The brain is the most complex organ in the human body. It is the center of the nervous system giving it control over other organs in the body. It is mainly composed of two types of cells; glial cells that provide support and neurons. The largest part of the brain, the cerebral cortex, is thought to contain 15-33 billion neurons. Neurons are critical because they send signals via neurotransmitters to other cells over large distances.

Electrochemical processes are also used by the brain for neuronal signaling and it is well-known that neurons must sustain an electrical charge in order to function properly. This causes the brain to require a substantial amount of energy, in fact, 20-25% of human basal metabolism is devoted to the brain, and most of this energy is provided by oxygen-dependent glucose metabolism.

Blood vessels supply the brain with oxygen and glucose and are also required for metabolic end product removal. In humans, the brain receives up to 20% of the blood flow from the heart. If blood flow to the brain stops, brain function ceases within seconds and neuronal damage may occur within minutes.

The normal neuronal-vascular relationship is vital to healthy brain functioning, and it is believed that nearly every neuron in the human brain has its very own capillary, composed of endothelial cells. Endothelial cell tight junctions within the blood brain barrier (BBB) limit the entry of molecules and immune cells into the brain from the blood, although beneficial small molecules such as glucose easily cross the BBB. Furthermore, toxins or certain immune cells that may cross the BBB and enter the brain due to injury, neurodegenerative processes, inflammation, or vascular disorder, often damage neurons. Interestingly, the brain was thought for many years to be an immune privileged (isolated from immune system) site, due to the presence of the BBB, however, it is now known that the brain is capable of mounting immune and inflammatory responses to a variety of insults including trauma (injury), stroke, infection, and toxins.
Within this paper, we review evidence from research funded by McCord Research that antioxidant phytonutrients found in Olivamine-containing supplements can help decrease the damage to mitochondria and endothelial cells caused by oxidative stress. As mentioned, antioxidant defense enzymes protect against oxidative damage. One antioxidant enzyme in particular, manganese superoxide dismutase (MnSOD), is critical for the removal of the ROS known as superoxide, generated from mitochondria. An increase in ROS levels and subsequent changes in mitochondrial morphology (associated with mitochondrial damage) of aged cells may result from an increase in the production of ROS or a decrease in ROS removal due to changes in cellular antioxidant capacity.14

Metabolically active brain cells have hundreds to thousands of mitochondria per cell.9 Mitochondria are the energy powerhouses that are vital for brain cell and other cell functioning. On the other hand, mitochondria also produce free radicals during energy metabolism, and can contribute substantially to oxidative stress when damaged or dysfunctional. Free radicals are unstable because they contain unpaired electrons resulting in their capacity to steal electrons from other molecules in a process known as oxidation. Oxidative stress results from the inability of cells to eliminate free radicals known as reactive oxygen species (ROS) using the natural antioxidant defense system that includes defense enzymes such as superoxide dismutases (SOD). Free radical damage can involve damage to cell membranes, DNA, proteins, lipids, and organelles including mitochondria. Oxidative stress has been linked with many inflammatory-associated diseases including Alzheimer’s and Parkinson’s disease.10

Cognitive decline and neurodegeneration are thought to result from many processes including vasculature reduction and/or endothelial dysfunction, mitochondrial dysfunction, oxidative stress and inflammation.7,11-13

As described previously by University of Iowa researcher, Dr. Ehab Sarsour, an established scientist funded by McCord research (for these studies), the important antioxidants, hydroxytyrosol and N-acetyl-L-cysteine found in Olivamine, activate the critical antioxidative defense enzyme, MnSOD.15,16 As shown in this paper, MnSOD was discovered to protect mitochondria from age-related oxidative damage.
In other studies, Olivamine was found to reduce levels of the inflammatory marker, C-reactive protein (CRP) by approximately 70%. In addition, recent results included in this paper from the laboratory of Dr. Thomas Karagiannis (a world-renowned researcher at the prominent Baker IDI Institute in Melbourne Australia) reveal that the phytoantioxidant sulforaphane substantially reduces inflammatory signaling molecules. Furthermore, as shown here, Olivamine was discovered by Dr. Karagiannis to decrease endothelial cell dysfunction and to increase angiogenesis, which may help improve brain vasculature.

Because levels of ROS increase with age and MnSOD protects against ROS, the hypothesis that MnSOD activity protects mitochondria from age-associated damage was investigated by Dr. Ehab Sarsour. It was demonstrated that over-expression of MnSOD protects mitochondria in normal human fibroblasts (NHF) from age-associated abnormalities (Figures 1 and 2).
Figure 2: A. MnSOD genotype MEFs were cultured in 4% oxygen environment and harvested for transmission electron microscopy visualization of mitochondria morphology; scales range from 100nm to 0.2µm. Representative of 2 or more experiments. B. MnSOD genotype MEFs cultured in 4% oxygen environment were incubated with MitoTracker (left panel), and MitoSox (right panel). Fluorescence was measured by flow cytometry. Asterisks (left panel) indicate significant difference in MitoTracker fluorescence in MnSOD (−/−) compared to (+/+ ) and (+/−) MEFs; asterisks in right panel represent significant difference in MitoSOX fluorescence in MnSOD (+/+) and (+/−) compared to MnSOD (−/−) MEFs; n=3, p<0.05.
L-Sulforaphane (LSF) Decreases the Secretion of Inflammatory Molecules from Normal PBMCs.

Figure 3: Sulforaphane (LSF) decreases the secretion of inflammatory molecules from normal PBMCs. Levels of IL-6 and TNF-α (cytokines), and MIP-1β and IP-10 (chemokines) in supernatants from PBMC samples stimulated with 1µM Trichostatin A (TSA), 10µM suberoyanilide hydroxamic acid (SAHA), 10mM sodium butyrate (NaB), 15µM LSF, 30µM LSF or PBS (untreated) were measured using a Milliplex® MAP Human cytokine/chemokine kit. The plate was read using a Luminex 100™ IS instrument and software package (Luminex Corporation, Texas, USA) and the mean fluorescent intensity data analyzed using a weighted 5-parameter logistic method to yield cytokine/chemokine concentrations (pg/mL) in the supernatants.
The effects of differential expression of MnSOD were further investigated in murine embryonic fibroblasts (MEFs) from mice genetically engineered to not express MnSOD (knockout mice). Knockout (gene is “knocked out”) mice that are homozygous are described as MnSOD (-/-), or heterozygous (+/-) if the gene in only one allele is knocked out. Consistent with the results from MnSOD overexpression, knockout and heterozygous mice showed varying levels of mitochondrial structural damage, whereas normal mice (MnSOD+/+) showed none. A mitochondrial-specific dye was used to measure the abundance of mitochondria, which was lower in MEFs with lower expression of MnSOD [MnSOD (+/-) and MnSOD (-/-)], suggesting that MnSOD protects mitochondria from damage and subsequent loss from cells.

**Olivamine improves cell migration by 60% in human umbilical vein endothelial cells, following treatment with high glucose**

(Pre-treatment for 72 hours with 30mM glucose)

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**Figure 4:** Olivamine improved cell migration and wound healing in human umbilical vein endothelial cells (HUVEC) pre-treated with high glucose (30 mM) for 72 hours. Hydroxytyrosol also improved wound healing compared to untreated cells.
Research funded by McCord Research from the laboratory of Dr. Thomas Karagiannis at the Baker IDI has revealed that the potent antioxidant, sulforaphane (LSF) also possesses important anti-inflammatory activities (Figure 3), as indicated by substantially decreased inflammatory immune signaling molecule (cytokine and chemokine) expression from normal peripheral blood mononuclear cells (PBMCs) stimulated with LSF compared to controls (TSA, SAHA, NaB).

Endothelial dysfunction has been associated with oxidative stress. Results from the laboratory of Dr. Karagiannis reveal that Olivamine (also referred to as the McCord Formula) decreases endothelial dysfunction induced by oxidative stress-associated high glucose or doxorubicin exposure of endothelial cells. In fact, cell migration was improved by 60%, (normal functioning levels, as measured by migration) following treatment with Olivamine.

Endothelial cells were also found to have increased vascular formation following treatment with Olivamine again suggesting that Olivamine is beneficial to vasculature such as capillaries that are associated with neurons in the brain.

Figure 5: Olivamine improves angiogenesis (vascular tube formation) in human umbilical vein endothelial cells (HUVEC). Mean ± standard deviations from a single experiment performed in triplicate are shown; total of 2 independent experiments tested.
In summary, small molecule antioxidants found in Olivamine supplements that easily cross the BBB such as hydroxytyrosol, N-acetyl-L-cysteine, and sulforaphane have important brain health enhancing properties including inflammation reducing capabilities, mitochondrial protective properties, endothelial function enhancing capabilities, and other properties that benefit vasculature, such as the ability to stimulate vascular growth (angiogenesis). In addition, other important Olivamine brain health supplement antioxidant ingredients that cross the BBB, including curcumin and taurine, have been shown to support neurons and mitochondria.\textsuperscript{18,19}

Furthermore, increasing evidence indicates that there is a connection between the brain and the digestive system or gut (sometimes referred to as the second brain), which has its own nervous system. It has been shown that the complex microbiome or community of microorganisms in the gut greatly influences the brain through several routes of communication including chemical signaling, neuronal signaling, and immune system interactions.\textsuperscript{20,21} The health of the gut is strongly connected to the health of the brain as they are greatly dependent upon one another. Therefore, Olivamine-containing supplement ingredients that are easily absorbed and bioavailable can enhance brain health on two important levels.

McCord Research’s investigation of the role of hydroxytyrosol, N-acetyl-L-cysteine, sulforaphane and Olivamine in brain health is far more extensive than has been revealed in this paper.

The purpose of this document is to present some of our basic research while protecting the most sensitive and protected findings. Olivamine represents high quality, but relatively low cost, scientifically-based, effective brain health support.

References