Matcha Tea and its Acute Effects on Postprandial Blood Glucose

by

Roni Romash

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Graduate Supervisory Committee:

Carol Johnston, Chair
Kathleen Dixon
Christy Lespron

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ABSTRACT

Many natural interventions have been effective at lowering postprandial glucose concentrations (PPG) in research trials and, theoretically, should have favorable effects on the prevention and management of T2DM. Natural interventions include vinegar, nuts and exercise. Green tea has been demonstrated to also possessing antiglycemic effects. Thus, green tea, and its most abundant catechin EGCG, are being consumed for its potential health benefits in cancer prevention and in its inhibitory effects on α-amylase. Many studies have found EGCG to inhibit α-amylase an enzyme needed in the breakdown of carbohydrates (CHO). Other studies have looked at EGCG and its potential for lowering PPG concentrations due to its inhibitory effects on α-amylase in both mice and humans. Yet there is no research on Matcha tea specifically. Matcha tea is green tea in powder form; hence, it is consumed in its entirety unlike traditional teas which are steeped in bags. The purpose of this study was to determine whether Macha tea impacts PPG concentrations in healthy adults. Twelve subjects completed this randomized controlled, single blinded, crossover study. On three separate occasions the twelve subjects consumed a bagel and jam with either water, Lipton green tea, or Macha tea. Fasting blood glucose was taken upon their arrival. Once the tea or water and bagel with jam were consumed PPG concentrations were measured every 30 minutes until 120 minutes were reached. Results showed no statistically significant effects on PPG concentrations in either test groups ($p=.960$). However, this study did not measure EGCG levels in the tea provided. Therefore, further research should be done with known EGCG amounts to see its effects on PPG concentrations to fully rule out its potential.

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INTRODUCTION

Diabetes is a growing problem worldwide. In 1995 it was estimated that there were 135 million people with diabetes with the expectation that it would grow to 300 million in 2025.[37, 52] Sadly, this estimation has underestimated the growing rate of diabetes worldwide as there are currently 422 million individuals with diabetes.[92] Recently diabetes had been estimated to increase up to 592 million in 2035.[62] Hence, it is important to develop strategies to slow this rate and help with the prevention of type 2 diabetes. One strategy is to lower postprandial blood glucose concentrations.

The body follows a tightly regulated glucose homeostasis. The brain is demanding and must have at least 200g/d of glucose. If blood glucose concentrations are below 40 mg/dL, coma or death could take place.[59] Conversely if blood glucose levels are too high, above 180 mg/dL, countinously for many years, renal failure and atherosclerosis may occur.[59] Glucose homeostasis includes two important pancreatic hormones insulin and glucagon which regulate blood glucose to keep it from being too low or too high. However, food and lifestyle choices can create many problems in this system. The Western diet is characterized by foods with a high glycemic index (GI).[23,59] When foods have a high glycemic index the sugar is absorbed fast, creating a spike of glucose in the blood. To compensate, insulin spikes as well to help lower the glucose levels and utilize the glucose. Postprandial blood glucose levels can indicate this response. High GI foods include refined starches like white bread, soda and instant rice. When a high GI meal is consumed instead of a low GI meal, postprandial hyperglycemia throws off the system

and can take a toll on the pancreas which can lead to insulin resistance.[59] Both postprandial hyperglycemia and insulin resistance increase the risk of type two diabetes.[18,37,67]

Many studies have taken place to find different ways that will help lower postprandial blood glucose concentrations specially when eaten with high GI Foods. Delaying gastric emptying and inhibiting enzymes to delay digestion and absorption are some ways to lower postprandial glucose. Numerous studies have found ways to potentially lower blood glucose levels through these mechanisms. This includes vinegar, nuts and exercise.[48, 50, 69, 71] There have also been a number of studies on dietary polyphenols and its effects on postprandial glucose. One example would be tea. The number two beverage consumed worldwide is tea.[13] Although there are different types of tea including white tea, black tea, oolong, and green tea they all come from one plant called *Camillia Sinensis.*[13] Of all teas, green tea is where most of the focus has been. Green tea has four main polyphenols also known as catechins these are, (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), (-)-epicatechin (EC), and (-)-epigallocatechin-3-gallate (EGCG).[13] Many animal and invitro studies have shown a strong link with green tea's most abundant catechin EGCG and its inhibitory effects on α-amylase.[32,34,62] There have been very few studies that have tested green tea consumption and its effect on postprandial glucose in humans.[48,67] One area that has been lacking is the study of Matcha tea and its effects on postprandial glucose. Matcha tea is green tea, but instead of seeping the leaves the leaves are in powder form and consumed whole. This will allow every unique nutrient of green tea to be completely consumed. Therefore, it is

possible that Matcha tea will help lower postprandial glucose concentration when consuming starches.

THE STUDY PURPOSE

The studies objective was to measure the acute effect of Matcha tea on postprandial blood glucose levels in healthy adults. In this randomized controlled cross-over study the subjects will visit on three different occasions. The subjects will randomly be assigned to consume three different drinks [water, Lipton green tea, or Matcha tea]. At each lab visit, one of the beverages stated above will be consumed with a bagel and jam. Blood glucose levels will be measured every 30 minutes to 120 minutes, including the measurement at time 0.

RESEARCH HYPOTHESES

Match tea consumption with a meal will lower postprandial glucose levels in healthy adults as compared to Lipton tea or water.

DEFINITION OF TERMS

- **Postprandial blood glucose-** The 2-hr blood glucose area-under-the-curve following meal ingestion
- **Insulin-** a hormone that functions to reduce blood glucose concentrations following meal ingestion
- **Glucagon-** a hormone that functions to increase blood glucose concentrations between meals
- **Glycemic index-** a ranking of different foods based on glycemic response, Scale from 0 to 100 those with High GI are foods that spike glucose in the blood at a

fast rate and are given a higher number, which is compared to the reference point of 100 which is pure glucose.

- **Polyphenol-** a chemical found in many plant foods that can alter metabolism; may play a role in the prevention of diseases like cancer

LIMITATIONS AND DELIMITATIONS

- Limitations of this study are small sample size and the possibility of inappropriate study adherence. Glucometers need to be used properly which includes calibrating the glucometer before each use. Lastly, the amount of EGCG was unknown in both Matcha tea and Lipton green tea, therefore we do not know how much EGCG the subjects consumed.
- Delimitations, this study excluded those with chronic diseases such as diabetes or cardiovascular disease and those on a weight loss program or taking any prescription medications for weight loss or any medications that may affect blood glucose levels. There were no imitations on weight. The ages of the subjects ranged from 20 to 31. These subjects are healthy adults therefore; this study may not be applicable for those with prediabetes or diabetes.

CHAPTER 2

REVIEW OF THE LITERATURE

This chapter will review the digestion and absorption of carbohydrates and glucose homeostasis. The glycemic index (GI) and glycemic load (GL) of foods will be defined and their effects on the body and disease states. Different interventions to help lower postprandial glucose concentration (PPG) including drugs, vinegar, nuts and exercise will be assessed. Finally, this chapter will highlight green tea and their healthful components, particularly the catechins. Specifically, Matcha tea will be highlighted with the focus on the catechin EGCG and its inhibitory effects on α-amylase and its potential for lowering PPG concentrations in humans.

CHARBOHYDRATE DIGESTION AND ABSORBTION

Carbohydrates (CHO) are made up of monosaccharides, basic unit of simple sugar. These include fructose, galactose, and the very important glucose. These monosaccharides can link together and form disaccharides. Lactose is composed of glucose and galactose and it is present in milk and dairy products. Sucrose is composed of glucose and fructose and is found in table sugar. Maltose is composed of two units of glucose and is found in molasses. Also, many glucose units can bond together and make polysaccharides. The common digestible polysaccharides are starch. Starch is found in plants and is made up of amylose and amylopectin which is composed of repeated glucose units that are bonded together.[40, 82] The glucose in amylose is bonded together in a linear form bonded at the α (1-4) glyosidic bonds.[40, 82] Amylopectin is also made up of glucose at α (1-4) glyosidic bonds, but then branch out, and these branch points are α (1-6) glyosidic bonds.[40, 82] Glycogen is also a polysaccharide and is stored in animal tissue

such as liver and skeletal muscle. Glycogen is a highly branched polysaccharide made up of glucose.[40] The importance of storing glycogen is to have glucose readily accessible when glucose levels become low in the blood. Enzymes cleave the bonds to release glucose molecules which are used for energy through glycogenolysis.[40] Lastly, cellulose is a polysaccharide found in plants cell walls. Cellulose is made up of many glucose molecules, but what makes it unique is how they bond together. Glucose is bonded at the β (1-4) glyosidic bonds, making it impossible to digest because humans do not possess the enzymes needed to break it down.[40] Therefore, cellulose is a source of dietary fiber (undigested polysaccharides) and gives the stool bulk.

Polysaccharide digestion begins in the mouth with the enzyme salivary α-amylase.[40, 55, 82] This enzyme breaks down the polysaccharides at the α (1-4) glyosidic bonds, and a few mono or disaccharides are released before swallowing.[40] Once the food enters the stomach the salivary α-amylase continues to do its job until gastric acid reduces enzymatic activity. The polysaccharides move from the stomach to the small intestine, duodenum and jejunum. At this point, pancreatic α-amylase is available to breakdown the polysaccharides amylose and amylopectin at the α (1-4) glyosidic bonds. [40,55, 82] This produces oligosaccharides, which contain three to ten monosaccharides. The oligosaccharides continue to be digested into monosaccharides by other enzymes found on the brush border, called lactase, sucrase, maltase, isomaltase, and trehalase. [40, 55, 82] Lactase takes lactose and breaks it down to glucose and galactose, sucrase breaks down sucrose to glucose and fructose, maltase breaks down maltose to produce two glucose molecules, and isomaltase breaks down the branch points of amylopectin into two glucose molecules.[40]

After the digestion of starches to monosaccharides and a few disaccharides, the absorption process can occur. The wall of the small intestine is made up of microvilli also known as the brush border.[40] The brush border helps in the absorption process. The main goal is to move the monosaccharides like glucose from the lumen of the small intestine into the intestinal mucosal cell or enterocytes for absorption into the blood.[40] Different transporters are used to move glucose, galactose, and fructose into the mucosal cell.[40] Glucose and galactose are absorbed into the enterocyte through active transportation. This transportation needs energy (ATP).[40] The glucose galactose receptor is known as sodium-glucose transporter 1 (SGLT1).[25, 40] This transporter takes glucose or galactose and a molecule of sodium into the enterocyte.[25] Glucose, and galactose can also be absorbed through GLUT2 into the enterocyte.[25, 40] Following a high CHO meal, the amount of glucose in the small intestine is elevated which leads to the activation of GLUT2 into the apical membrane. When this occurs, facilitated transport is used and ATP is not needed.[40, 50] Fructose absorption into the enterocytes is primarily through facilitated transport using GLUT5 receptor.[40, 50] All monosaccharides leave the enterocyte and enter the blood through GLUT2.[25, 40] Once in the blood the monosaccharides are taken to the liver. Fructose and galactose are metabolized by the liver. These monosaccharides can be converted into glucose and can be used in many ways:[40] stored as liver glycogen, circulate as blood glucose, or oxidized in the liver for energy. The glucose that has been sent to the liver can also go through all three processes stated above, but the surplus is sent into the systemic blood supply.[40]

Glucose is then utilized by other tissues like kidneys, brain, muscles and adipose tissue.[6, 40] Some tissues will require insulin for glucose uptake like skeletal muscle and

adipose cells through GLUT4 (insulin dependent).[40] While other tissues like liver, brain and kidney do not need insulin to obtain glucose from the blood. GLUT1 is expressed in the erythrocytes, central nervous system, the brain and placenta for glucose uptake.[40] GLUT2 can be found in the liver, kidney, small intestine and β cells of the pancreases.[87] GLUT3 can be found in the brain/neurons, placenta and preimplantation embryos.[6, 40] GLUT5 only for fructose uptake can be found in the intestine, kidney, brain, skeletal muscle and adipose tissue.[40, 50]

HOMIOSTASIS OF BLOOD GLUCOSE

Homeostasis of blood glucose concentrations is very important during fasting and postprandial period. If glucose falls below 40 mg/dL coma or death can occur.[59] If glucose levels become too high over 180 mg/dL continuously for many years, renal failure and atherosclerosis may occur.[59] Two important hormones involved in glucose homeostasis are insulin and glucagon. Insulin is a hormone secreted by the β-cells of the pancreas. A major manner for maintaining glucose homeostasis after a meal is the secretion of insulin when glucose concentration increases in the blood. Insulin will stop the production of glucose (gluconeogenesis) in the liver and lower the secretion of glucagon. Glycogen production from glucose is also stimulated. Insulin upregulates GLUT4 receptors in skeletal/cardiac muscles and adipose tissue. Facilitating glucose uptake by these cells to be used for energy. Glucagon is secreted by the α-cells of the pancreas when glucose concentration becomes too low during fasting or postoperative state. Glucagon sends a message to breakdown glycogen stores, this process is known as glycogenolysis to increase blood glucose concentrations.[40] A glucose tolerance test can help determine those with normal, impaired or diabetic PPG responses after a meal or

consumption of a high sugar beverage. Normal glucose concentrations are defined in table 1.

GLYCEMIC INDEX AND GLYCEMIC LOAD

The amount and type of dietary carbohydrates impact the control of blood glucose homeostasis. The glycemic index (GI) and glycemic load (GL) are used to portray the impact of dietary carbohydrates on glucose homeostasis after meal consumption.[59] When foods have a high glycemic index the carbohydrates are rapidly digested and absorbed quickly, creating a spike of glucose and insulin in the blood.[59, 91] When foods are digested and absorbed slowly they have a low glycemic index and a more moderate rise in glucose and insulin concentrations.[59, 91] To calculate the GI a subject consumes 50 g of glucose or 50 g of avalible carbohydrate in a test food on separate occasions. The test food can be grains, legumes, fruit or vegetables. The purpose is to get an idea of the effects of carbohydrates on PPG after 2 hours compared to the 50 g of glucose which has a GI of 100. Foods are classified as low GI [55 or less (oatmeal, pasta, sweet potato)] medium GI [56-69 (whole wheat bread, quick oats)] or High GI [70 or more (white bread or bagel, cornflakes)].[40, 68] Glycemic load is also important in glycemic response. GL is calculated using the GI multiplied by the grams of carbohydrate consumed this formula takes into account the quantity and quality of a carbohydrate.[91] GL is important when looking at different starches. That is, two starches may have a high GI but depending on how many carbohydrates that are available is important. For example, both watermelon and a doughnut have a GI of 76 which is high. However, one serving of watermelon is 11g of carbohydrates while a doughnut has 23g per serving.[69] When using the formula above the GL of watermelon and doughnut is 8 and 17 respectively. A high GL is ≥ 20, medium is

11-19 where the doughnut falls, and a low GL is ≤ 10, where the watermelon falls.[69] It is important to look at both GI and GL when it comes to the glycemic response, both will help determine its potential effects on PPG concentrations and insulin levels.

TYPE OF STARCH

A high or low glycemic index can depend on the type of starch. Starches can be classified into three categories which depends on how long digestion takes and the amount that can be digested. The three categories include rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS).[28, 30] RDS is quickly digested and absorbed in the small intestine. These starches include bread and potatoes and are converted into glucose in up to 20 minutes of enzymatic digestion.[79] SDS are digested slowly as well as completely in the small intestine. These starches can be found in cereals and take up to 100 minutes to be broken down into glucose.[79] RS is fermented by the gut microflora and not digested in the small intestine. This is the Starch that cannot be digested.[28] RS can be further broken down into 5 classes. Type 1 starches are resistant to digestion because they are trapped by cell walls and protein matrix making it impossible to utilize them. These starches include partly milled grains and legumes.[9, 28] Type 2 resistant starch is found in raw potatoes and green unripe bananas. When raw the RS cannot be digested due to their resistance to enzymatic hydrolysis.[9, 28] When a potato for example is cooked the starch significantly increases in digestibility due to starch gelatinization. Although, it is important to note that some starches in this classification may have a higher temperature needed for gelatinization to occur.[9] Type 3 starches are found in foods like potatoes and rice that, when cooked are gelatinized, but then when cooled the gelatinized starches are turned into RS. The process of digestible gelatinized

starched that are cooled and converted into RS is called retrogradation; these products cannot be broken down by pancreatic amylase.[9, 28, 79] Type 4 starches are modified by cross-linking or adding chemical derivatives. Both prevent enzymatic hydrolysis.[9, 28] Type 5 starches result from the interaction of lipids and starches to create a RS that can resist enzymatic hydrolysis, either by preventing amylase from breaking the starches down or by preventing gelatinization of a starch.[9] Therefore, starches with more SDS and RS will have a lower GI and will have a lesser effect on blood glucose concentrations.

The type of starch is not the only factor that effects the GI. Other factors include the acids and fat in foods which can slow down the process of stomach emptying. Slower stomach emptying can lead to a low GI because the rate of CHO digestion is slower. Particle size can affect the GI as well. If starchy foods are grounded up it can allow digestive enzymes to breakdown starches faster and therefore increase the GI.[28]

GLYCEMIC INDEX AND GLYCEMIC LOAD LINKED TO DIABETES AND ITS PREVENTION

Different theories exist in support of what mechanisms may cause type 2 diabetes. The two mechanisms include decrease pancreatic function from either excessive increase of insulin secretion or toxicity to the β cells from glucotoxicity and lipotoxicity. [59, 91] Both mechanisms point to the main culprit of a high GI/GL meals.

In the late postprandial period after a high GI meal there are low glucose and fatty acids in the blood which are important metabolic fuels, this can activate counter-regulatory hormones to fix these low levels of metabolites in the blood.[59, 91] Glycogenolysis (breakdown of glycogen to free glucose) and gluconeogenesis (production of glucose) are both stimulated which increase hepatic glucose output. The

stimulation of lipolysis (breakdown of adipose tissue to free fatty acids) elevates free fatty acids well above the levels of a low GI meal.[59] The increased levels of glucose and lipids in the blood from a high GI/GL meal may be toxic to the pancreas or cause pancreatic exhaustion from increased insulin demand. During the late postprandial period of a low GI meal no counter-regulatory hormones would occur, and there would be a balance of glucose and free fatty acids in the blood with no need to increase hepatic output.[59] The constant cycle of hyperglycemia, hyperinsulinemia and elevated free fatty acids from a high GI and GL diet could potentially damage the beta cells of the pancreas and increase the risk of T2DM.

The GI and GL of foods is the leading factor that effects postprandial glycemic response. Many studies have looked at diabetes prevention and lowering postprandial glycemic response. Studies have found that a low GI and GL diet and the associated lower postprandial glycemic response is linked with a decrease risk of diabetes.[10, 4, 49, 80, 91] In the Nursing Health Study which is a large prospective epidemiological study of women had found that women consuming foods from with the highest GL increased their risk of T2DM by 40% compared to those who consumed foods in the lowest GL ranges.[80, 91] One meta-analysis examined 8 studies which focused on diabetes risk factors, 6 of the studies found an association to diabetes and 2 did not.[10, 4] Findings in the meta-analysis showed that a high GI or GL was an independent factor in increasing the risk of T2DM.[10, 4] Other studies focused on nut consumption and T2DM. One study found that those who consume nuts had a lower risk of T2DM.[46] The relative risk was 0.78 for those who consumed nuts at least 5 times per week compared to those who did not consume

nuts.[46] This could be due to nuts effects on slowing down gastric emptying and therefore decreasing postprandial glucose concentrations.[10, 49]

DIABETES PREVELANCE

Table 1: Diabetes and Prediabetes Defined

Disease State	A1C	Fasting Plasma Glucose	Oral Glucose Tolerance Test (OGTT)
Diabetes	≥ 6.5%	≥ 126 mg/dl	≥ 200 mg/dl
Prediabetes	5.7 to 6.4%	100 mg/dl to 125 mg/dl	140-199 mg/dl
Normal	< 5.7%	< 100 mg/dl	< 140 mg/dl

diabetes.org

Type two diabetes mellitus (T2DM) has been growing at alarming rates. In 2013, 285 million people world-wide had T2DM. It had also been estimated that by 2030, 522 million worldwide will have T2DM.[7] In addition, prediabetes encompasses 79 million people in the US in 2013.[7] Diabetes is a huge problem due to increased risk of morbidity and mortality from microvascular and macrovascular complications. Microvascular complications include retinopathy, nephropathy, and neuropathy while macrovascular complications include heart attacks, strokes, and peripheral vascular disease. Hyperglycemia plays a large role in microvascular and macrovascular complications.[21] Healthcare cost are also greatly affected by this disease. Treating diabetes and its complications in 2007 cost $174 billion, a number that is estimated to double by 2050.[21] Early detection of T2DM is key because morbidity and mortality increase due to long term complications. For those diagnosed with prediabetes it is the optimal time to start an intervention and treatment plan to reverse pathophysiological defects that occur.[21] Diet and exercise is key in the prevention of insulin resistance, prediabetes, and diabetes.

INSULIN RESISTANCE

When a high carbohydrate meal is consumed and digested, the glucose in the lumen of the small intestine is absorbed using GLUT2 into the enterocyte.[40] The insulin that follows a spike in blood glucose concentrations down regulates GLUT2 to decrease the absorption of glucose. With insulin resistance, GLUT2 will remain upregulated and glucose will continue to absorb into the enterocyte and into the blood.[40] Muscles and adipose tissue are also affected. GLUT4 is an insulin dependent receptor since insulin is needed for GLUT4 to be brought up onto the cell membrane.[22, 40] If these cells are resistant to insulin it will prevent the uptake of glucose from the blood. Due to insulin resistance, glucose concentrations remain elevated in the blood. Gluconeogenesis and glycogenolysis both contribute to endogenous glucose production.[95] Both can be utilized in the fasted state and when glucose concentrations are low. Glycogenolysis is the breakdown of glycogen to glucose to increase blood glucose concentrations. Gluconeogenesis is when glucose is produced using lactate, pyruvate, glycerol and branched chain amino acids.[73] This occurs when glycogen stores are low. With insulin resistance increased gluconeogenesis occurs contributing to elevated fasting blood glucose concentrations.[76] Insulin resistance also affects adipose tissue. Hormone-sensitive lipase can be found in adipose tissue which breaks down fat stores into free fatty acids (FFA) to be used for energy. Insulin inhibits this enzyme in a healthy individual. For those with insulin resistance, insulin loses its effects on inhibiting hormone-sensitive lipase therefore increasing FFA levels circulate in the blood.[73, 85] Studies have found that FFA may have negative effects on insulin in the suppression of endogenous glucose production.[76] One study found that when FFA were increased in the blood

gluconeogenesis also increased and vice versa, which could be a factor in hepatic insulin resistance and increased endogenous glucose production.[95] The gold standard in measuring insulin resistance is a hyperinsulinemic-euglycemic clamp[51, 85] which measures the action of insulin and glucose metabolism.[51] Hyperinsulinemia is achieved with constant insulin infusion. Normal blood glucose concentrations are maintained via glucose infusion. The glucose infusion rate (GIR) is determined from blood glucose measurements taken every few minutes and adjusting the GIR.[2] Good insulin sensitivity requires a greater GIR because the cells are responding to the insulin and the glucose is being taken into cells to be utilized.[2] One study classified the different rates that indicate insulin resistance using the clamp. A glucose disposal rate (GDR) of < 5.6 mg/kg fat-free mass (FFM) + 17.7 · min has an 80% chance of insulin resistance.[85] The clamp is not normally used as a screening tool for individuals with prediabetes or diabetes, but rather commonly used in research. This is because the clamp is labor intensive and high in cost.[65] Although, the hyperinsulinemic-euglycemic clamp is the gold standard there are other tools to assess insulin resistance this includes the homeostatic model assessment of insulin resistance (HOMA-IR). Where insulin sensitivity is measured by fasting plasma insulin and glucose concentrations and is plugged into an equation. Therefore, determining if a person is insulin resistant.

PREDIABETES

During the prediabetic state a decrease in β-cell function has already taken place. There are many contributing factors such as genetics, insulin resistance, increased insulin secretory demand, glucotoxicity, and lipotoxicity as well as decreased β-cell mass that play a role in the progression of β-cell dysfunction in the prediabetic stage.[7] Together,

these factors can lead to β-cell failure and diabetes.[7] The diagnosis of prediabetes is based on impaired glucose tolerance (IGT) and/or impaired fasting glucose (IFG). IGT is characterized by early and late plasma insulin response when taking an oral glucose tolerance test (OGTT).[21] In those with prediabetes moderate to severe muscle insulin resistance may be present. When the muscles cells are not responding to insulin they are not able to take in glucose. Excessive levels of blood glucose concentrations can occur with insulin resistance in the muscles. This is due to its high amounts of glucose uptake. One study showed with a hyperinsulinemia-euglycemic clamp that the glucose uptake of skeletal muscle is about 80%. The study also found that with an increase in insulin infusion increasing glucose uptake occurred in the leg muscles.[22] Prediabetes is defined in table 1. Those with prediabetes also have altered triglyceride-rich lipoproteins metabolism. This manifests as high plasma triglyceride levels and low HDL-c. When there are higher amounts of triglycerides than needed the excess goes into the liver, skeletal muscle and pancreas which can result in lipotoxicity which contributes to β-cell dysfunction.[7] It has been estimated that 5-10% of those who are prediabetic develop diabetes annually and 70% of those with prediabetes will eventually develop diabetes in their lifetime.[7] Interventions that help reduce insulin demands and increase insulin sensitivity is important in preventing/delay the development of prediabetes to diabetes.[21]

THE PROGRESSION OF NORMAL GLUCOSE LEVELS TO PREDIEBETIC TO TYPE TWO DIABETES

Insulin resistance has been shown to start years before diabetes occurs. One study found that those who had diabetes showed increased glucose concentrations and decreased insulin sensitivity 13 years before they were diagnosed. Even though glucose

concentrations increased gradually they were still within the normal range until 2-6 years before diagnosis when there was a sudden increase in glucose levels.[84] By 3 to 4 years before diagnosis insulin secretions increased and then plummeted. These findings help support the idea that insulin resistance can take place long before diagnosis.[84] What may drive insulin resistance could be increase in weight, inactivity and genetics. There has been a 5-stage model in the development of diabetes by Weir. The first stage is called compensation where insulin resistance occurs and increased insulin secretion to keep glucose concentrations normal. This compensation of insulin most likely comes from increase in β-cell mass.[84, 90] The second stage is called stable adaption where β-cell are not able to fully compensate and fasting glucose and post prandial glucose levels are not truly normal although they fall within the normal range. Both compensation and stable adaption stages occur before the prediabetic stage.[84, 90] In stage three called unstable early decompensation, β-cells are not able to compensate and therefore glucose concentrations increase quickly. This stage most likely spreads from prediabetes to diabetes. The β-cells continue to show inadequate function, and glucose levels rise in a short period of time to stage 4 called stable decompensation. Further β-cell losses lead to stage 5 called sever decompensation which can include ketoacidosis and lead to insulin dependence.[90]

DIABETES, MICROVASCUALR AND MACROVASCULAR COMPLICATIONS

. Diabetes occurs when β-cell dysfunction has occurred, and insulin can no longer compensate for high glucose concentrations. Diabetes is defined in table 1. Diabetes is characterized by hyperglycemia which wreaks havoc on the body leading to many complications. These complications include microvascular like diabetic retinopathy and nephropathy and macrovascular complications like cardiovascular disease:

atherosclerosis, myocardial infractions, amputations and cerebrovascular disease like stroke.

Diabetic Retinopathy (DR) is the leading cause of blindness in developed countries and effects 100 million people worldwide and can only go higher with increased diabetes rates.[26, 81] DR develops as early as 7 years before the diagnosis of T2DM. DR may be related to the severity and duration of hyperglycemia. Hyperglycemia damage the capillaries and weaken them leading to the issues stated below.[36] There are two categories of DR the first is the early stage called nonproliferative diabetic retinopathy this stage includes visible characteristics such as microaneurysms which is swelling of a vessel and appear as red dots in the back of the eye, retinal hemorrhages where the vessels of the retina burst and bleed and can lead to blurry or loss of vision.[26, 36] Nonproliferative diabetic retinopathy can lead to a loss of functional capillaries and inadequate blood flow called ischemia and lack of oxygen to retinal neurons which then progresses to the next stage.[26] The advanced stage of DR is proliferative diabetic retinopathy where pathological preretinal neovascularization.[26] This lack of blood flow leads to the retinal cells angiogenic signals like vascular endothelial growth factor (VEGF) which stimulates the growth of new vessels form previous vessels. The problem with neovascularization is that these new vessels can be fragile, leak and even grow into the vitreous which is the space between the lens and retina. Neovascularization can lead to vitreous hemorrhage causing severe vision loss.[26] During both stages, diabetic macular edema (DME) takes place from the breakdown of the blood-retinal barrier due to diabetes due to leaky microvascular leakage and cause retinal thickening and edema of the macula and can cause vision loss.[26, 36] One current treatment used in proliferative diabetic

retinopathy is Anti-VEGF and has been shown to reduce vision loss and increase vision gains. Hyperglycemia triggers the expression of VEGF which is linked with the breakdown of BRB, increased vascular permeability, and new vessel growth. Using Anti-VEGF which inhibits VEGF has been shown to reduce vascular leakage and macular edema in those with diabetes.[27] Anti-VEGF can also reduce new vessel growth which can help reduce damage to the retina from these new vessels and the issues that come with it like leakage or vitreous hemorrhage. Treatment for early stages of DR are still needed, increased pathophysiology's for DR have been found but many treatments have not worked based on their findings.[81]

Diabetic Nephropathy (DN) is associated with three major changes in the glomeruli. Glomeruli is a cluster of capillaries that filters the blood. The major changes that take place include mesangial expansion, thickening of the glomerular basement membrane, and glomerular sclerosis. Mesangial expansion can potentially obstruct glomerular capillaries due to the expansion of mesangial matrix. Mesangial cells are important for maintaining the structure of glomerular capillary and regulate glomerular filtration by regulating blood flow though their ability to produce contractions.[24, 43] Hyperglycemia is linked with increased number and size of mesangial cells and increase in matrix production.[24] This could occur due to increased glucose levels in the cell. The excess glucose from hyperglycemia can lead to an accumulation of glycation end products (AGE) which is protein and lipids molecules that bind to glucose. Healthy kidneys excrete AGEs but in kidney disease AGEs build up in the tissue and can be one reason for mesangial expansion and can also affect glomerular basement membrane and podocytes.[24, 43, 83] Glomerular basement membrane is within the glomerulus. The

membranes lie between two cell types: glomerular endothelial cells and glomerular epithelial cells also known as podocytes which make up the glomerular filtration barrier.[60] The glomerular basement membrane plays a role in glomerular filtration barrier which is the process of selective filtration of the blood. The glomerular filtration barrier allows free permeability of water and small solutes while preventing macromolecules and cells from leaving the blood leading to protein free urine. Podocytes maintain the structure and function of glomerular basement membrane and any alterations in any of the layers of glomerular filtration barrier which includes podocytes and glomerular basement membrane can change glomerular permeability.[60] Hyperglycemia can increase the production of renin and angiotensin which increases angiotensin II. Angiotensin II has shown to increase activity in two growth factors VEGF-A and TGF-B. Both growth factors increase glomerular basement membrane thickening and mesangial expansion by depositing protein outside the cell and directly increasing proteinuria and disturbing glomerular filtration barrier.[15, 43] The kidneys naturally produce reactive oxygen species (ROS) (i.e., O_2^-,H_2O_2. NO, and $ONOO^-$) during metabolic activity. ROS production is usually corrected with antioxidant enzymes such as glutathioneperoxidase, catalase, and superoxide dismutase.[83] ROS production is stimulated from hyperglycemia effecting the balance between ROS and the body's ability to protect against ROS. ROS can greatly damage the kidneys, renal vasculature and potentially glomerular filtration barrier and podocytes through the damaging effects of ROS which include damage to the cells lipid membrane also known as peroxidation, oxidation of proteins, renal vasoconstriction and damage to DNA.[24, 83] These changes in the kidneys from the effects of hyperglycemia, leads to kidney damage, albuminuria, and diabetic kidney disease. Of those with diabetes

20 to 40% develop DN. This is the most common cause of end stage renal disease and required dialysis in the US. Dialysis is a costly process and not easy on the body. Those with diabetes on dialysis have a 22% higher mortality risk after the initiation of dialysis.[24]

Macrovascular complications occur from atherosclerosis which effects coronary arteries, peripheral arteries and carotid vessels. The formation of atherosclerosis is greatly affected by diabetes and increases the risk of its formation which can lead to coronary and peripheral artery disease as well as cerebrovascular disease. Cardiovascular disease is the leading cause of death in patients with diabetes because of the effects of atherosclerosis. Cardiovascular disease is responsible for 44% of deaths in those with diabetes.[61] Peripheral artery disease (PAD) occurs from the formation of atherosclerosis in the vessels of the lower extremities. PAD is linked to loss of function of the legs, increases the risk of myocardial infraction, stroke, and limb loss. In the US diabetes is the number one reason for non-traumatic lower extremity amputations.[5] The 12 million people in the US with PAD, about 20 to 30% have diabetes.[61] Glycemic control in patients with diabetes is an independent risk factor of PAD. Every 1% increase in HbA1C increases PAD risk by 28%.[61] Myocardial infraction increases the risk of recurrent myocardial infraction or cardiovascular death events by 18.8% in those who are nondiabetic whereas those who were diabetic had a 45% risk.[5] Those with diabetes have an increased risk of stroke (+150% to 400%). Poor glycemic control directly relates to stroke risk.[5] Cardiovascular disease and cerebrovascular disease are strongly driven by atherosclerosis. Diabetes exacerbates the rate of atherosclerosis increasing the risk of disability and death for patients with diabetes.

Atherosclerosis occur when lymphocytes and monocytes move to the intima of a vessel.[5] Monocytes which turn into macrophages take in oxidized LDL-c and become foam cells.[5] The accumulation of foam cells produces fatty streaks which is the beginning of atherosclerotic lesions. This pathological process is increased with diabetes. Hyperglycemia increases oxidative stress in the endothelium of the vessels and increases advanced glycation end products which leads to an increased production of nuclear factor $_{K}$B (NF-$_{K}$B).[5, 20] NF-$_{K}$B can be found in atherosclerotic lesions and plays a role in atherosclerosis due to its regulation of inflammatory gene expression of cytokines, chemokines, leukocyte-cell adhesion molecules and acute phase proteins.[5] The inflammatory gene expression brought on by the activation of NF-$_{K}$B recruits lymphocytes and monocytes into the intima of the vessels bringing about the possible production of foam cells and fatty streaks.[5, 20] Diabetes can also lead to plaque instability by decreasing collagen synthesis and increasing collagen break down. Collagen is very important in the stability of the fibrous cap. Studies have found that a thinner fibrous cap can increase the chances of plaque rupture.[3, 5] Plaque rupture can result in thrombus (blood clot).[3, 5, 61] The risk of thrombus is high in those with diabetes due to hyperglycemia and its effects on platelet activation. [5, 61] Hyperglycemia plays a role in atherosclerosis formation, plaque instability, platelet activation, and thrombus. Increasing the chances of blocking the flow of blood in a vessel leading to myocardial infraction, stroke or death.

INTERVENTIONS TO HELP PREVENT DIABETES

In diabetes, glycemic control is an issue characterized by hyperglycemia and insulin resistance. Glycemic control and fasting blood glucose are greatly affected when hepatic insulin resistance is present. Metformin is a drug that can be used in the prevention or treatment of diabetes. Metformin decreases fasting blood glucose concentrations by decreasing hepatic glucose production. Metformin suppresses gluconeogenesis and enhances the action of insulin to inhibit endogenous glucose production.[33, 35, 84] A randomized control trial evaluated the effects of metformin in subjects with IGT. IGT is a risk factor for T2DM.[73] This study looked at how metformin could positively affect the rate of those with IGT to the progression of diabetes. There were 29938 subjects who were investigated at Shougang Corporation using 75g oral glucose tolerance test (OGTT). Those with IGT based on WHO criteria (1985) with age ranging from 30 to 60 years were eligible for the study. Those with pre-existing diabetes, history of ischemic heart disease, renal or hepatic disorders, and those previously treated with metformin were excluded. Subjects were randomized into two groups: metformin (n=33) and placebo (n=37). Both groups averaged 50 years in age with close to normal weights and BMI. This was a double-blinded RCT; subjects in the metformin group received 250 mg three times a day. All subjects in both groups received education on diet, exercise, and healthy lifestyle every 3 months. The study ran for a year with follow up visits every 3 months. Using a venous blood sample, fasting plasma glucose, HbA1C and insulin were measured. Urine was also collected to measure urinary albumin excretion rate (UAE). After 12 months the 28 subjects receiving metformin reverted to

NGT (84.9%) compared to 19 in the placebo group (51.4%) (p-value =0.011). One subject in metformin group converted to T2DM (3.0%) while 6 subjects in the placebo group converted to T2DM (16.2%). By 12 months the placebo group displayed a decreased fasting blood glucose (mmol/l) from 7.3 ± 1.0 to 6.2 ± 1.3, and the metformin group displayed a decrease from 6.9 ± 0.9 to 5.0 ± 1.1 (P<0.01). HbA1c had a net fall of 0.7% in the metformin group with a p-value of 0.0001 whereas in the control group there was no significant decrease in HbA1C, 7.4 ± 0.8 to 7.3 ± 0.9. A decrease in HbA1c in the metformin group could be related to improved fasting glucose concentrations and improved glucose tolerance.[57] Insulin levels also decreased in the metformin group and significant improvements in insulin sensitivity was shown. Lastly, UAE for both groups were within normal limits, but those in the metformin group had a statistically significant decrease from baseline to 12 moths (P<0.05). Metformin did show improvements in fasting blood glucose concentrations, insulin levels and even decreased UAE. Also, the conversion of IGT to T2DM was only 1 person in the metformin group and 6 in the placebo group. This study shows improvements in FBG, insulin levels and HbA1c which is very important in the prevention of T2DM especially for those with prediabetes.[57]

Metformin and lifestyle modifications were looked at in another RCT. The study looked at those with IGT and the incidence of T2DM in Asian Indians. Subjects in this study who had IGT was determined using 75g OGTT and defined as FBG <126 mg/d and 2-h glucose 140-199 mg/d. Those who did show glucose concentrations that indicated IGT were tested twice to confirm. There were 532 subjects with IGT and were randomized into four different groups. Group 1 (n=136) the control, Group 2 (n=133) lifestyle modification (LSM), Group 3 (n=133) metformin (MET), and group 4 (n=129)

LSM and MET. Primary outcome was to see how many subjects developed diabetes which is either a FBG of ≥ 126 mg/dl and/or 2-h glucose ≥ 200 mg/dl using 75g OGTT. Subjects were given 250 mg two times a day of metformin initially. After two weeks, it was increased to 500 mg twice a day for 40 days and then brought back down to 250mg twice a day. Those subjects in the LSM groups received advice on a healthy diet and regular active. Diet modification included reduction of calories, refined CHO and fats as well as sugar and an increase in fiber-rich foods. This study took place for three years with an annual OGTT and anthropometric measurements. Those with blood levels that indicate diabetes were checked again. Results show that after three years the prevalence of diabetes was 55% in the control whereas the other groups did show a statistically significantly lower incident: LSM was 39.3%, MET was 40.5% and LSM + MET was 39.5%. Absolute relative risk was reduced significantly as well, LSM reduced risk 28.5%, LET +MET 28.2% and MET 26.4%. These data show that metformin reduced the risk and progression of T2DM.[75]

Other interventions have been found to help lower PPG concentrations. This is important due to its impact on the development of type 2 diabetes and cardiovascular disease. This includes both drugs and natural interventions. Drugs that helps lower PPG concentration are known as α-glucosidase inhibitors (AGIs).[23] One example of this drug is Acarbose. This drug inhibits α-glucosidase; these enzymes are found in the brush boarder of the gut.[23] They break down the oligosaccharides into monosaccharides to be absorbed into the enterocytes, so they can then enter the bloodstream.[23] The inhibition of these enzymes will greatly affect the amount of monosaccharides that can enter the bloodstream, lowering PPG concentrations.

A randomized double-blinded and placebo-controlled was done in Canada, Germany, Austria, Norway, Denmark, Sweden, Finland, Israel and Spain. Men and women between 40 and 70 years in high risk populations with a BMI ranging from 25 to 40 kg/m^2 were screened. Those who were eligible for the study were those with IGT (2-h PG $\geq$ 140 mg/dl and < 200 mg/dl and a FPG of 100-138 mg/dl). Subjects were randomly selected to placebo (n= 686) and acarbose (n= 682). Subjects receiving acarbose started at 50 mg a day and then slowly increased to 100 mg three times a day. Subjects were to record a 3-day food diary and physical activity during the last month of their annual visit. Subjects were seen every 3 months for pill count and distribution as well as measuring FPG. This study took 3.3 years and their primary outcome was development of diabetes (2-hr PG of $\geq$ 200 mg/dl after 75g OGTT). The subjects from Canada made up 40% of the subjects, 27% Germany and Austria, 24% Nordic countries, 5% each from Spain and Israel. Results showed that acarbose reduced the risk of the developing T2DM by 25%. Acarbose also increased the chances of IGT to revert to NGT ($p<0.0001$). The reason for these results could be explained by the findings of other studies where the higher the 2-hr PPG concentration after 75g OGTT in those with IGT the higher the risk for T2DM. Acarbose helps lower PPG concentrations and therefore reducing the development of T2DM.[16]

Natural interventions have also been able to lower PPG concentration as well. Vinegar is one natural intervention. The acetic acid in vinegar is effective in lowering PPG concentrations and insulin response in many studies.[47, 58, 70] This could be due to the acetic acid which delays gastric emptying rate.[58, 63] Therefore, polysaccharides enter the small intestine at a slower rate, preventing a spike in glucose. Other studies have also

found that acetic acid may inhibit disaccharidase.[63] These enzymes are found in the small intestine, its inhibition can result in decreased glucose absorption due to the inability to breakdown sucrose, maltose, and lactose into monosaccharides.[63] One study gave subjects a meal composed of a bagel with butter, juice and water. The GI of the bagel meal= 100. Before the meal was consumed, participants were given either a placebo drink or test drink that contained water and 20g of apple cider vinegar (close to a tablespoon) with 5% acetic acid.[47] At 60 minutes post meal PPG concentration was lowered by 54% in comparison with the control of no vinegar consumption.[47]

Another natural intervention is nut consumption and its effects on lowering PPG concentrations. In the study described above nuts were also examined. The test meal was the same, but instead of butter 25 grams of peanut butter was spread on the bagel.[47] PPG concentrations were lowered by 56% in comparison with the butter control.[47] Almonds also show similar effects when consumed with white bread in a different study.[49] White bread was consumed with different amounts of almonds: 0g (control) or 30g, 60g, or 90g.[49] The greater the number of almonds consumed with the white bread the lower the PPG concentrations.[49] The reason for the effects on nuts and PPG concentrations is due to its effects on gastric emptying. The higher the dose of almonds the slower the rate in gastric emptying and subsequently a decreased glycemic response.[49]

Exercise is another intervention that can lower PPG concentrations.[68,77] Two studies demonstrated that exercise for 90 minutes (moderate-intensity), 2 hours before or after meal, results in a 50% decrease of PPG concentrations.[68] Being physically active has been found to lower blood glucose concentrations for up to 24 hours or longer.[1, 77] The reason why exercise help lower glucose concentrations is due to its positive effects

on insulin sensitivity.[1] Increased sensitivity to insulin helps increase glucose uptake into muscle and moving it out of the blood stream.

It is important to find more ways to help lower PPG concentrations in the prevention of insulin resistance, type 2 diabetes and cardiovascular disease. The more interventions identified the more tools that will be available to the public. Everyone is different, and one intervention does not fit all; therefore, the more interventions are proven the more people that can be helped.

TEA

There have been a number of studies on dietary polyphenols and their effects on PPG concentrations. One example would be tea. The number two beverage consumed worldwide is tea.[13] Although there are different types of tea like white tea, black tea, oolong, and green tea they all come from one plant called *Camillia Sinensis.*[13] This evergreen tree can reach 98 feet tall. This tea tree has its roots in Xishuangbanna, China where it still grows today. There are 200 species of *Camillia, Camillia Sinensis* is solely for tea production.[38] The word Sinensis means from China, where tea was discovered.[38] The discovery of tea is explained in a mythical story of Emperor Shen Nung in 2737 B.C.E. The story follows, Emperor Shen Nung was a scholar and herbalist. He only consumed boiling water for hygienic purposes. One-day Shen Nung was sitting under a tea tree, when the breeze caught the tree. The leaves on the swaying branches fell into his boiling water. When he drank this new "beverage," he found it to be enjoyable as well as revitalizing. Thus, tea was discovered.[72]

TYPES OF TEA AND TEA PRODUCTION

There are many different types of tea which all have their own unique production process. White tea is the least handled. White tea can be just the downy buds of the plant which produce tea like silver needle. It can also be a mixture of the buds and leaves of the tea tree which produces tea like Shou Mei. [38] The Chinese process white tea by withering the leaves and buds, which are spread out on racks. They are left to air dry if weather permits for 12 to 24 hours, or, fans can be used as well. [38] Then the leaves are sorted to remove broken leaves and branches and other unwanted products. For green tea, leaves are plucked young with their buds attached while other green teas use mature leaves without buds. The leaves also go through the process of withering to dehydrate the plant to reduce moister from the leaves. [38] Dehydrating tea leaves can inhibit enzymatic activity and therefore slow down the oxidation of the leaves.[78] Tea leaves are left to air dry on racks or using a cylindrical machine that spins the leaves while blowing air on them.[38] Once the leaves are dried they go through the process of panning.[38] the leaves are heated once again to decrease oxidation or stop oxidation all together using vats. The heat causes enzymes, polyphenol oxidase and peroxidase, which are responsible for oxidation to denature.[39, 78] Pressing small batches of leaves to the bottom of the vat prevents the burning of the leaves. The leaves are subjected to three different heat/cooling cycles which gives the tea its unique properties like the release of the polyphenols and its aromatic smell. [38] The leaves are then rolled to release oils, also changing the shape of the leaves to twisted or bead-shaped. The leaves are then dried again so that the oils released from rolling will remain on the leaves and stabilize. Also, the drying process will further dehydrate the leaves leaving only 2 to 4% moister in the leaves. [38] The last part is sifting,

to remove any fine particles that could have happened from the leaves breaking, the leaves are then sorted into different sizes. [38] For black tea the withering of the leaves take place for 5 to 6 hours and are stirred often. When the leaves are mechanically processed they are heated by wood fires to give the tea a smoky aroma. Rolling takes place next to release enzymes that cause oxidation. This can be done using a machine to press the leaves down and release the enzymes. The oxidation of the leaves can take from 8 to 12 hours. They are spread on ground covered by wet cloth, temperature should reach 72 degrees Fahrenheit. [38] The leaves are then dried to eliminate moisture that remains in the leaves. This can be done using machines and blowing warm air on the leaves, or they can be added to another wood based heating process. Sorting and sifting of the leaves comes next. They separate the leaves into different grades while eliminating any unwanted product like branches. [38]

In Japan, green tea is processed differently. The leaves are plucked by machine and then steamed for about 20 to 80 seconds. This is important in terms of aromatic quality. Short bouts of steam produce a light taste reminiscent of green vegetables. [38] While longer steaming (40 to 80 seconds) is more traditionally done in Japan giving the tea an intense flavor and darker liquid. [38] The tea leaves are steamed or heated immediately to denature the enzymes that cause oxidation.[78] In turn the green color remains as it continues the step by step process.[78] The tea leaves are then cooled by air jets while leaves rotate in cylinders. The first drying takes place at 210° Fahrenheit for 45 minutes. Then they are moved into a different machine to be heated at 175° Fahrenheit and brought down to 105° Fahrenheit. The rotating cylinders used in this process are designed to brush the outside layer of the leave which determine the color of the leaves

and release the tannins. [38] The leaves are then rolled to release the oils and then a second drying takes place. This is done for 20 to 40 minutes at 80° Fahrenheit. After this 13% of water remain in the leaves. [38] Shaping is next, using machinery that give the leaves a needle shape which can last for 40 to 60 minutes at 158 to 248° Fahrenheit. [38] A third drying takes place on a conveyor belt for 30 minutes and heated to 185° Fahrenheit. [38] Shifting and sorting is done to take branches and other unwanted product out and to sort by size. [38] The final drying process depends on the final product that is desired. Leaves are heated to 105° Fahrenheit for aromatic purposes. The longer the leaves are heated the fresh grass aroma decreases and is replaced by a smell more like grilled nuts. Only 1 to 3% moister is left. [38]

Matcha tea started in China where it was custom to grind the leaves into a powder form. This method was adopted by the Japanese and became part of a ritual called Chanoyu. The high quality Matcha tea leaves come from Uji region of Japan. [38] The tea leaves go through the same process of Japanese green tea stated above but, the leaves are sorted strategically to permit grounding to a fine powder. [38] The preparation of Matcha tea is unique: **1)** 1 tsp of Matcha using a strainer, sift the tea to prevent lumps **2)** pour ¼ cup of water at temperature of 167 degrees Fahrenheit over tea then whisk. **3)** when foam begins to form, raise whisk carefully **4)** Drink the tea. To prepare the Matcha you will need a tea bowl, tea whisk, a teaspoon and a fine strainer. [38]

NUTRIENTS IN TEA

All tea has its own unique make up that draws attention to many health benefits. The main tea would be green tea for the abundant polyphenol content and its link to many health benefits. The chemical composition of green tea is based on dry weight 15 to 20%

proteins 1-4% of these proteins are composed of amino acids like, theanine, glutamic acid, tyrosine, valine, leucine, threonine, arginine, and lysine.[13] Carbohydrates 5-7%, including cellulose, glucose, fructose, sucrose, and pectins.[13] Lipids include linoleic and α-linolenic acids. Sterols for example stigmasterol can be found in green tea and vitamins such as B, C, and E. Minerals include Calcium, Magnesium, Chromium, Manganese, Iron, Copper, zinc, molybdenum, selenium, sodium, phosphorus, nickel, potassium, and Aluminum.[13] Studies have found different mineral content based on location of where the tea leaves have grown.[13] As tea leaves are processed to form different kinds of tea the nutrient composition changes. There are for major catechins in green tea which include (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), and (-)-epigallocatechin-3-gallate (EGCG).[38] Because green tea is the least oxidized these catechin's remain high. In black tea which is completely oxidized the catechin are decreased and form theaflavins and thearubigins.[56, 86] Tea also contains caffeine but in lower amounts compared to coffee. In 1 oz of coffee brewed prepared with water there is 12 mg of caffeine and for 4 oz of coffee there is 47 mg of caffeine.[88] In 1 oz of green tea brewed and unsweetened contains about 4 mg of caffeine and 16 mg of caffeine in 4 oz.[88]

GREEN TEA AND ITS POTENTIAL EFFECTS FROM ITS ANTIOXIDANTS

Green tea also contains polyphenols known as catechins. The four major catechins and their percentage of total catechin make are, (-)-epicatechin (EC) 6.4%, (-)-epigallocatechin (EGC) 19%, (-)-epicatechin-3-gallate (ECG) 13.6%, and (-)-epigallocatechin-3-gallate (EGCG) 59%.[38] The most abundant catechin in green tea is EGCG. Analysis of 31 commercial teas found that EGCG were greatest in old green tea leaves and then lessened with young leaves, oolong tea, and then black tea. It has also

been estimated that a cup of green tea which is 2.5 grams of tea leaves with 200 mL of water may contain 90 mg of EGCG.[13, 93]

The most abundant catechin in green teas, EGCG, has been a focal point of interest in many studies particularly cancer studies. It has also been found to inhibit the enzyme α-amylase.[32, 34]As discussed earlier α-amylase can be found in the saliva and pancreas. One study on EGCG and its effects on PPG concentrations in mice was conducted at Pennsylvania State University.[34] The male mice used were separated into groups based on body weight. The mice were given common corn starch, glucose, maltose or sucrose with 100 mg/kg of EGCG or without EGCG.[34] An α-amylase inhibition assay by EGCG and EGC was also conducted in vitro. The results found that, the mice receiving EGCG and CCS had reduced blood glucose concentrations as compared to mice that did not consume EGCG with CCS.[34] They found mean blood glucose concentrations decreased by 77 mg/dL within 30 minutes and 73 mg/dL lower after 60 minutes of consuming EGCG.[34] These findings showed a statistically significant ($p< 0.05$) decrease in blood glucose concentrations in the mice receiving EGCG.[34] There were no effects found from the glucose or maltose groups with the EGCG. The results from the α-amylase inhibition assay, showed EGCG and EGC did inhibit α-amylase, EGCG was found to have a stronger link in this. EGCG at 20 μM, inhibited α-amylase by 34%.[34] The study reports that the EGCG dosage was equivalent to 1.5 cups of green tea, which lowered PPG concentrations when taken in with common corn starch in mice.[34]

Another study also found that EGCG is the main catechin to inhibit α-amylase.[32] This is because EGCG has shown a higher inhibitory power compared to other polyphenols.[32] Using ultraviolet absorption spectroscopy and fluorescence emission

spectroscopy the interaction of EGCG and α-amylase were assessed. The interaction entailed bondage at the hydroxyl group of the polyphenol and the catalytic residues of the binging sites in α-amylase.[32] Hydrogen bonding and hydrophobic interactions bond the hydroxyl group of the polyphenol to the binding site on α-amylase.[32, 94] This forms a polyphenols-enzyme complex resulting in the inhibition of α-amylase.[32]

There have been few studies on green tea consumption and its effects on PPG concentrations in humans. There have also been very little or no studies on the effects of Matcha tea and PPG concentrations. Because there has been many animal and invitro studies assessing the inhibition of α-amylase from the catechin EGCG. It is important to further this knowledge and see if it can be applicable for human subjects. It is important to test Matcha tea, since instead of steeping the leaves for a few minutes in hot water and then removing the leaves, the leaves remain in the drink increasing the amount of polyphenols including EGCG that are ingested. Hence, since Matcha tea leaves are consumed whole more constituents are ingested which may facilitate efficacy. To study the effects of Matcha tea on PPG concentrations will add to the collection of tools in the prevention of these metabolic diseases.

GREEN TEA AND HEALTH CLAIMS

There are many health claims on green tea and its potential glucose lowering effects. Studies in mice may have shown the potential effects of EGCG extracts, but these results are not so comparable to green tea consumption in humans. Still, health claims have been made on the internet either adding miss-information or to help sell a product. One web article written on the website called Healthline, informs readers about green tea and diabetes management.[74] The web article states the potential of green tea and

prevention of diabetes. The research used to support this statement had found that those who consumed 6 or more cups of green tea daily were 33% less likely to develop diabetes.[42, 45] What this web article did not include was that this study also looked at coffee intake and found that 3 ≥ cups consumed daily was associated with a 42% lower risk for diabetes.[45] The study by researcher Hiroyasu Iso et al, was a retrospective cohort which collected self-reporting data on how much coffee, green tea, black tea and oolong are consumed daily. Based on their answers they categorized the amount of caffeine consumed. The subjects also self-reported if they were diagnosed with diabetes by a physician. Self-reporting could affect the study greatly since there could be individuals with diabetes, but may not have been diagnosed. The study did find an inverse relationship between consumption of higher levels of caffeine from either coffee or tea and a decreased risk of diabetes.[45] Although this study can be a stepping stone for research, more needs to be done. A randomized control trial is the gold standard and would provide more valid information. The web article also states that green tea may be able to help manage blood glucose levels in those with diabetes.[74] They support this with a comprehensive review that pulls many tea studies together and assess the power of tea collectively and its effects on fasting glucose, A1C levels, and fasting insulin levels. When looking into this comprehensive review over 10 of the studies used tea extract. Tea extract can be very different than consuming tea itself because the concentration of polyphenols like EGCG can be much higher. This information can be very misleading when supporting tea drinking to help manage diabetes. This article also explains the reasoning on why green tea can be so beneficial using information from Pacific College of Oriental Medicine.[71] The college talks about the potential effects of teas polyphenols.

Making claims that tea can help lower blood sugar concentrations supporting this with a study using tea extract, but not including any specific references to identify what study they wrote about.

Another web article written on everyday health titled "Why Drinking Tea May help prevent and manage Type 2 Diabetes."[12] This article also mentioned the retrospective cohort study by Hiroyasu Iso et al. This article only shared the finding of green tea but did not mention that only 3 cups of coffee rather than 6 cups of tea would have a higher percentage in decreasing diabetes risk.[45] This study was not a strong design since it was not a randomized control trial and relied on self-reporting. Also, the article makes a claim that polyphenols in green tea can help regulate glucose. There was no reference to support this statement. The only study that was mentioned was the one above and the only reason they found a decrease risk of diabetes is because of the caffeine content found in tea and coffee. Therefore, this article can be misleading and providing false information to its readers.

Lastly, one website makes many claims on Matcha tea for diabetes.[97] The web article mentions green tea and states that drinking Matcha tea is one way in preventing type 2 diabetes due to its high antioxidant content. The web article talks about Matcha tea being more potent and more effective in preventing and treating diabetes. Also, stating that Matcha helps absorb glucose and can reduce the amount of glucose in the blood. Although, there are no sources to support any of these statements. The web article also mentions catechins in green tea increase fat oxidation, improve exercise performance, and prevent obesity. The one study they referenced was by Venables et al. This study

used healthy men with above average VO2 max. The subjects were to take a placebo or green tea extract (GTE) 3 times before they would participate in 30 minutes of moderate-intense physical activity.[89] They found that when the men took GTE their fat oxidation was 35%, which was 5% higher than with the placebo.[89] This was a statistically significant difference. They did not study fat oxidation and GTE at rest. Stating that green tea can help increase fat oxidation is not accurate since it was only found during exercise in healthy men using GTE supplementation rather than drinking tea. Those who read this may be misled since the web article on 1001 tea fact is about drinking Matcha tea and, yet the study used GTE supplements. Those in the study were also healthy fit men, but the readers of this article may be sedentary, women or men, and may have a chronic disease. The information included in this article jumps from fact to fact with very little or no evidence to support the claims made. While providing many different links that sell green tea products.[97]

CHAPTER 3

METHODS

SUBJECT SELECTION

Subjects who were eligible for this study was 18+ years of age, healthy, and not on any medications that can alter blood glucose levels. An online survey was used to recruit ASU students and faculty. The study was approved by the IRB at ASU (see appendix A) and written consent was obtained (see appendix B for consent form). Once consent forms were signed, health history questionnaires were given, and anthropometrics was taken. (see appendix C for health history questionnaire)

STUDY DESIGHN AND PROCEDURE

This study was a 3-week, randomized controlled, single blinded, crossover study in 12 healthy subjects. The sample size was calculated using the Harvard sample size calculator (see appendix D). Subjects were block randomized for three treatments: control, Lipton green tea, and Matcha tea. All subjects were given a bagel to consume with dinner the night before. Participants were asked to not drink coffee or participate in high or moderate intensity exercise the day before the study. Subjects were asked to fast (no food or beverage with the exception of water) for 10 hours overnight. Subjects reported to the test site for about 2.5 hours. Subjects were in three different treatment groups. The first week some subjects consumed a bagel and water (control), a bagel and Lipton Green Tea, or a bagel and Matcha Tea. For the next two visits, they were in different groups to complete all treatments. The food and beverages used in this study include the commercially available Lipton tea (Lipton, Pure Green Tea, Unilever, Englewood Cliffs, NJ) one tea bag. The Matcha green tea used (Ceremonial Matcha, Marukyo-Koyamaen,

Uji, Kyoto, Japan). The following items were used: plain bagel (Chompies, Tempe, AZ), Strawberry Jam (Smucker's, Orrville, OH), and bottled water (Arrowhead, Chandler, AZ). Meals were prepared for all subjects which included a toasted bagel with 14g of jam and a water bottle. Lastly, depending on what group the subject was in that morning they receive either 240ml of water, 1 Lipton tea bag in 240 mL water or 1 teaspoon of Matcha in 240mL of water. To prepare the Lipton tea, a kettle with water was heated till the water boiled. The Lipton tea bag was added to a mug and 240 mL of the water was measured. Once the 240 mL of water was cooled at a temperature between 158-176°F, the water was poured over the teabag. A teaspoon of powdered Matcha was sifted through a fine strainer into a mug. Water was added to a kettle and brought to a boil. The 240 mL of the water was measured and once the temperature cooled to 158-176°F, the water was added to the bowl. The water and Matcha were mixed together with a bamboo whisk till the tea had a frothy layer on top. Subjects were assigned a calibrated glucometer (ACCU-CHEK, Aviva meter system, Indianapolis, IN). To calibrate the glucometer, turn the glucometer on, stick the test strip into glucometer. Using two different control solutions while wearing gloves add one drop of each solution to the fingertip and place test strip attached to glucometer towards the solution to be absorbed by the strip. The glucometer will read the liquid and the measurement should fall within the numbers that are on the bottle. Once this is done for both liquids the glucometer is calibrated. The subjects were assigned a glucometer using the number written on it. The glucometer was assigned to each subject, so each visit the same glucometer is used. Once the subject comes in they sat down, and their fasting blood glucose was taken. The finger was first cleaned with alcohol swab, and then pricked using a disposable lancing device.

The finger was massaged if needed to get the first drop of blood, which was then wiped away with a cotton swab. Then the finger was massaged again to get the second drop of blood which was tested using the test strip which was attached to the glucometer. Fasting blood glucose were recorded. The meals were then served to each subject blood glucose measurements were taken 30 mins after the first bite and continuing every 30 mins till 120 minutes was reached (see appendix E for flow chart).

STATISTACLE ANALYSIS

Data is presented Mean±SD, checked for normality, and transformed if necessary. The repeated measure ANOVA statistical test was used to assess differences between means over time using the Statistical Package for the Social Sciences (SPSS 2.0) software. Level of probability is $P = < 0.05$. The area-under-the curve for the glycemic response was computed using the Trapezoidal Rule and compared by treatment.

CHAPTER 4

RESULTS

Recruitment for subjects who were healthy men and women 18 years or older not on any medications that would alter blood glucose levels took place February 2017 at Arizona State University. Survey was open from February 3rd to February 15th, 2017. There were 28 people who answered survey monkey, and all respondents qualified but one who was vegan. Since bagels are not a vegan product, this volunteer would not qualify. Of the 27 subjects only 17 responded to the email and within two weeks 14 subjects were screened and signed the consent form. All subjects who were screened qualified for the study. Two subjects did not show on the day they were scheduled to come in therefore, there were only 12 subjects who participated and completed the study. Participant characteristics are displayed in Table 1. All participants were healthy, non-diabetic, and nonsmokers. Mean age was 23.8 ± 3.1 with a mean BMI (kg/m^2) of 23.9 ± 5.7, height (cm) 164.7 ± 6.8, weight (kg) 65.3 ± 20.2 and a waist circumference (cm) 76.5 ± 16.

Table 2. Sample Characteristics

Subject	**gender**	**age**	**Height (cm)**	**weight (kg)**	**BMI (Kg/m^2)**	**waist (cm)**
1	F	31	169.5	61.8	21.6	73.2
3	F	21	159.0	60.6	24.0	75.9
4	F	20	165.0	62.3	22.9	73.7
5	F	25	164.0	53.8	20.0	66.0
6	F	21	161.0	47.2	18.2	63.5
8	F	21	159.0	56.3	22.3	73.7
9	F	25	168.0	62.6	22.2	73.7
10	M	25	158.0	61.2	24.5	78.7

11	F	26	160.0	67.6	26.4	74.9
13	M	23	177.0	127.2	40.6	125.7
14	F	25	159.0	57.6	22.8	67.8
15	F	22	177	65.8	21	71.1
Mean		23.8 ± 3.1	164.7 ± 6.8	65.3 ± 20.2	23.9 ± 5.7	76.5 ± 16

Subjects were randomly selected to either water (Control), Matcha tea or Lipton tea (Treatment). This is a crossover study therefore all subjects consumed all three beverages about a week apart. The data collected can be seen in Table 2. Which includes the mean ± standard deviation of glucose concentrations for all three beverages at 0, 30, 60, 90, 120 minutes. At 0 minutes the mean ± SD of glucose concentration for water, Match tea and Lipton tea was 89.1 ± 6.2, 92.4 ±7.8, and 90.4 ± 7.8 respectively. At 30 minutes mean glucose concentration for water, Match tea and Lipton was 133.3 ± 15.5, 132.6 ± 17.8, and 131.0 ± 12.2 respectively. At 60 minutes mean glucose concentration for water, Match tea and Lipton tea was 119.5 ± 13.8, 125.3 ± 16. 9, and 125.0 ± 13.7 respectively. At 90 minutes mean glucose concentration for water, Match tea and Lipton was 112.5 ± 12.4, 115.8 ± 15.9, and 115.0 ± 13.1 respectively. At 120 minutes mean glucose concentration for water, Match tea and Lipton tea was 105.2 ± 9.6, 112.3 ± 18.1, and 106.3 ± 12.9 respectively. Using repeated measure ANOVA to assess differences between means over time. The area-under-the curve for the glycemic response was computed using the Trapezoidal Rule and compared by treatment. The glycemic curve for all three beverages showed no statistically significant difference with a p-value of 0.960. The glycemic curve which can be seen in table 3. Shows the glycemic response after consuming each beverage and a bagel. All curves showing a very similar glycemic

response to each beverage (p= 0.960). A p-value of 0.960 inhibits the ability to look at the differences between the different times.

Table 3. Mean Glucose Concentrations at 0, 30, 60, and 120 Minutes

minutes	**Water**	**Matcha**	**Lipton**	**p-value**
0	89.1 ± 6.2	92.4 ±7.8	90.4 ± 7.8	0.960
30	133.3 ± 15.5	132.6 ± 17.8	131.0 ± 12.2	
60	119.5 ± 13.7	125.3 ± 16. 9	125.0 ± 13.7	
90	112.5 ± 12.4	115.8 ± 15.9	115.0 ± 13.1	
120	105.2 ± 9.6	112.2 ± 18.1	106.3 ± 12.9	

*All means are reported as mean ± standard deviation. P value using repeated measure ANOVA. Significant at p<0.05.

Figure 1. Glycemic Curve for all Three Beverages

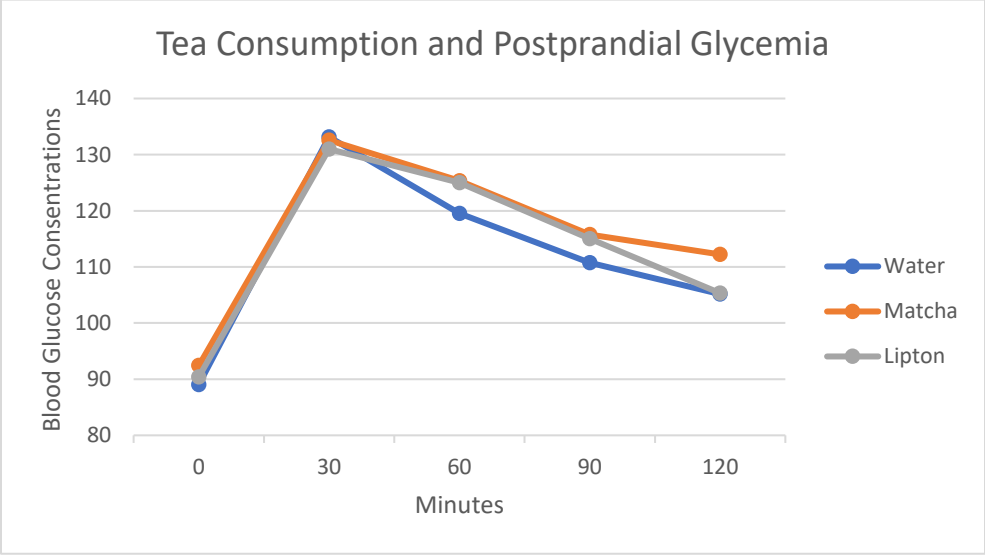

Figure 1. A comparison of the mean blood glucose measurements after consuming either water, Lipton tea, and Matcha tea at 0, 30, 60, 90, and 120 minutes. Data includes all subjects who have completed this study (n=12). Significant at p <0.05.

CHAPTER 5

DISCUSSION

The polyphenols in green tea, particularly EGCG, have been found to decrease postprandial glucose concentrations in mice. Many studies have seen inhibitory effects of EGCG on α-amylase, an important enzyme in the breakdown of carbohydrates.[32, 34, 61] This randomized controlled, single blinded, crossover study looked at the effects of Matcha tea as well as Lipton tea ingestion at meal-time on the 2-h postprandial glucose response. Twelve healthy subjects completed all arms of the crossover study. There were no statistically significant findings (P=0.960) between the test conditions which included the control (water) and the two treatment groups (Lipton tea and Matcha tea). A study by Forester et al, had shown that 100 mg/kg of EGCG given to mice consuming common corn starch decreased blood glucose concentrations by 73 mg/dl at 60 minutes compared to the control. Other studies in humans have not been able to show the same findings. One study in humans looked at green tea on postprandial glucose concentrations in 14 healthy subjects. The subjects consumed either 300 ml of water or tea and a test meal composed of white bread (50 g carbohydrate) and turkey.[48] The tea was prepared using 9.00 g of loose leaf green tea seeped in 300 ml of hot water.[48] There was no statistically significant decrease in postprandial glucose concentrations in either group. Hence, although direct supplementation with 100 mg/kg EGCG was associated with an antiglycemic response in mice, human trials indicate that green tea, when taken in customary amounts, does not possess antiglycemic properties at mealtime.

Matcha tea was used in this study since there are no studies to our knowledge on powdered green tea. Consuming the plant whole would provide all the nutritional

contents of tea; yet, this manner of ingestion did not provide any measurable effect. Limitations in this study included the small sample size. Moreover, the amount EGCG in 1 Lipton tea bag or 1 teaspoon of Matcha tea is not known. Based on one website called consumer lab, which had tested both Matcha tea and brewed green tea. They found that per 1 g of Matcha tea and brewed green tea there were 50-55 mg and 20 to 40 mg of EGCG respectively.[19] In this study 1 teaspoon of Matcha tea was used which is about 2 g. The 12 subjects could have consumed from 100-110 mg of EGCG in the Matcha tea. Still this is not an exact amount; and since the processing of these teas effects EGCG levels it would have been an important factor to determine in this study. Also, on a weight per weight basis, humans might need a higher dose of EGCG to produce the same effects in mice. Such a high amount would need to be provided as a supplement. Providing exact amounts of EGCG and including different dosage of EGCG for example 100 mg, 200 mg, 300 mg and so on could have helped strengthened this study.

Although, the inhibitory effects of EGCG on α-amylase has been found in many studies. Other studies have still not been able to consistently prove green tea to be effective in lowering postprandial glucose concentrations. Still, many websites make the claims that green tea helps in the management and prevention of diabetes. Some make the claim that it could help lower glucose concentrations, yet the studies used to support such claims talk about caffeine content or use green tea extract. These claims mislead those who read these web articles and do not look at the science behind such claims. These web articles are sending false information and/or selling green tea products. It is important for these individuals to get the accurate information so that they can make informed

decisions. There are other dietary strategies to lower postprandial glucose concentrations. These include vinegar, nuts, and exercise.

Vinegar has been found be very effective in lowering postprandial glucose concentrations in many studies. [47, 58, 70] The acetic acid delays gastric emptying and therefore slows down the digestion and absorption of a carbohydrate. [58, 63] Slower digestion and absorption lowers the GI of a carbohydrate and prevents a spike in glucose concentrations. In one study subjects were to drink a test drink (20g of apple cider vinegar) which provided 5% acetic acid or a placebo drink.[47] Once the beverage was consumed the subjects would eat a bagel with butter, juice and water. The GI of this meal was 100. After 60 minutes from consuming the meal the PPG concentrations were lowered by 54% compared to the placebo drink. Vinegar has shown to significantly decrease in PPG concentrations in this study and many other studies. Acetic acid has also been found to inhibit the enzymes in the brush boarder of the small intestine. These enzymes are known as disaccharidase. They are responsible for the breakdown of disaccharides sucrose, maltose, and lactose into monosaccharides. With an inhibition of these enzymes decreases the amount of glucose in the blood after a meal.[63]

Nuts also has been found to decrease PPG concentrations.[46, 47, 49] Nuts slow the rate of gastric emptying therefore slowing digestion and absorption of carbohydrates.[49] In one study subjects consumed either a bagel with butter or a bagel with 25g of peanut butter. PPG concentrations was lowered by 56% when consuming the bagel with peanut butter compared to just eating a bagel and butter.[47] Another study had subjects consume white bread and different amounts of almonds. For example, 0g (control) or 30g, 60g, 0r 90g.[49] The study found that the higher the dose of almonds the lower the PPG

concentrations due to slower gastric emptying and a decrease in the GI of the white bread.[49]

Exercise is another intervention that has effects on lowering PPG concentrations. Exercise for 90 minutes (moderate-intensity) 2-hours before or after a meal has been found to decrease PPG concentrations by 50%.[68] Physical activity has been shown to reduce PPG concentrations for up to 48 hours.[1, 77] This could be due to increased insulin sensitivity, which allows the cells of a muscle or adipose tissue to respond to insulin more efficiently and increasing the uptake of glucose.[1]

Although, Matcha tea which is essentially green tea in powder form has not shown an antiglycemic effects in this study. EGCG a polyphenol most abundant in green tea has been linked with cancer prevention. Many animal studies have found EGCG to inhibit tumorigenesis in the oral cavity, esophagus, stomach, small intestine, colon, liver, pancreas, lung, bladder, prostate, mammary glands and skin.[96] In more than 30 studies the catechins found in tea has inhibited tumorigenesis in genetically induced animals in the oral cavity, esophagus, stomach, small intestine and colon.[96] Oxidative stress is associated with tumorigenesis and phytochemicals like EGCG reduces oxidative stress therefore inhibiting tumorigenesis.[17] Oxidative stress has been linked to the development of cancer which includes three stages: initiation, promotion, and progression.[17] For example, oxidative stress can initiate cancer by causing mutations in oncogenes and tumor-suppressor genes.[17] One study looked at 133 smokers and the effects of green or black tea consumption on oxidative DNA damage. Smoking contains many compounds that can induce ROS production that causes oxidative damage to the DNA. The repair of oxidative damage to the DNA can be measured by the urinary excretion of 8-OHdG.[41]

Subjects consumed either 4 cups/d of green or black tea or water for 4 months. Urinary 8-OHdG was decreased by 31% in those consuming the green tea. Those consuming the black tea and water showed no difference. The study concluded that green tea consumption may protect smokers from oxidative damage and decrease the risk of cancer.[41] In a prospective cohort study of Japanese population which surveyed 8,552 individuals over 40 years old.[44] The study looked at relative risk of cancer incidents based on green tea consumption which showed a RR of .53 in non-smoker who consumed 10 or more cups of green tea a day and a RR of .73 was found in smokers who consumed 10 or more cups of green tea a day.[44] A study in Italy with 60 men who had high-grade prostate intraepithelial. Studies have found that those with high-grade prostate intraepithelial increased their chances of developing prostate cancer within 1 year by 30%.[8] For 12 months 30 subjects consumed a total of 600 mg/day of green tea catechins. Results found only one subject developed prostate cancer in this group, compared to 9 out of the 30 men who took the placebo.[8, 96] Still many human studies both epidemiological and RCT have found inconsistent results.[96]

EGCG acts as an antioxidant due to its ability to reduce reactive oxygen species (ROS) that can be linked to many diseases like cardiovascular disease. [96] EGCG's ability to act as an antioxidant makes drinking tea an important staple for our health. ROS is produced naturally in the body when we metabolize food which then produces energy which leads to ROS production. The removal of ROS is an important and necessary role for antioxidants. As ROS build up in the cell also known as oxidative stress it can damage the cells in the body due to its ability to attack polyunsaturated fatty acids in the cell membrane and DNA lipid and proteins which can lead to disease like cancer and

cardiovascular disease.[17, 96] In diabetes hyperglycemia can increase ROS production. If there are not enough antioxidants to reduce ROS in the cell causing oxidative stress it can lead to other diseases like cardiovascular disease, specifically atherosclerosis. Antioxidants are important in the prevention of such diseases by keeping ROS levels low.[96] EGCG has been found to reduce LDL-C as well. In one study EGCG was given at different doses from 107-857 mg/d in 1356 healthy subjects for 3 to 14 weeks. Those receiving the EGCG with either dosing showed a significant decrease in LDL-C.[64, 96] These findings show that decreasing LDL-C can help prevent cardiovascular disease, due to LDL-C large role in atherosclerosis.[64, 96] EGCG has also been found to inhibit NF-KB activation. NF-KB can be activated by ROS. NF-KB activates pro-inflammatory cytokines that play a role in atherosclerosis.[96]

In conclusion, even though this study did not find antiglycemic effects in Matcha tea consumption. There have been many studies that support the benefits of polyphenols specifically EGCG in tea which act as an antioxidant. Antioxidants are important in reducing oxidative stress and therefore green tea has protective properties to the body's cells and DNA. Green tea should be included in an individual's diet due to its potential anti-cancer effects and its ability to reduce oxidative stress.

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APPENDIX A

IRB APROVAL

ASU Knowledge Enterprise Development

APPROVAL: EXPEDITED REVIEW

Carol Johnston
SNHP: Nutrition
602/827-2265
CAROL.JOHNSTON@asu.edu

Dear Carol Johnston:

On 1/24/2017 the ASU IRB reviewed the following protocol:

Type of Review:	Initial Study
Title:	Matcha Tea and its Acute Effects on Postprandial Blood Glucose
Investigator:	Carol Johnston
IRB ID:	STUDY00005584
Category of review:	(2)(a) Blood samples from healthy, non-pregnant adults, (7)(b) Social science methods, (2)(b) Blood samples from others, (7)(a) Behavioral research
Funding:	None
Grant Title:	None
Grant ID:	None
Documents Reviewed:	• health history questionnaire, Category: Screening forms; • consent, Category: Consent Form; • online survey, Category: Recruitment Materials; • protocol, Category: IRB Protocol; • recruitment ad for flyers and list serves, Category: Recruitment Materials;

The IRB approved the protocol from 1/24/2017 to 1/23/2018 inclusive. Three weeks before 1/23/2018 you are to submit a completed Continuing Review application and required attachments to request continuing approval or closure.

If continuing review approval is not granted before the expiration date of 1/23/2018 approval of this protocol expires on that date. When consent is appropriate, you must use final, watermarked versions available under the "Documents" tab in ERA-IRB.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).

Sincerely,

IRB Administrator

cc:
Roni Romash

APPENDIX B

CONCENT FORM

MATCHA TEA AND ITS ACUTE EFFECTS ON POSTPRANDIAL BLOOD GLUCOSE

The purposes of this form are (1) to provide you with information that may affect your decision as to whether or not to participate in this research study, and (2) to record your consent if you choose to be involved in this study.

RESEARCHERS

Dr. Carol Johnston, a Nutrition professor at Arizona State University Downtown Campus, and Roni Romash, a nutrition graduate student, have requested your participation in a research study.

STUDY PURPOSE

The purpose of the research is to examine the effects of tea ingestion on post-meal rise in blood glucose. Blood glucose will be measured via finger pricks.

DESCRIPTION OF RESEARCH STUDY

You have indicated to us that you are 18 years of age and generally healthy. You have not been diagnosed with diabetes and you do not take diabetic medications including insulin. If female you have not recently been pregnant or lactating. Lastly, you are not gluten-sensitive. Participants will be asked to maintain their usual diet and physical activity level throughout the trial with the exception of the day prior to testing. This study will initially involve the completion of a brief medical history questionnaire to demonstrate the absence of medical conditions that may impact the study. Your weight, height, and girth will be measured at this time. This first meeting will take <20 minutes. There are three additional visits (e.g., the test days) that will last about 2.5 hours each and scheduled a week or two apart. The procedures on all test days are identical. On the day prior to testing you are asked to avoid exercise (normal activities such as walking to work or walking the dog is ok). You will be asked to eat a normal breakfast and to consume a bagel with lunch and with dinner. These bagels will be given to you. Following dinner, you will fast overnight and not consume any food or beverage with the exception of water. On test days, you will travel to ASU (the Nutrition labs at the ABC1 Building on the ASU Downtown campus) early in the morning. Your finger will be pricked for a blood sample. You will then consume either tea or water and a test meal (bagel with jam). Your finger will be pricked 4 more times over the next 2 hours. You may drink water during these two hours but you are not to consume any other food or beverage. You may read, study, or work on the computer at the test site. Once testing is complete, you may proceed with your normal activities. About 15 subjects will participate in this study.

Finger pricks will be conducted under sterile conditions using disposable, retractable lancets.

Blood samples will be analyzed for glucose.

RISKS

Bruising of the skin or a feeling of faintness is possible during the blood draws. A registered nurse will draw the venous blood sample under sterile conditions and is trained to minimize

these risks. For the finger pricks, disposable retractable lancets will be used and sterile conditions will be used.

BENEFITS

There is no direct benefit for participating in this trial. If desired, you will be given your blood glucose values.

NEW INFORMATION

If the researchers find new information during the study that would reasonably change your decision about participating, then they will provide this information to you.

CONFIDENTIALITY

All information obtained in this study is strictly confidential unless disclosure is required by law. The results of this research study may be used in reports, presentations, and publications, but your name or identity will not be revealed. In order to maintain confidentiality of your records, Dr. Johnston will use subject codes on all data collected, maintain a master list separate and secure from all data collected, and limit access to all confidential information to the study investigators. Plasma from blood samples will be stored for 5 years in freezers in the laboratories of the Nutrition Program at Arizona State University Downtown Campus after which time they will be disposed of as biohazard waste.

WITHDRAWAL PRIVILEGE

You may withdraw from the study at any time for any reason without penalty or prejudice toward you. Your decision will not incur negative treatment to you by the researchers.

COSTS AND PAYMENTS

The all test foods will be given to you during the study free of charge. You may need to pay for parking meters during the visits.

COMPENSATION FOR ILLNESS AND INJURY

If you agree to participate in the study, then your consent does not waive any of your legal rights. However, in the event of harm, injury, or illness arising from this study, neither Arizona State University nor the researchers are able to give you any money, insurance coverage, free medical care, or any compensation for such injury. Major injury is not likely but if necessary, a call to 911 will be placed.

VOLUNTARY CONSENT

Any questions you have concerning the research study or your participation in the study, before or after your consent, will be answered by Dr. Carol Johnston; 500 N. 3rd Street Phoenix, AZ 85004; 602-827-2265.

If you have questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Research Compliance Office, at 480-965 6788.

This form explains the nature, demands, benefits and any risk of the project. By signing this form you agree knowingly to assume any risks involved. Remember, your participation is voluntary. You may choose not to participate or to withdraw your consent and discontinue participation at any time without penalty or loss of benefit. In signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be given to you.

Your signature below indicates that you consent to participate in the above study.

_____	_____	_____
Subject's Signature	Printed Name	Date
_____	_____	
Contact phone number	Email	

INVESTIGATOR'S STATEMENT

"I certify that I have explained to the above individual the nature and purpose, the potential benefits, and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided the subject/participant a copy of this signed consent document."

Signature of Investigator_____

Date_____

APPENDIX C

HEALTH HISTORY QUESTIONAIRE

ID#_____

Height _____ft. ____in. Weight: _____lbs. Waist: _____ ins.

Age: _____
Gender: □ Male □ Female
Smoker: □Yes □ No

1. Have you been diagnosed with pre-diabetes or diabetes? Y N

2. Have you been diagnosed with other chronic diseases (such as heart disease, neurological disease, autoimmune disease, or cancer)? Y N

3. Do you take any medications regularly? Y N
Please list what kind and how frequently:

Medication Dosage Frequency

_____
_____
_____
_____
_____
_____
_____

4. Do you currently take supplements (vitamins, minerals, herbs, etc.)?
Y N
If yes, what supplements and how often?

_____
_____

_____
_____

_____
_____

_____
_____

_____
_____

5. Do you have medical conditions that you see a physician for on a regular basis? Y N

Please explain:

_____
_____

_____
_____

_____
_____

_____
_____

_____
_____

OVER ⟶

6. Do you have any food allergies including a gluten sensitivity?
Y N

If yes, please describe

_____

_____

7. Do you follow a special diet? (weight gain/loss, vegetarian, gluten-free etc.)
Y N

If yes, please describe

_____

_____

8. Will you have any problems fasting for 12 hours prior to testing sessions?
Y N

9. Do you have any difficulty chewing or swallowing?
Y N

10. Are you OK with drinking tea and eating bagels and strawberry jam?
Y N

11. Will you have a problem (such as fainting) providing blood samples? (5 finger pricks per test day)
Y N

12. If you drink alcohol or caffeine, will you be able to abstain from these beverages for the 24-hour periods prior to test days?
Y N

13. If you exercise regularly, will you be able to not exercise (other than basic walking and work activity) for the 24-hour period prior to testing?
Y N

14. Please circle the number of times you did the following kinds of exercises for **more than 15 minutes** last week.

Mild exercise (minimal effort):
Easy walking, golf, gardening, bowling, yoga, fishing, horseshoes, archery, etc.

Times per week: 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14+

Moderate exercise (not exhausting):
Fast walking, easy bicycling, tennis, easy swimming, badminton, dancing, volleyball, baseball, etc.

Times per week: 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14+

Strenuous exercise activities (heart beats rapidly):
Running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling, etc.

Times per week: 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14+

Please describe any other conditions or medical reasons that may affect your participation below:

APPENDIX D

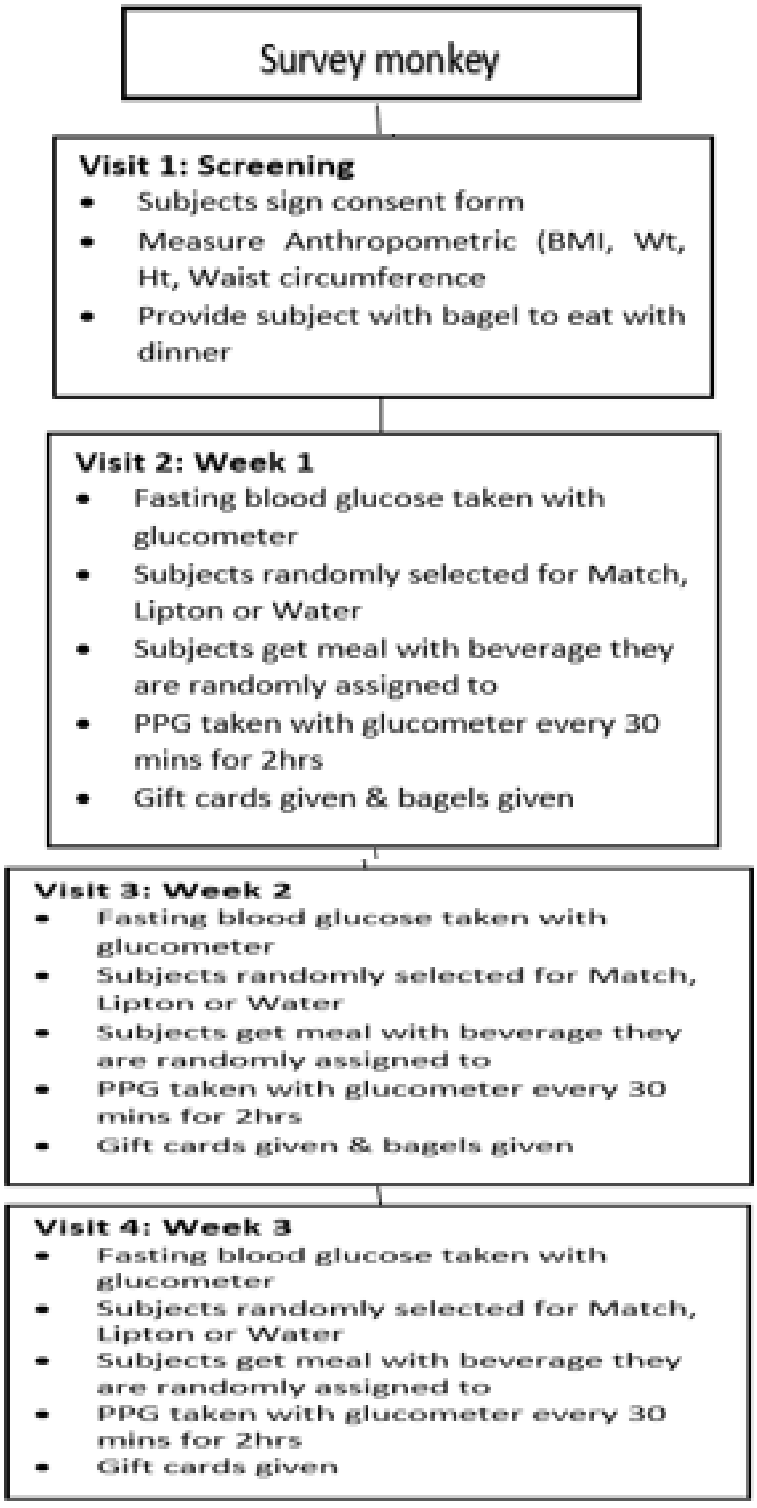
SAMPLE SIZE

Author	Year	PPG change	n per group	Calculated n per group	Age range	Subject State	Test
Nyambe-Silavwe et al	2015	27.4±7.52	16	6	22-30	Healthy	Low dose PFRF & PPG
Nyambe-Silavwe et al	2015	49±15.3	16	6	22-30	Healthy	High dose PFRF & PPG
Johnston et al	2015	54±1.98	11	4	25-31	Healthy	Vinegar PPG at 60mins
Johnston et al	2015	56±.66	11	4	25-31	Healthy	Peanuts PPG at 60mins
Average		**47±6.4**	**14**	**5**	**24-31**		

All data is represented as means ±SD

APPENDIX E

FLOW CHART


Survey monkey
Visit 1: Screening
Subjects sign consent form
Measure Anthropometric (BMI, Wt, Ht, Waist circumference
Provide subject with bagel to eat with dinner
Visit 2: Week 1
Fasting blood glucose taken with glucometer
Subjects randomly selected for Match, Lipton or Water
Subjects get meal with beverage they are randomly assigned to
PPG taken with glucometer every 30 mins for 2hrs
Gift cards given & bagels given
Visit 3: Week 2
Fasting blood glucose taken with glucometer
Subjects randomly selected for Match, Lipton or Water
Subjects get meal with beverage they are randomly assigned to
PPG taken with glucometer every 30 mins for 2hrs
Gift cards given & bagels given
Visit 4: Week 3
Fasting blood glucose taken with glucometer
Subjects randomly selected for Match, Lipton or Water
Subjects get meal with beverage they are randomly assigned to
PPG taken with glucometer every 30 mins for 2hrs
Gift cards given