Regulation of Postprandial Hyperglycemia with Green Tea

Honors Research Thesis

Presented in Partial Fulfillment of the Requirements for graduation
“with Honors Research Distinction in Human Nutrition” in the undergraduate college of
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Chapter 1: Introduction

Cardiovascular disease (CVD) is one of the leading causes of death in the United States [1]. Acute hyperglycemia is one of the risk factors for CVD-related mortality [2]. After a meal, the magnitude of postprandial hyperglycemia is inversely correlated to vascular function [3]. Vascular endothelial dysfunction is an early contributor to CVD. By lowering postprandial hyperglycemia, the risk of cardiovascular-related injury should decrease. Green tea has been seen to lower long-term CVD risk [4] and blood glucose levels [5]. The purpose of this study was to determine if a green tea extract (GTE)-containing confection would lower postprandial hyperglycemia that otherwise contributes to vascular dysfunction and increased risk of cardiovascular disease.

2 Vascular Endothelial Functions Relating to Cardiovascular Risk

Endothelial function is dependent on a balance between oxidants and antioxidants. It is usually defined at nitric oxide (NO) bioavailability and production [6]. With vasodilation, increased NO production is termed endothelium-dependent vascular reactivity [7]. Increased oxidative stress impairs vascular function by decreasing NO bioavailability. Vascular impairment will lead to vascular disease.

Chronic and acute elevations in blood glucose can increase risk for cardiovascular disease[7]. Increased extracellular glucose concentrations induce a dysregulation of reactive oxygen species and nitric oxide generation [8]. The mechanism for acute postprandial hyperglycemia impairing vascular function is not fully understood, but oxidative stress has been correlated to its pathogenesis [9].
The approach taken in this study to lower acute increases in blood glucose after a meal is to determine a method to lower them. Studies have shown that the bioactive catechins in green tea can decrease these glycemc levels following a starch-rich meal [10]. We investigated green tea as a dietary strategy to lowering postprandial glycemic levels and thereby protecting vascular function. The purpose of this study was to investigate a potential new dietary confection and generate evidence showing that green tea can regulate glucose and vascular response. Support of this would indicate further investigation for green tea as a dietary strategy for cardiovascular health.

3 Acute Postprandial Hyperglycemia

Acute hyperglycemia is a contributing factor in CVD risk. It is defined as plasma glucose levels of greater than 198 mg/dl, regardless if a patient has diabetes [11]. In a large-scale observational studies, 2 h blood glucose levels, rather than fasting, better predict CVD related mortality [2]. This cardiovascular risk created by short-term hyperglycemia is seen even in individuals without diabetes. Healthy individuals taking a glucose challenge were shown to have decreases brachial artery flow-mediated dilation (FMD) [9]. FMD is a noninvasive technique used to assess endothelial function. The magnitude of the PPH was also inversely correlated to vascular function ($r = -0.82; p>0.05$) [3]. It is known that this process is through an oxidative stress-dependent manner that reduces nitric oxide bioavailability. Nitric Oxide (NO) is important in regulating vascular endothelial function. NO is produced by nitric synthase in the endothelial cells. It causes vasodilation [7]. As endothelial flow increases from dilation, animal studies have linked increases in NO synthase expression and NO bioactivity [12].
Hyperglycemia disrupts nitric oxide homeostasis by increasing oxidative stress responses [13]. Results of one study indicate that PPH transiently induces vascular endothelial dysfunction in healthy people, which needs to be regulated in order to lower long-term vascular injury.

Although fasting blood glucose is often the target for therapy, postprandial hyperglycemia is a better predictor of CVD than fasting blood glucose[2] consistent with evidence that the magnitude of postprandial hyperglycemia is inversely correlated to vascular function.

Preventative measures to avoid this vascular impairment from a dietary standpoint should be explored. Just avoiding glucose-rich meals is difficult due to the fact that in many foods, glucose is present in different forms. This study looks at ways to lower glycemic levels after the consumption of starch and/or its downstream impairing responses that could lead to VED. Strategies to do this are necessary in lowering this vascular and eventual CVD risk.

4 Why Green Tea?

Green tea contains four major catechins: (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC) and (-)-epigallocatechin gallate (EGCG). Differences in structure between catechins are seen in Figure 1. Green tea typically contains 30 to 130 mg of EGCG per cup (237 ml) [14]. EGCG is the most predominate catechin [15], although the other catechins have more bioavailability in mice and humans [16]. The catechins experience hydrolysis by microflora in the body, and the resulting metabolites circulate the plasma. These metabolites have shown antioxidant, anti-inflammatory and enzyme inhibiting activity. [17]

Green tea consumption has been suggested as having a role in preventing an array of chronic diseases. Epidemiological evidence suggests that green tea at >5 cups/d lowers the risk for CVD-related mortality by 26% [4]. In an 11-year follow-up study in Ohsaki, Japan, 51,255
Japanese adults without history of CVD were compared to their cups of green tea consumed per day. Significantly reduced mortality of all causes was seen with consumption of green tea. The inverse association of cardiovascular disease mortality and green tea consumption was stronger than all-cause mortality for both sexes (p <0.01).

Blood glucose levels in healthy humans were measured after consumption of 1.5 g/body of green tea. Compared to participants drinking hot water, glucose tolerance was substantially improved. Blood glucose levels however did not significantly change in all participants. In the same study, streptozotocin (STZ)-diabetic mice blood glucose levels showed significant decrease with peroral administration of 300 mg/kg green tea. There were no significant changes observed in normal ddY mice. Green tea administration has an anti-hyperglycemic effect in diabetes in mice [5].

![Figure 1: Differing structures of four green tea catechins (adapted from Baletine et al [14])](image-url)
4.1 EGCG

There are four major green tea catechins. (-)-Epigallocatechin-3-gallate (EGCG) is the most abundant. EGCG treatment in high-fat obese mice showed reduced body weight gain, percent body fat and visceral fat weight (p < 0.05) compared to without treatment[18]. The same EGCG treatment (3.2 g/kg diet) in high-fat obese mice resulted in decreased fat weight and blood glucose (p <0.05).

In a feeding study of mice and common corn starch (CCS), EGCG treatment significantly reduced the postprandial spike in blood glucose levels. Mice were oral administered CCS and blood glucose levels were 77 and 73 mg/dL lower after 30 and 60 min in the EGCG-treated mice compared to control mice. EGCG treatment caused a significant (p < 0.05) decrease in blood glucose AUC in mice fed CSS. In the same study, mice oral administered sucrose had no significant reduction in blood glucose AUC. Mice fed glucose and maltose had an increase in blood glucose levels, with no significant effect from EGCG-treatment [19].

Treatment of rat inverted jejunal sacs with EGCG significantly (p <0.01) inhibited Na+-dependent glucose uptake[18]. Uptake decreased from 15.1 to 11.2 µmol/g (25% inhibition) in the presence of 1mM EGCG. Another green tea catechin, epicatechin gallate (ECG), showed a 36% inhibition with addition of 1mM ECG. Within the same study, observation of rabbit brush border membrane vesicle’s glucose uptake showed significant reduction by ECG 1 mM (53%, p < 0.001) and EGCG 1mM (35%, P < 0.005).
4.2 Pancreatic α-amylase activity

Green tea catechins have also been shown to inhibit pancreatic α-amylase activity, an enzyme that is essential in the hydrolysis of glucose from starch[20]. α-amylase is an enzyme that hydrolyzes inferior α-1,4- glucose linkages, aiding in the digestion of starch. There are two types, a salivary α-amylase and a pancreatic α-amylase.

Postprandial increases in blood glucose levels may be inhibited by green tea following a meal high in starch. Carbohydrate absorption is decreased by 25% after a carbohydrate white rice meal in healthy human subjects was seen with green tea consumption (0.1 g solid in warm water) [21].

Acute and chronic feeding studies of mice have shown this in CCS (common corn starch) ingestion, green tea catechins have reduced postprandial hyperglycemia through inhibition of pancreatic α-amylase activity. Through examination in a cell free system, at a concentration of 20 µM, EGCG inhibited α-amylase by 34%. Kinetic analysis showed significant (p < 0.01) reduction in $V_{\text{max}}$ but not $K_m$ (p=0.1). These results show EGCG inhibits in a noncompetitive method, with no regard to substrate concentration. This means that EGCG can inhibit pancreatic α-amylase, and increasing the amount of starch will not prevent this process. In a feeding study of rats, EGCG was seen to acutely lower postprandial elevation in blood glucose levels when fed CCS. The results when fed glucose or maltose showed no effect. This indicates that EGCG affects a facet of only starch metabolism, which supports evidence for the specific inhibition of pancreatic α-amylase activity [19]. From this evidence, it is viable to deduce that the consumption of green tea catechins would reduce VED in an acute setting.
Confections, like the gummy used in this study, are a food matrix that is low cost and high in consumer approval. Delivering bioactive food components, such as green tea, have been shown to be viable through these low-cost confections. Sessler et al. [22] successfully formulated a pectin-based confection that was shown to effectively release phenolics from its matrix. In this study, a corn starch-based confection containing green tea extract was created to attenuate their response to postprandial vascular function and glucose levels.

Co-administration of green tea (EGCG) and starch have been shown to be an effective way to lower postprandial glycemic elevations. This is shown to reduce the glycemic effect of foods high in starch. Taken together, their effects are more prevalent and therefore this method was chosen [19].

Hypothesis

The central hypothesis of this study that postprandial hyperglycemia was lowered by the consumption of a GTE-containing confection. The blood glucose area under the curve was expected to decrease with the consumption of green tea, indicating that green tea has an effect on acute post-prandial glycemic levels. The successful completion of this work will contribute to the understanding of an underway investigation examining the protective effects of a GTE-containing confection on nitric oxide-dependent vascular endothelial dysfunction by suppressing postprandial hyperglycemia.
Conclusion

This research addressed certain objectives. The focus of this thesis was on the relationship between green tea and acute postprandial hyperglycemia. The blood glucose levels of healthy men were tested before and after consumption of green tea and starch containing confection at trial 1. These results were compared with a control starch confection containing no GTE, consumed at trial 2. Lowering of postprandial blood glucose levels was expected after the consumption of green tea. Regulation of these acute hyperglycemic spikes will be influential in future research looking at reducing CVD risk. This is part of a study that focuses on vascular endothelial dysfunction and other biomarkers to determine how green tea could possibly be used as a preventative dietary measure in the future.
Chapter 2: Methods:

1. Study Design

Healthy men (n = 15 18-30 y; BMI: 19-25 kg/m²) completed a randomized cross-over study in which they ingested a starch-based confection (45 g starch) containing decaffeinated green tea extract at 0 or 1000 mg. The GTE-containing confection provides the equivalent of 4.5 servings of freshly brewed green tea. Plasma glucose was measured using a spectrophotometric kit prior to (0 h) and at 30 min intervals for 3 h following the ingestion of these test confections. Other biomarkers were examined as part of this study, but they are beyond the scope of this honor’s thesis. Indeed, vascular endothelial function and plasma insulin, triglyceride and cholesterol are being examined as part of a larger study testing the hypothesis that GTE protects against vascular endothelial dysfunction otherwise induced by the starch-based confection devoid of GTE.

2. Sampling

2.1 Recruitment:

Participants were recruited through flyers, e-mail, word of mouth and ResearchMatch through OSU Center for Clinical and Translational Science. The Institutional Review Board (IRB) at OSU approved the project in 2013. Posted flyers instructed potential participants to contact the study center (Bruno Laboratory, Department of Human Sciences) for further information. The graduate student on the study was available during phone-in hours to describe the study and determine if the potential participant qualified by conducting a scripted phone interview. This interview included questions like: do you take vitamins? do you smoke?. If the participant met preliminary study criteria, a screening meeting was scheduled. Before the screening, subjects
were asked to not consume anything but water for the previous 12 h. The screening meeting involved the full description of the study and the individual signing of an informed consent form. Also at the screening, the potential participant’s blood pressure, height, weight, waist circumference and a blood sample were collected. Within a 1-2 week period, the participant was notified if they qualified for the study.

2.2 Inclusion/Exclusion Criteria:
The determination of the subject’s “healthiness” of the 18-30 year old men (n=15) recruited was based on fasting serum clinical chemistries being within normal reference limits. Each participant was chosen based on BMI (18-24 kg/m²), diastolic blood pressure (< 90 mmHg), systolic blood pressure (<140 mmHg), glucose (<100 mg/dL), triglyceride (<150 mg/dL), cholesterol (<200 mg/dL), high-density lipoprotein (HDL) (>40 mg/dl), alanine aminotransferase (ALT) (<36IU/l), aspartate aminotransferase (AST) (<35IU/l), blood urea nitrogen (BUN) (<24 mg/dl) and creatinine (<1.5 mg/dl). Other information taken at the screening meeting was assessed for the following inclusion criteria. The participant had to be a non-dietary supplement user for greater than 2 months prior to the study. He also had to have not used any medications known to affect carbohydrate metabolism. The participant had to be a nonsmoker and have no history of cardiovascular disease or gastrointestinal disorders. Participants with allergies or aversions to green tea or cornstarch were excluded from the study, as well as men who consume more than three drinks of alcohol per day. Lastly, men could not be doing more than five hours per week of aerobic activity.

2.3 Screening Meeting
Potential participants, who satisfied the criteria for the study based on a telephone interview, were invited to the study center. The graduate student gave the participant a written consent form to sign and its contents were described to the participant. If the participant chose to join the study, his written consent was asked for. He was also informed that if his blood plasma levels and body measurements did not meet the above ‘healthy’ criteria, he will not be asked to participate. When the participant signed the consent form, and has fasted for 12 h. prior, a trained phlebotomist collected a blood sample (23 ml; 1.5T). Plasma chemistry assays was performed for blood lipids, glucose and ALT, AST, creatinine, BUN. Coding the samples ensured anonymity. Blood pressure, weight, height and waist circumference were also measured. If the blood pressure of the participant was >140/90 mm Hg, he was told his blood pressure was too high for this study. Also, if his body mass index did not fall within 18-24 kg/m², he was asked to not participate. Participants were contacted by phone or email based on their inclusion or exclusion in the study.

3. Intervention Procedures

3.1 Dietary Assessment

A dietary meeting with a Registered Dietitian from the research team was scheduled to inform the participant how to complete a dietary record accurately. For the 3 days prior to each visit, participants were asked to consume a diet of at least 150 g of carbohydrates. Participants were asked to completed a 3-day food diary and abstain from exercise for 24 h prior to visiting the study center.
3.3 Test Trials

Each subject participated in a randomized cross-over study. On 2 separate visits, separated by at least 7 d, participants reported in to the study center in the fasted state. Upon arrival, after a 12 hour fast, subject’s height, weight and two blood pressure measurements were taken. A flexible catheter was inserted into a forearm vein by a phleobologist after 15 min stabilization in the supine position and a baseline blood sample of 23 mL was collected into EDTA-containing tubes. Samples were then immediately placed on ice and centrifuged for plasma collection (1500 x g, 15 min, 4 degree C). Plasma aliquots were then snap frozen with liquid nitrogen and stored at -80 °C until analysis. Following the baseline blood collection the participant was given a starch-based confection (45 g) containing green tea extract (1000 mg) or a control (0 g). The participant was unaware of which confection had the green tea and the confections were identical for each trial. The confections were formulated to provide the equivalent of 4.5 servings of freshly brewed green tea. Time began after the confection was fully consumed. Thirty minutes after the time of consumption (30 min) and at 30 min intervals throughout the 3 h postprandial period proceeding to 3 h, a blood sample (approx. 23 ml) was collected and placed on ice. Plasma was obtained by centrifugation (1500 x g, 15 min, 4 C) and transferred to cryovials. These cryovials were then immediately frozen in liquid nitrogen and stored at -80C until analysis was completed.
4. Measurements (Sample Analysis)

4.1 Assays for Glucose Procedures:

For each 3 h session for the subject, plasma glucose was measured using the commercially assay (Pointe Scientific) at 0, 30, 60, 90, 120, 150 and 180 min following the ingestion of each test confection. The procedures were as follows: The commercially available reagent containing hexokinase 4000U/L, glucose-6-phosphate dehydrogenase (G6PDH) 4000 U/L, adenosine triphosphate (ATP) 6.0 mM, nicotinamide adenine dinucleotide (NAD) 3.0 mM, and buffer (pH +/- 0.1) was incubated for 3 min at 37 °C. This temperature is the optimal temperature for G6PDH catalytic activity. A 3 µl aliquot of plasma and 300 µl of incubated reagent were added to each well of a 96-well plate. Glucose in each plasma sample and standard was phosphorylated with adenosine triphosphate (ATP) by hexokinase. From this reaction, glucose-6-phosphate is oxidized by glucose-6-phosphate dehydrogenase with nicotinamide adenine dinucleotide (NAD), during which, NAD is reduced to NADH. The absorbance of NADH was read at 340 nm and is directly proportional to concentration of glucose in the sample.

A standard curve was generated using commercially available glucose standards (Pointe Scientific) which ranged from 3.125 mg/dL to 100 mg/dL (Table 1 and Figure 1). The slope and the y-intercept were used to calculate the concentration of glucose from each subject’s plasma.

Each batch of glucose measurement was performed together with a control plasma, which was determined to contain 80.25 mg/dL. Batch-to-batch variations were normalized using this control value. Coefficient of variation (CV) Absorbed is calculated to look at the variance between the three absorbance readings taken (CV Abs < 0.05).
Table 1: Data from Glucose Control

<table>
<thead>
<tr>
<th>Glucose Standard (mg/dL)</th>
<th>Abs 1</th>
<th>Abs 2</th>
<th>Abs 3</th>
<th>Avg Abs</th>
<th>StDev Abs</th>
<th>CV Abs</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0.489</td>
<td>0.491</td>
<td>0.495</td>
<td>0.492</td>
<td>0.0031</td>
<td>0.6%</td>
</tr>
<tr>
<td>50</td>
<td>0.356</td>
<td>0.355</td>
<td>0.365</td>
<td>0.359</td>
<td>0.0055</td>
<td>1.5%</td>
</tr>
<tr>
<td>25</td>
<td>0.283</td>
<td>0.279</td>
<td>0.28</td>
<td>0.281</td>
<td>0.0021</td>
<td>0.7%</td>
</tr>
<tr>
<td>12.5</td>
<td>0.249</td>
<td>0.256</td>
<td>0.248</td>
<td>0.251</td>
<td>0.0044</td>
<td>1.7%</td>
</tr>
<tr>
<td>6.25</td>
<td>0.229</td>
<td>0.23</td>
<td>0.234</td>
<td>0.231</td>
<td>0.0026</td>
<td>1.1%</td>
</tr>
<tr>
<td>3.125</td>
<td>0.223</td>
<td>0.224</td>
<td>0.227</td>
<td>0.225</td>
<td>0.0021</td>
<td>0.9%</td>
</tr>
</tbody>
</table>

Figure 1: Glucose Standard Curve

![Glucose Standard Curve](image-url)

\[ y = 0.0028x + 0.215 \]

\[ R^2 = 0.9992 \]

Table 2: Calculated Glucose from Control Plasma (mg/dL)

<table>
<thead>
<tr>
<th></th>
<th>Abs 1</th>
<th>Abs 2</th>
<th>Abs 3</th>
<th>Avg Abs</th>
<th>StDev Abs</th>
<th>CV Abs</th>
<th>Glucose (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ControlA</td>
<td>0.439</td>
<td>0.445</td>
<td>0.431</td>
<td>0.438</td>
<td>0.0070</td>
<td>1.6%</td>
<td>80.3</td>
</tr>
<tr>
<td>ControlB</td>
<td>0.439</td>
<td>0.431</td>
<td>0.444</td>
<td>0.438</td>
<td>0.0066</td>
<td>1.5%</td>
<td>80.2</td>
</tr>
</tbody>
</table>
5 Statistical Analysis:

All data was expressed as mean ± standard error using SPSS software. A repeated-measures ANOVA was performed to analyze the main effects of treatment, time and treatment x time interactions for postprandial glucose response. The trapezoidal rule was used to calculate glucose area under the curve throughout the 3 h postprandial period (AUC$_{0-3\text{ h}}$). A Student’s paired t test was used to compare the AUC$_{0-3\text{ h}}$ and baseline glucose levels between treatments. An $\alpha$-level of P < 0.05 was considered significant for all analyses.
Chapter 3: Results

1 Participants

Each participant’s information was taken at the screening meeting (Table 1). All values were within normal limits of each clinical measurement.

2 Plasma Glucose

Before 120 minutes, plasma glucose with green tea treatment curve is greater than placebo. At approximately 100 min, the green tea blood glucose levels decreased below the placebo. This decrease shows a hypoglycemic response below the normal amount after eating. Although this is observed, it is not significant. The main effect for treatment x time interaction was not significant (P = 0.537). Main effect for time, was significant (P < 0.01), but main effect for treatment was not (P = 0.994). Green tea treatment was significantly different than baseline at time points 30, 60 and 90 min than the placebo (P<0.05). Postprandial glucose significantly increased at 30 and 60 minutes for the treatment group and 60 minutes for the placebo. Then both statistically returned to baseline after these time points until 180 min (Figure 1).

As seen in Figure 3, each participant’s postprandial glucose response was highly variable. Also, figure 4 shows the variability in the participants change from baseline.
Figure 1: Plasma Glucose Levels for all participants

3 AUC

Postprandial glucose area under the curve (AUC) using time points every 30 min. The T-test for AUC between the treatments reported a p value of 0.8.
4 Correlations

Correlations between glucose values at each time point for each treatment and glucose AUC were looked at for significance with the clinical parameters of all participants. Placebo glucose time 0 min showed significance with diastolic blood pressure (P <0.01). Placebo glucose time 30 min showed highly significant correlation with BUN (P <0.01). Placebo glucose levels at 120 min showed significant correlation with systolic blood pressure (P<0.05). GTE glucose at 120 min showed significant negative correlation with cholesterol (P<0.05) and ALT (P<0.05). GTE glucose at 150 min and 180 min showed significant correlation with BUN (P<0.05).

Glucose AUC showed no significance with any of the clinical parameters looked at.

Figure 2: Blood Glucose AUC