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Effect of hydroxytyrosol supplementation on mitochondrial biogenesis, aerobic capacity and endurance exercise performance in healthy men

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Effect of hydroxytyrosol supplementation on mitochondrial biogenesis, aerobic capacity and endurance exercise performance in healthy men

by

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Abstract

Effect of hydroxytyrosol supplementation on mitochondrial biogenesis, aerobic capacity and endurance exercise performance in healthy men

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The University of Texas at Austin, 2011

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The purpose of this study was to investigate the effects of hydroxytyrosol (HT) supplementation on markers of mitochondrial biogenesis, aerobic capacity, and endurance exercise performance in recreationally active men. Sixty-one (n = 61) subjects (21.46 ± 0.22 yrs, 179.46 ± 0.79 cm, 78.91 ± 1.19 kg) consumed either a high dose (HI) HT supplement (150 mg HT), a low dose (LO) HT supplement (50 mg HT), or a placebo (PLA) every day for 6 weeks. Muscle biopsies from the vastus lateralis were obtained at baseline and after 6 weeks of supplement consumption and analyzed for markers of mitochondrial biogenesis: succinate dehydrogenase (SDH), citrate synthase (CS), and peroxisome proliferator-activated receptor ɣ coactivator (PGC)-1α. Subjects completed exercise testing on a bicycle ergometer at baseline and after 3 and 6 weeks of supplement consumption to measure changes in maximal aerobic

power (VO_{2MAX}), lactate threshold, respiratory exchange ratio (RER), substrate utilization, and endurance exercise performance on a 20 km time trial course. The primary findings were that HT supplementation increased muscle oxidative enzyme activity suggesting increased oxidative capacity. HT also increased time trial performance at midpoint and endpoint and this corresponded with an improvement in lactate threshold and a lower RER for the LO HT treatment. Time trial performance was also improved at endpoint for PLA, however, unlike LO an HI HT, this was accompanied by a significant increase in rating of perceived exercise (RPE) and not associated with improvements in muscle oxidative capacity. Our results indicate that HT ranging from 50 to 150 mg/day for 6 weeks can improve muscle oxidative capacity and aerobic performance, and suggests that HT may be used chronically to improve mitochondrial function. HT may be used as an effective means to increase mitochondria to improve exercise performance, and limit diseases associated with mitochondrial dysfunction such as cardiovascular disease, type II diabetes, and some cancers.

KEY WORDS: Hydroxytyrosol, mitochondrial biogenesis, aerobic metabolism, endurance exercise performance

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INTRODUCTION

Adherence to a Mediterranean diet may improve longevity and reduce the risk of diseases associated with mitochondrial dysfunction and aging, such as cardiovascular disease, type II diabetes, and cancer (Trichopoulou et al., 2003). It appears that hydroxytyrosol (HT) (*Figure 1*), a polyphenolic component of extra virgin olive oil, is partly responsible for these health benefits (Granados et al., 2010; Paiva-Martins et al., 2011). HT appears to exert its beneficial effects by increasing mitochondrial biogenesis and function inside the cell via the activation of 5'-AMP activated protein kinase (AMPK) and downstream transcription factor peroxisome proliferator-activated receptor ɣ coactivator (PGC)-1α (Hao et al., 2009; Zhu et al., 2010). The protective effects of HT have been clearly demonstrated in vitro and in animal models, but there is no research to-date showing HT-induced mitochondrial biogenesis in humans.

Most investigators agree that HT is biologically available to humans upon ingestion (Visioli et al., 2000; Pinto et al., 2006); however it is debated whether the amount of HT required to elicit its potential biological activities can be practically achieved in humans (Vissers et al., 2004; Khymenets et al., 2010). HT treatment of isolated cells at concentrations of as little as 1 µmol/L increased the expression of mitochondrial complexes I through IV in isolated adipocytes. These increases in mitochondrial complex protein expression were approximately 1.5- to 1.7-fold compared to control samples (Hao et al., 2009). HT administered to rats at a concentration of 0.5 mg/kg body weight 5 days per week for 6 weeks altered expression of genes related to apoptosis, cell cycle, proliferation, differentiation, survival and transformation pathways (Granados et al., 2011). Data from an unpublished HT supplementation study showed a significant difference on measured metabolic markers between placebo and 200 mg HT up to 90

minutes after supplementation with an observed effect size of .7 at 60 minutes and .85 at 30 min. Based on these findings, we sought to determine whether HT-induced mitochondrial biogenesis and improved endurance exercise performance could be demonstrated in humans.

Therefore, the purpose of this study was threefold: 1) to investigate if regular daily HT supplementation would increase markers of MT biogenesis and density in skeletal muscle: succinate dehydrogenase (SDH), citrate synthase (CS), and PGC-1α; 2) to determine if regular daily HT supplementation would increase maximal aerobic power (VO_{2MAX}), lactate threshold (LT), and endurance exercise time trial performance; and 3) to determine whether a regular high-dose (HI) HT supplement (150 mg/day) affects skeletal muscle markers of aerobic metabolism, VO_{2MAX}, LT, and time trial performance differently than a regular low-dose (LO) HT supplement (50 mg/day). All research was conducted on recreationally active, healthy males between the ages of 20 and 35 years.

METHODS

Experimental Design

This study was a double-blinded, parallel, randomized placebo-controlled study. Subjects consumed daily supplementation of a high dose (HI) of HT (150mg), a low dose (LO) of HT (50mg), or a placebo (PL) in capsule form for six weeks. Subjects refrained from consumption of specific foods including olive oil that contain high levels of antioxidants or polyphenols throughout the course of the study. Subjects were encouraged to maintain normal dietary and exercise habits.

Specific proteins related to mitochondrial biogenesis and function (SDH, CS, and PGC-1α) were measured directly by muscle biopsies at baseline and endpoint. Maximal aerobic capacity was determined by a VO_{2MAX} test on a bicycle ergometer at baseline, midpoint, and endpoint. Additionally, subjects completed a lactate threshold test as an indicator of aerobic capacity and exercise performance at baseline, midpoint, and endpoint. RER was measured during steady state exercise at baseline, midpoint, and endpoint to determine if there was a shift in substrate utilization at 65% of VO_{2MAX} over the course of the study. Endurance exercise performance, measured as time to complete a 20 km time trial on a bicycle ergometer, was also evaluated at baseline, midpoint, and endpoint (*Figure 2*).

Subjects

A total of 61 subjects were enrolled in this study: 3 groups, n = 19, 20, and 22. Recreationally active men between 20 and 35 years of age were recruited from The University of

Texas at Austin and the Austin, Texas community. Investigators posted flyers in UT buildings to recruit subjects from UT faculty, staff and students, and advertisements were posted on internet job databases to recruit additional subjects from the Austin, Texas community. Refer to *Table 1* for detailed subject characteristics.

This study was approved by the Institutional Review Board at The University of Texas at Austin prior to any subject recruitment or screening procedures. Upon an initial phone consultation, the individual came into the Fitness Institute of Texas (FIT), Bellmont Hall, The University of Texas at Austin. A detailed explanation of the experimental procedures and the potential risks of the study were given both verbally and in writing to all subjects. Subjects were given the opportunity to ask questions before signing the informed consent, according to the protocol described in the University of Texas at Austin's 'Institutional Review Board Procedures Manual for Faculty, Staff and Student Researchers with Human Subjects'. All potential subjects gave informed consent in writing prior to any biomedical research project-specific procedures.

After the informed consent was signed, the potential subject completed the *Participation Health Research Screening Form*. The volunteer was only invited to participate in the study if he answered "no" to questions on the *Participation Health Research Screening Form* pertaining to hypertension, circulatory, liver and kidney conditions and diabetes. Allergy, anti-depressant, and thyroid medications were acceptable only if the dosage had been constant for at least 2 months leading up to the screening. The potential subject was excluded if he was taking medication to treat cardiac, lipid, or hypertensive conditions or to control blood glucose.

The following tests and procedures were performed to determine if the subject qualified to participate:

- Measurement of weight, height, and calculation of body mass index (BMI). To be included in the study, BMI must have been less than 30 at the time of screening.
- Completion of the *Participation Health Research Screening Form*
- Review of medical history, medications and any dietary supplements that the potential subject was using at the time of screening
- Measurement of blood pressure. To be included in this study, blood pressure must have been lower than or equal to 140/90 mmHg at the time of screening.

If the potential subject qualified to participate in this study based on the results of the screening tests and procedures, the remaining visits were scheduled.

Study Supplement

HT is generally recognized as safe and non-toxic (Edwards et al., 2009). The study supplement was manufactured by DSM Nutritional Products (Kaiseraugst, Switzerland) through a purification process of waste water from olive oil processing. This protocol attempted to show the lowest efficacious chronic dose over 6 weeks in men consuming 50 mg HT per day. The high dose of 150 mg is slightly lower than the dose (200 mg pure extract) that has been used in a previous human study by Rietjens et al. (2011) and falls within acceptable daily intake (ADI) recommendations (Edwards et al., 2009).

Following is a description of the study supplement composition:

- Placebo (PLA): 0mg HT—3 capsules with each 333mg of modified starch
- Low-dose HT (LO): 50mg HT—1 capsule with each 333mg investigational product (50mg hydroxytyrosol) and 2 capsules with each 333mg modified starch

- High-dose HT (HI): 150mg HT—3 capsules with each 333mg investigational product (50mg hydroxytyrosol)

On the day of the baseline steady-state time trial test, each subject was randomly assigned to receive one of three double-blinded supplements: PLA (n=19), LO (n=20), or HI (n=22). Randomization was performed by Metronomia (Muenchen, Germany). Group assignments attempted to evenly distribute subjects by weight: if a subject entered the trial and weighed less than 80 kg, the lowest available medication number was assigned. If a subject entered the trial and weighed 80 kg or more, the highest available medication number was assigned.

Each subject was instructed to keep the supplement refrigerated at all times, and to consume 3 capsules at home in the morning with breakfast each day for the remainder of the study. On the days that the subject was scheduled to come to the laboratory for the endpoint muscle biopsy, he was instructed to consume that day's supplement at home precisely 1.5 hr before his appointment time. On the days that the subject was scheduled to come to the laboratory for an LT/VO_{2MAX} or SS/TT test, he was instructed to not consume that day's supplement at home; instead he was to bring the supplement into the laboratory and consume it in the laboratory under the supervision of a researcher. The time of supplement consumption was recorded, and exercise testing began 30 min from the time the supplement was consumed.

If the subject failed to consume a daily supplement dose by 2:00 PM for any reason, he was instructed to not consume that dose, and to leave the dose and its packaging intact in the box. The subject was instructed to return the empty and full supplement packaging to the laboratory at his first blood draw visit. The subject was allowed to miss no more than one dose per week and must have been at least 85% compliant (must have consumed at least 36 out of 42

provided supplement doses) over the entire study period. All study subjects met the minimum supplementation compliance requirements.

Dietary and Exercise Regimen

Each subject was required to refrain from consumption of foods or supplements containing high levels of antioxidants or polyphenols throughout the course of the study. Subjects were limited to 1 tbsp of olive oil per day, 1 cup of coffee or tea per day, 2 glasses of wine per week, and 2 chocolate bars per week.

The subject was required to avoid consuming coffee, tea, chocolate, wine, cherry tomatoes, broccoli, blueberries, and onions for at least 24 hr prior to each series of laboratory testing and on each day of laboratory testing, and to meet fasting requirements for each specific visit.

Additionally, the subject was required to avoid performing strenuous exercise beginning 24 hr prior to each series of laboratory testing (baseline, midpoint, and endpoint) and safety blood draw, and on each day of laboratory testing, with the exception of exercise performed in the laboratory as part of the study protocol.

Otherwise, the subject was required to maintain normal dietary and exercise habits over the course of the study. The subject was required to complete food/medication/exercise logs beginning 2 days prior to each series of laboratory testing (baseline, midpoint, and endpoint) and on each day of laboratory testing and to replicate his baseline diet and exercise for his subsequent midpoint and endpoint visits.

Daily Log Procedures

Each subject maintained a daily online log (Zoomerang, San Francisco, CA), in which he reported the time that he consumed his supplement each day, any exercise performed on the previous day, and any adverse events that may have occurred since the previous day. The log also contained a series of questions surveying his overall emotional and physical well-being for that day using a condensed version of the Profile of Mood States (POMS) scale (McNair and Droppelman, 1971). If the subject had not completed the log by 6:00 PM, he received a reminder to complete the log.

Subject Health and Safety Procedures

At each study visit, any newly occurring illnesses diseases or illnesses experienced by the subject that had worsened since the previous visit and any medications taken by the subject since the previous visit were documented by a researcher and reviewed by the study physician. If the study physician deemed that the subject's health might be in danger by continuing the study, or if the subject's condition or medication might interfere with the study results, the subject was disqualified from the study and referred to his primary care physician.

Familiarization

Familiarization occurred after the subject had met screening criteria and had signed the Informed Consent Form, and approximately 1 week prior to baseline testing. The purpose of the familiarization was to screen the subject for hepatitis B and C, as well as to allow the subject to

become accustomed to riding the bicycle ergometer and breathing though a Daniel's valve, and to acclimate to the time trial course, all of which would be utilized for experimental testing in subsequent visits. This trial simulated the experimental protocol, exclusive of a full-length steady state ride and blood draws.

For the familiarization, the subject reported to the Exercise Physiology and Metabolism Laboratory, Bellmont Hall, The University of Texas at Austin after a 2-hr fast, during which only water was consumed. If the subject met dietary and health compliance criteria, a researcher drew 4 ml of blood from a forearm vein, and the blood was sent to an outside diagnostic laboratory (Quest Diagnostics, Irving, TX) to be screened for hepatitis B and C. All subjects in this study tested negative for hepatitis B and C.

The subject's weight was measured and recorded, and the subject was fitted with a heart rate monitor (Cardiosport, Waterlooville, Hampshire, ENG) attached to an elastic strap around his chest, which communicated with a wrist receiver held by a researcher. The subject was given instructions regarding the SS/TT familiarization ride, which was similar to the subsequent SS/TT experimental rides. All exercise testing throughout the study was performed on a bicycle ergometer (VelotronDynafit Pro, Racermate, Seattle, WA; Velotron Coaching Software (CS) 2008, Version 1.6.458, Racermate Inc., Seattle, WA) equipped with an adjustable seat, adjustable handlebars, and pedals with straps to secure the subject's feet. The bicycle ergometer was fitted to the subject and the settings were recorded. The subject was equipped with a Daniel's valve which was connected to a computer-based metabolic system (ParvoMedics TrueOne 2400 (Sandy, UT)). This metabolic system was used throughout the study to calculate VO_2, VCO_2 and respiratory exchange ratio (RER). The metabolic system was calibrated prior to each testing

session. As a warm-up, the subject rode for 5 min at 65% of his estimated maximum heart rate (HR_{MAX}), based on the following regression equation (Tanaka et al., 2001):

$$\text{Estimated } HR_{MAX} \text{ (bpm)} = 208 - 0.7 * \text{Age (yrs)}$$

The Daniel's valve was removed and the subject then completed a custom 20 km time trial course (Velotron 3D 2008, Version 3, Racermate Inc., Seattle, WA) while being encouraged to complete this fixed distance as quickly as possible. The time trial course was designed with intermittent rolling hills and the ergometer was equipped with a toggle to allow the subject to shift gears at will throughout the course to adjust to the terrain. This time trial course was used for each time trial ride throughout the study. Water was provided *ad libitum*. Heart rate and RPE were collected at 10 km of the time trial ride, and again at 19.5 km of the time trial ride. Peak watts, average watts, average speed, and time to completion for the time trial ride was recorded. After completing the time trial course, the subject remained on the bicycle ergometer for a cool-down stage until his heart rate dropped below 120 bpm.

Muscle Biopsy

Over the course of the study, two muscle biopsies were collected from the vastus lateralis muscle of the thigh to measure succinate dehydrogenase (SDH), citrate synthase (CS) and PGC-1α. The first muscle biopsy was collected at baseline before the subject was randomized and provided with study supplement, and the second muscle biopsy was collected at endpoint after the subject had regularly consumed the study supplement for 39 days.

The subject reported to the laboratory in the morning following a 12-hr fast, during which only water was consumed. To prepare for each muscle biopsy, the subject was also instructed to shave both of his thighs to clear the muscle biopsy site of hair. If the subject met diet, exercise, and health compliance criteria, 15 ml of blood was drawn from a forearm vein to be analyzed for markers of immune system function, muscle damage, and general health (refer to *Appendix A - Safety Blood Draws*).

The subjects were then instructed to lie in a supine position on an examination table. The skin of the subject's mid-thigh was cleaned with 10% povidone-iodine and anesthetized locally with an injection of 1.5 cc of 1% Lidocaine-HCL into the skin. A 5-8 mm incision was made in the skin and subcutaneous fat, and approximately 100 mg of muscle tissue was removed with a Bergström biopsy needle (Dyna Medical, London, Ont. Canada). The incision site was sealed using butterfly bandages, and then the subject's thigh was wrapped with a pressure pack to minimize bruising.

Lactate Threshold/VO_{2MAX} Test

The LT/VO_{2MAX} test occurred three times over the course of the study. The baseline LT/VO_{2MAX} test was scheduled for the day after the first muscle biopsy, before the subject was randomized and provided with study supplement. The midpoint LT/VO_{2MAX} test was scheduled to occur after the subject had regularly consumed the study supplement for 20 days. The endpoint LT/VO_{2MAX} test was scheduled to occur the day after the last muscle biopsy, after the subject had regularly consumed his designated treatment for 40 days.

The subject reported to the laboratory following a minimum 2 hr fast, during which only water was consumed. If the subject met dietary, exercise, and health compliance criteria, his weight was measured and recorded and he was fitted with a heart rate monitor.

The LT/VO_{2MAX} test was performed on the bicycle ergometer. The protocol consisted of a 5-min warm-up at 60 watts, followed by a 20 or 30 watt increase every 2 min until the subject's increase in VO_2 was less than 0.2 $L \bullet min^{-1}$, or until the subject could not maintain a cadence of at least 60 RPM on the bicycle ergometer, or if the subject voluntarily stopped the test. The protocol was determined based on the subject's watts at 65% $HR_{MAX,}$ which was measured at the Familiarization visit: if average watts were 150 or below, the protocol increased in 20-watt increments, and if average watts were above 150, the protocol increased in 30-watt increments. Heart rate was measured and recorded during the final 30 sec of each work stage. During this test, the subject breathed through a Daniel's valve connected to the computer-based metabolic system which was used to measure average VO_2 ($L \bullet min^{-1}$), VCO_2 ($L \bullet min^{-1}$) and respiratory exchange ratio (RER) in 15 sec intervals for the entire test duration. The three highest 15 sec values were averaged to determine VO_{2MAX} ($L\ O_2 \bullet\ min^{-1}$).

During the VO_{2MAX} test, a researcher measured blood lactate by performing a finger prick blood collection within the final 30 seconds of each work stage. The subject's finger was cleaned with a sterile alcohol swab, allowed to dry, and pricked near the tip with a lancet. The first drop of blood was wiped off using sterile gauze, and the second drop was measured using the Lactate Pro handheld blood lactate analyzer (FaCT Canada Consulting Ltd., Quesnel British Columbia, Canada) and Lactate Pro test strips (ARKRAY Inc., Minami-Ku, Kyoto, Japan). The lactate analyzer was calibrated prior to each test session. Refer to *Figure 3* for an illustration of the LT/VO_{2MAX} test protocol.

Before leaving the laboratory, the subject was provided with 1 bottle of vanilla-flavored Ensure (Abbott Nutrition, Cleveland, OH) to drink exactly 10 hr before the steady state/time trial test to make certain that each subject consumed adequate calories so that lack of fuel availability was not a limiting factor in the strenuous time trial performance.

Steady State/Time Trial Test

The steady state/time trial (SS/TT) test occurred four times over the course of the study. The baseline SS/TT test was scheduled for the day after the baseline LT/VO_{2MAX} test, before the subject was randomized and provided with study supplement. The midpoint SS/TT test was scheduled for the day after the midpoint LT/VO_{2MAX} test, after the subject had regularly consumed the study supplement for 21 days. The first endpoint SS/TT test was scheduled to occur the day after the endpoint LT/VO_{2MAX} test, after the subject had regularly consumed the study supplement for 41 days. A second endpoint SS/TT test was scheduled for the day after the first endpoint SS/TT test, after the subject had regularly consumed the supplement for 42 days. The purpose of the second endpoint SS/TT test was to induce muscle damage and inflammation in the subject so that any potential protective or anti-inflammatory effects of the study supplement would be more conspicuous.

To prepare for each SS/TT test, the subject was required to consume the provided bottle of Ensure precisely 10 hr prior to his SS/TT test. The subject reported to the laboratory the day after the LT/VO_{2MAX} test, following a 10-hr fast, during which only water was consumed. If the subject met dietary, exercise, and health compliance criteria, he was fitted with a heart rate monitor and his weight was measured and recorded.

After the subject had sat quietly in a chair for 5 minutes, his resting heart rate was measured and recorded. A 20-guage Teflon catheter was inserted into a forearm vein, fitted with a three-way stopcock, extended with a catheter-extension, and taped in place. 10 ml of blood was drawn while the subject was seated, just before he began the steady state test.

The steady state test was performed on the bicycle ergometer and consisted of a 5 min warm-up steady state ride at watts equivalent to 50% VO_{2MAX}, followed by a 15 min steady state ride at watts equivalent to 65% VO_{2MAX}, based on the following regression equation adapted from Åstrand and Rodahl (1977):

> Work Rate (watts) = [VO_{2MAX} (ml $O_2 \bullet min^{-1}$) * (%VO_{2MAX} desired) – 300 (ml $O_2 \bullet min^{-1}$/watt)] / 12.5 (ml $O_2 \bullet min^{-1}$/watt)

During this test, the subject breathed through a Daniel's valve connected to the computer-based metabolic system which was used to measure average VO_2, VCO_2 and respiratory exchange ratio (RER) in 15 sec intervals during the final 10 min of the 15 min steady state period at 65% VO_{2MAX}. Carbohydrate and fat oxidation rates ($g \cdot min^{-1}$) were calculated from VCO_2, VO_2, and RER according to Frayn (1983). It was assumed that protein oxidation during exercise was negligible. Heart rate and Borg Rating of Perceived Exertion (RPE) was collected at 4 min into the 5 min 50% VO_{2MAX} steady state period and again at 10 min into the 15 min 65% VO_{2MAX} steady state period. Refer to *Figure 4* for an illustration of the steady state protocol. Upon completion of the steady state ride, the subject was allowed to dismount from the bicycle ergometer and rest for a period of 5 min.

The subject then completed the 20 km time trial while being encouraged to complete this fixed distance as quickly as possible. The subject was able to view and compete with his fastest

previous time trial performance. Average time to complete the time trial and the fastest time to completion across all subjects was also posted in the laboratory as another means of externally motivating the subjects to perform the time trial at maximum exercise capacity. Water was provided *ad libitum*. 0.05 ml of venous blood drawn from the forearm catheter during the time trial just prior to the subject's completion of the course (at 19.5 km) was used for analysis of blood lactate. Heart rate and RPE were also collected at 10 km into the course, and again at the time of the end blood draw. Peak watts, average watts, average speed, and time to completion for the time trial was recorded. Refer to *Figure 5* for an illustration of the time trial protocol.

After the subject completed the time trial, he remained on the bicycle ergometer for a cool-down period, until his heart rate dropped below 120 bpm. The subject then dismounted from the bicycle ergometer and was seated in a chair. A research assistant removed the catheter from the subject's arm, applied sterile gauze and a bandage to the site of the catheter puncture, and cleaned the skin around the area with a sterile alcohol swab. A light meal was then provided to the subject.

Blood Tissue Analysis

The blood sample collected at the end of the time trial (0.5 ml) was deposited into a storage tube containing 10% perchloric acid (PCA) (1 ml). The storage tube was centrifuged at 4˚ C for 10 min at 3,000 rpm with a HS-4 rotor in a Sorvall RC6 centrifuge (Kendro Laboratory Products, Newtown, CT). The supertanant was extracted from the storage tube and transferred to microcentrifuge tubes, then stored at -80˚C until further analysis.

Blood lactate. Blood lactate concentrations were measured from the PCA extracts using enzymatic analysis according to Hohorst. Samples were read at 340 nm using a Beckman DU640 Spectrophometer (Coulter, Fullerton, CA).

Muscle Tissue Homogenization

The extracted biopsy sample was trimmed of adipose and connective tissue and immediately frozen in liquid nitrogen, weighed, and stored in microcetrifuge tubes at -80^{o}C until further analysis.

SDH and CS. For the enzymatic analyses of SDH and CS, samples were homogenized in ice-cold buffer containing 0.17 M potassium phosphate (pH 7.4), 10 mM 2-mercaptoethanol (Sigma M6250), and 0.05% bovine serum albumin (ICN 105033) at a dilution of 1:30. Homogenization was performed on ice using 3 x 5 sec bursts with a size 20 tissue grinder (Kimble Chase, Vineland, NJ). A portion of the homogenate was immediately used for the SDH assay. The remaining homogenate was aliquoted to storage tubes and stored at -80°C for later analysis of CS.

PGC-1α. For determination of total PGC-1α content, the tissue samples were homogenized at a dilution of 1:10 in a modified RIPA buffer based on a previously described protocol (Wright et al., 2007) containing: 50 mM Tris-HCL (pH 7.4); 150 mM NaCl (pH 7.4); 1% each Igepal CA-630 and sodium deoxycholate; 1 mM each EDTA (pH 7.4), Na_3VO_4 (pH 10), NaF, and phenylmethylsulfonyl fluoride; 1 µg/ml each aprotinin, leupeptin and pepstatin. Homogenization was performed on ice using 4 bursts of 5 sec each with a Caframo RZRl Stirrer (Caframo Limited, Warton, Ontario, Canada). The homogenates were sonicated on ice for 10

sec and then centrifuged at 5,000 g for 20 min at 4°C. The supernatant was aliquoted to storage tubes and stored at -80°C for later analysis of PGC-1α.

Muscle Tissue Analysis

SDH. SDH content was determined according to the method of Lowry and Passonneau (1972). The amount of NADH produced during a 5 min incubation time was read on a Varian Cary Eclipse fluorometer with an excitation wavelength of 340 nm and emission wavelength of 450 nm (Varian, Inc., Palo Alto, CA) and corresponded to SDH activity in the sample. SDH activity was expressed as µmols/g/min protein.

CS. CS activity was determined according to the protocol of Srere (1969) on the homogenates after further dilution of 1:10 (wt/vol) with 0.1 M Tris-HCI and 0.4% Triton X-100 buffer (pH 8.3). The rate of appearance of DTNB was determined spectrophotometrically over 5 min at 412 nm and 37^{o} C using a Beckman DU 640 spectrophotometer (Fullerton, CA), and was proportional to CS activity. CS activity was expressed as µmols/g/min protein.

PGC-1α. PGC-1α content was determined by Western blotting. Total α-tubulin content was also determined as a housekeeping protein. Aliquots of homogenized muscle sample supernatants and standards were slowly thawed over ice and diluted 1:1 with sample buffer containing 1.25M Tris, pH 6.8, glycerol, 20% SDS, 2-mercaptoethanol, 0.25% bromophenol blue solution, and deionized water. Samples containing 100 µg of total protein were separated on 10% polyacrylamide gels by SDS-PAGE for 80 min at 185 V (Bio-Rad Laboratories, Hercules, CA.) After electrophoresis, the gels were electrotransferred using a wet transfer cell (Bio-Rad Laboratories, Hercules, CA.) using 90 V for 90 min to 0.4 µm polyvinylidine fluoride

(PVDF) membranes (Millipore, Bedford, MA). The membranes were blocked in TTBS (TBS, 50 mM Tris, 150 mM NaCl, containing 0.1% Tween-20), and 10% nonfat dry milk for 20 min at room temperature on a rocking platform at medium speed. The membranes were then washed in 1x TTBS 3 times for 5 min each wash. Using the molecular weight markers visible on the membranes as a guide, the membranes were cut at the 75 kD marker. The upper section was used for detection of PGC-1α, and the lower section was used to detect α-tubulin. Each membrane section was incubated overnight at 4°C on a rocking platform at low speed with antibodies directed against PGC-1α (no. 515667, EMD Calbiotech/Merck KGaA, Darmstadt, Germany), and α-tubulin (no. 2144, Cell Signaling, Danvers, MA). The antibodies were diluted 1:1000 for PGC-1α, and 1:900 for α-tubulin in TTBS containing 2% nonfat dry milk. Following the overnight incubation, membranes were washed three times with TTBS for 5 min each wash and incubated for 60 min with a secondary antibody (goat anti-rabbit, HRP-linked IgG, no. 7074, Cell Signaling, Danvers, MA). Dilutions were 1:900 for PGC-1α and 1:900 for α-tubulin. The immunoblots were visualized by enhanced chemiluminescence (Perkin Elmer, Boston, MA) using a Bio-Rad ChemiDoc detection system, and the mean density of each band was quantified using Quantity One 1-D Analysis software (Bio-Rad Laboratories, Hercules, CA.). A molecular weight ladder (Precision Plus Protein Standard, Bio-Rad) and a rodent internal control standard prepared from insulin-stimulated mixed skeletal muscle were also included on each gel. All blots were compared with the rodent control standard and the values of each sample were represented as a percent of standard for each blot.

Statistical Analysis

A power analysis was performed using G*Power3 (Faul et al., 2003) on the primary outcome parameter, Trolox equivalent antioxidant capacity (TEAC), to determine the number of subjects per group. Data from an HT supplementation study (Rietjens et al., 2011) showed a significant difference on measured metabolic markers between placebo and 200mg HT up to 90 minutes after supplementation with an observed effect size of .7 at 60 minutes and .85 at 30 minutes. Assuming a power of .8 and alpha =.05, and one tail, the sample size was estimated to be 20 subjects per group for 3 independent groups.

Statistical analysis of the data was performed using PASW 18.0 for Windows (SPSS Inc., Chicago, IL). A Levene test was performed to verify that variance was equal across groups. All overall within treatment (baseline, midpoint, and endpoint) variables were measured using repeated measures ANOVA. When significance was found, post hoc comparisons were performed using an LSD treatment with a manual adjustment to correct for positively-biased F values. Mean differences within treatments between timepoints were compared using paired-samples *t*-tests. A two-tailed Pearson correlation analysis was performed on the changes from baseline to endpoint in all variables across each treatment group. Differences were considered significant at $p < .05$. Data are presented as mean (M) ± SE.

RESULTS

SDH

SDH increased from baseline to endpoint within the HI treatment group by 9.4% ($p<.05$). SDH increased from baseline to endpoint within the LO treatment group by 9.6% and decreased in the PLA group by 1.3%, although neither change was significant. There were no significant overall time or treatment effects, nor was there a significant time by treatment interaction (*Figure 6*).

CS

CS increased from baseline to endpoint within the LO treatment group by 13.6% ($p<.05$) and within the HI treatment group by 17.8% ($p<.01$). CS increased in the PLA group by 1.0%, although this difference was not significant. There was a significant overall time effect. There was no significant treatment effect, nor was there a significant time by treatment interaction (*Figure 7*).

PGC-1α

There were no significant changes in PGC-1α within treatments over time, nor was there a significant overall time effect ($p=.056$), although all treatment groups increased from baseline to endpoint. There was no significant overall treatment effect, nor was there a time by treatment interaction (*Figure 8*).

α-tubulin was measured as a housekeeping protein of PGC-1α. There was no significant difference in α-tubulin across treatment or time. (*Figure 9*).

VO_{2MAX}

There was no significant overall treatment effect, nor a significant time by treatment interaction for VO_{2MAX}. Although VO_{2MAX} was significantly increased in the LO group from baseline to midpoint ($p<.05$) and from baseline to endpoint ($p<.01$), the magnitude of change was not physiologically relevant. There was a significant overall time effect ($p<.05$), with VO_{2MAX} increasing over the course of the six weeks of testing (*Figure 10*).

Lactate Threshold

VO_2 at lactate threshold increased from baseline to midpoint ($p<.05$) and from baseline to endpoint ($p<.01$) in the LO treatment group. Lactate threshold did not change over time in the PLA or HI treatment groups. There was a significant overall time effect ($p<.05$) and treatment by time interaction ($p<.01$). There was no significant overall treatment effect (*Figure 11*). The change in lactate threshold was positively correlated with the change in VO_{2MAX} ($r = .306$; $p<.05$), such as when VO_{2MAX} increased, lactate threshold increased.

RER at Steady State

RER at steady state decreased from baseline to midpoint ($p<.05$) and from baseline to endpoint ($p<.01$) in the LO treatment group. RER did not change over time in the PLA or HI

treatment groups. There were no significant overall time or treatment effects, nor was there a significant treatment by time interaction (*Figure 12*). The change in RER was negatively correlated with the change in VO_{2MAX} ($r = -.326$; $p<.01$), such as when VO_{2MAX} increased, RER decreased.

Time Trial Performance

Time trial (TT) time to completion improved from baseline to endpoint for all treatment groups (PLA: $p<.05$, LO: $p<.05$, HI: $p<.01$). TT time to completion improved from baseline to midpoint in LO ($p<.01$) and HI ($p<.01$) treatment groups. Furthermore, TT time to completion improved from midpoint to endpoint ($p<.01$) in the HI treatment group. The PLA group improved performance by 3.7%, the LO group improved performance by 2.4%, and the HI group improved performance by 4.7% from baseline to endpoint time trials. There was a significant overall time effect ($p<.01$). There was no significant overall treatment effect, nor was there a significant time by treatment interaction (*Figure 13*). The TT time to completion was negatively correlated with lactate threshold ($p<.01$), such as when lactate threshold increased, TT time to completion decreased.

TT blood lactate was greater at endpoint than at baseline ($p<.01$) in the HI treatment group. There was no change in TT blood lactate over time in the PLA or LO treatment groups. There was a significant overall time effect ($p<.05$). There was no significant overall treatment effect, nor was there a significant treatment by time interaction (*Figure 14*).

TT RPE was greater at endpoint ($p<.01$) and midpoint ($p<.01$) than at baseline, and greater at endpoint than at midpoint (<.01) for the PLA treatment group. Additionally, TT RPE was greater at endpoint than at midpoint ($p<.05$) for the PLA treatment group. TT RPE did not

change over time in the LO or HI treatment groups. There was a significant overall time effect ($p<.01$). There was no significant overall treatment effect, nor was there a significant time by treatment interaction. However, when the HI and LO HT treatment groups were combined and compared with PLA, there was a significant overall treatment effect ($p<.05$) with PLA higher than the HT combined treatments (*Figure 15*).

DISCUSSION

The purpose of this study was to investigate if regular daily hydroxytyrosol (HT) supplementation for 6 weeks would increase mitochondrial biogenesis and improve endurance exercise performance in recreationally-active males compared with a placebo (PLA). Additionally, a low-dose supplement (LO, 50 mg/day HT) was compared with a high-dose supplement (HI, 150 mg/day HT) to determine whether there was a dose-response. The primary findings were that HT supplementation increased oxidative enzymes SDH and CS, indicating activation of mitochondrial biogenesis. Time trial performance was improved at midpoint and endpoint in the LO and HI HT treatment groups. Improved time trial performance also occurred at endpoint in PLA, which was associated with an increased in RPE. RPE was not associated with improved time trial performance when HT was provided.

Adherence to a Mediterranean diet may improve longevity and reduce the risk for diseases associated with mitochondrial dysfunction and aging (Trichopoulou et al., 2003). Olive oil is the primary source of fat in the Mediterranean diet (Quaranta and Rotundo, 2000). Extra-virgin olive oil is rich in antioxidant and radical-scavenging polyphenolic compounds, which are thought to be responsible for the relatively stable characteristics of olive oil (Visioli and Bernardini, 2011; Sabatini, 2010; Tuck et al., 2002). Of these polyphenols, HT has been found in high concentrations in olive waste water, a by-product of olive oil manufacturing (Visioli et al., 1999). Recently, the health benefits associated with the Mediterranean diet have been credited in part to the HT content of olive oil due to its potential to increase mitochondrial density and function in the cells (Granados et al. 2010; Paiva-Martins et al., 2011). Mitochondria are the cellular framework by which active tissues utilize delivered oxygen via oxidative

phosphorylation to produce ATP, the form of energy required for muscular work and maintenance of vital cellular processes. Exercise and other conditions of high cellular oxidative demand (Vorobjev and Zorov, 1983; Nisoli and Carruba, 2006) induce mitochondrial biogenesis by activating AMPK (Hardie 2011) and the downstream mitochondrial transcription factor PGC-1α (Irrcher et al, 2003; Lee et al., 2006).

LO and HI HT caused increases in the oxidative enzymes SDH and CS, suggesting increased mitochondrial biogenesis. This was originally noted in studies with polyphenols similar to HT in structure, such as quercetin, and resveratrol (Howitz et al., 2003; Lagouge et al., 2006; Davis et al., 2009), and recent studies have found that HT at concentrations of 0.1 to 10 μmol/L are able to likewise induce mitochondrial biogenesis and increase oxidative enzymes in isolated cells and in vivo in rodents (Hao et al., 2009; Zhu et al., 2010; Lui et al., 2011). Although the increase in SDH in the LO treatment group was not statistically significant, the percentage increase of 9.6% was similar to the increase of 9.4% in the HI HT group. HT has been shown to activate AMPK (Hardie, 2011; Hao et al., 2009; Zhu et al., 2010; Lui et al., 2011), which regulates transcription of mitochondria by elevating the nuclear transcription factor peroxisome proliferator-activated receptor γ coactivator (PGC)-1α, a master regulator of mitochondrial biogenesis (Irrcher et al., 2003; Lee et al., 2006). However, the increase in oxidative enzymes in the HT groups was not associated with an increase in PGC-1α. Since the increase in mitochondrial oxidative enzymes was modest, it is possible that an increase in PGC-1α by HT was too small to detect.

Maximal aerobic power, VO_{2MAX}, was unaffected by HT. Although VO_{2MAX} was statistically higher in the LO HT group, such a small increase would not be expected to have physiologically relevant implications. HT should probably have little influence on VO_{2MAX},

considering that whole-organism VO_{2MAX} is limited by oxygen delivery rather than by mitochondrial volume. In fact, DiPrampero (1985) demonstrated that approximately 75% of VO_{2MAX} is determined by oxygen delivery, and the remaining 25% is determined by peripheral factors including mitochondrial volume. However, the VO_2 at which blood lactate begins to increase, lactate threshold, is highly related to mitochondrial density (Ivy et al., 1980).

Lactate is a product of anaerobic metabolism, which provides a rapid source of ATP via glycolysis in the cytosol of the cell. During high intensity exercise, the rate of lactic acid production eventually overwhelms the blood bicarbonate buffering system, leading to lactate accumulation and fatigue (Harrison and Pilcher, 1930; Holloszy et al., 1977). Increased mitochondria allow distribution of the oxidative demand, allowing an individual to rely primarily on aerobic metabolism at higher exercise intensities before anaerobic metabolism becomes the primary energy source (Holloszy and Coyle, 1984). In this regard, it was observed that lactate threshold increased in the LO HT group. In addition, RER was significantly lower at steady state exercise and time trial performance significantly improved for the LO HT group, indicating a shift of reliance from anaerobic to aerobic metabolism at a given exercise intensity (Hill et al., 1924; Harrison and Pilcher, 1930; Wasserman and McIlroy, 1964). This provides further evidence that there was an increase in mitochondria in the HT groups.

Although the HI HT group demonstrated increased SDH and CS, this increase was not accompanied by an increased lactate threshold or RER. It is possible that a type II error occurred, such as a treatment effect existed for lactate threshold that went undetected due to sampling error. The HI HT group entered the study with a higher mean lactate threshold and a lower RER at steady state than did the LO HT group. Perhaps the LO group experienced an

exaggerated response to HT supplementation because it was less trained and may have had lower intrinsic antioxidant capacity than the HI HT group (McArdle, 2000).

Time trial performance increased in each group over the course of the study, indicating a familiarization effect independent from the treatment. Performance improved at midpoint testing and at final testing in the HT groups, whereas performance in the PLA group did not improve until the final time trial. The increase in performance in the HT groups was accompanied by an increase in mitochondrial activity. PLA had no increase in mitochondrial activity. However, the increased performance in the PLA group was accompanied by a significant increase in RPE, whereas the LO and HI HT treatment groups did not demonstrate increased RPE. Collectively, these observations suggest that HT may have altered perception of effort during the time trial. No previous study has reported altered RPE during exercise in response to HT supplementation, although Alexander (2006) found that quercetin acts as an adenosine A_1 receptor antagonist in vitro, similarly to caffeine. HT may act similarly to other phenols such as caffeine and quercetin to limit CNS perception of effort during exercise. This theory is in agreement with Davis et al. (2009), who attributed increased voluntary running in mice after quercetin supplementation to decreased CNS perception of effort.

The improvement in time trial performance in the LO HT group was accompanied by an increased lactate threshold and a decreased RER, in addition to improved mitochondrial enzyme activity. However, LT and RER were unchanged in the HI HT group. Therefore, improvement in time trial performance may have involved factors other than cellular adaptations. When considering our results, psychological motivation should be taken into account. Each subject was able to view and compete with his fastest previous time trial performance. Average time to complete the time trial and the fastest time to completion across all subjects was also posted in

the laboratory as another means of externally motivating the subjects to perform the time trial at maximum exercise capacity. Lactate threshold has been found to be the primary determinant of endurance exercise performance in athletes since endurance racers typically perform at an intensity equal to lactate threshold, but below VO_{2MAX} (Allen et al., 1985). However, it appears that the psychological motivation of the subjects to beat previous performances was a stronger predictor of performance than was lactate threshold or RER. This is suggested by the performance of the PLA group, in which an increase in performance was accompanied by an increase in RPE. It is possible that a treatment effect may have been more pronounced if the subjects had not been provided with external psychological motivation, or if the study was examining a population typically accustomed to maintaining a race intensity equal to lactate threshold, such as competitive cyclists.

Our results indicate that 50 mg/day of HT, equivalent to 0.6 mg/kg body weight/day, for 6 weeks is as efficacious as 150 mg/day, equivalent to 1.9 mg/kg body weight/day, for improving muscle oxidative capacity and aerobic performance. This finding is supported by earlier research in rats which showed that 0.5 mg/kg/day of HT for 6 weeks improved mitochondrial function (Granados et al., 2010).

In summary, the present study demonstrated that HT supplementation for 6 weeks in recreationally-active males increased the activity of muscle oxidative enzymes, suggesting an increase in mitochondrial biogenesis. Time trial performance increased in the HT groups over the course of the study and was accompanied by an increase in LT in the LO HT group. Despite no increase in mitochondrial enzyme activity or LT, performance was improved after 6 weeks in the PLA group. This improvement was associated with an increase in RPE. RPE was not increased in either the LO or HI groups, although time trial performance was significantly

improved. It is possible that HT can improve performance by altering perception of effort, as well as by improving muscle oxidative capacity.

The present study is the first to show that HT is able to stimulate mitochondrial biogenesis, increase aerobic capacity, and decrease perception of effort in humans. These findings are highly relevant to endurance athletes and to individuals suffering from diseases related to mitochondrial dysfunction, such as cardiovascular disease, type II diabetes, and cancer. Olive oil waste water, which is rich in HT, may be transformed from a logistically and financially burdensome by-product of olive oil production into a valuable commodity. HT isolated from this waste water may be used as an effective means to increase mitochondria and gain benefits such as improved exercise performance, slowed aging, and decreased diseases associated with mitochondrial dysfunction.

Future research should strive to identify the specific mechanisms by which HT decreases perception of effort in humans. Additionally, acute effects of HT should be compared with chronic effects of HT, and the smallest efficacious acute dose of HT should be determined. Moreover, the effects of HT supplementation on endurance exercise performance should be examined in endurance athletes to determine if HT can increase oxidative enzymes, improve lactate threshold and RER, and decrease perceived effort in a trained population, and to determine whether any improvements translate into improved endurance exercise performance. Further research should be also pursued in a clinical population to determine whether HT is capable of decreasing diseases associated with mitochondrial dysfunction and aging such as cardiovascular disease, type II diabetes, and cancer.

References

Alexander SP. (2006). Flavonoids as antagonists at A_1 adenosine receptors. *Phytother Res, 20,* 1009–1012.

Allen W, Seals DR, Hurley BF, Ehsani AA, Hagberg JM. (1985). Lactate threshold and distance running performance in young and older endurance athletes. *Journal of Applied Physiology, 58*(4), 1281-1284.

Attardi G, Schatz G. (1988). Biogenesis of mitochondria. *Annual Review of Cellular Biology, 4*, 289-331.

Blair S, Kohl III, HW, Paffenbarger Jr RS, Clark DG, Cooper KH, and Gibbons LW. (1989). Physical fitness and all-cause mortality. *The Journal of the American Medical Association, 262*(17), 2395-2401.

Chapman AR, Vincenzino B, Blanch P, Hodges PW. (2007). Leg muscle recruitment during cycling is less developed in triathletes than cyclists despite matched cycling training loads. *Experimental Brain Research, 181*(3), 503-518

Davis M, Murphey EA, Carmichael MD, and Davis B. (2009). Quercitin increases brain and muscle mitochondrial biogenesis and exercise tolerance. *AJP - Regulatory Physiology, 296*(4), R1071-R1077.

Di Prampero, PE. (1985). Metabolic and circulatory limitations to VO_{2MAX} at the whole animal level. *J Exp Biol, 115,* 319-331.

Feng Z, Bai L, Yan J, Li Y, Shen W, Wang Y, Wertz K, Weber P, Zhang Y, Chen Y, Liu J. (2011). Mitochondrial dynamic remodeling in strenuous exercise-induced muscle and mitochondrial dysfunction: regulatory effects of hydroxytyrosol. *Free Radical Biology and Medicine, 50,* 1437-1446.

Granados S, Quiles JL, Ramirez-Tortosa CL, Sanchez-Rovira P, Ramirez-Tortosa MC. (2010). Hydroxytyrosol: from laboratory investigations to future clinical trials. *Nutrition Reviews, 68*(4), 16.

Hao J, Shen W, Yu G, Jia H, Li X, Feng Z, Wang Y, Weber P, Wertz K, Sharman E, Liu J. (2009). Hydroxytyrosol promotes mitochondrial biogenesis and mitochondrial function in 3T3-L1 adipocytes. *Journal of Nutritional Biochemistry, 21,* 634-644.

Hardie D. (2011). Sensing of energy and nutrients by AMP-activated protein kinase. *American Journal of Clinical Nutrition, 93*(4), 891-896.

Harrison TR, Pilcher C. (1930). Studies in congestive heart failure, II. The respiratory exchange during and after exercise. *Journal of Clinical Investigation, 8(3),* 291-315.

Hill AV, Long CNH, Lupton H. (1924). Muscular exercise, lactic acid, and the supply and utilization of oxygen. *Proc Roy Soc London, S. B. 96*, 84, 155, 438.

Holloszy J, Rennie MJ, Hickson RC, Conlee RK, Hagberg JM. (1977). Physiological consequences of the biochemical adaptations to endurance exercise. *Annals of the New York Academy of Sciences, 301*, 440-450.

Holloszy J, Coyle EF. (1984). Adaptations of skeletal muscle to endurance exercise and their metabolic consequenses. *Journal of Applied Physiology, 56*(4), 831-838.

Howitz K, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, Zipkin RE, Chung P, Kisielewski A, Zhang, L, Scherer B, and Sinclair DA. (2003). Small molecules activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature, 425,* 191-196.

Irrcher I, Adhihetty PJ, Joseph A-M, Ljubicic V, Hood DA. (2003). Regulation of mitochondrial biogenesis in muscle by endurance exercise. *Sports Medicine, 33*(11), 11.

Ivy JL, Withers RT, Van Handel PJ, Elger DH, Costill DL. (1980). Muscle respiratory capacity and fiber type as determinants of the lactate threshold. *Journal of Applied Physiology, 48(*3), 523-527.

Joyner M, Coyle EF. (2008). Endurance exercise performance: the physiology of champions. *The Journal of Physiology, 586*(1), 35-44.

Khymenets O, Fitó M, Touriño S, Muñoz-Aguayo D, Pujadas M, Torres JL, Joglar J, Farré M, Covas M-I, de la Torre R. (2010). Antioxidant activities of hydroxytyrosol main metabolites do not contribute to beneficial health effects after olive oil ingestion. *Drug Metab Dispos, 38*(9), 1417-1421. Published ahead of print June 1411, 2010.

Lagouge M AC, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Massadeq N, Milne J, Lambert P, Elliot P, Geny B, Laakso M, Puigserver P, and Auwerx J. (2006). Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1α. *Cell, 127*(6), 1109-1122.

Lee J, Kim M, Park H-S, Kim HS, Jeon MJ, Oh KS, Koh EH, Won JC, Kim M-S, Oh GT, Yoon M, Lee K-U, Park JY. (2006). AMPK activation increases fatty acid oxidation in skeletal

muscle by activating PPARα and PGC-1. *Biochemical and Biophysical Research Communications, 340*, 291-295.

Liu J, Raederstorff D, Wang-Schmidt Y, and Wertz K. (2011). United States Patent No. (Application) US 2011/0112201 A1.

Liu Z, Sun L, Zhu L, Jia X, Li X, Jia H, Wang Y, Weber P, Long J, Liu J. (2007). Hydroxytyrosol protects retinal pigment epithelial cells from acrolein-induced oxidative stress and mitochondrial dysfunction. *Journal of Neurochemistry, 103*, 2690-2700.

MacRae H, and Mefferd KM. (2006). Dietary antioxidant supplementation combined with quercitin improves cycling time trial performance. *Int J Sport Nutr Exercise Metab, 16*, 405-415.

McArdle A, Pattwell D, Vasilaki A, Griffiths RD, Jackson MJ. (2001). Contractile activity-induced oxidative stress: cellular origin and adaptive responses. *Am J Physiol Cell Physiol, 280*(3), C621-C627.

Nieman D, Dumke CI, Henson DA, McAnuity SR, McAnuity lA, Lind RH, and Murrow JD. (2003). Immune and oxidative changes during and following the Western States Endurance Run. *Int J Sports Med, 24*, 541-547.

Nisoli E, Carruba MO. (2006). Nitric oxide and mitochondrial biogenesis. *Journal of Cell Science, 119*(14), 8.

Paiva-Martins F, Rodrigues V, Calheiros R, Marques MPM. (2011). Characterization of antioxidant olive oil biophenols by spectroscopic methods. *J Sci Food Agric, 91*, 6.

Quaranta G, Rotundo V. (2000). Economic and commercial prospects for olive oil in view of the changes in the common market organisation (CMO) (Part one). *Olivae, 91*, 5.

Quiles J, Farquharson AJ, Simpson DK, Grant I, Wahle KWJ. (2002). Olive oil phenolics: effects on DNA oxidation and redox enzyme mRNA in prostate cells. *British Journal of Nutrition, 88*, 10.

Richter C. (1988). Do mitochondrial DNA fragments promote cancer and aging? *FEBS Letters, 241*(1,2), 5.

Rietjens J, Bast A, Haenen GRMM, and Heyden Van Der LCG. (2011). United States Patent No. (Application) US 2011/0052750 A1.

Sabatini N. (2010). Recent patents in olive oil industry: new technologies for the recovery of phenols compounds from olive oils, olive oil industrial by-products and waste waters. *Recent Pat Food Nutr Agric, 2*(2), 6.

Trichopoulou A, Costacou T, Bamia C, Trichopoulou D. (2003). Adherence to a Mediterranean diet and survival in a Greek population. *New England Journal of Medicine, 348*(26), 12.

Tuck K, Hayball PJ. (2002). Major phenolic compounds in olive oil: metabolism and health effects. *The Journal of Nutritional Biochemistry, 13*, 10.

Visioli F, Romani A, Mulinacci N, Zarini S, Conte D, Vincieri FF, Galli C. (1999). Antioxidant and other biological activities of olive mill waste waters. *J Agric Food Chem, 47*, 3397-3401.

Visioli F, Bernardini E. (2011). Extra virgin olive oil's polyphenols: biological activities. *Current Pharmaceutical Design, 17*(8), 19.

Vissers M, Zock PL, Katan MB. (2004). Bioavailability and antioxidant effects of olive oil phenols in humans: a review. *European Journal of Clinical Nutrition, 58*, 955-965.

Vorobjev I, Zorov DB. (1983). Diazepam inhibits cell respiration and induces fragmentation of mitochondrial reticulum. *FEBS Letters, 164*(2), 311-314.

Wasserman K, McIlroy MB. (1964). Detecting the threshold of anaerobic metabolism in cardiac patients during exercise. *American Journal of Cardiology, 14*(6), 844-852.

Zhang Y, Olivier M, Giulivi C, Ernster L, Davies KJA. (1990). The oxidative inactivation of mitochondrial electron transport chain components and ATPase. *The Journal of Biological Chemistry, 265*(27), 16330-16336.

Zhu L, Zhongbo L, Feng Z, Hao J, Shen W, Li X, Sun L, Sharman E, Wang Y, Wertz K, Weber P, Shi X, Liu J. (2010). Hydroxytyrosol protects against oxidative damage by simultaneous activation of mitochondrial biogenesis and phase II detoxifying enzyme systems in retinal pigment epithelial cells. *Journal of Nutritional Biochemistry, 21*, 1089-1098.

TABLE 1: Subject characteristics, *M* ± SE

	All (N=60)	**PLA (n=19)**	**LO (n=20)**	**HI (n=22)**
Age (yrs)	21.46 ± 0.22	21.68 ± 0.43	21.60 ± 0.41	21.14 ± 0.31
Height (cm)	179.46 ± 0.79	180.02 ± 1.07	181.57 ± 1.26	177.08 ± 1.52
Weight (kg)	78.91 ± 1.19	80.40 ± 2.29	79.48 ± 2.04	77.11 ± 1.93
BMI	24.46 ± 0.32	24.78 ± 0.64	24.08 ± 0.57	24.54 ± 0.47
VO_{2MAX} (L O_2•min^{-1})	3.39 ± 0.07	3.56 ± 0.14	3.24 ± 0.10	3.38 ± 0.13
Lactate Threshold (L O_2•min^{-1})	1.97 ± 0.09	2.07 ± 0.11	1.77 ± 0.08	2.07 ± 0.09
RER at 65% VO_{2MAX}	1.00 ± 0.01	0.98 ± 0.01	1.01 ± 0.01	1.00 ± 0.01

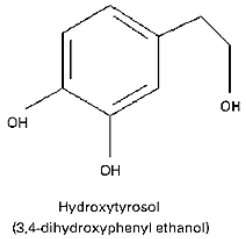

FIGURE 1: Molecular structure of hydroxytyrosol

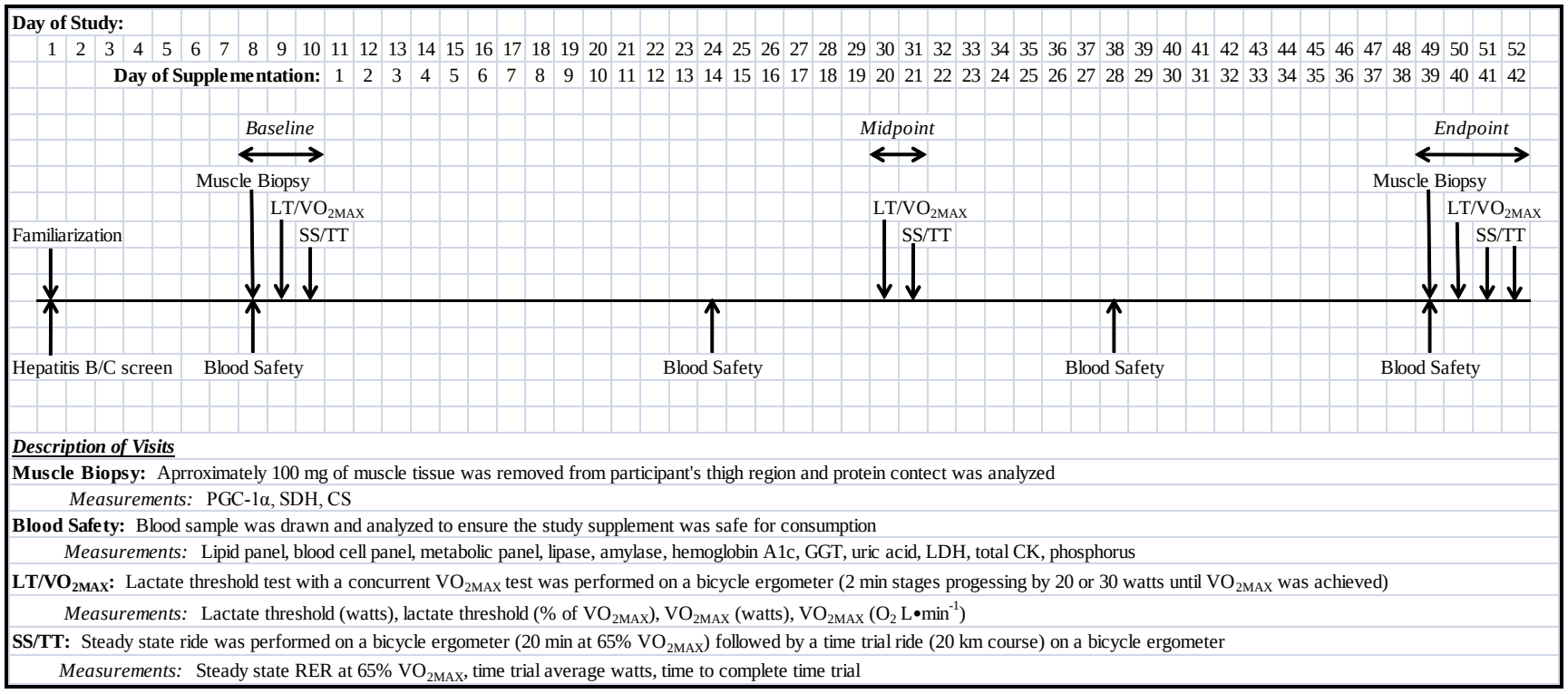

FIGURE 2: Study protocol

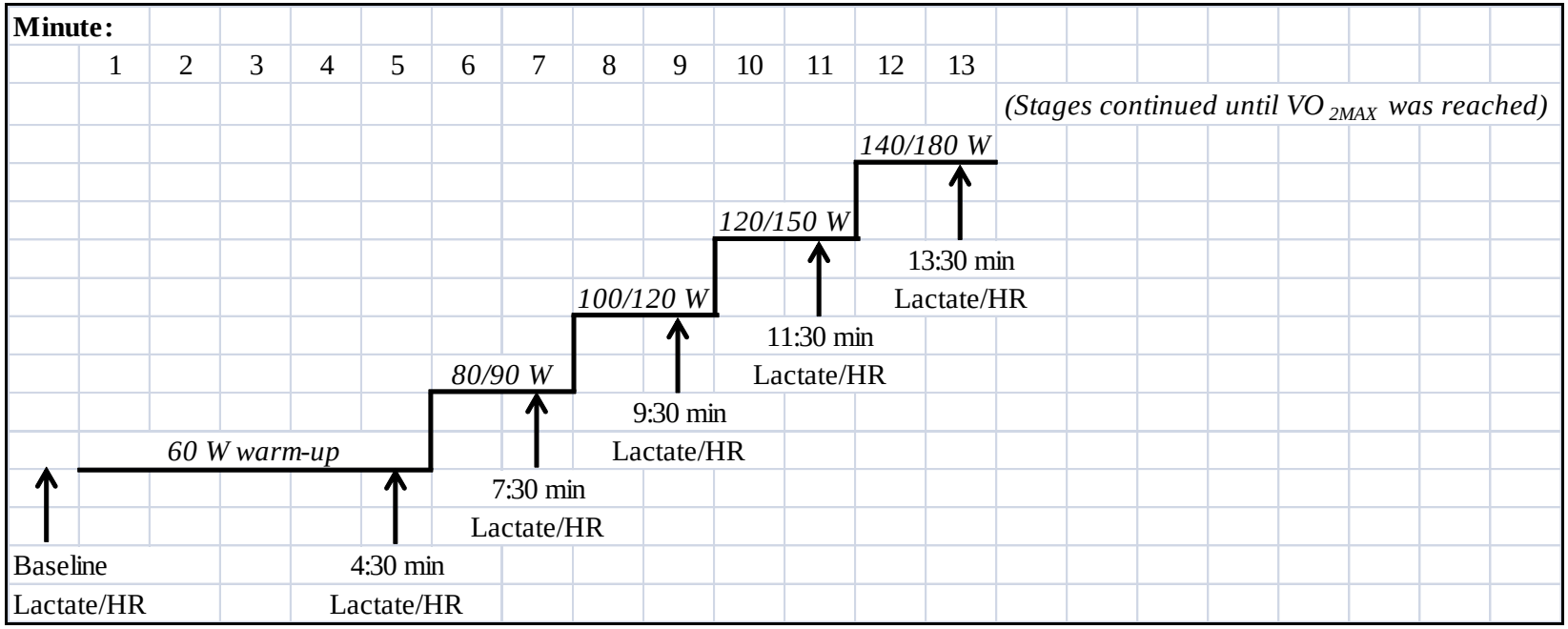

FIGURE 3: LT/VO_{2MAX} protocol

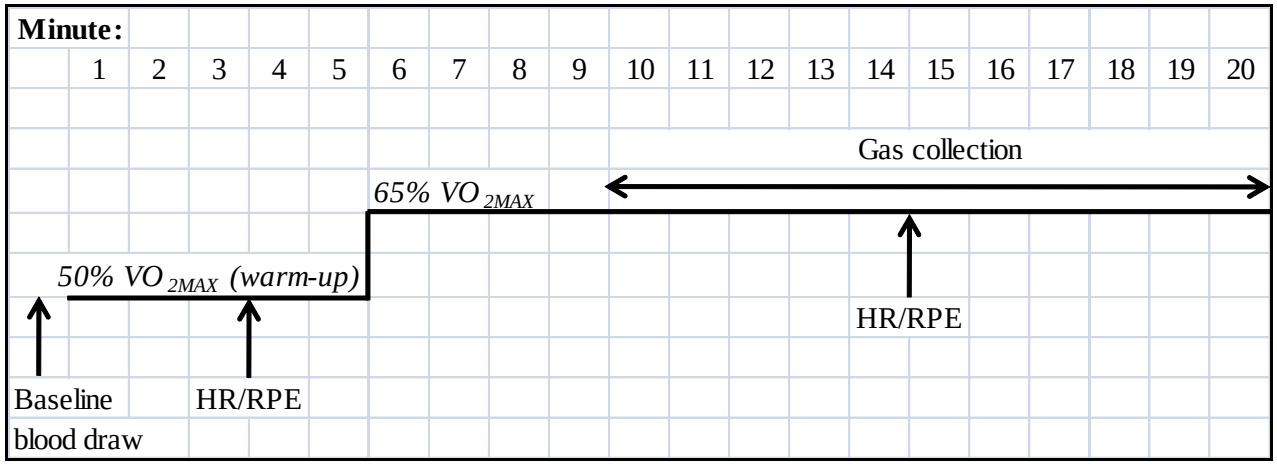

FIGURE 4: Steady state protocol

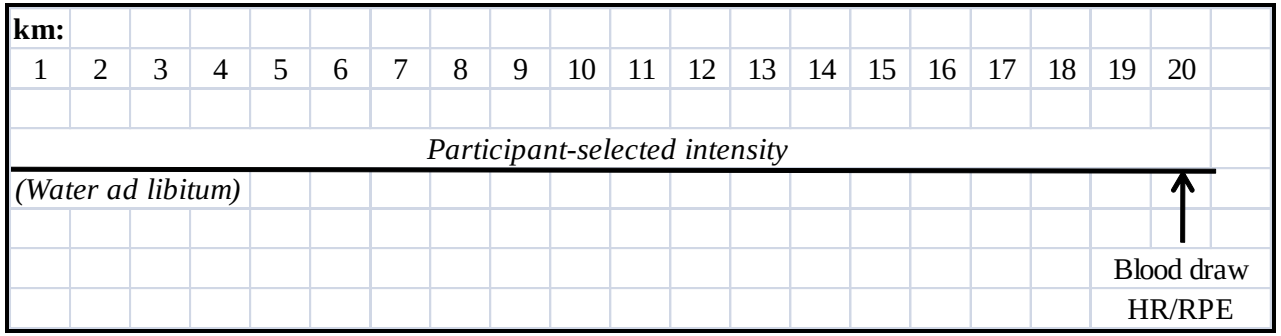

FIGURE 5: Time trial protocol

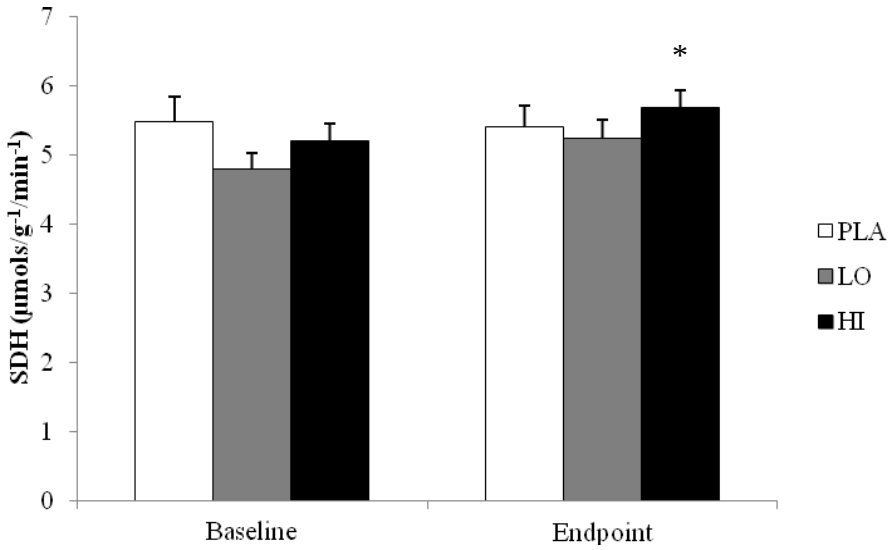

FIGURE 6: Succinate dehydrogenase (SDH). SDH changes within treatments over time were analyzed in tissue collected from muscle biopsies at baseline and endpoint. Values are presented as *M* ± SE. No significant overall treatment by time interaction. * Significant difference between baseline and endpoint in the HI treatment group (p=.05).

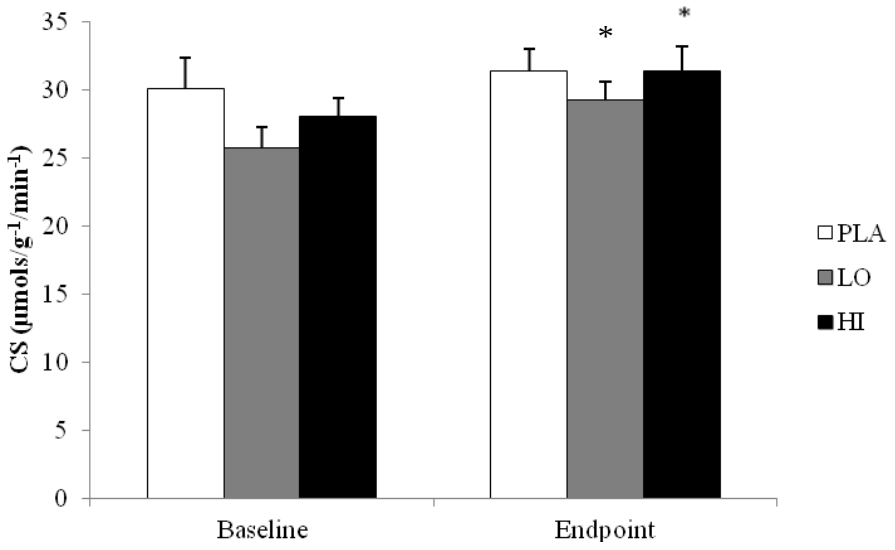

FIGURE 7: Citrate synthase (CS). CS changes within treatments over time were analyzed in tissue collected from muscle biopsies at baseline and endpoint. Values are presented as *M* ± SE. No significant overall treatment by time interaction. * Significant differences between baseline and endpoint in the LO ($p<.05$) and HI ($p<.01$) treatment groups.

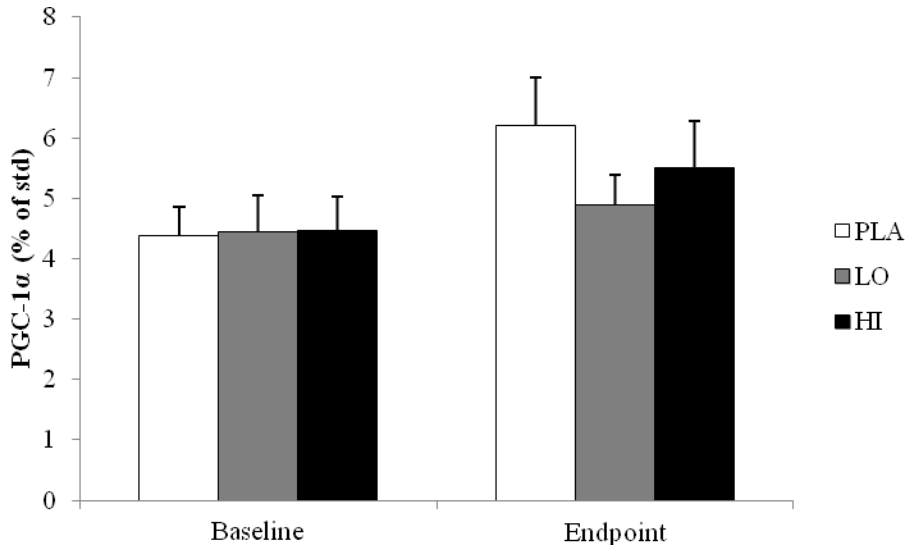

FIGURE 8: PGC-1α. PGC-1α changes within treatments over time were analyzed in tissue collected from muscle biopsies at baseline and endpoint. Values are presented as $M \pm$ SE. No significant overall treatment by time interaction. No significant differences between treatments over time.

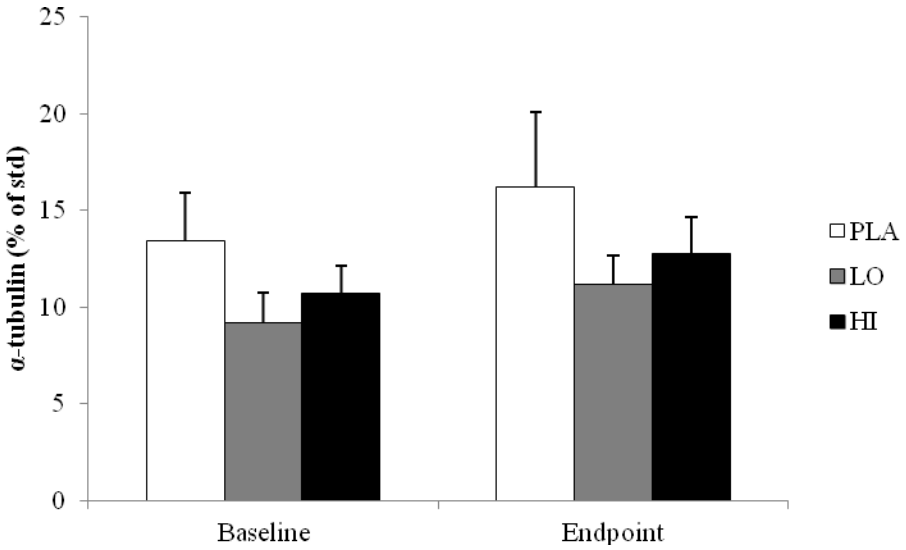

FIGURE 9: α-tubulin. α-tubulin changes within treatments over time were analyzed in tissue collected from muscle biopsies at baseline and endpoint. Values are presented as *M* ± SE. No significant overall treatment by time interaction. No significant differences between treatments over time.

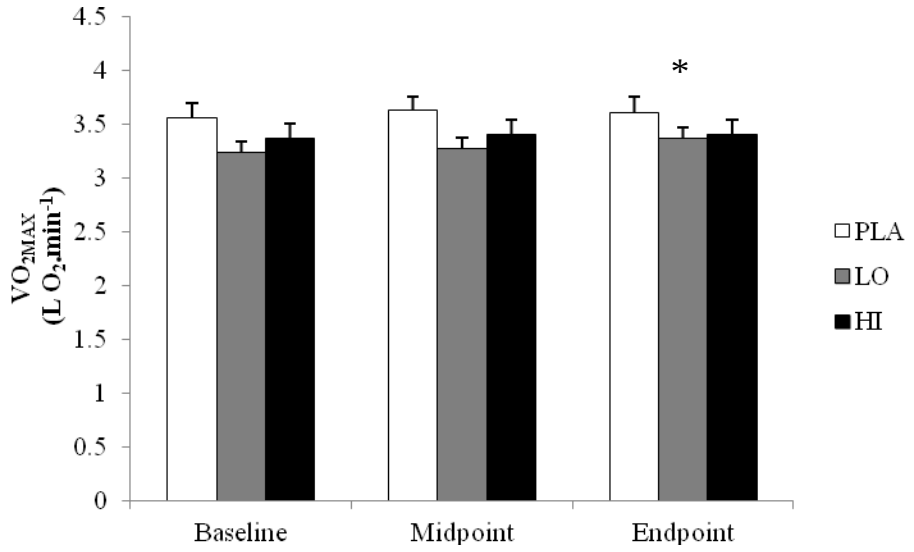

FIGURE 10: VO_{2MAX}. VO_{2MAX} changes within treatments over time. VO_{2MAX} was measured by a graded exercise test on a bicycle ergometer at baseline, midpoint, and endpoint. Values are presented as *M* ± SE. No significant overall treatment by time interaction. * Significant differences between baseline and endpoint ($p<.01$) and between midpoint and endpoint ($p<.05$) in the LO treatment group.

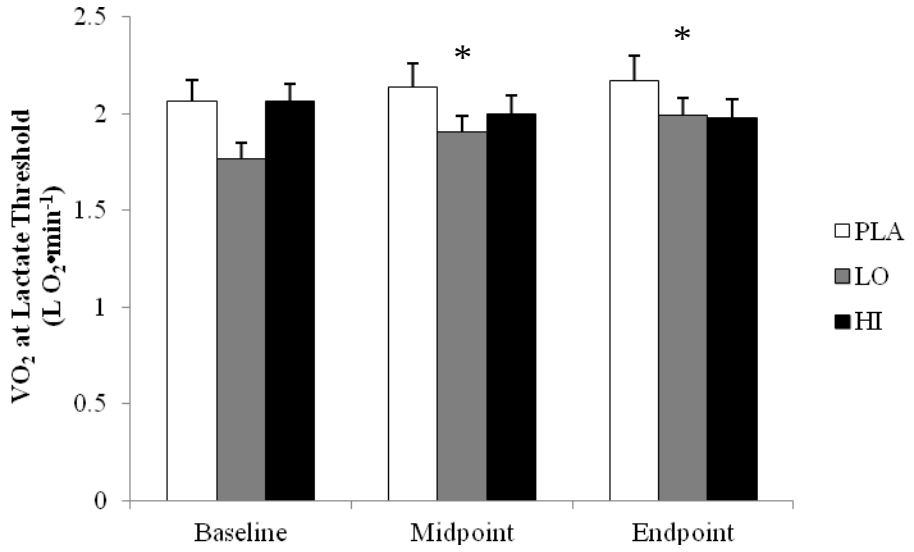

FIGURE 11: VO_2 at Lactate Threshold. Changes in VO_2 at lactate threshold within treatments over time. VO_2 at lactate threshold was measured by a graded exercise test on a bicycle ergometer at baseline, midpoint, and endpoint. Values are presented as $M \pm$ SE. Significant overall treatment by time interaction ($p<.01$). * Significant differences between baseline and midpoint ($p<.05$) and between baseline and endpoint ($p<.01$) in the LO treatment group.

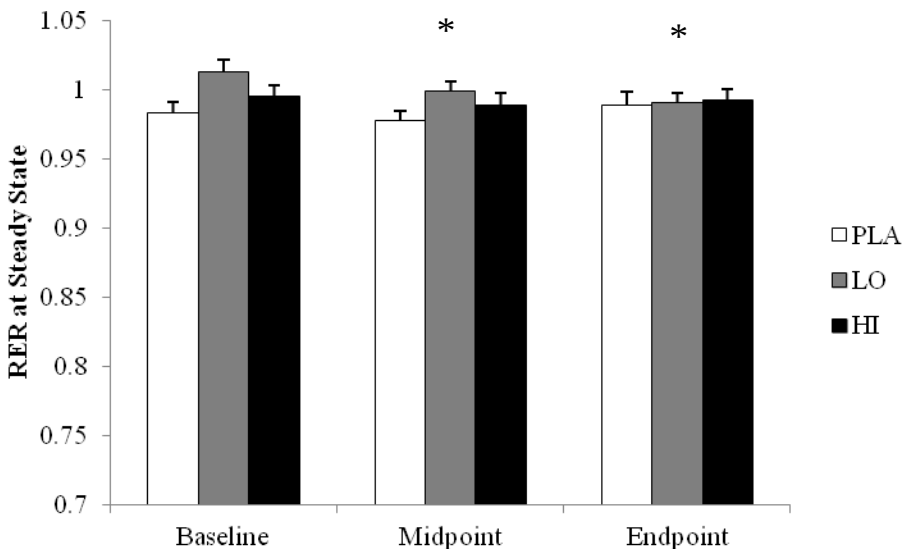

FIGURE 12: Respiratory exchange ratio (RER) at Steady State. Changes in RER at steady state within treatments over time. RER at steady state was measured during an exercise test at constant watts equivalent to 65% VO_{2MAX} on a bicycle ergometer at baseline, midpoint, and endpoint. Values are presented as $M \pm$ SE. No significant overall treatment by time interaction. * Significant differences between baseline and midpoint ($p<.05$) and between baseline and endpoint ($p<.01$) in the LO treatment group.

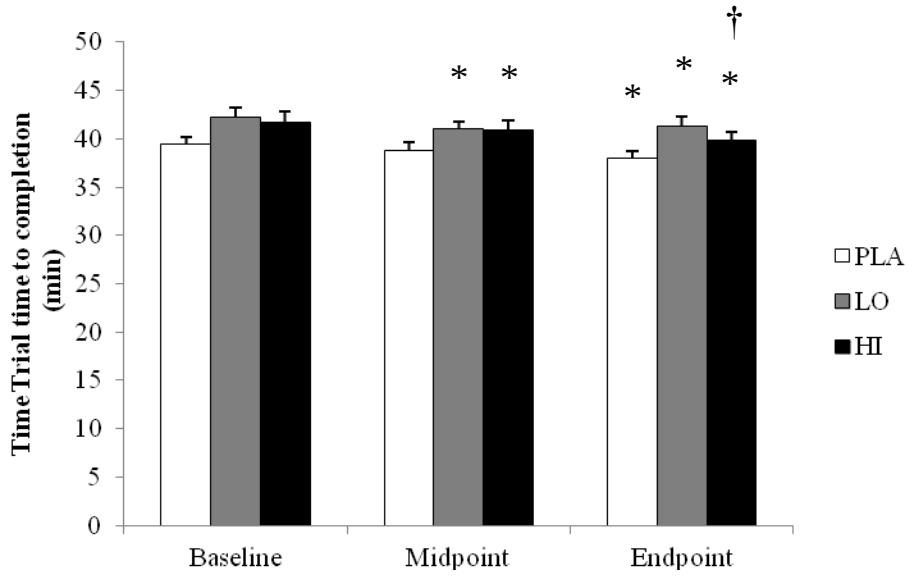

FIGURE 13: Time Trial (TT) time to completion. TT time to completion within treatments over time. TT time to completion was measured as time to complete a 20 km time trial course on a bicycle ergometer at baseline, midpoint, and endpoint. Values are presented as $M \pm$ SE. No significant overall treatment by time interaction. *Significant differences between baseline and endpoint ($p<.05$) and between midpoint and endpoint in the PLA treatment group ($p<.05$). *Significant differences between baseline and midpoint ($p<.01$) and between baseline and endpoint ($p<.05$) in the LO treatment groups. * Significant differences between baseline and midpoint ($p<.01$) and between baseline and endpoint ($p<.01$) in the HI treatment group. † Significant difference between midpoint and endpoint ($p<.01$) in the HI treatment group.

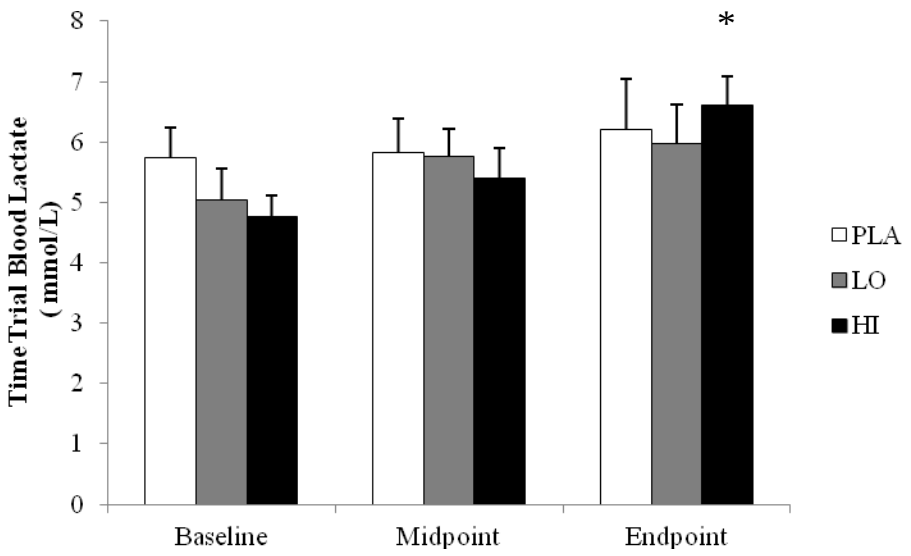

FIGURE 14: Time Trial (TT) blood lactate. TT blood lactate within treatments over time. TT blood lactate was measured in the final blood draw of baseline, midpoint, and endpoint time trials. Values are presented as $M \pm$ SE. No significant overall treatment by time interaction. *Significant difference between baseline and endpoint ($p<.01$) in the HI treatment group.

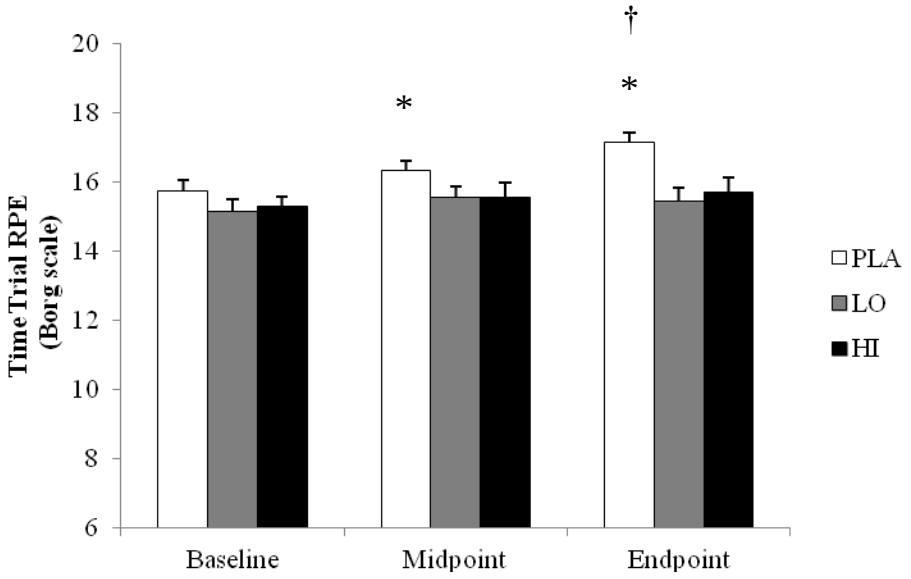

FIGURE 15: Time Trial (TT) ratings of perceived exertion (RPE). TT RPE within treatments over time. TT RPE was collected as the final blood draw of baseline, midpoint, and endpoint time trials. No significant overall treatment by time interaction. * Significant differences between baseline and midpoint ($p<.05$) and between baseline and endpoint ($p<.01$) in the PLA treatment group. † Significant difference between midpoint and endpoint ($p<.01$) in the PLA treatment group.

LITERATURE REVIEW

Introduction

Adherence to a Mediterranean diet may improve longevity and reduce the risk for diseases associated with mitochondrial dysfunction and aging, such as cardiovascular disease, type II diabetes, and cancer. A population-based investigation of 22,043 adults in Greece for a period of 44 months established inverse correlations with adherence to the traditional Mediterranean diet and total mortality, deaths due to coronary heart disease, and deaths due to cancer (Trichopoulou et al., 2003).

The traditional Mediterranean diet is characterized by high intake of fresh fruits and vegetables, fish, cheese, wine in moderation, low levels of red meat consumption, and olive oil as the predominant source of fat (Willett et al., 1995). Olive oil contributes to 71, 42, and 37% of the amount of vegetable fat obtained in the diet in Greece, Italy, and Spain, respectively (Quaranta and Rotundo, 2000). Olive trees possess over 30 potent antioxidant and radical-scavenging polyphenolic compounds that are protective against harsh heat and sun exposure of the Mediterranean basin (Visioli et al., 1998). Extra virgin olive oil is unique from other types of vegetable oils because it is rich in these phenolic compounds, which are thought to be responsible for the comparatively stable characteristics of olive oil (Visioli and Bernardini, 2011; Sabatini, 2010; Tuck et al., 2002).

The simple phenolic structure is characterized by a carbon aromatic ring linked to a hydroxyl group (Bravo, 1998). Hydroxytyrosol (3,4-dihydroxyphenyl ethanol) (HT) is a type of polyphenol which consists of a carbon aromatic ring linked to 2 hydroxyl groups and an ethanol group (Quiles et al., 2002). Recently, the health benefits associated with the Mediterranean diet

have been credited to the HT content of olive oil due to its potential to increase mitochondrial density and function in cells (Granados et al., 2010; Paiva-Martins et al., 2011). HT and its derivatives comprise approximately 50% of the phenolic compounds found in extra virgin olive oil (Raederstorff, 2009). Olive oil waste water, which is a product of olive oil manufacturing, is rich in HT. Currently, disposal of olive waste water represents a significant burden to olive oil manufactures, but if HT is found to evoke significant health benefits, this waste water may be a valuable commodity (Visioli et al., 1999).

Role of Mitochondria in Energy Production and Exercise Performance

Mitochondrial Metabolism

Mitochondria, located in the cytosol of most eukaryotic cells, are the cellular framework by which active tissues utilize delivered oxygen via oxidative phosphorylation. Mitochondria require the presence of oxygen to break down nutrients to produce adenosine triphosphate (ATP), the form of energy required for muscular work and maintenance of processes necessary for the cell to survive (Lardy and Ferguson, 1967). Acetyl CoA derived from the breakdown of fatty acids or carbohydrates enters the citric acid cycle (also known as the Krebs cycle) in the mitochondrial matrix and becomes a source of hydrogen to convert coenzymes nicotinamide adenine dinucleotide (NAD+) and flavin adenine dinucleotide (FAD) to reduced states NADH and to $FADH_2$, respectively. Hydrogens carried by NADH and $FADH_2$ are oxidized and the extracted electrons are passed along the respiratory chain of the mitochondria (Alberts, 2008).

The respiratory chain of the mitochondria consists of a series of cofactors, most of which are cytochromes (iron-protein electron carriers), that are grouped into five large respiratory

enzyme complexes. Hydrogens are donated from NADH + H^+ to complex I (NADH dehydrogenase) or from $FADH_2$ to complex II (succinate dehydrogenase (SDH)). These hydrogens are quickly split by the reduced coenzymes into protons (H^+) plus electrons. H^+ is pumped into the intermembrane space by the enzyme complexes while electrons continue to be transferred along the chain to complex III (cytochrome *c* reductase). Cytochrome *c*, a protein loosely associated with the mitochondrial membrane, transfers electrons from complex III to complex IV (cytochrome *c* oxidase), and finally the electrons are accepted by ½ O_2 to form H_2O as a by-product. Complex V of the respiratory chain is the inner membrane-associated enzyme complex ATP synthase, which harnesses the electrochemical gradient created by the proton pumps to drive the synthesis of ATP from mitochondrial matrix-associated ADP and an inorganic phosphate ion (Alberts 2008). The coupling of ATP to cellular proteins allows energetically-unfavorable reactions such as muscle contraction to occur.

Maximal Oxygen Consumption

A VO_{2MAX} test measures an individual's expired gases during a graded exercise test involving aerobic exercise such as running or cycling. As exercise intensity increases, the oxygen demand of the mitochondria increases; therefore, oxygen consumption increases proportionally to increasing workload (Barstow et al., 1993). Eventually, the maximal rate of aerobic metabolism no longer adequately matches oxygen demand, and VO_{2MAX} is reached when oxygen consumption does not increase despite an increase in workload. VO_{2MAX} is negatively associated with all-cause mortality (Blair et al., 1989) and is a major factor governing endurance exercise performance (Joyner and Coyle, 2008).

Lactate Threshold

Another main, and arguably the most important, determinant of endurance exercise performance is lactate threshold since endurance races are typically performed at an intensity equal to lactate threshold, but below VO_{2MAX} (Allen et al., 1985). Lactate threshold, which can be measured via blood sampling during a graded exercise test, is the point of lactate accumulation in the blood above baseline levels (Ivy et al., 1980).

Lactate is a product of anaerobic metabolism, which provides a rapid source of ATP via glycolysis in the cytosol of the cell. Stored muscle glycogen enters glycolysis and is eventually converted to pyruvate. Pyruvate can then be shuttled into the mitochondria, where it is converted to acetyl CoA and then enters the citric acid cycle and oxidative phosphorylation. However, during high intensity exercise, pyruvate cannot be shuttled into the mitochondria in proportion to the rate it is produced (Holloszy et al., 1977). Instead it is converted to lactic acid in the cytosol and then diffuses into the blood. The blood contains a bicarbonate buffering system that converts lactic acid into lactate and carbon dioxide (Harrison and Pilcher, 1930).

As the body continues to increase its reliance on anaerobic metabolism, lactic acid production from glycolysis begins to overwhelm the blood buffering system, and lactate accumulates in the blood and working skeletal muscle. Lactate accumulation eventually results in decreased blood and skeletal muscle pH, leading to fatigue and forcing the individual to decrease exercise intensity. Decreased skeletal muscle pH directly inhibits certain aerobic enzymes, further decreasing oxidative processes in the mitochondria (Jubrias et al., 2003).

Endurance Training Adaptations

Endurance training increases lactate threshold, allowing an individual to exercise at a higher intensity before experiencing lactate accumulation and fatigue (Allen et al., 1985). Typically, lactate threshold occurs at approximately 60% of VO_{2MAX} in untrained individuals, whereas in trained individuals, lactate threshold can occur at much higher exercise intensities—75 to 90% of VO_{2MAX} (Joyner and Coyle, 2008). Ivy et al., (1980) observed that lactate production is primarily influenced by the oxidative capacity of the muscle. Major training adaptations that are responsible for this relative shift in lactate threshold include increased mitochondrial density and function within the skeletal muscle (Henriksson, 1977). The shift from primarily anaerobic to aerobic metabolism at a given exercise intensity is accompanied by a shift in substrate utilization from primarily carbohydrates to fats, which can be measured by Respiratory Exchange Ratio (Hill et al., 1924; Harrison and Pilcher, 1930; Wasserman and McIlroy 1964). Increased mitochondria allow distribution of the oxidative demand, allowing an individual to rely primarily on aerobic metabolism at higher exercise intensities before anaerobic metabolism becomes the predominant energy source (Holloszy and Coyle, 1984).

Mitochondrial Biogenesis

Mitochondrial biogenesis is the term commonly used to describe the formation of new mitochondria through activation of specific transcription factors and signaling pathways inside the cell (Attardi and Schatz, 1988). Exercise and other conditions of high cellular oxidative demand (Vorobjev and Zorov, 1983; Nisoli and Carruba, 2006) induce mitochondrial biogenesis by activating 5'-AMP activated protein kinase (AMPK) in the cytosol of the cell (Brunk, 1981;

Moyes et al., 1997; Little et al., 2011). AMPK acts as a cellular energy sensor; it is activated by an increased cellular AMP:ATP ratio, which occurs at the onset of exercise or under conditions of low nutritional availability (Hardie, 2011).

AMP activates AMPK by binding to its regulatory subunit, which causes phosphorylation of the catalytic subunit by upstream kinases. Upon activation, AMPK switches on catabolic pathways that generate ATP such as glycolysis, fatty acid oxidation, and mitochondrial biogenesis; congruently it switches off anabolic pathways that consume ATP such as lipogenesis (Hardie, 2011). AMPK regulates transcription of mitochondria by elevating nuclear transcription factor peroxisome proliferator-activated receptor γ coactivator (PGC)-1α, a master regulator of mitochondrial biogenesis (Irrcher et al., 2003; Lee et al., 2006). PGC-1α expression is directly regulated by relative exercise intensity (Egan et al., 2010; Nordsborg et al., 2010). Furthermore, upregulation of PGC-1α coincides with increased mRNA expression of mitochondrial genes (Viollet et al., 2007; Little et al., 2011).

HT Promotes Mitochondrial Biogenesis and Function

Background Polyphenol Research

Nearly a decade ago, researchers began to examine the ability of polyphenols to induce mitochondrial biogenesis and enhance mitochondrial function. Resveratrol, a polyphenol found in the skin of grapes, originally gained notoriety for its ability to activate the silent information regulator two (Sir2) pathway, which is credited for lifespan extension during caloric restriction in various species (Howitz et al., 2003; Rogina and Helfand, 2004). Baur et al. (2006)

demonstrated that long-term resveratrol supplementation did indeed extend the lifespan of mice. Howitz et al., (2003) found that resveratrol increased the mammalian Sir2 homolog (SIRT1), an upstream activator of PGC-1α, in mouse skeletal muscle. Similarly, Lagouge et al. (2006) found that resveratrol treatment increased SDH, CS, and SIRT1 in mouse skeletal muscle.

Based on these findings, Davis and colleagues (2009) examined the effects of quercitin, a similar polyphenol derived from a wide variety of food plants including red onions, apples, and berries, on mitochondrial biogenesis and endurance exercise performance in mice. Mice were administered 12.5 or 25 mg/kg of quercitin supplement for 7 days. After 7 days, the mice were exercise tested, then killed and the soleus muscle and brain were analyzed for mRNA expression of PGC-1α. Quercitin increased brain and skeletal muscle mRNA expression of PGC-1α, accompanied by increased maximal endurance capacity and voluntary wheel running activity. Quiles attributed increased voluntary wheel running activity to decreased perception of effort. Alexander (2006) showed that phenols such as querctin act as adenosine A_1 receptor antagonists in vitro, similarly to caffeine. Additionally, quercetin supplementation was found by MacRae and Mefferd (2006) to improve 30 km cycling time trial performance by 3.1% in elite male cyclists. There was no increase in VO_{2MAX} observed in this study. Instead, the increase in performance was attributed to increased relative power output over the time trial course.

Hydroxytyrosol Promotes Mitochondrial Biogenesis

Because of its structural similarity other members of the polyphenolic family such as quercitin and resveratrol, researchers have recently examined the ability of HT to likewise increase mitochondrial biogenesis and enhance endurance exercise performance.

Hao et al. (2009) demonstrated for the first time that HT is capable of stimulating mitochondrial biogenesis via activation of PGC-1α in isolated rodent 3T3-L1 adipocytes. Cell cultures and differentiation of 3T3-L1 cells are commonly used as models of adipogenic differentiation and insulin resistance because they are sensitive to insulin, and display insulin-sensitive glucose uptake similarly to primary adipose cells. Cultured cells were incubated with HT at concentrations of 0.1, 1, 10, and 50 µmol/L for 24 hours. Treatment of cells with HT at 0.1-50 µmol/L resulted in dose-dependent stimulation of PGC-1α. HT treatment at a concentration of 1 µmol/L resulted in a significant increase in mtDNA. HT treatment at concentrations of 0.1, 1, and 10 µmol/L increased the expression of mitochondrial complexes I and II, while HT concentrations of 1 and 10 µmol/L increased expression of complexes III and IV. These increases in mitochondrial complex protein expression were approximately 1.5- to 1.7-fold compared to control samples. Consequently, mitochondria density increased and mitochondrial function was enhanced, indicated by increased activity of mitochondrial complexes I through IV, increased oxygen consumption, and decreased free fatty acid content of the cell. These findings were confirmed by Zhu et al. (2010), who likewise demonstrated that HT was capable of inducing mitochondrial biogenesis in vitro via activation of PGC-1α in isolated human ARPE-19 human retinal pigment epithelial cells.

To further examine the effects of HT on mitochondrial function and endurance exercise performance, Liu et al., (2011) supplemented mice with 50, 150, or 300 mg/kg body weight HT once per day for 3 weeks. After 3 weeks of treatment, maximal running distance on a treadmill was measured. HT increased the running distance to exhaustion in all treatment groups compared to placebo. The treatment group which was administered 300 mg/kg body weight HT

per day significantly increased run time to exhaustion compared to the 50 and 150 mg/kg body weight HT treatment groups.

In an unprecedented HT supplementation study in humans (Rietjens et al., 2011), eight healthy males (22.0 ± 1.9 years of age, 75.4 ± 3.3 kg, 1.8 ± 0.02 m, 22.7 ± 0.8 BMI) with no history of participation in a regular exercise program were given an olive oil supplement and exercise-induced lactate accumulation was evaluated. The subjects were instructed to avoid consumption of olives, olive oil, olive products, and other specific foods which contain high levels of antioxidants or polyphenols during the three days prior to and on the day of experimental testing, and to refrain from strenuous exercise during the study period. In a cross-over design, subjects were tested once after consumption of a supplement beverage containing 200 mg of HT, and once after consumption of a placebo. The supplement was consumed once at 8:00 PM on the night prior to experimental testing, and again 30 min prior the start of exercise testing. After a 5-min warm-up on a Stairmaster, subjects performed 8 sets of 10 repetitions on a horizontal leg press machine, followed by 8 sets of 10 repetitions on a leg extension machine. Both exercises were performed at 75% of the subject's individual 1 repetition maximum (RM) with 2-min rest intervals between sets. Blood samples were collected before the start of the exercise, during the exercise, and up to 2.5 hr after exercise. Analysis of plasma lactate concentrations indicated that plasma lactate considerably increased during exercise, but HT treatment attenuated the increase in plasma lactate (12.8-fold increase above baseline) compared to placebo (14.8-fold increase above baseline). Blood lactate was expressed as percent of baseline, however actual blood lactate values were not reported in this study. Although this attenuation of lactate was statistically significant, the physiological relevance is questionable.

In order to observe the protective effects of HT on exercise-induced cellular damage, Feng and colleagues (2011) strenuously exercised rats to induce cellular damage. While physical exercise is beneficial to health, excessive exercise can lead to chronic fatigue, muscle damage, and impaired immune function (Shephard 1994; Smith 2003). Rats which were treated with 25 mg/kg/day HT displayed enhanced endurance capacity and decreased immune system damage. HT treatment protected against inhibition of PGC-1α and enhanced mitochondrial complex I and II activities.

Hydroxytyrosol is an Antioxidant

Increased mitochondrial function may improve mitochondrial function by decreasing mitochondrial production of reactive oxygen species (ROS). The electron transport chain of the mitochondria is highly efficient, but electron leakage does occur in proportion to the rate of mitochondrial respiration, resulting in generation of ROS. ROS are oxygen radicals consisting primarily of superoxide anions ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($^{\bullet}OH$); these radicals are capable of damaging cellular deoxyribonucleic acid (DNA), lipids, and other molecules (Wallace, 2005). A low level of ROS production is necessary to control important cellular functions, but a high level of ROS production can cause mutations of the mitochondrial DNA (mtDNA) (Chan, 2006). Most mitochondrial proteins are encoded by the nuclear genome and imported into the mitochondria, but the mitochondria retains some DNA that is essential for its respiratory function (Wallace, 2005).

It has been proposed that mtDNA mutations decrease the efficiency of the electron transport chain, thus decreasing ATP production and further increasing production of ROS

(Richter, 1988; Zhang et al., 1990). This cycle of mtDNA mutation and disrupted respiratory function provides the basis for the theory that mitochondrial dysfunction plays a critical role in the aging process (Richter, 1995) and the development of degenerative diseases (Harman, 1956, 1992; Linnane et al., 1989; Lowell and Shulman, 2005; Murrow and Hoehn, 2010). Tissues which are highly dependent on cellular respiration such as brain, skeletal muscle, cardiac muscle, and endocrine are thought to be particularly susceptible to mitochondria-associated diseases (Wallace, 1999). High levels of ROS and oxidative damage to mtDNA have been consistently observed in age-associated diseases (Melov et al., 1999; Cooke et al., 2003; Pelicano et al., 2004; Zmijewski et al., 2005; Matthews, 2006). In fact, Barja and Herrero (2000) found that oxidative damage to mitochondria in the heart and brain of mammals was inversely related to maximum lifespan. Additionally, Nieman et al. (2003) found an association between ROS and fatigue in ultramarathon subjects.

Liu and colleagues (2007) examined the protective effects of HT on acrolein-induced toxicity in the human retinal pigment epithelial cell line ARPE-19, a cellular model of smoking- and age-related macular degeneration. Acrolein is an oxidant found in cigarette smoke, and is also a product of lipid oxidation in vivo. Treatment of cells with 75 µmol/L of acrolein for 24 hours induced mitochondrial dysfunction and increased ROS, indicated by decreased mitochondrial membrane potential, depressed activities of mitochondrial complexes, reduced number of viable mitochondria, decreased oxygen consumption, reduced factors for mitochondrial biogenesis, and increased intracellular calcium. Pre-treatment of the ARPE-19 cells with HT protected against ROS-induced damage and mitochondrial dysfunction. In a dose- and time-dependent manner, pre-treatment of cells with greater than 75 µmol/L of HT for 24 hours effectively protected cells, while 5 µmol/L of HT for 7 days showed a similar protective

effect. Thus, researchers concluded that HT supplementation may function as an effective therapeutic treatment of smoke-induced or age-related macular degeneration of human retinal pigment epithelial cells. This conclusion supports the findings of an earlier study by Quiles et al. (2002), which demonstrated that treatment of human prostate cells with HT for 24 hours at concentrations as low as 10 μM decreased ROS production and reduced ROS-induced mtDNA damage.

Hydroxytyrosol Improves Mitochondrial Control of the Cell Cycle

Not only can ROS damage mitochondrial enzymes necessary for oxidative phosphorylation, but high levels of ROS may also inhibit the mitochondria's control over the cell cycle (Brandon et al., 2006). In a properly-functioning cell, intracellular mechanisms which signal apoptosis are activated in response to cellular damage and dysfunction. High levels of ROS may oxidize certain pro-apoptotic intracellular proteins such as p53, inhibiting apoptosis and allowing survival and proliferation of malignant cells, possibly progressing to cancer (Hussain et al., 2003; Halliwell, 2007). It has been observed that animals with impaired ability to scavenge ROS have increased risk for developing cancers later in life (Halliwell, 1984; Kim et al., 2001; Sutton, 2003).

A novel study by Granados and colleagues (2011) demonstrated for the first time in animals that HT inhibits growth and cell proliferation of mammary tumors. The growth of these tumors in rats was induced by dimethylbenz[α]anthracene. The treatment group was administered 0.5 mg/kg body weight HT 5 days per week for 6 weeks. HT treatment altered

expression of genes related to apoptosis, cell cycle, proliferation, differentiation, survival and transformation pathways. These findings are in agreement with research by Notarnicola et al. (2011), which tested the effects of HT in two independent human colon cancer cell lines, HT-29 and SW620. HT had an anti-proliferative effect and was able to induce apoptosis in both cell lines. Bouallagui et al. (2010) showed similar results in MCF-7 human breast cancer cells.

Bioavailability and Safety of Hydroxytyrosol

A growing body of literature has made a valid case for the potential use of HT as a nutritional supplement to improve exercise tolerance and to treat mitochondria-associated diseases, though there has recently been disagreement in the literature involving the bioavailability of HT upon ingestion and its potential biological antioxidant activity in humans.

Because HT is a polar compound, it is likely to be excreted by the kidneys into the urine either in its unconjugate or conjugate form. Visioli and colleagues (2000) observed that after ingestion of 50 ml of an olive oil sample, the conjugate form of HT was increased in the urine in direct proportion to the amount of olive oil ingested, indicating that HT was dose-dependently absorbed in humans. To confirm this observation, Pinto et al. (2006) perfused cells of the jejunum and ileum of the small intestine with phenolic compounds including HT. They found that HT was indeed absorbed and metabolized by the cells of the small intestine, indicated by the presence of HT in its conjugate form.

In contrast, Vissers et al. (2004) reviewed available literature related to the bioavailability of olive oil phenols in animal and human studies. His group concluded that, of the studies reviewed, animals which were treated with olive oil that had high phenol content consistently

demonstrated improved markers of oxidation, but 5 out of 7 studies in humans did not demonstrate equivalent beneficial effects. Furthermore, Vissers suggested that 50 g of olive oil per day provides approximately 2 mg of HT equivalents per day. This amount of HT results in a blood concentration of 0.06 μmol/L; an amount lower than the minimum concentrations required to illicit antioxidant activity in vitro. Similarly, Khymenets et al. (2010) supplemented individuals with 50 mg of virgin olive oil and did not find significant antioxidant activity at the observed concentrations (0.01-10 μM).

According to preliminary safety testing (Edwards et al., 2009), HT is rapidly absorbed after oral intake, and is rapidly and widely spread throughout rat tissues, with no indication of accumulation in the tissues. The No Observed Adverse Effect Level (NOAEL) of HT supplementation in a 4-week trial in rats was a dosage of 560 mg/kg of body weight/day. In a 13-week clinical trial, the NOAEL of HT was 252 mg/kg body weight/day. HT dosage at 200 mg/day resulted in decreased sperm velocity in males. This condition was fully reversed upon the removal of the HT supplement, and did not result in any permanent dysfunction of sex organs or impairment of sperm development. Accordingly, the acceptable daily intake (ADI) of HT for males should be 150 mg/day. In an embryo toxicity study, the NOAEL of HT was 168 mg/kg body weight/day. An HT dosage of 504 mg/kg body weight/day resulted in a slight reduction of maternal and fetal bodyweight. Accordingly, the ADI of HT for females should be 100 mg/day. Furthermore, an Ames test confirmed that HT has no mutagenic potential (Edwards et al., 2009).

Collectively, these data indicate that HT is biologically available to humans upon ingestion and is generally non-toxic; however it is debated whether the amount of HT required to elicit its potential biological activities can be practically achieved in humans.

Conclusion

Adherence to a Mediterranean diet may improve longevity and reduce the risk for diseases associated with mitochondrial dysfunction and aging, such as cardiovascular disease, type II diabetes, and cancer. It appears that HT, a polyphenolic component of extra virgin olive oil, is responsible for these health benefits. HT may exert its beneficial effects by increasing mitochondrial biogenesis and function inside the cell. VO_{2MAX} and lactate threshold, both factors which dictate endurance exercise performance, are highly influenced by skeletal muscle mitochondrial volume and function. The rate of oxidative phosphorylation in the mitochondria is a major limiter of exercise performance. Increased mitochondria enhance exercise performance by decreasing reliance on glycolysis and increasing reliance on oxidative phosphorylation at a given exercise intensity. HT stimulates mitochondrial biogenesis through the activation of AMPK and downstream transcription factor PGC-1α. Dysfunctional mitochondria have been associated with decreased exercise tolerance, as well as age-associated diseases including type II diabetes, atherosclerosis, and cancer. A common theory for mitochondrial-based disease is the mutation of mtDNA by ROS. ROS are by-products of oxidative phosphorylation, but damage to the mitochondrial respiratory enzymes can increase ROS production, resulting in oxidation of mtDNA, which further compounds ROS production. HT protects isolated cell lines from ROS production and oxidation and enhances mitochondrial control of the cell cycle in isolated cell and animal models. The beneficial effects of HT have been clearly demonstrated in vitro and in animal models, but there is no research to-date showing HT-induced mitochondrial biogenesis in humans. Most investigators agree that HT is biologically available to humans upon ingestion; however it is debated whether the amount of HT required to elicit its potential biological activities can be practically achieved in humans.

References

Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. (2008). *Molecular Biology of the Cell* (Fifth ed.). 270 Madison Avenue, New York NY 10016, USA: Garland Science.

Allen W, Seals DR, Hurley BF, Ehsani AA, Hagberg JM. (1985). Lactate threshold and distance running performance in young and older endurance athletes. *Journal of Applied Physiology, 58*(4), 1281-1284.

Attardi G, Schatz G. (1988). Biogenesis of mitochondria. *Annual Review of Cellular Biology, 4*, 289-331.

Baar K. (2004). Involvement of PPARɣ co-activator-1, nuclear respiratory factors 1 and 2, and PPARα in the adaptive response to endurance exercise. *Proceedings og the Nutrition Society, 63*, 269-273.

Barja G, Herrero A. (2000). Oxidative damage to mitochondrial DNA is inversely related to maximum life span in the heart and brain of mammals. *The FASEB Journal, 14*, 312-318.

Barstow T, Casaburi R, and Wasserman K. (1993). O2 uptake kinetics and the O2 deficit as related to exercise intensity and blood lactate. *Journal of Applied Physiology, 72*(2), 755-762.

Bassett DJ, Howley, ET. (2000). Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Medicine & Science in Sports & Exercise, 32*(1), 70.

Baur J, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le Couteur D, Shaw RJ, Navas P, Puigserver P, Ingram DK,de Cabo R, and Sinclair DA. (2006). Resveratrol improves health and survival of mice on a high-calorie diet. *Nature, 444*, 337-342.

Blair S, Kohl III, HW, Paffenbarger Jr RS, Clark DG, Cooper KH, and Gibbons LW. (1989). Physical fitness and all-cause mortality. *The Journal of the American Medical Association, 262*(17), 2395-2401.

Bouallagui Z, Han J, Isoda H, Sayadi S. (2010). Hydroxytyrosol rich extract from olive leaves modulates cell cycle progression in MCF-7 human breast cancer cells. *Food and Chemical Toxicology, 49*, 179-184.

Brandon M, Baldi P, Wallace DC. (2006). Mitochondrial mutations in cancer. *Oncogene, 25*, 16.

Bravo L. (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews, 56*(11), 17.

Brunk C. (1981). Mitochondrial proliferation during myogenesis. *Experimental Cell Research, 136*, 305-309.

Chan D. (2006). Mitochondria: dynamic organelles in disease, aging, and development. *Cell, 125*, 12.

Cooke M, Evans MD, Dizdaroglu M, Lunec J. (2003). Oxidative DNA damage: mechanisms, mutation, and disease. *The FASEB Journal, 17*, 1195-1214.

Davis M, Murphey EA, Carmichael MD, and Davis B. (2009). Quercitin increases brain and muscle mitochondrial biogenesis and exercise tolerance. *AJP - Regulatory Physiology, 296*(4), R1071-R1077.

Edwards J, Beck M, and Bausch J. (2009). Water-soluable olive extract, olive 15% CWS outline safety summary: DSM Nutritional Products, R&D Human Nutrition and Health, NRD/CH Safety.

Egan B, Carson BP, Garcia-Roves PM, Chibalin AV, Sarsfield FM, Barron N, McCaffrey N, Moyna NM, Zierath, O'Gorman DJ. (2010). Exercise intensity-dependent regulation of peroxisome proliferator-activated receptor ɣ coactivator-1α mRNA abundance is associated with differential activation of upstream signalling kinases in human skeletal muscle. *The Journal of Physiology, 588*(10), 1779-1790.

Feng Z, Bai L, Yan J, Li Y, Shen W, Wang Y, Wertz K, Weber P, Zhang Y, Chen Y, Liu J. (2011). Mitochondrial dynamic remodeling in strenuous exercise-induced muscle and mitochondrial dysfunction: regulatory effects of hydroxytyrosol. *Free Radical Biology and Medicine, 50*, 1437-1446.

Granados S, Quiles JL, Ramirez-Tortosa CL, Sanchez-Rovira P, Ramirez-Tortosa MC. (2010). Hydroxytyrosol: from laboratory investigations to future clinical trials. *Nutrition Reviews, 68*(4), 16.

Granados S, Quiles JL, Ramirez-Tortosa Camacho-Corencia P, Sanchez-Rovira P, Vera-Ramirez L, Ramirez-Tortosa MC. (2011). Hydroxytyrosol inhibits growth and cell proliferation and promotes high expression of sfrp4 in rat mammary tumors. *Mol Nutr Food Res, 55*, 117-126.

Halliwell. (2007). Oxidative stress and cancer: have we moved forward? *Biochemical Journal, 401*, 1-11.

Halliwell B, Gutteridge JM. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemical Journal, 219*, 1-14.

Hao J, Shen W, Yu G, Jia H, Li X, Feng Z, Wang Y, Weber P, Wertz K, Sharman E, Liu J. (2009). Hydroxytyrosol promotes mitochondrial biogenesis and mitochondrial function in 3T3-L1 adipocytes. *Journal of Nutritional Biochemistry, 21*, 634-644.

Hardie D. (2011). Sensing of energy and nutrients by AMP-activated protein kinase. *American Journal of Clinical Nutrition, 93*(4), 891-896.

Harman D. (1956). Aging: a theory based on free radical and radiation chemistry. *Journal of Gerontology, 11*(3), 3.

Harman D. (1992). Free radical theory of aging. *Mutation Research, 275*, 10.

Harrison TR, Pilcher C. (1930). Studies in congestive heart failure, II. The respiratory exchange during and after exercise. *Journal of Clinical Investigation, 8(3),* 291-315.

Henriksson J. (1977). Training induced adaptation of skeletal muscle and metabolism during submaximal exercise. *J Physiol London, 270*, 661-675.

Hill AV, Long CNH, Lupton H. (1924). Muscular exercise, lactic acid, and the supply and utilization of oxygen. *Proc Roy Soc London, S. B. 96*, 84, 155, 438.

Holloszy J, Rennie MJ, Hickson RC, Conlee RK, Hagberg JM. (1977). Physiological consequences of the biochemical adaptations to endurance exercise. *Annals of the New York Academy of Sciences, 301*, 440-450.

Holloszy J, Coyle EF. (1984). Adaptations of skeletal muscle to endurance exercise and their metabolic consequenses. *Journal of Applied Physiology, 56*(4), 831-838.

Howitz K, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, Zipkin RE, Chung P, Kisielewski A, Zhang, L, Scherer B, and Sinclair DA. (2003). Small molecules activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature, 425*, 191-196.

Hussain S, Hofseth LJ, Harris CC. (2003). Radical causes of cancer. *Nature Reviews--Cancer, 3*, 10.

Hwang J-T, Kwon DY, Yoon SH. (2009). AMPK-activated protein kinase: a potential target for the diseases prevention by natural occuring polyphenols. *New Biotechnology, 26*(1/2), 17-22.

Irrcher I, Adhihetty PJ, Joseph A-M, Ljubicic V, Hood DA. (2003). Regulation of mitochondrial biogenesis in muscle by endurance exercise. *Sports Medicine, 33*(11), 11.

Ivy JL, Withers RT, Van Handel PJ, Elger DH, Costill DL. (1980). Muscle respiratory capacity and fiber type as determinants of the lactate threshold. *Journal of Applied Physiology, 48(*3), 523-527.

Joyner M, Coyle EF. (2008). Endurance exercise performance: the physiology of champions. *The Journal of Physiology, 586*(1), 35-44.

Jubrias S, Crowther GJ, Shankland EG, Gronka RK, Conlet KE. (2003). Acidosis inhibits oxidative phosphorylation in contracting human skeletal muscle *in vivo*. *The Journal of Physiology, 553*, 589-599.

Khymenets O, Fitó M, Touriño S, Muñoz-Aguayo D, Pujadas M, Torres JL, Joglar J, Farré M, Covas M-I, de la Torre R. (2010). Antioxidant activities of hydroxytyrosol main metabolites do not contribute to beneficial health effects after olive oil ingestion. *Drug Metab Dispos, 38*(9), 1417-1421. Published ahead of print June 1411, 2010.

Kim K, Rodriguez AM, Carrico PM, Melendez JA. (2001). Potential mechanisms for the inhibition of tumer cell growth by manganese superoxide dismutase. *Antioxid Redox Signal, 3*, 361-373.

Lagouge M AC, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Massadeq N, Milne J, Lambert P, Elliot P, Geny B, Laakso M, Puigserver P, and Auwerx J. (2006). Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1α. *Cell, 127*(6), 1109-1122.

Lardy H, Ferguson SM. (1969). Oxidative phosphorylation in mitochondria. *Annual Rev. Biochem., 38*, 991-1034.

Lee J, Kim M, Park H-S, Kim HS, Jeon MJ, Oh KS, Koh EH, Won JC, Kim M-S, Oh GT, Yoon M, Lee K-U, Park JY. (2006). AMPK activation increases fatty acid oxidation in skeletal muscle by activating PPARα and PGC-1. *Biochemical and Biophysical Research Communications, 340*, 291-295.

Leick L, Lyngby SS, Wojtasewski JFP, Pilegaard H. (2010). PGC-1α is required for training-induced prevention of age-associated decline in mitochondrial enzymes in mouse skeletal muscle. *Experimental Gerontology, 45*, 336-342.

Linnane A, Marzuki S, Ozawa T, Tanaka M. (1989). Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases. *The Lancet, 333*(8639), 4.

Lira V, Benton CR, Yan Z, Bonen A. (2010). PGC-1α regulation by exercise training and its influences on muscle function and insulin sensitivity. *Am J Physiol Endocrinol Metab, 299*, 145-161.

Little J, Safdar A, Birshop D, Tanopolsky MA, Gibala MJ. (2011). An acute bout of high-intensity interval training increases the nuclear abundance of PGC-1{alpha} and activates mitochondrial biogenesis in human skeletal muscle. *Am J Physiol Regu Integr Comp Physiol*, March 30, 2011. [Epub ahead of print].

Liu J, Raederstorff D, Wang-Schmidt Y, and Wertz K. (2011). United States Patent No. (Application) US 2011/0112201 A1.

Liu Z, Sun L, Zhu L, Jia X, Li X, Jia H, Wang Y, Weber P, Long J, Liu J. (2007). Hydroxytyrosol protects retinal pigment epithelial cells from acrolein-induced oxidative stress and mitochondrial dysfunction. *Journal of Neurochemistry, 103*, 2690-2700.

Lowell B, Shulman GI. (2005). Mitochondrial dysfunction and type 2 diabetes. *Science, 307*, 4.

MacRae H, and Mefferd KM. (2006). Dietary antioxidant supplementation combined with quercitin improves cycling time trial performance. *Int J Sport Nutr Exercise Metab, 16*, 405-415.

Matthews C. (2006). DNA precursor metabolism and genomic stability *The FASEB Journal*, *20*, 1300-1314.

Melov S, Schneider JA, Coskun PE, Bennet DA, Wallace DC. (1999). Mitochondrial DNA rearrangements in aging human brain and in situ PCR of mtDNA. *Neurobiol Aging, 20*, 565-571.

Mootha V, Lindgren CM, Eriksson K-F, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstrale M, Laurila E, Houstis N, Daly MJ, Patterson N, Mesirov JP, Golub TR, Tamayo P, Spiegelman B, Lander E, Hirschorn JN, Altshuler D, Groop LC. (2003). PGC-1α-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nature Genetics, 34*(3), 267-273.

Moyes C, Mathieu-Costello OA, Tsuchiya N, Filburn C, Hansford RG. (1997). Mitochondrial biogenesis during cellular differentiation. *American Journal of Cell Physiology, 272*(4), C1345-C1351.

Murrow B, Hoehn KL. (2010). Mitochondrial regulation of insulin action. *The International Journal of Biochemistry and Cell Biology, 42*, 4.

Nieman D, Dumke CI, Henson DA, McAnuity SR, McAnuity lA, Lind RH, and Murrow JD. (2003). Immune and oxidative changes during and following the Western States Endurance Run. *Int J Sports Med, 24*, 541-547.

Nisoli E, Carruba MO. (2006). Nitric oxide and mitochondrial biogenesis. *Journal of Cell Science, 119*(14), 8.

Nordsborg N, Lundby C, Leick L, Pilegaard H. (2010). Relative workload determines exercise-induced increases in PGC-1α mRNA. *Medicine & Science in Sports & Exercise, 42*(8), 1477-1484.

Notarnicola M, Pisanti S, Tutino V, Bocale D, Rotelli MT, Gentile A, Memeo V, Bifulco M, Perri E, Caruso MG. (2011). Effects of olive oil polyphenols on fatty acid synthase gene expression and activity in human colorectal cancer cells. *Genes Nutr, 6*.

Paiva-Martins F, Rodrigues V, Calheiros R, Marques MPM. (2011). Characterization of antioxidant olive oil biophenols by spectroscopic methods. *J Sci Food Agric, 91*, 6.

Pelicano H, Carney D, Huang P. (2004). ROS stress in cancer cells and therapeutic implications. *Drug Resistance Updates, 7*, 14.

Pinto J, Paiva-Martins F, Corona G, Debnam ES, Oruna-Concha MJ, Vauzour, Gordon MH, Spencer JPE. (2011). Absorption and metabolism of olive oil secoiridoids in the small intestine. *British Journal of Nutrition, 105*, 1607-1618.

Quaranta G, Rotundo V. (2000). Economic and commercial prospects for olive oil in view of the changes in the common market organisation (CMO) (Part one). *Olivae, 91*, 5.

Quiles J, Farquharson AJ, Simpson DK, Grant I, Wahle KWJ. (2002). Olive oil phenolics: effects on DNA oxidation and redox enzyme mRNA in prostate cells. *British Journal of Nutrition, 88*, 10.

Raederstroff D. (2009). Antioxidant activity of olive polyphenols in humans: a review. *Int J Vitam Nutr Res, 79*(3), 152-165.

Richter C. (1988). Do mitochondrial DNA fragments promote cancer and aging? *FEBS Letters, 241*(1,2), 5.

Richter C. (1995). Oxidative damage to mitochondrial DNA and its relationship to ageing. *International Journal of Cell Biology, 27*(7), 7.

Rietjens J, Bast A, Haenen GRMM, and Heyden Van Der LCG. (2011). United States Patent No. (Application) US 2011/0052750 A1.

Rogina BaHS. (2004). Sir2 mediates longevity in the fly through a pathway related to calorie restriction. *PNAS, 101*(45), 15998-16003.

Sabatini N. (2010). Recent patents in olive oil industry: new technologies for the recovery of phenols compounds from olive oils, olive oil industrial by-products and waste waters. *Recent Pat Food Nutr Agric, 2*(2), 6.

Schwerzmann. (1989). Oxidative capacity of muscle and mitochondria: correlation of physiological, biochemical, and morphometric characteristics. *PNAS, 86*(5), 1583-1587.

Shephard R, Rhind S, Shek PN. (1994). Exercise and the immune system. Natural killer cells, interleukins and related responses. *Sports Medicine, 18*(5), 340-369.

Smith L. (2003). Overtraining, excessive exercise, and altered immunity: is this a T helper-1 versus T helper-2 lyphocyte response? *Sports Medicine, 33*(5), 347-364.

Sutton A, Khoury H, Prip-Buus C, Cepances C, Pessayre D, Degoul F. (2003). The Ala16Val genetic dimorphism modulates the import of human manganese superoxide dismutase into rat liver mitochondria. *Pharmacogenetics, 13*, 145-157.

Taivassalo T, De Stefano N, Argov Z. (1998). Effect of aerobic training in patients with mitochondrial myopathies. *Neurology, 50*(4), 1055-1060.

Trichopoulou A, Costacou T, Bamia C, Trichopoulou D. (2003). Adherence to a Mediterranean diet and survival in a Greek population. *New England Journal of Medicine, 348*(26), 12.

Tuck K, Hayball PJ. (2002). Major phenolic compounds in olive oil: metabolism and health effects. *The Journal of Nutritional Biochemistry, 13*, 10.

Viollet B, Mounier R, Leclerc J, Yazigi A, Foretz M, Andreelli F. (2007). Targeting AMP-activated protein kinase as a novel therapeutic approach for the treatment of metabolic disorders. *Diabetes & Metabolism, 33*, 395-402.

Visioli F, Bellomo G, Galli C. (1998). Free radical-scavenging properties of olive oil polyphenols. *Biochemical and Biophysical Research Communications, 247*, 5.

Visioli F, Romani A, Mulinacci N, Zarini S, Conte D, Vincieri FF, Galli C. (1999). Antioxidant and other biological activities of olive mill waste waters. *J Agric Food Chem, 47*, 3397-3401.

Visioli F, Galli C, Bornet F, Mattei A, Patelli R, Galli G, Caruso D. (2000). Olive oil phenolics are dose-dependently absrobed in humans. *FEBS Letters, 468*, 159-160.

Visioli F, Bernardini E. (2011). Extra virgin olive oil's polyphenols: biological activities. *Current Pharmaceutical Design, 17*(8), 19.

Vissers M, Zock PL, Katan MB. (2004). Bioavailability and antioxidant effects of olive oil phenols in humans: a review. *European Journal of Clinical Nutrition, 58*, 955-965.

Vorobjev I, Zorov DB. (1983). Diazepam inhibits cell respiration and induces fragmentation of mitochondrial reticulum. *FEBS Letters, 164*(2), 311-314.

Wallace D. (1999). Mitochondrial diseases in man and mouse. *Science, 283*.

Wallace D. (2005). A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: A dawn for evolutionary medicine. *Annu Rev Genet., 39*(359), 52.

Wasserman K, McIlroy MB. (1964). Detecting the threshold of anaerobic metabolism in cardiac patients during exercise. *American Journal of Cardiology, 14*(6), 844-852.

Wenz T. (2011). Mitochondria and PGC-1α in aging and age-associated diseases. *Journal of Aging Research, 2011*, 1-12.

Willet W, Sacks F, Trichopoulou A, Drescher G, Ferro-Luzzi A, Helsing E, Trichopoulos D. (1995). Mediterranean diet pyramid: a cultural model for healthy eating. *The American Journal of Clinical Nutrition, 61*, 41.

Zhang Y, Olivier M, Giulivi C, Ernster L, Davies KJA. (1990). The oxidative inactivation of mitochondrial electron transport chain components and ATPase. *The Journal of Biological Chemistry, 265*(27), 16330-16336.

Zhu L, Zhongbo L, Feng Z, Hao J, Shen W, Li X, Sun L, Sharman E, Wang Y, Wertz K, Weber P, Shi X, Liu J. (2010). Hydroxytyrosol protects against oxidative damage by simultaneous activation of mitochondrial biogenesis and phase II detoxifying enzyme systems in retinal pigment epithelial cells. *Journal of Nutritional Biochemistry, 21*, 1089-1098.

Zmijewski J, Landar A, Watanabe N, Dickinson DA, Noguchi N, Darley-Usmar VM. (2005). Cell signalling by oxidized lipids and the role of reactive oxygen species in the endothelium. *Biochem Soc Trans, 33*(6), 1385-1389.

Zrelli H, Matsouka M, Kitazaki S, Zarrouk M, Miyazaki H. (2011). Hydroxytyrosol reduces intracellular reactive oxygen species levels in vascular endothelial cells by upregulating

catalase expression through the AMPK-FOXO3a pathway. *European Journal of Pharmacology*, *660*, 275-282.

APPENDIX A – Safety Blood Draws

Researchers performed safety blood analyses at baseline and at days 14, 28 and 39 of supplementation. Baseline blood safety parameters were measured at the baseline Muscle Biopsy visit, and subsequent blood safety parameters were measured at days 8, 14, 28 and 39 of supplementation and compared to baseline measures to ensure that the study supplement was safe to consume. For each safety blood draw, the subject reported to the laboratory in the morning after a 12 hr fast, during which only water was consumed. If dietary, exercise, and health criteria were met, a researcher drew 15 ml blood from a forearm vein and sent the blood sample the same day to an off-site diagnostic laboratory (Quest Diagnostics, Irving, TX) to measure the following markers:

- Lipid panel: triglycerides, total cholesterol, high density lipoprotein, low-density lipoprotein (calculated), cholesterol/high density lipoprotein ratio (calculated), non-high density lipoprotein (calculated), very low density lipoprotein
- Blood cell panel: white blood cells, red blood cells, hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, red blood cell distribution width, platelets
- Metabolic panel: albumin, albumin/globulin ratio (calculated), alkaline phosphatase, alanine transaminase, aspartate transaminase, blood urea nitrogen/creatinine ratio (calculated), calcium, carbon dioxide, chloride, creatinine, globulin (calculated), glucose, potassium, sodium, total bilirubin, total protein, urea nitrogen
- Lipase
- Amylase
- Hemoglobin A1c
- Gamma-glutamyl transpeptidase (GGT)
- Uric acid
- Lactate dehydrogenase (LDH)

- Total creatine kinase (CK)
- Phosphorus

All results from the diagnostic laboratory were reviewed by the study physician within 48 hr. If abnormal blood results were observed by the study physician, the subject was asked to return to the laboratory for a blood re-draw. The re-draw results were likewise sent to the diagnostic laboratory and reviewed by the study physician within 48 hr to determine if any persistent abnormalities were clinically significant. The study physician did not observe any clinically-significant abnormalities during the course of the study that would warrant excusing a subject for safety reasons.

APPENDIX B – Individual Subject Data

PGC-1α

PGC-1α (% of std)

	PLA		LO		HI	
Subject	**Base**	**End**	**Base**	**End**	**Base**	**End**
1	3.2	6.5	11.1	5.5	5.0	7.9
2	1.6	11.8	1.5	1.7	9.2	10.5
3	4.2	6.0	4.6	6.8	6.5	6.4
4	6.4	5.2	2.9	4.9	2.9	2.9
5	5.3	4.6	1.7	1.5	3.2	3.1
6	2.0	0.9	4.2	4.3	0.5	4.9
7	4.8	6.1	2.1	3.8	3.4	3.2
8	2.1	2.2	2.2	1.8	0.5	2.9
9	4.0	4.3	8.5	8.7	3.8	1.6
10	2.3	7.7	1.8	6.1	5.9	2.1
11	8.6	6.8	1.3	0.6	8.2	16.2
12	3.5	4.8	2.5	3.2	10.1	8.5
13	3.6	3.5	5.9	5.5	4.5	4.6
14	5.8	4.7	6.1	7.2	2.6	1.3
15	6.4	6.6	6.2	7.3	6.3	3.5
16	1.3	16.3	2.5	3.8	5.7	11.1
17	4.5	10.3	7.5	8.4	0.9	3.7
18	6.8	3.7	5.8	5.4	5.9	7.6
19	7.0	5.8	4.0	5.9	2.5	1.8
20			6.7	5.3	5.4	7.3
21					1.2	5.1
22					4.0	4.9
MEAN	**4.4**	**6.2**	**4.5**	**4.9**	**4.5**	**5.5**
SE	**0.5**	**0.8**	**0.6**	**0.5**	**0.6**	**0.8**

α-tubulin

α-tubulin (% of std)

	PLA		LO		HI	
Subject	**Base**	**End**	**Base**	**End**	**Base**	**End**
1	5.7	11.3	32.2	21.2	21.2	20.6
2	42.9	41.0	3.1	4.7	4.7	23.8
3	15.4	12.7	5.1	13.2	13.1	15.9
4	12.7	13.9	9.6	13.5	13.5	6.3
5	8.9	10.2	2.1	2.0	2.0	9.2
6	5.2	2.7	12.3	7.7	7.7	8.5
7	11.5	12.2	6.1	10.5	10.5	9.8
8	4.2	4.2	7.7	7.9	7.9	4.4
9	6.7	9.6	10.3	11.3	11.3	3.9
10	4.6	9.9	2.8	9.7	9.7	4.3
11	9.6	9.3	2.4	1.6	1.6	38.0
12	6.9	10.8	4.7	5.3	5.3	20.6
13	8.4	8.5	7.7	9.5	9.5	7.8
14	10.3	4.7	10.7	11.8	11.8	2.3
15	34.5	32.1	11.0	19.2	19.2	6.6
16	31.4	73.7	5.7	6.3	6.3	17.0
17	10.0	21.3	19.1	17.2	17.8	8.3
18	7.9	5.9	6.7	29.6	29.6	18.5
19	18.3	14.4	13.7	9.4	9.4	6.0
20			11.2	12.2	12.2	27.0
21					2.3	10.7
22					8.4	11.3
MEAN	**13.4**	**16.2**	**9.2**	**11.2**	**10.7**	**12.8**
SE	**2.5**	**3.9**	**1.5**	**1.5**	**1.4**	**1.9**

Succinate Dehydrogenase

SDH ($\mu mol \cdot g^{-1} \cdot min^{-1}$)

	PLA		LO		HI	
Subject	**Base**	**End**	**Base**	**End**	**Base**	**End**
1	4.250	3.903	3.817	5.208	4.176	3.928
2	9.164	7.963	4.858	3.742	3.182	5.329
3	3.583	3.894	4.894	5.091	4.814	5.791
4	5.469	3.753	4.301	4.553	3.258	3.961
5	7.908	6.599	4.844	5.288	6.163	5.844
6	5.577	5.219	4.260	5.577	7.417	7.101
7	5.574	6.579	4.012	7.702	7.507	6.939
8	3.862	4.897	4.538	4.353	4.983	6.545
9	3.747	4.512	4.399	5.100	5.726	5.366
10	4.534	6.058	1.765	5.482	6.592	7.151
11	5.956	7.283	4.660	3.870	5.322	5.763
12	5.860	5.550	5.454	6.362	6.348	6.998
13	6.307	6.879	5.585	5.619	3.815	4.307
14	7.202	5.254	5.857	5.155	5.300	6.590
15	4.655	4.639	5.927	2.653	5.330	4.652
16	5.648	5.012	5.701	5.760	4.348	5.857
17	2.498	3.254	5.356	4.850	5.591	7.407
18	6.054	6.500	3.954	4.306	3.977	3.714
19	6.449	5.251	4.744	7.387	5.718	4.148
20			6.957	6.842	3.797	6.676
21					5.979	5.122
22					5.047	5.904
MEAN	**5.489**	**5.421**	**4.794**	**5.245**	**5.200**	**5.686**
SE	**0.367**	**0.298**	**0.238**	**0.273**	**0.259**	**0.250**

Citrate Synthase

CS (µmol•g^{-1}•min^{-1})

	PLA		LO		HI	
Subject	**Base**	**End**	**Base**	**End**	**Base**	**End**
1	36.563	32.853	36.303	37.910	26.613	25.715
2	60.864	53.360	22.968	32.714	19.435	32.819
3	28.108	24.391	27.644	26.925	33.527	32.258
4	27.784	22.296	22.448	23.636	16.688	17.882
5	42.724	32.760	25.978	29.807	27.616	27.757
6	22.629	24.394	35.269	31.071	39.400	49.143
7	30.436	38.432	21.842	40.902	35.146	44.075
8	21.023	34.867	21.564	22.005	26.876	32.113
9	22.185	31.486	20.458	25.679	26.615	25.075
10	18.836	23.866	5.706	24.185	33.302	44.654
11	29.719	34.593	20.772	22.137	33.846	39.781
12	28.389	31.907	27.584	32.973	30.464	30.503
13	38.510	36.313	28.915	31.707	17.480	22.723
14	35.559	28.673	28.225	29.372	25.771	29.988
15	22.535	30.654	28.087	15.472	29.327	26.364
16	27.241	33.972	26.931	32.882	34.044	34.848
17	23.007	31.111	25.751	24.995	28.993	37.473
18	26.620	27.800	23.388	29.657	23.239	19.294
19	30.021	23.661	29.423	36.941	33.488	31.939
20			35.917	34.418	32.431	40.004
21					22.379	21.214
22					21.374	25.180
MEAN	**30.145**	**31.441**	**25.759**	**29.269**	**28.093**	**31.400**
SE	**2.240**	**1.629**	**1.505**	**1.388**	**1.307**	**1.807**

VO_{2MAX}

VO_{2MAX} (L O_2•min^{-1})

	PLA			LO			HI		
Subject	**Base**	**Mid**	**End**	**Base**	**Mid**	**End**	**Base**	**Mid**	**End**
1	3.52	3.69	3.28	3.71	3.52	3.82	2.4	2.19	2.23
2	3.65	3.61	3.49	2.8	2.94	2.83	2.83	2.9	2.9
3	3.18	3.11	3.09	2.27	2.49	2.17	3.56	3.45	3.66
4	2.83	2.79	2.79	2.65	3.01	3.18	2.73	2.87	2.77
5	3.52	3.69	3.58	3.52	3.72	3.66	2.92	2.85	2.68
6	3.16	3.39	3.43	3.33	3.24	3.58	2.81	2.88	3.01
7	3.00	2.90	3.04	3.47	3.6	3.55	2.96	3.4	3.23
8	2.88	2.87	2.74	2.83	2.83	2.91	2.77	2.69	2.84
9	2.86	3.33	2.84	2.9	3.03	3.09	2.92	3.02	3.16
10	3.63	3.86	3.93	2.87	2.69	3.09	3.27	3.27	3.38
11	4.48	4.60	4.65	3.28	3.13	3.27	4.03	4.1	3.9
12	4.39	4.04	4.23	3.54	3.63	3.91	3.97	3.95	4.07
13	4.20	4.40	4.26	4.04	3.7	3.69	3.42	3.42	3.45
14	3.50	3.45	3.72	3.61	3.52	3.64	2.92	2.98	3.01
15	3.82	3.73	3.98	2.66	2.82	2.86	4.09	3.86	3.68
16	3.68	3.76	3.74	3.49	3.88	3.8	4.19	4.11	4.14
17	2.71	3.03	2.91	2.96	2.93	3.1	4.72	4.96	5.26
18	4.05	4.35	4.49	3.42	3.64	3.6	3.42	3.66	3.41
19	4.67	4.45	4.46	3.75	3.77	3.76	3.47	3.45	3.63
20				3.68	3.53	3.84	2.97	3.03	2.91
21							3.85	3.63	3.67
22							4.04	4.27	3.94
MEAN	**3.56**	**3.63**	**3.61**	**3.24**	**3.28**	**3.37**	**3.38**	**3.41**	**3.41**
SE	**0.14**	**0.13**	**0.14**	**0.10**	**0.09**	**0.10**	**0.13**	**0.13**	**0.14**

VO_2 at Lactate Threshold

VO_2 at Lactate Threshold (L O_2•min^{-1})

	PLA			LO			HI		
Subject	**Base**	**Mid**	**End**	**Base**	**Mid**	**End**	**Base**	**Mid**	**End**
1	1.96	1.98	1.67	1.69	2.36	1.77	2.11	1.44	1.44
2	1.68	1.88	1.78	1.64	1.69	1.46	1.36	1.45	1.43
3	1.59	1.52	1.68	1.45	1.57	1.68	1.94	1.55	1.66
4	1.67	1.56	1.63	1.40	1.63	1.74	1.93	1.50	1.59
5	2.41	2.52	2.04	1.17	1.96	2.20	1.75	1.80	1.68
6	1.64	2.07	1.83	1.94	1.97	2.11	1.64	1.68	1.95
7	2.06	1.30	1.84	1.75	1.56	2.23	2.03	1.85	1.91
8	1.81	1.68	1.62	1.55	1.51	1.67	1.39	1.77	1.76
9	1.96	1.95	1.82	1.98	1.92	1.83	1.90	1.66	1.75
10	2.43	2.79	2.74	1.55	1.66	1.76	2.35	2.08	2.40
11	3.21	3.18	3.56	1.73	1.79	1.88	2.71	2.63	2.29
12	1.82	2.28	1.98	2.12	2.28	2.16	2.17	2.41	2.61
13	2.48	2.43	2.45	2.43	2.67	2.93	1.79	1.71	1.76
14	1.81	1.88	2.17	2.33	2.07	2.60	1.38	1.51	1.35
15	2.36	2.77	2.79	1.74	1.77	1.76	2.59	2.45	2.58
16	2.00	2.30	2.40	1.81	2.14	2.27	2.70	2.87	2.73
17	1.29	1.45	1.63	1.48	1.35	1.41	2.78	2.77	2.73
18	2.36	2.45	2.89	1.23	1.55	1.69	2.30	2.07	1.74
19	2.75	2.65	2.78	2.16	2.29	2.22	2.21	2.07	2.05
20				2.22	2.39	2.48	1.88	1.91	1.52
21							2.08	2.36	2.27
22							2.45	2.51	2.36
MEAN	**2.07**	**2.14**	**2.17**	**1.77**	**1.91**	**1.99**	**2.07**	**2.00**	**1.98**
SE	**0.11**	**0.12**	**0.13**	**0.08**	**0.08**	**0.09**	**0.09**	**0.10**	**0.09**

RER at Steady State

Respiratory Exchange Ratio (RER) at 65% VO_{2MAX}

	PLA			LO			HI		
Subject	**Base**	**Mid**	**End**	**Base**	**Mid**	**End**	**Base**	**Mid**	**End**
1	0.98	0.95	1.01	0.99	0.98	1.00	1.03	1.03	1.03
2	1.01	1.02	1.02	1.07	1.01	1.01	1.05	1.06	1.07
3	1.04	1.00	1.03	1.00	0.97	0.94	1.01	1.01	0.97
4	1.03	0.97	1.03		1.01	0.98	1.01	0.98	1.05
5	1.01	0.92	0.98	1.01	1.02	0.98	1.00	0.92	1.03
6	1.00	0.98	0.95	1.04	0.97	0.98	1.04	0.96	0.99
7	0.99	0.95	0.95	1.05	1.04	1.02	0.97	0.96	0.96
8	0.93	1.01	1.02	1.02	1.01	1.02	1.02	1.03	1.02
9	0.94	0.93	0.95	1.00	0.99	0.95	0.99	1.00	0.97
10	0.97	1.02	0.98	1.01	1.02	0.99	0.92	0.95	0.95
11	0.99	0.98	1.01	1.01	1.02	1.00	0.95	0.96	0.92
12	1.04		1.06	0.96	0.97	0.97	0.96	0.97	0.93
13	0.97	1.00	0.97	0.96	0.99	0.96	0.98	0.99	0.97
14	0.97	0.97	0.99	0.96	0.96	0.97	1.04	1.04	1.00
15	0.99		0.95	1.03	0.98	1.00	0.97	0.98	0.98
16	0.95	0.96	0.95	1.02	0.98	0.97	0.96	0.95	0.97
17	0.96	1.00	1.04	1.11	1.08	1.09	0.99	0.92	0.96
18	0.96	0.97	0.90	1.01	1.02	1.02	1.03	0.99	0.98
19	0.96	0.99	1.01	1.03	1.00	0.97	0.98	1.02	1.04
20				0.98	0.97	1.00	1.04	1.03	1.03
21							0.97	1.03	1.02
22							1.00	0.99	1.00
MEAN	**0.98**	**0.98**	**0.99**	**1.01**	**1.00**	**0.99**	**1.00**	**0.99**	**0.99**
SE	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**

Time Trial Performance

Time Trial time to completion (min)

	PLA			LO			HI		
Subject	**Base**	**Mid**	**End**	**Base**	**Mid**	**End**	**Base**	**Mid**	**End**
1	39.90	39.45	38.88	37.90	37.68	36.30	56.87	53.72	52.50
2	39.90	38.88	37.83	46.78	42.52	42.37	45.82	43.78	43.15
3	39.80	38.92	39.13	46.53	43.30	50.15	38.23	37.98	39.40
4	44.37	42.38	42.08	46.50	46.10	45.87	44.40	43.93	43.00
5	35.68	35.42	34.78	38.42	38.25		42.62	41.02	39.88
6	41.62	41.43	40.17	39.83	39.15	38.13	41.58	40.50	39.25
7	43.42	43.00	40.20	43.23	42.37	44.45	42.48	40.95	38.93
8	43.97	45.88	45.33	46.70	46.63	45.57	46.98	44.15	43.42
9	41.13	40.85	40.53	44.85	41.33	40.32	41.23	39.98	38.75
10	43.80	37.80	35.90	47.18	46.65	48.10	38.95	37.93	37.92
11	34.68	33.72	33.10	43.88	40.35	39.73	35.98	35.55	34.97
12	37.43		34.80	38.77	38.62	38.77	36.17	35.22	34.43
13	36.73	36.57	36.20	38.73	38.32	37.97	38.33	38.30	38.23
14	39.62	39.25	40.37	37.60	36.75	36.28	42.77	43.88	42.33
15	37.80	38.40	37.27	45.25	41.97	43.90	37.68	36.12	36.93
16	35.83	34.77	34.08	40.77	39.92	39.23	37.52	37.03	36.35
17	42.45	41.82	40.95	48.65	47.25	46.77	36.07	35.02	33.70
18	35.58	35.42	37.83	38.93	39.17	37.52		42.78	40.07
19	35.30	34.43	33.20	36.12	35.82	35.42	44.18	47.48	43.82
20				39.00	37.95	37.60	48.53	46.33	43.80
21							39.00	38.78	38.48
22							41.52	39.18	37.98
MEAN	**39.42**	**38.80**	**38.03**	**42.28**	**41.00**	**41.29**	**41.76**	**40.89**	**39.88**
SE	**0.75**	**0.79**	**0.75**	**0.90**	**0.78**	**1.03**	**1.10**	**0.98**	**0.88**

Time Trial Blood Lactate

Time Trial Blood Lactate (mmol/L)

	PLA			LO			HI		
Subject	**Base**	**Mid**	**End**	**Base**	**Mid**	**End**	**Base**	**Mid**	**End**
1	2.7	3.6	2.7	5.8	7.1	8.1	2.7	2.7	7.8
2	9.8	10.2	12.6	3.6	4.7	7.4	4.9	3.4	8.3
3	4.8	5.0	5.6	1.8	2.3	1.4	5.0	4.7	3.7
4	5.6	5.0	5.8	2.9	3.2	2.8	4.6	4.2	7.9
5	6.8	4.8	8.2	5.1	6.8	4.8	6.5	7.5	10.9
6	4.5	5.9	2.9	5.4	8.2	7.8	6.4	9.3	1.1
7	6.0	3.9	6.2	7.4	5.1	3.2	4.2	9.2	7.4
8	5.9	3.5	5.6	5.4	4.7	4.3	2.4	2.9	5.3
9	3.2	2.7	2.8	6.4	7.2	13.3	5.4	8.4	8.4
10	4.1	8.2	7.6	4.1	6.2	3.6	7.0	5.4	5.4
11	8.7	9.2	8.8	4.2	7.6	6.9	6.0	8.9	9.3
12	8.7		13.6	4.7	3.8	4.8	2.7	7.2	7.3
13	8.6	8.7	6.2	4.0	7.6	4.2	7.1	8.0	8.2
14	3.0	5.0	2.6	3.8	5.5	6.6	4.9	3.6	4.7
15	3.8	2.9	3.5	2.9	6.4	7.1	5.4	4.8	7.4
16	4.5	6.3	5.8	4.5	3.8	3.7	4.4	5.0	9.0
17	6.4	3.7	2.1	5.3	5.6	4.9	6.8	4.9	8.2
18	8.2	8.5	2.5	7.7		10.4		2.5	5.4
19	3.9	7.9	12.8	12.6	9.7	9.2	2.0	1.7	3.1
20				3.2	4.2	5.2	3.5	5.9	6.4
21							6.2	5.3	5.7
22							2.2	3.7	4.2
MEAN	**5.7**	**5.8**	**6.2**	**5.0**	**5.8**	**6.0**	**4.8**	**5.4**	**6.6**
SE	**0.5**	**0.6**	**0.8**	**0.5**	**0.4**	**0.6**	**0.4**	**0.5**	**0.5**

Time Trial RPE

Time Trial Rating of Perceived Exertion (Borg scale)

	PLA			LO			HI		
Subject	**Base**	**Mid**	**End**	**Base**	**Mid**	**End**	**Base**	**Mid**	**End**
1	14	16	15	18	19	20	14	14	14
2	18	17	16	13	15	15	14	13	15
3	16	16	17	14	17	17	17	19	19
4	15	15	15	13	16	13	16	17	16
5	17	18	19	16	15	15	14	14	14
6	17	16	17	16	18	17	14	15	16
7	16	18	17	14	15	13	16	18	19
8	16	17	17	17	15	17	13	14	16
9	15	16	16	16	16	15	17	18	15
10	17	18	19	15	15	14	17	17	18
11	17	16	18	14	15	15	18	19	20
12	18		18	14	15	15	14	15	16
13	15	17	18	12	13	13	15	16	15
14	14	16	17	17	17	18	15	15	14
15	15	16	17	16	15	16	16	18	16
16	15	17	19	15	15	15	16	16	16
17	15	15	17	17	16	15	15	12	13
18	13	13	16	14	16	16		12	13
19	16	17	18	17	14	14	14	14	15
20				15	14	16	17	15	14
21							14	15	16
22							15	16	16
MEAN	**15.7**	**16.3**	**17.2**	**15.2**	**15.6**	**15.5**	**15.3**	**15.5**	**15.7**
SE	**0.3**	**0.3**	**0.3**	**0.4**	**0.3**	**0.4**	**0.3**	**0.4**	**0.4**

COMPREHENSIVE BIBLIOGRAPHY

Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. (2008). *Molecular Biology of the Cell* (Fifth ed.). 270 Madison Avenue, New York NY 10016, USA: Garland Science.

Allen W, Seals DR, Hurley BF, Ehsani AA, Hagberg JM. (1985). Lactate threshold and distance running performance in young and older endurance athletes. *Journal of Applied Physiology, 58*(4), 1281-1284.

Alexander SP. (2006). Flavonoids as antagonists at A_1 adenosine receptors. *Phytother Res, 20*, 1009–1012.

Åstrand P, Rodahl K. (1977). *Textbook of work physiology: physiological bases of exercise*. New York: McGraw-Hill Book Company.

Attardi G, Schatz G. (1988). Biogenesis of mitochondria. *Annual Review of Cellular Biology, 4*, 289-331.

Baar K. (2004). Involvement of PPARɣ co-activator-1, nuclear respiratory factors 1 and 2, and PPARα in the adaptive response to endurance exercise. *Proceedings og the Nutrition Society, 63*, 269-273.

Barja G, Herrero A. (2000). Oxidative damage to mitochondrial DNA is inversely related to maximum life span in the heart and brain of mammals. *The FASEB Journal, 14*, 312-318.

Barstow T, Casaburi R, and Wasserman K. (1993). O2 uptake kinetics and the O2 deficit as related to exercise intensity and blood lactate. *Journal of Applied Physiology, 72*(2), 755-762.

Bassett DJ, Howley, ET. (2000). Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Medicine & Science in Sports & Exercise, 32*(1), 70.

Baur J, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le Couteur D, Shaw RJ, Navas P, Puigserver P, Ingram DK,de Cabo R, and Sinclair DA. (2006). Resveratrol improves health and survival of mice on a high-calorie diet. *Nature, 444*, 337-342.

Blair S, Kohl III, HW, Paffenbarger Jr RS, Clark DG, Cooper KH, and Gibbons LW. (1989). Physical fitness and all-cause mortality. *The Journal of the American Medical Association, 262*(17), 2395-2401.

Bouallagui Z, Han J, Isoda H, Sayadi S. (2010). Hydroxytyrosol rich extract from olive leaves modulates cell cycle progression in MCF-7 human breast cancer cells. *Food and Chemical Toxicology, 49*, 179-184.

Brandon M, Baldi P, Wallace DC. (2006). Mitochondrial mutations in cancer. *Oncogene, 25*, 16.

Bravo L. (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews, 56*(11), 17.

Brunk C. (1981). Mitochondrial proliferation during myogenesis. *Experimental Cell Research, 136*, 305-309.

Chan D. (2006). Mitochondria: dynamic organelles in disease, aging, and development. *Cell, 125*, 12.

Chapman AR, Vincenzino B, Blanch P, Hodges PW. (2007). Leg muscle recruitment during cycling is less developed in triathletes than cyclists despite matched cycling training loads. *Experimental Brain Research, 181*(3), 503-518

Cooke M, Evans MD, Dizdaroglu M, Lunec J. (2003). Oxidative DNA damage: mechanisms, mutation, and disease. *The FASEB Journal, 17*, 1195-1214.

Davis M, Murphey EA, Carmichael MD, and Davis B. (2009). Quercitin increases brain and muscle mitochondrial biogenesis and exercise tolerance. *AJP - Regulatory Physiology, 296*(4), R1071-R1077.

Di Prampero, PE. (1985). Metabolic and circulatory limitations to VO_{2MAX} at the whole animal level. *J Exp Biol, 115*, 319-331.

Edwards J, Beck M, and Bausch J. (2009). Water-soluable olive extract, olive 15% CWS outline safety summary: DSM Nutritional Products, R&D Human Nutrition and Health, NRD/CH Safety.

Egan B, Carson BP, Garcia-Roves PM, Chibalin AV, Sarsfield FM, Barron N, McCaffrey N, Moyna NM, Zierath, O'Gorman DJ. (2010). Exercise intensity-dependent regulation of peroxisome proliferator-activated receptor ɣ coactivator-1α mRNA abundance is associated with differential activation of upstream signalling kinases in human skeletal muscle. *The Journal of Physiology, 588*(10), 1779-1790.

Faul F, Erdfelder E, Buchner A, Lang AG. (2009). Statistical power analysis using G*Power 3.1: Tests for correlation and regression analyses. *Behavior Research Methods, 41*, 1149-1160.

Feng Z, Bai L, Yan J, Li Y, Shen W, Wang Y, Wertz K, Weber P, Zhang Y, Chen Y, Liu J. (2011). Mitochondrial dynamic remodeling in strenuous exercise-induced muscle and mitochondrial dysfunction: regulatory effects of hydroxytyrosol. *Free Radical Biology and Medicine, 50*, 1437-1446.

Frayn K. (1983). Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol, 55*, 628-634.

Granados S, Quiles JL, Ramirez-Tortosa CL, Sanchez-Rovira P, Ramirez-Tortosa MC. (2010). Hydroxytyrosol: from laboratory investigations to future clinical trials. *Nutrition Reviews, 68*(4), 16.

Granados S, Quiles JL, Ramirez-Tortosa Camacho-Corencia P, Sanchez-Rovira P, Vera-Ramirez L, Ramirez-Tortosa MC. (2011). Hydroxytyrosol inhibits growth and cell proliferation and promotes high expression of sfrp4 in rat mammary tumors. *Mol Nutr Food Res, 55*, 117-126.

Halliwell. (2007). Oxidative stress and cancer: have we moved forward? *Biochemical Journal, 401*, 1-11.

Halliwell B, Gutteridge JM. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemical Journal, 219*, 1-14.

Hao J, Shen W, Yu G, Jia H, Li X, Feng Z, Wang Y, Weber P, Wertz K, Sharman E, Liu J. (2009). Hydroxytyrosol promotes mitochondrial biogenesis and mitochondrial function in 3T3-L1 adipocytes. *Journal of Nutritional Biochemistry, 21*, 634-644.

Hardie D. (2011). Sensing of energy and nutrients by AMP-activated protein kinase. *American Journal of Clinical Nutrition, 93*(4), 891-896.

Harman D. (1956). Aging: a theory based on free radical and radiation chemistry. *Journal of Gerontology, 11*(3), 3.

Harman D. (1992). Free radical theory of aging. *Mutation Research, 275*, 10.

Harrison TR, Pilcher C. (1930). Studies in congestive heart failure, II. The respiratory exchange during and after exercise. *Journal of Clinical Investigation, 8(3)*, 291-315.

Hill AV, Long CNH, Lupton H. (1924). Muscular exercise, lactic acid, and the supply and utilization of oxygen. *Proc Roy Soc London, S. B. 96*, 84, 155, 438.

Holloszy J, Rennie MJ, Hickson RC, Conlee RK, Hagberg JM. (1977). Physiological consequences of the biochemical adaptations to endurance exercise. *Annals of the New York Academy of Sciences, 301*, 440-450.

Holloszy J, Coyle EF. (1984). Adaptations of skeletal muscle to endurance exercise and their metabolic consequenses. *Journal of Applied Physiology, 56*(4), 831-838.

Howitz K, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, Zipkin RE, Chung P, Kisielewski A, Zhang, L, Scherer B, and Sinclair DA. (2003). Small molecules activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature, 425*, 191-196.

Hussain S, Hofseth LJ, Harris CC. (2003). Radical causes of cancer. *Nature Reviews--Cancer, 3*, 10.

Hwang J-T, Kwon DY, Yoon SH. (2009). AMPK-activated protein kinase: a potential target for the diseases prevention by natural occuring polyphenols. *New Biotechnology, 26*(1/2), 17-22.

Irrcher I, Adhihetty PJ, Joseph A-M, Ljubicic V, Hood DA. (2003). Regulation of mitochondrial biogenesis in muscle by endurance exercise. *Sports Medicine, 33*(11), 11.

Ivy JL, Withers RT, Van Handel PJ, Elger DH, Costill DL. (1980). Muscle respiratory capacity and fiber type as determinants of the lactate threshold. *Journal of Applied Physiology, 48(*3), 523-527.

Joyner M, Coyle EF. (2008). Endurance exercise performance: the physiology of champions. *The Journal of Physiology, 586*(1), 35-44.

Jubrias S, Crowther GJ, Shankland EG, Gronka RK, Conlet KE. (2003). Acidosis inhibits oxidative phosphorylation in contracting human skeletal muscle *in vivo*. *The Journal of Physiology, 553*, 589-599.

Khymenets O, Fitó M, Touriño S, Muñoz-Aguayo D, Pujadas M, Torres JL, Joglar J, Farré M, Covas M-I, de la Torre R. (2010). Antioxidant activities of hydroxytyrosol main metabolites do not contribute to beneficial health effects after olive oil ingestion. *Drug Metab Dispos, 38*(9), 1417-1421. Published ahead of print June 1411, 2010.

Kim K, Rodriguez AM, Carrico PM, Melendez JA. (2001). Potential mechanisms for the inhibition of tumer cell growth by manganese superoxide dismutase. *Antioxid Redox Signal, 3*, 361-373.

Lagouge M AC, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Massadeq N, Milne J, Lambert P, Elliot P, Geny B, Laakso M, Puigserver P, and Auwerx J. (2006). Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1α. *Cell, 127*(6), 1109-1122.

Lardy H, Ferguson SM. (1969). Oxidative phosphorylation in mitochondria. *Annual Rev. Biochem., 38*, 991-1034.

Lee J, Kim M, Park H-S, Kim HS, Jeon MJ, Oh KS, Koh EH, Won JC, Kim M-S, Oh GT, Yoon M, Lee K-U, Park JY. (2006). AMPK activation increases fatty acid oxidation in skeletal muscle by activating PPARα and PGC-1. *Biochemical and Biophysical Research Communications, 340*, 291-295.

Leick L, Lyngby SS, Wojtasewski JFP, Pilegaard H. (2010). PGC-1α is required for training-induced prevention of age-associated decline in mitochondrial enzymes in mouse skeletal muscle. *Experimental Gerontology, 45*, 336-342.

Linnane A, Marzuki S, Ozawa T, Tanaka M. (1989). Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases. *The Lancet, 333*(8639), 4.

Lira V, Benton CR, Yan Z, Bonen A. (2010). PGC-1α regulation by exercise training and its influences on muscle function and insulin sensitivity. *Am J Physiol Endocrinol Metab, 299*, 145-161.

Little J, Safdar A, Birshop D, Tanopolsky MA, Gibala MJ. (2011). An acute bout of high-intensity interval training increases the nuclear abundance of PGC-1{alpha} and activates mitochondrial biogenesis in human skeletal muscle. *Am J Physiol Regu Integr Comp Physiol*, March 30, 2011. [Epub ahead of print].

Liu J, Raederstorff D, Wang-Schmidt Y, and Wertz K. (2011). United States Patent No. (Application) US 2011/0112201 A1.

Liu Z, Sun L, Zhu L, Jia X, Li X, Jia H, Wang Y, Weber P, Long J, Liu J. (2007). Hydroxytyrosol protects retinal pigment epithelial cells from acrolein-induced oxidative stress and mitochondrial dysfunction. *Journal of Neurochemistry, 103*, 2690-2700.

Lowell B, Shulman GI. (2005). Mitochondrial dysfunction and type 2 diabetes. *Science, 307*, 4.

Lowry O, Passonneau JV. (1972). *A flexible system of enzymatic analysis*. New York: Academic Press.

MacRae H, and Mefferd KM. (2006). Dietary antioxidant supplementation combined with quercitin improves cycling time trial performance. *Int J Sport Nutr Exercise Metab, 16*, 405-415.

Matthews C. (2006). DNA precursor metabolism and genomic stability *The FASEB Journal, 20*, 1300-1314.

McArdle A, Pattwell D, Vasilaki A, Griffiths RD, Jackson MJ. (2001). Contractile activity-induced oxidative stress: cellular origin and adaptive responses. *Am J Physiol Cell Physiol, 280*(3), C621-C627.

McNair D, Droppelman LF. (1971). *Manual for the profile of the mood states*. San Diego, CA: Educational and Industrial Testing Service.

Melov S, Schneider JA, Coskun PE, Bennet DA, Wallace DC. (1999). Mitochondrial DNA rearrangements in aging human brain and in situ PCR of mtDNA. *Neurobiol Aging, 20*, 565-571.

Mootha V, Lindgren CM, Eriksson K-F, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstrale M, Laurila E, Houstis N, Daly MJ, Patterson N, Mesirov JP, Golub TR, Tamayo P, Spiegelman B, Lander E, Hirschorn JN, Altshuler D, Groop LC. (2003). PGC-1α-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nature Genetics, 34*(3), 267-273.

Moyes C, Mathieu-Costello OA, Tsuchiya N, Filburn C, Hansford RG. (1997). Mitochondrial biogenesis during cellular differentiation. *American Journal of Cell Physiology, 272*(4), C1345-C1351.

Murrow B, Hoehn KL. (2010). Mitochondrial regulation of insulin action. *The International Journal of Biochemistry and Cell Biology, 42*, 4.

Nieman D, Dumke CI, Henson DA, McAnuity SR, McAnuity lA, Lind RH, and Murrow JD. (2003). Immune and oxidative changes during and following the Western States Endurance Run. *Int J Sports Med, 24*, 541-547.

Nisoli E, Carruba MO. (2006). Nitric oxide and mitochondrial biogenesis. *Journal of Cell Science, 119*(14), 8.

Nordsborg N, Lundby C, Leick L, Pilegaard H. (2010). Relative workload determines exercise-induced increases in PGC-1α mRNA. *Medicine & Science in Sports & Exercise, 42*(8), 1477-1484.

Notarnicola M, Pisanti S, Tutino V, Bocale D, Rotelli MT, Gentile A, Memeo V, Bifulco M, Perri E, Caruso MG. (2011). Effects of olive oil polyphenols on fatty acid synthase gene expression and activity in human colorectal cancer cells. *Genes Nutr, 6*.

Paiva-Martins F, Rodrigues V, Calheiros R, Marques MPM. (2011). Characterization of antioxidant olive oil biophenols by spectroscopic methods. *J Sci Food Agric, 91*, 6.

Pelicano H, Carney D, Huang P. (2004). ROS stress in cancer cells and therapeutic implications. *Drug Resistance Updates, 7*, 14.

Pinto J, Paiva-Martins F, Corona G, Debnam ES, Oruna-Concha MJ, Vauzour, Gordon MH, Spencer JPE. (2011). Absorption and metabolism of olive oil secoiridoids in the small intestine. *British Journal of Nutrition, 105*, 1607-1618.

Quaranta G, Rotundo V. (2000). Economic and commercial prospects for olive oil in view of the changes in the common market organisation (CMO) (Part one). *Olivae, 91*, 5.

Quiles J, Farquharson AJ, Simpson DK, Grant I, Wahle KWJ. (2002). Olive oil phenolics: effects on DNA oxidation and redox enzyme mRNA in prostate cells. *British Journal of Nutrition, 88*, 10.

Raederstroff D. (2009). Antioxidant activity of olive polyphenols in humans: a review. *Int J Vitam Nutr Res, 79*(3), 152-165.

Richter C. (1988). Do mitochondrial DNA fragments promote cancer and aging? *FEBS Letters, 241*(1,2), 5.

Richter C. (1995). Oxidative damage to mitochondrial DNA and its relationship to ageing. *International Journal of Cell Biology, 27*(7), 7.

Rietjens J, Bast A, Haenen GRMM, and Heyden Van Der LCG. (2011). United States Patent No. (Application) US 2011/0052750 A1.

Rogina BaHS. (2004). Sir2 mediates longevity in the fly through a pathway related to calorie restriction. *PNAS, 101*(45), 15998-16003.

Sabatini N. (2010). Recent patents in olive oil industry: new technologies for the recovery of phenols compounds from olive oils, olive oil industrial by-products and waste waters. *Recent Pat Food Nutr Agric, 2*(2), 6.

Schwerzmann. (1989). Oxidative capacity of muscle and mitochondria: correlation of physiological, biochemical, and morphometric characteristics. *PNAS, 86*(5), 1583-1587.

Shephard R, Rhind S, Shek PN. (1994). Exercise and the immune system. Natural killer cells, interleukins and related responses. *Sports Medicine, 18*(5), 340-369.

Smith L. (2003). Overtraining, excessive exercise, and altered immunity: is this a T helper-1 versus T helper-2 lyphocyte response? *Sports Medicine, 33*(5), 347-364.

Srere P. (1969). Citrate synthase: citrate oxaloacetate-lyase (CoA-acetylating). *Methods in Enzymology, 13*, 3-11.

Sutton A, Khoury H, Prip-Buus C, Cepances C, Pessayre D, Degoul F. (2003). The Ala16Val genetic dimorphism modulates the import of human manganese superoxide dismutase into rat liver mitochondria. *Pharmacogenetics, 13*, 145-157.

Taivassalo T, De Stefano N, Argov Z. (1998). Effect of aerobic training in patients with mitochondrial myopathies. *Neurology, 50*(4), 1055-1060.

Tanaka H, Monahan KD, Seals DR. (2001). Age-pedicted maximal heart rate revisited. *J Am Coll Cardiology, 37*, 153-156.

Trichopoulou A, Costacou T, Bamia C, Trichopoulou D. (2003). Adherence to a Mediterranean diet and survival in a Greek population. *New England Journal of Medicine, 348*(26), 12.

Tuck K, Hayball PJ. (2002). Major phenolic compounds in olive oil: metabolism and health effects. *The Journal of Nutritional Biochemistry, 13*, 10.

Viollet B, Mounier R, Leclerc J, Yazigi A, Foretz M, Andreelli F. (2007). Targeting AMP-activated protein kinase as a novel therapeutic approach for the treatment of metabolic disorders. *Diabetes & Metabolism, 33*, 395-402.

Visioli F, Bellomo G, Galli C. (1998). Free radical-scavenging properties of olive oil polyphenols. *Biochemical and Biophysical Research Communications, 247*, 5.

Visioli F, Romani A, Mulinacci N, Zarini S, Conte D, Vincieri FF, Galli C. (1999). Antioxidant and other biological activities of olive mill waste waters. *J Agric Food Chem, 47*, 3397-3401.

Visioli F, Galli C, Bornet F, Mattei A, Patelli R, Galli G, Caruso D. (2000). Olive oil phenolics are dose-dependently absrobed in humans. *FEBS Letters, 468*, 159-160.

Visioli F, Bernardini E. (2011). Extra virgin olive oil's polyphenols: biological activities. *Current Pharmaceutical Design, 17*(8), 19.

Vissers M, Zock PL, Katan MB. (2004). Bioavailability and antioxidant effects of olive oil phenols in humans: a review. *European Journal of Clinical Nutrition, 58,* 955-965.

Vorobjev I, Zorov DB. (1983). Diazepam inhibits cell respiration and induces fragmentation of mitochondrial reticulum. *FEBS Letters, 164*(2), 311-314.

Wallace D. (1999). Mitochondrial diseases in man and mouse. *Science, 283*.

Wallace D. (2005). A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: A dawn for evolutionary medicine. *Annu Rev Genet., 39*(359), 52.

Wasserman K, McIlroy MB. (1964). Detecting the threshold of anaerobic metabolism in cardiac patients during exercise. *American Journal of Cardiology, 14*(6), 844-852.

Wenz T. (2011). Mitochondria and PGC-1α in aging and age-associated diseases. *Journal of Aging Research, 2011*, 1-12.

Willet W, Sacks F, Trichopoulou A, Drescher G, Ferro-Luzzi A, Helsing E, Trichopoulos D. (1995). Mediterranean diet pyramid: a cultural model for healthy eating. *The American Journal of Clinical Nutrition, 61*, 41.

Zhang Y, Olivier M, Giulivi C, Ernster L, Davies KJA. (1990). The oxidative inactivation of mitochondrial electron transport chain components and ATPase. *The Journal of Biological Chemistry, 265*(27), 16330-16336.

Zhu L, Zhongbo L, Feng Z, Hao J, Shen W, Li X, Sun L, Sharman E, Wang Y, Wertz K, Weber P, Shi X, Liu J. (2010). Hydroxytyrosol protects against oxidative damage by simultaneous activation of mitochondrial biogenesis and phase II detoxifying enzyme systems in retinal pigment epithelial cells. *Journal of Nutritional Biochemistry, 21*, 1089-1098.

Zmijewski J, Landar A, Watanabe N, Dickinson DA, Noguchi N, Darley-Usmar VM. (2005). Cell signalling by oxidized lipids and the role of reactive oxygen species in the endothelium. *Biochem Soc Trans, 33*(6), 1385-1389.

Zrelli H, Matsouka M, Kitazaki S, Zarrouk M, Miyazaki H. (2011). Hydroxytyrosol reduces intracellular reactive oxygen species levels in vascular endothelial cells by upregulating catalase expression through the AMPK-FOXO3a pathway. *European Journal of Pharmacology, 660*, 275-282.

VITA

Marin Healy was born in Aurora, Colorado in 1988 to Donald R. and Leslie A. Nelson. She moved to Washington, Ohio, and finally to San Antonio, Texas in 1998. She later graduated *magna cum laude* from the University of Texas at San Antonio in 2008 with a Bachelor of Science in Exercise Physiology. Not only was she an athlete on the women's soccer team, but she was also a distinguished student. She was featured on the President's List and the Dean's List, was awarded the Carlos and Marlu Alvarez Special Opportunities Fund for outstanding undergraduate participation in research, and was a member of the National Society of Collegiate Scholars. After graduating, she was a data coordinator for CEDRA Clinical Research LLC, and later was a strength and conditioning coach for Competitive Athlete Training Zone, as well as a histology laboratory aid at Pathology Reference Laboratory, all located in San Antonio. In August 2009, she entered the graduate Exercise Physiology program at the University of Texas at Austin. After graduating with her Master of Science, she will pursue a Doctorate of Philosophy in the Molecular Medicine program at the University of Virginia with a focus on cancer research. She currently lives in Austin with her husband Bobby and their two adorable and occasionally well-behaved dogs.

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