GREEN TEA EXTRACT SUPPLEMENTATION AND CYCLING TIME-TRIAL PERFORMANCE AT MODERATE ALTITUDE

By

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ABSTRACT

Reactive oxygen species are unstable molecules that have been shown to cause muscle damage and possibly be involved in the skeletal muscle fatiguing process. Reactive oxygen species are generated by high intensity exercise and also exposure to altitude-related hypoxia, as well as various other conditions. Green tea extract is a known, powerful antioxidant, meaning it has the ability to scavenge reactive oxygen species, possibly decreasing muscle damage and fatigue. The purpose of this research was to determine the effects of green tea extract supplementation on reactive oxygen species production, oxidative stress, and endurance exercise performance at moderate altitude.

Ten subjects performed a graded exercise test to determine maximum oxygen consumption and power output. The subjects then participated in three time trials on a fixed gear cycle ergometer. These time trials corresponded with cycling at seventy five percent of their maximum power output for twenty minutes; if the subject pedaled faster, he completed the time trial in less than twenty minutes. One hour prior to performing in the second and third time trials, a blood sample was collected from each subject and a supplement was given. The supplement consisted of either a placebo (dextrose) or green tea extract. The supplementation was administered in a random, double blind fashion, to minimize bias of the results. One hour after completion of the time trial, a blood sample was again obtained.

The results of this study show an average twenty-nine second improvement in cycling time trial performance, and corresponding increase of approximately seven watts in mean power output, with green tea extract supplementation compared to placebo.

There were no significant differences in heart rate, percent of maximum heart rate, oxygen saturation, or rating of perceived exertion between the two different supplements. There also were no significant differences in the plasma malondialdehyde (marker of oxidative stress) between the two supplementations.

This study found that while green tea extract improves endurance exercise performance at moderate altitude, the mechanism by which this performance improved is not readily known, as there was no change in malondialdehyde concentrations. Further research should be completed to determine a better quantitative measure for oxidative stress and reactive oxygen species in the body.

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CHAPTER I

INTRODUCTION

Free radicals are highly reactive molecules containing an unpaired electron. These electrons are very unstable and constantly seeking to combine with other molecules to attain a more stable or less chemically active state (McBride & Kraemer, 1999; Sen & Packer, 2000). Free radicals in biological or living systems are known as reactive oxygen species (ROS). ROS consist of the superoxide anion, hydroxyl, alkoxyl, and peroxyl radical groups (Cooper, et al., 2002; McBride & Kraemer, 1999; Sen & Packer, 2000). Metabolic stress due to high intensity endurance exercise activities has been shown to generate ROS. (Cooper, et al., 2002).

It has been shown that ROS cause oxidative stress via lipid peroxidation to muscle membranes during exercise, causing damage to muscular tissues as well as blood (Viňa, et al., 2000). This damage to muscle tissues and blood is thought to be involved in the process of fatigue, or the inability to generate power. The oxidative damage has also been shown to alter the histochemistry of blood and cause muscle soreness (Dekkers, van Doornet, & Kemper, 1996; Kuipers, 1994). The increase in ROS with exercise may also affect aerobic energy pathways in the mitochondria, causing the onset of fatigue (Kendall & Eston, 2002).

Exposure to altitude and the associated decrease in blood oxygen, known as hypoxia, has been shown to increase production of ROS in rodent and human models (Yoshikawa, et al., 1982; Askew, 2002). Furthermore, ROS produced in hypoxic skeletal muscle leads to decreased force production (Mohanraj, et al., 1998).

Previous studies have utilized the antioxidants vitamin C and E, and have shown promise in decreasing ROS, possibly staving off the effects of fatigue (Powers, et al., 2004; Shafat, et al., 2004). Vitamins C and E, however, must be ingested and supplemented over the course of weeks to allow for peak concentrations, that are most effective in collecting ROS (Powers, et al., 2004; Shafat, et al., 2004).

Green tea contains polyphenols, namely catechins, which have antioxidant properties, similar to vitamins C and E (Benzie & Szeto, 1999; Yokozawa, et al., 1998). The four primary catechins in green tea extract are epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC) (Lee, et al., 2002). EGCG, EGC, and EC are known to be the most active as scavengers of free radicals (Lee, et al., 2002). Green tea catechins are water-soluble, which does not allow concentrations to build up in the body (Lee, et al., 2002). Since concentrations do not build up in the body, supplementation over an extended period of time is not necessary. Peak plasma EGCG occurs approximately 1 hour after ingestion of green tea extract, remains significantly elevated for several hours, and returns to baseline concentrations approximately 24 hours after ingestion (Chow, et al., 2003).

Green tea extracts (GTE) have been used to counteract the effects of ROS caused by a variety of cancers as well as conditions like diabetes. Green tea has also been shown to have significant effects in lowering low-density lipoproteins in patients with dyslipidemia (Orzechowski, 2003; Hodgson, et al., 2000; Yang, et al., 2000). Currently there are few studies that examine the antioxidant abilities of green tea catechins to collect exercise-induced ROS. Green tea infusions, however, have been shown to boost

the total antioxidant capacity, *in vivo,* in humans one to two hours after infusion (Sung, et al., 2000).

Previous well-controlled studies that have utilized antioxidant supplementation and measured ROS production and endurance exercise performance in humans are deficient and the existing data is ambiguous (Shepard, et al., 1974; Sumida, et al., 1989). There is even less data related to the effects of antioxidant supplementation on ROS production and exercise performance at high altitude. It has been shown that oxidative stress during muscular work at moderate altitude can be reduced via an antioxidant supplement consisting of beta-carotene, ascorbic acid, Vitamin E, selenium and zinc (Askew, 2002). To the researcher's knowledge, studies have not been designed to evaluate the possible beneficial effects of green tea catechins on muscular fatigue that is caused by ROS generated by exposure to moderate altitude.

Purpose of the study

The purpose of this study was to determine the effects of green tea extract supplementation on ROS production, oxidative stress, and endurance exercise performance at moderate altitude (8,200 feet).

Hypotheses

1. Supplementation with GTE, being a powerful antioxidant, will result in lower levels of markers of oxidative stress generated by exercise at moderate altitude compared to a placebo at moderate altitude.

2. Supplementation of GTE will result in increased endurance capacity, compared to placebo, at moderate altitude.
3. Improvements in endurance exercise capacity will be strongly associated with lower markers of oxidative stress after exercise at moderate altitude.

Definition of terms

The following terms are commonly used and are useful to the comprehension of this study. Therefore it is important to define their meaning.

1. Reactive oxygen species (ROS) – Chemical species in biological systems that contain an unpaired electron, which causes damage to muscle membranes, leading to muscular fatigue.
2. Hypoxia – A shortage of oxygen in the body, due to decreased pressure of oxygen at high altitude.
3. Endurance Exercise Performance – the amount of time required for a participant to complete a predetermined amount of work, assuming maximal effort.

Assumptions

The basic assumptions for this study are:

1. Subjects adhered to the dietary requirements of this study.
2. Subjects performed a full effort during the graded exercise test, and subsequent endurance time trials.

3. Measurement procedures allowed for detection of ROS in the body.
4. Increased performance in the endurance time trials following GTE ingestion was due to decreased fatigue from increased ROS.

Limitations

The limitations of this study are as follows:

1. Subjects may have experienced a learning effect over the course of the study, leading to increased performance in later endurance time trials.
2. The determination of a single best assay to detect ROS has not been described in the literature.

CHAPTER II

REVIEW OF LITERATURE

It has not yet been determined if green tea extract will have an antioxidant effect on endurance exercise performance at moderate altitude. This literature review will discuss the following: (1) the production and effects of ROS during exercise; (2) the production and effects of ROS in hypoxic conditions; (3) the effects of antioxidants on ROS generated in hypoxic conditions; and (4) the effects of antioxidants on ROS generated by high intensity exercise.

Reactive oxygen species production during exercise and fatigue

Reactive oxygen species are known to cause oxidative stress during exercise. The methods by which this occurs are widely debated and will be discussed here.

One of the most widely accepted mechanisms of ROS production is by the increase release of catecholamine hormones during exercise. The hormones oxidize automatically and are shown to form ROS (Cooper, et al., 2002). Catecholamines are used to handle the exercise response of increasing heart rate and blood pressure as well as allowing for increased blood flow to working muscles.

Another mechanism of ROS production during exercise is the production of mitochondrial superoxide, by reactions of flavin and/or ubisemiquinone radicals with oxygen (Sjodin, Hellsten Westing, & Apple, 1990; Ji, 1999). This mechanism is related to the increased aerobic metabolism occurring in the mitochondria during endurance exercise.

A third method of ROS production during exercise is by a process known as ischemia-reperfusion. Ischemia-reperfusion occurs at very high workloads, which causes blood and oxygen to be directed away from certain tissues and organs and towards working muscles and the skin. This condition causes relative, transient hypoxia in the non-working tissues. Hypoxia can also occur in muscle tissues at intensities higher than maximal oxygen consumption, at which point the energy demand exceeds the oxygen supply (Koyama, et al.,1999). At the cessation of exercise, the restoration of oxygen has been associated with the production of ROS (Packer, 1997; Koyama, et al., 1999). This reperfusion of oxygen leads to increased production of ROS by the conversion of xanthine dehydrogenase to xanthine oxidase. This conversion serves as the catalyst for the production of urea, post exercise. This mechanism of ROS production does not actually produce ROS during exercise, but rather several hours post exercise, but still form exercise-induced free radicals.

A final mechanism by which free radicals may be formed during exercise is by the oxidation of hemoglobin and myoglobin during exercise. Oxidation of these oxygen-carrying molecules has been observed during periods of high activity and is shown to produce ROS, both primarily, and by increasing the reactivity of ROS generated by other mechanisms (Reeder & Wilson, 2001). Oxidation of hemoglobin and myoglobin is dependant on the pressure of oxygen in the blood. The decreases in capillary and venous oxygen pressures associated with exercise leads to an increased production of ROS by the oxidation of hemoglobin and myoglobin (Svistynenko, et al., 1997).

Altitude, reactive oxygen species, and performance

Researchers have also shown that the hypoxic conditions associated with high altitude lead to an increased production of ROS and increased oxidative damage to tissues (Radak, et al., 1997; Kumar, et al., 1989; Moller, et al., 2001). This finding may be due to various reasons. The first of these reasons is the effect that high altitude has on the body's own antioxidant systems, while the second relates to the fact that high altitude associated hypoxic conditions cause higher production of ROS.

The body has its own antioxidant systems, which help to control ROS. These systems help to convert superoxides (powerful ROS) into less powerful hydrogen peroxide and eventually into water (Bakonyi & Radak, 2004). The body also produces vitamins like vitamin E & C, which are antioxidants themselves. It has been shown that the body's antioxidant capacities decrease with increasing altitude (Radak, et al., 1997). Conversely, it has been shown that antioxidant supplementation can help to restore the antioxidant capacity lost at higher altitudes and reduce oxidative damage to muscle tissues (Schmidt, et al., 2002). In this study an antioxidant mixture of vitamin E, beta-carotene, ascorbic acid, selenium, alpha-liporic acid, N-acetyl 1-cysteine, catechin, lutein, and lycopene was used. This antioxidant mixture must be built up in the body over a period of time to achieve its peak effectiveness.

The second mechanism by which ROS are increased at altitude relates to body systems that increase ROS production at high altitudes. One of the systems that produce ROS is the electron transport system of the mitochondria. ROS are generated because during hypoxia, less oxygen is available to be converted into water by cytochrome oxidase, which causes the accumulation of reducing agents for mitochondrial respiration.

This process is called reductive stress, and leads to ROS production by the oxidation of mitochondria complexes (Mohanraj, et al., 1998). Another mechanism by which ROS are produced during hypoxia is due to the previously described ischemia-reperfusion mechanism previously described. Hypoxia due to deficient atmospheric oxygen pressure can lead to tissue hypoxia, much like exercise-induced hypoxia. Again, this hypoxia leads to the production of ROS after the tissue becomes reoxygenated.

Antioxidant supplementation and performance at altitude

Previous studies have provided equivocal results as to the effects of antioxidant supplementation on performance at moderate and high altitudes. Chao, et al., 1999, showed that while antioxidants could help to control oxidative stress at high altitude, this effect was not completely controlled by traditional antioxidant supplements of vitamin E, vitamin A, beta-carotene, ascorbic acid, selenium, or zinc. Subudhi, et al., 2004, also showed that antioxidant supplementation did not significantly control oxidative stress or ROS that are associated with increased physical activity at high altitude. Pfieffer, et al., 1999, showed that oxidative stress increases with altitude despite elevated intake of dietary and supplemental antioxidants. Urso and Clarkson, 2003, detailed the effects of antioxidant supplementation on exercise and altitude and stated that the effect of antioxidants on increased oxidative stress is not yet clear. None of these studies have investigated GTE as a supplemental antioxidant.

Green tea extract supplementation and performance

Previously, few studies have been completed that investigate the effect of GTE supplementation on exercise performance, much less performance at moderate to high altitude. Murase, et al, completed a series of studies in the rat model, which showed that GTE improved endurance capacity and lipid oxidation during swimming and running exercise in the rat model. Dulloo, et al., 1999, and Berube-Parent, et al., 2005, both showed that green tea extract, when combined with caffeine, increased overall 24-hour energy expenditure and fat oxidation. These studies focused primarily on body composition rather than exercise performance. To the researcher's knowledge, previous studies on the effects of GTE on moderate altitude exercise performance have not been performed.

CHAPTER III

METHODS

Subjects

Subjects were limited to healthy, recreationally active, non-smoking male cyclists. All subjects rode 50-200 miles each week and had maximum oxygen consumption values between 45 and 65 ml/kg/min O_2. Only males were used as subjects in this study, since estrogen has been found to possess antioxidant qualities, during both the luteal and follicular phases of the menstrual cycle (Massafra, et al., 2000; Subbiah, et al., 1993). Subject age was limited to 18 and 36 years, since aging itself has been shown to play a role in increased production of ROS (Sen & Packer, 2000). Subjects were recruited via oral announcements (Appendix A) presented during classroom lectures for students enrolled in Health, Exercise, and Sport Sciences and Personal Fitness and Wellness courses conducted at Texas Tech University's Lubbock campus. All subjects were required to have had a physical exam within the previous 5 years. Subjects were asked to complete a health history questionnaire (Appendix B). Subjects with cardiovascular and/or other contraindications to exercise (ACSM's Guidelines For Exercise Testing and Prescription, 2006) were excluded from the study. Participation in the study was voluntary, and ten subjects participated. The Institutional Review Board of Texas Tech University approved this protocol for use.

Testing procedures

Timeline of the study

Completion of the study required 4 visits to the Exercise Physiology Lab housed within the Exercise Sciences Center on the Texas Tech campus. During visit 1, subjects filled out a health history questionnaire (Appendix B), completed an informed consent form (Appendix C), and performed a graded exercise test (GXT) on an electronically-braked cycle ergometer (Lode, Corival). Visit 2 followed the GXT by no less than 48 hours. During visit 2, the protocol was explained and subjects were familiarized with the testing equipment and procedures. The subject then performed a familiarization time trial (TT-F) without altitude simulation or supplementation. Following the GXT and TT-F (48-72 hours) each subject began a series of two cycle ergometer (Lode, Excalibur Sport) time trial tests (TT), with placebo or GTE supplementation, assigned in a randomized fashion. Each TT was separated by at least 48 hours and each visit to the laboratory required 2-2.5 hours of the subject's time.

Table 1. Timeline schematic of study protocol

Visit 1	Visit 2	Visit 3	Visit 4
Medical History Consent Form, GXT	TT-F	↓, S, TT-1↓	↓, S, TT-2 ↓

↓ = Blood Draw (One hour before and after the TT).
S = Supplementation (the supplement type and time trial condition was randomly assigned)

Graded exercise test

Each subject performed a GXT on a cycle ergometer (Lode, Corival). The test began with the subject pedaling between 60-80 rpm at a resistance of 0 watts for 4

minutes. Thereafter, the exercise intensity was ramped 25 watts each minute until the subject was unable to maintain 60 rpm or the subject desired to stop. At the completion of the GXT, the resistance on the cycle was reduced and the subject was allowed to "cool down" at his own cycling pace.

During the GXT, subjects breathed into a flexible rubber mouthpiece (inserted between the lips and teeth) and the nose was plugged to ensure the subject breathed into and out of the mouthpiece. The mouthpiece allows subjects to breathe in normal room air and exhale into a tube that connects to a gas analyzer and ventilation module. Expired respiratory gases were collected via open circuit spirometry during the GXT for the determination of oxygen consumption (VO_2), CO_2 production (VCO_2), minute ventilation (V_E) and other calculated variables. Expired gas analysis and ventilatory output were determined with the aid of an automated metabolic measurement cart (MedGraphics, CPX-D). Blood pressure (BP) was determined prior to, during (at the end of the warm-up and between stages 2 and 4), and following the GXT via auscultation. Heart rate and rhythm were monitored continuously before, during and after the GXT via electrocardiography (Quinton Model 4500). The maximum amount of watts that the subject reached during the GXT was recorded along with the maximum oxygen consumption (VO_2 max), which was calculated by averaging the breath by breath oxygen consumption over the last thirty seconds of the GXT.

Following the GXT, the subjects were monitored until heart rate and blood pressure returned to normal levels as suggested by ACSM (HR<100bpm; BP = pre-test values) to ensure subject safety upon leaving the testing site.

Supplementation

The GTE was a 400 mg in a capsule containing 82.67% catechins and 55.67% EGCG (Sunphenon 90 DCF, Taiyo Int., Minneapolis, MN). A low calorie dextrose capsule (similar packaging to the GTE) was administered as a placebo. Subjects were administered the GTE or placebo 1 hour before the performance of the TT in a double-blind fashion.

Dietary requirements

Subjects were asked to refrain from consuming the foods and/or beverages listed in Appendix D for three days prior to TT performance testing. These foods and beverages possess antioxidant properties, which may interfere with the green tea supplementation and confound the results (Manach, et al., 2004; Scalbert & Williamson, 2000). Otherwise, participants were asked to follow their normal diet. Subjects were asked to keep a three-day food intake log prior to any TT performance to ensure compliance with the banned food list. Subjects were asked to repeat the same diet the day preceding each of the TTs. Subjects were asked to refrain from using nutritional supplements at least 2 weeks prior to commencing the study and throughout the study. Multivitamins typically contain vitamins E and C, which are also considered antioxidants. Studies are conflicting on the amounts and preparations of these vitamins necessary to yield alterations in antioxidant status (Bloomer, Goldfarb, & McKenzie, 2006; Mastaloudis, et al., 2006). Since multivitamins vary in the amount and preparation of vitamins E and C, as well as many brands specifically adding other antioxidants, subjects were asked to discontinue usage of their multivitamin for the same time course. Subjects were asked to refrain from

using non-steroidal anti-inflammatory medications for 3 days prior to any TT performance. Subjects were asked not to eat food 3 hours prior to any exercise protocol.

Time trial performances

Based on the highest power output (Wmax) achieved on the GXT, each subject was assigned a specific amount of work in joules to be performed on the cycle ergometer (Lode, Excalibur). The total amount of work that the subjects performed was based on 75% of the subjects maximum wattage attained during the GXT. For each TT, the subject was asked to perform the cycle work as fast as possible. The workload is calculated using a modification of a formula originally proposed by Jeukendrup et al., (1996).

Total amount of work for time trial (J) = 0.75· Wmax· 1,200

The time trial workload was configured by setting 75% of the maximum wattage attained during the GXT to a specific pedaling cadence. The pedaling cadence was set to a value that each rider felt comfortable maintaining. If the rider pedaled faster than this cadence, he finished the time trial in under twenty minutes, and if the rider pedaled slower than this cadence, the time trial took longer than twenty minutes. Subjects were encouraged to pedal at least at the twenty minute pace.

Each subject completed a series of 2 randomized TTs each separated by at least 48 hours under 2 different conditions: Placebo + Simulated Moderate Altitude, and GTE + Simulated Moderate Altitude. The simulated moderate altitude TTs were performed in an air-conditioned tent (7'6" X 9'6" X 6', Colorado Altitude Training-CAT430) at a simulated altitude of 2500m (8,200 feet, Vail, CO). This altitude is similar to that of

Mexico City (7,400 feet), which was the site of the 1968 Olympics and also represents a common altitude where endurance performance competitions are performed in the US.

For all trials the GTE or placebo was ingested 1 hour prior to the TT. Before GTE ingestion and 60 minutes after each TT, a 10 ml (1-3 teaspoons) blood sample was drawn from an antecubital vein for the determination of ROS. The blood sample was taken one hour after the time trial since research has shown that markers of oxidative stress peak at this time (Michailidis, et al., 2007). Blood pressure (BP) was determined prior to and following the TT via auscultation. Heart rate, rhythm (Schiller-Cordovi/AT-10) and oxygen saturation levels (Biox) were continuously monitored prior to, during, and following all TT performances. Following the TT, the subjects were monitored until heart rate and blood pressure returned to normal levels as suggested by ACSM (HR<100bpm; BP = pre-test values) to ensure subject safety upon leaving the testing site.

Blood collection

As previously explained, immediately prior to supplementation of GTE and 1 hour following TT-1 and TT-2, approximately 10 ml of blood was collected via venipuncture from either the left or right antecubital vein. 5 ml of blood was placed in heprinized tubes, held on ice for 10 minutes, and spun in a centrifuge for ten minutes at 3,000 rpm to separate the plasma. The plasma was then be removed from the samples, and frozen at -80°C for further analysis.

Assay-Malondialdehyde

Lipid peroxidation was measured using a modified TBARS-MDA method. Butylated hydroxytouluene (BHT) was added before TBA reagents to prevent an increase of peroxidation during the assay procedure. BHT also allows for variations in sample lipid content and contamination from iron content of the sample. Plasma samples were thawed to room temperature and 250 microliters were placed into a microtube with 10 microliters of BHT. A 1:1 mixture of 500 microliters of phosphoric acid and TBA reagent was then added to the microtube. The microtube was then vortexed and incubated in a 60°C hot water bath for one hour. The sample was then centrifuged at 10,000 g for three minutes. Finally the sample was pipetted into a 96 well plate. The samples were analyzed on a plate reader (Spectromax 384 Plus, Molecular Devices, Sunnyvale,CA) at 532 nm. The absorbance readings were then converted to μM concentrations by an established procedure, multiplying the absorbance reading by a factor of 12.5.

Data analysis

Descriptive statistics were generated from the anthropometric and fitness data obtained during the GXT. To determine if group differences exist, data was analyzed via a 2x2 (condition by supplement) repeated measures ANOVA and paired t-tests. Student-Newman-Kuels post-hoc analysis was used to further test significance when necessary. Pearson correlation coefficients were generated to determine associations between variables of interest. All statistical tests were performed using SigmaStat for Windows

(Jandel Scientific Software, SPSS Inc., Chicago, IL.) For all statistical tests a p-value of <0.05 was considered significant.

CHAPTER IV

RESULTS

Time trial performance

Cycling time trial performance at moderate altitude was significantly improved ($p<0.01$) with GTE supplementation. The mean improvement in performance was a 28.9 (± 22.2) second improvement in trial time trial performance compared with the placebo trial (Figure 1). Correspondingly, the mean power output for the subjects was significantly higher ($p<0.01$) when supplemented with GTE (6.97 ± 3.93 W). All but one subject showed an improvement in performance time and mean power output with GTE supplementation (Figure 2).

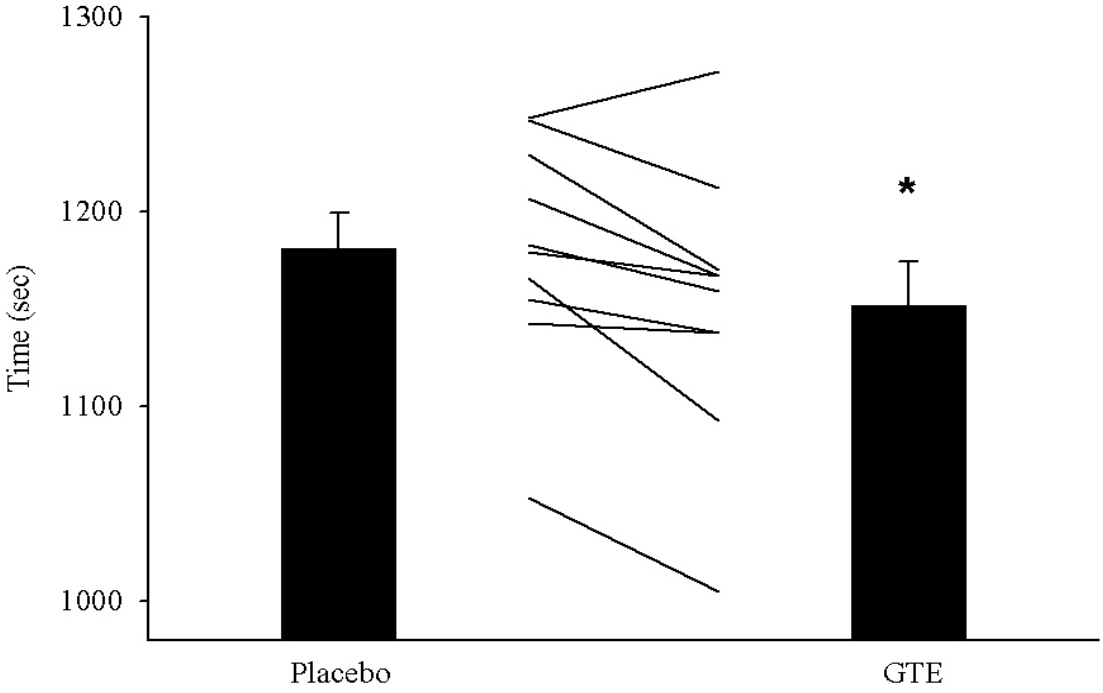

Figure 1. Time trial performance times by condition

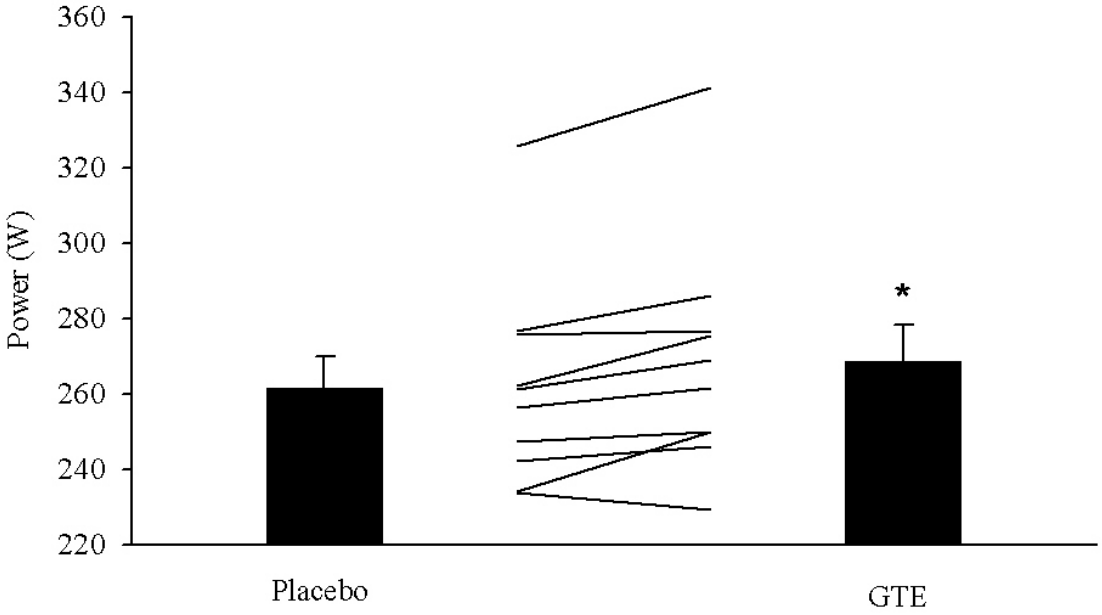

Figure 2. Time trial mean power output by condition

Cardiorespiratory measurements during the time trials

There were no significant differences in heart rate (HR) between the conditions (Table 2; Figure 4). HR did increase significantly ($p<0.05$) over the time course of both trials, but there were no significant differences between the subjects or the conditions.

There were also no significant differences found between subjects or conditions for percent heart rate max (%HRMax), calculated from the maximum HR attained during the GXT (Table 2; Figure 5). There was a significant ($p<0.05$) increase over the time course of the trials for this variable.

Oxygen saturation (SaO_2) significantly ($p<0.01$) decreased over the time course of the trials for both conditions, but there were not any significant differences between the subjects or the conditions (Table 2; Figure 6).

Ratings of Perceived Exertion (RPE) significantly ($p<0.05$) increased over each stage of the time trial, but no significant differences between the subjects or conditions were detected (Table 2).

Oxidative stress

No significant differences were found in plasma malondialdehyde (MDA) levels between the conditions or subjects (Figure 3). There were also no significant differences between the MDA levels prior to and after completing the time trial.

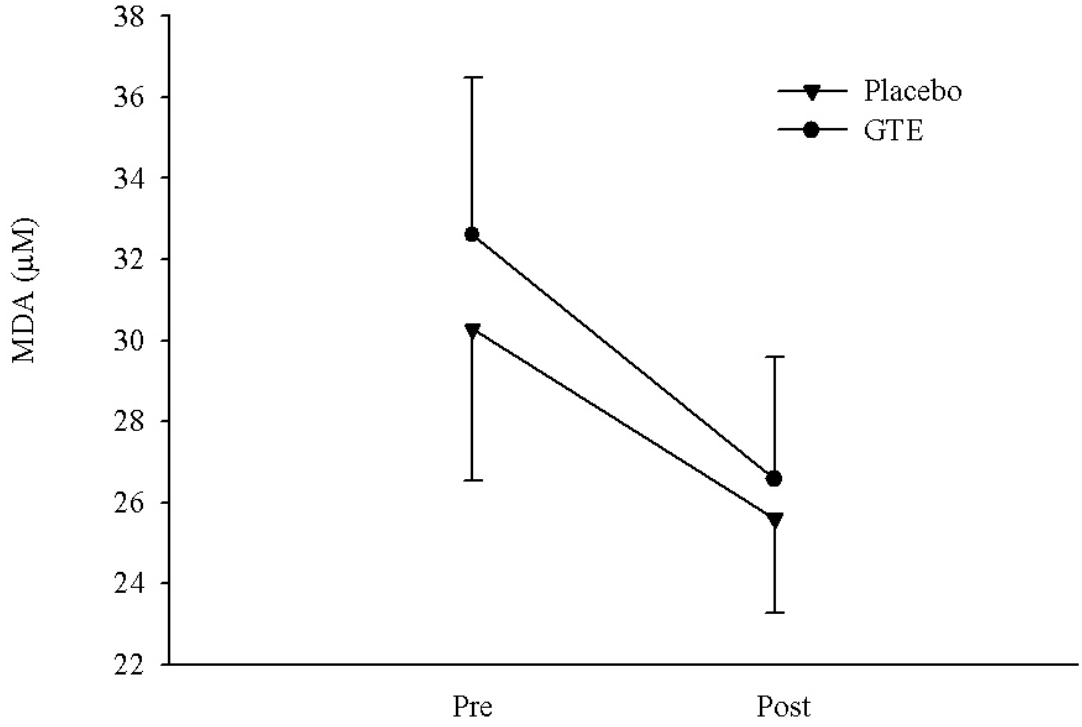

Figure 3. Malondialdehyde (MDA) concentration by condition and time

Associations between major variables

There were no significant correlations detected between the major variables of interest.

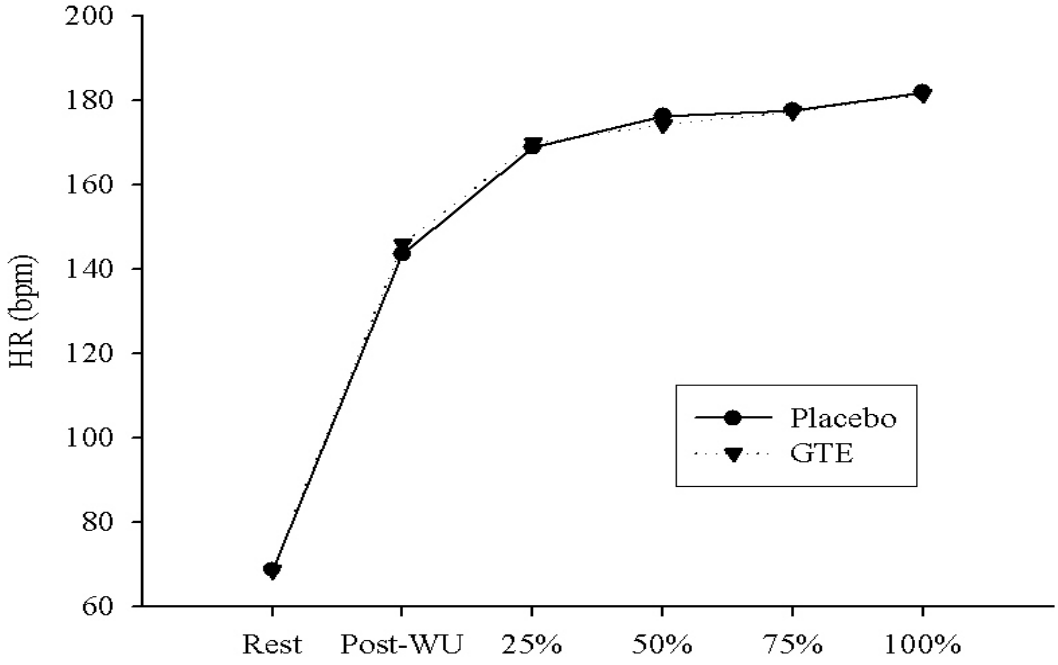

Figure 4. HR response

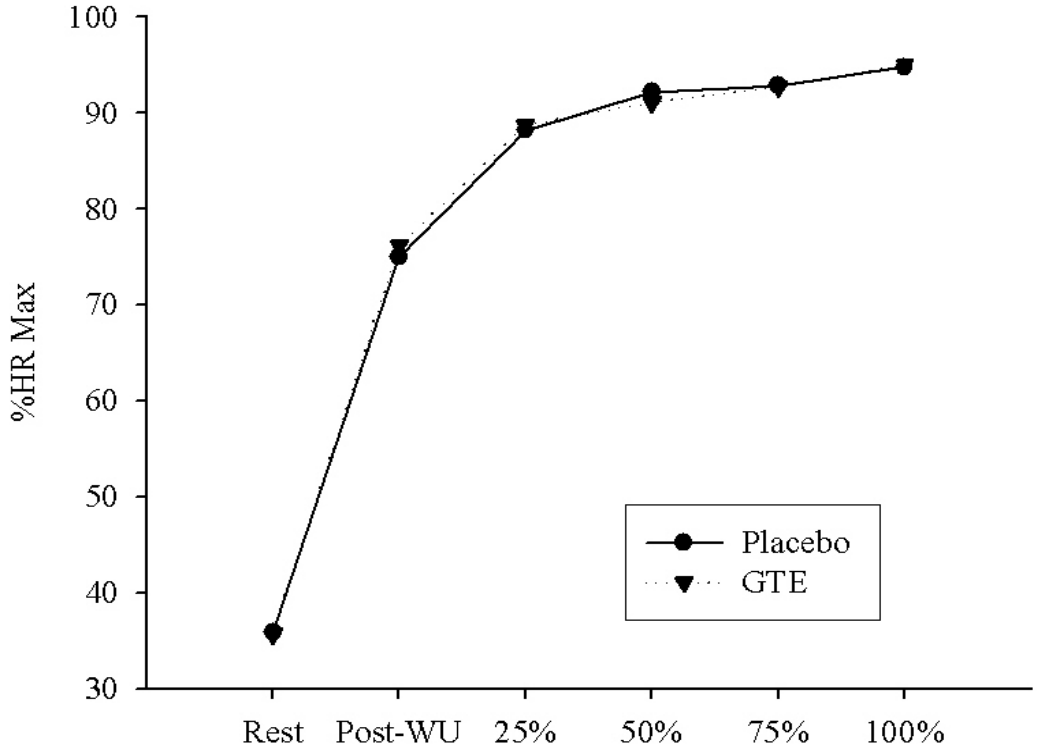

Figure 5. % HR Max vs. time

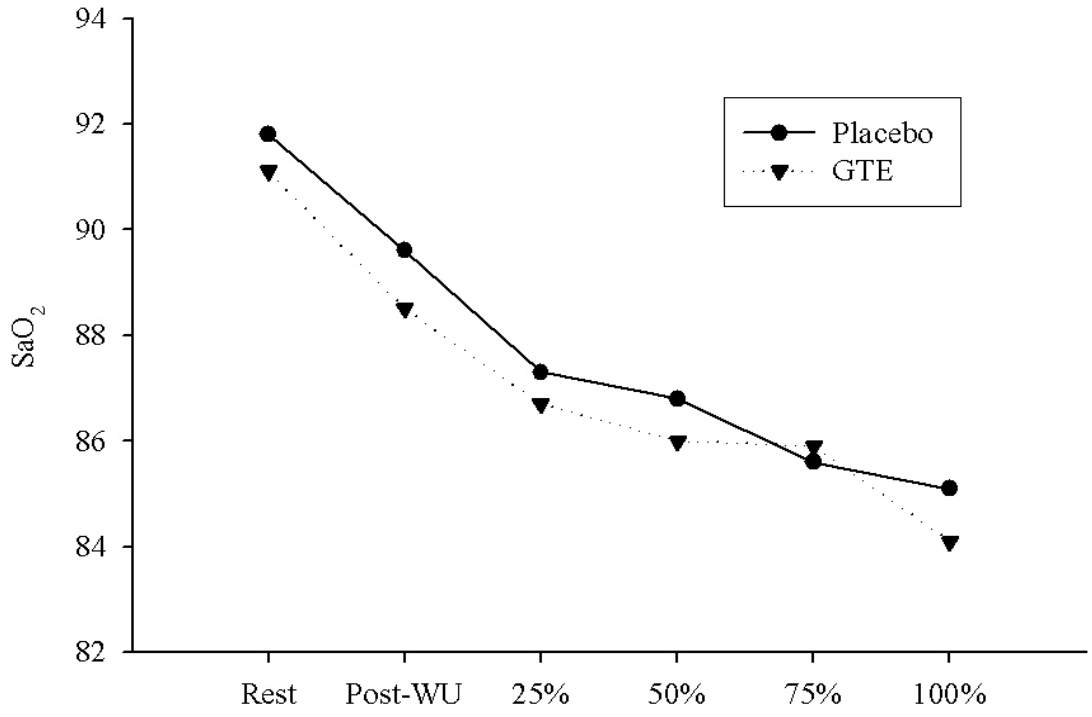

Figure 6. SaO_2 vs. time

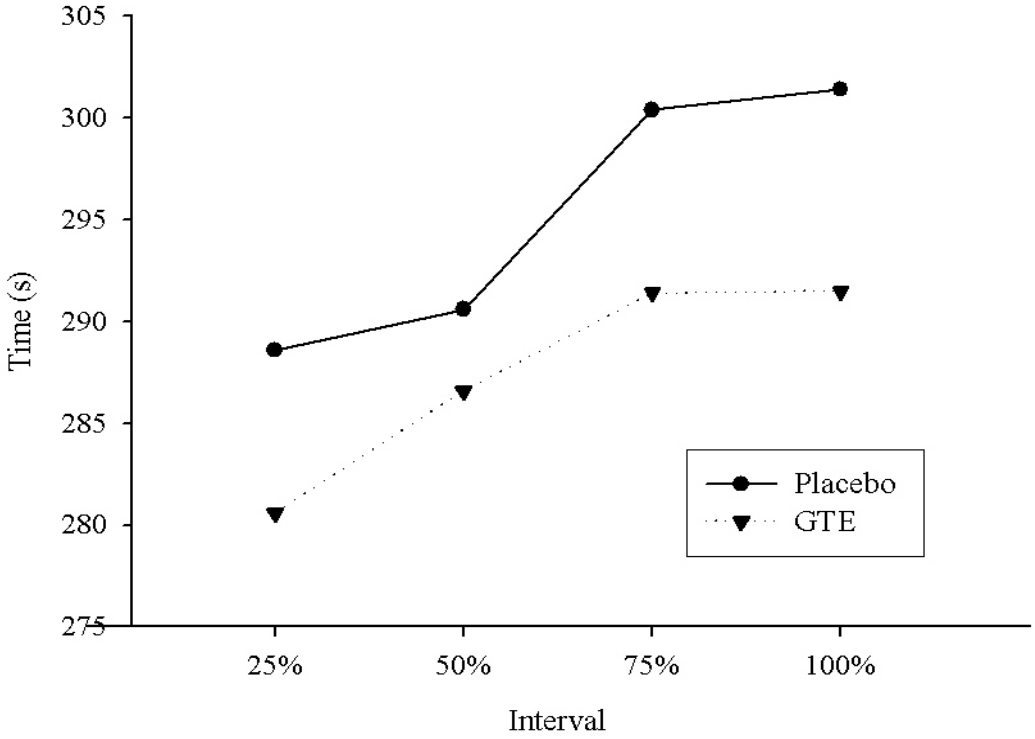

Figure 7. Time trial interval times

Table 2. Cardiorespiratory values

[All values= Mean (SE)]

Treatment-Time	HR	%HRMax	SaO_2	RPE	Time (s)
Placebo-Rest	68.8 (0.9)	35.9 (0.5)	91.8 (0.9)		
GTE-Rest	68.5 (0.8)	35.7 (0.4)	91.1 (0.6)		
Placebo-Post Warmup	143.5 (1.2)	75.0 (0.6)	89.6 (0.7)		
GTE-Post Warm-up	145.9 (1.5)	76.2 (0.8)	88.5 (0.6)		
Placebo-25% Complete	168.8 (0.8)	88.2 (0.5)	87.3 (0.9)	13.9 (0.6)	288.6 (4.3)
GTE-25% Complete	169.9 (0.8)	88.8 (0.4)	86.7 (0.6)	13.3 (0.8)	280.6 (7.6)
Placebo-50% Complete	176.3 (0.7)	92.2 (0.4)	86.8 (0.7)	16.3 (0.6)	290.6 (4.3)
GTE-50% Complete	174.2 (0.8)	91.1 (0.4)	86.0 (1.1)	15.2 (0.7)	286.8 (4.6)
Placebo-75% Complete	177.7 (0.7)	92.9 (0.4)	85.6 (0.6)	17.8 (0.5)	300.4 (5.8)
GTE-75% Complete	177.3 (0.7)	92.7 (0.4)	85.9 (1.1)	17.3 (0.5)	291.4 (5.8)
Placebo-100% Complete	181.9 (0.7)	94.8 (0.5)	85.1 (0.9)		301.4 (6.9)
GTE-100% Complete	181.4 (0.5)	95.0 (0.3)	84.1 (1.2)		291.5 (7.6)

CHAPTER V

DISCUSSION

The main finding of this study is the significant improvement in time trial performance associated with GTE supplementation at moderate altitude. (Figure 1). Subjects were able to ride at, and maintain, a higher intensity workload over the course of the study, as is evidenced by the increase in mean power output (Figure 2).

The cause for the improvement in time trial performance is not readily known. Table 2 indicates that for each exercise interval, the GTE riders were faster and had a higher mean power output; however this finding was not significant, though displays an interesting trend. Figure 7 indicates that along the course of the time trial, the riders slowed down gradually, presumably from the effects of skeletal muscle fatigue, though again this trend is not significant. Since there were no significant differences in HR, %HR Max, SaO_2, and RPE (Table 2), it is difficult to ascertain why the subjects were able to perform at a higher intensity while supplemented with GTE.

Usually a given workload is associated with a specific HR for each individual, thus the increase in performance without a corresponding increase in HR between the conditions of the study provides evidence that the results are possibly due to GTE staving off the effects of skeletal muscle fatigue.

The increase in HR and % HR Max over the course time course of the study (Figure 4, Figure 5) brings each of these values to near each of the subject's physiological limits, indicating that this was indeed very high intensity exercise, which should produce ROS. However, an increase in ROS or oxidative stress, as determined by MDA, was not apparent in this study (Figure 3).

The measured decrease in SaO_2 over the time course of the study is evidence that the subjects were exercising at a high intensity at a simulated high altitude (Table 2, Figure 6). This decrease in SaO_2 indicates that subjects were becoming desaturated with oxygen as the higher altitude caused the PO_2 in the surroundings to decrease and cause oxygen not to enter the bloodstream as readily. Higher intensity exercise also causes muscle tissue to pull more oxygen out of the blood to fuel aerobic energy pathways. SaO_2 levels have been shown not to decrease with exercise at sea level in untrained and moderately trained athletes, but may decrease slightly in highly trained athletes (Powers, et al, 1988;). The levels to which the subjects desaturated are below those associated with exercise-induced hypoxemia in elite athletes, demonstrating the effect of moderate altitude on oxygen saturation (Dempsey & Wagner, 1999).

Interestingly, MDA levels were not significantly different between the two trials, nor before or after the trials (Figure 3), which gives rise to the notion that MDA may not be the best marker for detecting ROS for this type of exercise and subject. MDA was used in this study because it is one of the most widely used markers for oxidative stress. It has been shown that ROS production increases with both high intensity exercise (Cooper, et al., 2002) and altitude associated hypoxia (Askew, 2002). Since we did not see a significant increase in MDA levels post exercise in the placebo trial, it is possible that MDA was not an appropriate marker for this type of exercise or population. There has been wide speculation about the time course for measuring MDA post exercise. Some studies have shown increased levels of MDA immediately after exercise (Childs, et al., 2001; Bloomer, Goldfarb, & McKenzie, 2006), while another study found that MDA levels did not increase until 42 hours after exercise (Close, et al., 2004). This study

utilized a one-hour time course for measuring MDA because of a study that found that one-hour post exercise was the ideal time course for measurement of MDA after endurance exercise (Michailidis, et al., 2007).

A review of the literature shows other markers that could have possibly been effected. Other common markers of oxidative stress include isoprostanes and protein carbonyls, and have shown promise in detecting oxidative stress and reactive oxygen species. Isoprostanes are formed by the breakdown of fatty acids associated with endurance exercise. Mass spectrometry is typically used to measure isoprostanes, but assay kits are also available to quantify isoprostane levels. Isoprostanes peak immediately after exercise and within one hour return to resting levels (Watson, et al., 2005). Protein carbonyls, formed from the breakdown of amio acids, have been shown to be elevated during and immediately after high intensity endurance exercise in humans (Radak, et al., 2003) as well as up to three hours after endurance exercise in rats (Nagasawa, et al., 2000). Eccentric anaerobic exercise has been shown to raise protein carbonyl levels up to 48 hours after exercise (Bloomer, Goldfarb, & McKenzie, 2006; Lee, et al., 2002). It is still not clear how the assay type is affected by the time course of sampling for markers of oxidative stress, or type of exercise performed.

A recent study (Bellinger, et al., 2008) has explored the notion that fatigue is actually due to ROS causing the sarcoplasmic reticulum to "leak" calcium instead of the controlled firing of the calcium ion. This type of ROS generated fatigue is not measureable by a blood assay, but would require muscle biopsy prior to, during, and after exercise for analysis. Figure 7 shows the fatigue process of the subjects over the course of the time trial. It is possible GTE supplementation was effective in preventing calcium

leak and thus was the mechanism by which performance improved, possibly without seeing differences in plasma MDA levels. GTE may have functioned to enhance performance through a mechanism independent of ROS production.

Studies exploring the effects of antioxidant supplementation on exercise performance have been varied and ambiguous. It has been shown that the antioxidants of vitamin A, beta-carotene, ascorbic acid, selenium, and zinc could help to control oxidative stress (measured using MDA, pentane, hydroxydeoxyguanasine, and lipid peroxide concentrations) at high altitude, but these effects were not enough to return levels of oxidative stress to those of much lower altitudes (Chao, et al., 1999). Another study showed that antioxidant supplementation did not control oxidative stress (measured by lipid peroxide and glutathione concentrations) caused by physical activity at high altitude (Subudhi, et al., 2004). Schmidt, et al, 2001, showed that supplementation with a "cocktail" of antioxidant supplementation can help to restore the body's antioxidant capacity that is lost at high altitude and reduce the associated oxidative muscle damage (measured with breath pentane, serum lipid hydroperoxides, and urine MDA concentrations). Conversely, Pfieffer, et al, 1999 saw significant increases in oxidative stress (measured via MDA, hydroxynonenal, hydrodeoxyguanosine, and lipid peroxide concentrations) associated with high altitude even though antioxidant intake was increased.

Given the demands of the endurance exercise and high altitude and previous literature, one would hypothesize that ROS were produced. It is possible that the marker (MDA) used in this study missed the increase in oxidative stress, since the blood draw was taken one hour post exercise, and as previously mentioned, the time course for peak

MDA levels is under debate. Isoprostanes and protein carbonyls may have had better success and been a better marker of oxidative stress for this type of exercise or population. Results also could have possibly been as expected if a different time course for MDA measurement, or other markers of oxidative stress, was used.

Further analysis should be completed to determine the time course of GTE supplementation to allow for optimum performance at moderate altitude. Research also must be completed to determine the best assay to evaluate oxidative stress in the human body.

CHAPTER VI

CONCLUSIONS

This study has demonstrated that GTE supplementation improves cycling time trial performance at moderate altitude, but does not pinpoint a mechanism by which this outcome is accomplished.

GTE supplementation was able to increase the mean power output each subject was able to maintain over the course of the time trial, and trends possibly indicate that these time trials were faster from the beginning. Again the MDA assay did not provide conclusive results as to the ROS scavenging effect of GTE. This study used a random, double blind design in an attempt to control for selection and experimental bias. Since the HR, SaO_2, and RPE responses were consistent between the two conditions, it lends credibility to the notion that this study was not biased by the researcher's input or "coaching".

Finding an assay or collection of assays suitable for measuring ROS production and concentration, as well as oxidative stress in the human body could possibly strengthen the position that GTE improves endurance exercise performance, if either by ROS species scavenging, or another mechanism completely. Research also should be completed to determine the time course for best measuring ROS and oxidative stress post exercise.

Further research should be completed to determine if GTE supplementation improves performance at lower altitudes; lower intensity, longer exercise duration; and when anaerobic exercise is performed.

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APPENDIX A

ORAL ANNOUNCEMENT

Drs. Robert Sawyer and Jim Williams are conducting a study for which we are seeking volunteers. The purpose of this study is to determine the effects of green tea antioxidants on exercise performance at an altitude of 3200 feet and at a simulated moderate altitude of 8200 feet in young healthy males. The results of this study will contribute to our understanding of antioxidant supplementation and exercise. Participation in this study will require performance of an oxygen consumption test and 4 cycle ergometer time trials and 8 breathing tests. In addition, you will be asked to provide 8 blood samples, 1 before and 1 after each time trial (~ 2 teaspoons/sample**).**

You will be asked to refrain from certain foods and drinks high in antioxidants 3 days prior to the test days and to discontinue use of multivitamins and any other nutrition supplements you may be using 2 weeks prior to any testing and for the duration of the study. A food journal will be kept for 3 days prior to the time trials.

You will be asked to maintain your normal activity habits for the duration of this study. In addition it will require six visits to the Exercise Physiology Laboratory housed within the Exercise Sciences Center on the Texas Tech campus. Each visit will require approximately 2-2.5 hours of your time. The first visit will include completion of a medical history questionnaire and consent form, and familiarization with the testing equipment and procedures. Visit 2 will consist of an oxygen consumption test. Visits 3-6 will be cycling time trials with either a green tea extract or placebo supplement, performed at our current altitude or at simulated moderate altitude in a large tent. A blood sample will be collected before and after the time trials.

Recruitment for this study is limited to young (18-35 yrs), healthy, male subjects who are recreationally active. Anyone willing to participate must be free of any known

disease, not currently experiencing any musculoskeletal problems, and be a non-smoker. You must have had a physical exam within the last 5 years to be a participant in the study. As a participant of this study, you will receive information regarding your current level of aerobic fitness. There is no monetary compensation or "extra credit" provided for participation in this study. There is absolutely no penalty in your courses at Texas Tech or from the HESS department should you decline to participate. Participation in this study is completely voluntary and subjects are free to quit the study at any time with no penalty.

Thank you for your attention and time. I will now respond to any questions you may have concerning this study and your interest in participating

APPENDIX B

HEALTH HISTORY QUESTIONNAIRE

Demographic Information
Last Name _____

First Name _____

Middle Initial _____

Date of Birth _____

Gender _____

Home Phone _____

Address _____

City _____

State _____

Zip Code _____

Work Phone _____

Family Physician _____

Section A
1. When was the last time you had a physical examination?

2. If you are allergic to any medications, foods, or other substances, please name them.

3. If you have been told that you have any chronic or serious illnesses, please list them.

4. Are you affected with hemophilia?

5. Give the following information pertaining to the last three times you have been hospitalized.

	Hospitalization 1	Hospitalization 2	Hospitalization 3
Reason for hospitalization			
Month and year of hospitalization			
Hospital			
City and State			

Section B

For question 1-13 have any of the following situations happened during the past 12 months?

1. Has a physician prescribed any form of medication for you? Yes__No__
   If yes were the medications for a cardiovascular conditions? Yes__No__ , were the medications related to joint problems? Yes__No__
2. Has your weight fluctuated more than a few pounds? Yes__No__
3. If yes, did you attempt to bring about this weight change through diet or exercise? Yes__No__
4. Have you experienced any faintness, light-headedness, or blackouts? Yes__No__
   If yes, what were the circumstances?
5. Have you occasionally had trouble sleeping? Yes__No__
6. Have you experienced any blurred vision? Yes__No__
7. Have you had any severe headaches? Yes__No__
8. Have you experienced chronic morning cough? Yes__No__
9. Have you experienced any temporary change in your speech pattern, such as slurring or loss of speech? Yes__No__
10. Have you felt unusually nervous or anxious for no apparent reason? Yes__No__
11. Have you experienced unusual heartbeats such as skipped beats or palpitations? Yes__No__
12. Have you experienced periods in which your heart felt as though it were racing for no apparent reason? Yes__No__
13. Have you experienced shortness or loss of breath while walking with others your own age? Yes__No__
    If yes, explain.

For questions 14-19 are you currently experiencing any of the following situations?

14. Do you experience sudden tingling, numbness, or loss of feeling in your arms, hands, legs, feet, or face? Yes__No__
15. Are your hands or feet sometimes feel cooler than other parts of your body? Yes__No__
16. Do you experience swelling of your feet and ankles? Yes__No__
17. Do you get pains or cramps in your legs? Yes__No__
18. Do you experience any pain or discomfort in your chest? Yes__No__
19. Do you experience any pressure or heaviness in your chest? Yes__No__
20. Have you ever been told that your blood pressure was abnormal? Yes__No__
21. Have you ever been told that your serum cholesterol or triglcyeride level was high? Yes__No__
22. Do you have diabetes? Yes__No__

If yes, how is it controlled? (Check One)

_Dietary means _Insulin injection _Oral medication _Uncontrolled

23. How often would you characterize your stress level as being high? (Check One)

_Occasionally _Frequently _Constantly

24. Have you ever been told that you have any of the following illnesses? Yes__No__
_Myocardial infarction _Arteriosclerosis _Heart disease
_Coronary thrombosis _Rheumatic heart _Heart Attack
_Coronary occlusion _Heart failure _Heart murmur
_Heart block _Aneurysm _Angina
_Heart arrhythmia
25. Have you ever had an injury to a knee? Yes__No___
26. Do you experience knee pain? Yes__ No__

Section C

1. Participants must be physically active healthy men between 18 and 35 years of age. Do you meet these study inclusion criteria? Yes__No__
2. If not, which criteria in #1, above, does not apply to you:

_____
_____
_____

3. Individuals who currently smoke, are overtly obese, have a history of known cardiac, respiratory or metabolic disease or musculoskeletal disease or injury that would limit exercise participation, are currently experiencing a major physical or mental illness, or are currently taking medication for a major physical or mental ailment will be excluded from participating.
Do any of the criteria in the list apply to you? Yes__No__
4. If yes, please specify which criteria in #3, above:

_____
_____
_____
_____

From Vivian H. Heyward, 2002, Advanced Fitness Assessment and Exercise Prescription, 4. ed. (Champaign. IL: Human Kinetics).

Exclusion Criteria

The following conditions will exclude the person from the study unless clarified or cleared by a physician.

Section A Q# 3, Section B #10; chronic or serious illnesses.

Section B Q#s
17, 25, 26; leg or knee pain or injury
18, 19; Chest pain, discomfort, pressure
20; Abnormal blood pressure
24; Heart disease or known symptoms of heart disease

Section C Q#1 No answer and Q#3 yes answer will also exclude participation in the study

Answers to other questions will help the researchers determine if the subject does not fit the apparently healthy criterion.

APPENDIX C

INFORMED CONSENT

You are being invited to participate in the research project entitled: Effects of Green Tea Supplementation on Endurance Exercise Capacity. The people responsible for this research project are: Drs. Robert Sawyer and Jim Williams of the Department of Health, Exercise, and Sport Sciences at Texas Tech University, (806) 742-3371.

I. Purpose and explanation of test:

The purpose of this research is to determine the antioxidant effects of green tea on free radical production and endurance exercise performance. Free radicals have been shown to damage muscle membranes and possibly limit exercise performance, especially at higher altitudes. The antioxidants in green tea may decrease the amount of free radicals produced during exercise and improve exercise performance.

You will be asked to complete the following tests and procedures:

1. A graded exercise test on a cycle ergometer (stationary bike) to determine oxygen consumption and power maintained at 60 rpm.

2. Two time trials on a cycle ergometer at an altitude of ~3200 feet (Lubbock TX) where you will ingest either a placebo (dextrose capsule) or a green tea supplement capsule 1 hour before the ride.

3. Two time trials on a cycle ergometer in a large tent that simulates moderate altitude (~8200 feet, Vail Co) where you will ingest either a placebo (sugar capsule) or green tea supplement capsule 1 hour before the ride.

4. A test to assess the strength of your breathing muscles before and after the time trials.

5. A blood draw (~2 teaspoons) immediately before and after each time trial performance.

All procedures for this study will be conducted in the Exercise Physiology Lab at Texas Tech University Exercise Sciences Center. The first visit will include a complete explanation of the study protocol, completion of the health history questionnaire and consent form, and familiarization with the testing equipment and procedures. This visit will last about 45 minutes. Visit 2 will consist of a graded exercise test (GXT) on a cycle ergometer and will take approximately 1 hour. Starting at least 24 hours after the GXT you will begin a series of cycling time trial tests on visits 3-6. Before and after each time trial you will have a blood draw of ~2 teaspoons taken from an arm vein. Before each time trial you will ingest either a sugar capsule or green tea extract capsule. Two of the time trials will be performed in an altitude tent that simulates oxygen levels at moderate altitude, and two of the time trials will be performed in the normal room environment. Before and after each time trial you will inhale and exhale into a tube to test the strength

of your breathing muscles. Each time trial will take ~2.5 hours and each time trial performance will be separated by ~48 hours.

Before you undergo any exercise procedures, you must certify to the researchers that you are in good health to the best of your knowledge. You must also certify that you have had a physical exam within the last 5 years. You will fill out a medical history questionnaire that will be reviewed by trained professionals prior to undergoing the tests. Based on the medical history you may be disqualified from the study because of increased risk to you during the exercise protocols. Consequently, it is important that you provide complete and accurate responses to the interviewer and recognize that your failure to do so could lead to possible unnecessary injury during the procedures.

During the course of this study you will complete a graded exercise test (GXT) on a cycle ergometer to determine your exercise power output. For the GXT you will cycle at low intensity for 5 minutes as a warm-up, thereafter the intensity will be increased every minute until you can no longer pedal at 60 rpm or higher. For the time trials a total amount of work to be performed will be calculated based on your performance on the GXT. The faster you pedal during the time trial the more work will be accumulated and the sooner the time trial will be completed. During the GXT or time trials you should immediately report to the investigators any symptoms such as fatigue, shortness of breath, or chest discomfort which may appear during the test. You may request that a test be stopped at any point if you feel unusual discomfort or fatigue, or simply choose to stop the test.

For the duration of the GXT you will breathe into a flexible rubber mouthpiece that fits between the teeth and lips (like a snorkel). The mouthpiece allows you to breathe in normal room air and exhale into a tube that connects to an analyzer. You will also have a clip on your nose to ensure that you breathe into the mouthpiece. Samples of your exhaled air during exercise will be collected to properly measure your oxygen consumption in the muscles and breathing capacity. Prior to beginning the GXT and each time trail you will have 4 electrodes put on your upper body that will be connected by 4 cables (leads) to an electrocardiogram recorder which will enable the program personnel to monitor your heart activity. A trained observer will monitor your exercise responses continuously during the course of the test and take readings of the electrocardiogram. Blood pressure will be monitored before and after each time trial, and before, during, and after the GXT.

II. Risks

There exists the possibility of adverse changes during the cycle test and time trial performances. These changes could include abnormal blood pressure, fainting, disorders of heart rhythm, stroke, and very rare instances of heart attack or even death. You may also experience muscle soreness following the cycle test and time trial performances. Every effort will be made to minimize these occurrences by precautions and observations taken during the test. Oxygen and personnel trained in CPR/AED will be available on site

during all exercise sessions. Side effects to green tea and placebo ingestion for some people include headache, nausea, and abdominal pain. There is the possibility of bruising and infection associated with the blood draws.

If this research project causes injury (physical, psychological, financial, etc), Texas Tech University or the Student Health Center, may not be able to treat your injury. You will have to pay for treatment from your own insurance. The university does not have insurance to cover such injuries. More information about these matters may be obtained from Dr. Kathleen Harris, Associate Vice President for Research, (806)742-3884, Room 203 Holden Hall, Texas Tech University, Lubbock, Texas 79409.

III. Benefits to be expected

The results of this test may or may not benefit you. Potential benefits relate mainly to your personal motives for taking the test, that is, knowing your exercise capacity in relation to the general population, understanding your fitness for certain sports and recreational activities, planning your physical conditioning program, or evaluating the effects of your recent physical activity habits.

IV. Confidentiality and use of information

All information obtained from these testing procedures will be treated as privileged and confidential and will consequently not be released or revealed to any person without your express written consent. By signing this form, you are agreeing to the use of any data recorded for research or statistical purposes so long as it does not provide facts that could lead to your identification. Your initials or a numeric code will be used as an identifier for statistical purposes and these will be deleted upon completion of the analysis. The information contained in the medical history form will be maintained with complete confidentiality during the course of the study and destroyed after 2 years of completion of the study. Any other information obtained, however, will be used only by the program staff to evaluate your exercise status or needs.

V. Inquiries and freedom of consent

Drs. Sawyer or Williams will answer any question that you may have about this study. For questions about your rights as a subject or about injuries caused by this research, you should contact the TTU Institutional Review Board for the Protection of Human Subjects, Office of Research Services, Texas Tech University, Lubbock, Texas 79409. Or you can call (806) 743-3884.

Participation in this research project is voluntary and refusal to participate involves no penalty or loss of benefits to which you may be entitled and you may discontinue participation at any time without penalty or loss of benefits.

By signing this form, you are acknowledging that you have read this document in its entirety and that the investigator reviewing this document has made certain that you understand it.

Participant's signature _____ Date _____

This consent form is not valid after _____.

APPENDIX D

FOODS AND BEVERAGES TO BE AVOIDED THREE DAYS PRIOR TO TESTING

Alcohol
Tea
Coffee
Orange
Pomegranate
Blueberry
Blackberry
Cherry
Soy Products
Beans
Chocolate

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Agree (Permission is granted.)

Student Signature	Date

Disagree (Permission is not granted.)

Michael Derek Pugh	June 23, 2008
Student Signature	Date