Milligan College

Ameliorating Effects of Virgin Coconut Oil Against Oxidative Stress

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499 Research Project

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INTRODUCTION

Mitochondria and free radical generation

The mitochondria is a vital organelle responsible for the majority of the cell's bioenergetic needs, as it produces energetic molecules of adenosine triphosphate (ATP) and facilitates catabolic and anabolic reactions.[1, 2] The mitochondria is composed of an outer and inner membrane, creating an intermembrane space between the inner and outer membranes and a matrix inside the inner membrane.[2, 3] The matrix contains many enzymes essential for biosynthetic and oxidative pathways as well as the mitochondrial DNA (mtDNA).[2, 3] The mtDNA is DNA unique to the mitochondria and is roughly 16 kilobases of circular DNA.[1] MtDNA encodes mitochondrial rRNAs, some proteins, and tRNA.[2, 3] Because the central nervous system (CNS) requires large amounts of ATP for innumerably frequent electrochemical messages, the CNS consumes 20% of oxygen in order to perform oxidative phosphorylation within mitochondria.[4] This high energy demand of the CNS is heavily dependent upon the efficient functioning of mitochondrial, thus neurons are highly sensitive to mitochondrial dysfunction.[4]

Via oxidative phosphorylation, reduced electron carriers (NADH) and ($FADH_2$) deposit electrons at complex 1 (NADH:ubiquinone oxidoreductase) and complex 2 (succinate dehydrogenase), respectively.[3] The electrons are transferred through the complexes via oxidative-reduction reactions, pumping protons across the inner membrane into the intermembrane space.[3] The accumulated protons generate a membrane potential within the intermembrane space of the mitochondria, and the ATP synthase enzyme uses the concentration gradient of the protons to phosphorylate adenosine diphosphate to ATP.[3, 5] The electrons are transferred through complex four and reduce molecular oxygen to water as the final product.[3]

Despite the efficiency of this respiratory pathway, mistakes when reducing molecular oxygen to water occur and produce cellular stress. Although the majority of molecular oxygen is properly reduced to water, about 1-2% of the molecular oxygen is only partially reduced, resulting in the superoxide free radical.[2, 6] This inappropriate reduction of molecular oxygen occurs most commonly at complex I and III (cytochrome bc1) during electron transfers through the chain of membrane proteins.[2, 6, 7] The favorable reduction of molecular oxygen within the ETC happens frequently due to the electron transporting molecules.[8] However, in normal conditions, this superoxide is converted by endogenous antioxidant defenses quickly to hydrogen peroxide, an oxidative molecule that does not contain a free radical.[9] The hydrogen peroxide is then either reduced to water or enters the Fenton reaction, a reaction that leads to the production of more free radicals, most notably hydroxyl radicals, a very potent reactive oxygen species (ROS).[3, 5, 10] The very powerful oxidant superoxide will actively seek interaction with other electrons, especially double bonds, disrupting proteins, lipids, and other vital biomolecules.[8]

Because the formation of ROS via oxidative phosphorylation occurs occasionally, endogenous antioxidant enzymes function to eliminate the oxidative stress induce by free ROS. Three main endogenous antioxidant proteins are superoxide dismutase, glutathione, and catalase.[11] Superoxide dismutase catalyzes the reaction of superoxide to molecular oxygen and hydrogen peroxide via the oxidation and reduction of its transition metal center.[11] Glutathione peroxidase is a small peptide with a necessary selenocysteine residue that functions to reduce hydrogen peroxide to water via the formation of disulfide bridges between two molecules of glutathione.[11] Catalase is an enzyme that catalyzes the reaction of hydrogen peroxide into water and molecular oxygen.[11] Hydrogen peroxide will either be reduced to water via glutathione or

catalase or enter the Fenton reaction, a reaction that leads to the production of more free radicals via metallic chemistry, most notably resulting in hydroxyl radicals.[10]

If the ROS levels overwhelm the cell, the oxidative stress initiates a degenerative pathway, beginning with the mitochondria and spreading to the complete death of the cell (**Figures 1a & b**).[5] This pathway with the activation of the mitochondrial permeability transition pore (mtPTP), an anionic channel that connects the inner and outer mitochondrial membranes.[5, 12] When this channel is opened and the permeability of the outer membrane is increased, the accumulated protons and other solutes within the very concentrated mitochondria diffuse into the cytosol, thus the mitochondria swells due to osmotic pressure.[5, 12] One key protein that leaves the mitochondria through the mtPTP is cytochrome c (cyt c), an electron transfer molecule that functions within oxidative phosphorylation.[5] Cyt c activates the caspase degenerative pathway that results in apoptosis.[5, 12, 13] Another key molecule that is implicated with apoptosis that is also harbored and released from the mitochondrial intermembrane space is apoptosis-inducing factor (AIF).[14] Upon its release into the cytosol, AIF will be transported to the nucleus where it will cause chromatin to condense and fracture; another independent function of AIF is its oxidative activity, thus AIF will decrease the levels of endogenous antioxidant molecules and generate ROS.[14]

Once cyt c enters the cytosol, it will bind apoptotic protease activating factor 1 (Apaf1) (forming the complex apoptosome), cleave procaspase 9 to activated caspase 9, and activate the caspase pathway of degeneration.[13, 15-17] After caspase 9 is activated, it will initiate the downstream activation of caspases 3, 7, 6, 2, 8, and 10, resulting in a cascade of death signals.[16] The most common pathway that cyt c utilizes to leave the mitochondria is via the mtPTP, but another apoptotic mechanism involves the mitochondrial apoptosis-induced channel (MAC), a

pore in the outer membrane of the mitochondria that opens independent of depolarization.[15] This pore has been identified to open in response to Bcl-2 associated X-protein (Bax), a proapoptotic, multidomain protein from the BCL-2 family.[15] The BCL-2 protein family includes three classes of apoptosis regulating proteins: the antiapoptotic (Bcl-2 and Bcl-xL), the multidomain, proapoptotic (Bax and Bcl-2 antagonist/killer-1 [Bak]), and the small, BH3 domain only proapoptotic (Bad and t-Bid).[15, 18] Bax and Bak are found in the cytosol in their inactive form as monomers and/or attached loosely to the mitochondrial membrane.[18] BH3-only proteins induce Bax/Bak to activate apoptosis, while Bcl-2 and Bcl-xL will inhibit this mechanism through binding to the BH3 death domain on Bax or Bak.[13, 15, 18] When uninhibited by the antiapoptotic proteins, Bax can cause the release of cyt c and second mitochondria-derived activator of caspase (Smac) through the MAC, leading to the activation of the caspase degeneration.[15, 18] Once the caspase cascade is activated, the caspases will cleave vital DNA-associated proteins, causing the characteristic apoptotic fragmentation of genomic DNA.[19] Caspase 3, in addition to activating caspases 2 and 6, will cleave and initiate caspase activated deoxyribosenuclease (CAD) that fragments the genomic DNA.[16, 20] Apoptotic morphological changes are carried out by the cleaving of lamin and fodrin by the caspase cascade.[20] Lamin is a necessary protein within the nuclear skeleton while fodrin of the cytosolic skeleton, thus the cleavage of these two proteins leads to decomposition of the nuclear membrane and formation of apoptotic bodies, respectively.[20] These apoptotic bodies are small, concentrated bundles of cytoplasm with or without genomic DNA fragments that are subsequently ingested by extracellular macrophages.[21] Even though apoptosis is due to cellular dysfunction, the overall process of apoptosis is contained within cytoplasmic vesicles that are quickly degraded by surrounding phagocytic cells.[21]

A known degenerative kinase c-Jun N-terminal kinase (JNK) has also been associated with the BCL-2 apoptotic protein regulation family.[22] JNK will phosphorylate the anchoring

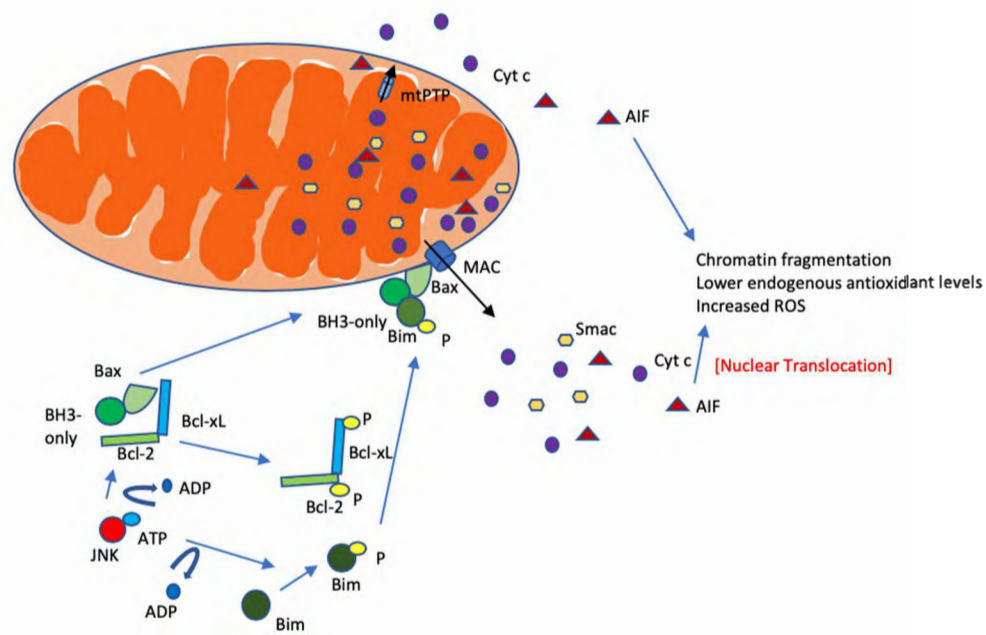


Figure 1a. The BCL-2 protein family and the degradation of the mitochondria. Bax and BH3-only protein are initially inhibited by anti-apoptotic proteins Bcl-2 and Bcl-xL. Upon JNK activation, Bcl-2 and Bcl-xL are phosphorylated and removed, and Bax moves to the mitochondrial membrane with phosphorylated Bim's assistance where the complex will associate with MAC. Activated MAC will release Smac, Cyt c, and AIF from the mitochondria, leading to nuclear degradation and caspase activation. MtPTP is activated via ROS accumulation and will release pro-apoptotic cyt c from the inner mitochondrial space. AIF is a protein factor that will initiate apoptotic nuclear changes including chromatin condensing and fracturing as well as increasing ROS production and decreasing endogenous antioxidant molecules.

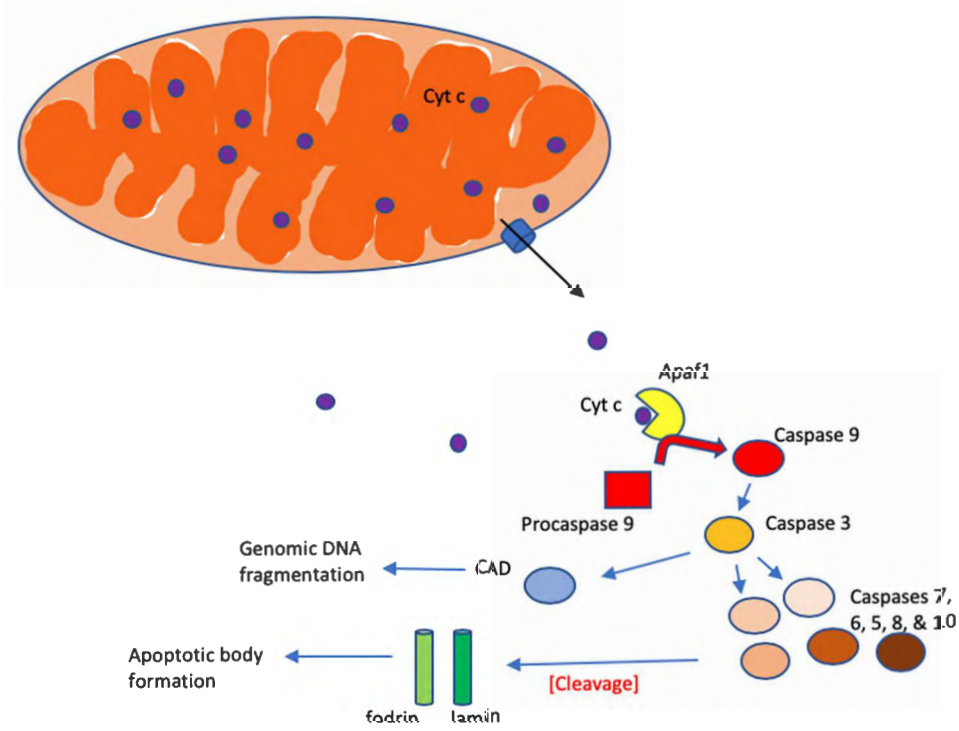


Figure 1b. The caspase cascade of apoptosis. Upon the release of cyt c through the ROS-activated mtPTP, cyt c will complex with Apaf1 to form the apoptosome which will subsequently convert procaspase 9 to caspase 9 and begin the activation of the following caspases (3, 7, 6, 5, 8, and 10). Caspase 3 will activate CAD to fragment DNA, and the protease activity of the caspases will cleave fodrin and lamin, microtubule proteins of the cytoskeleton and nuclear skeleton respectively. The degraded cytoskeleton will lead to apoptotic body formation in order to be processed and digested by extracellular macrophages.

proteins that sequester Bax and Bak to allow their translocation from the cytosol to the mitochondrial, in turn inducing the MAC to release cyt c.[22] However, JNK will directly phosphorylate Bcl-2-interacting mediator of cell death (Bim), another proapoptotic protein from the BCL-2 family rather than its sequestering proteins.[18, 22] In addition to activating these proapoptotic proteins, JNK will inhibit the function of antiapoptotic proteins Bcl-2 and Bcl-xL.[22]

When oxidative stress overwhelms the endogenous antioxidant defense systems, the ROS begin to accumulate within the cell and result in cellular dysfunction.[23] These free radical producing mistakes release free radicals that begin to alter the native structure of proteins and

DNA via the oxidation of carbonyl derivatives, leading to misfunctioning proteins and mutated DNA.[23] Mitochondrial DNA is especially suspect to oxidative damage due to the nearness to the source of the ROS, lack of histone proteins, and the primitive repair mechanisms of the mitochondria.[23-25] One study compared the oxidative stress induced mutations and damage to mitochondrial and nuclear DNA. The marker was significantly higher for the mitochondrial DNA than the nuclear DNA.[25] These levels of oxidative stress on the mitochondrial DNA were also shown to increase with age, with marker levels being significantly higher in elderly individuals as compared to younger individuals.[6] As more mitochondria are damaged, more ROS are released, creating a continuing cycle of producing damaging free radicals that are further mutating the mitochondrial DNA.[23] Hydroxyl free radicals are highly reactive to DNA nucleotides via the removal of an hydrogen atom.[26] The molecule 8-hydroguanine is a commonly oxidized form of damaged DNA that initiates the conversion of guanine to thymine and adenine to cytosine mutations.[26]

Not only does oxidative stress damage mitochondrial DNA but also oxidizes the lipids of the mitochondrial inner membrane. Lipid peroxidation causes the membrane to lose certain physiological characteristics require for normal mitochondrial activity and reduces the amount of bioenergy produced.[6] One particular example of this is exhibited in the oxidation of cardiolipin, a lipid necessary for proper cyt c functioning.[6] When cardiolipin is oxidized by a ROS, the cyt c molecule is not able to properly bind to the inner mitochondrial membrane, leading to apoptosis of the cell.[6] The mechanism by which lipids peroxidize follows three steps: initiation, propagation, and termination.[26] Initiation is characterized by the removal of hydrogens from a carbon of the fatty acid chain, which rearranges to a double bond with a radical.[26] An oxygen molecule is added, and the propagation step progresses when the oxygen removes a hydrogen

from a nearby lipid to continue the process.[26] Termination occurs when two of the lipid radicals react to result in paired electrons.[26] However, prior to termination, damage occurs when the lipid radicals break into reactive aldehydes that destabilize membranes, especially the mitochondria.[26] This cascade produces subsequent reactive species to continue this cycle of peroxidation and damage to unsaturated fatty acids within membranes.[27]

Because cellular respiration via mitochondria is vital to human life, the production of ROS are a normal result of the aging process.[23] The free radical theory of aging postulates that the accumulation of oxidative stress is a result of normal mitochondrial activity, leading to cellular damage associated with the elderly.[1, 23] When this theory was founded in the 1950's, the short life span of highly metabolic animals was believed to be the result of increased levels of ROS; however, this theory was rejected when some animals with high metabolic rates had much lower ROS levels than expected.[23] Individuals with mitochondrial diseases exhibit premature aging and symptoms that increase severity over time—another indicator that mitochondrial oxidative stress is associated with the aging process.[6] The accumulation of mitochondrial mutations associated with aging has also been implicated with increased apoptotic levels, thus the mitochondrial DNA damage is connected with further cellular death as a result of the formation of more ROS with age.[1, 25] However, this accumulation of ROS over time may lead to the greater risk for degenerative diseases associated with the elderly population.[5, 9]

The endoplasmic reticulum and oxidative stress

The endoplasmic reticulum (ER) is an organelle found in eukaryotic cells that is responsible for modifying proteins post-translationally and assisting in the folding process.[28] Additionally, the ER has a variety of other roles including redox and calcium ion homeostasis within the cytoplasm.[28] Because the ER is implicated in a large variety of cellular processes,

many intracellular signals are integrated here and initiate appropriate downstream signals in response.[29, 30] The ER closely monitors the protein folding process, and when proteins are misfolded, these aggregate and stimulate ER stress.[28-30] The process of protein folding is vital as misfolded proteins have many downstream consequences, including loss of normal function, formation of aggregates, preoccupation of normal protease activity.[31] ER stress leads to the unfolded protein response (UPR) which tries to repair the unfolded proteins but when dysfunction is too widely spread, UPR will activate the apoptosis pathway, leading to cellular programmed death (**Figure 3a & 3b**).[28, 30] UPR is initiated by the accumulation of misfolded proteins within the ER lumen and begins to activate specific transcription factors to tolerate the folding stress in order to allow more time to correct the misfolded proteins.[30-32] PKR-like ER kinase (PERK) and inositol-requiring protein-1 (IRE1) are kinases that are activated by the presence of misfolded proteins, and these kinases will activate downstream cascades resulting in the expression of more ER chaperones as well as transcription factors either reducing the ER stress or beginning degeneration.[31, 33, 34] It is the activation of PERK, IRE1, and activating transcriptional factor (ATF6) that results in the UPR.[31, 34]

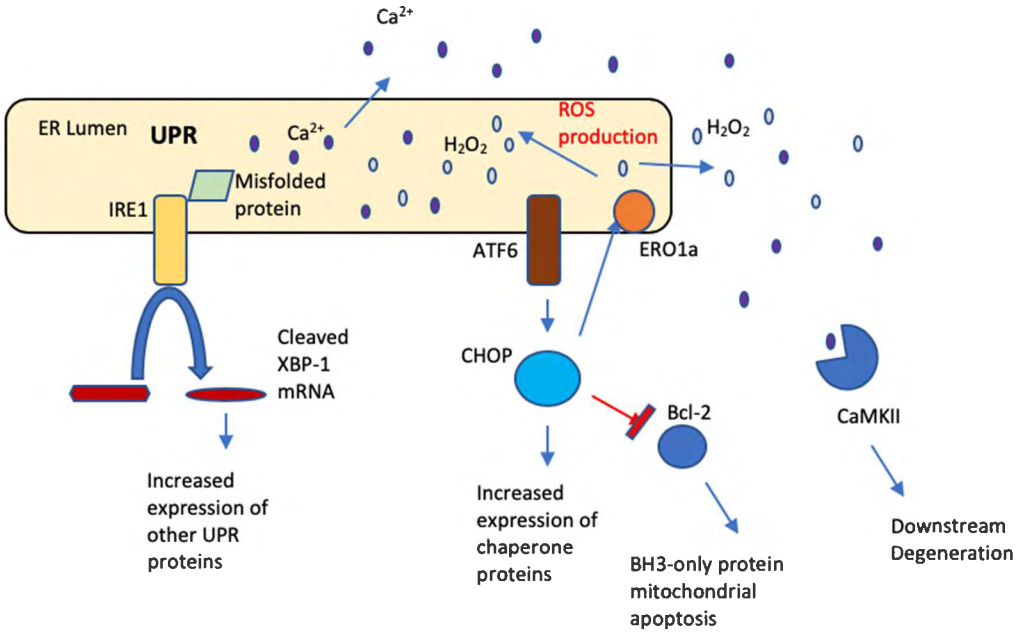


Figure 3a. The UPR cascade via ER transmembrane proteins IRE1 and ATF6. Upon the UPR within the ER lumen, misfolded proteins will bind to the lumen portion of the IRE1 protein, causing the cleavage of XBP-1 mRNA in order to increase the expression of other UPR proteins to resolve the UPR. When ATF6 is activated, CHOP will be activated to initiate the increased expression of chaperone proteins, inhibit anti-apoptotic Bcl-2, and cause ERO1a to increase the oxidative environment of the ER lumen. The excess oxidative stress of the lumen results in the seeping out of Ca^{2+} ions and H_2O_2 into the cytosol. CaMKII will bind the cytosolic Ca^{2+} ions to activate further downstream degenerative cascades.

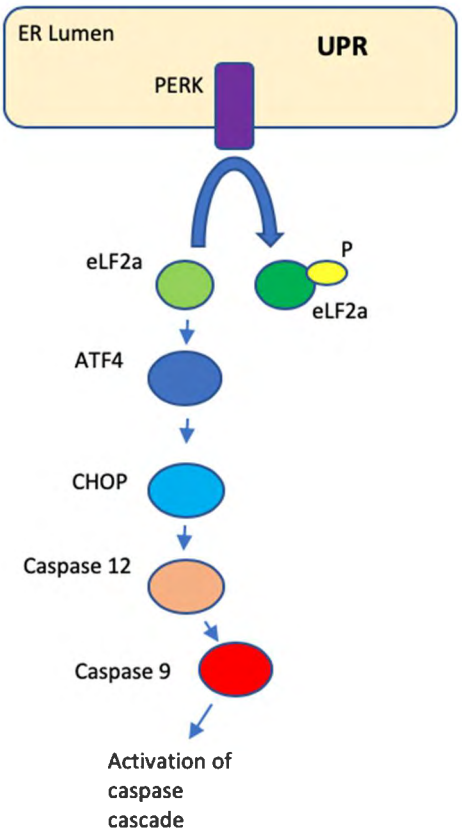


Figure 3b. PERK associated UPR that results in caspase activation. The UPR in the ER lumen will cause PERK to phosphorylate eLF2a. The decrease of unphosphorylated eLF2a leads to the activation of ATF4 and CHOP sequentially. CHOP will activate caspase 12, an ER specific caspase, that will activate the degenerative caspase cascade starting with caspase 9 and continuing through the previously mentioned sequence.

PERK is associated with the activation of the degenerative effects of the UPR as it initiates downstream expression of pro-degenerative transcription factors.[31, 34, 35] PERK will phosphorylate the alpha subunit of eukaryotic translation initiation factor 2 (eLF2a), but it is the decrease of the unphosphorylated eLF2 that initiates the activation of the C/EBP homologous protein (CHOP) promotor sequence via the upregulation of activating transcription factor 4 (ATF4).[34, 35] CHOP is associated with the ER stress induced apoptosis as it activates caspase 12,

which is specific to ER stress.[17, 31] Caspase 12 will then initiate caspase 9 and its associated downstream cascade via a different mechanism than the mitochondrial apoptotic pathway.[17]

The most conserved and oldest pathway of the UPR is the activation of IRE1. IRE1 is activated when misfolded proteins bind directly to IRE1 and when a protein chaperone dissociates due to the ER stress.[31, 34] Once activated, IRE1 cleaves an mRNA that encodes XBP-1, and the sliced form of XBP-1 causes the expression of other proteins involved in the UPR.[31] The third pathway uses ATF6, a factor that can also upregulate CHOP, to increase the expression of protein chaperones to combat the ER stress and initiate the UPR.[31, 34]

The upregulation of CHOP induces apoptosis by lowering the levels of a pro-survival protein Bcl-2, which in turn upregulates the levels of BH3-only proteins, a group of proteins involved in mitochondrial induced apoptosis.[34] CHOP is also responsible for the subsequent oxidation of the ER lumen which causes hydrogen peroxide to diffuse into the cytosol.[34] CHOP will induce ER oxidase 1a (ERO1a) to produce an over-oxidative environment.[34] CHOP will also affect the ER calcium levels, and as the calcium levels with the cytosol are increased, CaMKII, a calcium monitoring and apoptotic protein, is activated to initiate downstream degeneration.[34]

During ER stress, the thiol groups of cysteine residues are oxidized and reduced on unfolded proteins in an unregulated manner which leads to the formation of ROS and oxidative stress.[28] Similar to mitochondrial oxidative stress, the oxidative stress from misfolded proteins leads to more unfolded proteins, and the signal is again continued until apoptosis.[28] Under normal conditions, the protein disulfide isomerase enzymes (PDI) and ER oxidoreductin I (ERO1) are responsible for chaperoning the formation of disulfide bonds to stabilize tertiary and quaternary structures of proteins will produce ROS.[28, 29, 33] These two proteins function together to oxidize the cysteine residues to form disulfide bonds.[29] When the thiol groups are oxidized by

PDIs, ERO1 will then transfer the electrons on reduced PDI to molecular oxygen, forming hydrogen peroxide, the main ROS from the ER.[28, 29] Although the cell has endogenous antioxidant pathways that function to remove these ROS, the accumulation of unfolded proteins and the associated oxidative stress can easily overwhelm the antioxidant molecules, thus the ER is biased to oxidative stress.[28, 29] The ER's ratios of reduced glutathione to oxidized glutathione is significantly lower within the ER than compared to the cytosol due to the highly oxidizing environment of the ER lumen, a necessity for the oxidization of thiol groups on protein residues.[31] In addition to the endogenous antioxidant molecules like glutathione, the ER has adapted antioxidant pathways to reduce the effects of stress by increasing the cellular levels of reduced glutathione via UPR.[29] This increase of reduced glutathione within the ER during the UPR is at the expense of the cytosolic concentration, however, and reduces the intracellular concentration of reduced glutathione.[31]

When antioxidant stimulating pathways of the ER are unable to halt ER stress, inflammatory and degenerative pathways are initiated.[29] One pathway results in the activation of nuclear factor-κB, production of inflammatory cytokines, and activation of the JNK pathway.[29] Overall, the activation of these molecules result in increased inflammation and oxidative stress, leading to cellular dysfunction and apoptosis.[29] Inflammation initiated from different sources such as excess neurotransmitters or metabolic molecules will also disturb normal ER function, eliciting ER stress.[29]

In addition to regulating the proper folding of proteins, the ER is also responsible for calcium homeostasis by actively transporting calcium into the ER to create a 1,000 fold more concentrated calcium environment within the ER in comparison to the cytosol.[29] Calcium is also vital for chaperone function in properly folding proteins.[31] During ER stress, the increasing

levels of misfolded proteins within the ER lumen results in the leaking of calcium into the cytosol.[29] The mitochondria serves as a calcium buffer for the cell's intracellular calcium ion levels, thus the concentration of calcium within the mitochondria is typically strictly regulated.[36] Thus, the mitochondria is quick to absorb the excess cytosolic calcium from the stressed ER.[36] The calcium concentration within the cytosol is concentrated in the mitochondrial matrix, causing the mitochondrial membrane to depolarize.[29] The depolarization in turn will be combined with the increased levels of ROS, and both events will signal the mtPTP to open, causing mitochondrial swelling.[36] This cycle is amplified when mitochondrial ROS cause the ER to release more calcium and more proteins are misfolded. Each of these events activates the kinase cascades of degeneration.[29] In situations where ER stress is chronic and irreversible, inflammatory and cell death pathways are initiated in connection with mitochondrial distress.[37]

Metal-induced oxidative stress

Oxidative stress has both endogenous and exogenous sources, and many metals have been implicated as one of these exogenous sources in the formation of ROS within cells. Although many transition metals are essential for proper cellular function, their interaction with inappropriate binding sites can lead to dysfunction and toxicity.[38] Metals can be classified as redox active or inactive depending on their electron configuration, but both categories are involved in the production of free radicals.[39] For redox active metals, oxidative stress is facilitated by the Fenton reaction, resulting in the production of hydroxyl radicals.[39] However, the redox-inactive metals such as lead, mercury, and cadmium contribute to cellular dysfunction in less clear mechanisms.[39] The electron sharing abilities of heavy metals such as mercury, cadmium, and lead disrupt normal sulfhydryl bonds of antioxidant molecules and protein structure.[38] Glutathione is a protective molecule against ROS, but the covalent bonds available

through the sulfhydryl groups are attacked by ROS and redox inactive metals alike.[39] Therefore, the finite reserves of glutathione are reduced further in the presence of these heavy metal toxins, and oxidative stress increases.[38, 39]

Lead, one of the toxic, redox-inactive metals, interferes with sulfhydryl bonding in not only glutathione, but also in other antioxidant molecules, including superoxide dismutase and catalase.[38, 39] When these antioxidant molecules are pre-occupied with metal binding, ROS accumulate and lead to organellar stress and cell death.[39] One specific mechanism by which lead leads to the formation of ROS is through the accumulation of aminolaevulinic acid (ALA) as even low levels of lead will inhibit ALA dehydratase, an enzyme that converts ALA into a heme intermediate.[38] The tautomerization of ALA allows it to donate an electron to molecular oxygen, in turn generating superoxide and hydrogen peroxide.[38] As the levels of the natural antioxidant molecules decrease, lead causes an increase in lipid peroxidation, especially in the brain.[38] The lead induced ALA accumulation interferes with gamma-aminobutyric acid (GABA) neurotransmitter function, leading to the neurological impairments observed in lead poisoning.[38] The increase in ROS within the brain is also associated with increased intracellular calcium levels, leading to mitochondrial depolarization and inducing apoptosis as the mitochondria releases cyt c.[38]

Cadmium is unable to form ROS like the other heavy metals, but instead generates ROS through indirect mechanism.[2, 9, 38] Due to the ability of cadmium to be in the +2 oxidative state (Cd^{2+}), it readily replaces bound iron and copper from proteins such as ferritin, thus increasing the concentration of free iron and copper.[38] These elevated levels of free iron and copper undergo Fenton reactions, generating more ROS.[2, 9, 38] Cadmium is directly associated with lipid peroxidation of the mitochondrial membrane, but the mechanism is largely unknown.[39] It is

expected that the preoccupation of antioxidant molecules with cadmium allows ROS to accumulate and attack the double bonds within the membrane fatty acids, increasing the production of ROS from the mitochondria.[39] Cadmium is also especially mutagenic as it inhibits repair processes of DNA, causing an accumulation of mutations.[38]

Mercury interferes directly with oxidative phosphorylation as it functions as an uncoupling agent for complex IV.[39] Mercury will increase the rate of respiration while decreasing its efficiency, leading to increased production of superoxide and hydrogen peroxide.[39] Mercury is also implicated in lipid peroxidation similarly to cadmium; however, the effects of mercury toxicity are mitigated by selenium and vitamin E, both known antioxidants.[39] Similar to the other heavy metals, mercury decreases the levels of available antioxidant molecules, leading to accumulation of ROS as thiol groups on these proteins are preoccupied by the metal.[38]

Although iron holds vital biochemical functions within the body, increased levels of free iron is implicated in DNA and lipid damage, resulting in the production of cancer cells.[2] As iron is an essential mineral, the body has an endogenous method of storing the metal: the protein ferritin.[40] Ferritin holds about 4,000 units of iron(III), and must be reduced to iron(II) to be released from this iron core of the protein.[40] As superoxide enters the iron core of the ferritin molecule, the ROS will react with iron(III) to form iron(II) as well as hydroxyl radicals, continuing a cycle of ROS generation.[40] This reaction, as previously mentioned, is called the Fenton reaction.[40] The Fenton reaction generates many hydroxyl radicals through its cycles of reduction and oxidation of iron and copper (another divalent metal ion).[41] In addition, if an iron atom is associated with a membrane lipid, the superoxide and hydrogen peroxide molecules will target and peroxidize the lipid, leading to the product of more reactive lipid derivatives and a decrease in membrane fluidity and function.[40, 41]

Copper is a redox-active metal that normally functions as a metal center within antioxidant molecules such as superoxide dismutase; however, high copper levels induce tumor formation. Much like iron, copper can also peroxidize lipids of the membrane.[2] Copper is usually associated with DNA strands, especially with guanine, and the interaction between hydrogen peroxide and copper will cause DNA oxidative damage, such as breaking of DNA strands and disruption of bases.[42] Copper(I) is typically an intermediate along with hydrogen peroxide in the cycle of redox reactions resulting in DNA damage while copper(II) is associated with the specific breaking of the DNA strands.[42] Copper will oxidize hydroquinone (an electron transporting molecule) to form a ROS that forms DNA single and double strand breaks, resulting in carcinogenic effects.[42] Because copper is a divalent metal, it also holds the potential to undergo the Fenton reaction to generate a chain reaction of ROS after being activated by initial oxidative stress.[40]

Aluminum is a well-documented neurotoxin that is also one of the most abundant metals on earth, thus this metal is particularly relative within the discussion of oxidative stress-inducing components.[43] Although aluminum cannot alter its valence electrons as transition metals can, the metal does have an intrinsic ability to generate ROS through its interaction with iron.[44] Aluminum's presence within the cell will enact the following: altering the stability of DNA molecules, impairing DNA repair mechanisms, disrupting endogenous antioxidant activity, increasing the production of ROS, interfering with mitochondrial function, and inducing apoptosis through the JNK pathway.[43] Mechanistically, aluminum induces excess formation of ROS by causing the cell to sense a nonexistent iron storage, thereby causing the cell to concentrate an excess amount of iron atoms, leading to the activation of the ROS-producing Fenton reaction with iron and hydrogen peroxide.[43, 44] Aluminum also interferes with the activity

of the ETC, leading to another influx of superoxide, which is then converted to hydrogen peroxide to interact with the intracellular iron atoms.[43] Aluminum will displace the iron atom from its physiological bond state within the ETC molecules, allowing the freed iron atoms to participate in the Fenton reaction.[44] Even though aluminum lacks intrinsic capabilities to produce ROS, it will cause an accumulation of transition metals within the cell, allowing the Fenton reaction to occur at a high rate.[44]

As the brain ages, redox active metals (such as copper and iron) build up at the blood brain barrier (BBB), increasing susceptibility for oxidative stress via the Fenton reaction.[27] Individuals with genetic mutations that results in accumulations of metals must also be aware of the increased likelihood of developing a neurodegenerative disorder as a result of the BBB's affinity for metals and the oxidative activity of said metals.[27]

Alzheimer's disease and dysfunctional mitochondria

Alzheimer's disease (AD) is a neurodegenerative disease that is biochemically characterized by aggregates of amyloid beta (Aβ) plaques, tau neurofibrillary tangles, abnormal mitochondrial structure, and increased oxidative stress.[45] The cognitive decline of AD patients is strongly correlated with synaptic loss, which in turn is associated with the accumulation of Aβ.[46] Although many forms of AD exist, the types can be simplified into familial, early onset and sporadic, late onset.[45] Familial AD occurs when a mutated gene for the enzyme for that processes Aβ precursor protein (APP) is inherited.[45] The mechanisms for the sporadic cases of AD are less characterized, possibly occurring due to accumulation of chance mutations or environmental factors.[45]

Aβ arises within neurons via two mechanisms: amyloidogenic or non-amyloidogenic pathways.[46] In the amyloidogenic pathway, APP is cleaved into Aβ via β-secretase and γ-

secretase.[46] Early onset AD features genetic mutations that will activate the amyloidogenic secretase enzymes; on the other hand, late onset AD will activate β-secretase via age-dependent high ROS levels.[46] The non-amyloidogenic pathway is characterized by the cleavage of APP by α-secretase within the Aβ domain thus preventing the formation of Aβ.[46] This pathway is nonpathological.[46] In early AD prognosis, Aβ accumulates intracellularly, interfering with normal cellular function.[46] Upon the oligomerization of Aβ within the synaptic terminal, Aβ will be released from the synapse to accumulate extracellularly within the synapse.[46]

The mitochondrial theory of aging connects with the hypothesized pathogenesis of AD as mitochondrial oxidative stress is observed prior to the formation of Aβ aggregates, supporting the correlation of the age-dependent increase of Aβ in the CNS of AD patients.[46, 47] One hypothesis for the structural abnormalities in AD mitochondria is due to an increase in neurons deficient in cytochrome c oxidase (COX), the fourth ETC complex responsible for the reduction of O_2 to H_2O.[45] These deficiencies in COX possibly arise due to age related accumulation of mtDNA mutations within the sequence that codes for COX.[47] Structural abnormalities of the mitochondria include increased size, decreased quantity, broken cristae, and disrupted mitochondrial dynamics of fusion and fission.[48] The mitochondria without COX exhibits defects in respiration via oxidative phosphorylation.[45] As discussed in previous sections, issues with the ETC results in the production of ROS due to improper functioning of the redox electron carriers.[45] The accumulation of ROS results in damage to mitochondrial membrane molecules, essential proteins, and mitochondrial DNA.[45] The mutations within the mtDNA then continues the cycle of ROS production as mtDNA mutations will inhibit proper functioning of the ETC, causing electron transporters to be over-reduced, producing more ROS.[45] Ultimately, the high

levels of oxidative stress will activate the opening of the mtPTP, release cyt c, and initiate apoptosis of neurons.[45]

The Aβ plaques also contribute to this pathway of cell death and high oxidative stress production (**Figure 4**).[45] In normal levels, Aβ is present within neuronal synapses' mitochondria as an endogenous antioxidant defense system, but when increased oxidative stress results in higher levels of Aβ, Aβ will aggregate in synapses and mitochondria to become pro-oxidant toxins.[45, 49] As a soluble monomer in low levels, Aβ may perform a beneficial task within the mitochondria, but the toxic oligomeric form of the Aβ aggregates is damaging to mitochondria.[49] Aβ will also enter the mitochondria in order to interact with mitochondrial alcohol dehydrogenase, increasing the production of ROS, and again instigating the apoptotic pathway within neurons.[45] Aβ will also inhibit COX when within the mitochondria, further inhibiting the function of oxidative phosphorylation.[49] In late onset, the presence of the ApoE ε4 allele increases oxidative stress which then causes the spread of mtDNA mutations, resulting in more oxidative stress.[45, 46] Specifically, the ApoE ε4 allele is associated with Aβ degradation due to an increase in Aβ transportation via lysosomes, thus supporting the pathological effect of Aβ on synaptic mitochondria.[48] The mutation in the process of cleaving APP into Aβ results in an increase in the concentration of Aβ, leading to its accumulation and apoptosis.[45] The ROS that accumulate from Aβ and age will also activate β-secretase to secrete more Aβ, further perpetuating the decline of ATP production and ETC function within neuronal mitochondria.[46] In sporadic AD, one possible hypothesis is that chance somatic mtDNA mutations occur due to high ROS levels, leading to the overexpression and aggregation of Aβ which enter the mitochondria and initiate apoptosis.[45]

Other important proteins that impact mitochondrial function of AD patients are Presenilin 1 and Presenilin 2 (PS1 and PS2). These proteins have β- and γ-secretase function, thus work to cleave APP into the toxic Aβ peptides. Because these are mitochondrial proteins, they will interact with other mitochondrial proteins as well, including Bcl-2 and other apoptotic proteins to induce apoptosis. These complex proteins are also associated with mitochondrial-associated membranes (MAM's) that connect the ER with the mitochondria to function with calcium ion and lipid allocation. When the PS1 and PS2 proteins are pathological, calcium ions levels will influx within the mitochondria, sparking the apoptotic pathway via increased ROS and activated mtPTP.[49]

Damaged mitochondria containing Aβ aggregates are trafficked from the soma of the neuron to the synapse.[46] Mitochondrial trafficking is a normal physiological process to spread the production of ATP along the entire neuron.[46] However, the trafficking of degraded mitochondria to the synapse delivers low production of necessary ATP for proper synaptic function, thus leading to the degradation of the synapses.[46] In addition to mitochondrial trafficking, APP is also transported from the soma to the synapse where it is cleaved by β-secretase into monomeric Aβ.[46] Initially the Aβ is intracellularly contained, but the increased production of Aβ will complex with other Aβ, resulting in oligomeric Aβ that can enter mitochondria and leave the synapse.[46] The presence of Aβ within the mitochondria will then inhibit oxidative phosphorylation, produce ROS, lower ATP production, and initiate cell death via apoptosis.[46] Aβ oligomers also have the capacity to create an increase in ion channels, thus increasing the levels of Ca^{2+} and lead to mitochondrial dysfunction and eventual cell death.

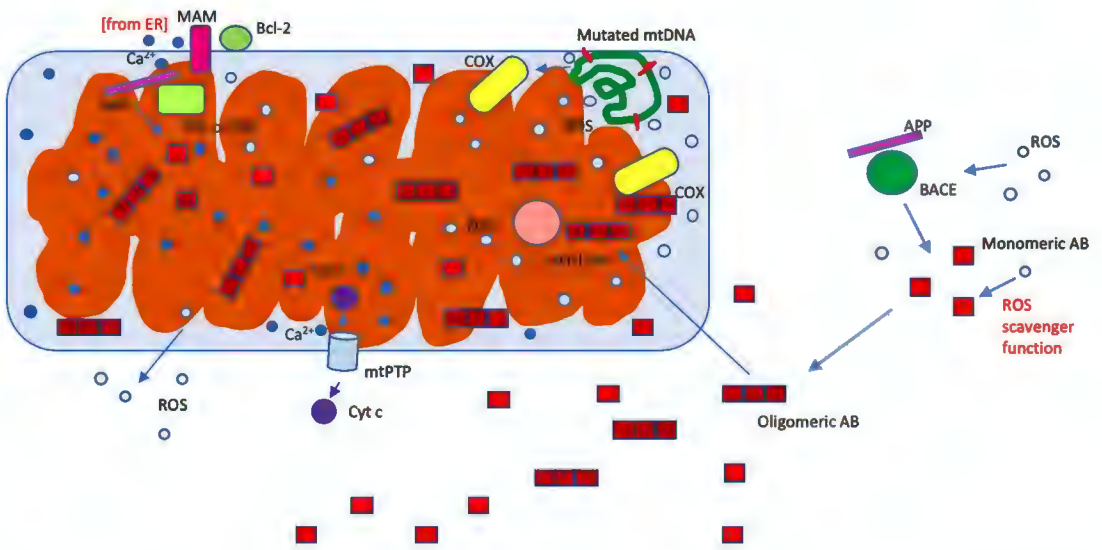


Figure 4. The Aβ cascade associated with increased oxidative stress and cell death initiation. Low levels of monomeric Aβ will act as endogenous ROS scavenger when APP is cleaved by β-secretase (BACE), but when ROS levels overwhelm normal Aβ levels, BACE will cleave an excess of Aβ, allowing it to aggregate and form the toxic oligomeric form. The oligomeric form will diffuse into the mitochondria where it will inhibit COX and alcohol dehydrogenase (EtOH DH), resulting in further ROS production. The increased ROS production will also mutate the mtDNA, possibly hitting the gene for COX to express mutated, ROS-producing COX. ROS will also activate the cleavage of APP within the mitochondria by PS1 and PS2. PS1/2 will also associate with MAM to absorb excess Ca^{2+} from the ER, activating the mtPTP to release cyt c when mitochondrial Ca^{2+} levels are high, and with Bcl-2 to inhibit its anti-apoptotic function.

The pathologically hyperphosphorylated neurofibrillary tangle tau is also associated with AD.[48, 49] Physiological tau is a microtubule-associated protein with phosphorylation sites, but in AD, hyperphosphorylated tau is at a much higher concentration than normal brains of the same age.[50] These hyperphosphorylated tau proteins will then aggregate to form neurofibrillary tangles (NFT).[50] Within late stages of NFT accumulation, the NFT aggregates will continue to grow through a process of enucleation where previous aggregates serve as the template by which more aggregates can form.[50] When tau is pathologically altered, axonal transportation becomes dysfunctional, with one of these transported molecules being mitochondria.[48] When complexed, tau and Aβ will inhibit proper mitochondrial function and dynamics.[49] When the caspase cascade

is initiated in apoptosis, the caspase cleavage of tau results in the oligomerization of pathological tau as well.[48] Together with Aβ, tau will also impair mitochondrial function, resulting in the continuation of the positive feedback loop of oxidative stress.[48]

Certain metals ions are suspected to play a role in the pathogenesis of aggregates in AD patients including aluminum (Al^{3+}), copper(II) (Cu^{2+}), iron(III) (Fe^{3+}), and zinc (Zn^{2+}).[51] This association between Al^{3+} and AD has been studied for almost a century as Al^{3+} even though the element has no physiological or enzymatic use within the body.[51] Due to its 3+ oxidative state, Al^{3+} can bind strongly to molecules that use phosphorus such as ATP, DNA, and enzymes that are phosphorylated in cell signaling, thus affecting genetic function, energy metabolism, and cellular communication.[50, 51] Within the brain especially, Al^{3+} will cause crosslinking and accumulation of proteins with metal-binding sites and high levels of phosphorylation such as microtubule-associated proteins like tau.[51] Al^{3+} will add to the Aβ cascade of accumulation by accelerating the rate at which Aβ will oligomerize and aggregate, increase the aggregates' preference for membranes, and carry higher toxicity than normal Aβ aggregates.[51] In addition to causing pathogenetic and degradable protein configurations, Al^{3+} will also elevate free Fe^{3+} levels due to its interaction with endogenous iron storage and chelating proteins, thus the metal-induced oxidative stress will increase with elevated free metal concentrations.[51] Al^{3+} is encountered through drinking water, with a concentration greater than 0.1 mg/Liter as a risk factor of dementia and AD.[52] Al^{3+} may also play a role in the formation of NFT that are associated with AD patient brains, or the NFTs may act as a "sink" that deposits the cation, stabilizing the NFT with covalent bonds.[50] Similar to other metals, Al^{3+} will also initiate the vicious cycle of oxidative stress through free radical propagation alongside Fe^{2+}, increasing not only hydrogen peroxide concentration but also levels of lipid peroxidation.[50]

Another metal that is associated with the accumulation of protein aggregates in AD is Cu^{2+}. APP plays a prominent role in Cu^{2+} homeostasis by containing a Cu^{2+} binding site that will reduce the cation to Cu^{1+}.[53, 54] APP will thus mitigate the toxic effects of free Cu in the cytosol, but when Cu^{2+} binds to APP rather than the preferred Cu^{1+}, APP will be signaled for processing and breakdown via the non-amyloidogenic pathway.[54] In AD patients, the cerebrospinal fluid levels of Cu were significantly greater than age-controlled group, thus Cu toxicity and its relation to APP is vital to understanding the pathogenesis of AD.[53] In addition to binding sites within APP, Cu also connected to increased accumulation of Aβ within AD brains.[55, 56] Specifically at lower pH values of 6.8, a value that is similar to *in vivo* mild inflammation of the CNS in AD, Cu induces Aβ aggregation.[56] When copper binds to Aβ monomers, the residue charges change from neutral to negative, thus resulting in the aggregation of Aβ.[56] The high affinity of copper to Aβ will reduce Cu^{2+} to Cu^{1+} as well as generate ROS and more aggregation.[54]

Iron is a particularly effective in the production of ROS due to its involvement in the Fenton reaction as discussed previously in this introduction. However, iron's oxidation is mitigated through iron sequestration via iron storage proteins such as ferritin as well as the export of iron from cells.[57] APP possesses ferroxidase activity that will prevent the formation of ROS via the Fenton reaction by catalyzing the oxidation of Fe^{2+} to Fe^{3+}.[57] When ferroxidase activity is decreased, Fe^{2+} can accumulate, enter the Fenton reaction cycle, and generate ROS within the cell.[57] Ferroxidase activity of APP is also connected to the exportation of iron out of the cell via transferrin as Fe^{2+} must be oxidized to Fe^{3+} to leave the cell.[57] In AD, Zn^{2+} complexes with Aβ aggregates in Aβ plaques, and Aβ can release free Zn^{2+} to inhibit the ferroxidase activity of functional APP.[57] In addition, Aβ possesses redox-active function that is able to reduce Fe^{3+} to Fe^{2+} in order to generate further ROS when Fe^{2+} interacts with H_2O_2 within the

Fenton Reaction. Thus, Fe^{2+} levels are increased within neurons, causing subsequent oxidative stress and cell death.

Zn^{2+} will inhibit some of the ROS generation caused by Aβ as it causes the aggregation of Aβ and subsequent repression of this redox activity of the soluble, neurotoxic form.[58] Zn^{2+} will compete with Cu^{2+} for binding sites upon the Aβ peptide in order to reduce the electron transfer associated with redox reactions.[58] When Cu^{2+} binds Aβ, Aβ will reduce the metal to Cu^{+} and initiate the formation of H_2O_2, but when Zn^{2+} binds, this activity will be decreased, even as the Aβ peptides aggregate into characteristic Aβ plaques.[58] AD patients exhibit increased levels of zinc, copper, and iron, but though the high levels of copper and iron are ROS producing, the increased presence of Zn^{2+} is possibly the result of cellular response to oxidative stress.[58] However, clinical studies that treated AD patients with zinc supplementation reported cognitive decline, suggesting that *in vivo* zinc may be associated with AD symptoms.[58] As previously reported, Zn^{2+} inhibits the ability of APP to oxidize and transfer Fe^{2+} out of the cell, thus the increased concentrations of Zn^{2+}, although protective versus the redox-activity of Aβ, still leads to the generation of ROS and neurodegeneration.[57]

Parkinson's disease

Parkinson's disease (PD) is another chronic neurodegenerative disease which is characterized by cell loss in the substantia nigra, high oxidative capacity, and hypersensitivity to mitochondrial dysfunction.[59] Clinical symptoms of PD include: bradykinesia, instability of posture, tremors, and rigidness.[48] Similar to AD, PD is also characterized by protein aggregations called Lewy bodies, made of α-synuclein and ubiquitin.[49] These Lewy bodies are cytoplasmic aggregates with dense, radiating filaments of α-synuclein.[60] Through alterations in certain protein levels and functions, α-synuclein aggregate leads to mitochondrial dysfunction as well as

caspase-dependent apoptosis.[61] α-synuclein will interact with pro-apoptotic Bad, causing it to form a heterodimer with Bcl-x1 to remove anti-apoptotic Bcl-2 and release cyt c from the mitochondria, initiating the cascade of apoptosis.[61] In addition, the presence of cytoplasmic cyt c can also bind to and facilitate the aggregation of more α-synuclein while initiating apoptosis.[61]

One particular measure of respiratory function is the presence of cytochrome c oxidase (COX) (complex IV) of the ETC.[59] With increased age, the ratio of COX-deficient to COX-functional cells increases, but in Parkinson's patients, this ratio is much greater.[59] These deficient mitochondria spread via clonal expansion, meaning the deficient mitochondria overcome the normal mitochondria in number within somatic cells.[59] In addition to COX-deficiency within these dysfunctional mitochondria, mutational deletions of the mitochondrial genome are occurring with age increases and disproportionately within Parkinson's patients.[59] A correlation has also been identified between deficiency of the ETC and the concentration of these random mitochondrial DNA deletions within the substantia nigra and somatic mitochondria as well.[59] These somatic mitochondrial DNA deletions accumulate rapidly via clonal expansion, leading to widespread respiratory dysfunction.[59] One possible mechanism by which these deletion mutations are occurring is through the accumulation of ROS within diseased neurons with dysfunctional mitochondria.[59] The high capacity of neurons within the substantia nigra results in higher levels of ROS, thus leading to the accumulation of mitochondrial DNA mutations and respiration deficiency.[59]

Several specific proteins are implicated in PD. The protein α-synuclein is found in both monomeric and oligomeric form within the inner membrane of the mitochondria, and it will inhibit Complex I function as well as mitochondrial fusion and fission dynamics.[48] With the inhibition of Complex I, electrons will inappropriately leak from the complex and form ROS.[61]

Thus, α-synuclein is causing oxidative stress which will in turn cause further aggregation of α-synuclein, sparking a positive feedback loop of further aggregation and oxidative stress characteristic of PD.[61] PTEN-induced kinase 1 (PINK1), a serine/threonine protein kinase found within the outer mitochondrial membrane, will coordinate with parkin, another mitochondrial protein, to regulate mitochondrial dynamics and mitophagy via lysosomes.[48, 49] In normal mitochondria, PINK1 is expressed in low levels.[48] However, once the mitochondrion is damaged or aged, the depolarized mitochondria membrane potential will inhibit the proteolysis of PINK1, causing it to accumulate and activate parkin.[48] Upon activation, parkin will ubiquitinate the affected mitochondria to mark it for lysosomal digestion.[48] When oxidative stress levels increase, the function of parkin is blocked, reducing the mitochondrial turnover and decreasing mitochondrial function.[48] Another important protein involved in the pathogenesis of PD is DJ-1, a small protein that acts as a ROS scavenger when oxidized.[48] Upon limited oxidative stress, specifically within the mitochondria, DJ-1's cysteine residues are oxidized and act as an effective endogenous antioxidant molecule.[48] DJ-1 is also rapidly transported to mitochondria during this stress and cause the mitochondria to elongate to combat the stress.[48] In PD, mutant forms of DJ-1 do not properly function as ROS scavengers and the mitochondria undergo lower energy production, increased sensitivity to ROS, and defective structural changes.[48]

Oxidative metals have also been connected to the onset of PD. Al^{3+} is not only increased in brain tissue of PD patients but also negatively affects the proper functioning of the dopaminergic system.[62] Although the exact pathogenesis of PD is unknown, heavy metals have been suspected as a possible cause due to etiology research in industry workers exposed to higher levels of heavy metals and subsequently, higher PD rates than the normal population.[60] In fact, brain autopsies have also revealed elevated levels of both aluminum and iron within Lewy

bodies in the substantia nigra.[60] The protein aggregate α-synuclein undergoes structural changes the presence of Al^{3+}.[60] The presence of metals will accelerate the formation of α-synuclein fibrils, typical of PD.[60] One possible mechanism by which Al^{3+} facilitates the aggregation of α-synuclein is through the competition with Na^{+} for cation binding sites within the protein, causing the proteins to aggregate via polyvalent ion bridging.[60] Overall, metals will facilitate the formation of protein aggregates specific to PD as well as lead to an accumulation of ROS, cultivating subsequent inflammation and cell death.

Another metal that is found in higher levels within PD patient brain autopsies is Fe^{3+}.[60] In addition, the levels of ferritin, a Fe^{3+} binding protein, are elevated and a shift in the Fe^{2+}/ Fe^{3+} ratio as higher levels of Fe^{3+} are observed in substantia nigra pars compacta (SNPC) neurons specifically.[60, 63] SNPC neurons contain melanin, and the interaction with free Fe^{3+} with melanin results in oxidative stress and neurodegeneration of SNPC neurons.[63] The levels of ROS that increase with the high levels of free Fe^{3+} will overwhelm H_2O_2 scavenger glutathione, allowing further ROS accumulation to occur.[63] Accumulated metals, particularly Fe^{3+} and Cu^{2+}, lead to an overwhelming increase in oxidative stress which will cause downstream cellular dysfunction and death—both of which are indicative of PD.[60]

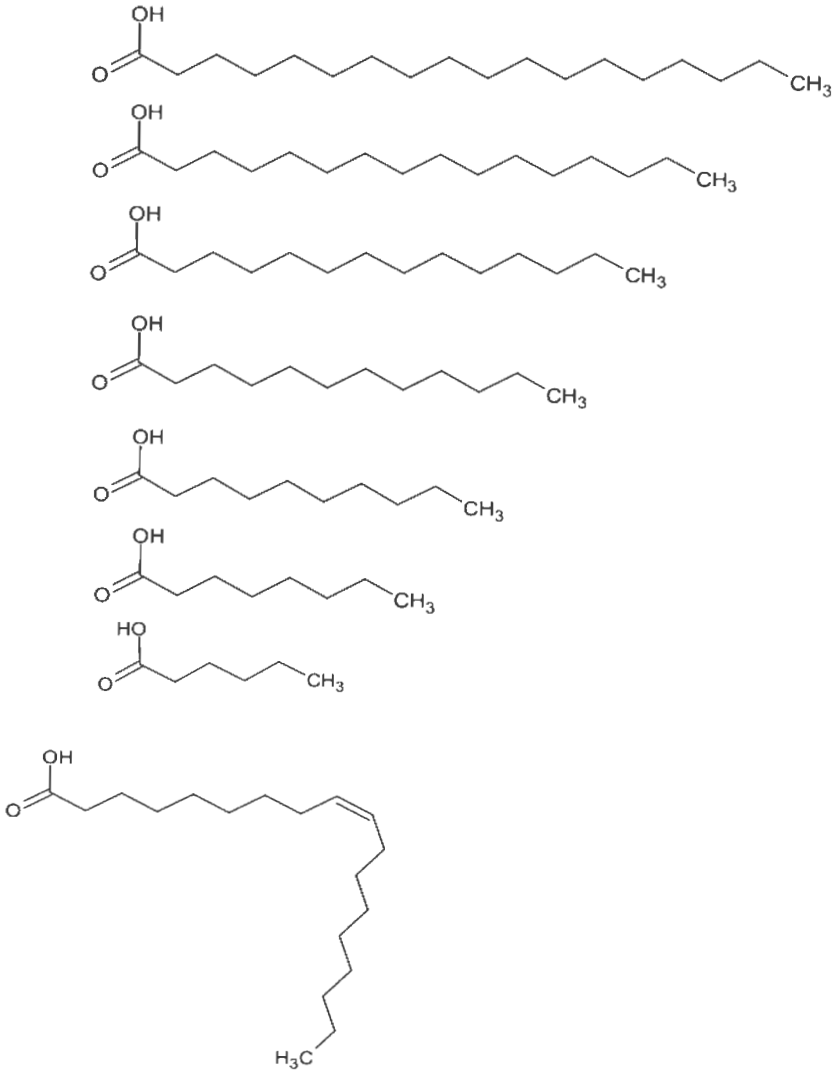
Copper metal is also implicated in the facilitation of α-synuclein aggregation within physiological levels.[64] Cu^{2+} will bind to cation-specific binding sites on the α-synuclein and cause the metal-bound α-synuclein to nucleate, that is, to form a "seed" for subsequent aggregation off this one protein.[64] Previously, *in vitro* studies showed that higher concentrations of Cu^{2+} were required for α-synuclein aggregation, but current studies have demonstrated that physiological levels are sufficient to cause aggregation.[64] Despite the aggregation of α-synuclein being dependent upon Cu^{2+} levels, reduced levels of Cu^{2+} within substantia nigra neurons, a

documented characteristic of PD, causes a decrease in the activity of SOD to scavenger ROS as Cu^{2+} is a necessary cofactor in normal SOD function.[65] Low levels of this endogenous antioxidant will also increase oxidative stress, α-synuclein aggregation, and cellular death characteristic of PD.[65]

Coconut oil as a potential ameliorating agent

With the rise of neurodegenerative diseases, any potential treatment that offers some possible solution should be investigated. Coconut oil (from *Cocos nucifera*) offers a possible avenue as it has the potential to improve cognitive function as associated with AD.[66, 67] Coconut oil is composed of mainly saturated fatty acids with the specific fatty acids including (in order of quantity) lauric, myristic, palmitic acid, caprylic acid, capric acid, stearic acid, caproic acid, and palmitoleic acid (**Figure 5**).[67] Of these saturated fatty acids, 70% are medium chain triglycerides (MCT).[66, 68] MCT has been shown in some early studies to improve cognitive functioning due to its easy metabolism and energy production.[66] MCTs are unique in that their size allows their utilization as energy rather than being stored as fat, and because MCTs are lipids, they can be converted into ketone bodies for easy uptake by the brain when in glucose shortage. Coconut oil also contains polyphenols and phenolics, molecules that have been studied as potential therapeutic options for a wide variety of ailments from AD to high blood pressure due to their antioxidant capacity.[68] Specifically, flavanones and dihydroflavanones are the polyphenol compounds present.[69] Polyphenols have been demonstrated to slow the normal aging process of memory loss through the reduction of oxidative stress in the brain.[70] These molecules function as ROS scavengers, regulators of cellular signaling, metal chelators, and lipid peroxidation preventors.[69] Polyphenols are highly conjugated systems with delocalized electrons that can be freely donated to ROS to neutralize the destruction chain reaction of ROS and peroxidized

lipids.[71] Polyphenols will also bind to Fe^{2+} in order to break the Fenton reaction of oxidative stress which leads to reduction of oxidative damage to DNA, mtDNA mutations, and lipid peroxidation as associated with neurodegenerative diseases.[71, 72] The lauric acid in coconut oil holds potential as an anti-inflammatory agent through the modulation of immune cell proliferation, and when used in high doses in mice studies, virgin coconut oil has been shown to significantly reduce inflammation.[73]



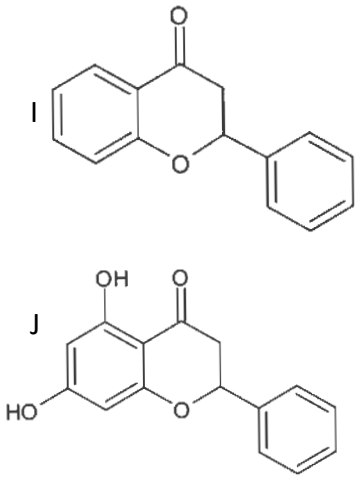


Figure 5. Composition of Coconut oil lipids and main polyphenols. **A.** Stearic acid **B.** Palmitic acid **C.** Myristic acid **D.** Lauric acid **E.** Capric acid **F.** Caprylic acid **G.** Caproic acid **H.** Palmitoleic acid **I.** Flavanone **J.** 5,7-dihydroxyflavanone

Coconut oil, though not yet thoroughly studied, holds unique promise for AD. Early studies have found that *in vitro* cultures of coconut oil and Aβ have improved conditions when compared to Aβ only controls.[66] In addition, coconut-oil has also modulated the Aβ-induced structural changes of neuronal mitochondria.[66] The high phenolic and polyphenol concentration of coconut oil also makes it a strong antioxidant.[66] The hydroxyl groups of the phenolic molecules will reduce the oxidative stress caused by the aggregation of Aβ and reduce the accumulation of Aβ by occupying the hydrogen bonding capacity of Aβ.[66] Caprylic acid has also been demonstrated to cross the BBB, a feat that makes it a molecule of interest due to the highly selective nature of the BBB.[66] The saturated fat makeup of coconut oil increases the amount of high density lipoproteins (HDL) cholesterol levels in the brain which is associated with longevity in elderly populations.[67] HDL is the "good" cholesterol as it is preventive against LDL oxidation and apoptosis, and saturated fatty acids increase HDL levels.[74] Low density lipoprotein (LDL) cholesterol is more susceptible to lipid peroxidation and the perpetuation of free radicals, but

antioxidant molecules (both endogenous and dietary such as vitamin E or coconut oil) are protective against this spreading oxidative stress by inhibiting the lipid peroxidation.[69] Virgin coconut oil, with higher levels of polyphenols and lower levels of free fatty acids, is also effective in increasing the levels of the endogenous antioxidant glutathione.[69] Due to the relevance of oxidative stress in relation to both AD and PD, coconut oil's antioxidant capacity through its phenolic and polyphenol composition presents a potential avenue for natural therapy.

Saccharomyces cerevisiae *as a model for degeneration*

Saccharomyces cerevisiae, or the budding yeast, is a model eukaryotic organism that undergoes similar responses to oxidative stress as human cells when in the presence of metals.[75] The yeast will increase expression of endogenous antioxidants, undergo mitochondrial stress, and initiate caspase dependent apoptosis within an hour.[75] *S. cerevisiae* has been used a model for a range of neurodegenerative disorders in order to study the process of oxidative stress induced apoptosis.[75]

Research question

Many neurodegenerative diseases involve mitochondrial dysfunction which generates free radicals as well as promotes cellular death, creating a vicious cycle of degeneration. Certain toxic metals such as aluminum and copper have been connected to inflammation and the generation of oxidative stress within the cell. Oxidative stress will be modeled within yeast and assayed for differences in mitochondrial activity. In addition to marking oxidative stress and damage to the mitochondria, the ameliorating effects of various antioxidant, anti-inflammatory oils such as coconut oil, fish oil, and olive oil will be examined as a possible therapeutic relief to ROS damage. Is there a regaining of health when the yeast is subjected to the oil after oxidative stress? Is there a preventive effect of the oil when the yeast is grown in it before being subjected

to oxidative stress? If there is a correlation between the oil and mitochondrial activity, what would be the possible mechanism?

Oxidative stress will be assessed via fluorochromes and Western blotting analysis in order to determine the difference in expression levels of proteins associated with oxidative stress and apoptosis. Spectroscopy will be utilized to determine mitochondrial activity. Coconut oil is expected to prevent and protect yeast from reactive oxygen species as proposed by research presented here.

MATERIALS AND METHODS

Maintaining yeast cultures

Saccharomyces cerevisiae (yeast) (strain HAO, *Carolina*) was grown in YEPD liquid media comprised of the following: 2% glucose (company), 1% yeast extract (company), and 2% bacto-peptone. The yeast was incubated at 37° C in 24 well plates (company) on a plate shaker (company). A stock of the yeast was grown on YPD solid media (YEPD media recipe with 2% agar [company]) and stored in a 4° C refrigerator. To obtain cell counts, OD600 was utilized to determine approximate population size of each well.

Determining ROS producing concentration of metal solutions within yeast cultures

Serial dilutions of sterile metal solutions were added to yeast cultures in order to determine an experimental concentration of metal solution that will result in oxidative stress-induced apoptosis. Grow cultures overnight. Measure cell population with OD600 in order to aliquot approximately 5 million cells per well. Expose to various concentrations (0.0001 M, 0.0005 M, 0.001 M, 0.005 M, 0.01 M, 0.05 M, 0.1 M, 0.15 M, 0.2 M, 0.25 M, and 0.3 M) of metal solutions ($CuSO_4$, $Al_2(SO_4)_3$, $Fe_2(SO_4)_3$ and $FeCl_2$), bring the total volume to 1 mL with fresh YEPD media, and incubate for 1 hour at 37° C. Pellet cells at 7 rpm for 8 minutes, discard supernatant, and resuspend in YEPD. Again, pellet cells at 7 rpm for 8 minutes, discard supernatant, and incubate in YEPD media for 3 hours, Measure OD600 to determine an appropriate metal concentration that indicates the cells are no longer in log phase but transitioning to the death phase. After analysis of the spectral data, the following concentrations of the same metals were used in treatment experimental trials: 0.005 M and 0.1 M $CuSO_4$; 0.1 M and 0.25 M $Fe_2(SO_4)_3$; 0.005 M and 0.05 M $FeCl_2$; and 0.05 M and 0.25 M $Al_2(SO_4)_3$.

Determining a beneficial concentration of coconut oil

Virgin coconut oil (VCO) has antifungal activity thus the coconut oil concentration used must not overwhelm the yeast, but instead be utilized by the yeast as an antioxidant against the metal induced oxidative stress. The same assay as with the metal solutions was performed to determine appropriate VCO concentration that will be effective versus oxidative stress; however, the concentration in which the VCO remains in solution and improves cell growth will be used as the concentration for further experiments.

Serial dilutions of sterile VCO were added to yeast cultures in order to determine an experimental concentration of VCO that will result in possible reduction of oxidative stress. Grow cultures overnight. Measure cell population with OD600 in order to aliquot approximately 5 million cells per well. Expose to various percent volume dilutions of VCO (1%, 5%, 10%, 25%, and 50%), bring total volume up to 1 mL with fresh YEPD media, and incubate for 1 hour at 37° C. Pellet cells at 7 rpm for 8 minutes, discard supernatant, and resuspend in YEPD. Again, pellet cells at 7 rpm for 8 minutes, discard supernatant, and incubate in YEPD media for 3 hours, Measure OD600 to determine an appropriate VCO concentration that allows cell population growth and not decline. After analysis of the spectral data, the 10% dilution of VCO was chosen as the treatment dosage for further trials.

Inducing oxidative stress with metal solutions

Measure cell population with OD600. Aliquot cell cultures to wells, centrifuge at 14,000 rpm for 3 minutes in 4° C. Discard supernatant and expose to 0.005 and 0.1 M $CuSO_4$, 0.05 M and 0.25 M $Al_2(SO_4)_3$, 0.01 M and 0.25 M $Fe_2(SO_4)_3$ and 0.005 M and 0.05 M $FeCl_2$ (bring volume up to 1 mL with fresh YEPD media) for 1 hour incubation. Wash with and incubate in YEPD media for 3 hours before measuring oxidative stress. For VCO trials, 10% of VCO is added to either the initial media (and removed prior to metal exposure), the post-exposure media, or both. As controls, add known ROS scavengers (10 mM DMTU [*N,N′*-dimethylthiourea], 10 nM MnTBAP [manganese(III)-tetrakis(4-benzoic acid)porphyrin], and 28 mM Tiron [4,5-dihydroxybenzene-1,3-disulfonate]) during post-exposure incubation, before metal exposure, or both before and after metal exposure.

Lysating harvested cell cultures

Harvest cells by centrifuging at 5000 xg for 3 minutes at 4° C. Wash in cold deionized water twice, and resuspend in lysis cocktail (100 mM Tris-HCl [pH 7.4], 20 µg/mL leupeptin, 10 µg/mL pepstatin A, 1 mM PMSF, and glycerol 10% [v/v]). Add 200 µL acid-washed glass beads, and centrifuge on high speed for 30 seconds and cool on ice between each pulse (repeat three times).

Quantification of reduced and oxidized glutathione levels

Incubate reaction buffer (0.1 M phosphate buffer [7 pH], 0.5 mM EDTA), lysate, and 3.0 mM 5-dithro-bis-(2-nitrobenzoic acid) at 30° C for 5 minutes. Add 0.4 mM NADPH and incubate the sample at 30° C for 20 minutes. Measure absorbance at 412 nm and compare with standard curve with various concentrations of reduced glutathione. [76]

RESULTS AND DISCUSSION

Before experimental trials could be conducted, appropriate metal concentrations had to be determined with serial dilutions. All data was collected in triplicates. A wide range of metal concentrations were assessed to find a concentration that would moderately inhibit cell population growth, indicating oxidative stress. However, the metals Fe^{3+}, Fe^{2+}, and Al^{3+} significantly increased the cell populations in comparison to the yeast-only control (**Figure 6**).

The Cu^{2+} trials were the only metal to significantly decrease the cell population size in comparison to the yeast only control (**Figure 6**).

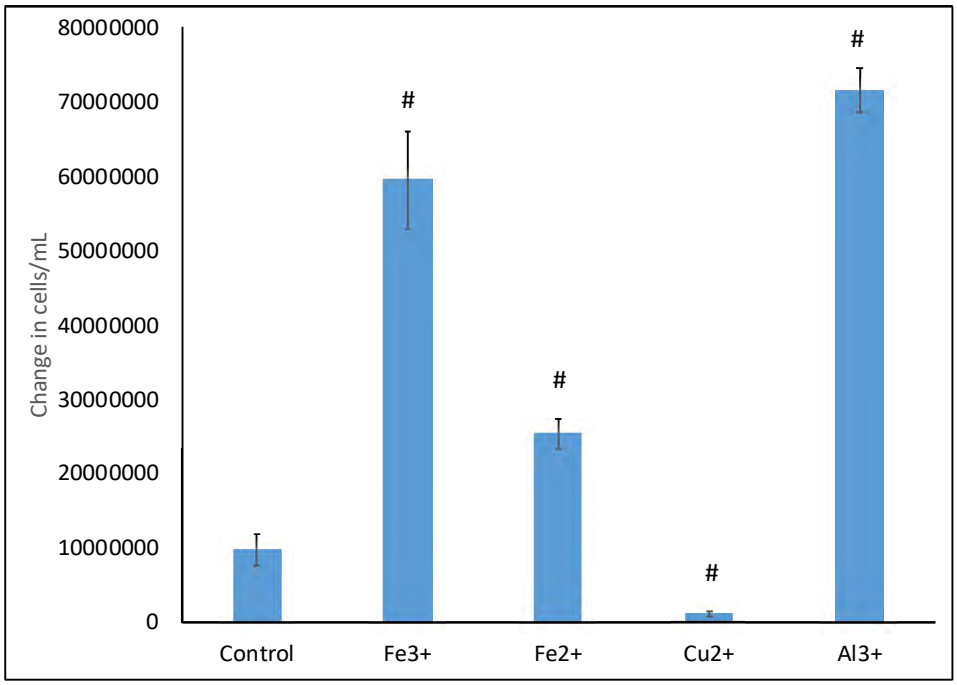


Figure 6. Change in cell population following metal incubation as measured with OD600 readings.

Concentration dependent increases in cell population size was also observed in the Fe^{3+}, Fe^{2+}, and Al^{3+} samples, but not in Cu^{2+} (**Figure 7**). Certain trends were also observed as concentrations increased. With Fe^{3+}, the cell population size plateaued between concentrations of 0.005 M, 0.01 M, and 0.05 M, but the population size increased steadily from 0.1 M up to 0.25 M before dipping slightly at the highest concentration of 0.3 M. Fe^{2+} trends were more subtle, but of the three metals that increased cell population size, Fe^{2+} increased the population the least. The concentrations of 0.005 M and 0.05 M of Fe^{2+} were chosen to use in later experiments due to the former concentration being the start of the increase in cell number and the latter being the concentration at which population increase plateaus. Al^{3+} population increases do not exponentially increase until the concentration of 0.05 M but continue to rise to the highest concentration. The concentration of 0.05 M was chosen for experiments due to it being the

turning point from slight increases in population to the large increases seen in higher concentrations, and the concentration of 0.25 M was chosen as it slightly plateaus at the higher end of the concentrations.

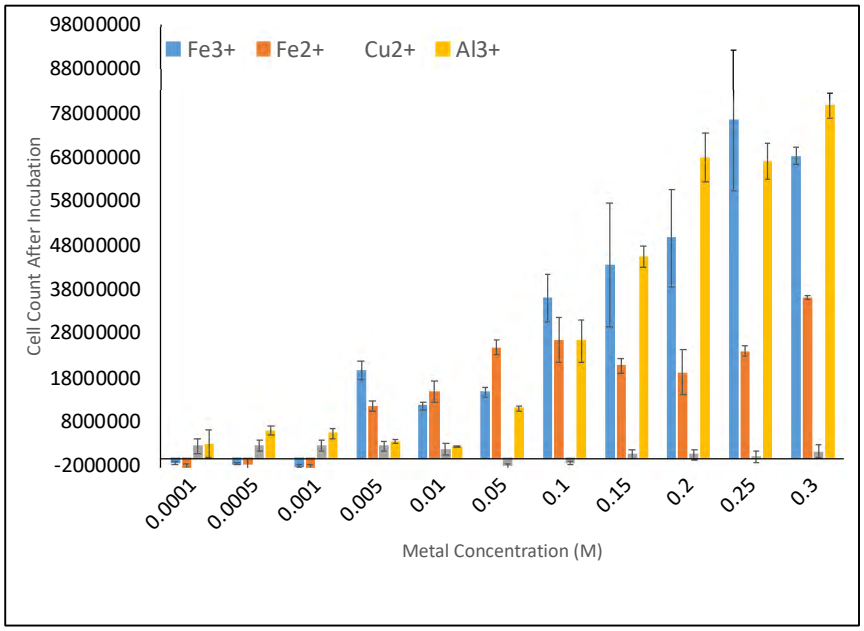


Figure 7. Change in cell population following metal incubation as measured with OD600 readings for concentrations of tested metals Cu^{2+}, Fe^{2+}, Fe^{3+}, and Al^{3+}.

In order to test for the presence of oxidative stress within the samples, a glutathione assay was performed. The absorbance at the 412 nm wavelength quantified the amount of thiolate anion present (2-nitro-5-thiobenzoate [TNB^{2-}]). The addition of 5,5'-dithiobis-(2-nitrobenzoic acid ($DTNB^{2-}$) to the lysate allowed the quantification of glutathione due to a reaction in which $DTNB^{2-}$ will oxidize reduced glutathione, forming a mixed disulfide with the glutathione organic chain attached and a thiolate anion, or TNB^{2-}, which is excited at the 412 nm wavelength (**Figure 8**). Rather than directly measuring the amount of ROS within the cells, this reaction measures the presence of reduced glutathione, thus higher absorbance values represent less oxidative stress within the cell. In oxidative environments, reduced glutathione is not as prevalent because it is an endogenous antioxidant that reduces the ROS to protect the cell.

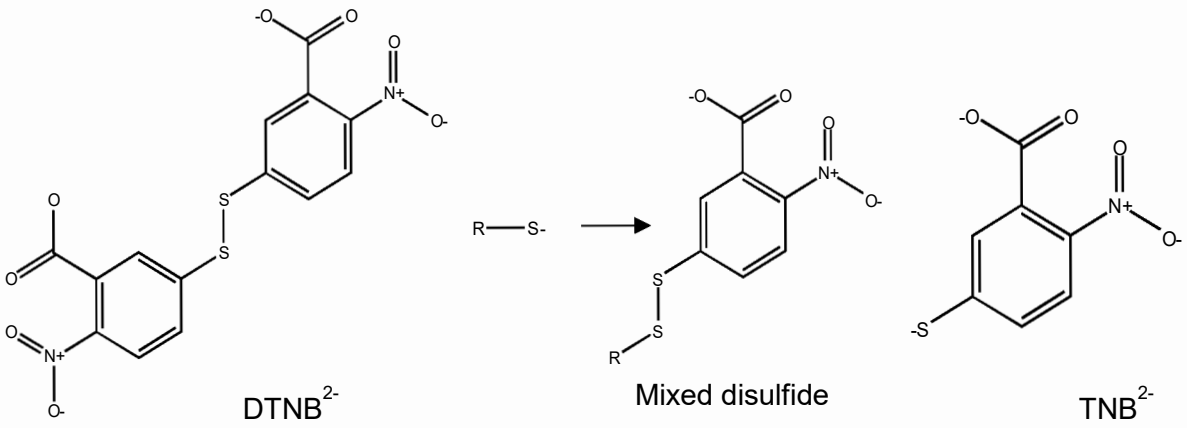


Figure 8. Reaction scheme of the formation of TNB^{2-} from the presence of reduced glutathione in order to detect ROS.

A cocktail of known ROS scavengers was used as a positive control treatment in order to determine if the ROS levels could be affected by scavengers within the metal induced oxidative stress mechanism. The control group without any treatment had around 0.00001 – 0.00002 moles of TNB^{2-}, indicating some oxidative stress as the levels of reduced glutathione are low (**Figure 9**). Significant scavenging of the ROS levels was observed in almost half of the treatment trials. Of these, the glutathione levels reached their peaks in the Fe^{3+} trials. The ROS scavenger cocktail was used in order to establish if the ROS levels could be rescued within the mechanism of metal-induced oxidative stress, and as significant increases demonstrate in **Figure 9**, the increase in reduced glutathione levels means that less oxidative stress was occurring, thus more reserved reduced glutathione is available. The cells were treated with ROS scavenger cocktail in the same manner as the coconut oil treatment: before the metal exposure, after the metal exposure, or both before and after metal exposure.

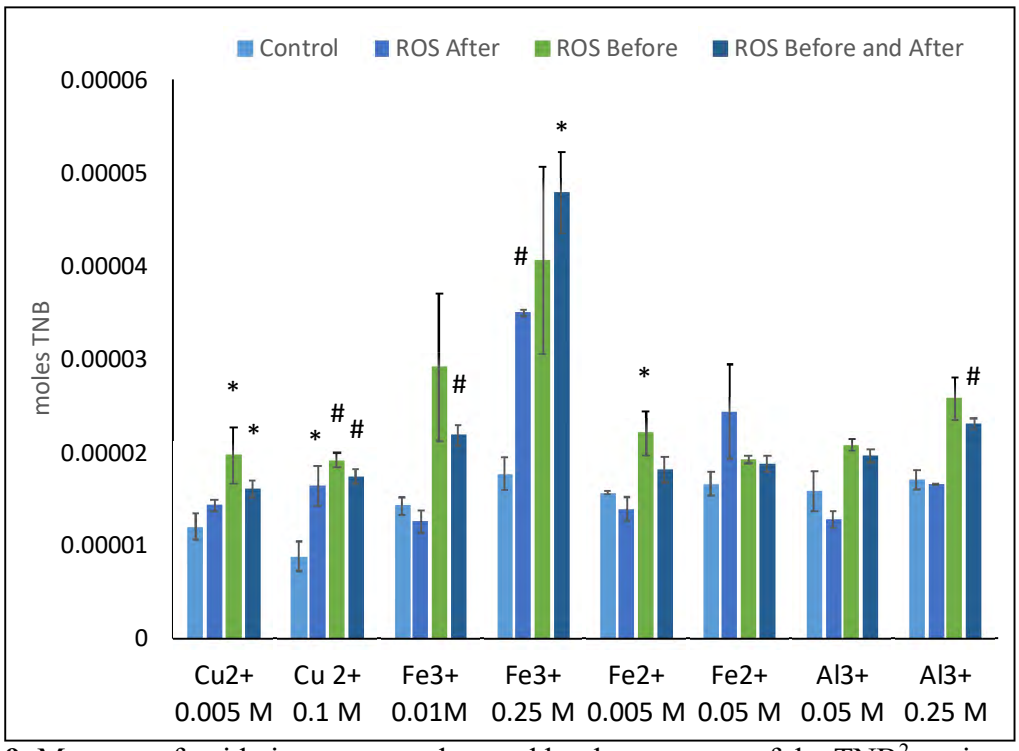


Figure 9. Measure of oxidative stress as detected by the presence of the TNB^{2--} anion presence in cell samples control ROS scavenger treatments. (* represents a p value < 0.05 and # represents a p value of < 0.01 using an one-tailed T-test)

In comparison to the no-VCO controls, VCO significantly increased the level of oxidative stress in Fe^{3+}, Fe^{2+}, and Al^{3+} in a couple treatments (**Figure 10**). For the other cases, there was insignificant increase in oxidative stress as well. However, in the Cu^{2+} exposed cells, the VCO treatments had significantly increased the levels of reduced glutathione, thus the VCO treatment significantly reduced oxidative stress. In the higher concentration of Cu^{2+}, all treatments of VCO reduced oxidative stress, but in the lower concentration of the Cu^{2+}, the VCO before metal exposure treatment was the only significant reduction of oxidative stress.

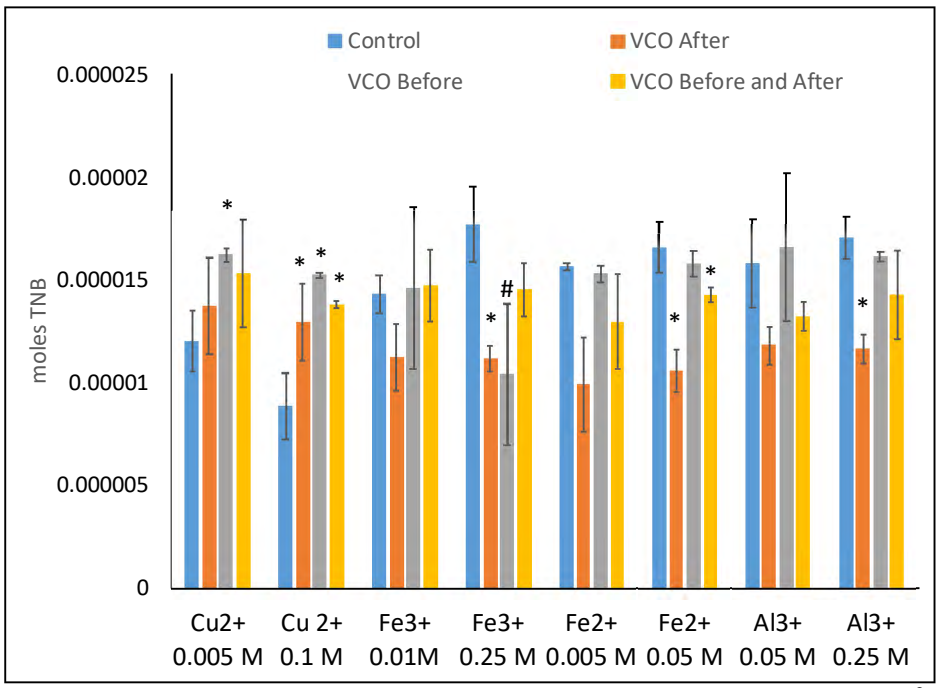


Figure 10. Measure of oxidative stress as detected by the presence of the TNB^{2-} anion presence in cell samples with VCO treatments. (* represents a p value < 0.05 and # represents a p value of < 0.01 using an one-tailed T-test)

In comparison with the VCO treatment and the ROS scavenger treatment of the 0.1 M Cu^{2+} exposed samples, all treatments exhibited a significant reduction of oxidative stress as represented by an increase in the reduced glutathione levels (**Figure 11**). Although the ROS scavengers were able to significantly reduce the oxidative stress in the other metal exposed samples, the VCO did not have the same effect and in some samples decreased the levels of reduced glutathione. Interestingly, with the Cu^{2+} trials, the VCO treated samples (VCO added after metal exposure) significantly rescued the decline of cell population as observed in untreated Cu^{2+} exposed samples (**Figure 12**).

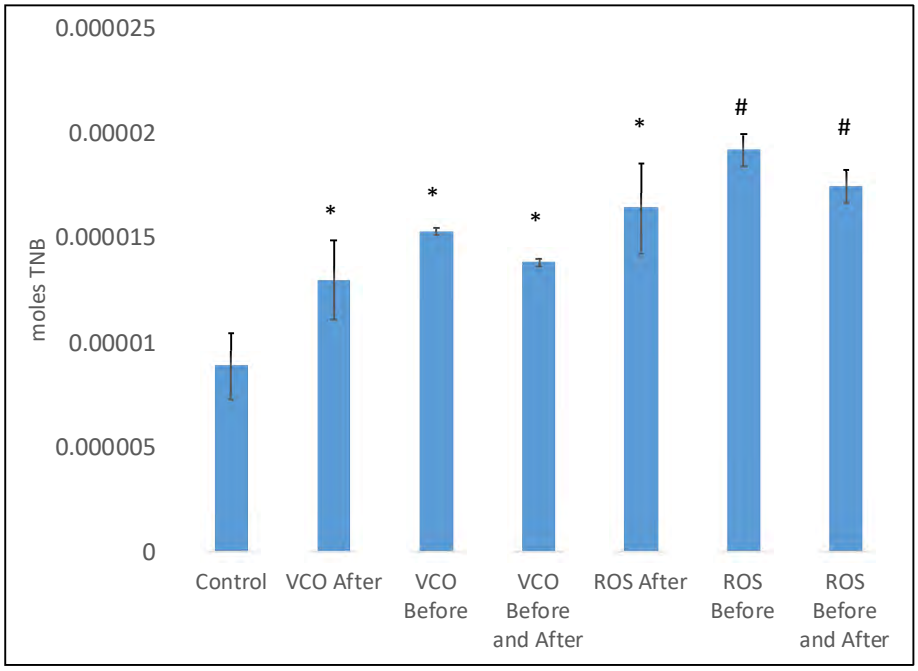


Figure 11. Comparison of VCO treatments and ROS scavenger controls with 0.1 M Cu^{2+} samples. (* represents a p value < 0.05 and # represents a p value of < 0.01 using an one-tailed T-test)

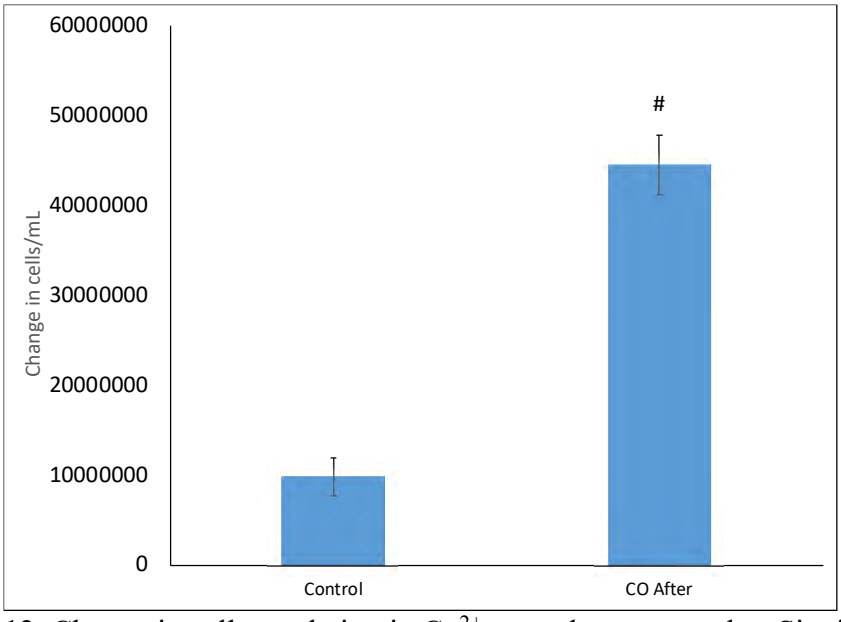


Figure 12. Change in cell population in Cu^{2+} treated yeast samples. Significant increase in cell population size observed in VCO treated after Cu^{2+} incubation. (* represents a p value < 0.05 and # represents a p value of < 0.01 using an one-tailed T-test)

CONCLUSION

Although it was originally hypothesized that VCO would reduce oxidative stress in all metal-exposed samples, the Cu^{2+} exposed trials were the only samples that exhibited VCO associated decline of ROS and increase in cell population size. More research should be conducted in order to identify the difference in yeast's utilization of Cu^{2+} as this was the only metal to inhibit the cell population growth while the other metals at the same concentration drastically increased the cell population. The initial dilutions were performed with solely the metals in order to determine a concentration that would reduce the rate at which the cell population increase, much like the Cu^{2+} exposed samples. However, the cell population size significantly increased in comparison to the yeast control without metal exposure. In further experiments, higher metal concentrations should be tested in order to determine if such a point exists in which the abundance of Fe^{2+}, Fe^{3+}, and Al^{3+} present in the cell would inhibit cell population growth.

The difference in how the yeast population reacted to the presence of each metals represents a difference in mechanistic processing of higher levels of the free metal cations. Yeast contain the ability to process iron and store it in transport and storage molecules, with higher levels of iron initiating the expression of more regulatory proteins to better store the iron.[77] With aluminum ions, the toxicity increases drastically with low pH environments, thus the toxic effects of aluminum on the yeast may have been mitigated.[76]

Even though copper is also a necessary metal for proper yeast health like the other metals, in excess the cation is highly toxic to the lipid membranes of yeast as well as other proteins, specifically by oxidizing side chains or destroying peptide bonds.[77] Although high

levels of redox-active metals like iron and copper will initiate the Fenton reaction, it is possible that less copper atoms are need to evoke a toxic effect on cell growth than with iron, maybe due the yeast's endogenous iron storage capabilities while copper will interfere with proper storage of iron, resulting in increased oxidative stress.[77] Due to these differences in mechanism, the amount of metal atoms needed to sufficiently overwhelm the yeast cells and inhibit growth drastically vary, with yeast having a lower tolerance for copper (**Figure 6**). Because copper toxic effect on yeast is exhibited mainly through the destruction of lipid membranes, the addition of VCO, a medium chain triglyceride, might have mitigated the damage by offering more source for the replacement of the membranes. Therefore, the inhibited growth of yeast when exposed to copper is rescued when treated with VCO (**Figure 12**).

The difference in reduction of oxidative stress when treated with known ROS scavengers may also vary due to the different effects the metal atoms have on the yeast. Different oxidative stress assays should be performed in order to better determine the mechanism by which the ROS scavenger and VCO treatments are functioning to raise and/or lower the oxidative stress. Glutathione redox is only one mechanism by which eukaryotic cells function to protect against oxidative stress, thus different mechanism maybe utilized to protect the yeast versus metal initiated stress such as catalase or endogenous storage molecules like ferritin. Further investigation should be conducted into the mechanism by which copper functions to inhibit cell growth as well as its rescue with VCO. Is this toxicity and rescue via VCO specific to Cu^{2+} exposed yeast samples or can this be translated to mammals?

In regard to VCO being a therapeutic option for neurodegenerative diseases such as AD and PD, more research must be conducted into the specific aspects of the oil is that is active in either reducing or possibly increasing oxidative stress. It is also possible that VCO functions to

provide therapeutic relief in a different manner such as in the reduction of the formation of toxic protein aggregates. Even though further studies are needed to evaluate the specific activity and function of VCO and other medium chain triglycerides upon neurodegenerative diseases, the use of these such health foods could be a helpful addition to the diet, assuming there is no interference with medications, in the meantime. However, if an active compound can be identified within VCO and concentrated, this could be a potential pharmaceutical treatment to alleviate the inflammatory and oxidative stress of neurodegenerative diseases.

REFERENCES

1. Kujoth, G.; Hiona, A.; Pugh, T.; Someya, S.; Panzer, K.; Wohlgemuth, S.; Hofer, T.; Seo, A.; Sullivan, R.; Jobling, W., Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. *Science* **2005,** *309* (5733), 481-484.
2. Sas, K.; Robotka, H.; Toldi, J.; Vécsei, L., Mitochondria, metabolic disturbances, oxidative stress and the kynurenine system, with focus on neurodegenerative disorders. *Journal of the neurological sciences* **2007,** *257* (1-2), 221-239.
3. Cox, M.; Nelson, D., *Lehninger Principles of Biochemistry*. 7th ed.; W.H. Freeman and Company: New York, NY, 2017.
4. Kann, O.; Kovács, R., Mitochondria and neuronal activity. *American Journal of Physiology-Cell Physiology* **2007,** *292* (2), C641-C657.
5. Kokoszka, J. E.; Coskun, P.; Esposito, L. A.; Wallace, D. C., Increased mitochondrial oxidative stress in the Sod2 (+/−) mouse results in the age-related decline of mitochondrial function culminating in increased apoptosis. *Proceedings of the National Academy of Sciences* **2001,** *98* (5), 2278-2283.
6. Wei, Y.-H.; Lu, C.-Y.; Wei, C.-Y.; Ma, Y.-S.; Lee, H.-C., Oxidative stress in human aging and mitochondrial disease-consequences of defective mitochondrial respiration and impaired antioxidant enzyme system. *Chinese Journal of Physiology* **2001,** *44* (1), 1-12.
7. Galkin, A.; Brandt, U., Superoxide radical formation by pure complex I (NADH: ubiquinone oxidoreductase) from Yarrowia lipolytica. *Journal of Biological Chemistry* **2005**.
8. Turrens, J. F., Mitochondrial formation of reactive oxygen species. *The Journal of physiology* **2003,** *552* (2), 335-344.
9. Valko, M.; Rhodes, C.; Moncol, J.; Izakovic, M.; Mazur, M., Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-biological interactions* **2006,** *160* (1), 1-40.
10. Chen, Q.; Chai, Y.; Mazumder, S.; Jiang, C.; Macklis, R.; Chisolm, G.; Almasan, A., The late increase in intracellular free radical oxygen species during apoptosis is associated with cytochrome c release, caspase activation, and mitochondrial dysfunction. *Cell death and differentiation* **2003,** *10* (3), 323.
11. Matés, J. M.; Pérez-Gómez, C.; De Castro, I. N., Antioxidant enzymes and human diseases. *Clinical biochemistry* **1999,** *32* (8), 595-603.
12. Giorgio, M.; Migliaccio, E.; Orsini, F.; Paolucci, D.; Moroni, M.; Contursi, C.; Pelliccia, G.; Luzi, L.; Minucci, S.; Marcaccio, M., Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis. *Cell* **2005,** *122* (2), 221-233.
13. Joza, N.; Susin, S. A.; Daugas, E.; Stanford, W. L.; Cho, S. K.; Li, C. Y.; Sasaki, T.; Elia, A. J.; Cheng, H.-Y. M.; Ravagnan, L., Essential role of the mitochondrial apoptosis-inducing factor in programmed cell death. *Nature* **2001,** *410* (6828), 549.
14. Miramar, M. D.; Costantini, P.; Ravagnan, L.; Saraiva, L. M.; Haouzi, D.; Brothers, G.; Penninger, J. M.; Peleato, M. L.; Kroemer, G.; Susin, S. A., NADH oxidase activity of mitochondrial apoptosis-inducing factor. *Journal of Biological Chemistry* **2001,** *276* (19), 16391-16398.
15. Dejean, L. M.; Martinez-Caballero, S.; Guo, L.; Hughes, C.; Teijido, O.; Ducret, T.; Ichas, F.; Korsmeyer, S. J.; Antonsson, B.; Jonas, E. A., Oligomeric Bax is a component of the putative cytochrome c release channel MAC, mitochondrial apoptosis-induced channel. *Molecular biology of the cell* **2005,** *16* (5), 2424-2432.
16. Slee, E. A.; Harte, M. T.; Kluck, R. M.; Wolf, B. B.; Casiano, C. A.; Newmeyer, D. D.; Wang, H.-G.; Reed, J. C.; Nicholson, D. W.; Alnemri, E. S., Ordering the cytochrome c–initiated caspase cascade: hierarchical activation of caspases-2,-3,-6,-7,-8, and-10 in a caspase-9–dependent manner. *The Journal of cell biology* **1999,** *144* (2), 281-292.

17. Morishima, N.; Nakanishi, K.; Takenouchi, H.; Shibata, T.; Yasuhiko, Y., An ER stress-specific caspase cascade in apoptosis: cytochrome c-independent activation of caspase-9 by caspase-12. *Journal of Biological Chemistry* **2002**.
18. Brunelle, J. K.; Letai, A., Control of mitochondrial apoptosis by the Bcl-2 family. *Journal of cell science* **2009,** *122* (4), 437-441.
19. Li, P.; Nijhawan, D.; Budihardjo, I.; Srinivasula, S. M.; Ahmad, M.; Alnemri, E. S.; Wang, X., Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* **1997,** *91* (4), 479-489.
20. Fan, T.-J.; Han, L.-H.; Cong, R.-S.; Liang, J., Caspase family proteases and apoptosis. *Acta biochimica et biophysica Sinica* **2005,** *37* (11), 719-727.
21. Elmore, S., Apoptosis: a review of programmed cell death. *Toxicologic pathology* **2007,** *35* (4), 495-516.
22. Gao, Y.; Signore, A. P.; Yin, W.; Cao, G.; Yin, X.-M.; Sun, F.; Luo, Y.; Graham, S. H.; Chen, J., Neuroprotection against focal ischemic brain injury by inhibition of c-Jun N-terminal kinase and attenuation of the mitochondrial apoptosis-signaling pathway. *Journal of Cerebral Blood Flow & Metabolism* **2005,** *25* (6), 694-712.
23. Finkel, T.; Holbrook, N. J., Oxidants, oxidative stress and the biology of ageing. *Nature* **2000,** *408* (6809), 239.
24. Prigione, A.; Fauler, B.; Lurz, R.; Lehrach, H.; Adjaye, J., The senescence-related mitochondrial/oxidative stress pathway is repressed in human induced pluripotent stem cells. *Stem cells* **2010,** *28* (4), 721-733.
25. Yakes, F. M.; Van Houten, B., Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. *Proceedings of the National Academy of Sciences* **1997,** *94* (2), 514-519.
26. Kupsco, A.; Schlenk, D., Oxidative stress, unfolded protein response, and apoptosis in developmental toxicity. In *International review of cell and molecular biology*, Elsevier: 2015; Vol. 317, pp 1-66.
27. Uttara, B.; Singh, A. V.; Zamboni, P.; Mahajan, R., Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Current neuropharmacology* **2009,** *7* (1), 65-74.
28. Cao, S. S.; Kaufman, R. J., Endoplasmic reticulum stress and oxidative stress in cell fate decision and human disease. *Antioxidants & redox signaling* **2014,** *21* (3), 396-413.
29. Zhang, K.; Kaufman, R. J., From endoplasmic-reticulum stress to the inflammatory response. *Nature* **2008,** *454* (7203), 455.
30. Walter, P.; Ron, D., The unfolded protein response: from stress pathway to homeostatic regulation. *Science* **2011,** *334* (6059), 1081-1086.
31. Santos, C. X.; Tanaka, L. Y.; Wosniak Jr, J.; Laurindo, F. R., Mechanisms and implications of reactive oxygen species generation during the unfolded protein response: roles of endoplasmic reticulum oxidoreductases, mitochondrial electron transport, and NADPH oxidase. *Antioxidants & redox signaling* **2009,** *11* (10), 2409-2427.
32. Travers, K. J.; Patil, C. K.; Wodicka, L.; Lockhart, D. J.; Weissman, J. S.; Walter, P., Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. *Cell* **2000,** *101* (3), 249-258.
33. Ryu, E. J.; Harding, H. P.; Angelastro, J. M.; Vitolo, O. V.; Ron, D.; Greene, L. A., Endoplasmic reticulum stress and the unfolded protein response in cellular models of Parkinson's disease. *Journal of Neuroscience* **2002,** *22* (24), 10690-10698.
34. Tabas, I.; Ron, D., Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress. *Nature cell biology* **2011,** *13* (3), 184.

35. Marciniak, S. J.; Yun, C. Y.; Oyadomari, S.; Novoa, I.; Zhang, Y.; Jungreis, R.; Nagata, K.; Harding, H. P.; Ron, D., CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. *Genes & development* **2004,** *18* (24), 3066-3077.
36. Sano, R.; Annunziata, I.; Patterson, A.; Moshiach, S.; Gomero, E.; Opferman, J.; Forte, M.; d'Azzo, A., GM1-ganglioside accumulation at the mitochondria-associated ER membranes links ER stress to Ca 2+-dependent mitochondrial apoptosis. *Molecular cell* **2009,** *36* (3), 500-511.
37. Hetz, C., The unfolded protein response: controlling cell fate decisions under ER stress and beyond. *Nature reviews Molecular cell biology* **2012,** *13* (2), 89.
38. Flora, S.; Mittal, M.; Mehta, A., Heavy metal induced oxidative stress & its possible reversal by chelation therapy. *Indian Journal of Medical Research* **2008,** *128* (4), 501.
39. Ercal, N.; Gurer-Orhan, H.; Aykin-Burns, N., Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. *Current topics in medicinal chemistry* **2001,** *1* (6), 529-539.
40. Trenam, C. W.; Blake, D. R.; Morris, C. J., Skin inflammation: reactive oxygen species and the role of iron. *Journal of investigative dermatology* **1992,** *99* (6), 675-682.
41. Cabiscol Català, E.; Tamarit Sumalla, J.; Ros Salvador, J., Oxidative stress in bacteria and protein damage by reactive oxygen species. *International Microbiology, 2000, vol. 3, núm. 1, p. 3-8* **2000**.
42. Li, Y.; Trush, M. A.; Yager, J. D., DNA damage caused by reactive oxygen species originating from a copper-dependent oxidation of the 2-hydroxy catechol of estradiol. *Carcinogenesis* **1994,** *15* (7), 1421-1427.
43. Wu, Z.; Du, Y.; Xue, H.; Wu, Y.; Zhou, B., Aluminum induces neurodegeneration and its toxicity arises from increased iron accumulation and reactive oxygen species (ROS) production. *Neurobiology of aging* **2012,** *33* (1), 199. e1-199. e12.
44. Bondy, S. C.; Kirstein, S., The promotion of iron-induced generation of reactive oxygen species in nerve tissue by aluminum. *Molecular and chemical neuropathology* **1996,** *27* (2), 185-194.
45. Coskun, P. E.; Beal, M. F.; Wallace, D. C., Alzheimer's brains harbor somatic mtDNA control-region mutations that suppress mitochondrial transcription and replication. *Proceedings of the National Academy of Sciences* **2004,** *101* (29), 10726-10731.
46. Reddy, P. H.; Beal, M. F., Amyloid beta, mitochondrial dysfunction and synaptic damage: implications for cognitive decline in aging and Alzheimer's disease. *Trends in molecular medicine* **2008,** *14* (2), 45-53.
47. Swerdlow, R. H.; Burns, J. M.; Khan, S. M., The Alzheimer's disease mitochondrial cascade hypothesis. *Journal of Alzheimer's Disease* **2010,** *20* (s2), S265-S279.
48. Yan, M. H.; Wang, X.; Zhu, X., Mitochondrial defects and oxidative stress in Alzheimer disease and Parkinson disease. *Free Radical Biology and Medicine* **2013,** *62*, 90-101.
49. Coskun, P.; Wyrembak, J.; Schriner, S. E.; Chen, H.-W.; Marciniack, C.; LaFerla, F.; Wallace, D. C., A mitochondrial etiology of Alzheimer and Parkinson disease. *Biochimica et Biophysica Acta (BBA)-General Subjects* **2012,** *1820* (5), 553-564.
50. Walton, J., Aluminum in hippocampal neurons from humans with Alzheimer's disease. *Neurotoxicology* **2006,** *27* (3), 385-394.
51. Kawahara, M.; Kato-Negishi, M., Link between aluminum and the pathogenesis of Alzheimer's disease: the integration of the aluminum and amyloid cascade hypotheses. *International journal of Alzheimer's disease* **2011,** *2011*.
52. Rondeau, V.; Commenges, D.; Jacqmin-Gadda, H.; Dartigues, J.-F., Relation between aluminum concentrations in drinking water and Alzheimer's disease: an 8-year follow-up study. *American Journal of Epidemiology* **2000,** *152* (1), 59-66.
53. Multhaup, G.; Schlicksupp, A.; Hesse, L.; Beher, D.; Ruppert, T.; Masters, C. L.; Beyreuther, K., The amyloid precursor protein of Alzheimer's disease in the reduction of copper (II) to copper (I). *Science* **1996,** *271* (5254), 1406-1409.

54. Barnham, K. J.; McKinstry, W. J.; Multhaup, G.; Galatis, D.; Morton, C. J.; Curtain, C. C.; Williamson, N. A.; White, A. R.; Hinds, M. G.; Norton, R. S., Structure of the Alzheimer's disease amyloid precursor protein copper binding domain A regulator of neuronal copper homeostasis. *Journal of Biological Chemistry* **2003,** *278* (19), 17401-17407.
55. Sparks, D. L.; Schreurs, B. G., Trace amounts of copper in water induce β-amyloid plaques and learning deficits in a rabbit model of Alzheimer's disease. *Proceedings of the National Academy of Sciences* **2003,** *100* (19), 11065-11069.
56. Syme, C. D.; Nadal, R. C.; Rigby, S. E.; Viles, J. H., Copper binding to the amyloid-beta (Aβ) peptide associated with Alzheimer's disease. Folding, coordination geometry, pH dependence, stoichiometry and affinity of Aβ (1-28); insights from a range of complementary spectroscopic techniques. *Journal of Biological Chemistry* **2004**.
57. Duce, J. A.; Tsatsanis, A.; Cater, M. A.; James, S. A.; Robb, E.; Wikhe, K.; Leong, S. L.; Perez, K.; Johanssen, T.; Greenough, M. A., Iron-export ferroxidase activity of β-amyloid precursor protein is inhibited by zinc in Alzheimer's disease. *Cell* **2010,** *142* (6), 857-867.
58. Cuajungco, M. P.; Goldstein, L. E.; Nunomura, A.; Smith, M. A.; Lim, J. T.; Atwood, C. S.; Huang, X.; Farrag, Y. W.; Perry, G.; Bush, A. I., Evidence that the β-amyloid plaques of Alzheimer's disease represent the redox-silencing and entombment of Aβ by zinc. *Journal of Biological Chemistry* **2000,** *275* (26), 19439-19442.
59. Bender, A.; Krishnan, K. J.; Morris, C. M.; Taylor, G. A.; Reeve, A. K.; Perry, R. H.; Jaros, E.; Hersheson, J. S.; Betts, J.; Klopstock, T., High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. *Nature genetics* **2006,** *38* (5), 515.
60. Uversky, V. N.; Li, J.; Fink, A. L., Metal-triggered structural transformations, aggregation, and fibrillation of human α-synuclein a possible molecular link between parkinson' s disease and heavy metal exposure. *Journal of Biological Chemistry* **2001,** *276* (47), 44284-44296.
61. Hsu, L. J.; Sagara, Y.; Arroyo, A.; Rockenstein, E.; Sisk, A.; Mallory, M.; Wong, J.; Takenouchi, T.; Hashimoto, M.; Masliah, E., α-Synuclein promotes mitochondrial deficit and oxidative stress. *The American journal of pathology* **2000,** *157* (2), 401-410.
62. Bondy, S. C., The neurotoxicity of environmental aluminum is still an issue. *Neurotoxicology* **2010,** *31* (5), 575-581.
63. Youdim, M. B.; Stephenson, G.; Shachar, D. B., Ironing iron out in Parkinson's disease and other neurodegenerative diseases with iron chelators: a lesson from 6-hydroxydopamine and iron chelators, desferal and VK-28. *Annals of the New York Academy of Sciences* **2004,** *1012* (1), 306-325.
64. Rasia, R. M.; Bertoncini, C. W.; Marsh, D.; Hoyer, W.; Cherny, D.; Zweckstetter, M.; Griesinger, C.; Jovin, T. M.; Fernández, C. O., Structural characterization of copper (II) binding to α-synuclein: Insights into the bioinorganic chemistry of Parkinson's disease. *Proceedings of the National Academy of Sciences* **2005,** *102* (12), 4294-4299.
65. Davies, K. M.; Bohic, S.; Carmona, A.; Ortega, R.; Cottam, V.; Hare, D. J.; Finberg, J. P.; Reyes, S.; Halliday, G. M.; Mercer, J. F., Copper pathology in vulnerable brain regions in Parkinson's disease. *Neurobiology of aging* **2014,** *35* (4), 858-866.
66. Fernando, W. M. A. D. B.; Martins, I. J.; Goozee, K.; Brennan, C. S.; Jayasena, V.; Martins, R. N., The role of dietary coconut for the prevention and treatment of Alzheimer's disease: potential mechanisms of action. *British Journal of Nutrition* **2015,** *114* (1), 1-14.
67. Kappally, S.; Shirwaikar, A.; Shirwaikar, A., Coconut oil–a review of potential applications. *Hygeia JD Med* **2015,** *7*, 34-41.
68. Lockyer, S.; Stanner, S., Coconut oil–a nutty idea? *Nutrition Bulletin* **2016,** *41* (1), 42-54.
69. Nevin, K.; Rajamohan, T., Virgin coconut oil supplemented diet increases the antioxidant status in rats. *Food chemistry* **2006,** *99* (2), 260-266.

70. Farr, S. A.; Price, T. O.; Dominguez, L. J.; Motisi, A.; Saiano, F.; Niehoff, M. L.; Morley, J. E.; Banks, W. A.; Ercal, N.; Barbagallo, M., Extra virgin olive oil improves learning and memory in SAMP8 mice. *Journal of Alzheimer's Disease* **2012,** *28* (1), 81-92.
71. Tsao, R., Chemistry and biochemistry of dietary polyphenols. *Nutrients* **2010,** *2* (12), 1231-1246.
72. Perron, N. R.; Hodges, J. N.; Jenkins, M.; Brumaghim, J. L., Predicting how polyphenol antioxidants prevent DNA damage by binding to iron. *Inorganic Chemistry* **2008,** *47* (14), 6153-6161.
73. Intahphuak, S.; Khonsung, P.; Panthong, A., Anti-inflammatory, analgesic, and antipyretic activities of virgin coconut oil. *Pharmaceutical biology* **2010,** *48* (2), 151-157.
74. Nevin, K.; Rajamohan, T., Beneficial effects of virgin coconut oil on lipid parameters and in vitro LDL oxidation. *Clinical biochemistry* **2004,** *37* (9), 830-835.
75. Chin, P.-j., Analysis of Cellular Responses to Heavy Metal-induced Stress in Saccharomyces cerevisiae. **2014**.
76. Ezaki, B.; Gardner, R. C.; Ezaki, Y.; Kondo, H.; Matsumoto, H., Protective roles of two aluminum (Al)-induced genes, HSP150 and SED1 of Saccharomyces cerevisiae, in Al and oxidative stresses. *FEMS microbiology letters* **1998,** *159* (1), 99-105.
77. Shanmuganathan, A.; Avery, S. V.; Willetts, S. A.; Houghton, J. E., Copper-induced oxidative stress in Saccharomyces cerevisiae targets enzymes of the glycolytic pathway. *FEBS letters* **2004,** *556* (1-3), 253-259.