, J. C. Lambert, P. Amouyel and D. Campion (2011). "APOE and Alzheimer disease: a major gene with semi-dominant inheritance." Mol Psychiatry **16**(9): 903-907.

Giatromanolaki, A., M. I. Koukourakis, H. M. Sowter, E. Sivridis, S. Gibson, K. C. Gatter and A. L. Harris (2004). "BNIP3 Expression Is Linked with Hypoxia-Regulated Protein Expression and with Poor Prognosis in Non–Small Cell Lung Cancer." Clinical Cancer Research **10**(16): 5566-5571.

Gibson, G. (1989). "Causes of cell damage in hypoxia/ischemia, aging and Alzheimer's disease." Neurobiol Aging **10**(5): 608-609; discussion 618-620.

Gibson, G. E., J. A. Hirsch, P. Fonzetti, B. D. Jordan, R. T. Cirio and J. Elder (2016). "Vitamin B1 (thiamine) and dementia." Ann N Y Acad Sci **1367**(1): 21-30.

Gibson, G. E., J. A. Luchsinger, R. Cirio, H. Chen, J. Franchino-Elder, J. A. Hirsch, L. Bettendorff, Z. Chen, S. A. Flowers, L. M. Gerber, T. Grandville, N. Schupf, H. Xu, Y. Stern, C. Habeck, B. Jordan and P. Fonzetti (2020). "Benfotiamine and Cognitive Decline in Alzheimer's Disease: Results of a Randomized Placebo-Controlled Phase IIa Clinical Trial." J Alzheimers Dis **78**(3): 989-1010.

Gibson, G. E., K. F. Sheu, J. P. Blass, A. Baker, K. C. Carlson, B. Harding and P. Perrino (1988). "Reduced activities of thiamine-dependent enzymes in the brains and peripheral tissues of patients with Alzheimer's disease." Arch Neurol **45**(8): 836-840.

Gibson, G. E. and H. Zhang (2002). "Interactions of oxidative stress with thiamine homeostasis promote neurodegeneration." Neurochemistry International **40**(6): 493-504.

Gidday, J. M., J. C. Fitzgibbons, A. R. Shah and T. S. Park (1994). "Neuroprotection from ischemic brain injury by hypoxic preconditioning in the neonatal rat." Neuroscience Letters **168**(1): 221-224.

Gleadle, J. M. and P. J. Ratcliffe (1997). "Induction of Hypoxia-Inducible Factor-1, Erythropoietin, Vascular Endothelial Growth Factor, and Glucose Transporter-1 by Hypoxia: Evidence Against a Regulatory Role for Src Kinase." Blood **89**(2): 503-509.

Goate, A., M. C. Chartier-Harlin, M. Mullan, J. Brown, F. Crawford, L. Fidani, L. Giuffra, A. Haynes, N. Irving, L. James and et al. (1991). "Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease." Nature **349**(6311): 704-706.

Gold, M., M. F. Chen and K. Johnson (1995). "Plasma and Red Blood Cell Thiamine Deficiency in Patients With Dementia of the Alzheimer's Type." Archives of Neurology **52**(11): 1081-1086.

Gold, M., R. A. Hauser and M. F. Chen (1998). "Plasma thiamine deficiency associated with Alzheimer's disease but not Parkinson's disease." Metab Brain Dis **13**(1): 43-53.

Gold, P. E. (2005). "Glucose and age-related changes in memory." Neurobiology of Aging **26**(1, Supplement): 60-64.

Goldman, S. A., W. A. Pulsinelli, W. Y. Clarke, R. P. Kraig and F. Plum (1989). "The effects of extracellular acidosis on neurons and glia in vitro." Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism **9**(4): 471-477.

Goodman, J. C. (2015). "Neurological Complications of Bariatric Surgery." Curr Neurol Neurosci Rep **15**(12): 79.

Graybeal, J. J., P. L. Bozzelli, L. L. Graybeal, C. M. Groeber, P. E. McKnight, D. N. Cox and J. M. Flinn (2015). "Human ApoE ε4 alters circadian rhythm activity, IL-1β, and GFAP in CRND8 mice." J Alzheimers Dis **43**(3): 823-834.

Greijer, A. E. and E. van der Wall (2004). "The role of hypoxia inducible factor 1 (HIF-1) in hypoxia induced apoptosis." Journal of Clinical Pathology **57**(10): 1009-1014.

Groeneveld, O. N., L. J. Kappelle and G. J. Biessels (2016). "Potentials of incretin-based therapies in dementia and stroke in type 2 diabetes mellitus." J Diabetes Investig **7**(1): 5-16.

Gudala, K., D. Bansal, F. Schifano and A. Bhansali (2013). "Diabetes mellitus and risk of dementia: A meta-analysis of prospective observational studies." Journal of diabetes investigation **4**(6): 640-650.

Guo, C., L. J. Hao, Z. H. Yang, R. Chai, S. Zhang, Y. Gu, H. L. Gao, M. L. Zhong, T. Wang, J. Y. Li and Z. Y. Wang (2016). "Deferoxamine-mediated up-regulation of HIF-1α prevents

dopaminergic neuronal death via the activation of MAPK family proteins in MPTP-treated mice." Exp Neurol **280**: 13-23.

Guo, C., T. Wang, W. Zheng, Z. Y. Shan, W. P. Teng and Z. Y. Wang (2013). "Intranasal deferoxamine reverses iron-induced memory deficits and inhibits amyloidogenic APP processing in a transgenic mouse model of Alzheimer's disease." Neurobiol Aging **34**(2): 562-575.

Guo, C., Y. X. Zhang, T. Wang, M. L. Zhong, Z. H. Yang, L. J. Hao, R. Chai and S. Zhang (2015). "Intranasal deferoxamine attenuates synapse loss via up-regulating the P38/HIF-1α pathway on the brain of APP/PS1 transgenic mice." Front Aging Neurosci **7**: 104.

Guo, M., L. P. Song, Y. Jiang, W. Liu, Y. Yu and G. Q. Chen (2006). "Hypoxia-mimetic agents desferrioxamine and cobalt chloride induce leukemic cell apoptosis through different hypoxia-inducible factor-1alpha independent mechanisms." Apoptosis **11**(1): 67-77.

Haass, C., C. A. Lemere, A. Capell, M. Citron, P. Seubert, D. Schenk, L. Lannfelt and D. J. Selkoe (1995). "The Swedish mutation causes early-onset Alzheimer's disease by beta-secretase cleavage within the secretory pathway." Nat Med **1**(12): 1291-1296.

Habeb, A. M., S. E. Flanagan, M. A. Zulali, M. A. Abdullah, R. Pomahačová, V. Boyadzhiev, L. E. Colindres, G. V. Godoy, T. Vasanthi, R. Al Saif, A. Setoodeh, A. Haghighi, A. Haghighi, Y. Shaalan, C. International Neonatal Diabetes, A. T. Hattersley, S. Ellard and E. De Franco (2018). "Pharmacogenomics in diabetes: outcomes of thiamine therapy in TRMA syndrome." Diabetologia **61**(5): 1027-1036.

Halterman, M. W. and H. J. Federoff (1999). "HIF-1α and p53 Promote Hypoxia-Induced Delayed Neuronal Death in Models of CNS Ischemia." Experimental Neurology **159**(1): 65-72.

Ham, P. B., 3rd and R. Raju (2017). "Mitochondrial function in hypoxic ischemic injury and influence of aging." Prog Neurobiol **157**: 92-116.

Hanberry, B. S., R. Berger and J. A. Zastre (2014). "High-dose vitamin B1 reduces proliferation in cancer cell lines analogous to dichloroacetate." Cancer chemotherapy and pharmacology **73**(3): 585-594.

Hanson, L. R. and W. H. Frey, 2nd (2008). "Intranasal delivery bypasses the blood-brain barrier to target therapeutic agents to the central nervous system and treat neurodegenerative disease." BMC neuroscience **9 Suppl 3**(Suppl 3): S5-S5.

Hansson, L. O., A. Friedler, S. Freund, S. Rüdiger and A. R. Fersht (2002). "Two sequence motifs from HIF-1α bind to the DNA-binding site of p53." Proceedings of the National Academy of Sciences **99**(16): 10305-10309.

Harman, D. (1956). "Aging: A Theory Based on Free Radical and Radiation Chemistry." Journal of Gerontology **11**(3): 298-300.

Harman, D. (1972). "The Biologic Clock: The Mitochondria?" Journal of the American Geriatrics Society **20**(4): 145-147.

Harper, A. E., R. H. Miller and K. P. Block (1984). "Branched-chain amino acid metabolism." Annu Rev Nutr **4**: 409-454.

Haupt, Y., R. Maya, A. Kazaz and M. Oren (1997). "Mdm2 promotes the rapid degradation of p53." Nature **387**(6630): 296-299.

Hazell, A. S., L. McGahan, W. Tetzlaff, A. M. Bedard, G. S. Robertson, Y. Nakabeppu and A. M. Hakim (1998). "Immediate-early gene expression in the brain of the thiamine-deficient rat." J Mol Neurosci **10**(1): 1-15.

Hazell, A. S., P. Pannunzio, K. V. Rama Rao, D. V. Pow and A. Rambaldi (2003). "Thiamine deficiency results in downregulation of the GLAST glutamate transporter in cultured astrocytes." Glia **43**(2): 175-184.

Hazell, A. S., K. V. Rao, N. C. Danbolt, D. V. Pow and R. F. Butterworth (2001). "Selective down-regulation of the astrocyte glutamate transporters GLT-1 and GLAST within the medial thalamus in experimental Wernicke's encephalopathy." J Neurochem **78**(3): 560-568.

Hazell, A. S., D. Sheedy, R. Oanea, M. Aghourian, S. Sun, J. Y. Jung, D. Wang and C. Wang (2010). "Loss of astrocytic glutamate transporters in Wernicke encephalopathy." Glia **58**(2): 148-156.

Hazell, A. S., K. G. Todd and R. F. Butterworth (1998). "Mechanisms of neuronal cell death in Wernicke's encephalopathy." Metab Brain Dis **13**(2): 97-122.

Hazell, A. S. and C. Wang (2005). "Downregulation of complexin I and complexin II in the medial thalamus is blocked by N-acetylcysteine in experimental Wernicke's encephalopathy." J Neurosci Res **79**(1-2): 200-207.

Hekimi, S., J. Lapointe and Y. Wen (2011). "Taking a "good" look at free radicals in the aging process." Trends Cell Biol **21**(10): 569-576.

Helton, R., J. Cui, J. R. Scheel, J. A. Ellison, C. Ames, C. Gibson, B. Blouw, L. Ouyang, I. Dragatsis, S. Zeitlin, R. S. Johnson, S. A. Lipton and C. Barlow (2005). "Brain-Specific Knock-Out of Hypoxia-Inducible Factor-1α Reduces Rather Than Increases Hypoxic-Ischemic Damage." The Journal of Neuroscience **25**(16): 4099-4107.

Hendrie, H. C., M. S. Albert, M. A. Butters, S. Gao, D. S. Knopman, L. J. Launer, K. Yaffe, B. N. Cuthbert, E. Edwards and M. V. Wagster (2006). "The NIH Cognitive and Emotional Health Project. Report of the Critical Evaluation Study Committee." Alzheimers Dement **2**(1): 12-32.

Héroux, M. and R. F. Butterworth (1988). "Reversible alterations of cerebral gamma-aminobutyric acid in pyrithiamine-treated rats: implications for the pathogenesis of Wernicke's encephalopathy." J Neurochem **51**(4): 1221-1226.

Hewitt, J., L. Castilla Guerra, M. D. C. Fernández-Moreno and C. Sierra (2012). "Diabetes and stroke prevention: a review." Stroke research and treatment **2012**: 673187-673187.

Hillbom, M., P. Saloheimo, S. Fujioka, Z. K. Wszolek, S. Juvela and M. A. Leone (2014). "Diagnosis and management of Marchiafava-Bignami disease: a review of CT/MRI confirmed cases." Journal of neurology, neurosurgery, and psychiatry **85**(2): 168-173.

Hirai, K., G. Aliev, A. Nunomura, H. Fujioka, R. L. Russell, C. S. Atwood, A. B. Johnson, Y. Kress, H. V. Vinters, M. Tabaton, S. Shimohama, A. D. Cash, S. L. Siedlak, P. L. Harris, P. K. Jones, R. B. Petersen, G. Perry and M. A. Smith (2001). "Mitochondrial abnormalities in Alzheimer's disease." The Journal of neuroscience : the official journal of the Society for Neuroscience **21**(9): 3017-3023.

Hiwatashi, Y., K. Kanno, C. Takasaki, K. Goryo, T. Sato, S. Torii, K. Sogawa and K. Yasumoto (2011). "PHD1 interacts with ATF4 and negatively regulates its transcriptional activity without prolyl hydroxylation." Exp Cell Res **317**(20): 2789-2799.

Hoffman, R. (2016). "Thiamine deficiency in the Western diet and dementia risk." British Journal of Nutrition **116**(1): 188-189.

Hou, Y., S. Lautrup, S. Cordonnier, Y. Wang, D. L. Croteau, E. Zavala, Y. Zhang, K. Moritoh, J. F. O'Connell, B. A. Baptiste, T. V. Stevnsner, M. P. Mattson and V. A. Bohr (2018). "NAD(+) supplementation normalizes key Alzheimer's features and DNA damage responses in a new AD mouse model with introduced DNA repair deficiency." Proc Natl Acad Sci U S A **115**(8): E1876-E1885.

Hsiao, K., P. Chapman, S. Nilsen, C. Eckman, Y. Harigaya, S. Younkin, F. Yang and G. Cole (1996). "Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice." Science **274**(5284): 99-102.

Hu, X., L. Wei, T. M. Taylor, J. Wei, X. Zhou, J. A. Wang and S. P. Yu (2011). "Hypoxic preconditioning enhances bone marrow mesenchymal stem cell migration via Kv2.1 channel and FAK activation." Am J Physiol Cell Physiol **301**(2): C362-372.

Huckabee, W. E. (1958). "Relationships of pyruvate and lactate during anaerobic metabolism. III. Effect of breathing low-oxygen gases." The Journal of clinical investigation **37**(2): 264-271.

Hynd, M. R., H. L. Scott and P. R. Dodd (2004). "Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer's disease." Neurochemistry International **45**(5): 583-595.

Iadecola, C., F. Zhang, R. Casey, H. B. Clark and M. E. Ross (1996). "Inducible nitric oxide synthase gene expression in vascular cells after transient focal cerebral ischemia." Stroke **27**(8): 1373-1380.

Iqbal, K., F. Liu, C. X. Gong and I. Grundke-Iqbal (2010). "Tau in Alzheimer disease and related tauopathies." Current Alzheimer research **7**(8): 656-664.

Isenberg-Grzeda, E., A. J. Hsu, V. Hatzoglou, C. Nelso and W. Breitbart (2015). "Palliative treatment of thiamine-related encephalopathy (Wernicke's encephalopathy) in cancer: A case series and review of the literature." Palliat Support Care **13**(5): 1241-1249.

Iyalomhe, O., S. Swierczek, N. Enwerem, Y. Chen, M. O. Adedeji, J. Allard, O. Ntekim, S. Johnson, K. Hughes, P. Kurian and T. O. Obisesan (2017). "The Role of Hypoxia-Inducible Factor 1 in Mild Cognitive Impairment." Cellular and molecular neurobiology **37**(6): 969-977.

Jauch-Chara, K. and K. M. Oltmanns (2014). "Glycemic control after brain injury: boon and bane for the brain." Neuroscience **283**: 202-209.

Jeyasingham, M. D., O. E. Pratt, A. Burns, G. K. Shaw, A. D. Thomson and A. Marsh (1987). "The activation of red blood cell transketolase in groups of patients especially at risk from thiamin deficiency." Psychol Med **17**(2): 311-318.

Jhala, S. S. and A. S. Hazell (2011). "Modeling neurodegenerative disease pathophysiology in thiamine deficiency: Consequences of impaired oxidative metabolism." Neurochemistry International **58**(3): 248-260.

Jian, K. T., Y. Shi, Y. Zhang, Y. M. Mao, J. S. Liu and F. L. Xue (2015). "Time course effect of hypoxia on bone marrow-derived endothelial progenitor cells and their effects on left ventricular function after transplanted into acute myocardial ischemia rat." Eur Rev Med Pharmacol Sci **19**(6): 1043-1054.

Johnson, A. B. and N. R. Blum (1970). "Nucleoside phosphatase activities associated with the tangles and plaques of alzheimer's disease: a histochemical study of natural and experimental neurofibrillary tangles." J Neuropathol Exp Neurol **29**(3): 463-478.

Jones, N. M. and M. Bergeron (2001). "Hypoxic preconditioning induces changes in HIF-1 target genes in neonatal rat brain." J Cereb Blood Flow Metab **21**(9): 1105-1114.

Jonus, H. C., B. S. Hanberry, S. Khatu, J. Kim, H. Luesch, L. H. Dang, M. G. Bartlett and J. A. Zastre (2018). "The adaptive regulation of thiamine pyrophosphokinase-1 facilitates malignant growth during supplemental thiamine conditions." Oncotarget **9**(83): 35422-35438.

Jung, F., L. A. Palmer, N. Zhou and R. A. Johns (2000). "Hypoxic regulation of inducible nitric oxide synthase via hypoxia inducible factor-1 in cardiac myocytes." Circ Res **86**(3): 319-325.

Jung, S. H., M. L. Brownlow, M. Pellegrini and R. Jankord (2017). "Divergence in Morris Water Maze-Based Cognitive Performance under Chronic Stress Is Associated with the Hippocampal Whole Transcriptomic Modification in Mice." Front Mol Neurosci **10**: 275.

Kaelin, W. G., Jr. (2005). "ROS: really involved in oxygen sensing." Cell Metab **1**(6): 357-358.

Kalaria, R. N. (2016). "Neuropathological diagnosis of vascular cognitive impairment and vascular dementia with implications for Alzheimer's disease." Acta neuropathologica **131**(5): 659-685.

Kalaria, R. N., R. Akinyemi and M. Ihara (2016). "Stroke injury, cognitive impairment and vascular dementia." Biochimica et biophysica acta **1862**(5): 915-925.

Karuppagounder, S. S., H. Xu, D. Pechman, L. H. Chen, L. A. DeGiorgio and G. E. Gibson (2008). "Translocation of amyloid precursor protein C-terminal fragment(s) to the nucleus precedes neuronal death due to thiamine deficiency-induced mild impairment of oxidative metabolism." Neurochem Res **33**(7): 1365-1372.

Karuppagounder, S. S., H. Xu, Q. Shi, L. H. Chen, S. Pedrini, D. Pechman, H. Baker, M. F. Beal, S. E. Gandy and G. E. Gibson (2009). "Thiamine deficiency induces oxidative stress and exacerbates the plaque pathology in Alzheimer's mouse model." Neurobiology of aging **30**(10): 1587-1600.

Kaule, G., R. Timpl, F. Gaill and V. Günzler (1998). "Prolyl hydroxylase activity in tissue homogenates of annelids from deep sea hydrothermal vents." Matrix Biology **17**(3): 205-212.

Ke, Z.-J., L. A. Degiorgio, B. T. Volpe and G. E. Gibson (2003). "Reversal of Thiamine Deficiency-Induced Neurodegeneration." Journal of Neuropathology & Experimental Neurology **62**(2): 195-207.

Kern, W., A. Peters, B. Schultes, E. Deininger, J. Born and H. L. Fehm (2001). Improving Influence of Insulin on Cognitive Functions in Humans.

Kerns, J. C., C. Arundel and L. S. Chawla (2015). "Thiamin deficiency in people with obesity." Adv Nutr **6**(2): 147-153.

Kerridge, C., D. I. Kozlova, N. N. Nalivaeva and A. J. Turner (2015). "Hypoxia Affects Neprilysin Expression Through Caspase Activation and an APP Intracellular Domain-dependent Mechanism." Frontiers in neuroscience **9**: 426-426.

Kiernan, E. A., S. M. C. Smith, G. S. Mitchell and J. J. Watters (2016). "Mechanisms of microglial activation in models of inflammation and hypoxia: Implications for chronic intermittent hypoxia." The Journal of Physiology **594**(6): 1563-1577.

Kim, H. L., E. J. Yeo, Y. S. Chun and J. W. Park (2006). "A domain responsible for HIF-1alpha degradation by YC-1, a novel anticancer agent." Int J Oncol **29**(1): 255-260.

Kim, S.-Y., J.-S. Choi, C. Park and J.-W. Jeong (2010). "Ethyl pyruvate stabilizes hypoxia-inducible factor 1 alpha via stimulation of the TCA cycle." Cancer Letters **295**(2): 236-241.

Kimelberg, H. K., E. Rutledge, S. Goderie and C. Charniga (1995). "Astrocytic swelling due to hypotonic or high K+ medium causes inhibition of glutamate and aspartate uptake and increases their release." J Cereb Blood Flow Metab **15**(3): 409-416.

Kimura, M. and Y. Itokawa (1990). "Cooking losses of minerals in foods and its nutritional significance." J Nutr Sci Vitaminol (Tokyo) **36 Suppl 1**: S25-32; discussion S33.

Kolobova, E., A. Tuganova, I. Boulatnikov and K. M. Popov (2001). "Regulation of pyruvate dehydrogenase activity through phosphorylation at multiple sites." The Biochemical journal **358**(Pt 1): 69-77.

Koshiji, M., Y. Kageyama, E. A. Pete, I. Horikawa, J. C. Barrett and L. E. Huang (2004). "HIF-1α induces cell cycle arrest by functionally counteracting Myc." The EMBO Journal **23**(9): 1949-1956.

Kothari, S., J. Cizeau, E. McMillan-Ward, S. J. Israels, M. Bailes, K. Ens, L. A. Kirshenbaum and S. B. Gibson (2003). "BNIP3 plays a role in hypoxic cell death in human epithelial cells that is inhibited by growth factors EGF and IGF." Oncogene **22**(30): 4734-4744.

Koumenis, C., R. Alarcon, E. Hammond, P. Sutphin, W. Hoffman, M. Murphy, J. Derr, Y. Taya, S. W. Lowe, M. Kastan and A. Giaccia (2001). "Regulation of p53 by hypoxia: dissociation of transcriptional repression and apoptosis from p53-dependent transactivation." Mol Cell Biol **21**(4): 1297-1310.

Kubli, D. A., M. N. Quinsay, C. Huang, Y. Lee and A. B. Gustafsson (2008). "Bnip3 functions as a mitochondrial sensor of oxidative stress during myocardial ischemia and reperfusion." Am J Physiol Heart Circ Physiol **295**(5): H2025-2031.

Kubli, D. A., J. E. Ycaza and A. B. Gustafsson (2007). "Bnip3 mediates mitochondrial dysfunction and cell death through Bax and Bak." The Biochemical journal **405**(3): 407-415.

Kuhl, D. E., E. J. Metter, W. H. Riege and M. E. Phelps (1982). "Effects of human aging on patterns of local cerebral glucose utilization determined by the [18F]fluorodeoxyglucose method." J Cereb Blood Flow Metab **2**(2): 163-171.

Kunze, R., W. Zhou, R. Veltkamp, B. Wielockx, G. Breier and H. H. Marti (2012). "Neuron-specific prolyl-4-hydroxylase domain 2 knockout reduces brain injury after transient cerebral ischemia." Stroke **43**(10): 2748-2756.

Labay, V., T. Raz, D. Baron, H. Mandel, H. Williams, T. Barrett, R. Szargel, L. McDonald, A. Shalata, K. Nosaka, S. Gregory and N. Cohen (1999). "Mutations in SLC19A2 cause thiamine-responsive megaloblastic anaemia associated with diabetes mellitus and deafness." Nat Genet **22**(3): 300-304.

Lane, D. J. R., S. Ayton and A. I. Bush (2018). "Iron and Alzheimer's Disease: An Update on Emerging Mechanisms." J Alzheimers Dis **64**(s1): S379-S395.

Langlais, P. J., G. Anderson, S. X. Guo and S. C. Bondy (1997). "Increased cerebral free radical production during thiamine deficiency." Metabolic Brain Disease **12**(2): 137-143.

Lanoiselée, H.-M., G. Nicolas, D. Wallon, A. Rovelet-Lecrux, M. Lacour, S. Rousseau, A.-C. Richard, F. Pasquier, A. Rollin-Sillaire, O. Martinaud, M. Quillard-Muraine, V. de la Sayette, C. Boutoleau-Bretonniere, F. Etcharry-Bouyx, V. Chauviré, M. Sarazin, I. le Ber, S. Epelbaum, T. Jonveaux, O. Rouaud, M. Ceccaldi, O. Félician, O. Godefroy, M. Formaglio, B. Croisile, S. Auriacombe, L. Chamard, J.-L. Vincent, M. Sauvée, C. Marelli-Tosi, A. Gabelle, C. Ozsancak, J. Pariente, C. Paquet, D. Hannequin, D. Campion and C. N. R. M. A. J. p. collaborators of the (2017). "APP, PSEN1, and PSEN2 mutations in early-onset Alzheimer disease: A genetic screening study of familial and sporadic cases." PLOS Medicine **14**(3): e1002270.

Lanoiselée, H. M., G. Nicolas, D. Wallon, A. Rovelet-Lecrux, M. Lacour, S. Rousseau, A. C. Richard, F. Pasquier, A. Rollin-Sillaire, O. Martinaud, M. Quillard-Muraine, V. de la Sayette, C. Boutoleau-Bretonniere, F. Etcharry-Bouyx, V. Chauviré, M. Sarazin, I. le Ber, S. Epelbaum, T. Jonveaux, O. Rouaud, M. Ceccaldi, O. Félician, O. Godefroy, M. Formaglio, B. Croisile, S. Auriacombe, L. Chamard, J. L. Vincent, M. Sauvée, C. Marelli-Tosi, A. Gabelle, C. Ozsancak, J. Pariente, C. Paquet, D. Hannequin and D. Campion (2017). "APP, PSEN1, and PSEN2 mutations in early-onset Alzheimer disease: A genetic screening study of familial and sporadic cases." PLoS Med **14**(3): e1002270.

Larner, A. J. and M. Doran (2006). "Clinical phenotypic heterogeneity of Alzheimer's disease associated with mutations of the presenilin-1 gene." J Neurol **253**(2): 139-158.

Lee, H.-K., P. Kumar, Q. Fu, K. M. Rosen and H. W. Querfurth (2009). "The Insulin/Akt Signaling Pathway Is Targeted by Intracellular β-Amyloid." Molecular Biology of the Cell **20**(5): 1533-1544.

Lee, W.-T., S.-H. Tai, Y.-W. Lin, T.-S. Wu and E. J. Lee (2018). "YC-1 reduces inflammatory responses by inhibiting nuclear factor-κB translocation in mice subjected to transient focal cerebral ischemia." Molecular medicine reports **18**(2): 2043-2051.

Lee, W. T., S. H. Tai, Y. W. Lin, T. S. Wu and E. J. Lee (2018). "YC-1 reduces inflammatory responses by inhibiting nuclear factor-κB translocation in mice subjected to transient focal cerebral ischemia." Mol Med Rep **18**(2): 2043-2051.

Lee, Y., D. A. Kubli, R. A. Hanna, M. Q. Cortez, H. Y. Lee, S. Miyamoto and B. Gustafsson Å (2015). "Cellular redox status determines sensitivity to BNIP3-mediated cell death in cardiac myocytes." Am J Physiol Cell Physiol **308**(12): C983-992.

Li, F., N. Y. Calingasan, F. Yu, W. M. Mauck, M. Toidze, C. G. Almeida, R. H. Takahashi, G. A. Carlson, M. Flint Beal, M. T. Lin and G. K. Gouras (2004). "Increased plaque burden in brains of APP mutant MnSOD heterozygous knockout mice." J Neurochem **89**(5): 1308-1312.

Li, L., P. Saliba, S. Reischl, H. H. Marti and R. Kunze (2016). "Neuronal deficiency of HIF prolyl 4-hydroxylase 2 in mice improves ischemic stroke recovery in an HIF dependent manner." Neurobiol Dis **91**: 221-235.

Li, L., X. Zhang, D. Yang, G. Luo, S. Chen and W. Le (2009). "Hypoxia increases Aβ generation by altering β- and γ-cleavage of APP." Neurobiology of Aging **30**(7): 1091-1098.

Li, Y., W. Zhou, Y. Tong, G. He and W. Song (2006). "Control of APP processing and Abeta generation level by BACE1 enzymatic activity and transcription." FASEB J **20**(2): 285-292.

Liang, X., S. W. Yee, H. C. Chien, E. C. Chen, Q. Luo, L. Zou, M. Piao, A. Mifune, L. Chen, M. E. Calvert, S. King, F. Norheim, J. Abad, R. M. Krauss and K. M. Giacomini (2018). "Organic cation transporter 1 (OCT1) modulates multiple cardiometabolic traits through effects on hepatic thiamine content." PLoS Biol **16**(4): e2002907.

Liu, D., Z. Ke and J. Luo (2017). "Thiamine Deficiency and Neurodegeneration: the Interplay Among Oxidative Stress, Endoplasmic Reticulum Stress, and Autophagy." Mol Neurobiol **54**(7): 5440-5448.

Liu, Y., F. Liu, I. Grundke-Iqbal, K. Iqbal and C. X. Gong (2011). "Deficient brain insulin signalling pathway in Alzheimer's disease and diabetes." J Pathol **225**(1): 54-62.

Liu, Y., F. Liu, K. Iqbal, I. Grundke-Iqbal and C.-X. Gong (2008). "Decreased glucose transporters correlate to abnormal hyperphosphorylation of tau in Alzheimer disease." FEBS letters **582**(2): 359-364.

Llewellyn, D. J., I. A. Lang, K. M. Langa and D. Melzer (2011). "Vitamin D and cognitive impairment in the elderly U.S. population." J Gerontol A Biol Sci Med Sci **66**(1): 59-65.

Llewellyn, D. J., I. A. Lang, K. M. Langa, G. Muniz-Terrera, C. L. Phillips, A. Cherubini, L. Ferrucci and D. Melzer (2010). "Vitamin D and risk of cognitive decline in elderly persons." Arch Intern Med **170**(13): 1135-1141.

Lonsdale, D. (2007). "Three Case Reports to Illustrate Clinical Applications in the Use of Erythrocyte Transketolase." Evidence-Based Complementary and Alternative Medicine **4**: 854132.

Lonsdale, D. (2018). "Thiamin." Adv Food Nutr Res **83**: 1-56.

Lövheim, H., F. Elgh, A. Johansson, H. Zetterberg, K. Blennow, G. Hallmans and S. Eriksson (2017). "Plasma concentrations of free amyloid β cannot predict the development of Alzheimer's disease." Alzheimers Dement **13**(7): 778-782.

Lu'o'ng, K. and L. T. Nguyen (2011). "Role of thiamine in Alzheimer's disease." Am J Alzheimers Dis Other Demen **26**(8): 588-598.

Lu, H., C. L. Dalgard, A. Mohyeldin, T. McFate, A. S. Tait and A. Verma (2005). "Reversible inactivation of HIF-1 prolyl hydroxylases allows cell metabolism to control basal HIF-1." J Biol Chem **280**(51): 41928-41939.

Lueptow, L. M. (2017). "Novel Object Recognition Test for the Investigation of Learning and Memory in Mice." Journal of visualized experiments : JoVE(126): 55718.

Lukienko, P. I., N. G. Mel'nichenko, I. V. Zverinskii and S. V. Zabrodskaya (2000). "Antioxidant properties of thiamine." Bulletin of Experimental Biology and Medicine **130**(9): 874-876.

Luong, K. V. Q. and L. T. H. Nguyễn (2013). "The beneficial role of thiamine in Parkinson disease." CNS neuroscience & therapeutics **19**(7): 461-468.

Lương, K. v. q. c. and L. T. H. Nguyê~n (2012). "Thiamine and Parkinson's disease." Journal of the Neurological Sciences **316**(1): 1-8.

Macheda, T., K. Roberts, D. N. Lyons, E. Higgins, K. J. Ritter, A.-L. Lin, W. J. Alilain and A. D. Bachstetter (2019). "Chronic Intermittent Hypoxia Induces Robust Astrogliosis in an Alzheimer's Disease-Relevant Mouse Model." Neuroscience **398**: 55-63.

MacKenzie, E. D., M. A. Selak, D. A. Tennant, L. J. Payne, S. Crosby, C. M. Frederiksen, D. G. Watson and E. Gottlieb (2007). "Cell-permeating alpha-ketoglutarate derivatives alleviate pseudohypoxia in succinate dehydrogenase-deficient cells." Mol Cell Biol **27**(9): 3282-3289.

Maguire, D., D. Talwar, P. G. Shiels and D. McMillan (2018). "The role of thiamine dependent enzymes in obesity and obesity related chronic disease states: A systematic review." Clin Nutr ESPEN **25**: 8-17.

Maiuri, M. C., E. Zalckvar, A. Kimchi and G. Kroemer (2007). "Self-eating and self-killing: crosstalk between autophagy and apoptosis." Nat Rev Mol Cell Biol **8**(9): 741-752.

Makin, S. (2018). "The amyloid hypothesis on trial." Nature **559**(7715): S4-S7.

Marcus, C., E. Mena and R. M. Subramaniam (2014). "Brain PET in the diagnosis of Alzheimer's disease." Clinical nuclear medicine **39**(10): e413-e426.

Markowicz-Piasecka, M., J. Sikora, A. Szydłowska, A. Skupień, E. Mikiciuk-Olasik and K. M. Huttunen (2017). "Metformin - a Future Therapy for Neurodegenerative Diseases : Theme: Drug Discovery, Development and Delivery in Alzheimer's Disease Guest Editor: Davide Brambilla." Pharmaceutical research **34**(12): 2614-2627.

Marshall, A. J., M. Rattray and P. F. T. Vaughan (2006). "Chronic hypoxia in the human neuroblastoma SH-SY5Y causes reduced expression of the putative α-secretases, ADAM10 and TACE, without altering their mRNA levels." Brain Research **1099**(1): 18-24.

Mastrogiacoma, F., L. Bettendorff, T. Grisar and S. J. Kish (1996). "Brain thiamine, its phosphate esters, and its metabolizing enzymes in Alzheimer's disease." Ann Neurol **39**(5): 585-591.

Mattsson, N., H. Zetterberg, S. Janelidze, P. S. Insel, U. Andreasson, E. Stomrud, S. Palmqvist, D. Baker, C. A. Tan Hehir, A. Jeromin, D. Hanlon, L. Song, L. M. Shaw, J. Q. Trojanowski, M. W. Weiner, O. Hansson and K. Blennow (2016). "Plasma tau in Alzheimer disease." Neurology **87**(17): 1827-1835.

McLure, K. G., M. Takagi and M. B. Kastan (2004). "NAD+ modulates p53 DNA binding specificity and function." Molecular and cellular biology **24**(22): 9958-9967.

Meador, K., Loring, D., Nichols, M., Zamrini, E., Rivner, M., Posas, H., … Moore, E. (1993). "Preliminary Findings of High-Dose Thiamine in Dementia of Alzheimer's Type." Journal of Geriatric Psychiatry and Neurology **6**(4): 222-229.

Mellor, H. R. and A. L. Harris (2007). "The role of the hypoxia-inducible BH3-only proteins BNIP3 and BNIP3L in cancer." Cancer Metastasis Rev **26**(3-4): 553-566.

Mendez, M. F. (2017). "Early-Onset Alzheimer Disease." Neurol Clin **35**(2): 263-281.

Meng, Y., Y. Yong, G. Yang, H. Ding, Z. Fan, Y. Tang, J. Luo and Z.-J. Ke (2013). "Autophagy alleviates neurodegeneration caused by mild impairment of oxidative metabolism." Journal of Neurochemistry **126**(6): 805-818.

Mergenthaler, P., U. Lindauer, G. A. Dienel and A. Meisel (2013). "Sugar for the brain: the role of glucose in physiological and pathological brain function." Trends in neurosciences **36**(10): 587-597.

Mi, D. J., S. Dixit, T. A. Warner, J. A. Kennard, D. A. Scharf, E. S. Kessler, L. M. Moore, D. C. Consoli, C. W. Bown, A. J. Eugene, J. Q. Kang and F. E. Harrison (2018). "Altered glutamate clearance in ascorbate deficient mice increases seizure susceptibility and contributes to cognitive impairment in APP/PSEN1 mice." Neurobiol Aging **71**: 241-254.

Miller, B. A., R. S. Perez, A. R. Shah, E. R. Gonzales, T. S. Park and J. M. Gidday (2001). "Cerebral protection by hypoxic preconditioning in a murine model of focal ischemia-reperfusion." Neuroreport **12**(8): 1663-1669.

Mimori, Y., H. Katsuoka and S. Nakamura (1996). "Thiamine therapy in Alzheimer's disease." Metab Brain Dis **11**(1): 89-94.

Mitchell, A. B., T. E. Ryan, A. R. Gillion, L. D. Wells and P. Muthiah (2019). "Vitamin C and Thiamine for Sepsis and Septic Shock." Am J Med.

Mojsilovic-Petrovic, J., D. Callaghan, H. Cui, C. Dean, D. B. Stanimirovic and W. Zhang (2007). "Hypoxia-inducible factor-1 (HIF-1) is involved in the regulation of hypoxia-stimulated expression of monocyte chemoattractant protein-1 (MCP-1/CCL2) and MCP-5 (Ccl12) in astrocytes." Journal of Neuroinflammation **4**(1): 12.

Molina, J. A., F. J. Jiménez-Jiménez, A. Hernánz, E. Fernández-Vivancos, S. Medina, F. de Bustos, C. Gómez-Escalonilla and Y. Sayed (2002). "Cerebrospinal fluid levels of thiamine in patients with Alzheimer's disease." J Neural Transm (Vienna) **109**(7-8): 1035-1044.

Moloney, A. M., R. J. Griffin, S. Timmons, R. O'Connor, R. Ravid and C. O'Neill (2010). "Defects in IGF-1 receptor, insulin receptor and IRS-1/2 in Alzheimer's disease indicate possible resistance to IGF-1 and insulin signalling." Neurobiol Aging **31**(2): 224-243.

Moniz, S., J. Biddlestone and S. Rocha (2014). "Grow(2): The HIF system, energy homeostasis and the cell cycle." Histology and histopathology **29**.

Moraes, J. O., S. D. C. Rodrigues, L. M. Pereira, R. C. N. Medeiros, C. A. S. de Cordova and F. M. de Cordova (2018). "Amprolium exposure alters mice behavior and metabolism in vivo." Animal Model Exp Med **1**(4): 272-281.

Morimoto, B. H. and D. E. Koshland, Jr. (1990). "Induction and expression of long- and short-term neurosecretory potentiation in a neural cell line." Neuron **5**(6): 875-880.

Mosconi, L. (2005). "Brain glucose metabolism in the early and specific diagnosis of Alzheimer's disease. FDG-PET studies in MCI and AD." Eur J Nucl Med Mol Imaging **32**(4): 486-510.

Mosconi, L., R. Mistur, R. Switalski, W. H. Tsui, L. Glodzik, Y. Li, E. Pirraglia, S. De Santi, B. Reisberg, T. Wisniewski and M. J. de Leon (2009). "FDG-PET changes in brain glucose metabolism from normal cognition to pathologically verified Alzheimer's disease." European journal of nuclear medicine and molecular imaging **36**(5): 811-822.

Mosconi, L., A. Pupi and M. J. De Leon (2008). "Brain glucose hypometabolism and oxidative stress in preclinical Alzheimer's disease." Annals of the New York Academy of Sciences **1147**: 180-195.

Mosconi, L., W. H. Tsui, K. Herholz, A. Pupi, A. Drzezga, G. Lucignani, E. M. Reiman, V. Holthoff, E. Kalbe, S. Sorbi, J. Diehl-Schmid, R. Perneczky, F. Clerici, R. Caselli, B. Beuthien-Baumann, A. Kurz, S. Minoshima and M. J. de Leon (2008). "Multicenter standardized 18F-FDG PET diagnosis of mild cognitive impairment, Alzheimer's disease, and other dementias." Journal of nuclear medicine : official publication, Society of Nuclear Medicine **49**(3): 390-398.

Moskowitz, A., L. W. Andersen, M. N. Cocchi, M. Karlsson, P. V. Patel and M. W. Donnino (2017). "Thiamine as a Renal Protective Agent in Septic Shock. A Secondary Analysis of a Randomized, Double-Blind, Placebo-controlled Trial." Ann Am Thorac Soc **14**(5): 737-741.

Moskowitz, A. and M. W. Donnino (2020). "Thiamine (vitamin B1) in septic shock: a targeted therapy." Journal of thoracic disease **12**(Suppl 1): S78-S83.

Muñoz, S. S., B. Garner and L. Ooi (2019). "Understanding the Role of ApoE Fragments in Alzheimer's Disease." Neurochem Res **44**(6): 1297-1305.

Nabokina, S. M., K. Inoue, V. S. Subramanian, J. E. Valle, H. Yuasa and H. M. Said (2014). "Molecular identification and functional characterization of the human colonic thiamine pyrophosphate transporter." J Biol Chem **289**(7): 4405-4416.

Ndubuizu, O. I., J. C. Chavez and J. C. LaManna (2009). "Increased prolyl 4-hydroxylase expression and differential regulation of hypoxia-inducible factors in the aged rat brain." Am J Physiol Regul Integr Comp Physiol **297**(1): R158-165.

Nelson, A. R., M. D. Sweeney, A. P. Sagare and B. V. Zlokovic (2016). "Neurovascular dysfunction and neurodegeneration in dementia and Alzheimer's disease." Biochimica et biophysica acta **1862**(5): 887-900.

Nepal, B., L. J. Brown and K. J. Anstey (2014). "Rising midlife obesity will worsen future prevalence of dementia." PloS one **9**(9): e99305-e99305.

Nordberg, A., J. O. Rinne, A. Kadir and B. Långström (2010). "The use of PET in Alzheimer disease." Nature Reviews Neurology **6**(2): 78-87.

Nordström, A. and P. Nordström (2018). "Traumatic brain injury and the risk of dementia diagnosis: A nationwide cohort study." PLoS medicine **15**(1): e1002496-e1002496.

O'Brien, R. J. and P. C. Wong (2011). "Amyloid precursor protein processing and Alzheimer's disease." Annual review of neuroscience **34**: 185-204.

Oakley, H., S. L. Cole, S. Logan, E. Maus, P. Shao, J. Craft, A. Guillozet-Bongaarts, M. Ohno, J. Disterhoft, L. Van Eldik, R. Berry and R. Vassar (2006). "Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation." The Journal of neuroscience : the official journal of the Society for Neuroscience **26**(40): 10129-10140.

Oddo, S., A. Caccamo, J. D. Shepherd, M. P. Murphy, T. E. Golde, R. Kayed, R. Metherate, M. P. Mattson, Y. Akbari and F. M. LaFerla (2003). "Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction." Neuron **39**(3): 409-421.

Olin, J., L. Schneider, A. Novit and S. Luczak (2001). "Hydergine for dementia." Cochrane Database Syst Rev(2): CD000359.

Ollat, H., B. Laurent, S. Bakchine, B. F. Michel, J. Touchon and B. Dubois (2007). "[Effects of the association of sulbutiamine with an acetylcholinesterase inhibitor in early stage and moderate Alzheimer disease]." Encephale **33**(2): 211-215.

Oltmanns, K. M., H. Gehring, S. Rudolf, B. Schultes, S. Rook, U. Schweiger, J. Born, H. L. Fehm and A. Peters (2004). "Hypoxia causes glucose intolerance in humans." Am J Respir Crit Care Med **169**(11): 1231-1237.

Onal, H., S. Bariş, M. Ozdil, G. Yesil, G. Altun, I. Ozyilmaz and T. Celkan (2008). "Thiamine-responsive megaloblastic anemia: Early diagnosis may be effective in preventing deafness." The Turkish journal of pediatrics **51**: 301-304.

Onyango, I. G., J. Dennis and S. M. Khan (2016). "Mitochondrial Dysfunction in Alzheimer's Disease and the Rationale for Bioenergetics Based Therapies." Aging and disease **7**(2): 201-214.

Pan, X., Z. Chen, G. Fei, S. Pan, W. Bao, S. Ren, Y. Guan and C. Zhong (2016). "Long-Term Cognitive Improvement After Benfotiamine Administration in Patients with Alzheimer's Disease." Neurosci Bull **32**(6): 591-596.

Pan, X., G. Fei, J. Lu, L. Jin, S. Pan, Z. Chen, C. Wang, S. Sang, H. Liu, W. Hu, H. Zhang, H. Wang, Z. Wang, Q. Tan, Y. Qin, Q. Zhang, X. Xie, Y. Ji, D. Cui, X. Gu, J. Xu, Y. Yu and C. Zhong (2015). "Measurement of Blood Thiamine Metabolites for Alzheimer's Disease Diagnosis." EBioMedicine **3**: 155-162.

Pan, X., N. Gong, J. Zhao, Z. Yu, F. Gu, J. Chen, X. Sun, L. Zhao, M. Yu, Z. Xu, W. Dong, Y. Qin, G. Fei, C. Zhong and T.-L. Xu (2010). "Powerful beneficial effects of benfotiamine on

cognitive impairment and β-amyloid deposition in amyloid precursor protein/presenilin-1 transgenic mice." Brain **133**(5): 1342-1351.

Pan, X., S. Sang, G. Fei, L. Jin, H. Liu, Z. Wang, H. Wang and C. Zhong (2017). "Enhanced Activities of Blood Thiamine Diphosphatase and Monophosphatase in Alzheimer's Disease." PloS one **12**(1): e0167273-e0167273.

Pannunzio, P., A. S. Hazell, M. Pannunzio, K. V. Rao and R. F. Butterworth (2000). "Thiamine deficiency results in metabolic acidosis and energy failure in cerebellar granule cells: an in vitro model for the study of cell death mechanisms in Wernicke's encephalopathy." J Neurosci Res **62**(2): 286-292.

Pardanani, A., C. Harrison, J. E. Cortes, F. Cervantes, R. A. Mesa, D. Milligan, T. Masszi, E. Mishchenko, E. Jourdan, A. M. Vannucchi, M. W. Drummond, M. Jurgutis, K. Kuliczkowski, E. Gheorghita, F. Passamonti, F. Neumann, A. Patki, G. Gao and A. Tefferi (2015). "Safety and Efficacy of Fedratinib in Patients With Primary or Secondary Myelofibrosis: A Randomized Clinical Trial." JAMA Oncology **1**(5): 643-651.

Parker, W. D., C. M. Filley and J. K. Parks (1990). "Cytochrome oxidase deficiency in Alzheimer's disease." Neurology **40**(8): 1302-1302.

Paroni, G., P. Bisceglia and D. Seripa (2019). "Understanding the Amyloid Hypothesis in Alzheimer’s Disease." Journal of Alzheimer's Disease **68**: 493-510.

Pekny, M. and M. Nilsson (2005). "Astrocyte activation and reactive gliosis." Glia **50**(4): 427-434.

Pfeiffer, A., M. Jaeckel, J. Lewerenz, R. Noack, A. Pouya, T. Schacht, C. Hoffmann, J. Winter, S. Schweiger, M. K. E. Schäfer and A. Methner (2014). "Mitochondrial function and energy

metabolism in neuronal HT22 cells resistant to oxidative stress." British journal of pharmacology **171**(8): 2147-2158.

Piaceri, I., B. Nacmias and S. Sorbi (2013). "Genetics of familial and sporadic Alzheimer's disease." Front Biosci (Elite Ed) **5**: 167-177.

Pluta, R., M. Barcikowska, M. J. Mossakowski and I. Zelman (1998). "Cerebral accumulation of beta-amyloid following ischemic brain injury with long-term survival." Acta Neurochir Suppl **71**: 206-208.

Pluta, R., L. Ouyang, S. Januszewski, Y. Li and S. J. Czuczwar (2021). "Participation of Amyloid and Tau Protein in Post-Ischemic Neurodegeneration of the Hippocampus of a Nature Identical to Alzheimer's Disease." International journal of molecular sciences **22**(5): 2460.

Provenzano, F. A., J. Muraskin, G. Tosto, A. Narkhede, B. T. Wasserman, E. Y. Griffith, V. A. Guzman, I. B. Meier, M. E. Zimmerman, A. M. Brickman and I. Alzheimer's Disease Neuroimaging (2013). "White matter hyperintensities and cerebral amyloidosis: necessary and sufficient for clinical expression of Alzheimer disease?" JAMA neurology **70**(4): 455-461.

Pugazhenthi, S., L. Qin and P. H. Reddy (2017). "Common neurodegenerative pathways in obesity, diabetes, and Alzheimer's disease." Biochimica et biophysica acta. Molecular basis of disease **1863**(5): 1037-1045.

Pulsinelli, W. A., S. Waldman, D. Rawlinson and F. Plum (1982). "Moderate hyperglycemia augments ischemic brain damage: a neuropathologic study in the rat." Neurology **32**(11): 1239-1246.

Quinsay, M. N., R. L. Thomas, Y. Lee and Å. B. Gustafsson (2010). "Bnip3-mediated mitochondrial autophagy is independent of the mitochondrial permeability transition pore." Autophagy **6**(7): 855-862.

Quirós, P. M., M. A. Prado, N. Zamboni, D. D'Amico, R. W. Williams, D. Finley, S. P. Gygi and J. Auwerx (2017). "Multi-omics analysis identifies ATF4 as a key regulator of the mitochondrial stress response in mammals." J Cell Biol **216**(7): 2027-2045.

Raj, V., S. Ojha, F. C. Howarth, P. D. Belur and S. B. Subramanya (2018). "Therapeutic potential of benfotiamine and its molecular targets." Eur Rev Med Pharmacol Sci **22**(10): 3261-3273.

Ratan, R. R. (2019). "Does iron loading of oxygen-sensing prolyl hydroxylases rather than random Fenton-driven radical formation drive programmed ferroptosis and degeneration in neurological diseases?" Current Opinion in Physiology **7**: 60-65.

Raval, R. R., K. W. Lau, M. G. Tran, H. M. Sowter, S. J. Mandriota, J. L. Li, C. W. Pugh, P. H. Maxwell, A. L. Harris and P. J. Ratcliffe (2005). "Contrasting properties of hypoxia-inducible factor 1 (HIF-1) and HIF-2 in von Hippel-Lindau-associated renal cell carcinoma." Mol Cell Biol **25**(13): 5675-5686.

Ray, R., G. Chen, C. Vande Velde, J. Cizeau, J. H. Park, J. C. Reed, R. D. Gietz and A. H. Greenberg (2000). "BNIP3 heterodimerizes with Bcl-2/Bcl-X(L) and induces cell death independent of a Bcl-2 homology 3 (BH3) domain at both mitochondrial and nonmitochondrial sites." J Biol Chem **275**(2): 1439-1448.

Reger, M. A., G. S. Watson, P. S. Green, C. W. Wilkinson, L. D. Baker, B. Cholerton, M. A. Fishel, S. R. Plymate, J. C. S. Breitner, W. DeGroodt, P. Mehta and S. Craft (2008). "Intranasal insulin improves cognition and modulates β-amyloid in early AD." Neurology **70**(6): 440.

Reiman, E. M., R. J. Caselli, L. S. Yun, K. Chen, D. Bandy, S. Minoshima, S. N. Thibodeau and D. Osborne (1996). "Preclinical Evidence of Alzheimer's Disease in Persons Homozygous for the ε4 Allele for Apolipoprotein E." New England Journal of Medicine **334**(12): 752-758.

Resende, L. S., A. M. Ribeiro, D. Werner, J. M. Hall and L. M. Savage (2012). "Thiamine deficiency degrades the link between spatial behavior and hippocampal synapsin I and phosphorylated synapsin I protein levels." Behav Brain Res **232**(2): 421-425.

Rodrigue, K. M., K. M. Kennedy and D. C. Park (2009). "Beta-amyloid deposition and the aging brain." Neuropsychology review **19**(4): 436-450.

Rosenthal, J. M., S. A. Amiel, L. Yágüez, E. Bullmore, D. Hopkins, M. Evans, A. Pernet, H. Reid, V. Giampietro, C. M. Andrew, J. Suckling, A. Simmons and S. C. R. Williams (2001). "The Effect of Acute Hypoglycemia on Brain Function and Activation." Diabetes **50**(7): 1618.

Roses, A. D. (1997). "Apolipoprotein E, a gene with complex biological interactions in the aging brain." Neurobiol Dis **4**(3-4): 170-185.

Rzymski, T., M. Milani, L. Pike, F. Buffa, H. R. Mellor, L. Winchester, I. Pires, E. Hammond, I. Ragoussis and A. L. Harris (2010). "Regulation of autophagy by ATF4 in response to severe hypoxia." Oncogene **29**(31): 4424-4435.

Saad, M., Z. Brkanac and E. M. Wijsman (2015). "Family-based genome scan for age at onset of late-onset Alzheimer's disease in whole exome sequencing data." Genes Brain Behav **14**(8): 607-617.

Safavi, M., A. Hosseini-Sharifabad, Y. Seyed-Yousefi and M. Rabbani (2020). "Protective Effects of Citicoline and Benfotiamine Each Alone and in Combination on Streptozotocin-induced Memory Impairment in Mice." Clinical psychopharmacology and neuroscience : the official scientific journal of the Korean College of Neuropsychopharmacology **18**(1): 81-92.

Sandau, U. S. and R. J. Handa (2007). "Glucocorticoids exacerbate hypoxia-induced expression of the pro-apoptotic gene Bnip3 in the developing cortex." Neuroscience **144**(2): 482-494.

Sang, S., X. Pan, Z. Chen, F. Zeng, S. Pan, H. Liu, L. Jin, G. Fei, C. Wang, S. Ren, F. Jiao, W. Bao, W. Zhou, Y. Guan, Y. Zhang, H. Shi, Y. Wang, X. Yu, Y. Wang and C. Zhong (2018). "Thiamine diphosphate reduction strongly correlates with brain glucose hypometabolism in Alzheimer's disease, whereas amyloid deposition does not." Alzheimers Res Ther **10**(1): 26.

Sapin, E., C. Peyron, F. Roche, N. Gay, C. Carcenac, M. Savasta, P. Levy and M. Dematteis (2015). "Chronic Intermittent Hypoxia Induces Chronic Low-Grade Neuroinflammation in the Dorsal Hippocampus of Mice." Sleep **38**(10): 1537-1546.

Sarkar, S., S. Liachenko, M. G. Paule, J. Bowyer and J. P. Hanig (2016). "Brain endothelial dysfunction following pyrithiamine induced thiamine deficiency in the rat." NeuroToxicology **57**: 298-309.

Savage, L. M., J. M. Hall and L. S. Resende (2012). "Translational rodent models of Korsakoff syndrome reveal the critical neuroanatomical substrates of memory dysfunction and recovery." Neuropsychol Rev **22**(2): 195-209.

Schoonen, W., Y. Wanamarta, J. M. Moorsel, C. Jakobs and H. Joenje (1990). "Respiratory failure and stimulation of glycolysis in Chinese hamster ovary cells exposed to normobaric hyperoxia." The Journal of biological chemistry **265**: 1118-1124.

Schroeder, J. P. and M. G. Packard (2003). "Systemic or intra-amygdala injections of glucose facilitate memory consolidation for extinction of drug-induced conditioned reward." Eur J Neurosci **17**(7): 1482-1488.

Schroeder, J. P. and M. G. Packard (2004). "Facilitation of memory for extinction of drug-induced conditioned reward: role of amygdala and acetylcholine." Learn Mem **11**(5): 641-647.

Schubert, D., T. Soucek and B. Blouw (2009). "The induction of HIF-1 reduces astrocyte activation by amyloid beta peptide." The European journal of neuroscience **29**(7): 1323-1334.

Sechi, G. and A. Serra (2007). "Wernicke's encephalopathy: new clinical settings and recent advances in diagnosis and management." Lancet Neurol **6**(5): 442-455.

Semenza, G. (2002). "Signal transduction to hypoxia-inducible factor 1." Biochem Pharmacol **64**(5-6): 993-998.

Semenza, G. L. (2003). "Targeting HIF-1 for cancer therapy." Nat Rev Cancer **3**(10): 721-732.

Semenza, G. L. (2012). "Hypoxia-inducible factors in physiology and medicine." Cell **148**(3): 399-408.

Semenza, G. L., M. K. Nejfelt, S. M. Chi and S. E. Antonarakis (1991). "Hypoxia-inducible nuclear factors bind to an enhancer element located 3' to the human erythropoietin gene." Proc Natl Acad Sci U S A **88**(13): 5680-5684.

Sharma, A., R. Bist and P. Bubber (2013). "Thiamine deficiency induces oxidative stress in brain mitochondria of Mus musculus." Journal of Physiology and Biochemistry **69**(3): 539-546.

Shen, J. and R. J. Kelleher, 3rd (2007). "The presenilin hypothesis of Alzheimer's disease: evidence for a loss-of-function pathogenic mechanism." Proc Natl Acad Sci U S A **104**(2): 403-409.

Shin, D. H., J.-H. Kim, Y.-J. Jung, K.-E. Kim, J. M. Jeong, Y.-S. Chun and J.-W. Park (2007). "Preclinical evaluation of YC-1, a HIF inhibitor, for the prevention of tumor spreading." Cancer Letters **255**(1): 107-116.

Shively, S., A. I. Scher, D. P. Perl and R. Diaz-Arrastia (2012). "Dementia resulting from traumatic brain injury: what is the pathology?" Archives of neurology **69**(10): 1245-1251.

Smirnova, N. A., D. M. Hushpulian, R. E. Speer, I. N. Gaisina, R. R. Ratan and I. G. Gazaryan (2012). "Catalytic mechanism and substrate specificity of HIF prolyl hydroxylases." Biochemistry (Moscow) **77**(10): 1108-1119.

Soucek, T., R. Cumming, R. Dargusch, P. Maher and D. Schubert (2003). "The regulation of glucose metabolism by HIF-1 mediates a neuroprotective response to amyloid beta peptide." Neuron **39**(1): 43-56.

Speer, R. E., S. S. Karuppagounder, M. Basso, S. F. Sleiman, A. Kumar, D. Brand, N. Smirnova, I. Gazaryan, S. J. Khim and R. R. Ratan (2013). "Hypoxia-inducible factor prolyl hydroxylases as targets for neuroprotection by “antioxidant” metal chelators: From ferroptosis to stroke." Free Radical Biology and Medicine **62**: 26-36.

Steen, E., B. M. Terry, E. J. Rivera, J. L. Cannon, T. R. Neely, R. Tavares, X. J. Xu, J. R. Wands and S. M. de la Monte (2005). "Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease--is this type 3 diabetes?" J Alzheimers Dis **7**(1): 63-80.

Stobart, J. L. and C. M. Anderson (2013). "Multifunctional role of astrocytes as gatekeepers of neuronal energy supply." Frontiers in cellular neuroscience **7**: 38-38.

Stover, K. R., M. A. Campbell, C. M. Van Winssen and R. E. Brown (2015). "Early detection of cognitive deficits in the 3xTg-AD mouse model of Alzheimer's disease." Behav Brain Res **289**: 29-38.

Stroh, C., F. Meyer and T. Manger (2014). "Beriberi, a severe complication after metabolic surgery - review of the literature." Obesity facts **7**(4): 246-252.

Strowitzki, M. J., E. P. Cummins and C. T. Taylor (2019). "Protein Hydroxylation by Hypoxia-Inducible Factor (HIF) Hydroxylases: Unique or Ubiquitous?" Cells **8**(5): 384.

Sulistijo, E. S., T. M. Jaszewski and K. R. MacKenzie (2003). "Sequence-specific dimerization of the transmembrane domain of the "BH3-only" protein BNIP3 in membranes and detergent." J Biol Chem **278**(51): 51950-51956.

Sulistijo, E. S. and K. R. MacKenzie (2006). "Sequence dependence of BNIP3 transmembrane domain dimerization implicates side-chain hydrogen bonding and a tandem GxxxG motif in specific helix-helix interactions." J Mol Biol **364**(5): 974-990.

Sun, X., G. He, H. Qing, W. Zhou, F. Dobie, F. Cai, M. Staufenbiel, L. E. Huang and W. Song (2006). "Hypoxia facilitates Alzheimer's disease pathogenesis by up-regulating BACE1 gene expression." Proceedings of the National Academy of Sciences of the United States of America **103**(49): 18727-18732.

Sunderland, T., N. Mirza, K. T. Putnam, G. Linker, D. Bhupali, R. Durham, H. Soares, L. Kimmel, D. Friedman, J. Bergeson, G. Csako, J. A. Levy, J. J. Bartko and R. M. Cohen (2004). "Cerebrospinal fluid beta-amyloid1-42 and tau in control subjects at risk for Alzheimer's disease: the effect of APOE epsilon4 allele." Biol Psychiatry **56**(9): 670-676.

Sweeney, M. D., A. P. Sagare and B. V. Zlokovic (2018). "Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders." Nature Reviews Neurology **14**: 133+.

Sweet, R., A. Paul and J. Zastre (2010). "Hypoxia induced upregulation and function of the thiamine transporter, SLC19A3 in a breast cancer cell line." Cancer Biology & Therapy **10**(11): 1101-1111.

Sweet, R. L. and J. A. Zastre (2013). "HIF1-α-mediated gene expression induced by vitamin B1 deficiency." Int J Vitam Nutr Res **83**(3): 188-197.

Swerdlow, R. H. and S. M. Khan (2004). "A "mitochondrial cascade hypothesis" for sporadic Alzheimer's disease." Med Hypotheses **63**(1): 8-20.

Tabarki, B., A. Al-Hashem and M. Alfadhel (1993). Biotin-Thiamine-Responsive Basal Ganglia Disease. GeneReviews(®). M. P. Adam, H. H. Ardinger, R. A. Pagon et al. Seattle WA, © 1993-

2020, University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle.

Tallaksen, C. M., A. Sande, T. Bøhmer, H. Bell and J. Karlsen (1993). "Kinetics of thiamin and thiamin phosphate esters in human blood, plasma and urine after 50 mg intravenously or orally." Eur J Clin Pharmacol **44**(1): 73-78.

Tamagno, E., P. Bardini, A. Obbili, A. Vitali, R. Borghi, D. Zaccheo, M. A. Pronzato, O. Danni, M. A. Smith, G. Perry and M. Tabaton (2002). "Oxidative stress increases expression and activity of BACE in NT2 neurons." Neurobiol Dis **10**(3): 279-288.

Tapias, V., S. Jainuddin, M. Ahuja, C. Stack, C. Elipenahli, J. Vignisse, M. Gerges, N. Starkova, H. Xu, A. A. Starkov, L. Bettendorff, D. M. Hushpulian, N. A. Smirnova, I. G. Gazaryan, N. A. Kaidery, S. Wakade, N. Y. Calingasan, B. Thomas, G. E. Gibson, M. Dumont and M. F. Beal (2018). "Benfotiamine treatment activates the Nrf2/ARE pathway and is neuroprotective in a transgenic mouse model of tauopathy." Hum Mol Genet **27**(16): 2874-2892.

Todd, K. and R. F. Butterworth (1999). "Mechanisms of selective neuronal cell death due to thiamine deficiency." Ann N Y Acad Sci **893**: 404-411.

Todd, K. G. and R. F. Butterworth (1998). "Evaluation of the role of NMDA-mediated excitotoxicity in the selective neuronal loss in experimental Wernicke encephalopathy." Exp Neurol **149**(1): 130-138.

Todd, K. G. and R. F. Butterworth (1999). "Early microglial response in experimental thiamine deficiency: An immunohistochemical analysis." Glia **25**(2): 190-198.

Todd, K. G. and R. F. Butterworth (1999). "Mechanisms of Selective Neuronal Cell Death due to Thiamine Deficiency." Annals of the New York Academy of Sciences **893**(1): 404-411.

Todd, K. G., A. S. Hazell and R. F. Butterworth (1999). "Alcohol-thiamine interactions: an update on the pathogenesis of Wernicke encephalopathy." Addict Biol **4**(3): 261-272.

Toffanello, E. D., A. Coin, E. Perissinotto, S. Zambon, S. Sarti, N. Veronese, M. De Rui, F. Bolzetta, M. C. Corti, G. Crepaldi, E. Manzato and G. Sergi (2014). "Vitamin D deficiency predicts cognitive decline in older men and women: The Pro.V.A. Study." Neurology **83**(24): 2292-2298.

Toledo, R. A., Y. Qin, S. Srikantan, N. P. Morales, Q. Li, Y. Deng, S.-W. Kim, M. A. A. Pereira, S. P. A. Toledo, X. Su, R. C. T. Aguiar and P. L. M. Dahia (2013). "In vivo and in vitro oncogenic effects of HIF2A mutations in pheochromocytomas and paragangliomas." Endocrine-Related Cancer **20**(3): 349-359.

Tönnies, E. and E. Trushina (2017). "Oxidative Stress, Synaptic Dysfunction, and Alzheimer's Disease." Journal of Alzheimer's disease : JAD **57**(4): 1105-1121.

Tracy, K., B. C. Dibling, B. T. Spike, J. R. Knabb, P. Schumacker and K. F. Macleod (2007). "BNIP3 is an RB/E2F target gene required for hypoxia-induced autophagy." Mol Cell Biol **27**(17): 6229-6242.

Trebukhina, R. V., Y. M. Ostrovsky, G. N. Mikhaltsevich, M. G. Velichko and V. N. Tumanov (1983). "Transketolase, pyruvate and oxoglutarate dehydrogenase activities and [14C]thiamin turnover in tissues of mice fed thiamin-deficient diet." J Nutr **113**(7): 1285-1291.

Tretter, L. and V. Adam-Vizi (2005). "Alpha-ketoglutarate dehydrogenase: a target and generator of oxidative stress." Philos Trans R Soc Lond B Biol Sci **360**(1464): 2335-2345.

Trotti, D., N. C. Danbolt and A. Volterra (1998). "Glutamate transporters are oxidant-vulnerable: a molecular link between oxidative and excitotoxic neurodegeneration?" Trends Pharmacol Sci **19**(8): 328-334.

Ułamek-Kozioł, M., S. J. Czuczwar, J. Kocki, S. Januszewski, J. Bogucki, A. Bogucka-Kocka and R. Pluta (2019). "Dysregulation of Autophagy, Mitophagy, and Apoptosis Genes in the CA3 Region of the Hippocampus in the Ischemic Model of Alzheimer's Disease in the Rat." Journal of Alzheimer's Disease **72**: 1279-1286.

Van Hoecke, M., A. S. Prigent-Tessier, P. E. Garnier, N. M. Bertrand, R. Filomenko, A. Bettaieb, C. Marie and A. G. Beley (2007). "Evidence of HIF-1 functional binding activity to caspase-3 promoter after photothrombotic cerebral ischemia." Mol Cell Neurosci **34**(1): 40-47.

Vangeison, G., D. Carr, H. J. Federoff and D. A. Rempe (2008). "The good, the bad, and the cell type-specific roles of hypoxia inducible factor-1 alpha in neurons and astrocytes." The Journal of neuroscience : the official journal of the Society for Neuroscience **28**(8): 1988-1993.

Vannucci, R. C., J. Towfighi and S. J. Vannucci (1998). "Hypoxic Preconditioning and Hypoxic-Ischemic Brain Damage in the Immature Rat: Pathologic and Metabolic Correlates." Journal of Neurochemistry **71**(3): 1215-1220.

Varela-Nallar, L., M. Rojas-Abalos, A. C. Abbott, E. A. Moya, R. Iturriaga and N. C. Inestrosa (2014). "Chronic hypoxia induces the activation of the Wnt/beta-catenin signaling pathway and stimulates hippocampal neurogenesis in wild-type and APPswe-PS1DeltaE9 transgenic mice in vivo." Front Cell Neurosci **8**: 17.

Vasagiri, N. and V. K. Kutala (2014). "Structure, function, and epigenetic regulation of BNIP3: a pathophysiological relevance." Mol Biol Rep **41**(11): 7705-7714.

Vassar, R., D. M. Kovacs, R. Yan and P. C. Wong (2009). "The beta-secretase enzyme BACE in health and Alzheimer's disease: regulation, cell biology, function, and therapeutic potential." The Journal of neuroscience : the official journal of the Society for Neuroscience **29**(41): 12787-12794.

Velliquette, R. A., T. O'Connor and R. Vassar (2005). "Energy inhibition elevates beta-secretase levels and activity and is potentially amyloidogenic in APP transgenic mice: possible early events in Alzheimer's disease pathogenesis." The Journal of neuroscience : the official journal of the Society for Neuroscience **25**(47): 10874-10883.

Vemuganti, R., H. Kalluri, J.-H. Yi, K. K. Bowen and A. S. Hazell (2006). "Gene expression changes in thalamus and inferior colliculus associated with inflammation, cellular stress, metabolism and structural damage in thiamine deficiency." European Journal of Neuroscience **23**(5): 1172-1188.

Verkhratsky, A., M. Olabarria, H. N. Noristani, C.-Y. Yeh and J. J. Rodriguez (2010). "Astrocytes in Alzheimer's disease." Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics **7**(4): 399-412.

Vignisse, J., M. Sambon, A. Gorlova, D. Pavlov, N. Caron, B. Malgrange, E. Shevtsova, A. Svistunov, D. C. Anthony, N. Markova, N. Bazhenova, B. Coumans, B. Lakaye, P. Wins, T. Strekalova and L. Bettendorff (2017). "Thiamine and benfotiamine prevent stress-induced suppression of hippocampal neurogenesis in mice exposed to predation without affecting brain thiamine diphosphate levels." Molecular and Cellular Neuroscience **82**: 126-136.

Vora, B., E. A. E. Green, N. Khuri, F. Ballgren, M. Sirota and K. M. Giacomini (2020). "Drug-nutrient interactions: discovering prescription drug inhibitors of the thiamine transporter ThTR-2 (SLC19A3)." Am J Clin Nutr **111**(1): 110-121.

Wacker, B. K., J. L. Perfater and J. M. Gidday (2012). "Hypoxic preconditioning induces stroke tolerance in mice via a cascading HIF, sphingosine kinase, and CCL2 signaling pathway." Journal of neurochemistry **123**(6): 954-962.

Wall, A. M., A. E. Corcoran, K. D. O'Halloran and J. J. O'Connor (2014). "Effects of prolyl-hydroxylase inhibition and chronic intermittent hypoxia on synaptic transmission and plasticity in the rat CA1 and dentate gyrus." Neurobiol Dis **62**: 8-17.

Wang, C., G. Fei, X. Pan, S. Sang, L. Wang, C. Zhong and L. Jin (2018). "High thiamine diphosphate level as a protective factor for Alzheimer's disease." Neurological Research **40**(8): 658-665.

Wang, G. L. and G. L. Semenza (1993). "General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia." Proc Natl Acad Sci U S A **90**(9): 4304-4308.

Wang, J. J., Z. Hua, H. M. Fentress and C. K. Singleton (2000). "JNK1 is inactivated during thiamine deficiency-induced apoptosis in human neuroblastoma cells." J Nutr Biochem **11**(4): 208-215.

Warner, T. A., J. Q. Kang, J. A. Kennard and F. E. Harrison (2015). "Low brain ascorbic acid increases susceptibility to seizures in mouse models of decreased brain ascorbic acid transport and Alzheimer's disease." Epilepsy Res **110**: 20-25.

Watanabe, I. and S. Kanabe (1978). "Early edematous lesion of pyrithiamine induced acute thiamine deficient encephalopathy in the mouse." J Neuropathol Exp Neurol **37**(4): 401-413.

Watanabe, I., T. Tomita, K. S. Hung and Y. Iwasaki (1981). "Edematous necrosis in thiamine-deficient encephalopathy of the mouse." J Neuropathol Exp Neurol **40**(4): 454-471.

Weinreb, O., T. Amit, S. Mandel, L. Kupershmidt and M. B. Youdim (2010). "Neuroprotective multifunctional iron chelators: from redox-sensitive process to novel therapeutic opportunities." Antioxid Redox Signal **13**(6): 919-949.

Wen, Y., O. Onyewuchi, S. Yang, R. Liu and J. W. Simpkins (2004). "Increased beta-secretase activity and expression in rats following transient cerebral ischemia." Brain Res **1009**(1-2): 1-8.

Westermann, B. (2009). "Nitric Oxide Links Mitochondrial Fission to Alzheimer's Disease." Science Signaling **2**(69): pe29-pe29.

Whitfield, K. C., M. W. Bourassa, B. Adamolekun, G. Bergeron, L. Bettendorff, K. H. Brown, L. Cox, A. Fattal-Valevski, P. R. Fischer, E. L. Frank, L. Hiffler, L. M. Hlaing, M. E. Jefferds, H. Kapner, S. Kounnavong, M. P. S. Mousavi, D. E. Roth, M.-N. Tsaloglou, F. Wieringa and G. F. Combs, Jr. (2018). "Thiamine deficiency disorders: diagnosis, prevalence, and a roadmap for global control programs." Annals of the New York Academy of Sciences **1430**(1): 3-43.

Wick, A., W. Wick, J. Waltenberger, M. Weller, J. Dichgans and J. B. Schulz (2002). "Neuroprotection by hypoxic preconditioning requires sequential activation of vascular endothelial growth factor receptor and Akt." The Journal of neuroscience : the official journal of the Society for Neuroscience **22**(15): 6401-6407.

Winkler, E. A., Y. Nishida, A. P. Sagare, S. V. Rege, R. D. Bell, D. Perlmutter, J. D. Sengillo, S. Hillman, P. Kong, A. R. Nelson, J. S. Sullivan, Z. Zhao, H. J. Meiselman, R. B. Wendy, J. Soto, E. D. Abel, J. Makshanoff, E. Zuniga, D. C. De Vivo and B. V. Zlokovic (2015). "GLUT1 reductions exacerbate Alzheimer's disease vasculo-neuronal dysfunction and degeneration." Nature neuroscience **18**(4): 521-530.

Wiśniewski, H., R. D. Terry and A. Hirano (1970). "Neurofibrillary pathology." J Neuropathol Exp Neurol **29**(2): 163-176.

Wolters, F. J., H. I. Zonneveld, A. Hofman, A. v. d. Lugt, P. J. Koudstaal, M. W. Vernooij and M. A. Ikram (2017). "Cerebral Perfusion and the Risk of Dementia." Circulation **136**(8): 719-728.

Wu, H. and Q. Chen (2015). "Hypoxia activation of mitophagy and its role in disease pathogenesis." Antioxid Redox Signal **22**(12): 1032-1046.

Xie, X., Y. Gao, M. Zeng, Y. Wang, T. F. Wei, Y. B. Lu and W. P. Zhang (2019). "Nicotinamide ribose ameliorates cognitive impairment of aged and Alzheimer's disease model mice." Metab Brain Dis **34**(1): 353-366.

Xu, W. L., A. R. Atti, M. Gatz, N. L. Pedersen, B. Johansson and L. Fratiglioni (2011). "Midlife overweight and obesity increase late-life dementia risk: a population-based twin study." Neurology **76**(18): 1568-1574.

Xue, S., L. Jia and J. Jia (2006). "Hypoxia and reoxygenation increased BACE1 mRNA and protein levels in human neuroblastoma SH-SY5Y cells." Neuroscience letters **405**: 231-235.

Yan, J., B. Zhou, S. Taheri and H. Shi (2011). "Differential effects of HIF-1 inhibition by YC-1 on the overall outcome and blood-brain barrier damage in a rat model of ischemic stroke." PloS one **6**(11): e27798-e27798.

Yang, Z., J. Ge, W. Yin, H. Shen, H. Liu and Y. Guo (2004). "[The expression of p53, MDM2 and Ref1 gene in cultured retina neurons of SD rats treated with vitamin B1 and/or elevated pressure]." Yan ke xue bao (2016) **20**(4): 259-263.

Ye, J. and C. Koumenis (2009). "ATF4, an ER stress and hypoxia-inducible transcription factor and its potential role in hypoxia tolerance and tumorigenesis." Curr Mol Med **9**(4): 411-416.

Yeaman, S. J., E. T. Hutcheson, T. E. Roche, F. H. Pettit, J. R. Brown, L. J. Reed, D. C. Watson and G. H. Dixon (1978). "Sites of phosphorylation on pyruvate dehydrogenase from bovine kidney and heart." Biochemistry **17**(12): 2364-2370.

Yeh, W.-L., D.-Y. Lu, C.-J. Lin, H.-C. Liou and W.-M. Fu (2007). "Inhibition of Hypoxia-Induced Increase of Blood-Brain Barrier Permeability by YC-1 through the Antagonism of HIF-1α Accumulation and VEGF Expression." Molecular Pharmacology **72**(2): 440-449.

Yook, Y. H., K. H. Kang, O. Maeng, T. R. Kim, J. O. Lee, K. I. Kang, Y. S. Kim, S. G. Paik and H. Lee (2004). "Nitric oxide induces BNIP3 expression that causes cell death in macrophages." Biochem Biophys Res Commun **321**(2): 298-305.

Youdim, M. B. (2013). "Multi target neuroprotective and neurorestorative anti-Parkinson and anti-Alzheimer drugs ladostigil and m30 derived from rasagiline." Exp Neurobiol **22**(1): 1-10.

Young, B., L. Ott, R. Dempsey, D. Haack and P. Tibbs (1989). "Relationship between admission hyperglycemia and neurologic outcome of severely brain-injured patients." Annals of surgery **210**(4): 466-473.

Yu, Q. and C. Zhong (2018). "Membrane Aging as the Real Culprit of Alzheimer's Disease: Modification of a Hypothesis." Neuroscience Bulletin **34**(2): 369-381.

Yuan, L. B., H. L. Dong, H. P. Zhang, R. N. Zhao, G. Gong, X. M. Chen, L. N. Zhang and L. Xiong (2011). "Neuroprotective effect of orexin-A is mediated by an increase of hypoxia-inducible factor-1 activity in rat." Anesthesiology **114**(2): 340-354.

Zastre, J. A., B. S. Hanberry, R. L. Sweet, A. C. McGinnis, K. R. Venuti, M. G. Bartlett and R. Govindarajan (2013). "Up-regulation of vitamin B1 homeostasis genes in breast cancer." The Journal of Nutritional Biochemistry **24**(9): 1616-1624.

Zastre, J. A., R. L. Sweet, B. S. Hanberry and S. Ye (2013). "Linking vitamin B1 with cancer cell metabolism." Cancer & Metabolism **1**(1): 16.

Zera, K. and J. Zastre (2017). "Thiamine deficiency activates hypoxia inducible factor-1α to facilitate pro-apoptotic responses in mouse primary astrocytes." PloS one **12**(10): e0186707-e0186707.

Zera, K. and J. Zastre (2018). "Stabilization of the hypoxia-inducible transcription Factor-1 alpha (HIF-1alpha) in thiamine deficiency is mediated by pyruvate accumulation." Toxicol Appl Pharmacol **355**: 180-188.

Zera, K. and J. Zastre (2018). "Stabilization of the hypoxia-inducible transcription Factor-1 alpha (HIF-1α) in thiamine deficiency is mediated by pyruvate accumulation." Toxicol Appl Pharmacol **355**: 180-188.

Zhang, C. E., X. Yang, L. Li, X. Sui, Q. Tian, W. Wei, J. Wang and G. Liu (2014). "Hypoxia-induced tau phosphorylation and memory deficit in rats." Neurodegener Dis **14**(3): 107-116.

Zhang, F., L. Niu, S. Li and W. Le (2019). "Pathological Impacts of Chronic Hypoxia on Alzheimer's Disease." ACS Chemical Neuroscience **10**(2): 902-909.

Zhang, H., M. Bosch-Marce, L. A. Shimoda, Y. S. Tan, J. H. Baek, J. B. Wesley, F. J. Gonzalez and G. L. Semenza (2008). "Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia." J Biol Chem **283**(16): 10892-10903.

Zhang, H., P. Gao, R. Fukuda, G. Kumar, B. Krishnamachary, K. I. Zeller, C. V. Dang and G. L. Semenza (2007). "HIF-1 inhibits mitochondrial biogenesis and cellular respiration in VHL-deficient renal cell carcinoma by repression of C-MYC activity." Cancer Cell **11**(5): 407-420.

Zhang, Q., Y. Zhang, S. Diamond, J. Boer, J. J. Harris, Y. Li, M. Rupar, E. Behshad, C. Gardiner, P. Collier, P. Liu, T. Burn, R. Wynn, G. Hollis and S. Yeleswaram (2014). "The Janus Kinase 2 Inhibitor Fedratinib Inhibits Thiamine Uptake: A Putative Mechanism for the Onset of Wernicke's Encephalopathy." Drug Metabolism and Disposition **42**(10): 1656.

Zhang, S., Z. Zhang, G. Sandhu, X. Ma, X. Yang, J. Geiger and J. Kong (2007). "Evidence of oxidative stress-induced BNIP3 expression in amyloid beta neurotoxicity." Brain research **1138**: 221-230.

Zhang, S., Z. Zhang, G. Sandhu, X. Ma, X. Yang, J. D. Geiger and J. Kong (2007). "Evidence of oxidative stress-induced BNIP3 expression in amyloid beta neurotoxicity." Brain Res **1138**: 221-230.

Zhang, Z., X. Yang, S. Zhang, X. Ma and J. Kong (2007). "BNIP3 Upregulation and EndoG Translocation in Delayed Neuronal Death in Stroke and in Hypoxia." Stroke **38**(5): 1606-1613.

Zhang, Z., L. Yao, J. Yang, Z. Wang and G. Du (2018). "PI3K/Akt and HIF-1 signaling pathway in hypoxia-ischemia (Review)." Molecular medicine reports **18**(4): 3547-3554.

Zheng, X., B. Zhai, P. Koivunen, S. J. Shin, G. Lu, J. Liu, C. Geisen, A. A. Chakraborty, J. J. Moslehi, D. M. Smalley, X. Wei, X. Chen, Z. Chen, J. M. Beres, J. Zhang, J. L. Tsao, M. C. Brenner, Y. Zhang, C. Fan, R. A. DePinho, J. Paik, S. P. Gygi, W. G. Kaelin, Jr. and Q. Zhang (2014). "Prolyl hydroxylation by EglN2 destabilizes FOXO3a by blocking its interaction with the USP9x deubiquitinase." Genes Dev **28**(13): 1429-1444.