2020, Volume 3 (Issue 3): 13 OPEN ACCESS

Effects of Purple Tea on Muscle Hyperemia and Oxygenation, Serum Markers of Nitric Oxide Production and Muscle Damage, and Exercise Performance

Original Research

Kyle Cesareo[1], Tim Ziegenfuss[1], Betsy Raub[1], Jennifer Sandrock[1], Hector Lopez[1,2]

[1]The Center for Applied Health Sciences, Canfield, OH USA
[2]Supplement Safety Solutions, Bedford, MA USA

Abstract

Introduction: Purple tea exhibits a unique composition of chemical constituents that may exert favorable outcomes related to recovery from muscle damage, improvements in blood flow, perfusion, and recovery. The purpose of this study was to examine the impact of a brief oral dosing period of purple tea in exercising humans after stressful, damaging exercise.

Methods: Using a randomized, placebo-controlled, double-blind, crossover study design, 30 healthy men (33.5 ± 11.4 years, 178.4 ± 7.6 cm, 92.5 ± 13.3 kg) completed an eight day supplementation regimen consisting of either a maltodextrin placebo or 100 mg of purple tea extract (PurpleForce™, Oryza Oil & Fat, Ltd.) interspersed with a two week washout period. After five and eight days of supplementation, changes in muscle oxygenation, body composition, reactive hyperemia, visual analog responses, exercise performance, and muscle damage markers were assessed. Data were analyzed using mixed factorial ANOVA, t-tests with 95% confidence intervals, and effect sizes (ES).

Results: Lactate dehydrogenase was significantly reduced (p = 0.04) in PT in comparison to PLA after eight days of supplementation and exercise performance challenge. In comparison to PT, arm circumference increased in PLA after five days of supplementation (p=0.04) and tended to be greater after eight days (p=0.06). Significantly greater decreases in impedance were observed in PT (p=0.02) while between-group differences in oxygen saturation post-leg extension exercise were greater in PT 30s into recovery (p=0.04) and tended to be greater 60s after recovery (p=0.06). Total bench press repetitions completed were greater in purple tea than PLA (p = 0.001). Total leg extension repetitions completed tended to be different between groups (p=0.09) while the total number of repetitions completed in purple tea increased from day five to day eight (p<0.001) with no change in PLA (p=0.37). No between-group changes were observed in the visual analog scales; however, only the PT condition experienced a significant improvement in Willingness to Exercise (p=0.02).

Conclusions: Acute supplementation of PT decreased lactate dehydrogenase, a marker of muscle damage, while also improving lower body muscle endurance performance.

Key Words: blood flow, dietary supplement, nitrates

Corresponding author: Hector Lopez, MD, hl@appliedhealthsciences.org

Published September 9, 2020

Introduction

Consumption of tea and variations of the tea plant (*Camellia sinensis*) can be traced back thousands of years. In present day, black, oolong, and green tea are all commonly consumed with each type of plant being touted for the composition of nutrients found within its roots, stems, and most commonly the leaves. Purple tea, a relatively new cultivar of tea, was developed by the Tea Research Foundation of Kenya. Unique growing conditions (i.e., grown typically at 1,500 – 2,500 meters above sea level resulting in high exposure to ultraviolet light) contribute to this tea variation presenting with colorful red-purple leaves. These leaves are rich in anthocyanins and polyphenols such as epigallocatechin gallate (EGCG) and epicatechin gallate (ECG), compounds that are valued for their caffeine content and ability to function in a variety of biological roles [1]. Purple tea has a higher proportion (16.5%) of polyphenols by weight of dry tea leaves in comparison to green tea (9.1%), oolong tea (7.4%), and black tea (10.1%) while also containing lower amounts of caffeine [2,3]. Purple tea also contains various anthocyanidins (malvidin, peralgonodin and cyanidin 3-O-galactoside) and also contains 1,2-di-O-galloyl-4,6-O- (S)-hexahydroxydiphenoyl-β-D-glucose (GHG), a hydrolyzable tannin (Figure 1) that is not found in other commonly consumed forms of tea. Non-published, internal 'proof of concept' experiments highlighted GHG's potential to impact body weight, skin health, and blood flow.

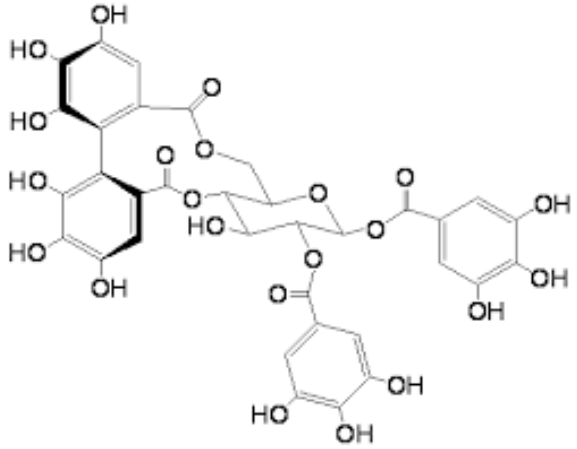

Figure 1. Chemical structure of 1,2-di-Galloyl-4,6-Hexahydroxydiphenoyl-β-D-Glucose, a unique constituent found in parts of the purple tea plant.

Additional work by Shimoda and colleagues [4] have reported that purple tea ingestion (two times per day from a 1.5 gram portion of tea leaves into 100 – 200 mL of water) by humans over four weeks may aid in reducing body weight, body mass index, and body fat. In animals, a 200 mg/kg dose significantly suppressed gains in body weight, abdominal fat, and triglycerides while also increasing the expression of carnitine palmitoyltransferase I [4]. Beyond its potential weight loss benefits, *in vitro* work has highlighted the ability of purple tea to improve nitric oxide production, assist exercise recovery, and mitigate muscle damage through anti-oxidant and anti-inflammatory mechanisms [5,6]. A narrative review by Harty and colleagues [7] highlighted previous research which examined the potential for tea supplementation and/or its constituents to impact various aspects of the exercise recovery process such as reductions in creatine kinase [8], oxidative stress [6,9], and perceived soreness [8,10]. Because not all research supports these outcomes, more research is needed to clarify potential benefits. Currently, no controlled scientific investigations using human participants have been published in peer-reviewed literature investigating the impact of purple tea ingestion on changes in exercise performance, exercise recovery, and components of the muscle damage process. Therefore, the purpose of this study was to examine the ability of oral Purple Tea supplementation (PurpleForce™, Oryza Oil & Fat Chemical. Co. Ltd, Japan) to impact muscle hyperemia and oxygenation, serum markers of nitric oxide production and muscle damage, and exercise performance in healthy human participants. It was hypothesized that purple tea supplementation may modulate the inflammatory response and enhance recovery from intense (muscle-damaging) exercise stress.

Methods

Overview of Study Design

The study design employed for this protocol was a randomized, double-blind, placebo-controlled, crossover investigation where each study participant supplemented for eight days with each treatment. Each participant completed five study visits. The first visit was for screening purposes and consisted of signing an IRB-approved consent form, completing a medical history, evaluating the presence of inclusion and exclusion criteria, and assessing routine blood work (comprehensive metabolic panel, complete blood panel, lipid panel) and resting vitals (heart rate and blood pressure). Participants were randomized into one of two groups, and subsequently consumed their respective treatment for eight days. After five and eight days of supplementation within each condition, participants returned for study visits and. Participants then completed a two-week washout and switched to the other treatment and subsequently consumed the alternate treatment for eight days while completing study visits and (after five and eight days of supplementation, respectively). Each study visit consisted of measuring vitals (resting heart and blood pressure), collection of venous blood for assessment of muscle damage and inflammation, nitric oxide markers, body composition (via DEXA), reactive hyperemia (via circumference measurements), fluid shifts (segmental BIA), muscle oxygenation (via NIRS), muscular endurance (via bench press and leg extension exercises), perceived recovery questionnaire, and visual analog scales for energy, willingness to exercise, muscle soreness, and sleep quality. A summary of the research design is provided in Table 1. To facilitate replication for all measured endpoints, study participants were asked to replicate their diet (including abstention from caffeine and alcohol) for 24 hours prior to each study visit, fast for 10 hours prior to each visit, and refrain from exercise for 48 hours prior to each study visit. Participants were given a standardized workout on of supplementation (i.e. general calisthenics using body weight exercises) and instructed to not participate in physical activity outside the study procedures during both periods of supplementation, in order to avoid conflating study outcomes.

Table 1. Overview of Study Design

Test Day	Screening	Day 5	Day 8	Day 27	Day 30
Visit	1	2	3	4	5
Screening Procedures:					
Informed Consent	X				
Inclusion/Exclusion Criteria	X				
Medical History	X				
Physical	X				
Height	X				
Weight	X				
Vitals (BP and HR)	X	X	X	X	X
Safety Screen[8]	X				
Phlebotomist/Blood Sampling	X	X	X	X	X
Concomitant Medications	X	X	X	X	X
Testing Procedures:					
Changes in lean mass (DEXA)		X		X	
Reactive Hyperemia		X	X	X	X
Bioelectrical Impedance		X	X	X	X
Muscular Endurance		X	X	X	X
Perceived Recovery Scale		X	X	X	X
Nitrate Assessment		X	X	X	
Muscle Damage		X	X	X	X
Muscle Oxygenation		X	X	X	X
VAS Muscle Soreness		X	X	X	X
Food Frequency		X	X	X	X
Diet Records/Analysis		X	X	X	X
Protocol Compliance		X	X	X	X
Dispense Test Product		X	X	X	X
Adverse Events Monitoring		X	X	X	X

*The safety screen included a metabolic/clinical chemistry panel, complete blood count, and a lipid panel.

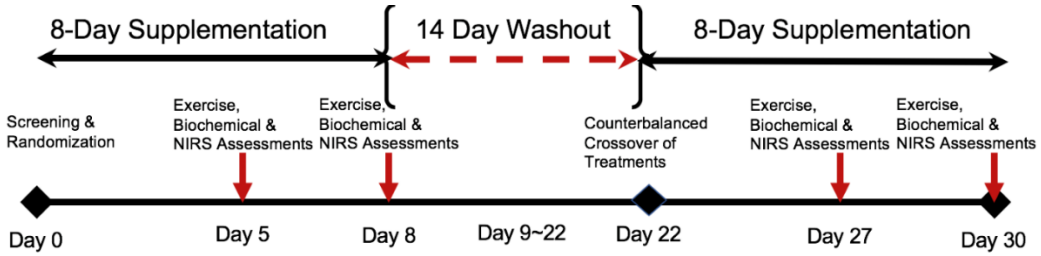

Figure 2. Timeline Schematic of Study Design and Procedures

Participants

Recreationally active men (i.e., intensive resistance training between 1-2 days per week) were recruited as participants in this study primarily from a local suburban community in Ohio. Complete demographics of all study participants can be found in Table 2. All participants read and signed an IRB-approved informed consent form prior to participating in the study (Integreview, Austin, TX, Protocol # Oryza-001-2019, Approval date: June 4, 2019). All study participants were required to be in good health as determined by review of their medical history and routine blood chemistries by the study physician. Inclusion criteria indicated that all participants were between the ages of 18 – 55, had body mass index levels between 25 – 34.99 kg/m^2, were normotensive (systolic pressure between 100 – 139 mm Hg and diastolic pressure between 65 – 89 mm Hg) with a normal resting heart rate (≤90 beats/min), had not used a sports supplement product in the four weeks prior to screening, and agreed to abide by all requirements of the study protocol.

Table 2. Baseline Characteristics of all Study Participants.

	Mean ± SD	Minimum	Maximum
Age (years)	33.5 ± 11.4	18	52
Height (cm)	178.4 ± 7.6	165	193
Weight (kg)	92.5 ± 13.3	73.7	126.2
Body Mass Index (kg/m^2)	29.0 ± 3.4	24.2	34.0
Systolic BP (mm Hg)	127.4 ± 9.4	104	143
Diastolic BP (mm Hg)	78.3 ± 7.1	64	90
Resting Heart Rate (beats/min)	64.2 ± 9.0	50	82

Alternatively, participants were excluded if they indicated use of an anabolic/anti-catabolic dietary supplement products for four weeks prior to testing, excluding a multivitamin and low dose (< 3 g/day) fish oil. Additionally, any participant with a history of diabetes, malignancy in the previous five years except for non-melanoma skin cancer, prior gastrointestinal bypass surgery, chronic inflammatory condition or disease, or any other known gastrointestinal or metabolic diseases that might impact nutrient absorption, e.g. short bowel syndrome, diarrheal illnesses, history of colon resection, gastro paresis, and inborn errors of metabolism were excluded. Participants with a concomitant use of corticosteroids or testosterone replacement therapy were excluded. A CONSORT diagram is provided in Figure 3 for all study participants through the study protocol.

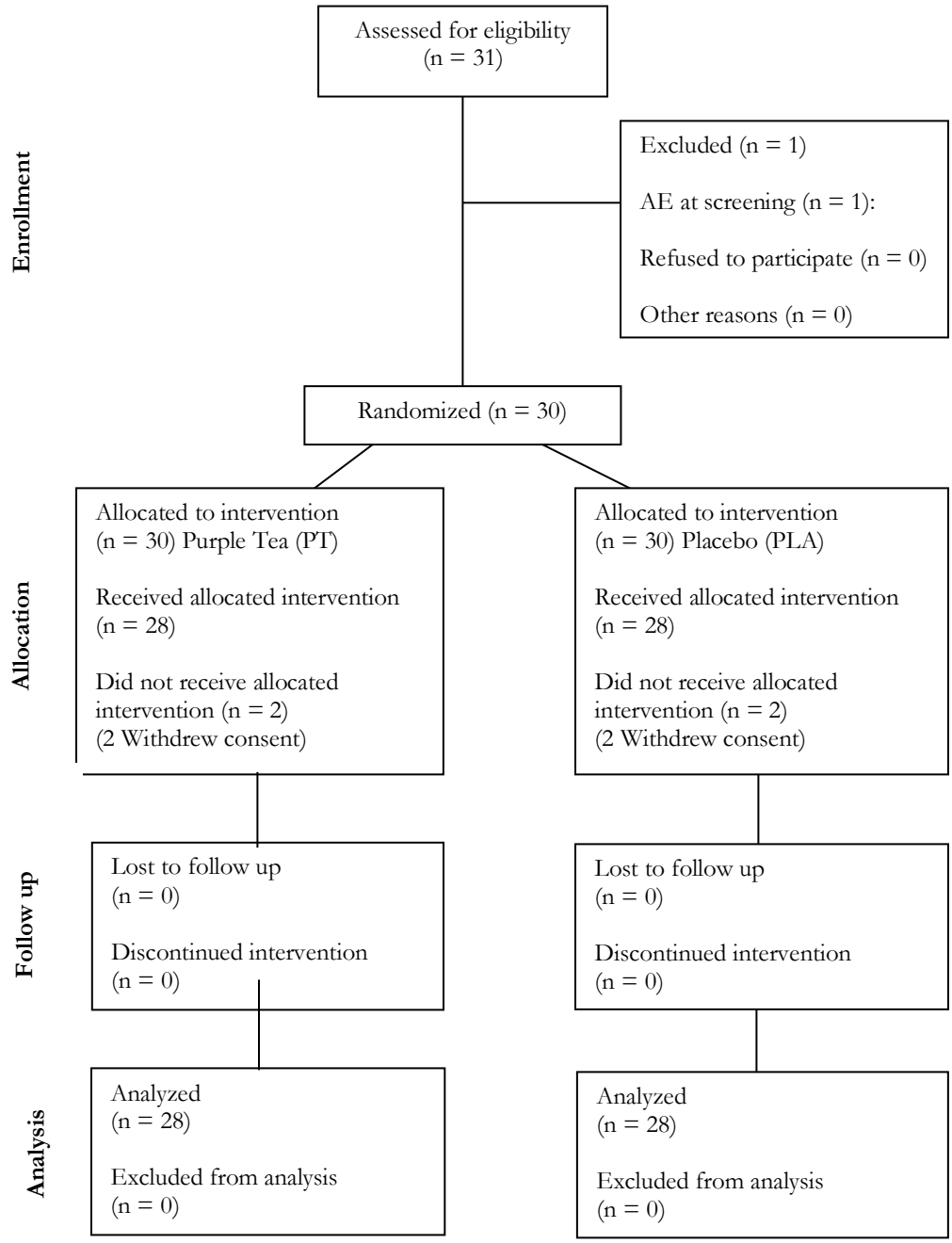

Figure 3. Consolidated Standards of Reporting Trials (CONSORT) diagram.

Protocol

Height, Weight, Heart Rate, Blood Pressure

Standing height was determined using a wall-mounted stadiometer with each study participant in their socks with heels together. Body weight was measured (±0.5 kg) using a Seca 767™ Medical Scale (Hamburg, Deutschland). Resting heart rate and blood pressure was measured in a sitting position after resting quietly for approximately ten minutes using an automated blood pressure cuff (Omron HEM-780).

Venous Blood Collection and Processing

Whole blood and serum samples were collected using standard phlebotomy techniques at all study visits. Whole blood samples were collected into K_2-EDTA treated Vacutainer tubes. Upon collection, each sample was slowly inverted ten consecutive times prior to immediate refrigeration. Serum samples were collected in serum separation tubes and allowed to clot for 30 minutes at room temperature prior to being centrifuged (Horizon mini E Centrifuge, Drucker Diagnostics, Port Matilda, PA) for 15 minutes at 3,200 rpm. An 800 µl aliquot of serum was placed polypropylene centrifuge tubes and frozen at -80°C for later analysis of total nitrates.

Biochemical Analysis

For screening purposes, blood collected at visit 1 was analyzed for a comprehensive metabolic panel, complete blood count with platelet differentials, and lipid panel. Components of the comprehensive metabolic panels consists of glucose, blood urea nitrogen [BUN], creatinine, aspartate aminotransaminase [AST], alanine aminotransaminase [ALT], total bilirubin, alkaline phosphatase [ALP], sodium, chloride, calcium, potassium, carbon dioxide, total protein, albumin, and globulin. Complete blood counts were analyzed for absolute cell number and percentage of each cell type contributing to the total sample for neutrophils, eosinophils, basophils, lymphocytes, and monocytes in addition to overall white blood cell and red blood cell count, hemoglobin, hematocrit, mean corpuscle volume, mean corpuscle hemoglobin, red cell dimension width, and mean corpuscle hemoglobin content. Lipid panel components consist of triglycerides [TG], total cholesterol [TC], LDL cholesterol, HDL cholesterol. All analyses were completed using automated clinical chemistry analyzers (LabCorp, Dublin, OH branch) and can be found in Supplementary Data Table 1. In addition, pre-exercise creatine kinase, lactate dehydrogenase, and C-reactive protein were analyzed on days 5 and 8 using an automated clinical chemistry analyzer at the same commercial diagnostic laboratory (See Table 3). All aforementioned blood samples were batch-analyzed with test-retest reliabilities commonly reported using internal quality control data from clinical laboratories and associated automated analyzers within a range of 5–7%. Total nitrate content was determined using a microplate-based approach. During batch analysis, serum was de-proteinized using 30 kD cutoff filter tubes and centrifugation at 12,000 g at 4°C for 20 minutes. Filtered serum was then analyzed in duplicate for total nitrates using a commercially-available kit (kit #780001, Cayman Chemical, Ann Arbor, MI, USA), and absorbance was read at 545 nm using a spectrophotometer (BioTek Synergy H1; BioTek, Winooski, VT, USA). All samples were within the linear standard curve range, and duplicate coefficient of variation values were less than 5%.

Circumference Measurements

To measure reactive hyperemia of the active muscle group, circumference measurements were taken around the upper arm and thigh pre and post testing at visits 2 - 5 with a Gulick spring loaded tape measure. Specifically, arm measurements were taken at half the distance between the acromion process and the olecranon process, and leg measurements were taken at half the distance between the inguinal crease and the proximal border of the patella. Arm circumference was obtained pre-bench press and immediately post-bench press of the last set, and leg circumference was obtained pre-leg extension and post-leg extension of the last set. All measurements were taken on the left side of the body. In our laboratory, ICC for repeated measures of thigh girth are >0.85.

Body Composition

Total and regional lean mass, fat mass, and % fat were determined by dual-energy x-ray absorptiometry (DEXA; General Electric Lunar DPX Pro). All DEXA scans were performed by the same technician and analyzed by the manufacturer's software (enCORE version 13.31). Briefly, participants were positioned in the scanner according to standard procedures and remained motionless for approximately 15 minutes while scans were being completed. DEXA segments for the trunk and upper and lower limbs were demarcated using standard anatomical landmarks. Percent fat was calculated by dividing total fat mass by total scanned mass. Lean to fat mass ratio was computed using a simple ratio between the two values. Quality control calibration procedures were performed prior to all scans using a calibration block and procedures provided by the manufacturer. Prior to this study, we determined test–retest reliability for repeated measurements of lean mass, bone mineral content, and fat mass using this DEXA using intra-class correlation coefficients; all values were $\geq$0.98

Bioelectrical Impedance Analysis

To measure changes in segmental fluid shifts of intra/extracellular fluids, a bioelectrical impedance analysis (BIA) of the thigh was used (ImpediMed SFB7). The SFB7 model scans 256 frequencies between 3 kHz and 1000 kHz and, using Cole modelling with Hanai mixture theory, determines total body water, extracellular fluid and intracellular fluid. Initial placement of electrodes was measured and recorded for each participant to replicate positioning during future visits. Four electrodes were placed on the left side of the body, two injecting and two receiving. The first injecting electrode was placed medially 5 cm below the medial malleolus of the tibia. The second injecting electrode was placed medially 5 cm below the styloid process of the ulna. The first receiving electrode was placed medially 5 cm above the proximal border of the patella. The second receiving electrode was placed medially 5 cm below the iliac crest. BIA was administered twice at both timepoints (pre and post) to measure resistance at a frequency of zero hertz (R zero, which reflects changes in extracellular fluid resistance), resistance at infinity frequency (R infinity, which reflects changes in intracellular fluid resistance), and resistive index (RI = stature in meters divided by resistance of each arm). The average value was used for data analysis. ICCs for this procedure are >0.97.

Muscle Oxygenation

A Moxy Monitor™ (www.moxymonitor.com, Fortiori Design, LLC, Hutchinson, MN) was used to measure muscle oxygen saturation (SmO_2) and total hemoglobin/ myoglobin (THMb) using near-infrared spectroscopy. For bench press testing, the Moxy Monitor was placed on the lateral head of the triceps, equidistant between the olecranon process of the ulna and acromion process of the scapula, and for leg extension testing it was placed on the middle portion of the vastus lateralis, equidistant between the superior border of the patella and iliac crest. Both sites were on the right side of the body and adhered with an elastic, adhesive wrap (Coban®, 3M, St. Paul, MN) to keep secure. The Moxy Monitor was paired with a Garmin Fenix 5 watch to display SmO_2 and THMb. Muscle oxygenation was measured pre and post exercise. Specially, resting values were recorded for 60 seconds prior to the first set on the bench press and leg extension. Lowest saturation values were recorded immediately after each set, and recovery values were obtained at 30 and 60 seconds following the bench press and leg extension for both sets.

Muscular Endurance

Muscular endurance was assessed for the upper body using a Smith machine bench press, and the lower body using a bilateral leg extension exercise. To standardize the exercises, a relative load was used. Specifically, 65% of body weight for the bench press, and 30% for the leg extension was used to assess muscular endurance. Participants were instructed to perform five repetitions with the Smith machine bar for a warm-up. Following a brief rest and return to baseline SmO_2 levels, the participant then completed as many repetitions to failure (RTF). Following one minute of seated rest, a final set of RTF was performed. A complete repetition was considered from full extension of the elbows with bar held over the chest to where the bar touches the sternum. Procedures for the leg extension were identical to the bench press whereby a complete repetition went from approximately 90 degrees of knee flexion to approximately 180 degrees of knee extension. No more than two seconds were allowed between repetitions. A trained researcher was present at all times to ensure safety, proper form and execution of each repetition.

Visual Analog Scales

Visual analog scales (VAS) were completed by each study participant before performance testing. All visual analog scales were similarly constructed using a 100-mm line anchored by "Lowest Possible" and "Highest Possible" to assess subjective ratings of energy, willingness to exercise, muscle soreness, and sleep quality. The validity and reliability of VAS to assess fatigue and energy have been previously established [11] and our methods have been published elsewhere [12-15].

Dietary Intake and Physical Activity Monitoring

No changes in dietary habits were prescribed as part of this study investigation. As a result, all participants were instructed to continue their typical diet throughout the entire study protocol. During baseline screening, participants were asked to complete 24-hour dietary recall. Dietary records were analyzed for average daily energy and macronutrient intake by trained study investigators and NutriBase IX (Clinical Edition) software (CyberSoft, Inc. Phoenix, AZ).The recalled log of food and fluid intake was copied and

provided back to the study participant. Study participants were then instructed to duplicate their food and fluid intake for the 24 hours prior to each subsequent study visit.

Supplementation

After screening, all participants were randomly assigned in a double-blind fashion to one of two supplementation groups: placebo (maltodextrin) or Purple Tea. All doses were consumed on a daily basis in the morning. On testing days, the dose was instructed to be consumed approximately 30 – 60 minutes prior to testing. Participants consumed one capsule containing 100 mg of purple tea extract as PurpleForce™ (Oryza Oil & Fat Chemical. Co. Ltd, Japan) per manufacturer recommendations. Placebo capsules contained maltodextrin and were consumed using an identical timing and dosing schedule. All study materials were prepared following current good manufacturing practices (cGMP) according to Code of Federal Regulations of US Food and Drug Administration Title 21 CFR part 111 in blinded capsules and packaged in coded generic containers for double-blind administration. Compliance to the supplementation regimen was monitored by daily logs, communication with study participants at each study visit, and counting all capsules at each subsequent study visit. Purity and potency of the test products were verified by an independent laboratory.

Adverse Events

During weekly phone calls, the frequency and intensity of local and systemic non-serious and serious adverse events (AEs) were recorded by study team members. All reported events were coded using the Medical Dictionary for Regulatory Activities (MedDRA) while the intensity of recorded adverse events were graded using standardized criteria.

Statistical Analysis

All data were entered into two separate Microsoft Excel spreadsheets (i.e. manual double-key data entry) and compared to assure data quality prior to analysis. SPSS 23 (Armonk, NY USA) was used for all analyses. Normality assumptions were checked on all variables using a one-sample Shapiro-Wilk test. Non-normal distributions were transformed using natural logarithms, cubed, and square root transformations. Outliers were checked via visual inspection of studentized calculations on the residuals (threshold value of ± 3 SD) of each dependent variable. Separate mixed factorial ANOVA with repeated measures on time were assessed for all outcomes. When the sphericity assumption was not met, the Huynh-Feldt correction was applied when epsilon was greater than 0.75 and the Greenhouse-Geiser correction was applied when epsilon was less than 0.75. In addition, delta values were computed and independent t-tests were completed to assess group differences. Mean differences of the change scores and 95% confidence intervals were calculated on the difference between groups. Within-group effects were compared using paired samples t-test. Effect sizes (ES) were also used to assess the magnitude of change, and values of 0.2, 0.5 and 0.8 were considered small, medium and large effects, respectively. All data are presented as means ± standard deviations. Results were considered statistically significant at $P \leq 0.05$ and trends were declared at $0.051 \leq p \leq 0.10$.

Results

Participant demographics for all study participants is provided in Table 2. Additionally, weekly compliance checks by the research study team revealed >95% compliance to the supplementation (data not shown) regimen. A summary table of adverse events (AEs) is provided (See Table 3).

Table 3. Summary of Adverse Events.

	Active (n=28)	Placebo (n=28)	Screened subjects prior to allocation (n=31)
Severity			
Mild	1	1	1
Moderate			
Severe			
Relationship to Test Article			
Not related			1
Possible	1	1	
Definite			
Gastrointestinal			
Nausea		1	
Nervous System			
Sleep disturbance; Abnormal dreams	1		
Surgical & Medical Procedures			
Presyncope; Vagal Reaction			1
Total Number of Adverse Events Experienced During Study	1	1	1
Total Number of Subjects Experiencing AEs: n (%)	1/28 (3.5%)	1/28 (3.5%)	1/31 (3%)

The data provided in this table are counts of each respective category.

Clinical Safety Markers, Muscle Damage, Inflammation, and Nitrate Content

All variables measured as part of complete blood counts with platelet differentials and comprehensive metabolic panels were analyzed as part of eligibility screening at the beginning of the study protocol and are provided in Table 4. No significant group x time interaction or within-group changes were identified for creatine kinase and C-reactive protein (Table 5). A significant group x time interaction (95% CI: 2.36, 18.29 U/L, ES = 0.34, $p = 0.01$) was found for lactate dehydrogenase. Within-group analysis revealed a significant reduction in lactate dehydrogenase concentrations in PT ($p = 0.04$) from day five to day eight while no change was shown for PLA ($p = 0.16$). No significant group x time interaction or within-group changes were identified for changes in total nitrate concentrations (Table 5).

Table 4: Serum and whole blood metabolic and hematological markers collected at baseline prior to supplementation.

Variables	Mean ± SD	Minimum	Maximum
White Blood Cell Count (cells/L)	5.5 ± 1.2	3.4	9.4
Red Blood Cell Count (cells/L)	5.1 ± 0.3	4.6	5.8
Hemoglobin (grams/dL)	15.7 ± 0.7	14.3	17.1
Hematocrit (%)	44.9 ± 1.9	41.6	48.5
Glucose (mg/dL)	93.0 ± 8.0	74	116
Blood Urea Nitrogen (BUN) (mg/dL)	15.5 ± 3.5	9	24
Creatine (mg/dL)	1.00 ± 0.17	0.65	1.43
BUN: Creatinine Ratio	15.8 ± 3.4	8	25
Sodium (mEq/L)	141 ± 1.60	137	143
Potassium (mEq/L)	4.3 ± 0.2	4	4.9
Chloride (mEq/L)	103 ± 2.1	99	107
Carbon Dioxide (mEq/L)	23.8 ± 1.6	21	27
Calcium (mg/dL)	9.5 ± 0.22	8.9	9.9
Protein (g/dL)	7.1 ± 0.3	6.5	7.9
Albumin (g/dL)	4.7 ± 0.2	4.3	5.1
Globulin (g/dL)	2.5 ± 0.3	1.7	3.1
Albumin: Globulin Ratio	1.9 ± 0.3	1.5	2.9
Bilirubin (mg/dL)	0.6 ± 0.3	0.2	1.7
Alkaline Phosphatase (IU/L)	68.7 ± 18.9	38	128
Aspartate Aminotransferase (U/L)	22.9 ± 5.2	14	35
Alanine Aminotransferase (U/L)	27.1 ± 12.0	6	58
Total Cholesterol (mg/dL)	175.5 ± 26.3	117	245
Triglycerides (mg/dL)	133 ± 112	36	582
HDL Cholesterol (mg/dL)	48.4 ± 11.1	30	74
VLDL Cholesterol (mg/dL)	23.3 ± 14.2	7	69
LDL Cholesterol (mg/dL)	102.6 ± 22.1	62	165

Table 5: Markers of Muscle Damage, Inflammation, and Nitrate Content.

Group	Day 5	Day 8	Delta	Within-Group p-value	Group x Time 95% CI	*p*
Creatine Kinase (U/L)						
PLA	202 ± 267	262 ± 256	60.8 ± 288	0.27	(-147, 151)	*0.98*
PT	191 ± 137	250 ± 209	58.7 ± 193	0.12		
Lactate Dehydrogenase (U/L)						
PLA	170 ± 22	174 ± 28	4.8 ± 17.3	0.16	(2.36, 18.29)	*0.01*
PT	175 ± 26	170 ± 28	-5.5 ± 13.5	0.04		
C-Reactive Protein (mg/L)						
PLA	1.24 ± 1.41	1.21 ± 1.28	-0.03 ± 0.79	0.85	(-0.60, 0.39)	*0.66*
PT	1.01 ± 0.94	1.09 ± 1.11	0.08 ± 0.71	0.56		
Nitrates (μM)						
PLA	43.9 ± 13.1	41.1 ± 15.6	-2.8 ± 13.6	0.28	(-13.5, 14.3)	*0.95*
PT	48.3 ± 32.4	45.0 ± 19.5	-3.3 ± 31.5	0.59		

95% CI = 95% confidence interval calculated on the differences between conditions

Circumferences, Body Composition, and Impedance Data

No statistically significant changes were reported in either group from day five to day eight (Table 6). Changes in arm circumference between conditions (PLA vs. PT) were significantly different after five days of supplementation ($p = 0.04$) and tended to be different after eight days of supplementation ($p = 0.06$). In both cases, PLA experienced greater increases in arm circumference than PT, presumably due to swelling. Changes in DXA lean mass in the legs between conditions tended ($p = 0.07$) to be different after eight days of supplementation with PT experiencing larger increases than what was observed in PLA.

Changes in bioimpedance data (Average R Infinity) between conditions tended (p = 0.07) to be different after eight days of supplementation. Changes in average RI between conditions was significantly different after eight days of supplementation (p=0.02). In both outcomes, PT experienced greater decreases than what was observed in PLA.

Table 6: Circumferences and Body Composition

		Day 5		Day 8	
		Pre	Post	Pre	Post
Arm Circumference	PLA	33.5 ± 2.5	34.0 ± 2.9†	33.3 ± 2.3	33.9 ± 2.7†
(cm)	PT	32.8 ± 2.6	33.1 ± 2.8	33.2 ± 2.7	33.3 ± 2.8
		Pre	Post	Pre	Post
Thigh Circumference	PLA	53.7 ± 4.1	54.3 ± 4.2	53.4 ± 4.8	54.5 ± 4.0
(cm)	PT	53.3 ± 4.6	54.0 ± 4.3	53.7 ± 4.3	54.0 ± 4.1
		Pre	Post	Pre	Post
DXA Lean-Arms	PLA	8.2 ± 1.2	8.2 ± 1.2	8.2 ± 1.2	8.2 ± 1.1
(kg)	PT	8.1 ± 1.3	8.3 ± 1.4	8.1 ± 1.1	8.2 ± 1.2
		Pre	Post	Pre	Post
DXA Lean-Legs	PLA	21.7 ± 3.0	21.7 ± 2.9	21.5 ± 3.3	21.7 ± 3.0
(kg)	PT	21.7 ± 2.9	21.7 ± 2.9	21.8 ± 3.1	22.0 ± 3.1
		Pre	Post	Pre	Post
R Zero	PLA	42.0 ± 6.2	40.3 ± 5.9	41.9 ± 7.8	40.5 ± 7.2
(ohms)	PT	41.1 ± 6.4	40.1 ± 6.9	42.0 ± 8.0	40.8 ± 7.2
		Pre	Post	Pre	Post
R Infinity	PLA	25.3 ± 3.7	24.7 ± 3.6	25.0 ± 4.5	26.0 ± 5.2
(ohms)	PT	25.4 ± 4.0	24.5 ± 4.1	25.7 ± 4.3	25.0 ± 3.9
		Pre	Post	Pre	Post
RI Average	PLA	65.6 ± 14.0	65.4 ± 14.5	64.7 ± 14.5	63.8 ± 14.4
(ohms)	PT	67.3 ± 15.0	64.0 ± 13.5	68.0 ± 12.7	64.6 ± 13.1†

† = Different between groups at designated time point (day 5 or day 8). No statistically significant group x time interactions were observed between groups from day 5 to day 8.

Muscle Oxygenation

As expected, widespread within-group changes were observed in both groups for both THMb and SmO_2 skeletal muscle tissue oxygenation. No pattern of between-group changes were observed for either THMb or SmO_2 for bench press (Table 7). Changes between-groups in day five THMb values during the bench press exercise tended to be different after 60s of recovery (95% CI: -0.01, 0.14, $p = 0.10$) while day five SmO_2 levels during the bench press also tended to be different (95% CI: -17.03, 1.27, $p = 0.09$) immediately after exercise. Changes between-groups in day five SmO_2 values during the leg extension exercise (see Table 8) were not different immediately after exercise (95% CI: -0.97, 8.87, $p = 0.11$), but were different after 30s of recovery (95% CI: 0.25, 14.3, $p=0.04$), and tended to be different after 60s of recovery (95% CI: -0.16, 9.97, $p = 0.06$).

Table 7. Muscle Oxygenation – Bench Press

TOTAL HEMOGLOBIN/ MYOGLOBIN (THMb)					Group x Time	
Day 5 Bench Press						
	Pre	Immediate Post	Group	Time	95% CI	*p*
PLA	11.90 ± 0.46	12.22 ± 0.53	0.60	<0.001	(-0.12, 0.19)	*0.68*
PT	12.02 ± 0.46	12.25 ± 0.53				
	Pre	30s Recovery				
PLA	11.90 ± 0.46	12.17± 0.41	0.54	<0.001	(-0.04, 0.12)	*0.31*
PT	12.02 ± 0.46	12.20 ± 0.39				
	Pre	60s Recovery				
PLA	11.90 ± 0.46	12.19 ± 0.38	0.66	<0.001	(-0.01, 0.14)	*0.10*
PT	12.02 ± 0.46	12.19 ± 0.39				
Day 8 Bench Press						
	Pre	Immediate Post	Group	Time	95% CI	*p*
PLA	12.02 ± 0.46	12.25 ± 0.53	0.11	<0.001	(-0.07, 0.12)	*0.55*
PT	11.92 ± 0.45	12.14 ± 0.57				
	Pre	30s Recovery				
PLA	12.02 ± 0.46	12.20 ± 0.39	0.09	<0.001	(-0.09, 0.11)	*0.78*
PT	11.92 ± 0.45	12.09 ± 0.41				
	Pre	60s Recovery				
PLA	12.02 ± 0.46	12.19 ± 0.39	0.12	<0.001	(-0.11, 0.08)	*0.71*
PT	11.92 ± 0.45	12.11 ± 0.38				
MUSCLE TISSUE OXYGEN SATURATION (SmO_2)						
Day 5 Bench Press						
	Pre	Immediate Post	Group	Time	95% CI	*p*
PLA	70.00 ± 10.49	20.02 ± 17.32	0.98	<0.001	(-17.03, 1.27)	*0.09*
PT	66.10 ± 11.79	24.05 ± 22.22				
	Pre	30s Recovery				
PLA	70.00 ± 10.49	73.48 ± 15.11	0.65	0.004	(-11.56, 1.41)	*0.12*
PT	66.10 ± 11.79	74.69 ± 14.70				
	Pre	60s Recovery				
PLA	70.00 ± 10.49	84.38 ± 6.04	0.22	<0.001	(-7.36, 3.16)	*0.42*
PT	66.10 ± 11.79	82.62 ± 9.46				
Day 8 Bench Press						
	Pre	Immediate Post	Group	Time	95% CI	*p*
PLA	66.38 ± 11.08	19.83 ± 12.98	0.50	<0.001	(-7.23, 4.85)	*0.69*
PT	67.00 ± 13.03	73.29 ± 13.59				
	Pre	30s Recovery				
PLA	66.38 ± 11.08	72.67 ± 12.08	0.69	0.001	(-5.44, 5.44)	*1.00*
PT	67.00 ± 13.03	67.03 ± 13.02				
	Pre	60s Recovery				
PLA	66.38 ± 11.08	82.67 ± 7.43	0.35	<0.001	(-6.18, 3.65)	*0.60*
PT	67.00 ± 13.03	84.55 ± 6.95				

Table 8. Muscle Oxygenation – Leg Extension

TOTAL HEMOGLOBIN/ MYOGLOBIN (THMb)				Group x Time		
Day 5 Leg Extension						
	Pre	Immediate Post	Group	Time	95% CI	*p*
PLA	12.49 ± 0.48	12.61 ± 0.50	0.82	0.01	(-0.03, 0.12)	0.23
PT	12.50 ± 0.49	12.58 ± 0.50				
	Pre	30s Recovery				
PLA	12.49 ± 0.48	12.54 ± 0.48	0.85	0.50	(-0.08, 0.16)	0.48
PT	12.50 ± 0.49	12.50 ± 0.50				
	Pre	60s Recovery				
PLA	12.49 ± 0.48	12.53 ± 0.46	0.82	0.50	(-0.04, 0.12)	0.27
PT	12.50 ± 0.49	12.50 ± 0.48				
Day 8 Leg Extension						
	Pre	Immediate Post	Group	Time	95% CI	*p*
PLA	12.50 ± 0.44	12.62 ± 0.46	0.71	0.01	(-0.03, 0.10)	0.24
PT	12.50 ± 0.45	12.59 ± 0.44				
	Pre	30s Recovery				
PLA	12.50 ± 0.44	12.62 ± 0.42	0.81	<0.001	(-0.06, 0.11)	0.53
PT	12.50 ± 0.45	12.59 ± 0.45				
	Pre	60s Recovery				
PLA	12.50 ± 0.44	12.55 ± 0.40	0.85	0.12	(-0.04, 0.08)	0.46
PT	12.50 ± 0.45	12.53 ± 0.41				
MUSCLE TISSUE OXYGEN SATURATION (SmO_2)						
Day 5 Leg Extension						
	Pre	Immediate Post	Group	Time	95% CI	*p*
PLA	52.62 ± 9.28	14.67 ± 11.60	0.48	<0.001	(-0.97, 8.87)	0.11
PT	55.86 ± 8.50	13.95 ± 8.82				
	Pre	30s Recovery				
PLA	52.62 ± 9.28	49.71 ± 17.11	0.88	0.01	(0.25, 14.3)	0.04
PT	55.86 ± 8.50	45.69 ± 14.36				
	Pre	60s Recovery				
PLA	52.62 ± 9.28	67.57 ± 13.52	0.19	<0.001	(-0.16, 9.97)	0.06
PT	55.86 ± 8.50	65.90 ± 10.36				
Day 8 Leg Extension						
	Pre	Immediate Post	Group	Time	95% CI	*p*
PLA	55.04 ± 8.68	16.07 ± 15.42	0.08	<0.001	(-4.38, 7.24)	0.61
PT	53.48 ± 7.96	13.07 ± 10.26				
	Pre	30s Recovery				
PLA	55.04 ± 8.68	46.19 ± 17.28	0.14	0.003	(-5.17, 7.51)	0.71
PT	53.48 ± 7.96	43.45 ± 18.45				
	Pre	60s Recovery				
PLA	55.04 ± 8.68	64.86 ± 14.00	0.42	0.001	(-5.87, 4.02)	0.70
PT	53.48 ± 7.96	64.21 ± 17.58				

Physical Performance

No significant group x time interaction were found in the number of bench press repetitions completed during either set or for the total number of repetitions completed (Table 9). The purple tea condition was able to perform approximately 1.28 ± 3.1 more total bench press repetitions from day five to day eight (p = 0.05, ES = 0.11). A significant group x time interaction effect was observed (95% CI: -7.34, -0.01, p=0.05, ES = 0.34) for repetitions completed during set 1 of the leg extension exercise. The purple tea condition was able to complete approximately 4.6 ± 4.4 more repetitions from day five to day eight (p = 0.001, ES = 0.18) while no change was observed in the PLA group. Total leg extension repetitions completed between groups tended to be different (95% CI: -8.62, 0.70, p=0.09, ES = 0.29). Again, the total repetitions completed in the purple tea condition significantly increased from day five to day eight (p < 0.001, ES = 0.44) while no change was observed in PLA (p = 0.37, ES = 0.10).

Table 9: Physical Performance.

	Day 5	Day 8	Delta	Within-Group p-value	Group x Time 95% CI	Group x Time *p*-value
Bench Press Reps to Fatigue – Set 1						
PLA	19.5 ± 8.6	20.0 ± 8.9	0.52 ± 1.7	0.13	(-1.66, 0.77)	*0.46*
PT	18.7 ± 7.7	19.6 ± 8.2	1.00 ± 2.8	0.09		
Bench Press Reps to Fatigue – Set 2						
PLA	8.5 ± 3.3	8.6 ± 3.8	0.11 ± 1.9	0.77	(-1.38, 1.01)	*0.75*
PT	8.9 ± 4.0	9.2 ± 3.7	0.30 ± 1.8	0.40		
Bench Press Total Reps						
PLA	28.0 ± 11.2	28.7 ± 12.3	0.63 ± 2.4	0.19	(-2.12, 0.86)	*0.39*
PT	27.6 ± 11.1	28.8 ± 11.2	1.28 ± 3.1	0.05		
Leg Extension Reps to Fatigue – Set 1						
PLA	34.9 ± 13.8‡	35.8 ± 12.3	0.92 ± 7.2	0.53	(-7.34, -0.01)	*0.05*
PT	29.1 ± 8.0	33.7 ± 8.4	4.6 ± 4.4	<0.001		
Leg Extension Reps to Fatigue – Set 2						
PLA	20.8 ± 4.2	21.4 ± 4.2	0.68 ± 3.1	0.28	(-2.48, 1.92)	*0.80*
PT	20.9 ± 5.6	21.8 ± 5.0	0.96 ± 3.7	0.21		
Leg Extension Reps to Fatigue – Total Reps						
PLA	55.7 ± 16.2‡	57.3 ± 15.5	1.60 ± 8.7	0.37	(-8.62, 0.70)	*0.09*
PT	50.0 ± 12.5	55.6 ± 12.7	5.56 ± 5.7	<0.001		

Visual Analog Scales

No significant group x time interaction or within-group changes were observed for any of the visual analog scales (Table 10). The 'Willingness to Exercise' scale did exhibit a tendency (95% CI: -0.95, 0.09, p = 0.10, ES = 0.29) to improve in the purple tea condition from day five to day eight. When changes across time were viewed individually for each group, the purple tea condition experienced a significant increase in this scale (p = 0.02).

Table 10. Visual Analog Scales

Group	Day 5	Day 8	Delta	Within-Group p-value	Group x Time 95% CI	*p*-value
Perceived Recovery Scale						
PLA	8.04 ± 1.86	7.93 ± 1.96	-0.11 ± 2.54	0.83	(-1.84, 1.19)	*0.67*
PT	8.14 ± 1.56	8.36 ± 1.25	0.21 ± 1.95	0.57		
Energy						
PLA	6.89 ± 1.48	7.21 ± 1.28	0.31 ± 1.26	0.20	(-0.40, 0.46)	*0.89*
PT	6.96 ± 1.37	7.25 ± 1.47	0.29 ± 0.95	0.12		
Willingness to Exercise						
PLA	7.26 ± 1.54	7.32 ± 1.50	0.06 ± 1.03	0.77	(-0.95, 0.09)	*0.10*
PT	7.39 ± 1.61	7.88 ± 1.34	0.49 ± 1.06	0.02		
Soreness						
PLA	2.49 ± 2.54	2.88 ± 2.67	0.38 ± 2.81	0.48	(-1.67, 1.42)	*0.87*
PT	2.35 ± 2.12	2.85 ± 2.36	0.51 ± 2.21	0.23		
Sleep						
PLA	6.85 ± 1.65	6.68 ± 1.94	-0.17 ± 1.87	0.63	(-1.39, 0.48)	*0.32*
PT	6.76 ± 1.64	7.05 ± 1.74	0.29 ± 1.37	0.28		

Discussion

This randomized, double-blind, placebo-controlled, crossover investigation determined the effects of a standardized purple tea extract (Purple Force, Oryza Oil & Fat Chemical, Ltd.) on clinical safety, body composition, hyperemia, muscle damage, exercise performance, and muscle oxygenation. Key findings from this project revealed an increase in the number of leg extension repetitions completed after one set and a tendency for more repetitions to be completed across the exercise protocol in addition to an improvement (i.e. reduction) in the circulating levels of lactate dehydrogenase, a commonly assessed marker of muscle damage. Additionally, PT supplementation led to an increase in the rating of 'Willingness to Exercise'. Supplementation was well tolerated as assessed by the general lack of change exhibited by adverse events, side-effect profiles, clinical assessments during study visits, and blood-based markers of health and safety.

Several variations of the tea plant (*Camellia sinensis*) are consumed worldwide including black, Oolong, and green tea. Each variation contains compounds such as catechins, polyphenols, and anthocyanins (among others) that have been purported and, in many instances, demonstrated to have healthy attributes. For example, tea ingestion has been shown to improve metabolism, increase fat oxidation, and bolster anti-oxidant, anti-inflammatory, and other cytoprotective functions [16,17]. Limited human research, however, has been completed examining the ability of purple tea to impact health, performance, and recovery. Purple tea is a unique cultivar of the tea plant, primarily being grown in regions of Eastern Kenya. Reports have highlighted that purple tea has higher amounts of polyphenols and anthocyanins when compared to other variations of tea [2,3] and is the only cultivar that contains the hydrolysable tannin, 1,2-di-O-galloyl-4,6-O- (S)-hexahydroxydiphenoyl-β-D-glucose (GHG). Well-controlled research using purple tea, however, is limited. Shimoda and colleagues [4] previously published outcomes that indicated human ingestion of purple tea (two times per day from 1.5 gram portion of tea leaves into 100 – 200 mL of water) for four weeks improved reductions in body weight loss, body mass index, and body fat. In the same paper, animal model data were published, and a 200 mg/kg dose suppressed gains in body weight, abdominal fat, and triglycerides while also increasing the expression of a key fat metabolism enzyme (carnitine palmitoyltransferase I).

The ability of purple tea exposure to stimulate nitric oxide production was demonstrated *in vitro* whereby human umbilical vein endothelial cells were cultured with either control sera, or sera containing 1, 10, and 100 uM of purple tea. Production of nitrite, nitrate, and total nitrate increased with purple tea exposure (data not published) which led to the need for a more rigorous investigation to see if purple tea could impact exercise performance and recovery from stressful exercise. Not surprisingly, results from the present study contrast previous data published by Shimoda et al. [4] which demonstrated a reduction in body mass, body mass index, and percent fat. The dosing used between the two investigations were different with Shimoda using a tea beverage preparation (1.5 grams of tea leaves in 100 – 200 mL of water,

two times per day) while the present study used a 100 mg dose in a capsulated formulation. The largest difference between studies was the length of investigation. To this point, the Shimoda study provided their beverage for a total of four weeks while the present study used an eight-day supplementation regimen. While other *in vitro* and *in vivo* work has documented the ability of tea, extracts, and constituents catechins to impact energy expenditure and mechanistic targets of lipolysis [18-20], more research is needed in an exercising population over the course of several months (12 – 16 weeks) of supplementation in conjunction with a hypoenergetic dietary regimen and exercise program to better understand the impact of purple tea's ability to impact changes in various body composition parameters.

Catechins and polyphenols have been well studied for their ability to impact cellular responses to stress, particularly oxidative stress. In light of these potential functions, purple tea administration was investigated in the present study for its potential ability to improve performance and recovery from challenging exercise. Upper-body exercise performance did not change, but the number of repetitions performed using the leg extension exercise was improved. Additionally, circulating levels of lactate dehydrogenase, a marker of muscle damage, were decreased while no changes in creatine kinase, another marker of muscle damage and C-reactive protein (a systemic marker of inflammation), were observed. Scant research has been completed examining the ability of tea or various catechins to impact performance and no published research is available that has examined purple tea's ability to impact exercise performance and recovery. From a human performance perspective, the majority of research has focused upon the catechin's ability to function in an anti-oxidant fashion. In this respect, previous work by Kerksick and colleagues [10] used an isokinetic muscle damage model in healthy college-aged men after supplementing for 14 days with 1,800 mg of epigallocatechin gallate (EGCG) to identify EGCG's ability to improve recovery and mitigate muscle damage, inflammation, and apoptosis. Performance was not impacted by ECGG and while changes in creatine kinase and various markers of oxidative stress, inflammation, and apoptosis changed in response to the damage bout, no impact of EGCG was observed. While these findings do not align with those of the current study, the differences in supplementation and exercise model made it challenging to closely compare outcomes between the two investigations.
Another area of interest for the present study was to examine more closely if purple tea could enhance nitrate production and improve muscle oxygenation parameters. These outcomes were of interest due to previous *in vitro* work in cultured cells that demonstrated purple tea's ability to increase nitrate production and subsequently stimulate nitric oxide release, which has been shown to improve blood flow. The findings from this study suggest that the lower SmO_2 values at 30s recovery post-leg extension exercise bout with five days of PT supplementation may have been linked to improved vastus lateralis muscle oxygen extraction and/or utilization (Table 8). It is generally well accepted that changes in the dynamic balance between skeletal muscle O_2 delivery and O_2 utilization alter intracellular metabolism, metabolite accumulation and ultimately contractile muscle function and tolerance during exercise (21, 22). Previous studies have used the measure of muscle NIRS to assess tissue oxygenation, blood flow and microcirculation during resistance training, and our exercise induced SmO_2 decrease from baseline data as a time effect are similar to those previously presented (22,23). A short period of PT supplementation over five days, as demonstrated by the delta of SmO_2 from baseline to 30s and 60s recovery post-leg extensor exercise, may evoke a more rapid adjustment in O2 supply and delivery to match demand of exercising muscle. The SmO_2 data in our present study lend support to one potential mechanism for the improved lower body muscular endurance and tolerance. Improved oxygen extraction and utilization from the skeletal muscle tissue vascular bed would be expected to spare the finite anaerobic energy reserves, and attenuate the accumulation of fatigue-related metabolites, thereby promoting enhanced exercise tolerance.

No changes, however, were identified in the present study for total plasma nitrate-related outcomes. In light of these findings, future work should examine more sensitive measures to evaluate blood flow such as ultrasound and flow-mediated dilation approaches, while also examining more closely if changing the supplementation regimen may impact outcomes from these areas. In summary, eight days of supplementing with a purple tea extract resulted in an improvement in lower body muscular endurance and reductions in circulating levels of a commonly used marker of muscle damage. While preliminary, this evidence points towards the ability of oral supplementation with a purple tea extract in healthy previously active men to facilitate recovery from stressful exercise and enhance the ability to perform a maximal number of exercise repetitions. Future research should expand on these outcomes using well-

controlled studies that explore in more detail any potential impact on the type of exercise performance, different athletes, and dosing regimens.

Media-Friendly Summary

Eight days of supplementing with a purple tea extract at a dose of 100 mg resulted in an improvement in lower body muscular endurance and reductions in circulating levels of a commonly used marker of muscle damage. Supplementation was well tolerated with no side effects being reported throughout the investigation. Overall, these findings provide preliminary evidence that oral supplementation with a purple tea extract in healthy previously active men may help to facilitate recovery from stressful exercise and enhance the ability to perform a maximal number of exercise repetitions. Clearly more research is needed using well-controlled randomized, double-blind, placebo-controlled approaches that better identify what type of exercise performance may be impacted the most, if different type of athletes will be impacted differently, and what dosing regimen will yield beneficial outcomes.

Acknowledgements

The supplement (Purple Force™), placebo, and funding for this project was received through a restricted external grant from Oryza Oil & Fat Chemical. Co. Ltd, Japan. The authors would like to thank the study participants who completed the study protocol. Publication of these results should not be considered as an endorsement of any product used in this study by the Center for Applied Health Sciences or any of the organizations where the authors are affiliated.

Funding

Funding was provided by Oryza Oil & Fat Chemical. Co. Ltd, Japan through a restricted grant to The Center for Applied Health Sciences. Outside of initial discussions, the sponsor played no part in designing the study. Further, the sponsor had no part in collecting the data, analyzing the data, or preparing the manuscript for publication.

Conflict of Interests

Raub, Cesareo, Kedia, and Sandrock all report no conflicts of interest.

Lopez is an officer and member of The Center for Applied Health Sciences, a privately held contract research organization that has received external funding from companies that do business in the dietary supplement, natural products, medical foods and functional foods and beverages industries. He is the co-founder and member of Supplement Safety Solutions, LLC., serving as an independent consultant for regulatory compliance, safety surveillance and Nutravigilance to companies in the dietary supplement and functional foods industry, but not the sponsor of the current research. Lopez is also co-inventor on multiple patents within the field of dietary supplements, applied nutrition and bioactive compounds.

Ziegenfuss is an officer and member of The Center for Applied Health Sciences, a privately held contract research organization that has received external funding from companies that do business in the dietary supplement, natural products, medical foods and functional foods and beverages industries. Ziegenfuss has received grants and contracts to conduct research on dietary supplements; has served as a paid consultant for industry; has received honoraria for speaking at conferences and writing articles about functional foods and dietary supplements; receives royalties from the sale of several sports nutrition products (none related to the product examined in the present study); and has served as an expert witness on behalf of the plaintiff and defense in cases involving dietary supplements. Ziegenfuss is also co-inventor on multiple patents within the field of dietary supplements, applied nutrition and bioactive compounds.

Authors' Contributions

HLL and TNZ designed the study, secured funding for the project, and assisted with manuscript preparation. KC and TZ wrote the initial draft. BR, KC, and JS carried out subject recruitment, data collection, coordination of the study and compliance. TZ coordinated the statistical analysis. AWK provided medical oversight. All authors read and approved the final manuscript.

References

1. Westerterp-Plantenga MS. Green tea catechins, caffeine and body-weight regulation. *Physiol Behav*. 2010;100(1):42-46.
2. Cherotich L, Kamunya S, Alakonya A, et al. Variation in catechin composition of popularly cultivated tea clones in East Africa (Kenya). Paper presented at: 2013 JKUAT Annual Scientific Conference Proceedings2013; Nairobi, Kenya.
3. Kilel EC, Faraj AK, Wanyoko JK, Wachira FN, Mwingirwa V. Green tea from purple leaf coloured tea clones in Kenya- their quality characteristics. *Food Chem*. 2013;141(2):769-775.
4. Shimoda H, Hitoe S, Nakamura S, Matsuda H. Purple Tea and Its Extract Suppress Diet-induced Fat Accumulation in Mice and Human Subjects by Inhibiting Fat Absorption and Enhancing Hepatic Carnitine Palmitoyltransferase Expression. *Int J Biomed Sci*. 2015;11(2):67-75.
5. Jowko E, Sacharuk J, Balasinska B, et al. Effect of a single dose of green tea polyphenols on the blood markers of exercise-induced oxidative stress in soccer players. *Int J Sport Nutr Exerc Metab*. 2012;22(6):486-496.
6. Panza VS, Wazlawik E, Ricardo Schutz G, Comin L, Hecht KC, da Silva EL. Consumption of green tea favorably affects oxidative stress markers in weight-trained men. *Nutrition*. 2008;24(5):433-442.
7. Harty PS, Zabriskie HA, Erickson JL, Molling PE, Kerksick CM, Jagim AR. Multi-ingredient pre-workout supplements, safety implications, and performance outcomes: a brief review. *J Int Soc Sports Nutr*. 2018;15(1):41.
8. Herrlinger KA, Chirouzes DM, Ceddia MA. Supplementation with a polyphenolic blend improves post-exercise strength recovery and muscle soreness. *Food Nutr Res*. 2015;59:30034.
9. Jowko E, Dlugolecka B, Makaruk B, Cieslinski I. The effect of green tea extract supplementation on exercise-induced oxidative stress parameters in male sprinters. *Eur J Nutr*. 2015;54(5):783-791.
10. Kerksick CM, Kreider RB, Willoughby DS. Intramuscular adaptations to eccentric exercise and antioxidant supplementation. *Amino Acids*. 2010;39(1):219-232.
11. Lee KA, Hicks G, Nino-Murcia G. Validity and reliability of a scale to assess fatigue. *Psychiatry research*. 1991;36(3):291-298.
12. Ziegenfuss TN, Habowski SM, Sandrock JE, Kedia AW, Kerksick CM, Lopez HL. A Two-Part Approach to Examine the Effects of Theacrine (TeaCrine(R)) Supplementation on Oxygen Consumption, Hemodynamic Responses, and Subjective Measures of Cognitive and Psychometric Parameters. *Journal of dietary supplements*. 2017;14(1):9-24.
13. Ziegenfuss TN, Kedia AW, Sandrock JE, Raub BJ, Kerksick CM, Lopez HL. Effects of an Aqueous Extract of Withania somnifera on Strength Training Adaptations and Recovery: The STAR Trial. *Nutrients*. 2018;10(11).
14. Ziegenfuss TN, Lopez HL, Sandrock JE, Kedia AW, Habowski S, Kerksick C. Effect of a Multi-Nutrient Over-the-Counter Supplement on Changes in Metabolic Rate and Markers of Lipolysis. *Journal of dietary supplements*. 2017;14(3):288-302.
15. Lopez HL, Cesareo KR, Raub B, et al. Effects of Hemp Extract on Markers of Wellness, Stress Resilience, Recovery and Clinical Biomarkers of Safety in Overweight, But Otherwise Healthy Subjects. *Journal of dietary supplements*. 2020:1-26.
16. Malongane F, McGaw LJ, Mudau FN. The synergistic potential of various teas, herbs and therapeutic drugs in health improvement: a review. *Journal of the science of food and agriculture*. 2017;97(14):4679-4689.
17. Naveed M, BiBi J, Kamboh AA, et al. Pharmacological values and therapeutic properties of black tea (Camellia sinensis): A comprehensive overview. *Biomed Pharmacother*. 2018;100:521-531.
18. Hodgson AB, Randell RK, Jeukendrup AE. The effect of green tea extract on fat oxidation at rest and during exercise: evidence of efficacy and proposed mechanisms. *Advances in nutrition*. 2013;4(2):129-140.
19. Vazquez Cisneros LC, Lopez-Uriarte P, Lopez-Espinoza A, Navarro Meza M, Espinoza-Gallardo AC, Guzman Aburto MB. Effects of green tea and its epigallocatechin (EGCG) content on body weight and fat mass in humans: a systematic review. *Nutr Hosp*. 2017;34(3):731-737.

20. Turkozu D, Tek NA. A minireview of effects of green tea on energy expenditure. *Crit Rev Food Sci Nutr.* 2017;57(2):254-258.