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The Interconnectivity of Parkinson's disease and Type Two Diabetes Mellitus

Erica Olfson
ericaolfson@yahoo.com

THE INTERCONNECTIVITY OF PARKINSON'S DISEASE AND TYPE TWO DIABETES MELLITUS

Erica Olfson

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Nick Magdich Date 4/7/21

Thesis Director

Date 4/6/21

Committee Member

Chris Banton Date 4/6/21

Committee Member

Accepted for the Honors Council and Honors Program:

_____ Date _____

Dr. Bonnie Smith Whitehouse, Director
The Honors Program

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1. Introduction- Parkinson's disease and Type II Diabetes Mellitus Pathology

PARKINSON'S DISEASE

Parkinson's disease (PD), the most prevalent neurodegenerative disease among elderly people, is caused by the degradation and eventual death of dopamine neurons in the substantia nigra pars compacta (Manoharan et al, 2016). Neurons are specialized, yet fundamental, cells in the brain, and one of two main types of cells in the central nervous system. Neurons are also referred to as nerve cells. There are also glial cells in the brain, which outnumber the neurons. However, the neurons are the most important cells in the brain because of the offensive role they hold, in comparison to the supportive role glial cells have (Woodruff, 2019). Dopaminergic neurons are the main source of dopamine, a specific neurotransmitter, in humans. Neurotransmitters are chemical messengers which are transmitted from a neuron's synapse to another neuron, cell, or gland. Neurotransmitters are used to signal an action or response by the receiver, and dopamine specifically is a monoamine neurotransmitter. Dopamine is known for its role in reward and motivation pathways. Dopamine also plays a pivotal role in motor movement pathways, cognitive function, and even reproductive behaviors. Normally, dopamine is release from an axon, and it then binds to G protein-coupled receptors. From there, dopamine signals a specific action or passes on a message to the postsynaptic neuron. Most dopaminergic neurons are found in the mesencephalon, specifically the nigrostriatal system, which is the region where the substantia nigra pars compacta is located. The dopaminergic neurons located specifically within the substantia nigra pars compacta are both indirectly and directly involved in voluntary and involuntary muscle motor movement.

When dopaminergic neurons are degraded, or eventually die, many issues arise due to the shortage, or lack entirely, of dopamine. As dopaminergic neurons degrade, they are less able to

produce dopamine. And when the dopamine neurons completely die, they no longer produce dopamine entirely. Dopamine is very important, and for this reason a deficiency of dopamine causes the movement difficulties commonly linked with Parkinson's disease. When there is a lack of dopamine, the thalamus is less able to be regulated. When there is decreased regulation of the thalamus, the motor cortex is regulated less as well, and this in turn leads to the movement issues associated with Parkinson's disease. Although many symptoms are associated with the diagnosis of Parkinson's disease, by far the most common is the loss of muscle control. Specifically, the muscular and movement symptoms include inability to switch directions, difficulties with balance, tremors, stiffness, inability to coordinate movements together, and bradykinesia. After diagnosis, many patients also struggle with mental health difficulties, memory loss, irregular blood pressure and heart rate, and other common symptoms associated with dementia and rapid aging (Parkinson's disease, 2017). The reason for many of these other symptoms is that patients with Parkinson's disease also suffer with a loss of the nerve endings that produce norepinephrine. Norepinephrine is critical to the sympathetic nervous system, which oversees blood pressure, heart rate, and other necessary, automatic functions of the body (Parkinson's disease, 2017).

Parkinson's disease is often times spoken of in conjunction with dementia and Alzheimer's disease. Dementia, the general memory loss and thinking difficulty that some people encounter with old age, is always linked to Alzheimer's disease (What is dementia?, 2019). However, not all patients who are diagnosed with Parkinson's disease will experience dementia. While dementia is not one single disease, the term dementia does include a wide range of neurological conditions such as Alzheimer's Disease. Alzheimer's Disease, which is the most common cause of dementia, is linked to the buildup of plaques, as well as tangles of tau protein,

in the brain (What is dementia?, 2019). When there is a buildup of plaques, brain cells are damage, and thus the brain does not function normally. Cellular damage leads to memory loss, difficulties with communication, and irregular behavior, many of which are common symptoms of Alzheimer's disease patients. Specifically in Alzheimer's disease, cells in the hippocampus are damaged, which is incredibly troublesome as the hippocampus is the headquarters for learning and memory in the human brain. Whereas dementia is one of the first symptoms of Alzheimer's disease, dementia develops slower and further on in the disease progression for patients with Parkinson's disease. Additionally, if a patient with Parkinson's disease develops dementia, their life is impacted considerably more than a patient with only Alzheimer's disease, as this dementia is heaped onto motor behavior issues as well. Overall, dementia in both Parkinson's disease and Alzheimer's disease is developed due to change in the structure and functioning of cells in the human brain. When cells are damaged in the brain and unable to function properly or fully, the patient unfortunately faces various severe complications.

GENESIS OF PARKINSON'S DISEASE

There are some cases of Parkinson's disease which are due to genetic mutations being passed on through familial lines, but there are also many cases of Parkinson's disease which are caused by idiopathic mechanisms, meaning that the cause is spontaneous and not related to inherited genes (National Institute on Aging, 2017). In regards to genetic inheritance of Parkinson's disease, the disease can be inherited through an autosomal dominant pattern, or through an autosomal recessive pattern. The autosomal dominant pattern is seen in the alteration of the genes *LRRK2* or *SNCA*. *LRRK2* is a gene that codes for the formation of the protein dardarin. Dardarin has kinase activity, and is known to interact with other proteins because of its "leucine-rich region" (*LRRK2* gene: MedlinePlus Genetics, 2020). There have been over 100

mutations identified in the *LRRK2* gene which causes single amino acids to be replaced, thus altering the structure and function of dardarin. A majority of these mutations happen near the kinase domain, and thus influence the protein's ability to transfer phosphate groups. It is still unknown how these gene mutations lead to the symptoms of Parkinson's disease. As far as *SNCA*, this gene instructs the cell on how to make "alpha synuclein" which is an abundant protein in the brain (*SNCA* gene: MedlinePlus Genetics, 2020). Alpha-synuclein is mainly located in the tips of neurons in presynaptic terminals, which is where neurotransmitters are released from synaptic vesicles. Alpha-synuclein is thought to be involved in maintaining the synaptic vesicles in the presynaptic terminals. The correct distribution and acceptance of neurotransmitters by neurons is incredibly important to the healthy functioning of the brain. However, when there is a mutation in *SNCA*, of which there are at least 30 known ones, and alpha synuclein is not properly formed, there is not proper maintenance of synaptic vesicles, and thus the neuronal pathway is harmed. Overall, because these genes are autosomal dominant, it only requires one copy of the mutated gene to be passed onto an offspring for the offspring to experience the effects of the mutated gene.

The autosomal recessive pattern is slightly different in that it is linked to mutations in *PARK7, PINK1,* and *PRKN*. *PARK7* gene tells the cell how to make the DJ-1 protein which is thought to provide protection to cells from oxidative stress (*PARK7* gene: MedlinePlus Genetics, 2020)). When one of the over 25 known mutations in this gene occurs, a smaller DJ-1 protein is formed, and this makes the entire protein unstable and unable to function properly. The *PINK1* gene is only slightly different than the *PARK7* gene in that it instructs the cell on how to make PTEN induced putative kinase 1, which is found all throughout the body. Similarly to DJ-1, PTEN induced putative kinase 1 protects the cell, but specifically from cellular stress (*PINK1*

gene: MedlinePlus Genetics, 2020). Lastly, *PRKN* is one of the largest and most commonly expressed human genes, and it instructs the cell on how to make parkin. Parkin is pivotal to the breaking down and tagging of unnecessary and excess proteins (*PRKN* gene: MedlinePlus Genetics, 2020). Whenever parkin suffers from one of its over 200 mutations, it is made to be smaller or with a different structure. Because of the mutation, parkin is now unable to tag and breakdown the necessary proteins, and thus is nonfunctional and degraded by cells. Overall, because these genes are autosomal recessive, it takes a copy to be passed from the father and another copy to be passed from the mother in order for the offspring to display the recessive phenotype.

Idiopathic mechanisms are causes for diseases that are not related to inherited genetics but are spontaneous, having nothing to do with the genes that were passed on to the patient. Additionally, there is recent research stating that there are many toxic chemicals which are known to cause Parkinson's disease, such as paraquat. Although familial causes and idiopathic causes are innately different, both relate back to oxidative stress and the role of reactive oxygen species in the brain. In the book *Ending Parkinson's Disease: A Prescription for Action,* significant exposure to paraquat, a weed killer, causes people to be "2.5 times more like to have Parkinson's than those who" were not exposed to it (Dorsey, Bloem, Okun, & Sherer, 76) Another very common, and very harmful toxic chemical linked to Parkinson's disease is Trichlorethylene (TCE). TCE is a colorless liquid chemical which, when ingested, was found to cause numerous different types of cancers, miscarriages, and Parkinson's disease (Dorsey, Bloem, Okun, & Sherer, 89). TCE is very commonly found in paint removers, stain removers, and various degreasing products (Dorsey, Bloem, Okun, & Sherer, 89). Although there are many other chemicals and toxins which are linked to the onset and progression of Parkinson's disease,

the main point is that repeated or long-term exposure to, and especially ingestion of, toxic substances leads to major cellular damage in the brain, which leads to dopaminergic damage and death. This then leads to dopamine deficiency, and then the beginning of and continuation of Parkinson's disease. In regards to spontaneous, non-environmental causes of Parkinson's disease, "mild traumatic brain [injuries]" in veterans are known to also "increase the risk of developing Parkinson's by more than 50%" (Dorsey, Bloem, Okun, & Sherer, 101). The connection between brain injury and Parkinson's disease is also exhibited in athletes. When the brain undergoes a traumatic injury, causing cellular neuronal damage, the neurons are then unable to function properly, not producing the correct neurotransmitters, such as dopamine or glutamate, and leading to a malfunctioning of the injured area of the brain. In conclusion, there are many causes of Parkinson's disease, but all causes and pathways to the onset of the disease ultimately result in degraded or dead dopaminergic neurons, thus a deficiency of dopamine, and finally major motor movement issues.

DIABETES (GENERAL)

Diabetes is a medical condition, of which there are three major types, which is characterized by hyperglycemia, or high blood sugar. Glucose, or sugar, exists in our blood as the main source of energy in humans, and the glucose is taken out of the foods we ingest every day. Normally, insulin, which is a hormone, assists in the process of taking the glucose in our bloodstream which comes in the food we eat, and transporting the glucose into our cells to be used as energy. However, in patients with diabetes, their bodies do not produce enough insulin, any insulin at all, or their cells have become resistant to insulin's actions. Thus, these patients simply have all of this free glucose, taken from their food, floating in their blood, causing their blood sugar concentration to be higher than normal. Not only is this an issue because the

concentration of glucose in the blood should not be higher than normal as this can lead to further problems within the body, but also this means that the cells in the patient's body are not getting the glucose from the food properly, or in adequate enough supplies, to function properly and complete their many cellular processes.

The three types of diabetes, type 1, type 2, and gestational, differ mainly in regard to the issues with insulin. Type I diabetes mellitus is considered an autoimmune disorder, as the body attacks the insulin producing cells, Islet beta cells, in the pancreas, and thus this person does not produce insulin at all (Type 1 diabetes, 2020). Because of this, patients with type 1 diabetes mellitus rely completely on insulin injections to decrease the amount of free sugar in the bloodstream. Gestational diabetes, the third type of diabetes, is seen only in pregnant women, and occurs when the body cannot keep up with the increased amount of insulin needed during pregnancy (Symptoms & causes of gestational diabetes, 2017). In addition to the inability to produce enough insulin to keep up with the body's requirements, many pregnant women also suffer from insulin resistance which is caused by the special hormones, specifically human placental lactogen (HPL) and human chorionic gonadotropin (hCG), which are produced in the body during pregnancy. These hormones unfortunately cause the cells to not utilize the insulin available to them as well as normal, and thus they develop insulin resistance (Symptoms & causes of gestational diabetes, 2017). Most women do experience insulin resistance but are able to overcome this condition by producing enough insulin. However, the women who are not able to overcome it will develop gestational diabetes for the duration of their pregnancy.

TYPE TWO DIABETES MELLITUS

Type 2 diabetes mellitus (T2DM) is one of three major forms of diabetes, and it is the most common type of diabetes. T2DM is characterized by the patient's body not utilizing insulin

properly, or not making enough insulin in the first place (JDRF). Furthermore, patients with T2DM can become resistant to insulin, in a similar manner to those patients with gestational diabetes. This insulin resistance is an even greater problem, as even if the body is producing enough insulin, the cells are simply not using the insulin effectively. Type 2 diabetes is different from type 1 diabetes in that it typically does not emerge until later in life, and it is additionally very preventable. Type 2 diabetes does not typically develop until later on in life is because it is mostly caused by lifestyle factors such as physical activity level, diet and nutrition, obesity, and excess body fat. Most patients who are diagnosed with T2DM are 45 or older, and have extra belly fat, or fall into the categories of overweight or obese (National Institute of Diabetes and Digestive and Kidney Disease, 2017).

Regardless of whether or not the patient's body is not making enough insulin, or just not using the insulin effectively, the glucose the patient consumes from their food stays in the patient's bloodstream and is not able to be utilized by the cells of the body. The inability of glucose to be utilized by the cells is problematic, as these cells need the energy provided by the glucose to carry out various cellular functions. Additionally, these glucose molecules build up in the bloodstream, leading to high blood sugar levels. High blood sugar levels are dangerous as they can lead to cardiovascular diseases, nerve and kidney damage, bone and joint issues, and much more (Mayo Clinic Staff, 2018). For many people, type 2 diabetes can be prevented, or at least managed, by altering diet and nutritional intake, exercising, managing blood pressure and cholesterol levels, and taking medications. Because excess weight causes the insulin resistance seen in patients with type 2 diabetes, losing some weight and regaining a healthy body mass index can be a very effective treatment against type 2 diabetes mellitus. Most medications, such as insulin injections and metformin, lower blood sugar and help the body better handle glucose

(National Institute of Diabetes and Digestive and Kidney Disease, 2017). Additionally, some patients may also have to take medications to treat the high blood pressure, high cholesterol, and other conditions which can be caused by type 2 diabetes. In conclusion, type 2 diabetes differs significantly from type 1 diabetes and gestational diabetes in that it is preventable, and mainly linked to lifestyle decisions which can cause an excess of glucose in the blood stream, or insulin resistance.

2. **Reactive Oxygen Species (ROS), Glycation, and Advanced Glycation End-products (AGEs) in the context of Parkinson's disease**

Oxidative stress, which is a common thread in diseases such as cancer, Alzheimer's disease, and heart disease, in the substantia nigra leads to dopaminergic degeneration due to its severe effects on the dopamine neurons. The damage which oxidative stress causes is a characteristic attribute of people with Parkinson's disease. Oxidative damage, which is caused by oxidative stress, is mainly seen due to three chemical imbalances: the first being the accumulation of free radicals which react with other compounds to produce reactive oxygen species (ROS), the second being a decrease in available and active antioxidants, and the third being the accumulation of advanced glycation end products (AGEs) due to glycation (Hwang, 2013). Oxidative damage is so detrimental to the brain because it, over time, destroys the dopaminergic neurons due to the toxicity of the compounds, and this destruction of the neurons means that the dopamine pathway does not function properly. Although there is not a selectively toxic effect of oxidative damage only to dopaminergic neurons, dopaminergic neuron damage is the cause for the symptoms and disease state of Parkinson's disease. When the dopamine pathway in the brain does not function as it is supposed to, that is when patients begin to develop locomotive issues, and when they have issues with muscle control in general. Eventually, the

dopaminergic neurons will die due to the amount of harm the oxidative damage has had on them, and the full loss of dopaminergic neurons leads to the progression of the symptoms associated with Parkinson's disease, and the progression of the actual condition of the disease.

FREE RADICALS AND REACTIVE OXYGEN SPECIES

In a normally healthy body, there are both free radicals and antioxidants present. The formation of these free radicals is an "inevitable byproduct of metabolism" and the main source in humans of these species is the mitochondrial respiratory chain which produces an abundance of free electrons which can be transferred to molecular oxygen, forming the superoxide anion, the most common free radical (Nowotny, Junh, Höhn, Weber, & Grune, 2015). Free radicals are specific kinds of molecular species which contain unpaired electrons in one of their orbitals, and when they interact with other compounds, they produce reactive oxygen species (ROS) (Lobo, Patil, Phatak, & Chandra, 2010). Because of these unpaired electrons, free radicals are highly reactive and highly unstable. Free radicals can either be electron donors, or electron gainers, and because of this they are capable of reacting with many different species. However, when free radicals react with other species in cells, they cause damage to these other species which can be "molecules such as DNA, proteins, carbohydrates, and lipids" (Lobo, Patil, Phatak, & Chandra, 2010). These macromolecules will be oxidized by the free radicals, due to their unpaired electrons, and will slowly accumulate in the brain. The oxidized biomolecules, which were created through reaction with free radicals, are reactive oxygen species. The accumulation of oxidized macromolecules, the ROS, will cause significant harm to the neurons they come in contact with, and this is the most common mechanism of free radicals, and thus ROS, causing oxidative damage and stress. A few of the most common free radicals in the brain include

hydroxyl radical, superoxide anion radical, hydrogen peroxide, and oxygen singlet (Lobo, Patil, Phatak, & Chandra, 2010).

Normally, there is a large enough amount of antioxidants to then counteract the damage done by the free radicals which react with the other macromolecules, and this balance is a part of homeostasis. However, when a patient is diagnosed with Parkinson's disease, they almost always have an imbalance of antioxidants and increased levels of "oxidized lipids, proteins, and DNA" in their substantia nigra (Hwang, 2013). This means that patients with Parkinson's disease either have a plethora of free radicals, or a deficit in their amount of antioxidants, which means that they are unable to maintain this careful balance between the harm of the free radicals and the positive action of the antioxidants. Instead, the dopaminergic neurons in the substantia nigra are damaged to a point beyond repair, and over time this damage causes the signs and symptoms associated with the diagnosis, and progression, of Parkinson's disease.

ANTIOXIDANTS

Antioxidants are compounds which are found in various different foods such as berries and artichokes, and are also made in the body. Antioxidants work to counteract natural aging and any damage to cells all over the body, but especially in the brain (How do antioxidants lend themselves to brain health?, 2019). In regards to the brain, antioxidants will offset the damage done by free radicals, which are the acting agents of oxidative stress. When the levels of antioxidants are reduced in the brain, there are less molecules to fight back against the free radicals, which means more oxidative damage is done to the neurons in the brain and there is nothing to counteract this damage. Although not all of the body's antioxidants come from food sources, the cells of the brain do not produce antioxidants on their own, and thus consuming antioxidants and ensuring bodily levels of antioxidants remain high is very important. In addition

to the already low levels of antioxidants in the brain of Parkinson's disease patients, there is a decreased antioxidant ability that is seen after the progression of the oxidative damage, after diagnosis (Chen et al., 2009). Even if there are plenty of antioxidants available to balance out the damage done by ROS, and the reactions they initiate, there is seen to be a decreased capacity for these antioxidants to fully fight off the damage due to the conditions and environment in a brain that has developed Parkinson's disease.

DNA DAMAGE

In addition to the negative correlation of antioxidants in connection with oxidative damage, there is also an increase in the amount of reactive oxygen species in the brain of patients with Parkinson's disease. The accumulation of these elements has a toxic effect on the brain. The damage done by the ROS produced through free radicals reacting with biomolecules leads to dopaminergic degeneration and eventual neuron death. More specifically, damage to the DNA in the dopamine neurons which is caused by free radicals interacting with other macromolecules, is apparent in patients who are diagnosed with Parkinson's disease (Manoharan et al, 2016). Although this damage is not exclusively limited to the dopaminergic neurons, the harm done to dopaminergic neurons by an antioxidant deficiency, and an abundance of oxidative stress, leads to the symptoms and diseased condition of Parkinson's disease. Overall, the buildup of free radicals, which then interact with biomolecules to form ROS and cause harm, which are toxic to the brain due to their effects on DNA and cellular functions, leads to neurodegeneration in the substantia nigra of the brain. ROS, with their many effects, are also caused by numerous mechanisms.

GLYCATION

One of the many mechanisms through which ROS are produced is through glycation. ROS, as discussed prior, lead to oxidative damage and eventual neurodegeneration. All throughout the body, glycation is occurring. Through many different mechanisms, such as lipid peroxidation which is caused by free radicals interacting with lipids, and glycolysis, there are varying reactive carbonyl species which are formed. These reactive carbonyl species are “agents of glycation” and can then lead to advanced glycation-end products (AGEs) (Scheckhuber, 2019). Glycation is the name for a “ reaction between reducing sugars… and proteins, lipids or nucleic acids” (Gkogkolou and Böhm, 2012). A reducing sugar is any carbohydrate that has a free aldehyde or ketone group that is available to interact with another molecule. Glycation is thus when a carbohydrate brings in, adducts, another biomolecule which may be a DNA molecule, a protein, or a lipid (Glycation). Glycation may be enzymatic, which is considered glycosylation, or non-enzymatic, and glycation is also done by varying sugars from monosaccharides to polysaccharides. Glycation is also reversible, however they are many “irreversible chemical [modifications]” which occur in the reactions that may follow the glycation reaction (Glycation). An example of one of the irreversible compounds formed after a glycation reaction is seen below in Figure 1 (Glycation).

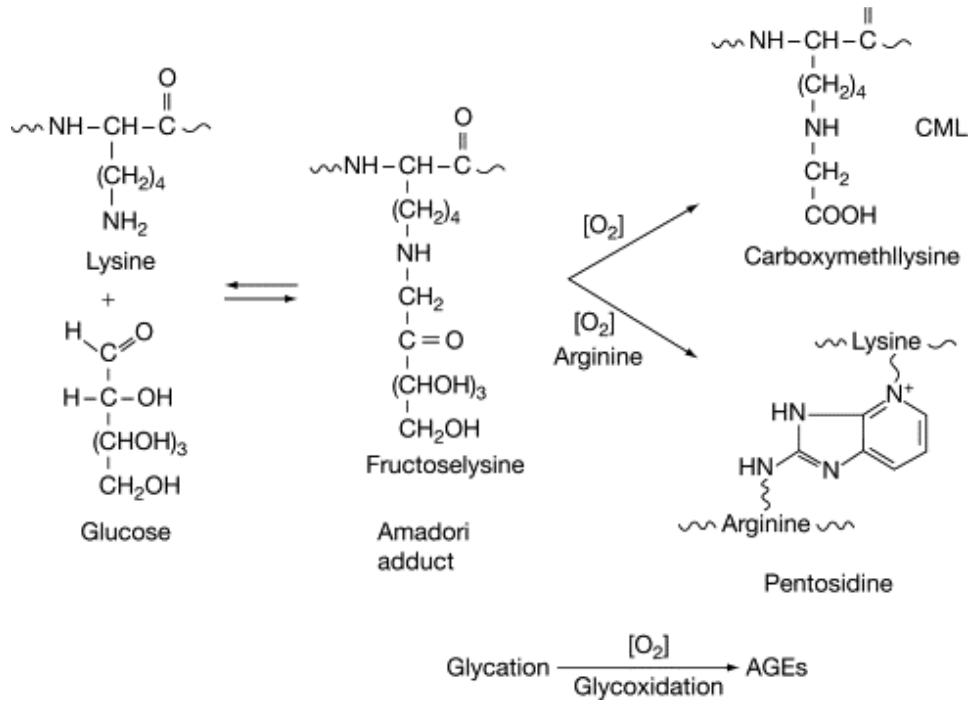

Figure 1: Glucose reacting with lysine, forming a Schiff base (not pictured in the figure), and then rearranging into fructoselysine. The rearrangement creates an unstable product, fructoselysine, that undergoes oxidation to yield both pentosidine, an advanced glycation end-product (AGE), and carboxymethllysine.

In the case of figure 1, the reaction between glucose, a reducing sugar, and lysine, an amino acid, is reversible, and yield a Schiff base. However, after this, the Schiff base is rearranged into an Amadori adduct, fructoselysine, because the Schiff base is unstable. Next, oxidation occurs and this is the irreversible portion which yields pentosidine, an AGE. Nearly all AGEs are formed through a very similar combination of a glycation reaction with an oxidation reaction, and as these reactions occur over time, their products, which are called glycoxidation products, build up in the brain.

Glycation occurs in the brain due to an unusual buildup of carbohydrates and elevated levels of available sugars. Specifically, this is seen in tissues and areas of the body experiencing

hyperglycemia, oxidative stress due to ROS, or inflammation. In tissues of patients who are hyperglycemic, the blood glucose levels are elevated meaning that there are more reducing sugars available, and not absorbed into cells, to react with other biomolecules, undergoing a glycation reaction, and then being oxidized to form AGEs. AGEs lead to ROS formation in cells by stimulating the production of a "superoxide anion" and compromising the cell's antioxidant defense mechanisms (Gkogkolou and Böhm, 2012). Additionally, when AGEs bind to the receptor of advanced glycation end-products (RAGE), NADPH oxidases are activated. NADPH oxidases "catalyze the transfer of electrons from NADPH to molecular oxygen", which creates a free radical, which is now available to create a ROS, due to the unpaired, unstable electrons (Nowotny, Junh, Höhn, Weber, & Grune, 2015). In the brain, AGEs alone are extremely reactive molecules which can lead to further processes which may be toxic to the dopaminergic cells of the substantia nigra. In addition to AGEs causing cellular damage, the AGEs also impair mitochondrial function. The impaired mitochondrial function leads to mitochondrial damage, dysregulated pathways, and further neurodegeneration (Schlotterer et al., 2009). The impaired mitochondrial function that AGEs may cause will lead to ROS production, which loops back to the original effects of ROS on the dopaminergic neurons of the substantia nigra. Although the impact of oxidative stress and damage is not exclusively limited to the dopaminergic neurons, the noticeable symptoms and effects of Parkinson's disease are seen in the harm and loss of function seen in the dopaminergic neurons.

3. Hyperglycemia in the context of T2DM

Is it estimated that as of 2020, about 8.2% of the United States population has diagnosed diabetes, and thus suffers from hyperglycemia (Centers for Disease Control and Prevention, 2020). Hyperglycemia is a serious condition because not only is it a sign that the human body is

not functioning properly in relation to glucose breakdown and insulin's role in that process, but hyperglycemia can also lead to many more serious conditions, or cause an already present disease to worsen. Hyperglycemia is defined as high blood glucose levels in the blood. Typically, blood glucose levels lower than 200 milligrams per deciliter, on a random blood sugar test and not a fasted blood sugar test, are considered healthy and safe. However, when a blood sugar level reads above 200 milligrams per deciliter, then the patient is considered to have diabetes (Diabetes, 2020). These high blood glucose levels are caused by many processes, but they are all tied to malfunction of the mechanism for absorbing glucose.

BREAKDOWN OF CARBOHYDRATES

After each meal, the body begins to break down the carbohydrates that were consumed. These complex polysaccharides are slowly broken down through digestion by the salivary amylase molecules in the mouth. Once the polysaccharides are fully broken down into monosaccharides, the body begins the process of cellular respiration. The first step of cellular respiration is glycolysis, when glucose is oxidized in order to produce ATP, the body's main source of energy (Lumen Learning). However, in patients with hyperglycemia, they are unable to start glycolysis as the glucose they have received from their food cannot be taken into the cells, and instead remains in the bloodstream. The reason for this is because in order to be taken into the cells from the bloodstream, glucose must receive the help of insulin, which is an important hormone that the pancreas secretes. Normally, after a meal is eaten, the body's natural blood glucose levels begin to rise as the salivary amylase breaks down the polysaccharides and the glucose molecules, which are monosaccharides, are absorbed into the bloodstream. The rise in the glucose levels in the blood is a signal to the pancreas to release insulin, which is then supposed to work to transport the glucose across the plasma membranes of cells. Because the

plasma membrane of cells is impermeable to carbohydrates, the glucose must use a transport system in order to successfully make it into the cell. There are many versions of these transporters, with GLUT 4 being one of the most common and the transporter which functions with insulin (Szablewski, 2011). Depending on what kind of diabetes the patient suffers from, the transporter either is desensitized to insulin, the body does not make enough insulin, or the body does not make any insulin at all. Insulin is necessary in order to activate the transporter in order to get the glucose across the cell membrane. However, in the case of insulin sensitivity, the receptor, GLUT 4 for muscle cells, is no longer responding to the insulin's signals and thus the glucose is not taken into the cell properly. Regardless, because of the inability to get the glucose from the blood stream, across the cell's plasma membrane, and into the cell, the glucose will remain in the bloodstream and will slowly build up over time. Glucose remaining in the blood stream is problematic because not only does it mean that the cells are not breaking down the glucose into energy for their processes as they are supposed to, but it also means that the glucose will begin to accumulate and cause further issues and difficulties.

Insulin has many other important roles in regard to hyperglycemia, and blood glucose levels, aside from just functioning to get the glucose past the plasma membranes of cells. Insulin also stimulates glycogenesis, which is the conversion of glucose to glycogen for storage in the body, and inhibits glycogenolysis, which is the breakdown of glycogen into glucose. Insulin also inhibits gluconeogenesis, which is the breakdown of other macromolecules for glucose by the liver and kidneys, and slows the breakdown of fats to fatty acids, and eventually ketones. Lastly, insulin stimulates the storage of fats (Alic, 2020). Overall deficiency of insulin in the body, as seen in type one and type two diabetes, means that not only does the glucose build up in the

bloodstream because it cannot get past the plasma membrane, but also more glucose is created through these various cellular processes.

SYMPTOMS AND COMPLICATIONS OF HYPERGLYCEMIA

Despite the fact that hyperglycemia can become extremely dangerous, many of the symptoms of hyperglycemia go unnoticed for a long time. Frequent urination and increased thirst are two of the most noticeable symptoms, and patients can also experience extreme fatigue. Eventually, if the hyperglycemia remains undetected, and thus untreated, the patient will experience more severe and life-threatening symptoms including having fruity-smelling breath, weakness, and shortness of breath (Hyperglycemia in diabetes, 2020). Furthermore, having fruity smelling breath can be an indication that diabetic ketoacidosis is occurring. Diabetic ketoacidosis is a life-threatening and extremely toxic condition when the body begins to improvise for energy resources. Since there is not enough sugar available inside the cells to be used for cellular processes, the body begins to breakdown ketone bodies to use. Up until a certain level, the broken down ketone bodies can be reabsorbed by the glomerular tubules in the kidneys (Guo et al., 2018). However, a level can be reached where the ketone bodies are no longer reabsorbed and instead build up in the bloodstream, creating a state of ketoacidosis, which is a very acidic pH in the blood of the body. Regardless of whether or not the cells are able to take in the glucose and then break it down, the human body still needs energy in order to function and carry out its' many processes. Because of this, the human body will begin to break down fat for energy if it is not receiving the necessary energy from carbohydrates (Hyperglycemia in diabetes, 2020). However, the process of breaking down fat creates ketones, which are toxic acids, as a bi-product. These toxic acids will accumulate in the blood, just like the glucose, and can eventually cause a person to fall into a coma. Overall, diabetes, and hyperglycemia as a whole, is typically

noticed when a patient complains of frequent urination coupled with extreme thirst, or when a doctor performs a routine urine test. Unfortunately though, some patients discover that they have diabetes and extreme hyperglycemia after a near-death experience due to the severe side effects of having high blood glucose levels.

In addition to the emergency complications, and symptoms, that can develop due to hyperglycemia, there are also many long-term complications and co-morbidities that can develop alongside hyperglycemia and diabetes. Because of the kidney's inability to process and handle the glucose, and sometimes ketone, levels in the urine, kidney damage and failure is a long-term complication of hyperglycemia (Hyperglycemia in diabetes, 2020). Additionally, advanced glycation end products are a factor in the diabetes neuropathy which is a common long-term complication of hyperglycemia. After large periods of time when high blood sugar levels remain untreated, there can be oxidative stress in the nerves of all limbs, caused by advanced glycation end-products (Yagihashi, Mizukami, & Sugimoto, 2010). The AGEs and oxidative stress will cause peripheral nerve injury in the limbs, most commonly the feet, and this nerve damage can lead to severe infections and ulcerations as the nerve damage prevents the patient from feeling any sort of cut or abrasion on the foot, which can grow to be infected. Overall, there are a plethora of long-term complications that are associated with patients with untreated or unmanaged hyperglycemia. However, with prevention or medicated management of hyperglycemia, and the greater condition of diabetes, these long-term complications are avoidable.

MANAGEMENT AND TREATMENT OF HYPERGLYCEMIA

Because of how much a patient's blood sugar levels can vary depending on the time of day, it is important that all patients monitor their blood sugar levels frequently. Depending on the

severity of the hyperglycemia, the monitoring may need to occur multiple times a day, but a diabetic who has their condition under control can push that back to multiple times a week. Additionally, patients need to stay on top of taking their prescribed medications. For type two diabetics, most of their medications function to lower insulin levels, lower glucose production by the liver through gluconeogenesis, or increase the body's sensitivity to insulin. Metformin, one of the most common medications for type two diabetics, functions to both decrease the rate at which gluconeogenesis is occurring, and improve the body's sensitivity to insulin (Type 2 diabetes, 2021). Metformin assists the body by decreasing the rate at which gluconeogenesis is occurring, ensuring that the body is not adding to the already large amount of glucose in the bloodstream. The other mechanisms of Metformin helps the cells to respond more accurately to insulin signaling at the GLUT 4 transporter, which is supposed to function to transport the glucose across the plasma membrane. Another branch of medications is Sulfonylureas which assist the patient's body in secreting more insulin in order to be able to increase the amount of glucose that is taken in by cells and decrease the amount of glucose which is in the bloodstream (Type 2 diabetes, 2021). Separate from medications, most patients can also get their hyperglycemia under control by eating a more balanced diet and exercising more. By tracking their meals, and monitoring their blood sugar in the hours following, patients can determine what foods and what food groups their body reacts best to. Additionally, it is recommended that patients with hyperglycemia eat smaller amount of food at a time, in order to not spike the blood glucose levels after huge meals. As for exercise, the act of physically exercising a patient's body will use up some of the glucose which is accumulating in the patient's blood stream.

4. The impact of nutritional intake on Parkinson's disease and T2DM

Nutrition and dietary consumption is an aspect of health that is consistently mentioned as something everyone needs to have under control in order to prevent many obesity related illnesses such as stroke, heart disease, cancer, high blood pressure, and diabetes (Center for Disease Control and Prevention). However, Parkinson's disease is now emerging as a another possible disease that is connected to what the patient eats regularly in the years leading up to, and after, diagnosis.

TYPE 2 DIABETES AND NUTRITION

For decades, physicians and scientists have recommended that patients preventatively control their diet in order to not develop type 2 diabetes, and that they modify their diet after a diagnosis in order to prevent the progression and lessen the severity of the disease. Type 2 diabetics should watch the type and amount of fat they consume, along with the type and amount of carbohydrates they consume. In regard to fat consumption, the diet should be kept low-fat in general, as high-fat diets lead to insulin resistance when coupled with high carbohydrate consumption. In regard to the type of fat consumed, "saturated, monounsaturated and polyunsaturated fats, excluding n-3 fatty acids, have caused insulin resistance" when consumed in large amounts (Steyn et al., 2004). This is because the high fat intake is associated with higher fasting glucose and insulin levels, which is seen in patients with type 2 diabetes. Most patients who replaced saturated fat with unsaturated fats saw a decrease in their blood glucose levels, and they were better able to manage their diabetes. When analyzing the total amount of carbohydrates consumed, it is recommended that patients with the propensity to develop type 2 diabetes, or patients with a diagnosis of type 2 diabetes, eat a moderately low carbohydrate diet. When carbohydrates are consumed, insulin must be secreted in order to properly breakdown the

carbohydrate so that it may be absorbed through the plasma membrane and into the cell. However, when there are large amounts of carbohydrates consumed, more and more insulin is needed. Patients with type 2 diabetes suffer from issues with insulin, whether it is resistance, an inability to produce insulin, or not enough insulin being produced to keep up. By lowering the total amount of carbohydrates consumed, patients are able to naturally lower their blood sugar, and lessen the strain on the pancreas to produce so much insulin. If patients are unable to produce insulin at all, this will assist in the amount of insulin they must acquire from injections in order to keep their blood sugar at a reasonable level. When analyzing the type of carbohydrates consumed, it is important to look at the amount of dietary fiber, a type of carbohydrate, being eaten. Dietary fiber is comprised of "cellulose, hemicelluloses, pectins, hydrocolloids, resistant starches and resistant oligosaccharides" and foods that contain these compounds are difficult to breakdown and digest (Steyn et al., 2004). The difficulty, and ultimate impossibility, in digesting fiber is beneficial to the body as it burns energy and since fiber cannot be broken down fully, it passes through the body undigested. Because it goes undigested, the blood sugar levels do not rise with the consumption of this type of carbohydrate. Additionally, there is no need for insulin to be released in regards to fibrous foods.

PARKINSON'S DISEASE AND NUTRITION

Much more recently, physicians and scientists have discovered that diet changes, and different nutrition levels, can impact the development, and progression, of Parkinson's disease. There are correlations between many different nutrients, such as dairy, antioxidants, and caffeine, as well as macromolecules, such as carbohydrates and fat, and suppressing the onset or progression of Parkinson's disease, or oppositely, increasing the risk of Parkinson's disease developing. Starting with the nutrients, consumption of large amounts of dairy is positively

associated with risk for developing Parkinson's disease due to both the presence of pesticides in milk, as well as the low serum uric acid levels which are present in many dairy products (Seidl, Santiago, Bilyk, & Potashkin, 2014). Low serum uric acid levels are characteristic in patients with Parkinson's disease. Uric acid typically functions as an antioxidant, and because there are lower levels, it has less of an impact and is less equipped to counteract the harm of ROS (Sakuta et al., 2016). In regard to the relation with antioxidants, there is no research supporting consuming increased levels of antioxidants, as food that is consumed gets distributed throughout the entire body (Filograna, Beltramini, Bubacco, & Bisaglia, 2016). However, the dopaminergic neurons would benefit from antioxidant therapy directly in the substantia nigra or directly to individual dopaminergic neurons. That being said, there is not a strong correlation between low levels of antioxidants being consumed by humans, and more of a risk of developing Parkinson's disease (Filograna, Beltramini, Bubacco, & Bisaglia, 2016). Lastly, there is a strong protective relationship between caffeine and Parkinson's disease. Caffeine has been shown to reduce the toxicity of species and compounds of dopaminergic neurons and it can actually slow the progression of Parkinson's disease "through antagonism of adenosine A_{2A} receptors", which are involved in the release of dopamine in the brain (Seidl, Santiago, Bilyk, & Potashkin, 2014). By antagonizing and inhibiting these receptors, the caffeine provides a neuroprotective effect and relieve some of the motor symptoms which are commonly associated with Parkinson's disease (Seidl, Santiago, Bilyk, & Potashkin, 2014).

There are also relationships between the consumption of macromolecules, and the onset or progression of Parkinson's disease. In regard to fat consumption, a slight positive correlation is seen between those who consume a large amount of animal fat, and those who are at a greater risk for developing Parkinson's disease. There are some conflicting results, and there needs to be

more research done to narrow down what kinds of fats are more detrimental, and lead to a great risk of developing Parkinson's disease. In regard to carbohydrates, carbohydrates have the ability to "increase [dopamine] production in the brain by allowing easier passage of the [dopamine] precursor, tyrosine, through the blood-brain barrier into cerebrospinal fluid" (Seidl, Santiago, Bilyk, & Potashkin, 2014). There are also conflicting results showing that there is a positive association between increase carbohydrate consumption, and increased risk of developing Parkinson's disease. Overall, the greatest tie between carbohydrates and Parkinson's disease is seen in the recent studies showing that type 2 diabetics are shown to have an increased risk of developing Parkinson's disease (Seidl, Santiago, Bilyk, & Potashkin, 2014). The hyperglycemia associated with type 2 diabetes impacts the amount of glycation which occurs in the brain, and thus the amount of AGEs produced and the amount of oxidative damage done, through the abundance of available reducing sugars for interactions in the bloodstream of diabetics.

FAD-DIETS

In this era of fad-diets, there have been studies showing how a low carbohydrate, high protein diet is much healthier for most people, and especially those who have been diagnosed with Parkinson's disease and type 2 diabetes (Lange et al., 2019). The ketogenic diet satisfies much of what a low carbohydrate, high protein diet looks like, and has been shown to decrease ROS, and lower blood sugar levels, due to the smaller supplies of monosaccharides on hand. In regard to type 2 diabetes, a ketogenic diet can assist with both the prevention and progression of the disease. People who know they are predisposed, due to both lifestyle decisions and genetics, to type 2 diabetes can lower their risk of developing type 2 diabetes by using the ketogenic diet to keep their blood sugar levels low. Because people on the ketogenic diet eat low carbohydrate, these people's bodies will have less carbohydrates to process, and thus less of a need for insulin.

This is helpful because if the person is insulin resistant, their body does not make insulin at all, or they just do not make enough insulin, they will be asking their body to use less insulin anyway as they are consuming less carbohydrates that require insulin to get the glucose past the plasma membrane. In regard to those who have received a diagnosis of type 2 diabetes, most physicians and scientists believe that the disease can be managed by simple lifestyle changes such as altering their diet. In altering their diet to be low carbohydrate, type 2 diabetics may be able to lower their blood glucose to a level where they can lower their medication or lower the amount of injections they are receiving. Lowering their hyperglycemia to this range is beneficial as it is both healthier for the patient, and saves the patient money they would be spending on medications or insulin injections.

In reference to Parkinson's disease, glycation has been proved to be a critical mechanism for producing AGEs, and then oxidative stress and damage. By lowering the amount of carbohydrates consumed through following the ketogenic diet, a patient who has Parkinson's disease may be able to prevent further damage done to their dopaminergic neurons through glycation, AGEs, and then oxidative damage. By preventing further damage, some of the most harsh symptoms of Parkinson's disease can be halted and patients who have Parkinson's disease may be able to live and behave on a more normal spectrum for longer. Although the ketogenic diet has not been shown to prevent Parkinson's disease altogether, it can definitely prevent the worsening of the disease through less of a buildup of AGEs and less oxidative damage (Gaenslen, Gasser, and Berg, 2008).

ANTIOXIDANT THERAPY

Another potential treatment to combat the oxidative damage that causes Parkinson's disease is antioxidant therapy. Although there are no clinical trials that show definite results from

antioxidant therapy as of now, there are a few that show promising results to build further research on. One of the largest issues in treating patients diagnosed with Parkinson's disease with antioxidant therapy is that by the time of diagnosis, about "50% of dopaminergic neurons are lost" (Snow et al., 2010). In this case, at the point in time when Parkinson's disease is clinically evident, the disease is too progressed for antioxidant therapy to be beneficial. However, if people who know they are genetically or environmentally predisposed to develop Parkinson's disease, antioxidant therapy may be a viable option for preventing Parkinson's disease, or at least preventing the disease from an early onset or the highest levels of severity. Specifically, "mitochondria-targeted antioxidant MitoQ" therapy is a promising treatment option for those with the known genetic mutations in the *PARK7*, *PINK1*, *PRKN*, *LRRK2* or *SNCA* genes.

5. The impact of fructose on both Parkinson's disease and Type II Diabetes Mellitus

Fructose is one of the most highly consumed carbohydrates by Americans. Biochemically, fructose is a monosaccharide, however it is commonly combined with glucose to create sucrose, which is table sugar (Vos et. al, 2008). Naturally, fructose is in many different fruits and vegetables. However, Americans also consume fructose in many processed foods that contain high-fructose corn syrup, a sweetener that is processed from corn, and it is in table sugar, also known as sucrose (Vos et. al, 2008). In 2010, the average American consumer 54.7g of fructose a day, which is nearly 20g more than the average recommended quantity a day: 37g (Vos et. al, 2008). Although awareness of the dangers associated with, and harm done by, fructose has grown since 2010, it is still included in most soft drinks, packaged sweets, candy, and other highly processed sweet foods.

Fructose is also known to be associated with many different diseases and conditions in humans. Short-term studies show that high consumption of fructose is correlated with hyperlipidemia, which is associated with cardiovascular disease, and insulin resistance, which is linked with type 2 diabetes. In reference to type 2 diabetes, fructose is associated with this disease as nearly 20% of Americans' daily carbohydrate consumption comes from fructose consumption. Because of this fact, it is correlated with many of the issues Americans have with controlling their blood sugar, which leads to type 2 diabetes. By decreasing the amount of fructose, and specifically high fructose corn syrup, they consume, patients are able to prevent the onset, or delay the progression, of type 2 diabetes. One of the most common alterations to diet that physicians will suggest to patients who have been diagnosed with type 2 diabetes is cutting out many of the processed, sugary foods that patients consume, much of which contain high fructose corn syrup, or table sugar.

The relationship between Parkinson's disease and fructose is less clear. As discussed earlier, glycation and the damage done by AGEs, is a common mechanism for dopaminergic damage in the substantia nigra. Fructose plays a very similar role to glucose in the production of AGEs as it can undergo glycation by reacting with other biomolecules. Although there is no difference between fructose reacting with other biomolecules through glycation, and glucose reacting with other biomolecules through glycation, fructose is nonetheless another source of carbohydrates which can lead to the production of AGEs, and further, oxidative damage in the brain. Additionally, fructose can be converted to glucose within the cell, so consumption of large amounts of fructose will also act to raise the blood sugar. Overall, patients with diagnoses of Parkinson's disease, and or type 2 diabetes, can benefit from monitoring, and lowering, the amount of fructose in their diet.

6. Parkinson's disease in *C. elegans*

Caenorhabditis elegans (*C. elegans*) are a type of microscopic nematode with a simple nervous system, and a genome that has been completely mapped and is quite similar to the human genome. Structurally, *C. elegans* are unsegmented worms that are generally about 1mm in length and have a long cylindrical body (What is C. elegans?, 2021). *C. elegans* can either be hermaphrodites, meaning they are able to self-fertilize and thus do not need a mate, or male. In the wild, *C. elegans* feed on microbes in soil. However, in the laboratory, *C. elegans* live on a plate and get their food from a bacterial lawn on the agar in the plate. For about two decades now, *C. elegans* have been used when researching human diseases and conditions because *C. elegans* and humans both contain about 20,500 genes (Why use the worm in research?, 2015). Additionally, not only are *C. elegans* cheap, but they also reproduce in only three days, and both of these attributes make them great model organisms in a laboratory. Another attractive element of *C. elegans* is that they are "non-hazardous, non-infectious, non-pathogenic, non-parasitic [organisms]" making them incredibly safe to use in laboratory settings, not putting any scientists at risk (What is *C. elegans*?, 2021).

NERVOUS SYSTEM OF *C. ELEGANS* AND EFFECT OF 6-OHDA

In regards to their biological characteristics, *C. elegans* have a very simple nervous system consisting of their brain, the circumpharyngeal nerve ring, and then 302 neurons (What is *C. elegans*?, 2021). Because of their simple nervous system. *C. elegans* are very useful for modeling neurological disorders, such as Parkinson's disease. Out of the four main neurotransmitters found in the *C. elegans* nematode, dopamine is produced by eight of the neurons in the hermaphrodite. In the males, there are also six additional dopaminergic neurons in the tail. In *C. elegans,* dopamine functions similarly to its purpose in humans by controlling

locomotion changes due to environmental changes, and movement behavior. Specifically, *C. elegans* exhibit dopamine signaling, allowing them to slow when they find a bacterial lawn, or speed up in search for a new food source (Chase & Koelle, 2007). However, when the eight dopaminergic neurons of the *C. elegans* are damaged, the dopamine signaling function does not work properly, and thus the worms are not able to move properly in order to find their food source. There are many ways in which dopaminergic neurons can be damaged in humans, but there is only one main way we are able to stimulate that in *C. elegans.*

6-Hydroxydopamine (6-OHDA) is a neurotoxin that is used around the world to induce Parkinson's disease like conditions in many human model organisms. Although 6-OHDA does not produce all of the symptoms associated with Parkinson's disease, it does induce the oxidative stress and reactive oxygen species, which leads to the neurodegeneration and neuronal death that is largely present in Parkinson's disease. 6-OHDA is a dopamine analogue, meaning that it can be uptaken by dopamine transporters in the substantia nigra (Hernandez-Baltazar, Zavala-Flores, & Villanueva-Olivo, 2017). When the 6-OHDA is taken up by the dopamine transporters, which assume that the neurotoxin is actually dopamine, one of three cytotoxic mechanisms take place: 1. 6-OHDA is oxidized, causing it to produce hydrogen peroxide, superoxide, and other toxic hydroxyl radicals, 2. Hydrogen peroxide is formed by monoamine oxidase, or 3. The mitochondrial respiratory chain complex I is inhibited. Altogether, or even separately, these three mechanisms lead to the production of ROS which results in oxidative stress within the dopaminergic cells. Ultimately, if the amount of ROS produced becomes too much for the cell to handle, the cell will induce its own death through apoptosis.

MOVEMENT OF C. ELEGANS

Normally, *C. elegans* move sinusoidally across a bacterial lawn on a plate in the laboratory. When they encounter a drop of liquid, they will thrash back and forth for the duration that they remain in the liquid, and then once they are out of the liquid, they will return to sinusoidal movement (Chase & Koelle, 2007). However, when the dopaminergic neurons in the *C. elegans* are damaged by 6-Hydroxydopamine (6-OHDA), the worm is unable to return to sinusoidal movement after encountering the drop of liquid. This is due to the fact that the 6-OHDA has entered into the dopamine neurons through the dopamine transporters (DAT), creates reactive oxygen species (ROS) and damaged the neurons to the point that the nematode is no longer able to move and control its locomotion properly. The movement patterns of *C. elegans* can be analyzed and depending on whether or not the worm is able to return to normal sinusoidal movement after encountering a drop of liquid dictates whether or not the worm is determined to have a Parkinson's like disease (Chase & Koelle, 2007). If the worm can return to normal sinusoidal movement, it is determined to not have a Parkinson's like disease, however if it cannot return to normal sinusoidal movement, it is determined to indeed have a Parkinson's like disease. Overall, because of the impact of 6-OHDA on the DAT in *C. elegans*, these nematodes have become incredibly reliable models for humans with Parkinson's disease.

7. Fructose impact in *C. elegans* research

C. elegans, although they are low maintenance and extremely cost efficient, do require a very specific procedure in order to be at the right level of maturity to be treated by the 6-OHDA, and for the proper effects to be seen. First, the worms had to be maintained and properly husbanded on the nematode growth medium (NGM) which has a lawn of OP50 bacteria on it as their food source, as seen in Figure 2. The *C. elegans* were chunked to these clean plates where they are left

to grow and mature for three days. After three days, the *C. elegans* would undergo a hypochlorite synchronization protocol where all of the immature *C. elegans* were spun out and eliminated and only the eggs remained. The hypochlorite synchronization protocol was done so the age could be the same, and the variable of age could be eliminated from the experiment. Once the protocol had been completed, the worms were spun for one full day, and then poured on new NGM plates on the following day. These new NGM plates had OP50 as the worms' food source, and the worms grew here again for three days. After three more days of growth, the *C. elegans* were chunked onto fructose plates for the first time. These fructose plates were of five varying concentrations: 50mM, 100mM, 200mM, 400mM, and 800mM, as shown in Figure 2. The fructose plates also had OP50 on them as the worms' food source. The fructose concentrations were determined from previous scientific research done using *C. elegans* and fructose.

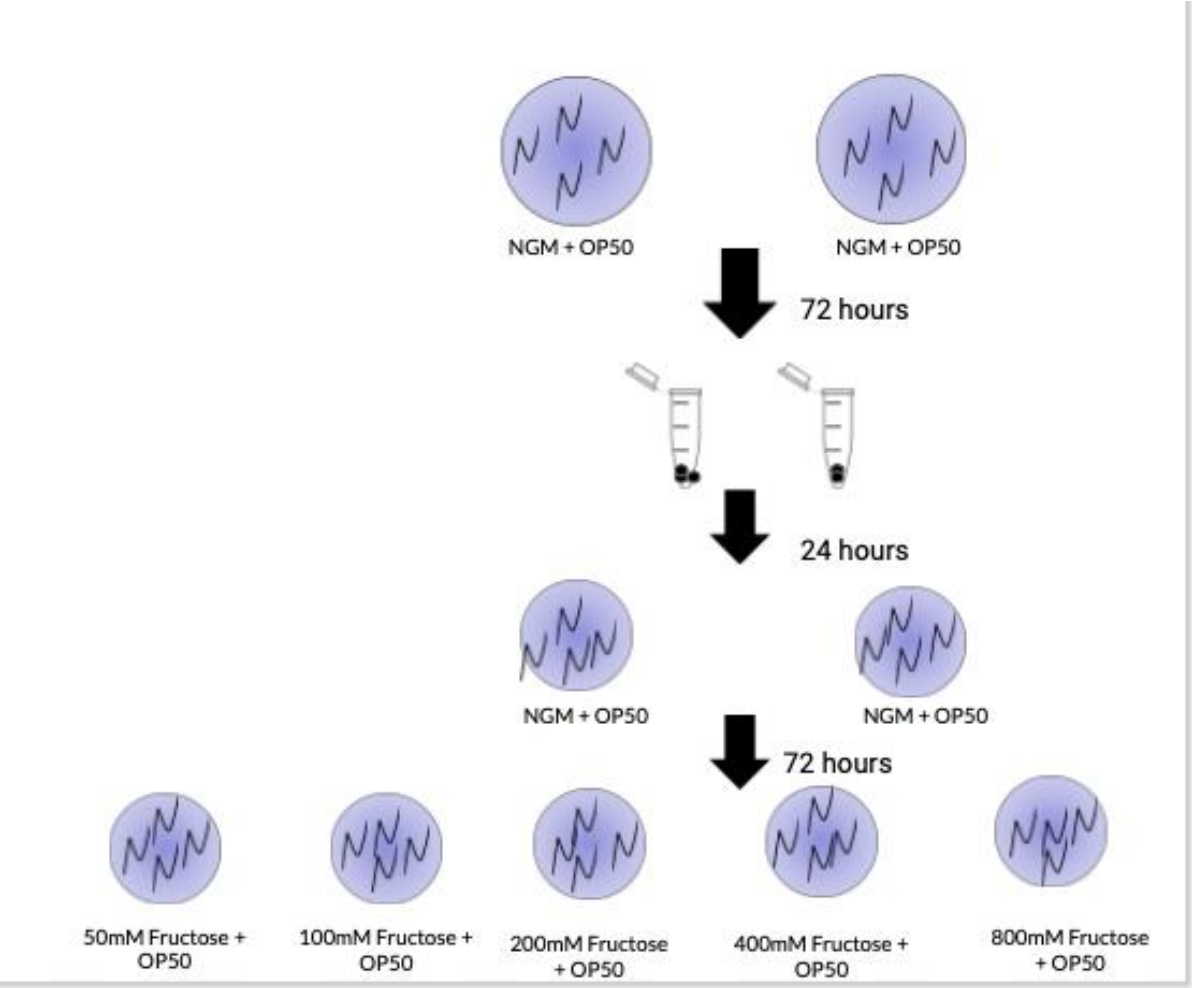

Figure 2: This shows the first four steps of *C. elegans* husbandry. First, the worms must be grown on NGM with OP50 food source for 3 days. After that, all of the worms from the plates undergo a hypochlorite synchronization step. Third, the worms are transferred to NGM with OP50 plates to grow for another 3 days. Fourth, the worms are transferred to the Fructose plates, of varying concentrations, with OP50 food source.

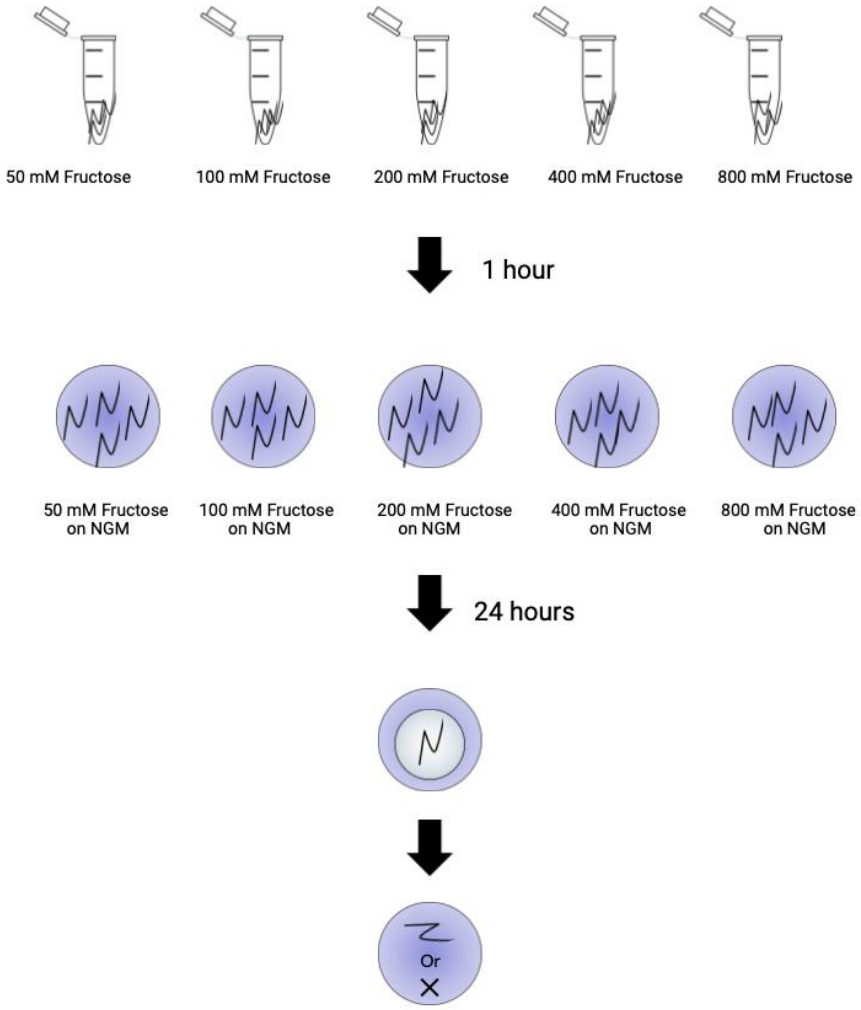

Figure 3: This shows the last four steps of *C. elegans* husbandry. After growth on the Fructose plates for three days, the worms are treated with 6-OHDA for an hour, with each fructose concentration in a separate test tube. Next, the worms are transferred to an NGM plate for one day. Lastly, the worms undergo the mobility test using a drop of S-BASAL to test if they return to sinusoidal movement, or if they continue to thrash or move unusually.

Once the worms grew on the fructose plates for three days, they were then ready to be treated with the 10 mM 6-OHDA, as seen in Figure 3. The treatment with the 6-OHDA lasted one hour and was done separately for each of the varying fructose concentrations that the worms

had been plated at. After the 6-OHDA treatment, the worms were then transferred to new fructose NGM plates, without OP50, for 24 hours, for the last time. The next day, the worms were ready to be analyzed using the mobility pattern test. For this, one single worm was taken from the fructose plate, and it was transferred to a plain NGM plate with nothing else on it. On this plate, a drop of S-BASAL liquid was dropped onto the *C. elegans*, the worm would thrash for a minute, the liquid would be collected using a Kem-wipe, and then the ability, or inability, for the worm to return to sinusoidal movement was analyzed. If the worm could return to normal sinusoidal movement, it was determined to not have a Parkinson's like disease, however if it could not return to normal sinusoidal movement, it was determined to indeed have a Parkinson's like disease. The question that I was asking in this experiment was will excess fructose increase the neurotoxicity of 6-OHDA? I hypothesized that increasing the fructose concentration will significantly increase the neurotoxicity of 6-OHDA in *C. elegans.*

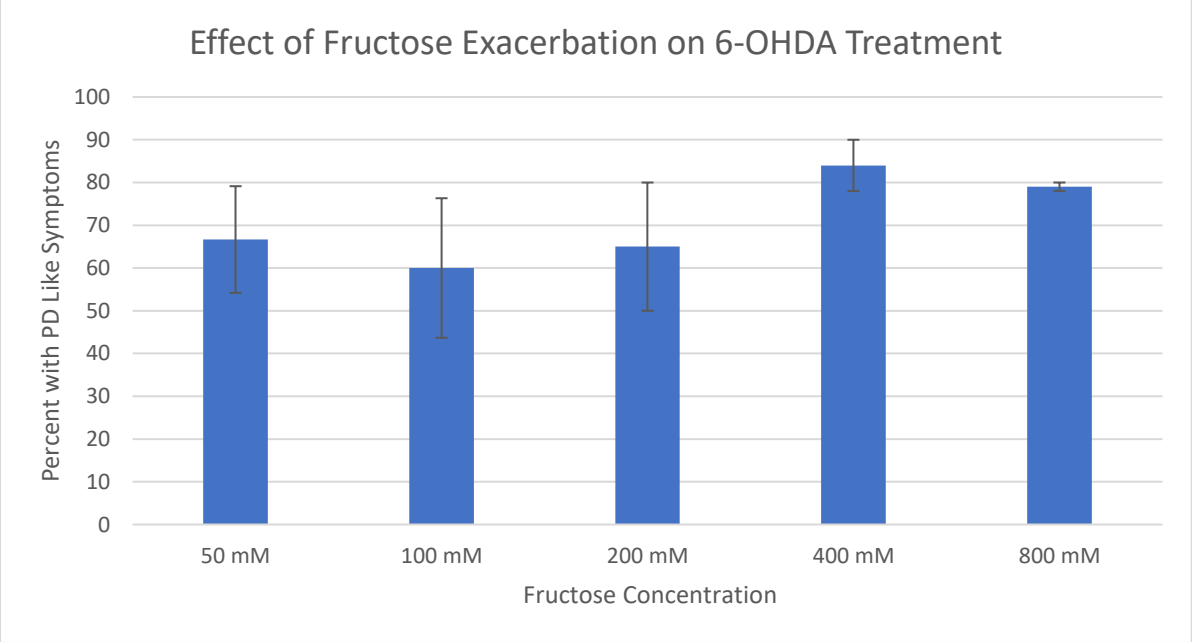

Figure 4: This graph shows the effect of Fructose exacerbation on 6-OHDA treatment of *C. elegans*. The data is statistically insignificant, however, there is a general trend of a greater percent of PD like symptoms seen at the higher fructose concentrations.

Despite the fact that there is a general trend that can be seen in Graph 1 of increasing percent of *C. elegans* with Parkinson's disease like symptoms at the higher concentrations of fructose, there is not a scientifically significant trend. Treating the *C. elegans* with increasing concentration of fructose did not significantly exacerbate the effect of 6-OHDA on the DAT, and the fructose plates did not ultimately lead to greater oxidative stress and thus more worms with Parkinson's disease like symptoms. The one way ANOVA showed that $F(4,7)= 0.91$ and the p-value of the data is equal to 0.51. The error bars are due to the fact that we had a few outliers, and the fact that my experimental groups were very small, meaning that the variability in my data effected the average percent greatly.

8. Conclusion

NUTRITION AND FRUCTOSE IN REGARD TO DIABETES AND PARKINSON'S

In regards to nutrition, both Parkinson's disease and type 2 diabetes have been shown to be impacted by the macronutrients, micronutrients, and compounds consumed in food. Specifically, carbohydrate consumption greatly impacts blood sugar levels, and thus the characteristic condition of hyperglycemia that is found in all type 2 diabetics. When thinking of Parkinson's disease, carbohydrate consumption has an effect on the prevention and progression of the locomotive symptoms as the amount of carbohydrates in the body directly impacts the amount of carbohydrates available in the brain to undergo glycation which produces AGEs which cause oxidative damage. By lowering the amount of carbohydrates eaten on a daily basis, patients with both Parkinson's disease and type 2 diabetes may be able to control their diseased

condition and many of the severe symptoms and or long-term effects of the disease. Additionally, if these patients decide to move fully to a ketogenic diet, they may be able to keep their carbohydrate levels under control and this could lead to a regression or prevention of progression of the locomotive issues associated with Parkinson's disease, or a possible decrease in the medications and insulin injections required for patients with type 2 diabetes.

POTENTIAL FUTURE TREATMENTS

In reference to the human diet, fructose is in many of the processed foods and drinks consumed by Americans on a daily basis. Because fructose accounts for nearly 20% of Americans' daily carbohydrate consumption, just focusing on limiting the amount of sucrose, which is table sugar, and high fructose corn syrup consumed can positively impact a patient with both Parkinson's disease and type 2 diabetes. In regard to potential future treatments that need to be investigated, antioxidant therapy looks like a promising way of preventing the onset, or early onset, of Parkinson's disease. Although antioxidant therapy cannot help much once dopaminergic neurons have already been damaged, antioxidant therapy can help significantly prior to dopaminergic neurons being damaged, as the therapy increases the levels of antioxidants available to fight the oxidative damage and stress being done by the reactive oxygen species due to free radicals. This is especially helpful for patients who have the known mutations in the *PARK7, PINK1*, *PRKN*, *LRRK2* or *SNCA* genes.

C. ELEGANS RESEARCH

In prior experiments, Dr. Ragsdale's lab found that treating the *C. elegans* with 10mM 6-OHDA for 1 hour, just as we did, and growing them on plates with just OP-50 food source, but no additional sugar source, led to 80% of the worms showing Parkinson's disease like symptoms. In our experimentation with the fructose plates, we saw 84% of the 400 mM Fructose worms

exhibiting Parkinson's disease like symptoms with 1 hour of 6-OHDA treatment. Despite the fact that the 84% is greater than the prior determined 80%, the difference is not statistically significant, but more of a general trend. In the future, there needs to be more research done with more concentrations of fructose gradually increasing from about 200 mM to 800mM, where the impact of the fructose can actually be seen. Additionally, thought needs to be taken on having more control groups, that way the effects of the fructose are able to be separated from the effects of the 6-OHDA and are also able to be separated from the effects of the 6-OHDA and fructose combined. By decreasing the concentration of 6-OHDA used, and altering that while changing the concentration of fructose, the effects of the two compounds may be better delineated and separated. Also, future research needs to contain larger sample sizes that way there can be more statistically significant data that is not altered so drastically by outliers.

However, we also witnessed that there was a slight dip in the generally increasing trend, in the percent of *C. elegans* with Parkinson's disease like symptoms down to 79% at the 800mM fructose group. One reason for this decrease could be that there is a point when the fructose concentration exacerbates the effects of the 6-OHDA, perhaps at 400mM fructose, and then there could be a point that the fructose concentration no longer exacerbates the effects of the 6-OHDA, but rather inhibits the 6-OHDA from having its full neurotoxic effect, or even provides benefits to the worm in evading the impact of the 6-OHDA. This is a possibility as we saw a small dip in our own data in the percentage of worms with Parkinson's disease like symptoms at the highest concentration of fructose we used in our experimentation. In the future, additional research needs to be done to determine at what point that fructose exacerbates the symptoms, and at what point it switches to having a different effect on the *C. elegans*, possibly blocking the effects of the 6-OHDA treatment.

Overall, from the research done with the *C. elegans*, I believe that these results are promising and indicate a possible exacerbation of the effect of 6-OHDA with used in tandem with fructose concentrations around 400 mM. From this, scientists and physicians may be able to better recommend specific amounts of fructose, and overall sugar, consumption that is safe for patients with Parkinson's disease. They also might be better equipped to define at what point, and at what level of consumption, do sugars, specifically fructose, exacerbate the movement and locomotive symptoms of Parkinson's disease.

OVERALL BIOCHEMICAL CONNECTION

Overall, there is are many mechanisms that overlap between the disease states and progression of both type 2 diabetes and Parkinson's disease. Specifically, hyperglycemia causes the many associated diseases and risks of type 2 diabetes. Additionally, having high blood glucose levels means that there is an abundance of glucose, a carbohydrate, in the blood which can be used in the brain for glycation. With an abundance of glycation occurring, more AGEs are produced and thus more oxidative damage is done. I believe that it is through glycation and hyperglycemia that type 2 diabetes and Parkinson's disease are biochemically intertwined, leading to many alterations in diet and lifestyle that can positively influence patients with either diagnosis, or both diagnoses. In the future, I think more research needs to be done to see if having type 2 diabetes puts you more at risk to developing Parkinson's disease, and vice versa. It is important to the future of patients with both Parkinson's disease and T2DM that this interconnectivity is investigated further. Overall, I have concluded that through hyperglycemia, glycation, nutrition, and even fructose, there is a link between type 2 diabetes and Parkinson's disease.

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