GREEN TEA EXTRACT, EXERCISE METABOLISM AND GLYCEMIC CONTROL

# THE INTERACTIVE EFFECTS OF GREEN TEA EXTRACT SUPPLEMENTATION AND EXERCISE ON METABOLISM AND GLYCEMIC CONTROL IN HUMANS

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#### LAY ABSTRACT

Tea is one of the most popular beverages in the world. Compared to other teas, green tea has a greater abundance of catechins, compounds that have been associated with health benefits particularly related to the metabolism of sugars and fats. This unique property of green tea could partly explain its longstanding medicinal role in some Asian cultures. Extensive research on green tea has increased its popularity over the past three decades. Studies involving both humans and other animals have shown improvements in weight control and glycemic control. In response to these findings green tea is often touted as having "anti-obesity" and anti-diabetic" properties. This dissertation examined the interaction between green tea extract supplementation and exercise on metabolism with a particular focus on blood sugar control. We observed that supplementation with green tea extract improved the response to sugar ingested after exercise. This finding has important implications for improving the control of ingested sugar in humans.

#### ABSTRACT

Green tea contains high concentrations of polyphenolic compounds known as catechins. Studies in animal models suggest several potential mechanisms for specific metabolic effects at rest and during exercise, including improved glycemic control, altered activity of several glucose transporter proteins and improved endurance capacity. In humans, green tea extract (GTE) supplementation has been associated with improved glycemic control under resting conditions and increased fat oxidation during exercise. This dissertation examined the potential interactive effects of GTE supplementation and exercise on metabolism in humans with a focus on glycemic control. In Study 1, we demonstrated that GTE increased lipolysis and reduced heart rate during steady-state exercise in recreationally active men. Although substrate oxidation was not affected, GTE appeared to lower postprandial glucose under resting conditions. We hypothesized that the effects of GTE on exercise metabolism and glycemic control would be more apparent in humans with reduced exercise tolerance and impaired glucose tolerance. Thus, in Study 2, we examined the effects of GTE in sedentary overweight men. There were no differences in any metabolic or physiological responses during exercise; however, following exercise, GTE supplementation reduced [glucose] and insulinemia in response to an oral glucose load. Based on the findings of Study 2, the aim of Study 3 was to elucidate potential mechanisms for the alterations in glycemic response. Through the use of a dual-glucose tracer method, we demonstrated that GTE did not affect the rate of appearance of glucose in plasma in sedentary men; however, GTE supplementation allowed for the same glucose clearance rate despite a reduced insulinemia. We also observed lower carbohydrate oxidation during exercise with GTE. These findings suggest that GTE has an insulin-sensitizing effect during recovery from exercise, possibly due to enhanced glucose transporter activity; however, this hypothesis warrants further investigation in humans.

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# LIST OF ABBREVIATIONS

AMPK	5'AMP-activated protein kinase
AUC	area under the curve
cAMP	cyclic adenosine monophosphate
CAT	catechins
CGM	continuous glucose monitoring
COMT	catachol-O-methyl transferase
CK	creatine kinase
EC	epicatechin
ECG	epicatechin gallate
EGC	epigallocatechin
EGCG	epigallocatechin-3-gallate
FPG	fasting plasma glucose
FPI	fasting plasma insulin
GLUT	glucose transporter
GTC	green tea catechins
GTE	green tea extract
GXT	graded exercise test
HOMA	homeostatic model assessment
HR <sub>max</sub>	maximal heart rate
M <sub>ISI</sub>	Matsuda insulin sensitivity index
OGTT	oral glucose tolerance test
PI3K	phosphoinositide 3-kinase
PLA	placebo
Ra <sub>Endo</sub>	rate of endogenous glucose appearance
$Ra_{Exo}$	rate of exogenous glucose appearance
Ra <sub>Total</sub>	rate of total glucose appearance
Rd	rate of glucose disappearance
REE	resting energy expenditure
RER	respiratory exchange ratio
SGLT-1	Sodium-dependant glucose transporter
VO <sub>2</sub> peak	peak oxygen consumption
VO <sub>2</sub> max	maximal oxygen consumption
W <sub>max</sub>	maximal workload

#### **DECLARATION OF ACADEMIC ACHIEVEMENT**

#### FORMAT AND ORGANIZATION OF THESIS

This thesis is prepared in the "sandwich" format. It includes a general introduction, three independent studies prepared in journal article format, and an overall discussion. The candidate is the first author on all three manuscripts. At the time of the thesis preparation, Chapters 2 and 3 were published in a peer-reviewed journals and a first revision of Chapter 4 was in review with *J Appl Physiol*, September 2016.

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# CHAPTER 1

# INTRODUCTION

#### PREAMBLE

Teas are categorized by their method of preparation, either fermented (e.g., black tea), semi-fermented (e.g., oolong tea), or non-fermented (e.g., green tea). The method of preparation influences the chemical composition of the teas. Lack of fermentation in the preparation of green tea results in a higher concentration of polyphenolic compounds, which have been associated with health benefits (1). The most characteristic polyphenols in green tea are compounds known as catechins, which are a subclass of flavonoids (2). There are four main catechins in green tea: epigallocatechin (EGC), epicatechin gallate (EGCG), epicatechin (EC), and epigallocatechin gallate (EGCG) (3). Of the four main green tea catechins (GTC), EGCG comprises the largest percentage, typically accounting for  $\sim$ 50-80% (4).

Once ingested, catechins are hydrolyzed and either methylated, glucuronidated, or sulfated, forming conjugates and producing metabolites that are biologically active (5, 6). One of the more recognized roles is their antioxidant potential and ability to scavenge reactive oxygen species (ROS), while also preventing peroxidative reactions (7, 8). Certain catechins have also been shown to bind to specific proteins, enzymes, and receptors leading to altered biological activities (9). Differences in bioavailability and the range of potential actions of individual catechins make it challenging to assess their specific effects.

A typical cup of green tea contains  $\sim$ 300 mg of tea solids,  $\sim$ 40% being catechins (10). Thus, one cup of green tea would provide  $\sim$ 120 mg of total catechins, of which  $\sim$ 60 mg would be EGCG. Although EGCG is more abundant and believed to be the most biologically active GTC, it is also the least bioavailable (11). With a green tea dose of  $\sim$ 700 mg, only 0.07% of EGCG appeared in the plasma, whereas 0.75% of EC was present (12). This variability in catechin absorption along with a short half-life of  $\sim$ 4 hours make comparing studies using various dosing regimens and catechin compositions difficult (13). Consumption of multiple as opposed to single catechins, as occurs with green tea extract (GTE) ingestion, is believed to enhance their purported effectiveness (14).

The increasing prevalence of chronic diseases such as diabetes and obesity has prompted research into alternative approaches for treatment. Research on GTC has shown numerous potential benefits for treating diseases, such as metabolic syndrome, cardiovascular disease, and cancer (15). Another well-known approach to treating and/or preventing chronic disease has been from the positive metabolic effects of exercise (16). Considering the potential benefits of GTC supplementation as well as exercise, combining the two approaches may potentiate those achieved individually. Despite this potential, knowledge of the role of GTC when combined with exercise in humans remains limited.

#### **REVIEW OF LITERATURE**

Tea is one of the most popular beverages in the world (1, 3, 4, 17), second only to water. The use of tea originated thousands of years ago in China, where the leaves from the *Camellia sinensis* plant were first chewed for their purported medicinal qualities. Soon the leaves began to be infused with hot water leading to the making of the current day tea beverage. Tea cultivation quickly developed and tea consumption gained global popularity. Subsequently, research in the field of tea and its potential uses gained momentum.

#### 1.1. Epidemiological Studies and Green Tea Catechins

Tea consumption is inversely associated with both obesity and diabetes. Epidemiological studies have suggested that high intakes of tea are independently associated with lower BMI, improved insulin sensitivity and reduced risk of diabetes (2). A recent meta-analysis that examined the associations between health indices and coffee, decaffeinated coffee and tea consumption indicated that individuals who drank more than three to four cups of tea per day had approximately one-fifth lower risk of diabetes than those consuming no tea (18).

#### 1.1.1. Obesity and BMI

A cross-sectional analysis performed as part of the National Health and Nutrition Examination (NHANES) survey examined the association between hot and iced tea beverages over three years in 6,472 participants with markers for metabolic syndrome (19). An inverse relationship was found between hot tea consumption and obesity, assessed via waist circumference and BMI. Interestingly, iced tea consumption was associated with increased BMI and waist circumference. The authors concluded that the apparent benefits of tea consumption might only be present when consumed in the traditional manner, as well, iced tea is traditionally sweetened, which might have negated any effect of the tea on BMI. Moreover, there is evidence that carbohydrates, such as the sugar added to the tea, might reduce the already low bioavailability of tea catechins (13). Another cross-sectional epidemiological study in 2003 found a 19.6% decrease in body fat and a 2.1% lower waist:hip ratio in a group of 1,103 Taiwanese tea drinkers when compared to those who did not consume tea (20). Lastly, another study also observed an inverse relationship between BMI and tea consumption in 4,280 participants over a 14-year period in The Netherlands Cohort (21).

#### 1.1.2. Diabetes Risk

The Japan Collaborative Cohort Study for Evaluation of Cancer Risk assessed tea and coffee consumption in 17,413 participants aged 40 to 79 over a two year period (22). Iso et al. (22) reported a reduction in the risk of developing diabetes with both tea and coffee consumption. However, with regards to green tea, they found that the relationship was only present in those who drank six or more cups per day. The Singapore Chinese Health Study analyzed data from 36,908 participants on the consumption of coffee, black tea and green tea and the association to type 2 diabetes risk over a five-year period (23). They reported no relationship between reduced diabetes risk and green tea consumption, and only a small reduction in those drinking black tea. However, compared to the amount of tea ingestion reported by Iso et al. (22), the amount of tea consumed per day was much smaller, less than three cups per day. Similarly, The Whitehall II cohort study examining the relationship between tea and coffee and the risk for diabetes found no association between tea or coffee consumption and diabetes risk (24). However, the authors did report a small reduction in diabetes risk with the combined ingestion of both tea and coffee. Interestingly, they also reported smaller quantities of beverage consumption compared to those by Iso et al. (22). It is possible a "dose-response" relationship might exist with regards to the effects of tea catechins and diabetes risk. Although prospective studies assessing the associations between tea and diabetes risk is less conclusive than those examining the effects on obesity, given the close association between obesity and diabetes it is likely that individuals maintaining a healthier weight would also have better glycemic control. The presence of confounding factors is an inherent limitation of observational studies, however, the benefits observed from more controlled clinical trials appear more conclusive, which will be discussed in the proceeding chapters.

#### 1.2. Mechanisms for the Metabolic Effects of Green Tea Catechins

The mechanisms by which GTC might induce favourable metabolic effects have primarily been investigated in animal models. The mechanisms suggested to occur in animals provide insight into potential actions that may provide beneficial effects observed in humans. However, there are no studies that have examined these potential mechanisms in humans. Figure 1. On the following page provides an overview of evidence from rodent models providing relevant mechanisms related to their potential effects on obesity, diabetes and when combined with exercise.

#### 1.2.1. Bioenergetics

Several potential mechanisms have been proposed for the numerous effects on energy regulation from consuming tea catechins. Tea catechins, particularly EGCG, have been shown to alter energy homeostasis by inducing effects through either regulation of energy stores and/or altering utilization of substrates. Potential mechanisms include: 1) inhibition of fat absorption; 2) inhibition of carbohydrate absorption; 3) inhibition of catechol-O-methyl transferase (COMT), an enzyme that aids in controlling catecholamine concentrations and inhibiting fat oxidation in brown adipose tissue; and 4) increases in activation of 5'-AMP-activated protein kinase (AMPK), a sensor for cellular energy metabolism (1, 25).



Figure 1. Potential mechanisms of GTC in diabetes and obesity. glucose transporter 4 (GLUT4), phosphoinositide 3-kinase (PI3K), 5' AMP-activated protein kinase (AMPK), sodium-dependent glucose transporter 1 (SGLT1), and nuclear factor- $\kappa$ B, (NF- $\kappa$ B).

#### 1.2.1.1. Energy Regulation

Two possible ways tea catechins might induce an effect on weight loss or weight maintenance is through either increased energy expenditure and/or reductions in energy intake. For example, tea catechins might stimulate thermogenesis through inhibition of COMT, which would lead to increases in catecholamines such as epinephrine (EPI) and norepinephrine (NE) (26). Prolonged adrenergic stimulation can lead to increases in energy expenditure. Caffeine, which is naturally present in tea, might also affect adrenergic stimulation via inhibition of phosphodiesterase, an enzyme that degrades cyclic adenosine monophosphate (cAMP) released in response to NE (27). Thus, GTC might induce thermogenesis through synergistic actions of both GTC inhibition of COMT, as well as prolonging actions of cAMP. With regards to reductions in energy intake, researchers reported reduced fat absorption from the gut in mice on high-fat diets supplemented with catechins from oolong tea (28). The authors contributed the reduced fat absorption to an inhibition of lipase activity in the pancreas from the tea catechins. Other similar mechanisms are related to the potential for tea catechins to alter fat oxidation.

#### 1.2.1.2. Substrate Metabolism

Increased fat oxidation may occur via induced thermogenesis from COMT inhibition. With inhibition of COMT, the resulting increase in adrenergic stimulation could lead to greater lipolysis and availability of substrates for oxidation. Another potential site for increased fat oxidation is through greater respiration of brown adipose tissue. *In vitro* treatment of brown adipose tissue with EGCG led to small but significant increases in respiration, potentiated by the addition of caffeine (29). Lastly, obese rats treated with green tea over a 12-week period had a reduction in body weight, adipose tissue, and hyperlipidemia (30). The researchers suggested that increased activation of AMPK modulated these positive effects observed in the obese rats.

#### 1.2.2. Glycemic Regulation

The "anti-diabetic" effect of tea catechins might be related to any number of mechanisms observed in studies conducted in rodents. Tea catechins have shown potential to inhibit carbohydrate absorption in the intestines, as well as improve glucose uptake by insulin sensitive tissues. Other potential effects include suppression of glucose release from the liver and modulating transport of intracellular glucose transporters. Through their antioxidant activity, tea catechins may inhibit advanced glycation end-products and relieve ROS induced inhibition of the insulin-signaling pathway. EGCG and ECG have been shown to inhibit intestinal glucose uptake from the gut, possibly via inhibition of the sodium-dependant glucose transporter (SGLT-1). Using ex vivo and in vivo methods in high-fat fed rats, SGLT-1 inhibition has been observed following both acute (30 min) and chronic GTC exposure (six weeks) (31). Similarly, Kobayashi et al. (32) exposed sections of rat and rabbit jejunum to EGCG and ECG in the presence of glucose, and observed that the sections containing the catechins reduced glucose uptake by 36% with ECG and 25% with EGCG.

Several studies have provided evidence of GTC modulating the expression and activity of glucose transporters (GLUT). For example, rats receiving green tea for three weeks showed increases in skeletal muscle GLUT-4 translocation, with a concomitant reduction in GLUT-4 translocation in adipose tissue (33). Similarly, acute in vitro EGCG treatment in muscle cells of rats, as well as 7-days of EGCG supplementation in rats showed increases in GLUT-4 translocation in skeletal muscle (34). Rats fed a high fructose diet while being treated with GTE showed increases in the mRNA expression of GLUT-1 and GLUT-4 in the liver compared to those rats not receiving the GTE (35). The anti-inflammatory properties of EGCG have also been shown to reverse fatty acid-induced impairments in the insulin-signaling pathway (36), which improved downstream translocation of GLUT-4 in skeletal muscle and adipose tissue.

Several GTC, particularly EGCG, were recently shown to have an inhibitory effect on glucose-stimulated insulin secretion in pancreatic  $\beta$ -cells (37). Glucose-stimulated insulin secretion from pancreatic  $\beta$ -cells requires the transport of glucose into the pancreas via GLUT-2, leading to an increase in ATP and consequently inactivation of ATP-sensitive K<sup>+</sup> channels and release of Ca<sup>2+</sup> from voltage-dependant channels (38). The researchers suggested the effect of the GTC was likely from activation of the K<sup>+</sup> channels, thus inhibiting release of Ca<sup>2+</sup>. Conversely, other in vitro evidence suggests GTC might improve the transport of glucose through GLUT-2 activations (31). Taken together these mechanisms highlight the potential effects of tea catechins to improve glycemic regulation.

#### 1.2.3. Exercise and Green Tea Catechins

Studies in exercising mice supplemented with GTE have shown consistent findings regarding increases in fat oxidation and other improved metabolic functions. Mice on a high-fat diet combined with GTE, EGCG, or PLA exercised 3x/week for 10 weeks; following training, the GTE and EGCG groups showed greater  $\beta$ -oxidation activity compared to the PLA group (39). Additionally, the EGCG group showed greater elevations in FAT/CD36 mRNA concentrations and gene expression compared to the GTE and EGCG groups. Improvements in energy substrate use from lipid sources is partially regulated by FFA transport through the plasma membrane via FAT/CD36; thus, the authors believed these increases in FAT/CD36 potentially influenced the enhanced βoxidation, as well as the endurance improvements they observed in the exercising mice (39). Although the GTE group did not show changes to FAT/CD36, they still showed improvements in  $\beta$ -oxidation and endurance capacity, suggesting other mechanisms might be contributing to the improvements. Another study conducted by the same group subjected mice to a 10-week protocol in which the animals swam 3x/wk for 30 min while supplementing with GTE. Following training, the mice receiving the GTE showed reductions in carbohydrate utilization, with a concomitant increase in fat oxidation, as well as reduced lactate and increases in plasma FFA during exercise when compared to the PLA group (40). Similarly, mice on a high-fat diet and GTE for 15 weeks participating in running 3x/week also reduced their carbohydrate oxidation and increased fat oxidation when compared to a PLA group (41). A common feature from many of the rodent studies is an apparent dose-response; findings were more robust with higher doses of GTC. However, it is not clear if these improvements necessitate such long supplementation periods. For example, a similar response was observed in mdx mice (a mouse model for muscular dystrophy) following three weeks of GTE supplementation and 24-hour voluntary wheel running (42). When compared to the PLA group, the GTE group showed increases in  $\beta$ -oxidation activity along with greater citrate synthase activity, which was independent of running.

The various mechanisms reviewed above provide a brief overview of the evidence for improvements in exercise metabolism from studies using rodent models. Although these findings have yet to be replicated in human subjects, they show the potential benefits that may exist with GTC supplementation combined with exercise. The studies discussed above demonstrate the potential efficacy of tea as an "anti-obesity" and "antidiabetic" agent. Although similar effects on bioenergetics and glycemic control have been observed in humans, similar mechanisms have yet to be investigated.

#### 1.3. Human Interventions – Green Tea Catechins at Rest

Intervention trials in humans ingesting GTC have shown benefits with regard to increased fat loss, increased total energy expenditure, improved insulin sensitivity, and lower fasting glucose concentrations (1). The findings have been mostly favourable following both acute and chronic treatments with GTC supplements or drinking brewed tea. Many of the mechanisms reported above are believed to contribute to the findings discussed in the following sections.

#### 1.3.1. The "Anti-Obesity" Effect

There is a growing body of evidence that GTC are a beneficial dietary strategy to reduce the prevalence of obesity. Several studies have reported reduced body weight and body fat following GTC consumption via increases in energy expenditure and/or reductions in respiratory exchange ratio (RER) (43). The temporal effects of GTC are still questionable; studies that have supplemented for short periods have shown less consistent results than those administering GTC over several weeks to months. Unfortunately, the evidence for the chronic effects of GTC on obesity and obesity-related outcomes is limited.

A relatively low dose of GTE (141 mg + 87 mg caffeine) administered over a 12week period in overweight Thai subjects increased resting energy expenditure (REE) and reduced RER at eight weeks of supplementation (44). Significant body weight loss was also observed at both the eight and 12-week follow-ups. Although RER returned to normal at the 12-week follow-up, the authors suggested a possible compliance issue might have confounded the measurements at the end of the trial. Another possible explanation is that the GTE was less effective after the participants lost weight over the 12-week period. Similar findings were observed following short-term supplementation with 300 mg of EGCG daily for two days in overweight men (45). Although no effect was observed on REE, they did observe a lower RER in the postprandial state. Conversely, a similar dose of EGCG, ~250 mg (GTE, 500 mg) prior to REE measurements in healthy normal weight men showed no effect on REE or RER (46). However, they did report a significant 6% increase in energy expenditure with a 50 mg dose of caffeine, which shows how caffeine could be a confounding factor in GTE supplementation trials.

A study conducted in overweight Japanese men ingesting a decaffeinated GTE (548 mg) beverage over a 12 week period resulted in reduced body weight and body fat compared to a group consuming a PLA (47). One of the earlier studies conducted by Dulloo et al. (48) administered a single dose of GTE (375 mg + 150 mg caffeine), and observed a small but significant 4% increase in energy expenditure over a 24 hour period with the GTE + caffeine, whereas the same dose of caffeine alone had no effect. These results provide evidence of the potential metabolic alterations associated with ingestion of GTE in non-exercising humans. However, given that other trials have failed to show an effect of EGCG or GTE without the addition of caffeine, the combination might be more effective in resting trials. A recent meta-analysis suggests the effects on obesity and obseity-related outcomes are greater when the two compounds are combined (49).

#### 1.3.2. The "Anti-Diabetic" Effect

The acute and short-term administration of GTC has been shown to reduce [glucose] and insulinemia following ingestion of a glucose load. In one study, healthy participants received a single dose of green tea powder (1.5 g) containing ~110 mg of total catechins, ~85 mg EGCG + ~60 mg of caffeine. Following ingestion, researchers performed an oral glucose tolerance test (OGTT) and observed significantly lower plasma glucose concentrations compared to a PLA (50). Similarly, Venables et al. (51) administered three doses of decaffeinated GTE (dose = 340 mg polyphenols, 136 mg EGCG) over a 24-hour period and reported a lower insulin area under the curve (AUC) and improved Matsuda insulin sensitivity index (M<sub>ISI</sub>) from an OGTT. Interestingly, the difference in the length of supplementation between these two studies appeared to alter the effect of GTC; the acute administration lowered glucose while the short-term administration reduced insulinemia. Studies contrasting these temporal effects have yet to be conducted. Although these observations were made in healthy participants, they provide insight into the potential utility GTC might have with improving glycemic control in a diabetic or insulin resistant population.

Researchers examining the effects of EGCG supplementation (300 mg•day<sup>-1</sup>) for 12-weeks in healthy participants reported reduced fasting [glucose] and insulinemia (52). However, participants lost weight over the 12-week period, which might have confounded the results. This raises a particular concern when trying to evaluate the efficacy of an intervention over several months, which might have an effect on both weight loss and glycemic control. However, a similar study utilized oolong tea, also having a high concentration of EGCG, to assess the effects of catechins in a diabetic population (53). In a randomized crossover design, researchers had participants consume the equivalent of 5 cups of oolong tea per day for 4 weeks, after which they observed lower fasting levels of glucose and fructosamine compared to the control trial. Another study among healthy participants showed that a GTE (300 mg EGCG) + 10 g sucrose beverage ingested with a mixed meal containing 50 g of carbohydrate reduced intestinal absorption by 25% (54). The differences occurred at each hour from the 2-8 hour

measurement period. Taken together, this evidence supports an "anti-diabetic" effect of GTC, and the findings are supported by the mechanisms discussed previously.

#### 1.4. Human Interventions – Green Tea Catechins during Exercise

Nutritional interventions can alter the acute and chronic responses to exercise (55). For example, certain foods or supplements ingested at particular times can alter substrate use during exercise, improve training-induced adaptations, improve performance and/or enhance the recovery process following exercise. Studies have investigated the potential benefits of GTC and exercise in humans in all of the above areas. The following section discusses those studies, which have provided evidence for or against these potential benefits.

#### 1.4.1. Exercise Substrate Metabolism

One of the first studies to investigate the metabolic effects of GTE during exercise in humans was by Venables et al. in 2008 (51). Healthy men ingested three doses of ~300 mg of GTE over 24 h (GTE = 890 mg•day<sup>-1</sup> and EGCG = 366 mg•day<sup>-1</sup>), and subsequently performed 30 min of steady-state exercise at 50% of their maximal power output ( $W_{max}$ ). A 17% increase in fat oxidation rates was observed when compared to a PLA. Interestingly, this was accompanied by increased plasma glycerol and a trend towards increased FFA. Several other studies have failed to observe similar findings and inconsistencies with regard to dosing, exercise intensities, and subject population makes it difficult to draw conclusions on the efficacy of supplementation. For example, Eichenberger et al. (56) administered 160 mg of GTE once daily for 3 weeks in trained subjects and did not observe metabolic or physiological effects during exercise. Similarly, Dean et al. (57) examined the effects of 6 days of once daily EGCG (270 mg) or EGCG + caffeine (270 mg + 3mg•kg<sup>-1</sup>) vs. PLA during 60 min of steady-state cycling, and did not observe changes in substrate oxidation.

Methodological differences between studies may in part explain the equivocal findings. First, Venables et al. (51) provided a much larger dose (~300 mg) three times over a 24-hour period, whereas both Eichenberger et al. and Dean et al. (57) administered 160 mg of GTE and 270 mg EGCG just once a day, respectively. Second, the subject's training status might have contributed to the different findings. Subjects in all three studies mentioned above had mean VO2peak's between 50-55 ml•kg<sup>-1</sup>•min<sup>-1</sup>, however in the study by Venables et al. (51) participants were defined as "healthy", whereas both Eichenberger et al. (56) and Dean et al. (57) defined their participants as "trained". The "sports adrenal medulla" is an adaptation in trained individuals that affects the release of EPI during exercise (58). Thus, given the potential COMT mechanism, the effects of GTE might have been attenuated in the trained individuals, whereas differences might be observed in those unaccustomed to exercise. However, a recent study by Roberts et al. (59) is the only study to have investigated the chronic effects of a larger dose of decaffeinated GTE (571 mg•day<sup>-1</sup>) over four weeks. The authors reported that the trained individuals increased fat oxidation during steady-state exercise at two and for weeks of supplementation.

Although there are several other possibilities for the equivocal findings, the COMT mechanism is believed to be the most likely candidate for the effects on energy expenditure and substrate oxidation (48). However it has not been thoroughly investigated in humans. Moreover, the effect of GTC administration on actual catecholamine concentrations is very limited. Those that have investigated the effects have reported conflicting findings (48, 60). Acute administration of three doses of GTE + caffeine (375 mg catechins + 150 mg caffeine) over 24 hours resulted in higher 24-hour urinary NE concentrations in the GTE group when compared to a PLA (48). Conversely, Hodgson et al. (60) reported no effects of a single dose of GTE + caffeine (559 mg catechins + 120 mg caffeine) on metabolites indicative of increased catecholamines.

#### 1.4.2. Exercise Performance

The evidence for a performance enhancing effect from GTC in humans is limited. However, in addition to the mechanisms discussed earlier, one of the rationales for potential performance improvements stems from the initial study by Venables et al. (51), which observed increased fat oxidation during 30 min of steady-state cycling following GTE ingestion. Thus, if an exercise session were to last long enough to challenge glycogen stores and one could preserve those stores though the use of GTC, then enhanced performance could be gained in the latter stages of that session. In the study by Dean et al. (57) previously mentioned, once daily EGCG administration over 6 days did not improve performance on a 40 km cycling time-trial. However, the time-trial in this case was not performed under conditions that might have been affected by reduced glycogen stores. In the study by Roberts et al. (59), participants performed a 40 min cycling time-trial subsequent to a one-hour steady-state ride following GTE administration. Under these conditions (increases in fat oxidation during the steady-state portion), increases in the distance covered in the 40 min performance ride were observed.

Another possibility is improved performance from increases in VO<sub>2</sub>peak, which has been observed in one human study. Richards et al. (61) had participants perform an incremental cycling test until volitional fatigue following 48 hours of EGCG (405 mg•day<sup>-1</sup>) supplementation. Authors reported an ~4.4% increase in VO<sub>2</sub>peak with EGCG compared to a PLA, which was not evidenced by a concomitant increase in cardiac output. Considering O<sub>2</sub> consumption is dependent on both arterial-venous O<sub>2</sub> difference ([a-v]O<sub>2</sub> diff) and cardiac output, the authors concluded the increase in VO<sub>2</sub>peak was likely a consequence of improved [a-v]O<sub>2</sub> diff. Since exercise performance is partially determined by VO<sub>2</sub>peak, GTC might offer some performance improvements through greater O<sub>2</sub> uptake.

#### 1.4.3. Post-Exercise

From the available literature on the effects of GTC during post-exercise recovery, most studies have focused on the ability of GTC to reduce inflammation, ROS, and/or reduce markers of muscle damage. For example, in the study by Eichenberger et al. (56), lower creatine kinase (CK) concentrations were observed in trained men following three weeks of supplementation with 160 mg of GTE. Lower CK prior to performing any exercise suggests that in the days prior to the actual trials, CK was lowered, likely in

response to the participant's regular training. The authors suggested the GTE might have attenuated free radicals and/or improved cellular integrity resulting in decreased CK leakage following a muscle-damaging exercise bout. Another study had participants performing resistance exercise following a seven-day regimen of drinking an actual green tea beverage three times per day (GTC = ~415 mg•day<sup>-1</sup>). They reported reductions in CK, markers of oxidative stress, lipid hydroperoxides, xanthine oxidase, as well as increases in one endogenous ROS scavenger, glutathione (62). Similarly, improvements in endogenous antioxidant capacity and reductions in oxidative stress biomarkers were observed in participants ingesting a single dose of GTC (780 mg) and performing a cycling endurance test to exhaustion (63).

Despite these potential improvements in oxidative stress and muscle damage, it is well accepted that the adaptation to strenuous exercise is partially dependent on cyclic perturbations in muscle damage, oxidative stress, and inflammation (64). Adaptations to a physiological stress is known as hormesis, and the dose-response of the physiological stress is suggested to be in the form a U-shape where too much stress is as counter productive as none at all (65). It has been shown that a high dose of antioxidants during exercise training blunts the adaptive response from the exercise stress (66). However, others have shown that antioxidant supplementation during exercise training does not blunt the adaptive response (67). It is plausible that ingestion of GTC during exercise might reduce the adaptive response, although this has yet to be investigated. In certain populations, such as in overweight and sedentary individuals, the initial stages of beginning physical activity could possibly induce excessive amounts of inflammation and oxidative stress. Thus, in these cases, GTC might be beneficial.

#### **1.5.** Purpose of Thesis

The global objective of this dissertation was to assess the effects of GTE supplementation combined with an exercise stimulus in an effort to advance our understanding of the potential benefits of tea catechins. Although numerous efforts have been made to provide evidence for the efficacy of GTC supplementation and identify an ideal dosing or supplementation regimen, the findings have been equivocal. The literature review presented here clearly identifies the difficulty in assessing the data between diverse methodologies. The numerous variations in study design (i.e. dose of GTC, caffeinated or decaffeinated, supplementation pattern, form of exercise and intensity, and the participant population) make it difficult to draw conclusions and ascertain the relative efficacy of supplementation. Therefore, in an effort to minimize extraneous variables, the present dissertation utilized the same decaffeinated GTE supplementation, as well as the same supplementation patterns and similar exercise protocols throughout each study.

Considering GTC have been shown to have metabolic effects both at rest and during exercise, Study 1 sought to assess several aspects of GTE supplementation on energy expenditure and substrate oxidation both before and during 60 min of steady-state exercise, as well as on a time-trail performed following the steady-state session. We hypothesized that GTE would increase REE at rest, as well as fat oxidation during the steady-state exercise session while conserving glycogen and leading to improved timetrial performance. This study was performed in recreationally active men to compare our findings with a majority of the published literature. However, there is no evidence of the effects of GTC during exercise in a sedentary overweight population. Thus, Study 2 attempted to fill this void by examining the effects of GTE in this population, while using similar methodologies applied in Study 1. In addition, considering the potential healthrelated disparities between an active and sedentary population and the potential for GTC to improve glycemic control, Study 2 included additional measures of glycemic control. We hypothesized that the metabolic effects of GTE during exercise would be more robust in participants with reduced exercise tolerance, and supplementation would improve the glycemic response to an oral glucose load at rest and during post-exercise recovery. Finally, Study 3 was designed to build on both Study 1 and 2, but in a sedentary normal weight population to contrast between the three studies, as well as provide more detail into understanding the effects on glycemic control. We hypothesized that GTE treatment would increase fat oxidation during exercise and improve the glycemic response to an oral glucose load in the post-exercise recovery period. The effects on [glucose] and insulinemia would not be due to reduced intestinal absorption or hepatic output, but will be suggestive of improved uptake by insulin sensitive tissues.

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# CHAPTER 2

# No Effect of Short-term Green Tea Extract Supplementation on metabolism at rest or during exercise in the fed state

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No effect of short-term green tea extract supplementation on metabolism at rest or during exercise in the fed-state

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## Abstract

Supplementation with green tea extract (GTE) in animals has been reported to induce numerous metabolic adaptations including increased fat oxidation during exercise and improved performance. However, data regarding the metabolic and physiological effects of GTE during exercise in humans are limited and equivocal. PURPOSE: To examine the effects of short-term GTE treatment on resting energy expenditure (REE), whole-body substrate utilization during exercise and time trial performance. METHODS: Fifteen active men (24  $\pm$  3 y; VO<sub>2peak</sub> = 48  $\pm$  7  $ml \cdot kg \cdot min^{-1}$ ; BMI = 26 ± 3 kg \cdot m^{2(-1)}) ingested GTE (3 x/d = 1,000 mg/d) or placebo (PLA) for 2 d in a double-blind, crossover design (each separated by a 1 wk wash-out period). REE was assessed in the fasted state. Subjects then ingested a standardized breakfast (~5.0 kcal•kg<sup>-1</sup>) and 90 min later performed a 60 min cycling bout at an intensity corresponding to individual maximal fat oxidation (44 ± 11% VO<sub>2peak</sub>), followed by a 250 kJ TT. RESULTS: REE, wholebody oxygen consumption (VO<sub>2</sub>) and substrate oxidation rates during steadystate exercise were not different between treatments. However, mean heart rate (HR) was lower in GTE vs. PLA (115  $\pm$  16 vs. 118  $\pm$  17 beats•min<sup>-1</sup>; main effect, P = 0.049). Mixed venous blood [glycerol] was higher during rest and exercise after GTE vs. PLA (P = 0.006, main effect for treatment) but glucose, insulin and free-fatty acids were not different. Subsequent time trial performance was not different between treatments (GTE =  $25:38 \pm 5:32$  vs. PLA =  $26:08 \pm 8:13$ min:sec; P = 0.75). CONCLUSION: GTE had minimal effects on whole-body substrate metabolism but significantly increased plasma glycerol and lowered heart rate during steady-state exercise, suggesting a potential increase in lipolysis and a cardiovascular effect that warrants further investigation.

Keywords: GTE, Tea Catechins, Fat Oxidation, Substrate Utilization

## Introduction

Tea is one of the most common beverages in the world (Cabrera, Artacho, & Gimenez, 2006; Kao, Chang, Lee, & Chen, 2006; Khan & Mukhtar, 2007; Schneider & Segre, 2009). All teas contain polyphenols that are believed to be responsible for its purported effects on physiologic function (Kao, et al., 2006). Unlike other teas, the preparation of green tea does not involve a fermentation process, resulting in a higher polyphenol concentration that has made it the preferred choice for medicinal use by Asian cultures for centuries (Cabrera, et al., 2006; Khan & Mukhtar, 2007). Four specific compounds known as catechins (CAT) make up the majority of polyphenols in green tea: epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC), and epigallocatechin gallate (EGCG) (Cabrera, et al., 2006). Of the four green tea CAT (GTC), EGCG comprises the largest percentage of the four GTC, typically accounting for ~50-80% (Khan & Mukhtar, 2007).

GTC administration to mice has consistently been shown to increase running distance and time to exhaustion (Call, et al., 2008; Murase, Haramizu, Shimotovodome. Nagasawa, & Tokimitsu, 2005; Murase. Haramizu. Shimotoyodome, & Tokimitsu, 2006; Murase, Haramizu, Shimotoyodome, Tokimitsu, & Hase, 2006; Shimotoyodome, Haramizu, Inaba, Murase, & Tokimitsu, 2005). The improved endurance capacity has been attributed to increased lipid utilization during exercise, as evidenced by reductions in respiratory exchange ratio (RER) and greater muscle glycogen content following exercise (Murase, et al., 2005). In contrast, data regarding the metabolic and physiological effects of GTC supplementation during exercise in humans are equivocal.

Venables, Hulston, Cox, & Jeukendrup, (2008) provided three doses of ~300mg GTE over 24 h to healthy men (GTE = 890 mg•d<sup>-1</sup> and EGCG = 366 mg•d<sup>-1</sup>), and observed reductions in RER during 30 min of cycling at 50% of their maximal power ( $W_{max}$ ). This was associated with a ~17% increase in fat oxidation rates compared to a placebo. The subjects also showed elevated plasma glycerol concentrations and a trend towards elevated free fatty acids (FFA). Richards, Lonac, Johnson, Schweder, & Bell, (2010) also found a beneficial effect of EGCG administration and reported that a dose of 405 mg•d<sup>-1</sup> for 2 d to healthy men and women increased maximal oxygen uptake ( $VO_{2max}$ ). In contrast, Eichenberger, Colombani, & Mettler (2009), observed that a daily dose of 160 mg of GTE for 3 wk did not alter metabolic or physiological markers during steady-state exercise or improve subsequent time trial performance in trained subjects. Similarly, Randell et al., (2013) examined the effects of a 1 and 7 d treatment with GTE and found no change in fat utilization during moderate intensity (~54% VO<sub>2max</sub>) steady-state cycling in moderately-trained individuals.

The divergent findings among human studies are likely related in part to differences in experimental design. For example, Eichenberger et al., (2009) employed a relatively low dose of GTE (160 mg), administered once daily for 3

wk. Similarly, Dean, Braakhuis, & Paton (2009), examined the effects of a daily dose of EGCG (270 mg) with and without caffeine for 6 d. However, neither Eichenberger et al., (2009) nor Dean et al., (2009) observed a metabolic of physiological effect during exercise. In contrast, Venables et al., (2008) and Richards et al., (2010) both employed a larger dose of GTC administered more frequently (3 x/d). Conversely, the study by Randell et al., (2013) employed a very large dose of GTE administered 2 x/d. Although, considering the GTE treatment utilized contained caffeine the authors discussed the potential effect the caffeine might have had on blunting fat oxidation through increased glycolysis.

In the present study, we employed dosing regimens similar to those used by both Venables et al., (2008) and Richards et al., (2010) to investigate the metabolic and physiological effects of GTE at rest, during steady-state exercise, and on time trial performance in adult humans. Previous studies have examined the effects of GTC in the fasted state, considering this we chose to examine the effects of GTE in the fed state, which is more practical to real life and would potentially blunt steady-state fat utilization allowing a difference to be observed during the GTE treatment. We hypothesized that, compared to placebo, 2 d of GTE administered would increase REE and fat oxidation during steady-state exercise, concomitantly preserving carbohydrate and improving subsequent time trial performance. These alterations would be evidenced by reductions in RER and accompanied by altered blood markers of fat catabolism.

#### Methods

<u>Subjects</u>: Fifteen young healthy men between the ages of 18-45 completed the protocol in its entirety, except for one subject who was unable to complete the first time trial due to exhaustion from the previous 60 min of steady-state exercise. Thus, all time trial data is out of 14 subjects. Participants were healthy, non-habitual GTC users ( $\leq 2 \text{ x/wk}$ ), non-tobacco users and habitually performed aerobic activity on a recreational basis ( $\geq 3 \text{ x/wk}$ ). The research protocol and potential risks were explained to each participant prior to obtaining written, informed consent. Based on differences in fat oxidation rates from a previously published study (Venables et al., 2008), with 80% power and an alpha level of 0.05, we estimated a sample size of 15 subjects per group was required to detect statistical significance.

<u>Overview of Research Design</u>: A double blind, placebo-controlled, crossover design was used to assess the effects of GTE ingestion for 2 d on REE and substrate utilization during exercise. The study design incorporated dosing protocols, which appear to be effective at improving either physiologic or metabolic processes in exercising humans (Richards, et al., 2010; Venables, et al., 2008). Subjects visited the laboratory on four occasions in total for baseline testing, a familiarization session and two experimental trials. Each session was conducted in the morning at approximately the same time. Subjects ingested a standardized breakfast 90 min prior to exercise, which consisted of a granola bar,

banana and apple juice (approximately 385 kcal which was comprised of 79 g CHO, 6 g fat, and 6 g protein). Although pharmacokinetic evidence (Lee, et al., 2002) suggests rapid excretion of CAT (undetectable after 24 h), a 1 wk washout period separated the two experimental trials in order to eliminate any potential confounding influences or order effect.

Supplementation: Subjects were provided a total of 7 capsules containing either a high quality decaffeinated GTE powder (Sunphenon®, 90D, Taiyo International Inc. Minneapolis MN) or placebo (corn flour). Subjects received 1,000 mg of total polyphenols/d, the equivalent of  $\sim$ 2-3 cups of green tea ( $\sim$ 300 mg) 3 x/d. There were no adverse effects of supplementation and all subjects reported administering the treatments as prescribed. Table 1 displays the concentrations of CAT in the GTE, determined by the supplier through HPLC analysis. Capsules were opague and of identical color, shape, and size to assure the contents of the GTE and placebo remained indiscernible to subjects and researchers. Capsules were provided in a standard prescription pill container, subjects were asked to return the empty containers to verify all capsules were consumed. Pharmacokinetic evidence suggests greater bioavailability of CAT ingested in the fasted state (Chow, et al., 2005) and a half-life of ~4 h (Lee, et al., 2002). Considering this, subjects supplemented 3 x/day, 1 h before meals (breakfast, lunch, and dinner) for 48 h, ingesting each capsule with 500 ml of water. Participants were reminded via text or email on the morning supplementation was to begin and to confirm they understood the instructions.

## Experimental Protocol:

*Baseline testing:* During the first visit, height (cm) and body mass (kg) were recorded. The BOD POD® (COSMED Inc., Concord, CA) was used to measure body composition via air-displacement plethysmography. Subjects then completed a graded exercise test (GXT) on the cycle ergometer (Lode Excalibur Sport V 2.0, Groningen, The Netherlands) to determine the intensity eliciting maximal fat oxidation rates (Fat<sub>max</sub>). Previous work suggests high inter-individual variability in Fat<sub>max</sub> (Meyer, Gassler, & Kindermann, 2007). However, a previously established time-efficient protocol has been shown to effectively assess Fat<sub>max</sub> (Achten, Gleeson, & Jeukendrup, 2002). Therefore, as an alternative to using a set percentage of maximal workload, a modified GXT protocol was used to individually assess the workload eliciting Fat<sub>max</sub>. Subjects performed a 5 min warm-up, cycling at 50 watts (W), after which intensity increased to 95 W for 3 min, then increased 35 W every 3 min until volitional fatigue. All participants completed the Fat<sub>max</sub> assessment to exhaustion, eliciting steady-state workloads for Fat<sub>max</sub> between 50 and 165 W, which corresponded to 44 ± 11 % VO<sub>2peak</sub>.

Continuous heart rate (HR) was recorded throughout the session via a telemetry chest strap and wireless receiver (Polar Electro Oy, Kempele, Findland). Respiratory gases were assessed throughout the test using an on-line gas collection system (Moxus modular oxygen uptake system, AEI Technologies, Pittsburgh, PA). Oxygen consumption ( $VO_2$ ) and carbon dioxide production ( $VCO_2$ ) were used in stoichiometric equations to calculate fat oxidation during

exercise (1.695 · VO2 – 1.701 · VCO2) as previously described (Frayn, 1983; Jeukendrup & Wallis, 2005).

*Familiarization Session:* This session was conducted as an actual experimental trial (detailed below) to allow subjects to become familiarized with the trials prior to actual data collection. This session also allowed for verification of the previously determined intensity for eliciting Fat<sub>max</sub>, and to determine the appropriate flow rate for the canopy system used for REE (detailed below).

*Experimental Trials:* Subjects arrived for testing, fasted, 1 h after ingesting their last capsule. After body mass was measured, subjects laid down in a darkened and quiet room for 30 min of respiratory gas collection to assess REE. A canopy system attached to an on-line gas collection unit (Moxus modular oxygen uptake system, AEI Technologies, Pittsburgh, PA) was used for REE data collection. With subjects in a relaxed supine position, the canopy was positioned over the subject's head and a tight seal was created using a plastic skirt attached to the canopy. The canopy flow was adjusted to the rate ( $\pm$  100 ml) determined during the familiarization session. The chosen flow rate elicited mixed CO<sub>2</sub> concentrations of 0.65-0.85% per manufactures instructions. The first 10 min of REE data was discarded to avoid using data influenced by the initial application of the collection equipment. Stoichiometric equations were used to calculate resting EE (0.550 · VCO2 – 4.471 · VO2) and fat oxidation rates (1.67 · VO2 – 1.67 · VCO2) from VO<sub>2</sub> consumption and VCO<sub>2</sub> production (Frayn, 1983; Jeukendrup & Wallis, 2005).

Following REE data collection, subjects received the standardized breakfast and began the exercise portion of the trial 1.5 h later. A 5 min warm-up (50 W) preceded 1 h of cycling at the individually determined intensity for eliciting Fat<sub>max</sub>. To assure hydration was maintained, water (1.5 ml•kg<sup>-1</sup>) was provided after each blood collection. Respiratory gases were collected for 5 min at 10 min intervals throughout steady-state exercise, with the last 2 min of each collection period used for analysis. Following the 1 h of cycling and 5 min of rest, subjects performed a 250 kJ time trial. During the time trial, resistance and cadence were self-selected, participants were instructed to give a maximal effort each time and were aware of the quantity of work completed (kJ) during the bout but blinded to other data, i.e. HR and time. Method reproducibility for the time trial (coefficient of variation) was 2.6% in our laboratory when eight active but untrained individuals were tested 1 wk apart with no intervening intervention (Burgomaster, Heigenhauser, & Gibala, 2006). Both experimental trials were performed identical for each subject. Figure 1 displays the experimental trial timeline.

<u>Blood Sampling</u>: Mixed venous blood was collected during the two trials via an intravenous catheter and blood collection port (BD Angiocath<sup>™</sup> 20 G x 1.25 in and Q-SYTE<sup>™</sup>, Becton, Dickinson and Company, Franklin Lakes, NJ) placed in an adequate upper extremity vein. Approximately 10 ml of blood was collected pre-prandial, pre-exercise, after 15, 30 and 45 min of exercise, immediately post-exercise and immediately after the performance trial. Samples were immediately

placed into appropriate collection tubes (BD Vacutainer®: SST, EDTA, or Fluoride, Becton, Dickinson and Company, Franklin Lakes, NJ). Catecholamines were analyzed from fasting blood samples only, and in 14 subjects only due to inadequate plasma volume in one sample.

Dietary/Exercise Controls: Subjects were asked to maintain their current activity and dietary routines throughout the study period and ceased consumption of any tea or products containing tea CAT. To maintain consistency, subjects recorded dietary intake for 48 h prior to the first baseline testing then and used this to replicate nutritional intake prior to the familiarization session and each supplementation period. Additionally, 72 h prior to baseline testing, familiarization session, and experimental trials, subjects refrained from: physical activity, consuming alcohol or substances with stimulatory effects (not including caffeine). and ingesting food and beverages containing high levels of CAT. Although caffeine might act synergistically with GTC (Dulloo, et al., 1999; Dulloo, Seydoux, Girardier, Chantre, & Vandermander, 2000), the withdrawal effects in habituated users suggests potential reductions in lipolysis during exercise (Hetzler, Warhaftig-Glynn, Thompson, Dowling, & Weltman, 1994). The results from a similar investigation (Van Soeren & Graham, 1998) disagree with the latter, however, considering the possible conflict we did not have subjects refrain from caffeine except during the 12 h preceding each session. Lastly, subjects completed a general questionnaire to identify nutrition, supplementation, and medication patterns that might influence the effects of the GTE on fat oxidation.

<u>Blood Analysis:</u> Blood collected for serum (insulin and FFA) was allowed to clot for 30 min after collection then separated by centrifuging (10 min at 4000 rpm) and stored at -40 °C for later analysis. Plasma obtained from either EDTA (glycerol and catecholamines) or fluoride (glucose and lactate) tubes were immediately centrifuged (10 min at 4000 rpm) and stored at -40 °C for later analysis. Plasma glucose was analyzed using a glucose (hexokinase) reagent kit (Pointe Scientific, Canton MI) and serum insulin was measured through an ELISA (ALPCO Diagnostics, Salem NH). Plasma glycerol (Sigma-Aldrich CO. LLC. St. Louis, MO) and serum FFA (WAKO Diagnostics, Richmond, VA) were determined with enzymatic colorimetric assays. Plasma lactate was measured using a lactate reagent set (Pointe Scientific, Canton MI). Lastly, the University Health Network Laboratory (Toronto, ON) analyzed plasma epinephrine (EPI) and norepinephrine (NE) using HPLC methods.

<u>Statistical Analysis</u>: A 2 factor repeated measures ANOVA (time x condition) was used to compare blood variables, HR, and fat oxidation during exercise. A student's paired *t* test was used to assess differences in resting data, catecholamines, and time trial data. Significance was accepted with a P < 0.05 (2-tailed). All data was analyzed using Statistical Package for Social Sciences (version 20.0, SPSS Inc, Chicago, IL).

## Results

<u>Resting Measures:</u> There was no difference between treatments in resting HR (GTE = 61 ± 8 vs. PLA = 60±8 beats•min<sup>-1</sup>, P = 0.67), SBP (GTE = 121 ± 12 vs. PLA = 122 ± 11 mm•hg<sup>-1</sup>, P = 0.68), or DBP (GTE = 70 ± 10 vs. 71±10 mm•hg<sup>-1</sup>, P = 0.57). Similarly, REE (GTE = 1.4 ± 0.20 vs. PLA = 1.5 ± 0.24 kcal•min<sup>1</sup>, P = 0.17), fat oxidation (GTE = 0.12 ± 0.03 vs PLA = 0.12 ± 0.04 g•min<sup>-1</sup>, P = 0.71) and RER was not different between treatments (GTE = 0.77 ± 0.05 vs. PLA = 0.77 ± 0.06, P = 0.90).

<u>Steady-state Exercise:</u> GTE supplementation did not alter O<sub>2</sub> uptake (GTE = 1.66  $\pm$  0.50 vs. PLA = 1.66  $\pm$  0.47 l•min<sup>-1</sup>, P = 0.83) or CO<sub>2</sub> production (GTE = 1.46  $\pm$  0.47 vs. PLA = 1.46  $\pm$  0.42 l•min<sup>-1</sup>, P = 0.86) during steady-state exercise. Fat oxidation rates (0.33  $\pm$  0.05 vs. 0.33  $\pm$  0.08 g•min<sup>-1</sup>, P = 0.83), and RER were also similar between GTE vs. PLA (0.88  $\pm$  0.03 vs. 0.88  $\pm$  0.03, P = 0.67). However, mean exercise heart rate was lower (Fig. 2) during GTE vs. PLA (115  $\pm$  16 vs. 118  $\pm$  17 beats•min<sup>-1</sup>; main effect, P = 0.049). The within subject coefficient of variation, determined using the method error (Sale, 1991) and based on data collected during the familiarization and placebo trials, was 5% for VO<sub>2</sub>, 5% for VCO<sub>2</sub>, 2% for RER and 2.5% for HR.

<u>Hematologic Measures:</u> Blood [glycerol] was higher in GTE vs. PLA during steady-state exercise (Fig. 3-A) and following the time trial ( $0.22 \pm 0.17$  vs.  $0.17 \pm 0.10 \text{ mmol} \cdot \text{L}^{-1}$ , P = 0.02), but not different at rest ( $0.08 \pm 0.15$  vs  $0.05 \pm 0.05 \text{ mmol} \cdot \text{L}^{-1}$ , P = 0.056) GTE did not affect FFA (Fig. 3-B) glucose (GTE = 5.71 ± 0.52 vs. PLA 6.05 ± 0.66 mmol \cdot \text{L}^{-1}, P = 0.09), insulin (GTE = 4.45 ± 2.17 vs. PLA = 4.64 ± 2.44  $\mu$ IU · L<sup>-1</sup>, P = 0.66) or lactate (GTE = 1.96 ± 0.27 vs. PLA = 1.96 ± 0.23 mmol · L<sup>-1</sup>, P = 0.88) during steady-state exercise. Lastly, NE at rest was not different between treatments (GTE = 0.79 ± 0.34 vs. PLA = 1.00 ± 0.49 nmol · L<sup>-1</sup>, P = 0.14). EPI at rest did not rise above the reference value of 0.8 nmol · L<sup>-1</sup> and reported as no change.

<u>*Time Trial Performance*</u>: There was no difference in time required to complete the 250 kJ time trial (GTE =  $25:38 \pm 5:32$  vs. PLA =  $26:08 \pm 8:13$  min:sec; P = 0.75), nor mean HR during this test (GTE =  $160 \pm 13$  vs. PLA  $160 \pm 14$ , beats•min<sup>-1</sup>, P = 0.65).

## Discussion

The present study examined the metabolic and physiological effects of short-term GTE supplementation at rest and during exercise in healthy active men. This is the first study to investigate the effects GTE during exercise in the fed state, in addition to employing a  $Fat_{max}$  protocol to individually determine steady-state exercise intensity. The main novel finding was that, while GTE did not alter oxygen uptake or substrate utilization, heart rate was slightly but significantly reduced during steady-state exercise. Based on first principles, the reduced HR response despite similar VO<sub>2</sub> is suggestive of either a slightly increased stroke volume or enhanced rate of skeletal muscle oxygen extraction

during exercise after GTE. While we are the first to report such an effect, Richards et al., (2010) previously reported that a dose of 405 mg·d<sup>-1</sup> of EGCG for 2 d increased VO<sub>2max</sub> by ~4.4% in healthy adult men and women. These researchers did not detect changes in HR or stroke volume (SV) and thus the potential mechanism to explain the increased VO<sub>2max</sub> was unclear. Although the previous study would suggest a potential performance improvement, in the present study we hypothesized that improved time trial performance would occur due to increased availability of carbohydrate following 60 min of steady-state exercise. Since we did not observe improved fat utilization during the steady-state exercise, substrate availability would likely not have been influenced during the time trial, thus explaining the lack of performance improvement we have reported.

The most often-cited mechanism by which GTC exert metabolic and physiological effects following acute supplementation is through inhibition of catechol-O-methyl transferase (COMT), an enzyme responsible for the degradation of catecholamines, such as NE (Dulloo, et al., 1999; Lu, Meng, & Yang, 2003). Inhibition of COMT could result in greater plasma concentrations of NE and thus potentially increase sympathetic stimulation through adrenergic receptors. The increased NE would increase adrenergic drive, and potentially the subsequent effects on HR and glycerol observed in the present study. Although our findings are supported by this potential mechanism, Richards et al., (2009) did not observe a concomitant effect on HR or SV with increased VO<sub>2max</sub>, and suggested the increase might have resulted from enhanced arterial-venous O<sub>2</sub> difference. Interestingly, GTC supplementation in mice was recently shown to promote increases in muscle capillarity after exercise training (Nogueira, et al., 2011). However, these findings were observed after 15 days of supplementation, long enough to allow for potential adaptations, unlike the acute nature of this study and the one by Richards et al. (2009). Indeed there is evidence, which suggests GTC might have the potential to enhance O<sub>2</sub> delivery to exercising muscles. For example, GTC have been shown to increase activation of endothelia nitric oxide synthase following both acute in-vitro treatment (Ramirez-Sanchez, Maya, Ceballos, & Villarreal) and long-term administration in mice (Ihm, et al.). However, supporting evidence has yet to be examined in exercising humans. Therefore, the mechanisms to explain the findings observed following acute supplementation with GTE are unclear.

Although the increased adrenergic stimulation, which might result from the potential inhibition of COMT following GTC supplementation is commonly cited as a potential mechanism, only a few studies have attempted to measure catecholamine concentrations. Dulloo et al., (1999) observed increased EE with concomitant elevation in 24 h urinary NE excretion following a 24 h GTE+caffeine intervention. Conversely, a study by (Berube-Parent, Pelletier, Dore, & Tremblay, 2005) also observed increased EE following administration with EGCG, yet reported no effect on 24 h urinary NE. Consistent with another recent report (Hodgson, Randell, Boon, Garczarek, Mela, & Jeukendrup, 2012), in the present

study we did not observe an effect of GTE on resting catecholamine concentrations.

Previous studies suggest a sedentary lifestyle reduces sensitivity of  $\beta$ adrenergic receptors (Bell, et al., 2001; Stob, et al., 2007). If GTC potentially improve this sensitivity, it might explain why resting studies, using sedentary participants, observe more consistent improvements in EE then exercise studies, which generally use trained or recreationally active subjects. These differences are summarized in Table 2, which depicts several studies that have examined the effects of various GTC at rest and during exercise in humans. In the present study we found evidence of increased lipolysis, similar to those reported previously (Venables et al., 2008), which was believed to contribute to the increased fat utilization observed during steady-state exercise in healthy men in that study. However, we, and others (Hodgson, et al., 2012; (Randell, et al., 2013; Dean et al., 2009) did not observe any change in substrate use during steady-state exercise in active or trained subjects. It is possible that other disparities, such as exercise intensity, might have contributed to the divergent effects on fat utilization during exercise. For example, the steady-state exercise intensity was much lower in the present study (~45% VO<sub>2peak</sub>) compared to the study conducted by Venables et al., (2008) (~60% VO<sub>2max</sub>). However, as Randell et al., (2012), and Dean et al., (2009) had their subjects exercise at similar intensities (~54% and 60% VO<sub>2max</sub>, respectively) this is unlikely. Moreover, considering the current study largely replicated aspects of the design by Venables et al., (2008) we do not believe the conflicting results would be related to the GTE treatment regimen.

There are limitations to the present study that should be addressed. First, as previously noted, the steady-state exercise intensity was lower than utilized in previous studies. Considering the work by Venables et al., (2008), if fat utilization was improved through an increase in lipolysis and lipolysis is not limited at low exercise intensities, than an increase in fat oxidation might not have occurred due to the lower exercise intensity utilized in the present study. Additionally, although having exercise performed in the fed state was a novel aspect of the present study, it has been shown that a pre-exercise meal reduces fat utilization and increases carbohydrate oxidation (Coyle, Coggan, Hemmert, Lowe, & Walters, 1985). Thus, the combined effects of feeding and relatively low intensity exercise might have blunted the potential metabolic effects of the GTE, as well the high carbohydrate feeding might have further blunted the potential effects on substrate use during the time trial. Second, since we examined the effects of GTE both at rest and during exercise in the fed state, this limited our ability to assess either condition when CAT have been shown to peak (~2 h). Thus, the timing from ingestion of the last capsule to collection of data might have influenced our ability to detect potential metabolic changes. Lastly, while we took steps to promote subject compliance with the supplementation protocol, we did not measure plasma catechins, which would have helped to verify the participants indeed ingested the GTE as prescribed.

In summary, the present study found that 2 d of GTE supplementation did not alter REE or substrate utilization during steady-state exercise in healthy active men. However, we did observe a lower HR during steady-state exercise and evidence of increased lipolysis. These findings, in conjunction with previous data showing that short-term EGCG supplementation increased VO<sub>2max</sub> in healthy adults (Richards et al., 2010) suggest potential physiological effects of GTC that warrant further investigation.

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ITEM	Concentration %	mg/day
Polyphenols	95 %	1,000 mg
Catechins	90 %	900 mg
EGCG	50 %	450 mg
EGC	20 %	180 mg
EC	10 %	90 mg
ECG	7 %	63 mg
Caffeine	< 1%	< 9 mg

## Table 1. GTE catechin composition.

**Table 2. GTC in humans during sedentary and exercising conditions.** EE, energy expenditure; RER, respiratory exchange ratio; ISI, insulin sensitivity index; TFA, total fat area; FOX, fat oxidation.

Researchers	Population	GTC (dose)	Frequ ency	Durati on	Result	Change
Sedentary						
Dulloo et al	Healthy	GTE (125 mg)	3x/d	24 h	↑EE/↓RER	10%↑/24 h
Boschmann et al	Overweight	EGCg (135 mg)	3x/d	48 h	↓RER	8%
Venables et al	Healthy	GTE (300 mg)	3x/d	24 h	∱ISI	13%
Lonac et al	Healthy	EGCg 135 mg)	3x/d	48 h	_	No change
Exercise						
Richards et al	Healthy	EGCg (135 mg)	3x/d	48 h	↑VO <sub>2max</sub>	4.4%
Venables et al	Healthy	GTE (300 mg)	3x/d	24 h	↑FOX, ↑Glycerol	17%, P<0.05
Eichenberger et al	Endurance	GTE (160 mg)	1x/d	3 wk	_	No change
Dean et al	Endurance	EGCg (270 mg)	1x/d	6 d		No change
Hodgson et al	Active	GTE (600 mg)	2x/d	7 d	*↑FOX – Rest ↓FOX - Exercise	_
Randell et al	Active	GTE (600 mg)	2x/d	7 d	↑Glycerol	P<0.05













GTE = open circles O, PLA = closed squares  $\blacksquare$ . A. Free Glycerol (mmol/L) B. FFA (mmol/L). \*denotes significance (P  $\le$  0.05). All values are means ± SEM. -5, Pre-exercise; 15, 30, 45, 60 min during steady-state exercise.

## **CHAPTER 3**

# Short-term green tea extract supplementation attenuates the postprandial blood glucose and insulin response following exercise in overweight men. Published in *Appl Physiol Nutr Metab*, DOI 10.1139/apnm-2016-0169, 2016.

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Short-term green tea extract supplementation attenuates the postprandial blood glucose and insulin response following exercise in overweight men.

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#### **ABSTRACT**

Green tea extract (GTE) ingestion improves glucose homeostasis in healthy and diabetic humans, but the interactive effect of GTE and exercise is unknown. The present study examined the effect of short-term GTE supplementation on the glycemic response to an oral glucose load at rest and following an acute bout of exercise, as well as substrate oxidation during exercise. Eleven sedentary, overweight men with fasting plasma glucose (FPG)  $\geq$ 5.6 mmol/L (34±13 y; BMI=32±5 kg•m<sup>-2</sup>; FPG=6.8±1.0; mean±SD) ingested GTE  $(3x \cdot d^{-1}, 1050 \text{ mg} \cdot d^{-1} \text{ total})$  or placebo (PLA) for 7-d in a double-blind, crossover design. The effects of a 75g glucose drink were assessed on 4 occasions during both GTE and PLA treatments: On days 1 and 5 at rest, and again following an acute bout of exercise on days 3 and 8. The glycemic response was assessed via an indwelling continuous glucose monitor (CGM) and venous blood draws. At rest, 1-h CGM glucose AUC was not different (P>0.05), but the post-exercise response was lower after GTE vs. PLA (330 $\pm$ 53 and 393 $\pm$ 65 mmol $\cdot$ L<sup>-1</sup> $\cdot$ min<sup>-1</sup>, main effect of treatment, P<0.05). The 1-h postprandial peaks in venous blood glucose ( $8.6\pm1.6$  and  $9.8\pm2.2$  mmol·L<sup>-1</sup>) and insulin  $(96\pm59 \text{ and } 124\pm68 \text{ } \mu\text{IU}\text{\cdot}\text{ml}^{-1})$  were also lower post-exercise with GTE vs. PLA (time x treatment interactions, P<0.05). In conclusion, short-term GTE supplementation did not affect postprandial glucose at rest; however, GTE was associated with an attenuated glycemic response following a post-exercise oral glucose load. These data suggest that GTE might alter skeletal muscle glucose uptake in humans.

#### Key words

green tea extract; catechins; glucose metabolism; insulin; exercise metabolism

#### **INTRODUCTION**

Green tea, the unfermented leaves from the *Camellia sinensis* plant, is a rich source of catechins, a class of plant polyphenols that has been linked to human health and lower disease risk (1). Asian populations have used green tea medicinally for centuries, which has prompted extensive research into the health effects and its purported benefits, including anti-diabetic and anti-obesity properties (2, 3). Green tea extract (GTE) is a concentrated form of catechins and is typically high in epigallocatechin gallate (EGCG) (2), which accounts for ~50-80% of the total catechin content in green tea and is believed to be the most biologically active molecule in GTE (3). Other major catechins include epigallocatechin, epicatechin gallate and epicatechin.

Green tea catechin (GTC) supplementation in humans has been reported to improve indices of metabolic syndrome, including insulin sensitivity, and to reduce adiposity and cholesterol (4-7). Specifically with respect to glycemic control, two recent meta-analyses concluded that GTC ingestion lowered fasting plasma glucose (8, 9). Acute GTC administration has also been shown to improve insulin sensitivity and to reduce insulin area under the curve (AUC) during an oral glucose tolerance test (OGTT) (10). Several mechanisms have been proposed to account for the observed effect of GTC on glycemic control, based largely on cell culture models and rodent studies (11). Ueda et al. (12) demonstrated that physiological doses of EGCG in rats (7 d of supplementation) and in L6 myotubes (15 min of treatment) increased skeletal muscle GLUT-4 translocation in bothnormal and insulin resistant skeletal muscle. Another study reported alterations in several glucose transporters, including GLUT-2 and GLUT-4, in various tissues following both acute and chronic administration of a green tea decoction or a combination of EGCG and other catechins (13). Lastly, three weeks of green tea consumption was shown to reduce adipose GLUT-4 translocation and concomitantly increase skeletal muscle GLUT-4 translocation (14).

Considering the large increase in energy expenditure and the changes in glucose flux associated with exercise, the effect of GTC on glucose metabolism during exercise could be greater than that seen at rest, but this possibility has not been studied. The primary purpose of the present study was to examine the effect of short-term GTE supplementation on indices of glycemic control at rest and following exercise in response to an oral glucose load. We hypothesized that, compared to placebo (PLA), GTE would reduce the glucose and insulin response to a 75 g glucose beverage, at rest and following exercise. Recently, Jagannathan et al. examined various diagnostic methods used to assess the level of dysglycemia in humans, concluding that the 1-h post-load plasma glucose (PG) correlated well with the values obtained from a standard 2-h OGTT (15). Thus, our aim was to use the 1-h peak glucose and insulin as a proof of principle and temporal assessment regarding length of supplementation to provide evidence for further research. A secondary purpose, given the equivocal data on the effects of GTC on fat oxidation during exercise (10, 16-20), was to examine the potential for short-term GTE supplementation to alter whole-body energy expenditure and substrate utilization during an acute bout of exercise. We hypothesized that GTE would increase fat oxidation during an incremental exercise test designed to examine maximal rates of fat oxidation.

# METHODS

#### Subjects

Eleven healthy but sedentary (< 1 h of physical activity/week) overweight men were recruited. Participant baseline characteristics are presented in Table 1. Participants completed a general history questionnaire to identify the presence of chronic diseases or medical conditions, medication use, as well as dietary habits, which would preclude them from participation. Participants did not habitually consume green tea beverages, supplements or medications that might influence the effects of GTE, and agreed not to ingest any such products over the course of the study. The Hamilton Integrated Research Ethics Board approved the experimental protocol, which was explained to each participant prior to obtaining written, informed consent. All procedures were performed at McMaster University.

#### **Experimental Protocol**

<u>Overview of Study Design:</u> A double blind, crossover design was used to compare the effects of GTE compared to a PLA. Following an initial familiarization visit, subjects performed two experimental trials in random order. Each trial involved a 7-day supplementation protocol, with measurements performed both under free-living conditions at rest, as well as during and following an acute bout of exercise. Figure 1 displays an overview of the study design. All trials were conducted in the morning, following an overnight fast. The first trial was initiated at least three days following the familiarization. This was followed by a 2-week washout period prior to the start of the second trial.

<u>*Familiarization:*</u> During the initial visit to the laboratory, anthropometric data were collected and body composition was determined via air-displacement plethysmography BOD POD® (COSMED Inc., Concord, CA). Subjects then completed a graded exercise test (GXT) on a cycle ergometer (Lode Excalibur Sport V 2.0, Groningen, The Netherlands) in order to become familiar with the exercise test that was employed during the main experimental trials. The protocol involved a 5-min warm-up at 25 watts (W), after which the workload was increased by 25 W every 3 min until volitional fatigue to determine the maximal rate of fat oxidation (1). Heart rate (HR) (Polar Electro Oy, Kempele, Findland) and respiratory gases (Moxus modular oxygen uptake system, AEI Technologies, Pittsburgh, PA) were assessed throughout the test. Oxygen consumption (VO<sub>2</sub>) and carbon dioxide production (VCO<sub>2</sub>) were used in stoichiometric equations for calculation of substrate oxidation: fat oxidation = 1.695 •  $VO_2 - 1.701 \cdot VCO_2$ , and carbohydrate oxidation = 4.210 •  $VCO_2 - 2.962 \cdot VO_2$  (21)

<u>Continuous Glucose Monitoring</u>: Following familiarization and prior to each trial, subjects were fitted with a continuous glucose monitor (CGM) device (iPro<sup>TM</sup>, Medtronic, Northridge, CA, USA), which provides interstitial glucose readings at 5 min intervals. The CGM was connected to a glucose sensor (Sof Sensor, Medtronic), which was inserted into the interstitial soft tissue on the anterolateral flank. Subjects were also provided with a glucose meter (OneTouch® UltraMini®, Lifescan, Inc., Milpitas, CA, USA) and instructed to record capillary glucose measurements prior to each meal for CGM calibration purposes, per the manufacturer's instructions. CGM data are derived

from n = 10, owing to sensor malfunctions in one participant.

<u>Supplementation</u>: Subjects were provided with capsules prior to each trial that contained either decaffeinated GTE powder (Sunphenon®, 90D, Taiyo International Inc. Minneapolis MN) or PLA (cellulose). Capsules were opaque and of identical color, shape, and size to assure the contents of the GTE and PLA remained indiscernible to subjects and researchers. Given pharmacokinetic evidence that the bioavailability of ingested catechins is greater in the fasted state (22), and considering a half-life of ~4 h (23), subjects were instructed to ingest the capsules with 500 ml of water ~1 h before the glucose beverage (detailed below) or breakfast, lunch and dinner each day. Subjects were asked to bring their capsule bottle to each visit to assure adherence to the supplementation regimen. Each capsule in the GTE condition provided 300 mg of catechins, the equivalent of ~2-4 cups of green tea (24). Table 2 describes the composition of the GTE used in the current study.

Experimental Trials: Subjects were tested twice at rest and twice in response to an acute bout of exercise during each trial, after varying durations of supplement ingestion. For resting measurements, subjects ingested a 75 g oral glucose beverage (Glucodex, Rougier Pharma, Mirabel, OC, Canada) under free-living conditions, in a fasted state on the first and sixth day of each intervention, after having consumed a single capsule or a total of 16 capsules, respectively. CGM data were collected and analyzed over a 1-h period immediately following glucose ingestion. For exercise measurements, subjects arrived at the laboratory in the fasted state on the third and eighth day of each trial, after consuming a total of 7 and 22 capsules, respectively. A catheter was placed into an antecubital vein for blood sampling via a collection port (BD Angiocath® 20 G x 1.25 in and O-SYTE<sup>TM</sup>, Becton, Dickinson and Company, Franklin Lakes, NJ). Blood (10 mL) was collected and placed into appropriate collection tubes (BD Vacutainer®: SST, EDTA, or Fluoride, Becton, Dickinson and Company, Franklin Lakes, NJ) for subsequent analysis. After a resting blood sample was obtained, participants performed a GXT, which was conducted as described for the familiarization visit. A second blood sample was obtained immediately following the GXT, prior to ingestion of a 75 g oral glucose drink. Subjects then rested for 1 h before a final blood sample was obtained. CGM data was again collected and analyzed over a 1-h period immediately following glucose ingestion.

<u>Nutritional Controls</u>: Subjects were provided with all meals for 48 h prior to the measurements made in response to the acute bouts of exercise during each trial (i.e., on the third and eighth days). Energy need was based on BOD POD® estimations ( $\pm$  100 kcal) for sedentary individuals, which uses the Nelson Prediction Equation (25). The meals were individualized for each subject and provided 2,262  $\pm$  216 kcal·day<sup>-1</sup> (protein = 2.5  $\pm$  0.3, carbohydrate = 5.4  $\pm$  0.7, and fat = 1.6  $\pm$  0.1, g•kg<sup>-1</sup>). Subjects were also instructed to refrain from physical activity and alcohol during the periods of controlled nutrition, supplementation, and medication patterns that might influence the effects of the GTE.

<u>Blood Analyses</u>: Blood collected for serum (insulin) was allowed to clot for 30 min after collection then separated by centrifugation (10 min at 1,300 g) and stored at -80

°C for later analysis. Plasma obtained from either EDTA (glycerol and free fatty acids (FFA)) or fluoride (glucose) tubes were immediately centrifuged (10 min at 1,300 g) and stored at -80 °C for later analysis. Plasma glucose was analyzed using a glucose (hexokinase) reagent kit (Pointe Scientific, Canton MI), and serum insulin was measured through an ELISA (ALPCO Diagnostics, Salem NH). Plasma glycerol (Sigma-Aldrich CO. LLC. St. Louis, MO) and plasma FFA (WAKO Diagnostics, Richmond, VA) were determined with enzymatic colorimetric assays.

#### **Statistical Analyses**

A 3-way repeated measures ANOVA (time x day x treatment) was used to analyze venous blood data. All blood analyses were conducted in duplicate with coefficient of variations of: 8.2, 4.5, 7.8, and 1.4 % for glucose, insulin, FFA, and glycerol, respectively. A 2-way repeated measures ANOVA (day x treatment) was used for all CGM data and exercise variables (HR, VO<sub>2</sub>, VCO<sub>2</sub>, fat and carbohydrate oxidation). Significant interactions were analyzed using pairwise comparisons for simple interactions. All assumptions were met and significance was accepted with a P < 0.05 (2-tailed). All data were analyzed using Statistical Package for Social Sciences (SPSS, version 20.0, Inc, Chicago, IL).

#### RESULTS

There were no day x treatment interactions for any variable; thus, all data reported are the means  $\pm$  SD across the two days of each experimental trial for all variables.

#### **Resting Measures**

There was no effect of GTE on glucose AUC in response to the 75 g glucose drink, when ingested under fasted, resting conditions (GTE =  $394 \pm 70$  vs. PLA =  $409 \pm 78$ , mmol/L• 60 min, P = 0.51). Fasting, pre-exercise blood glucose, insulin, glycerol and FFA concentrations were also not different with GTE vs. PLA (P > 0.05, Table 3). The homeostatic model assessment of insulin resistance (HOMA-IR) was also not different between treatments (GTE =  $3.8 \pm 3.4$  vs.  $4.6 \pm 2.8$ , P = 0.21).

#### Exercise

Peak oxygen uptake during the GXT was not different between treatments (GTE =  $24.2 \pm 4.2$  vs. PLA =  $23.7 \pm 5.0$  mL•kg<sup>-1</sup>•min<sup>-1</sup>, P = 0.43). Peak fat oxidation was similar with GTE vs. PLA ( $0.32 \pm 0.10$  vs.  $0.31 \pm 0.11$  g•min<sup>-1</sup>, P = 0.67), with no difference in the intensity that elicited peak fat oxidation (GTE =  $45 \pm 25$  and PLA =  $45 \pm 18$  W, P = 1.0). Similarly, there were no differences in the mean carbohydrate oxidation during the GXT with GTE vs. PLA ( $1.36 \pm 0.21$  vs.  $1.35 \pm 0.29$  g•min<sup>-1</sup> P = 0.83). Supplementary Figure S1, displays the fat and carbohydrate oxidation rates at each workload during the GXT, which is truncated to the highest workload that all participants achieved. Lastly, mean exercise heart rate did not differ between treatments (GTE =  $131 \pm 17$  vs. PLA =  $130 \pm 17$  beats•min<sup>-1</sup>; P = 0.65).

#### **Post-exercise**

Immediately following exercise, blood glucose, insulin, glycerol and FFA

concentrations were not different (P > 0.05, Table 3). In response to the 75 g glucose beverage during exercise recovery, the 1-h glucose AUC from the CGM was lower with GTE vs. PLA ( $330 \pm 53$  vs.  $393 \pm 65$  mmol/L•60 min, main effect of treatment, P < 0.001; Fig. 2). Peak blood glucose and insulin were both lower with GTE vs. PLA (time x treatment interactions, P = 0.04 and 0.01, respectively; Fig. 3). Lastly, 1-h post-exercise glycerol and FFA concentrations were not different between treatments (P > 0.05, Table 3).

#### **DISCUSSION**

The major novel finding from this study was that GTE attenuated the glucose and insulin response to an oral glucose load following acute exercise but had no effect on these variables under resting conditions. GTE or PLA was administered 3 times•day<sup>-1</sup> over a 7-day period with assessments made after 2 and 7 days of supplementation. For each trial, we examined the effects of GTE on the glycemic response to oral glucose ingestion at rest and during exercise recovery. GTE attenuated the postprandial rise in glucose and insulin following exercise, but the effect on day 7 was similar to that on day 2. Thus, the duration of short-term GTE supplementation did not influence the magnitude of the response. Notably, we did not observe any effects of GTE under resting conditions, and the effect of GTE was only apparent when combined with an exercise stimulus.

While we are the first to report an effect of GTE on blood glucose and insulin post-exercise, GTE was previously shown to acutely improve insulin sensitivity and the insulinemic response following an OGTT at rest. Venables et al. (10) provided participants with three doses (dose = 340 mg polyphenols, 136 mg EGCG) of GTE over 24 h, with the last dose at least 1 h prior to the OGTT, which lowered insulin AUC and increased insulin sensitivity relative to PLA. Similarly, a green tea beverage (1.5 g green tea powder), administered 10 min prior to an OGTT lowered glucose levels at 30 and 120 min post glucose ingestion vs. PLA (26). The effects of green tea have also been observed following chronic supplementation. A recent study that investigated the effects of a high polyphenol diet over 8 weeks, supplemented partially by green tea, observed a significant reduction in post-load glucose AUC and improved insulin sensitivity (27). In contrast, 8 weeks of EGCG supplementation (400 mg, 2 times  $day^{-1}$ ), with the last dose taken the evening prior to an OGTT, did not improve insulin sensitivity or lower glucose when compared to a PLA (5). Two notable differences between these studies, aside from the duration of the treatments, is the treatment itself (GTE vs. EGCG) and the timing of the last dose. Firstly, although EGCG is the most bioactive of the four main catechins, it is potentially more effective when ingested as a component with other catechins, such as with GTE, than when ingested alone (28). Secondly, the half-life of tea catechins in the blood is very short (~4 h) (23), and thus the timing of the last ingested dose between these studies is a potential reason for the differences. As we also did not report an effect of GTE at rest, even with a catechin-rich GTE supplement and short duration of time between the final dose and the measurement, the differences among studies could indicate a relatively small effect size of GTE on resting glucose metabolism.

The lower blood glucose and insulin concentrations observed following glucose ingestion during recovery from exercise in the present study is suggestive of either decreased glucose absorption, enhanced glucose uptake from the blood, or both. Randomized controlled trials have shown lower fasting blood glucose and glycated hemoglobin (HbA1c) from GTC supplementation and green tea consumption over several weeks to months (8, 9). However, a 1-h PG  $\geq$  8.6 mmol/L following ingestion of a 75 glucose beverage was recently identified as a better identifier of high-risk individuals than HbA1c (15), further supporting to the relevant nature of the findings from the present study. Although insight into potential mechanisms for these effects is only available from rodent models, one putative mechanism observed following GTC treatment is an increased translocation of glucose transporters. For example, GLUT-4 translocation increased in rat skeletal muscle following 7 days of supplementation as well as in L6 myotubes following an acute 15-min treatment (24). EGCG has also been shown to reverse fatty acid induced impairments in the insulin-signaling pathway (29), which improved downstream translocation of GLUT-4 in skeletal muscle and adipose tissue. These mechanisms, if also present in humans, would explain the lower insulin AUC observed by Venables et al. (10) as well as the data from the current study. Interestingly, others have shown that EGCG increases activation of 5'-AMP-activated protein kinase (AMPK) (30), which is a target of the common diabetic medication, Metformin. GTC might also improve insulin sensitivity through reductions in ROS-generating inflammatory markers (31). Overall, several mechanisms have been put forward to explain the ability of GTC to influence glycemic control in humans, either at rest or combined with exercise, but more mechanistic studies of humans are needed.

There is evidence of metabolic and physiologic effects of GTC during exercise in humans, which could have contributed to the differences we observed on glucose and insulin during exercise recovery. It has been shown that GTC increased fat oxidation during exercise, improved VO<sub>2</sub>max, lowered steady-state exercise HR, and increased markers of lipolysis (17, 20, 32, 33). The most often-cited mechanism by which GTC might exert these effects during exercise is through inhibition of catechol-O-methyl transferase (COMT), an enzyme responsible for the degradation of catecholamines, such as epinephrine (EPI) and norepinephrine (NE) (34, 35). Indeed, GTC are purported to increase adrenergic drive through increased beta-receptor stimulation. However, we recently reported no effect of GTE on either EPI or NE following 48 h of GTE supplementation, when compared to a PLA. (17). Additionally, Hodgson et al. (36) observed no differences in metabolites indicative of increases in catecholamines following 1 or 7 days of GTE administration. Considering that we concurrently examined the effects of GTE on exercise substrate use and markers of fat catabolism (FFA and glycerol), neither of which were different between treatments, our data do not support an effect of GTE on fuel use in exercising humans. Consequently, it is unlikely that an interaction between GTE supplementation and the exercise stimulus on substrate use contributed to the changes observed in glucose or insulin during the post-exercise period.

There are limitations to the present study that should be considered. First, using CGM under free-living conditions instead of more direct measurements of blood glucose and insulin might have limited our ability to observe an effect of GTE on the response to

the oral glucose beverage at rest. Although CGM data is clinically accurate (37), and it was a novel means of examining the effects of GTE under free-living conditions, it does not allow us to make conclusions on the effects of GTE on insulin at rest, and it is not completely equivalent to performing an OGTT with venous blood draws. Secondly, while the post-exercise data is strengthened by similar glucose responses measured with CGM and in blood, these data only allow us to conclude that glucose and insulin concentrations were lower with GTE; however, these data do not provide mechanistic insight to explain these results. Specifically, we do not have data to demonstrate whether GTE alters glucose absorption or uptake. Lastly, while these data are applicable to sedentary, overweight men, there is limited data on the effects of GTE in a female population. Thus, possible sex-based differences might exist with regard to exercise substrate oxidation and glycemic control in a female population.

The data presented in this study suggest that GTE could have positive health benefits. Given the common practice of eating in the post-exercise period, consuming GTE regularly might enhance glucose uptake and/or reduce spikes in glucose and insulin concentrations, which would have implications for both active populations, as well as those with impaired glycemic control. For example, this effect would be especially advantageous in overweight populations beginning an exercise routine, where the normal postprandial response increases insulin and reduces lipolysis and potentially post-exercise fat oxidation (38). Interestingly, one study previously showed that GTE reduced respiratory exchange ratio at rest, but only in the postprandial period (39). As we are the first to test the effects of GTE on postprandial glucose responses following exercise, more research is needed to confirm our results and to identify the physiological mechanism(s) involved.

In summary, the present study found that 2 or 7 d of GTE supplementation attenuated the glucose and insulin responses to an oral glucose load following acute exercise. We did not observe any effects of GTE on glucose metabolism under resting conditions or whole-body substrate utilization during exercise. Our findings, along with previous evidence showing that GTE improves insulin sensitivity in humans (10) and alters glucose transporters in rodents (12), suggest potential metabolic effects of GTE that warrant further investigation.

#### **Conflict of Interest**

The authors declare no conflicts of interest

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| Variable   | Mean ± SD      |
|--|----------------|
| Age (y)  | 34 ± 13        |
| Weight (kg)  | $98 \pm 18$    |
| BMI ( $kg \cdot m^2$ )                                       | $32 \pm 5$     |
| Body fat (%)   | $27 \pm 7$     |
| Vo <sub>2</sub> peak (ml•kg <sup>1</sup> •min <sup>1</sup> ) | $24.2 \pm 4.2$ |
| PPO (watts)  | $157 \pm 34$   |
| FPG (mmol• $L^{-1}$ )  | $6.8 \pm 1.0$  |

Table 1. Baseline participant characteristics

BMI: body mass index, PPO: peak power output, FPG: fasting plasma glucose

Ingredient	<b>Concentration (%)</b>	Supplementation dose (mg/day)
Polyphenols	95	1,000
Catechins	90	900
EGCG	50	450
Epigallocatechin	20	180
Epicatechin	10	90
Epicatechin gallate	7	63
Other Catechins	13	117
Caffeine	< 1	< 9

Table 2. The composition of the green tea extract used in the present study.

Variable Pre-exercise		Post-exercise		1-hour post-exercise		
variable	GTE	PLA	GTE	PLA	GTE	PLA
Glucose	$6.6 \pm 0.9$	6.7 ± 0.9	$6.7 \pm 0.7$	$6.8 \pm 1.0$	8.6 ± 1.6 *	$9.8 \pm 2.2$
(mmol/L)						
Insulin (µIU/ml)	$13 \pm 8$	$15 \pm 8$	$10 \pm 6$	$12 \pm 6$	96 ± 59 *	$124 \pm 68$
Free fatty acids	$0.48\pm0.14$	$0.51\pm0.11$	$0.44\pm0.10$	$0.43\pm0.09$	$0.27\pm0.09$	$0.29\pm0.14$
(mmol/L)						
Glycerol	$0.14\pm0.06$	$\textbf{0.16} \pm \textbf{0.10}$	$0.17\pm0.06$	$0.18\pm0.07$	$0.09\pm0.05$	$0.13\pm0.14$
(mmol/L)						

Table 3. Effects of green tea extract (GTE) on blood measurements, pre-exercise	e,
post-exercise, and 1 h post-exercise in overweight, sedentary men.	

GTE (green tea extract), PLA (placebo). All values are means  $\pm$  SD. \*denotes significance, GTE vs. PLA (P  $\leq$  0.05).

# **Figure captions**

**Figure 1. An overview of the experimental trials.** Panel A depicts the overall design of the study. Subjects consumed GTE or PLA for 7 d periods, which were randomized in order and separated by a 2-week washout. The supplementation regimen and the timing of rest and exercise measurements are shown. "Rest" and "Exercise" indicate the timing of the resting and exercise measurements, which are detailed further in panels B and C.

Figure 2. Effects of an oral glucose beverage on 1-h post-exercise continuous glucose monitor-based glucose area under the curve (AUC) following 2 and 7 days of green tea extract (GTE) or placebo (PLA) supplementation. All values are means  $\pm$  SD. \*denotes the 1-h glucose AUC was significance lower with GTE vs. PLA (P ≤ 0.05).

Figure 3. Effects of an oral glucose beverage on 1-h peak glucose and insulin postexercise, following 2 and 7 days of green tea extract (GTE) or placebo (PLA) supplementation. Panel A depicts the blood glucose concentration ( $\text{mmol} \cdot \text{L}^{-1}$ ) pre-, post-, and 1-hr post-exercise. Panel B depicts the blood insulin concentration ( $\mu$ IU·ml<sup>-1</sup>) pre-, post-, and 1-hr post-exercise. All values are means  $\pm$  SD. \*denotes postprandial rises in glucose (panel A) and insulin (panel B) were significantly lower with GTE vs. PLA (P  $\leq$  0.05).



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# **CHAPTER 4:**

Green tea extract does not affect exogenous glucose appearance but reduces insulinemia with glucose ingestion in exercise recovery Submitted to Am J Physiol Endocrinol Metab. July, 2016. Green tea extract does not affect exogenous glucose appearance but reduces insulinemia with glucose ingestion in exercise recovery

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Running head: Green tea extract and glycemic control

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### ABSTRACT

We reported that supplementation with green tea extract (GTE) lowered the glycemic response to an oral glucose load following exercise but via an unknown mechanism. Here we examined the effect of supplementation with GTE on plasma glucose kinetics upon ingestion of a glucose beverage during exercise recovery. Eleven healthy, sedentary men  $(21\pm2 \text{ y}; \text{BMI}=23\pm4 \text{ kg}\cdot\text{m}^{-2}, \text{VO}_2\text{peak}=38\pm7 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}; \text{mean}\pm\text{SD})$  ingested GTE (350 mg) or placebo (PLA) thrice daily for 7-d in a double-blind, crossover design. In the fasted state, a primed constant infusion of  $[U^{-13}C_6]$  glucose was started, and 1-h later, subjects performed a graded-exercise test (25 Watts/3 min) on a cycle ergometer. Immediately post-exercise, subjects ingested a 75g glucose beverage containing 2g of [6,6-<sup>2</sup>H<sub>2</sub>] glucose, and blood samples were collected every 10-min for 3-h of recovery. The rate of carbohydrate oxidation was lower during exercise after GTE vs. PLA (1.26±0.34 vs. 1.48±0.51 g•min<sup>-1</sup>, P=0.04). Glucose area under the curve (AUC) was not different between treatments after drink ingestion (GTE=1067±133 vs. PLA=1052±91 mM•180 min, P=0.91). Insulin AUC was lower after GTE vs. PLA (5673±2153 vs. 7039±2588 µIU•180 min, P=0.05), despite similar rates of glucose appearance  $(GTE=0.42 \pm 0.16 \text{ vs. PLA}=0.43 \pm 0.13, \text{ g} \cdot \text{min}^{-1}, \text{ P}=0.74)$  and disappearance  $(GTE=0.43 \pm 0.13, \text{ g} \cdot \text{min}^{-1}, \text{ P}=0.74)$  $\pm$  0.14 vs. PLA=0.44  $\pm$  0.14, g•min<sup>-1</sup>, P=0.57). We conclude that short-term GTE supplementation did not affect glucose kinetics following ingestion of an oral glucose load post-exercise; however, GTE was associated with attenuated insulinemia. These findings suggest GTE lowers the insulin required for a given glucose load during postexercise recovery, which warrants further mechanistic studies in humans.

Keywords: green tea extract, glycemic control, insulin sensitivity, catechins, polyphenols

### **NEW & NOTEWORTHY**

Using a dual glucose tracer approach, we examined the effect of green tea extract (GTE) supplementation on blood glucose kinetics in response to the post exercise ingestion of a glucose beverage. Our study provides novel data showing that short-term GTE supplementation attenuates the insulin response to a 75 g oral glucose load given in the post-exercise period. To our knowledge this is the first study to provide potential mechanisms of GTE in humans.

#### **INTRODUCTION**

Green tea is produced from unfermented leaves of the *Camellia sinensis* plant. The lack of fermentation results in a higher concentration of polyphenolic compounds than found in other teas (22). Green tea extract (GTE) has a high concentration of polyphenols, comprised mostly of four green tea catechins (GTC): epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC), and epigallocatechin gallate (EGCG) (8). These polyphenolic compounds are thought to have a number of actions including binding to specific proteins, enzymes, and receptors leading to altered biological activities. EGCG is reported to be the most biologically active GTC and most abundant accounting for ~50-80% of all GTC by content (23). Avenues of research into the effects of GTC have been conducted in rested and exercising humans, including their potential anti-obesity and anti-diabetic properties (8, 23).

Studies have shown improvements in indices related to metabolic syndrome, such as weight loss and improved glycemic control following supplementation with GTE (5-7, 9, 12, 42). For example, Venables et al. reported improved insulin sensitivity and reduced insulin area under the curve (AUC) during an oral glucose tolerance test (OGTT) following acute GTE administration (45). In contrast, Brown et al. concluded that 8 weeks of supplementation with EGCG did not affect insulin sensitivity (7), suggesting that EGCG might not be as effective alone versus when ingested with other GTC. Following supplementation with GTE, we recently observed an attenuated increase in plasma glucose and insulinemia following an oral glucose load during post-exercise recovery (31); however, these effects were not apparent under resting conditions.

While several other studies have examined the metabolic and physiological effects of supplementation with GTC, during exercise, the findings are equivocal. For example, during acute exercise, an early study reported that short-term GTE supplementation increased fat oxidation during steady-state exercise (43), and another study reported increased maximal oxygen uptake (VO<sub>2max</sub>) from short-term EGCG supplementation (39, 45). In contrast, several other studies have failed to observe similar findings following GTC supplementation (11, 31, 32, 37, 38). Methodological differences, such as the dose of GTE, length of supplementation, and/or intensity of exercise between many of these studies might partially explain some of the varied findings.

Data derived from rodent and cell culture studies provides mechanistic support for some of the observations made in humans regarding the effects of GTC on glycemic control. Administration of a green tea decoction or a combination of EGCG and other catechins altered the expression of several glucose transporters in both rat jejunum (SGLT-1, GLUT-2) and adipose tissue (GLUT-4), which was associated with improvements in glucose regulation (40). Similarly, rats supplemented with green tea showed increased skeletal muscle GLUT-4 translocation, while reducing adipose GLUT-4 translocation under resting conditions (3). Supplementation with EGCG in rats, as well as treatments of L6 myotubes with EGCG, increases skeletal muscle GLUT-4 translocation, in both normal and insulin resistant skeletal muscle (44). Taken together, these data suggest that supplementation with GTC may modulate postprandial glucose regulation in skeletal muscle; however, to our knowledge, no studies have clarified whether these potential mechanisms explain the effects of GTE on glycemic control in humans.

We aimed to examine the effects of GTE on glycemic control following exercise in response to an oral glucose load and to elucidate the mechanisms of this effect using tracer methodology in humans. Specifically, we sought to determine whether the attenuated glycemic response previously observed following an oral glucose load during post-exercise recovery after GTE supplementation in humans (31) was related to reduced glucose uptake by the gut, reduced hepatic glucose output and/or greater glucose uptake post-absorption. Considering both insulin-independent and dependent glucose uptake during exercise recovery and the potential effect of GTE on GLUT transporters the glycemic response might be more apparent under these conditions than at rest. We also sought to examine the potential effects of GTE on substrate use during exercise, as both a means to assess equivocal data on this topic but also to verify that observations in the post-exercise period were not a function of potentially greater carbohydrate oxidation during exercise. We hypothesized that supplementation with GTE would reduce the glucose and insulin responses following an oral glucose load administered in the postexercise period, corresponding to greater rates of glucose disposal with GTE. These findings will provide novel data with regard to the effects of GTE on glycemic control and glucose kinetics during exercise recovery in humans.

#### **METHODS**

Participants. Eleven sedentary but otherwise healthy men were recruited (21±2 y; BMI=23±4 kg•m<sup>-2</sup>, VO<sub>2</sub>peak=38±7 ml•kg<sup>-1</sup>•min<sup>-1</sup>, mean±SD). Based on differences in plasma glucose and insulin in a previous study from our lab (31), with 80% power and an alpha level of 0.05, sample size calculations estimated 10 participants were required to detect statistical significance. We attempted to recruit and test 12 to account for potential dropouts with one participant unable to complete both trials. Participants completed a general questionnaire to assess activity levels and identify nutrition, supplementation, and medication patterns that might influence the effects of the GTE. Identification of any nutritional practices, which might alter the measured variables, precluded participation. Participants were deemed sedentary and healthy if they did not perform any structured exercise sessions per week and they were free of chronic disease and medications, respectively. Participants did not routinely consume green tea beverages or dietary supplements that might influence the effects of GTE. The Hamilton Integrated Research Ethics Board approved the experimental protocol, which was explained to each participant prior to obtaining written, informed consent. All procedures were performed at McMaster University.

*Experimental Protocol.* A randomized, double-blind, crossover design was employed to assess the effects of GTE compared to a placebo (PLA). Sample and data collection consisted of baseline testing and familiarization followed by two experimental trials. At least 3 d following baseline testing and familiarization, subjects initiated the GTE or PLA ingestion protocol, and the experimental trials were conducted following 7-d of supplementation. The 7-d supplementation period was based on a previous study from our lab that also examined the effects of GTE on glycemic control (31). Experimental trials were separated by a 2-week washout and were conducted in the morning at approximately

the same time. A schematic illustration of the experimental design can be seen in Figure 1.

Baseline testing and familiarization. Participants initially reported to the laboratory in the fasted state for the determination of mass and body composition, via air-displacement plethysmography (BOD POD®, COSMED Inc., Concord, CA). Participants then completed a modified, graded-exercise test on a cycle ergometer (Lode Excalibur Sport V 2.0, Groningen, The Netherlands). The familiarization session was performed until volitional exhaustion; however, to maintain consistency during experimental trials, participants only exercised until the highest stage completed in full during the familiarization session. As an alternative to using a set percentage of maximal workload, this modified protocol was used to assess the effects of GTE on fat oxidation at varying intensities. A previously established protocol used to assess maximal fat oxidation (1) was modified to suit a sedentary population. Participants performed a 5-min warm-up, cycling at 25 watts (W), after which intensity increased by 25 W every 3 min until volitional fatigue. Heart rate (HR) (Polar Electro Oy, Kempele, Findland) and respiratory gases (Moxus modular oxygen uptake system, AEI Technologies, Pittsburgh, PA) were assessed throughout the test with data from the final minute of each stage used for analysis. Oxygen consumption (VO<sub>2</sub>) and carbon dioxide production (VCO<sub>2</sub>) were used in stoichiometric equations to calculate fat and carbohydrate oxidation rates during exercise, as previously described (14).

Fat oxidation =  $(1.695 \cdot VO_2) - (1.701 \cdot VCO_2)$ 

Carbohydrate oxidation =  $(4.210 \cdot VCO_2) - (2.962 \cdot VO_2)$ 

*Dietary and Exercise Controls.* Participants were asked to maintain their current activity and dietary routines throughout the study period and to not consume tea beverages or products containing tea catechins. To minimize the influence of dietary intake on experimental variables, participants were provided with 1 d of meals, snacks and beverages prior to each experimental trial. Daily nutritional intake consisted of various frozen entrees, granola bars, fruit cups, chips, and bottled juices. Energy needs were based on BOD POD® estimations ( $\pm$  100 kcal), which uses the Nelson Prediction Equation (34). The meals were individualized for each subject and provided 1,863  $\pm$  436 kcal•day<sup>-1</sup> (protein = 1.8  $\pm$  0.4, carbohydrate = 3.6  $\pm$  0.8, and fat = 1.0  $\pm$  0.2, g•kg<sup>-1</sup>). During the first supplementation period, participants were provided with a diet log to record times of food intake, allowing them to replicate their diet during the second treatment period. Participants were asked to refrain from physical activity and the consumption of alcohol during each period of the controlled diet.

Supplementation. Participants were provided with 22 capsules of either a high quality decaffeinated GTE powder (Sunphenon®, 90D, Taiyo International Inc. Minneapolis MN) or PLA (microcrystalline cellulose). Subjects received 900 mg of total catechins/day, the equivalent of ~2-4 cups of green tea (300 mg) 3 times•d<sup>-1</sup> (17). The catechin composition of the GTE, provided by the supplier was determined through

HPLC analysis. Table 1 provides respective dosages and concentrations of individual components of the GTE. Capsules were opaque and of identical color, shape, and size to assure the contents of the GTE and PLA remained indiscernible to participants and researchers. Pharmacokinetic evidence suggests greater bioavailability of catechins ingested in the fasted state (10) and a half-life of ~4 h (27). Participants therefore ingested capsules 3 times•d<sup>-1</sup> with 500 mL of water, approximately 1 h before meals (breakfast, lunch, and dinner) for 7-d. The final capsule was ingested on the morning of the experimental trials, immediately prior to the start of the glucose tracer infusion.

*Experimental Trials.* Participants reported to the laboratory at ~0700 following an overnight fast. They rested in a semi-supine position during which an intravenous catheter was placed into an antecubital vein of both arms for repeated blood sampling and tracer infusion. A resting blood sample was obtained followed by the administration of a primed (13.5  $\mu$ mol•kg<sup>-1</sup>), constant (0.35 $\mu$ mol•kg<sup>-1</sup>•min<sup>-1</sup>) infusion of [U-<sup>13</sup>C<sub>6</sub>] glucose (Cambridge Isotope Laboratories, Tewksbury, MA). After 60 min of infusion, participants performed the GXT, during which respiratory gases and HR were assessed in the same manner as described above. Immediately following the GXT, a blood sample was obtained and subjects ingested a 73 g oral glucose beverage (80.3 g dextrose monohydrate, CanadianProtein.com) with an additional 2 g of [6,6-<sup>2</sup>H<sub>2</sub>] glucose (Cambridge Isotope Laboratories). Participants then rested in bed while blood was sampled at 10 min intervals over 180 min.

Blood Analysis. Blood samples (~6 mL) were obtained at each time point and placed into EDTA collection tubes (BD Vacutainer®: EDTA, Becton, Dickinson and Company). Samples were immediately centrifuged (10 min at 1500 x g), and plasma aliquots were obtained and stored at -80°C pending further analysis. Plasma glucose was analyzed using an automated glucose analyzer (YSI STAT 2300 plus, Yellow Springs, OH). Plasma insulin concentration was analyzed via a commercially available ELISA kit (ALPCO Diagnostics, Salem NH). Methods for the analysis of isotopic enrichments of  $[U^{-13}C_6]$ glucose and  $[6,6^{-2}H_2]$  glucose were adapted from those previously described (26). Briefly, 10 µL of plasma was deproteinized using 1:1 0.3N barium hydroxide (150 µL) and 0.3N zinc sulphate (150  $\mu$ L). The precipitated proteins were subsequently centrifuged (30 min at 1500 x g), after which 200  $\mu$ L of the supernatant was passed over ion-exchange resins (Dowex® 50WX8-200, and 1X8-200-400, Sigma Aldrich, St. Louis, MO), dried and resuspended in a 1:1 solution of 2% methoxamine in pyridine (50 µL) and N,O-Bis(trimethylsilyl)trifluoroacetamide with trimethylchlorosilane (BSTFA + TMCS, 99:1) (50  $\mu$ L). Plasma enrichments of [6,6-<sup>2</sup>H<sub>2</sub>] and [U-<sup>13</sup>C<sub>6</sub>] were measured by GCMS (Agilent Technologies, GC 7890A and MSD 5975C, Santa Clara, CA) using electron ionization. Chemstation software (Agilent Technologies, CA, USA) was used for data analysis. Ions were selectively monitored at mass to charge ratios (m/z) 319 (M+0), 321 (M+2), and 323 (M+4). The 325 (m+6) ions were not monitored as the predominant ion was 323 amu and contained only 4 of the 6<sup>13</sup>C-labelled atoms. Plasma glucose enrichments for each ion were expressed relative to enrichments at 319 (M+0). The baseline blood sample was used to ascertain background isotopic plasma enrichments. and standard curves were created from known amounts of  $[U^{-13}C_6]$  and  $[6,6^{-2}H_2]$  to determine the relative enrichment of each monitored ion.

*Calculations*. The effects of GTE on glucose kinetics were first assessed from calculating the rate of glucose appearance ( $Ra_{Total}$ ; equation 1) and whole-body glucose disposal (Rd; equation 2) using the single-pool, non-steady-state Steele equation (41) adapted for stable isotope methodology (47). In order to account for any adverse impact of analytical variability on glucose kinetics (47), when appropriate, data were curve fitted as previously described (16, 47):

$$Ra_{Total} = \frac{F - pV[(C_2 + C_1/2)][(E_2 - E_1)/(t_2 - t_1)]}{(E_2 + E_1/2)}$$
(1)  

$$Rd = Ra_{Total} - pV\left(\frac{C_2 - C_1}{t_2 - t_1}\right)$$
(2)

where *F* is the infusion rate ( $\mu$ mol·kg<sup>-1</sup>·min<sup>-1</sup>), *pV* is the volume of distribution for glucose calculated as 18% body weight (kg), *E*<sub>1</sub> and *E*<sub>2</sub> represent [U-<sup>13</sup>C<sub>6</sub>] glucose enrichments at *t*<sub>1</sub> and *t*<sub>2</sub>, respectively, and *C*<sub>1</sub> and *C*<sub>2</sub> represent plasma [glucose] at *t*<sub>1</sub> and *t*<sub>2</sub>, respectively. A rearranged version of the single-pool non-steady-state Steele equation (35) and the [6,6-<sup>2</sup>H<sub>2</sub>] enrichment of the glucose beverage (E<sub>3</sub>) along with the Ra<sub>Total</sub> calculated from *Eq.* 1 were used to calculate the exogenous glucose rate of appearance (Ra<sub>Exo</sub>) of the ingested [6,6-<sup>2</sup>H<sub>2</sub>] glucose; where *E*<sub>1</sub> and *E*<sub>2</sub> are now represented by plasma enrichments of [6,6-<sup>2</sup>H<sub>2</sub>] glucose:

$$Ra_{Exo} = Ra_{Total} \bullet \left[ \frac{(E_2 + E_1)/2 + (C_2 + C_1)/2 \bullet (E_2 - E_1)}{(t_2 - t_1) \bullet pV} \right] / E_3$$
(3)

Endogenous glucose rate of appearance ( $Ra_{Endo}$ ) was calculated as the difference between  $Ra_{Total}$  and  $Ra_{Exo}$ , as shown in *Eq.* 4:

$$Ra_{Endo} = Ra_{Total} - Ra_{Exo} \tag{4}$$

The effects of GTE on insulin sensitivity were measured using the Matsuda insulin sensitivity index (ISI) (33) and the homeostatic model assessment (HOMA-IR) (33). Where FPG is fasting plasma glucose, FPI is the fasting plasma insulin, and  $\overline{I}$  and  $\overline{G}$  are the mean insulin and glucose from the OGTT, respectively.

$$Matsuda - ISI = \frac{1000}{\sqrt{(FPG \bullet FPI) \bullet (\bar{I}) \bullet (\bar{G})}}$$
(5)

$$HOMA - IR = \frac{FPG \bullet FPI}{22.5} \tag{6}$$

Statistical Analysis. A two-way repeated measures ANOVA (time x treatment) was used

to compare all blood (glucose and insulin), glucose kinetics ( $Ra_{Total}$ ,  $Ra_{Exo}$ ,  $Ra_{Endo}$  and Rd) and exercise variables (HR, VO<sub>2</sub>, VCO<sub>2</sub>, RER, fat and carbohydrate oxidation). The coefficient of variation for insulin was 5.7%. A paired t-test was used to compare all AUC calculations. All assumptions were met and significance was accepted with a P  $\leq$  0.05 (2-tailed). All data were analyzed using Statistical Package for Social Sciences (SPSS, version 20.0, Inc, Chicago, IL). All data are reported as the mean  $\pm$  standard deviation.

#### RESULTS

*Exercise variables.* During exercise, there was no interaction between intensity and treatment, thus data were averaged across intensities during the GXT. Exercise heart rate did not differ between treatments (GTE =  $131 \pm 15$  vs. PLA =  $129 \pm 10$  beats•min<sup>-1</sup>; P = 0.46). Mean whole-body oxygen uptake was also similar between GTE vs. PLA ( $1.44 \pm 0.31$  vs.  $1.51 \pm 0.35$  L•min<sup>-1</sup>; P = 0.08). Carbohydrate oxidation rate was lower throughout exercise with GTE vs. PLA ( $1.26 \pm 0.34$  vs.  $1.48 \pm 0.51$  g•min<sup>-1</sup>, main effect of treatment, P = 0.04, Figure 2A); however, a concomitant significant increase in fat oxidation rate from the GTE was not detected (GTE =  $0.22 \pm 0.10$  vs. PLA =  $0.17 \pm 0.11$ , g•min<sup>-1</sup>, P = 0.09, Figure 2B), but this may be a type 2 error. Cohen's d calculations of 0.51 for carbohydrate oxidation and 0.48 for fat oxidation suggest intermediate and small effect sizes, respectively. Lastly, RER was not different between GTE and PLA ( $0.90 \pm 0.04$  vs.  $0.92 \pm 0.05$ , P = 0.07).

*Plasma glucose and insulin.* The mean plasma glucose and insulin concentrations are provided in Table 2. Fasting plasma glucose and insulin concentrations were similar between GTE and PLA (P = 0.14 and 0.09, respectively), and the HOMA-IR calculated from fasting samples also was not different between treatments (P = 0.07). There was no effect of GTE on the mean [glucose] or AUC over the 180 min post-exercise period (Figure 3A, P = 0.91 and 0.87, respectively). The mean plasma insulin and insulin AUC from a standard 75 g glucose, 7-sample OGTT during post-exercise recovery were lower with GTE vs PLA (Figure 3B, main effect of treatment, P = 0.05 for both measures). Cohen's d score for insulin suggests an intermediate effect size of 0.61. The observed effects of GTE on the insulin response post-exercise contributed to a 32% increase in Matsuda ISI with GTE vs. PLA ( $6.49 \pm 3.53$  vs.  $4.93 \pm 2.91$ , P = 0.04, Figure 4).

*Glucose tracer kinetics.* Glucose  $Ra_{Total}$ ,  $Ra_{Exo}$  and  $Ra_{Endo}$  and Rd over 180 min of postexercise recovery are shown in Figure 5 (A, B, C, and D, respectively). The  $Ra_{Exo}$  peaked at 90-100 min in both conditions, and the response over the 180 min was similar for both GTE and PLA (P = 0.28). Glucose  $Ra_{Endo}$ , which decreased rapidly in the first 50 min as  $Ra_{Exo}$  increased and had its slowest rate correspond to the peak in  $Ra_{Exo}$ , was not different between GTE and PLA (P = 0.65). Lastly, the Rd displayed a similar response in both GTE and PLA (P = 0.57), peaking at 100 min, and matching  $Ra_{Total}$  at ~90 min in both conditions (P = 0.74). The mean glucose tracer values are displayed in Table 2.

#### DISCUSSION

The novel finding from this study was that GTE lowered insulinemia following an oral glucose load ingested immediately following an acute bout of exercise, which led to

a 32% increase in Matsuda-ISI. Despite this response, glucose disappearance was similar under both conditions. Utilizing two glucose tracers, we examined the effects of 7-d of supplementation with GTE or PLA on glucose kinetics. For each trial, the glucose kinetics and the glycemic response was assessed in the post-exercise recovery period via a primed-constant infusion of  $[U-{}^{13}C_6]$  glucose and ingestion of 75g oral glucose (including 2g of  $[6,6-{}^{2}H_2]$  glucose). Although supplementation with GTE attenuated postprandial insulinemia, tracer analysis revealed that the response was not a consequence of reduced glucose Ra<sub>Exo</sub> or Ra<sub>Endo</sub>. We also observed reduced carbohydrate oxidation during the acute exercise session with GTE compared to PLA.

There is growing evidence of the health benefits of GTC, including effects on glycemic control. Numerous mechanisms have been proposed to explain these potential benefits, based mainly on rodent and cell models, with little investigation in humans. In a recent study, which utilized the same exercise protocol and similar GTE treatment regimen as the present study, we concluded that GTE attenuated the glycemic response to an oral glucose load at 1 h post-exercise (31). Interestingly, this improved glycemic response was not evident under resting conditions, which suggests the effects of GTE on glycemic control might be enhanced during exercise recovery. However, whether this improvement was a function of reduced intestinal absorption or increased uptake of glucose could not be determined. Thus, in the present study we used glucose tracers to ascertain if the improvement in glycemic control in humans was due to a GTE-induced reduction in intestinal absorption. Well-supported and recognized mechanisms provided by rodent models suggest alterations in several of the of glucose transporters, including the inhibition of SGLT-1. When sections of rat and rabbit jejunum were exposed to ECG or EGCG in the presence of glucose, glucose uptake was reduced by 36% with ECG and 25% with EGCG, mediated through inhibition of SGLT-1 (25). Similar SGLT-1 inhibition has also been observed acutely (30 min) and chronically (6 wk) using ex vivo and in vivo methods in high-fat fed rats (40). Taking into account that we did not observe a change in the Ra<sub>Exo</sub> in the present study, our data shows that GTE did not affect glucose uptake from the gut following exercise in humans. Additional data in mice support these findings with no effect of GTE on the expression of GLUT-2 or SGLT-1 (13). However, the researchers did report an effect of GTE on plasma glucose following ingestion of cornstarch, which would suggest GTE might affect glucose tolerance differently with glucose ingestion vs. starch. Secondly, in the present study, we did not observe an effect of supplementation with GTE on glucose concentrations or the Ra<sub>Endo</sub> over the 180-min period post-exercise, which indicates no effect on hepatic glucose release in humans. Although findings from the present study were during the post-exercise period, a very similar glucose and insulin response has been observed under resting conditions, following the acute administration of a very similar dose of GTE in humans (45). Taken together these findings suggest GTE might be affecting glycemic control through improved peripheral insulin sensitivity.

Preclinical and cell-based models have suggested an effect of GTC on the translocation of GLUT-4 to the plasma membrane, both in muscle and adipose tissue. For example, acute EGCG treatment in L6 myotubes and 7 days of treatment in rats both showed increases in GLUT-4 translocation in skeletal muscle (44). Similarly, EGCG has

also been shown to reverse fatty acid-induced impairments in the insulin-signaling pathway (28), which improved downstream translocation of GLUT-4 in skeletal muscle and adipose tissue. Lastly, a 3-wk green tea treatment in rats increased skeletal muscle GLUT-4 translocation, while reducing adipose GLUT-4 translocation (3). Evidence of these mechanisms has yet to be shown in humans. However, if GTE is acting via glucose transporters in tissues such as skeletal muscle, findings from the present study and those reported previously (31, 45) would suggest this response is insulin mediated. Thus, less insulin is required to clear a given amount of glucose (i.e., improved insulin sensitivity).

There are several potential mechanisms that might attenuate the release and/or appearance of insulin from the pancreatic beta cells (15). One of these is the effect of catecholamines, such as epinephrine (EPI) and norepinephrine (NE), where both  $\alpha$  and  $\beta$  receptors inhibit or stimulate insulin secretion, respectively. GTE might influence catecholamine concentrations through inhibition of catechol-O-methyl transferase, an enzyme responsible for the degradation of catecholamines, such as EPI and NE (12, 29). Thereby, GTE would modulate insulin secretion via adrenergic stimulation. However, Hodgson et al. (18) observed no differences in metabolites indicative of increases in catecholamines following 1 or 7-d of GTE administration. Moreover, we have also reported no effect of GTE on either EPI or NE at rest, following 48 h of GTE supplementation, when compared to a PLA (32). Although the effects observed in the present study could have been influenced by catecholamine release during exercise, the effects of GTE on glycemic control observed by Venables et al. (45) were at rest, which suggests the improved insulin sensitivity observed in the present study might not have been due to a synergistic effect of GTE and the exercise stress.

Although there are numerous other potential effects of GTC on glycemic control, which could lead to the reduced insulin and improved insulin sensitivity, many are beyond the scope of this paper, but have been reviewed (24). One possibility is the effect of insulin sensitivity on insulin secretion, or disposition index (4). The disposition index, as described by Bergman et al. (4) and others (2, 21) can be represented by a hyperbolic function, where insulin secretion increases with reduced insulin sensitivity. This response can clearly be seen in the initial stages of developing insulin resistance, where, through an effort to maintain glucose homeostasis, the pancreatic  $\beta$  cells become more sensitive, increasing insulin secretion for a given glucose load. Conversely, improved insulin sensitivity reduces  $\beta$  cell sensitivity, and increases peripheral insulin clearance and hepatic extraction (2). Therefore, the lower insulin observed in the present study could be the result of increased insulin sensitivity. When a green tea beverage (1.5 g green tea powder) was administered to human participants just 10 min prior to an OGTT, they observed lower glucose levels at 30 and 120 min but no effect on insulin (43). Such an acute response suggests that GTC are improving insulin sensitivity but before adjustments are made to the disposition index, described above.

We observed that GTE lowered carbohydrate oxidation during the graded exercise test. Several studies have investigated the effects of GTC on metabolic and physiological function during exercise. It has been shown that GTC increased fat oxidation during exercise, improved VO<sub>2</sub>max, lowered steady-state exercise HR, and increased markers of lipolysis (32, 36, 39, 46); however, these findings remain equivocal, as many others have

been unable to replicate the findings (11, 18, 19, 31, 32, 37, 38). As previously stated, GTC are purported to increase adrenergic drive through increased beta-receptor stimulation (12, 29). And again, data from investigations examining these effects of GTE on catecholamines in humans have failed to show an effect (18, 32). Although several other mechanisms have been observed in rodent models (20), none of these mechanisms has been followed up in humans. Thus, these potential adaptations are still questionable.

There are some limitations of the present study that must be recognized. First, our data are suggestive of an insulin-sensitizing effect of GTE that we proposed is potentially muscle-specific; however, we cannot conclude that the glucose disappearance, which occurred under a reduced insulin concentration, resulted from increased uptake into skeletal muscle. Secondly, although it is unlikely that an interaction between GTE and the reduced carbohydrate oxidation contributed to the decreased insulin concentration and improved insulin sensitivity, we cannot confirm that substrate utilization was not altered during post-exercise recovery

In summary, the present study found that 7-d of GTE supplementation attenuated the insulin response to an oral glucose load ingested post-exercise. We did not, however, observe any indications that GTE altered the  $Ra_{Exo}$ ,  $Ra_{Endo}$  or Rd of glucose. Our findings, along with previous evidence showing that GTE improves insulin sensitivity in humans (45) and alters skeletal muscle glucose transporters in rodents (44), suggests GTE might improve insulin action, requiring less insulin to clear a given amount of glucose; however, this hypothesis warrants further investigation.

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# Disclosures

All authors report no potential conflicts of interest, financial or otherwise.

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Ingredient	Concentration (%)	Dose (mg/day)
Polyphenols	95	1,000
Catechins	90	900
EGCG	50	450
Epigallocatechin	20	180
Epicatechin	10	90
Epicatechin gallate	7	63
Other Catechins	13	117
Caffeine	< 1	< 9

Table 1. The composition of the green tea extract used in the present study.

Variable	GTE	PLA
Insulin (µIU•mL <sup>-1</sup> )	$17.3 \pm 6.3*$	$21.7 \pm 8.1$
Glucose (mM)	$5.7 \pm 1.0$	$5.7 \pm 0.9$
$Ra_{Total}(g \cdot min^{-1})$	$0.42\pm0.16$	$0.43 \pm 0.13$
$Ra_{Exo}(g \cdot min^{-1})$	$0.34\pm0.12$	$0.36 \pm 0.11$
$Ra_{Endo}(g \cdot min^{-1})$	$0.08\pm0.09$	$0.07\pm0.07$
$Rd(g \cdot min^{-1})$	$0.43 \pm 0.14$	$0.44 \pm 0.14$

Table 2. Plasma glucose and insulin data and glucose kinetics for GTE and PLA trials.

Data presented are the insulin from seven time points for 120 min OGTT and [glucose] across the 180 min collection period. Glucose rates of appearance (Ra<sub>Total</sub>) and disappearance (Rd) for [6,6-<sup>2</sup>H<sub>2</sub>] and [U-<sup>13</sup>C<sub>6</sub>] and their respective rates of exogenous (Ra<sub>Exo</sub>) and endogenous (Ra<sub>Endo</sub>) glucose production across the 180 min are shown. Values are the means  $\pm$  SD. \* denotes GTE significantly different compared to PLA (P  $\leq$  0.05)

# **Figure Captions**

**Figure 1.** An overview of the experimental trials. After an initial blood draw, subjects consumed a final capsule, and then received a primed-constant infusion of U-<sup>13</sup>C<sub>6</sub> glucose. Exercise began 60 min after the glucose infusion and was followed immediately by ingestion of a glucose beverage containing  $6,6^{-2}H_2$  glucose.

Figure 2. Effects of a 7-day treatment with green tea extract (GTE) or placebo (PLA) on carbohydrate (panel A) and fat (panel B) oxidation rates during a graded exercise test. All values are means  $\pm$  SD. \*denotes lower carbohydrate oxidation with GTE vs. PLA,  $P \le 0.05$ .

Figure 3. Effects of a 7-day treatment with green tea extract (GTE) or placebo (PLA) on plasma insulin concentration (panel A) and plasma glucose concentration (panel B) following ingestion of a 75g glucose beverage during post-exercise recovery. All values are means  $\pm$  SD. \*denotes insulin AUC was significantly lower with GTE vs. PLA,  $P \le 0.05$ .

Figure 4. Effects of a 7-day treatment with green tea extract (GTE) or placebo (PLA) on Matsuda-insulin sensitivity index (ISI) following a 7 time-point OGTT during post-exercise recovery. All values are means  $\pm$  SD. \*denotes Matsuda-ISI was significantly higher with GTE vs. PLA, P  $\leq$  0.05.

Figure 5. Effects of a 7-day treatment with green tea extract (GTE) or placebo (PLA) on glucose kinetics over a 180 min OGTT during post-exercise recovery. Total rate of plasma glucose appearance ( $Ra_{Total}$ ) (panel A), contribution of exogenous glucose appearing from gut ( $Ra_{Exo}$ ) (panel B), rate of endogenous glucose production ( $Ra_{Endo}$ ) (panel C), and rate of glucose disappearance from plasma (Rd) (panel D) are shown. All values are means  $\pm$  SD.



**Figure 1. An overview of the experimental trials.** After an initial blood draw, subjects consumed a final capsule, and then received a primed-constant infusion of  $U^{-13}C_6$  glucose. Exercise began 60 min after the glucose infusion and was followed immediately by ingestion of a glucose beverage containing  $6,6^{-2}H_2$  glucose.



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# CHAPTER 5: GENERAL DISCUSSION

#### 5.1. Overview

The main objective of the present dissertation was to advance our understanding of the metabolic, physiological and health-related effects of GTE supplementation before, during, and after exercise. In Study 1 (Chapter 2) we investigated whether two days of GTE supplementation would alter REE, substrate oxidation during exercise or performance in recreationally active men. The work was distinguished from other studies (1, 2) in that we provided a relatively large dose of decaffeinated GTE three times per day. Although this treatment regimen was similar one previously shown to be effective at increasing fat oxidation during exercise (3), we did not observe any effect of GTE on exercise substrate utilization. Surprisingly, the dose of GTE provided in Study 1 far exceeded that which has previously been shown to increase REE (4). These findings or lack thereof, when compared to the literature provided some potential areas for contrast. For example, a majority of the literature has utilized healthy active men and activity level and health status of the individuals might modulate the effects of GTE. In Study 2 (Chapter 3), we attempted to advance our understanding of the influence that subject characteristics and duration of treatment might have on the effect of GTE supplementation. In particular, we focused on the potential interactive effect of GTE supplementation and exercise on glycemic control. Following a seven-day treatment period in sedentary overweight men, we observed attenuated [glucose] and insulinemia at one-hour following ingestion of 75 g of glucose in the post-exercise period with GTE compared to a PLA; however, we did not observe an effect of GTE on substrate use during exercise. These findings support those that have previously shown GTE to improve glycemic control (3, 5); however, this was the first study to investigate the potential interaction of GTE and glycemic control following exercise. Lastly, Study 3 (Chapter 4) built on the findings of Study 2 and animal literature that has examined mechanisms of glycemic control. We employed a design similar to Study 2 but incorporated stable isotope tracer technology to examine glucose kinetics in sedentary normal weight men. Despite comparable effects of GTE on insulinemia, following a 75 g glucose load ingested during post-exercise recovery, we did not show an effect on plasma glucose. Additionally, we observed lower carbohydrate oxidation during exercise, which was not apparent in studies one or two. Taken together, the series of studies in the present dissertation present novel insights into the potential for GTE to improve glycemic control, and potentially exercise substrate use in certain populations. This chapter integrates the findings from all three studies with the current literature while highlighting any limitations and future directions.

# 5.2. No Effect of Green Tea Extract on Resting Energy Expenditure

Although tea is one of the most common beverages in the world (6-9) and its consumption has been inversely associated with obesity (10-12), the effect of GTC on REE in humans is still limited. The thermogenic effect of GTC is believed result from increased adrenergic stimulation via increased catecholamines from the inhibition of COMT (13, 14). However, empirical evidence for this mechanism is also limited. A component of study one of this dissertation explored the potential for GTE to influence REE and substrate oxidation under resting conditions in the fasted state. We did not

observe an effect on either REE or substrate use, which supports the findings from other investigations (7, 8). Moreover, we also did not observe an effect on the catecholamines EPI or NE, which is supported by one other study (15).

Others have shown an effect of GTE on REE and substrate use. For example, Dulloo et al. (13) reported increased REE, reduced RER and increased urinary catecholamine excretion over a 24 hour measurement period with a relatively small dose of GTE + caffeine when compared to an equivalent dose of caffeine or PLA. Although their treatment contained caffeine, which potentially had a synergistic effect with the GTE, another study observed similar findings from an EGCG supplement without caffeine. Boschmann et al. (16) provided 135 mg of EGCG over 48 hours and observed an 8% lower RER when compared to a PLA. Interestingly, the effects observed by Dulloo et al. (13) were only apparent in the diurnal portion of the trial and the findings by Boschmann et al. (16) were only significant in the postprandial period. This would suggest the effects on REE and RER might be potentiated by the thermic effect of feeding. One possibility for this increased thermogenesis is from an increase in catecholamines following a meal, thus leading to a greater adrenergic response from COMT inhibition. However, Lonac et al. (17) specifically examined the effects of a twoday treatment with EGCG on resting metabolic rate and the thermic effect of feeding and did not observe any differences when compared to a PLA.

Caffeine is one potential reason for equivocal findings in some studies, but another possibility is the magnitude of the effects and sensitivity of the measurement. For example, Dulloo et al. (13) reported a small, although significant increase in REE of  $\sim 4\%$ over 24 hours in a respiratory chamber. This small change would equate to  $\sim$ three kcal per hour. Over a 24 hour period this increase is meaningful and would offset the typical yearly decline in REE with aging (17); however, given the typical measurement period in studies are  $\sim$ one hour, a per hour difference of  $\sim$ three kcal would be far under the day-today variability in humans. Thus, it is very likely that these small changes at rest could only be ascertained from 24 hour collection periods in a chamber.

#### 5.3. Effects of Green Tea Extract during Exercise

Green tea has gained its popularity due to it being particularly high in polyphenols mainly in the form of flavonoids known as catechins (7). These GTC have been shown to inhibit COMT, an enzyme responsible for control of catecholamines (18). Considering the importance of catecholamines and their effect in controlling thermogenesis and fat oxidation it is possible that GTC, through COMT inhibition, results in a prolonged adrenergic response leading to increased energy expenditure and/or greater fat oxidation when combined with exercise. Although the COMT mechanism is the most often cited when referring to the potential for GTC to alter the metabolic and/or physiological effects when combined with exercise, several other possibilities have been proposed through *in vivo* and *in vitro* rodent studies. There are several other potential functions mediated through GTC when combined with exercise that will be highlighted and further discussed in this section
## 5.3.1. Physiological Effects

Although a majority of the GTC and exercise literature has focused on the potential for supplementation to alter metabolism, Richards et al. showed that short-term EGCG supplementation increased VO<sub>2</sub>max in healthy young men and women (19). In Study 1 of this dissertation, we observed a small but significantly lower heart rate during the steady-state exercise portion of the trial. To our knowledge, this is the first and only study to report an effect on heart rate following GTE or GTC supplementation. Rodent studies that have investigated the physiological effects of GTC suggest the potential for improved O<sub>2</sub> extraction. One, in particular, is a study by Nogueire et al., which assessed the effects of a 15-day treatment with epicatechin on several measures of exercise function and adaptations in one-year-old mice (20). The authors reported a larger increase in muscle capillarity in the mice receiving epicatechin when compared to a control group. Interestingly, although this response was more robust in mice receiving epicatechin + exercise training, it was also present in the non-exercising epicatechin group. These findings if present in humans could explain the effects on heart rate observed in study one, as well as those observed by Richards et al.

Richards et al. observed an increased VO<sub>2</sub>max during a standard GXT and thus if the participants in study one were exercising at a lower percentage of their VO<sub>2</sub>max, heart rate could be lower for a given workload. Through the COMT mechanism, it is plausible the GTE had an inotropic effect, thus increasing stroke volume and reducing heart rate. However, Richards et al. assessed stroke volume and reported no differences between the EGCG and PLA trials. The authors suggested the effect might have been through enhanced  $[a-v]O_2$  diff, which would improve  $O_2$  extraction at the muscle level and potentially increase VO<sub>2</sub>max via similar mechanisms explained above.

Study 2 and Study 3 of this dissertation did not observe an effect on heart rate during the exercise session; however, these studies also involved a GXT opposed to the steady-state exercise used in study one, which might have precluded our ability to observe an effect on heart rate. And, although the GXT used in Studies 2 and Study 3 were designed to improve our ability to observe effects on substrate oxidation, participants still exercised until volitional fatigue and we did not observe an increase in VO<sub>2</sub>peak. Therefore, the potential for GTC to alter physiological aspects associated with exercise in humans remains unclear.

#### 5.3.2. Metabolic Effects

GTE ingestion has been shown to reduce RER and increase fat oxidation during exercise in humans (3, 15, 21). Similar findings have been observed in exercising rodents, which have been further supported by evidence of increased ability to transport and utilize lipids (e.g. increased FAT/CD36 and increased beta-oxidation activity) (22-24). One overarching focus of this dissertation was to specifically examine the potential for GTE to alter substrate oxidation during exercise. To control for as many extraneous variables as possible, all three studies utilized a dosing regimen deemed to optimize a potential effect (i.e., comparatively large and administered 3  $x \cdot day^{-1}$ ) and controlled for variability in dietary intake, supplements, and medications.

Given the effects of GTC on exercise metabolism in humans remains equivocal, Study 1 emulated specific methodologies as the study by Venables et al. (3), which was the first to show an effect of GTE on substrate oxidation during exercise. In this earlier study, participants deemed "healthy" supplemented with three doses of GTE (340 mg polyphenols, 136 mg EGCG) over 24 hours prior to 30 min of steady-state exercise in the fasted state. While all three studies of this dissertation utilized a similar dose of GTE (350 mg polyphenols, 150 mg EGCG), studies one and two did not observe any alterations in substrate oxidation between the GTE and a PLA. To contrast with the study by Venables et al., Study 1 extended the supplementation from 24 hours to 48 and utilized a 60 min steady-state exercise session vs. 30 min and in recreationally active men vs. "healthy" men. Similar to their findings, we did observe evidence of greater lipolysis from increased plasma glycerol concentrations with GTE compared to a PLA. However, since we did not observe a similar effect on substrate oxidation, in Study 2 we altered some of the methodologies in an attempt to potentiate the effects on exercise substrate oxidation and measures of glycemic control.

Some differences, which might have led to conflicting findings between study one and the study by Venables et al. (REF), include the group studies, fasted vs. fed state comparison, and the intensity of the exercise session. First, sedentary and overweight individuals possess reduced beta-adrenergic sensitivity compared to those who are active and/or normal weight (25, 26). Although substrate oxidation can be altered by any of these three differences, specifically with regard to the COMT mechanism of GTC, it is possible that using sedentary individuals could augment the adrenergic response. Several others have also not observed an effect of GTC on substrate use during exercise and utilizing trained or active individuals (1, 2, 27). Second, it is well known that exercise in the fed vs. fasted state blunts fat oxidation during exercise (28), thus testing participants following an overnight fast could augment the effect of the GTE on fat oxidation. Conversely, it could be argued that the already high rate of fat oxidation in the fasted state might diminish the ability to see a small effect from the GTE. Lastly, the exercise intensity performed in study one was much lower than that assigned by Venables et al. (~45% vs. ~60%, respectively).

In Study 2, we utilized sedentary overweight men and had them exercise in the fasted state. Also, as opposed to a steady-state exercise bout, we utilized a GXT designed to assess fat oxidation rates at increasing intensities to determine the fat oxidation rates at varying intensities. We also extended the supplementation period to seven days to contrast any findings from 48 hours to those following seven days. Yet, similar to the findings from study one, there was no effect of GTE on substrate oxidation; however, unlike study one the GTE treatment had no effect on markers of lipolysis. The effect of the GTE on markers of lipolysis, in this case, might have been masked by an already high concentration of plasma glycerol and FFA in this population. Similarly, an increase in lipolysis is one route the GTE might enhance exercise fat oxidation and an already high availability of substrate might have limited any potential effect of supplementation in this population. Considering these weaknesses study three sought to emulate study two but in a sedentary normal weight population.

Similar to Study 2, the participants in study three supplemented for seven days. However, due to other measurements being examined in this study, only one exercise trial was performed at the end of each seven-day supplementation period. In study three we observed a significant reduction in carbohydrate oxidation with GTE compared to PLA and although not statistically significant this was mirrored by a small concomitant increase in fat oxidation. Taken together, these three studies along with current literature, suggest GTE might improve substrate oxidation in sedentary normal weight men and this effect is potentially due to an enhanced adrenergic response. However, additional research is necessary to elucidate if this response is catecholamine-induced or due to some other mechanism in humans.

#### 5.3.3. Performance Effects

Supplementation with GTC has shown improvements in time-trial performance (29) and increases in VO2max (19). Study one examined the potential for GTE to improve time-trial performance when performed after 60 min of steady state cycling. We hypothesized that if the GTE increased fat oxidation while concomitantly conserving carbohydrate, time-trial performance would be improved when performed immediately after the steady-state bout. While we did not observe an effect of the GTE on performance, as stated above GTE also did not alter substrate oxidation. Based on similar rationale, Roberts et al. did report improved performance during a 40 min time-trial when performed after a 60 min steady-state bout (29). However, in this study, the authors also observed increased fat oxidation and reduced carbohydrate oxidation. While Roberts et al. had their subjects perform the steady-state and performance trials in the fasted state, in study one subjects were in the fed state, which might have precluded us from observing an effect on substrate oxidation and performance. Another mechanism for improved performance is through increased VO<sub>2</sub>max. Richards et al. observed an increase in VO2max in healthy men and women supplementing with EGCG for 48 hours (19). Considering exercise performance is partially based on VO<sub>2</sub>max GTC might offer performance improvements through this route. Although researchers have reported improved swimming and running times to exhaustion after supplementing rodents with GTE, similar trials have vet to be conducted in humans (24, 30).

#### 5.4. Green Tea Extract and Glycemic Control under Resting Conditions

Short-term GTC intake has been shown to improve both the glucose (5) and insulinemic (3) response to an oral glucose load under resting conditions. Additional evidence from a recent meta-analysis suggests that chronic intake of GTC lowers fasting plasma glucose in type-2 diabetics (31). Unpublished data from Study 1 suggested a potential effect on plasma glucose following a high carbohydrate mixed meal. A novel and unique component of Study 2 was the focus on a potential effect of GTE on the glucose response to high glucose loads as well as mixed meals. These measurements were collected under free-living conditions via an indwelling continuous glucose monitor, which participants wore for the entire seven-day treatment period. During this seven-day period, we examined the effects of a 75 g oral glucose beverage on glucose excursions following one dose of GTE and after five days of supplementation. Additionally, glucose

control was also monitored over 48 hour periods in response to controlled meals, which were identical between treatment periods. In response to the oral glucose beverage, the one-hour peak glucose, and area under the glucose curve was not different between GTE and PLA and was not affected by the length of supplementation. Additionally, GTE did not affect the glucose concentrations in response to mixed meals throughout the treatment periods.

In contrast to the findings reported by Tsuneki et al. (5), we did not observe an effect of GTE on glucose control, at least as reflected by an acute effect of GTE treatment on glucose concentrations. Conversely, if we consider the short-term effects observed by Venables et al. (3), GTE supplementation altered insulin AUC under resting conditions and not [glucose]. Moreover, considering Tsuneki et al. (5) reported lower glucose following acute administration of GTC suggests the only time GTE might affect glucose concentrations is under very acute conditions. This would imply our only opportunity to observe an effect was following the first dose of GTE on day one. It could be argued that the use of continuous glucose monitoring limited our ability to detect differences; however, additional data from study two (discussed in section 5.5) suggests an unequivocal mirrored response between plasma glucose concentrations and those provided by continuous glucose monitors. Another potential consideration is that the small effect the GTE might have had on glucose under these conditions could have been masked by a lack of control over subjects and their ability to reproduce accurate timing of ingested capsules, glucose beverages, and meals. Therefore, at least from data provided from study two, acute or short-term GTE supplementation does not affect glucose control under free-living resting conditions.

#### 5.5. Green Tea Extract and Glycemic Control During Exercise Recovery

A majority of the literature on GTC during exercise recovery has examined its effects on inflammation, muscle damage, or oxidative stress (2, 32-34). While a large portion of this dissertation focused on the post-exercise period, a key objective was to examine the interactive effects of GTE and exercise on glycemic control, an area which has been unexplored until now. As previously discussed, study two examined the effects of GTE on glucose control at rest; however in addition to this we assessed the effects of GTE on glycemic control during post-exercise recovery. Similar to the resting measures in study one, the temporal response was also examined, comparing 48 hours of supplementation with seven days. On trial days participants ingested a 75 g glucose immediately following the GXT. Blood collected at one hour of recover revealed attenuated [glucose] and insulinemia with the GTE compared a PLA at the one-hour postexercise time point. Additionally, these data were further confirmed via the indwelling continuous glucose monitor, which was in agreement with the plasma values obtained. The seven-day response appeared more robust than 48 hours although this was not statistically different. Although there was an apparent affect of the GTE on glycemic control, mechanisms provided from studies conducted in rodents provide a few possibilities to explain these findings.

According to Kobayashi et al. (35), GTE inhibits SGLT-1, an intestinal glucose transporter, which would reduce the relative glucose load, lowering [glucose] and

insulinemia, such as we observed during post-exercise recovery in Study 2. Another possibility is reduced hepatic glucose production from the down-regulated expression of gluconeogenic genes (36). The last and probably most well supported mechanism is an effect of GTC on increased GLUT-4 translocation and content observed ex vivo and in vitro in rodent models (37-39). Considering these possibilities, the major purpose of study three was to further investigate the glycemic response of GTE during post-exercise recovery and attempt to elucidate a likely mechanism/s leading to this response in humans. Largely emulating study two, study three examined the effects of GTE following seven days of treatment only; however, in addition to examining the post-exercise glycemic response, a dual-isotopic glucose method was utilized to asses for the effect of GTE on gut absorption, hepatic output, and uptake by insulin sensitive tissues. While study two was novel by assessing the effects of GTE on glycemic control during exercise recovery, study three was the first to highlight the potential mechanisms related to the effects of GTE on glycemic control in humans. What we observed was that while the GTE again improved glycemic control through reduced insulinemia leading to a 32% in M<sub>ISI</sub>, this effect was not due to reduced gut absorption or hepatic glucose output. Thus, we concluded GTE appears to sensitize insulin sensitive tissues leading to similar glucose disposal despite a reduced insulinemia.

## 5.6. Limitations and Future Directions

There are a few limitations to the series of studies discussed in this dissertation. First, although the samples size in all three studies were based on power calculations largely derived from other studies examining similar variables, some statistical power was inevitable lost and possibility limited our ability to observe significant differences in certain measures. Secondly, without measurements of plasma catechin concentrations we cannot conclude with certainty all capsules were taken as directed. However, participants ingested a final GTE capsule upon arrival to the lab on the mornings of exercise trial days, which assured at least the acute delivery of catechins at that time. Considering the short half-life of catechins in plasma (40), a confirmatory blood draw on the morning of trials would not have been conclusive regarding adherence to supplementation in the days prior. Lastly, data from study three along with the mechanisms discussed previously are suggestive of enhanced skeletal muscle glucose uptake, likely due to the effects of GTE on GLUT-4. A limitation to this suggestion is the absence of actual evidence for this GLUT-4 mechanism.

Although the enhanced GLUT-4 translocation observed in rodent studies offer insight into the improved glycemic control observed in studies two and three, this has not been examined during post-exercise recovery. Considering data from studies two and three, with no affect of GTE on glucose control at rest, it appears the effects of the GTE on glycemic control is potentiated following exercise. Insulin signaling is temporarily impaired following muscle damaging exercise through oxidative stress and inflammation (41). And, GTC have been shown to reduce markers of muscle damage, oxidative stress, and inflammation (2, 32, 42), thus, during post-exercise recovery GTE might be improving insulin-stimulated glucose uptake by relieving inhibition on the insulin-signaling pathway. Rapid replenishment of glycogen is of great importance to athletes

and this potential mechanism for the findings observed from this dissertation offers direction for future human studies to investigate the effects of GTE on post-exercise glycemic control.

## 5.7. Conclusion

In summary, the series of studies presented in this dissertation offer insight into the potential metabolic and physiological effects of GTE in humans and in particular the potential interactive effect of GTE supplementation and exercise on metabolic regulation in humans. The control of hyperglycemia and hyperinsulinemia can help reduce the risk of type-2 diabetes and our findings suggest GTE is effective in improving glycemic control following exercise, which could have important implications in reducing insulin spikes in sedentary individuals as well as potentially improving glycogen replenishment in athletes. Despite evidence from rodent studies, study three did not support an effect of GTE reducing glucose uptake in the gut, suggesting GTE has an insulin-sensitizing effect post-absorption. While the diverse methodologies in the literature make it difficult to ascertain the effectiveness of GTC on substrate oxidation, the present dissertation allowed a comparison from all three studies in three vastly different populations while using similar methods and utilizing the same dosing regiment. This contrast showed that GTE altered substrate oxidation during exercise in sedentary men but not active or sedentary overweight men. Research in the form of larger randomized clinical trials is necessary to further address the potential for GTC to improve glycemic control and alter substrate oxidation during exercise in humans.

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