UNIVERSITY OF CENTRAL FLORIDA

PROTOCOL TITLE:

Effects of Post-Exercise Recovery Drink Composition on Subsequent Performance in Masters Class Athletes

PRINCIPAL INVESTIGATOR:

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VERSION NUMBER/DATE:

3.0 – 3-31-21 (revised); 4-1-21 (IRB approval)

REVISION HISTORY

Revision #	Version Date	Summary of Changes	Consent Change?

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1.0 Study Summary

Study Title	Effects of Post-Exercise Recovery Drink Composition on Subsequent Performance in Masters Class Athletes
Study Design	Randomized, Double-blind, placebo-controlled between-subject
Primary Objective	The purpose of this study is to demonstrate that performance in a subsequent bout of high-intensity glycogen depleting exercise will not be significantly different regardless of post-exercise recovery drink (carbohydrate vs. carbohydrate and protein vs. placebo).
Secondary Objective(s)	Assess the level of sports nutrition knowledge and total daily carbohydrate and protein intake in a group of endurance-trained masters class athletes.
Research Intervention(s)/ Investigational Agent(s)	Erica Goldstein, MS, RDN Jeffrey Stout, PhD David Fukuda, PhD Adam Wells, PhD Maxine Furtado, BS
IND/IDE #	
Study Population	Male Athletes, Age 35-59 years
Sample Size	50
Study Duration for individual participants	14 days; 3-4 total visits
Study Specific Abbreviations/ Definitions	Graded Exercise Test (GXT), Maximal Oxygen Consumption (VO_2max), Plateau in Oxygen Uptake (VO_2peak), High-Intensity Aerobic Interval Exercise (INT), Time to Exhaustion (TTE), Heart Rate Variability (HRV), Sweat Rate (SR), Carbohydrate (CHO), Carbohydrate-Protein (CHO-P), Placebo (PLA), Masters Class Athletes (MCA's)

2.0 Objectives*

2.1 The purpose of this investigation is to examine the effects of carbohydrate vs. carbohydrate-protein co-ingestion vs. placebo on a subsequent bout of performance following high-intensity exercise and a 2-hour recovery period in Masters Class Athletes.

2.2 It is hypothesized that:

- Carbohydrate will improve subsequent performance significantly better than the placebo post-exercise in male Masters Class Athletes.
- Carbohydrate and protein will improve subsequent performance significantly better than PLA post-exercise in male Masters Class Athletes.
- Carbohydrate and Protein will not improve subsequent performance significantly better than CHO post-exercise in male Masters Class Athletes.

3.0 Background*

3.1 The rate of participation by masters class athletes (MCA's) in organized endurance and ultra-endurance (>6 hours) events has increased exponentially over the past 10-15 years (Lepers et al. 2013; Lepers and Stapley 2016). MCA's are generally classified as ≥35 years of age who meticulously train for and compete in organized sport (Ransdell et al., 2009; Lepers and Cattagni 2012; Lepers and Stapley 2016; Fien et al. 2017). Carbohydrate and protein are key nutritional factors that promote glycogen and protein synthesis, support recovery and allow for repetitive high-intensity efforts. Few studies, however, have examined short-term (2-6 hours) post-exercise recovery nutrition specific to MCA's (Doering et al. 2016a, 2017).

MCA's (cyclists) maintain weekly mileage of approximately 250 – 350 miles per week, with no significant difference between younger (35-44 years) and older (≥ 55) age groups. Endurance exercise at this level of competition requires prolonged high-intensity activity of ~70-90% VO_2max, which relies quite heavily on carbohydrate oxidation (Romijn et al. 1993; Spriet 2007). Competitive athletes rely on their ability to both store and mobilize glucose from muscle. The storage form of glucose in muscle is glycogen. Typical glycogen depleting protocols in studies that have examined the difference between carbohydrate (CHO) ingestion or carbohydrate-protein (CHO-P) co-ingestion have been running

or cycling to fatigue at 70% VO2max or intermittent exercise at 70%-90% VO2max. These protocols have been sufficient for depleting muscle glycogen content to ~100 – 200 mmol/kg/dw (Tsintzas et al. 1995; Jentjens et al. 2001; Alghannam et al. 2016; Dahl et al. 2020). Conversely, both CHO (1.2 g/kg) and CHO-P co-ingestion (0.8/0.4 g/kg) were found to be equally effective for promoting glycogen resynthesis during varying periods of acute recovery (3, 4, and 5 hours) (van Loon et al. 2000; Jentjens et al. 2001; Alghannam et al. 2016; Dahl et al. 2020).

Consuming carbohydrates immediately post-exercise has been shown to increase glycogen resynthesis rate by three times as compared to delaying carbohydrates for 2-hours (Ivy et al. 1988). Ivy et al. (1988) demonstrated the rate of glycogen synthesis to be 45% slower when delaying carbohydrate intake by 2 hours. Consequently, muscle glycogen concentration was also significantly less when carbohydrate intake was delayed by 2 hours following high-intensity intermittent exercise (Ivy et al. 1988). A similar pattern was shown for leg protein synthesis when a CHO-P supplement (10 g protein; 8 g carbohydrate; 3 g fat) was provided immediately post-exercise in comparison to 3 hours later (Levenhagen et al. 2001).

Two different isoenergetic options have been shown to effectively promote glycogen resynthesis post-exercise: CHO only (1.2 g/kg) or CHO-P co-ingestion (0.8 g/kg/0.4 g/kg). Several studies had demonstrated an equivalent increase in muscle glycogen content (van Loon et al. 2000; Jentjens et al. 2001; Alghannam et al. 2016) when these recovery beverages were consumed beginning immediately post-exercise and at 30-min intervals after that for 3, 4, and 5-hour recovery periods, or an increase in favor of CHO-P co-ingestion when consumed immediately-post and at 1-hour intervals during the first 2 hours (Berardi et al. 2006).

MCA’s may train twice per day, with limited time between bouts of exercise; therefore, an effective recovery strategy is paramount. On two occasions, CHO-P co-ingestion has been associated with either increased performance or an attenuated decrement to performance during a subsequent bout of endurance exercise in younger athletes (Berardi et al. 2008; Dahl et al. 2020). Appropriate post-exercise nutrient intake is vital for MCA’s as several studies have reported slower recovery from muscle-damaging exercise, even when moderate amounts of protein are consumed (Doering et al. 2016a, 2017).

Post-exercise nutrient intake in a group of Australian (endurance) MCA's was reported to be well below the suggested amount for CHO (~0.7 g/kg) and slightly below the recommended threshold for protein (~0.3 g/kg) (Doering et al. 2016b). Furthermore, the MCA's surveyed in this study demonstrated poor or no knowledge of post-exercise nutritional practices for CHO and protein intake (Doering et al. 2016b). It has been suggested that while endurance athletes are familiar with the need for carbohydrates in the diet, the importance of meeting total daily protein needs as well as the incorporation of protein within the immediate-post recovery period may be less well understood (Vitale and Getzin 2019). Inadequate nutrient intake in the acute post-exercise recovery period may compromise the rate of both glycogen and protein synthesis. On a cellular level, this may negatively affect skeletal muscle remodeling and glycogen storage, promoting fatigue and poor training outcomes and performance (Ivy et al. 1988; Breen et al. 2011; Kruseman et al. 2020).

In conclusion, the rate of MCA participation in elite-level endurance competition is exponentially increasing. In contrast, the level of nutritional knowledge and recovery practices was reportedly poor in one group of surveyed endurance MCA's (endurance) athletes. Additionally, a critical gap in post-exercise recovery research exists specific to MCA's. Even less research exists specific to the MCA and short-term (2 hours or less) recovery. This is a problem as slower recovery rates between subsequent bouts of exercise have been demonstrated, and higher protein needs post-exercise have been indicated to promote muscle remodeling. A unique opportunity exists to examine CHO-P co-ingestion in a 2-hour recovery period to promote optimization of post-exercise glycogen resynthesis and protein remodeling to support successive bouts of training or competition.

4.0 Study Endpoints*

4.1 The study will be completed when participants have finished all visits to the laboratory. It is anticipated that the total testing for each individual should be completed within 14 days or 3-4 total visits.

5.0 Study Intervention/Investigational Agent

1.1 Participants will consume either a CHO beverage (1.2 g/kg of bw), a CHO-P beverage (0.8 g/kg bw CHO + 0.4 g/kg bw protein), or PLA beverage matched for taste and appearance between exercise bouts to elucidate which drink moderates

performance decrements most effectively. The dietary source of CHO will be Gatorade®, the PLA will be Gatorade® G Zero, and the protein will be provided via BiPro Elite whey protein isolate.

6.0 Procedures Involved*

6.1 To test the hypothesis, this study will utilize a randomized, double-blind, placebo-controlled between-subject design to examine the effects of CHO and CHO-P supplementation on short-term recovery following aerobic interval exercise and time to exhaustion testing in MCA's. After completing the informed consent and screening process (Visit #1), each participant will be assessed for their anthropometric measures as well as body composition and will complete a GXT to volitional exhaustion on a cycle ergometer to determine VO_2peak. Participants will also be provided with the option to return to the lab on a separate date for the GXT and VO_2peak testing. Forty-eight to 72 hours after Visit #1, or VO_2peak testing, participants will return to the lab for familiarization with the INT exercise protocol and TTE (Visit #2). Within five to nine days after familiarization (Visit #2), participants will return to the lab for Visit #3 to complete INT exercise and TTE, a 2-hour passive recovery period including the consumption of one of three treatments (CHO, CHO-P, PLA), followed by repeated INT exercise and TTE, HRV and SR determination. Participants will also complete the Nutritional Recovery Practices, Knowledge, and Beliefs of Australian Triathletes survey during the 2-hour recovery period. Participants will be asked to complete the ASA24®, an automated self-administered 24-hour dietary assessment tool during the 5-9-day period between familiarization (Visit #2) and the experimental protocol (Visit #3).

Testing Procedures.

- Participants will report to the testing location on 3 separate occasions.
- Visit #1: The study will be explained to the participant by the investigators. Participants will be asked to provide their written, informed consent. The participants will then be asked to complete the American College of Sports Medicine Exercise Preparticipation Health Screening Questionnaire for Exercise Professionals (PHSQEP), Physical Activity Readiness Questionnaire for Everyone (PAR-Q+), and Medical Health and Activity Questionnaire.

Following the written consent and screening process, anthropometrics and body composition will be measured, and the VO_2peak test outlined in section 6.3 will be completed. Following informed consent, screening, and anthropometrics and body composition, if necessary (i.e., due to time constraints), participants may choose to return to the lab on a separate occasion for VO_2peak testing.

- Visit #2: Participants will report to the testing location for familiarization to the testing protocols. Based on VO2peak data obtained during Visit #1, participants will perform the INT and TTE protocol outlined in section 6.4 to familiarize them with the protocol for the experimental trial (Visit #3), and teach them how to respond to the rating of perceived exertion RPE measures.
- Visit #3: To ensure participants are hydrated prior to the start of testing, they will be asked to consume 500 ml of water 2 hours before their arrival to the laboratory for testing (Moreno et al. 2013; Vanderlei et al. 2015). Participants will complete a warm-up followed by the INT and TTE protocol, as outlined in section 6.4. Immediately following the INT and TTE testing, the participant will be given an isocaloric CHO (1.2 g/kg of bw), CHO-P (0.8 g/kg bw CHO + 0.4 g/kg bw protein), or PLA beverage that have a similar appearance and taste and will be blinded to the contents. Participants will rest for two hours and then repeat the INT and TTE testing (6.4). Participants will be weighed in their cycling bib shorts immediately before and after the first and second bouts of INT exercise and TTE testing. Sweat rate will be determined based on the difference between pre-and post-weight changes for each bout of exercise, the amount of fluid consumed during the recovery period, and total exercise time in minutes (Casa 2019). Participants will also be asked to report total sleep in number of hours and minutes for the night prior to testing. Immediately upon completion of the second exercise bout, participants will rest for an additional 30 minutes for HRV recording. Completion of Visit #3 will conclude all required testing.

6.2 Anthropometric measurements:

- Participants will be assessed for body weight, height, and body composition.

- Height will be assessed using a stadiometer (Health-o-meter Professional Patient Weighing Scale, Model 500 KL, Pelstar, Alsip, IL, USA), and body composition and weight will be assessed using Bio-electrical Impedance Analysis (InBody 770, Biospace Co, Ltd. Seoul, Korea). Participants will be asked to remove any jewelry, their footwear, including socks, and wear only light athletic attire. Then they will be asked to stand on a platform while holding two handles out to the side. They will hold this position as the bioelectrical impedance analysis sends a minute electrical current (that is safe and cannot be seen or felt) through the body, to determine body composition. There are no risks or discomforts associated with the use of bioelectrical impedance analysis. Participants should be two-hours fasted for this assessment.

6.3 Determination of VO_2peak

- All participants will perform a graded exercise test (GXT) to volitional exhaustion on a cycle ergometer (Lode, Corival cpet, Groningen, The Netherlands) to determine VO_2peak and peak power output. Prior to testing, each participant will be provided with a Polar heart rate monitor (chest strap and sensor; Polar H10, Polar Electro Oy, Kempele, Finland) to record heart rate. Participants will be asked to place the heart rate monitor just below the sternum. Participants will complete a five-minute warm-up on the cycle ergometer at a self-selected intensity and cadence. The test will consist of 2-minute stages, beginning at an initial workload of 50 watts (W), then 100W, then 150W followed by an increases of 30W every 2 minutes until the participants can no longer maintain 60 revolutions per minute (rpm) (Beltz et al. 2016; McCarthy and Spriet 2019). VO_2peak will be determined as the highest peak value achieved during the last completed stage of the test, and if it coincides with at least two of the following three parameters: heart rate (HR) within 10% of age-predicted maximal HR; respiratory exchange ratio (RER) of 1.15 or higher; a plateau in oxygen consumption despite an increase in exercise intensity. Open-circuit spirometry will be used to estimate VO2peak ($l \cdot min^{-1}$) with a calibrated metabolic cart (True One 2400® Metabolic Measurement System, Parvo-Medics Inc., Sandy, UT) by sampling and analyzing the breath-by-breath

expired gases. The metabolic cart software continuously records ventilation and expired gases, with averages every 15s, calculates VO_2, and determines the VO_2peak value. The highest power output achieved will be recorded as peak power output (PPO) in watts.

6.4 High-Intensity Aerobic Intervals and Time to Exhaustion

- All participants will warm-up on a cycle ergometer for 3-minutes at 50W, 2-minutes at 100W, and 1-minute at 75W followed by a 3-minute rest. Participants will then perform 5 x 4-minute high-intensity aerobic intervals at 70-80% of individual PPO, as determined by the familiarization trial. Two-minutes of low-intensity cycling at 50W will separate intervals. Immediately following the fifth interval, participants will cycle at a work rate (watts) that corresponds to 90% PPO until volitional exhaustion or when rpm falls below 60 for 10 seconds. Following the endurance trial (INT and TTE), participants will cycle with no resistance for 5 minutes to cool down. Revolutions per minute will be the only performance measure visible to participants, and minimal verbal encouragement will be provided throughout the aerobic intervals and time to exhaustion. Rating of perceived exertion (RPE) will be recorded using the Borg 10 scale during the last 30 seconds of each aerobic and rest interval and once immediately before completion of the TTE.

6.5 Recovery Slope of Heart Rate Variability

- R-R intervals recorded via the heart rate monitor and Elite HRV will be downloaded and analyzed using a laptop with commercially available HRV analysis software (Kempele, Finland; Kubios HRV Analysis v 3.3, Kuopio, Finland). R–R interval series will be filtered using the software's automatic artifact correction to detect and replace artifacts from the normal sinus arrhythmia. Replaced R–R periods will not exceed 5% of total heartbeats. Each window will be analyzed for the time-domain values of root mean square of the successive differences of R–R intervals (RMSSD). RMSSD reactivation following exercise will be reported to behave linearly during the first 30-minutes post-exercise (Orellana et al. 2019). Therefore, RMSSD values will be recorded during the 90% TTE and then post-exercise every 5 min. Using

simple linear regression, a slope value for RMSSD values vs. time of recording (end of TTE, 5min, 10min, 15min, 20min, 25min, 30min) for each participant will be performed.

6.6 Dietary Analysis

- To understand if MCA are meeting carbohydrate and protein intake recommendations, participants will be asked to complete the ASA24®. The ASA24® is a validated, automated self-administered 24-hour dietary assessment tool developed by the National Cancer Institute (Bethesda, Maryland) (Park et al. 2018). Each participant will be instructed on the use of the ASA24® during Visit #1, either by a registered dietitian or a trained volunteer research assistant. Participants will be asked to report a typical daily intake for days that they train. Following completion of VO_2peak testing participants will be emailed a sample of a detailed diet recall and an individual username and password to access the ASA24® system. They will be asked to report a 24-hour intake that is typical for days that they train. The sample diet recall will demonstrate a level of detail that includes all foods and fluids consumed upon waking until bedtime, including foods and/or supplements consumed pre, during, and immediately post-exercise. Participants will be asked to complete the electronic diet recall during the 5-9-day period between familiarization (Visit #2) and the experimental protocol (Visit #3). After data collection, the dietary intake will also be analyzed for total energy (kilocalorie [kcal]) and macronutrient distribution (carbohydrate, protein, and fat). An Excel spreadsheet of each participant's macro-and micronutrient analysis will be downloaded from the ASA24® website.

6.7 Postexercise Nutritional Practices Survey

- The Nutritional Recovery Practices, Knowledge and Beliefs of Australian Triathletes survey is an instrument previously designed to assess knowledge of postexercise nutritional recommendations and recovery practices in endurance athletes (Doering et al. 2016b*)*. Participants will complete the survey electronically during the 2-hour recovery period (Visit #3). Masters class athletes in this study will be asked to complete a series of 34 total questions assessing their postexercise nutrition knowledge and recovery

practices. Questions 1 through 10 will be omitted as the current study is not specific to triathletes.

6.8 Outcome Variables

- VO_2peak Test
  - VO_2peak
  - Peak Power
- INT and TTE Protocol
  - Peak Power
  - Average Power
  - RPE
  - Total time to exhaustion at 90% VO_2peak
- Anthropometrics
  - Height
  - Weight
  - Age
  - Body Fat Percentage
- HRV
  - Root mean square of the successive differences of R–R intervals (RMSSD)
- Sweat Rate Determination
  - Individual sweat rate pre-post exercise

7.0 Data and Specimen Banking*

7.1 Data Storage.

All informed consents, medical history questionnaires and screening paperwork will be stored in a locked cabinet in the Physiology of Work and Exercise Response (POWER) Laboratory, and data collection sheets will be stored in a locked cabinet in the Kinesiology and Teaching Laboratory during and following the investigation; all electronically entered data will be saved in an encrypted file. Data from VO_2peak tests and performance tests will be collected and managed using Trueone 2400 software on a secure computer. The computer is password-protected, and the data stored is deidentified and password protected.

Participant folders will be marked with an ID number to protect against a breach of confidentiality; participant names

and ID numbers will be stored separately in a digitally encrypted file.

7.2 Data Access.

Access to research-related data, paperwork, and records will be limited to appropriate laboratory personnel only.

7.3 Data disposal.

All participant records and paperwork (e.g., informed consent forms, data collection sheets) will be destroyed six years from the end of the study. Password protected electronic file containing participants' names will be discarded after the completion of data analysis.

7.4 Data dissemination

The results of this study will be published as a group as part of a scientific publication. No individual results will be published or shared with any third person or party.

8.0 Sharing of Results with Subjects*

8.1 Individual results will remain confidential and will only be relayed to the participant upon request. All information attained from the medical history questionnaire will be held in strict confidence.

9.0 Study Timelines*

9.1 Describe:

- All visits will be separated by 48-72 hours. Visit #1 and Visit #2 will take approximately 60-90 min each. Visit #3 will take approximately 4 hours.
- We anticipate the participant recruitment and enrollment will be completed within two months and estimate that data collection will be completed within approximately 6 to 8 months.
- The estimated date for completion of primary analysis will be December 2021.

10.0 Inclusion and Exclusion Criteria*

10.1 Individuals will be screened for eligibility using the American College of Sports Medicine Exercise Preparticipation Health Screening Questionnaire for Exercise Professionals (PHSQEP), Physical Activity Readiness Questionnaire for Everyone (PAR-Q+), and confidential Medical Health and Activity Questionnaire to determine the need for medical clearance from a health care professional. Participants presenting

any sign or symptoms suggestive of cardiovascular, metabolic, or renal disease will be asked to seek medical clearance in order to participate.

10.2 Inclusion Criteria.

- Men between the ages of 35 and 59 years
- Free of any physical limitations as determined by the Exercise Preparticipation Health Screening Questionnaire for Exercise Professionals (PHSQEP), and a Physical Activity Readiness Questionnaire for Everyone (PAR-Q+)
- Regularly engaged in endurance exercise (running, cycling, swimming) for a minimum of three years, a weekly training volume of 5-10 hours, and a maximal oxygen uptake of 45.00 $ml \cdot kg^{-1} \cdot min^{-1}$ or higher as determined by completion of VO_2peak assessment (Visit #1).

10.3 Exclusion Criteria

- Recent musculoskeletal injuries or surgeries
- Any chronic illness that requires continuous medical care
- Current incarceration, cognitive impairment or inability to provide consent
- Present or past use of performance-enhancing drugs

10.4 Indicate specifically whether you will include or exclude each of the following special populations:

- Adults unable to consent: exclude
- Individuals who are not yet adults (infants, children, teenagers): exclude
- Pregnant women: exclude
- Prisoners: exclude

11.0 Vulnerable Populations*

11.1 N/A: This research does not involve any vulnerable populations.

- No pregnant women will be included.
- No prisoners will be included.
- No children will be included.
- No cognitively Impaired Adults will be included.

12.0 Local Number of Subjects

12.1 All participants will be recruited from the University of Central Florida and from within the surrounding Central Florida area.

12.2 Participants will be recruited and screened until a maximum of 50 individuals are enrolled. Participants will be considered enrolled only after an informed consent document has been signed, a medical history questionnaire, PHSQEP and PAR-Q+ have been completed, and anthropometric data have been recorded. To account for individuals who do not meet the inclusion criteria, the number of recruited individuals will likely exceed the number of enrolled participants. However, no more than 50 participants will be recruited.

13.0 Recruitment Methods

13.1 Participants will be recruited at the University of Central Florida and in surrounding locations through flyers, posters, information sheets, notices, and internet postings and media to recruit participants. The flyer will be posted on social media. Participant recruitment will begin upon approval of the IRB protocol and all supporting documents.

13.2 Subjects will primarily be recruited from the University of Central Florida and surrounding locations. Participants will be eligible to participate if they hear about the study through flyers, posters, information sheets, notices, internet postings, and/or media, and word of mouth.

13.3 Participants will be recruited by flyers, posters, information sheets, notices, and internet postings, which will detail the study objectives and a brief explanation of the inclusion and exclusion criteria.

13.4 There will not be any compensation to participants for participating in this study

14.0 Withdrawal of Subjects*

14.1 Participants have the right to discontinue participation without penalty, regardless of their status in the study. Participants will also be instructed that participation in the study may be terminated at any time by the researchers in charge of the project. Possible reasons for subject withdrawal without their consent include inability or unwillingness to adhere to the study protocol, failure to adhere to any requirements (including exercising vigorously within the 24-hours prior to the testing visit), and refusal to participate in testing trials

14.2 If a participant is asked to withdraw without their consent, they will be told that they can no longer participate in the study and the reasoning for this will be explained privately. Additionally, study personnel will explain the destruction of their data to them.

14.3 If a participant is asked to withdraw or voluntarily withdraws from the study, their data will be removed from the study results and destroyed.

15.0 Risks to Subjects*

15.1 The exercise-based assessments carry the same inherent risks as participating in any physical activity, such as muscle soreness, and fatigue and possibly muscle strains, and/or joint sprains, elevated heart rate, feelings of nausea and possible episodes of emesis, arrhythmia, myocardial infarction, and death. To minimize these risks, participants will be instructed on the appropriate technique for the performance assessments and will be required to complete a warm-up prior to completing the assessments. Furthermore, participants will be informed that they can quit at any time before or during the test. All personnel involved in this study are CPR certified. Furthermore, all research personnel involved in data collection are experienced in the administration of the proposed assessments, and all Doctoral students involved in this study are Certified Strength and Conditioning Specialists through the National Strength and Conditioning Association. Participants will be instructed to immediately stop and report any injury or discomfort associated with the performance assessments to a member of the investigative team. The extent of the injury/discomfort, as well as the ability of the participant to continue with the study, will be subsequently be determined by the investigative team. If it is deemed that the discomfort/injury will prevent the participant from completing the study, or if the injury/discomfort may be exacerbated by further participation in the study, the investigative team will suspend the individual's participation in the study.

15.2 This study is recruiting for male participants only. Therefore, subjects who are pregnant will not be enrolled.

15.3 There are no risks to others in the study who are not participants.

16.0 Potential Benefits to Subjects*

16.1 There is no direct benefit of participation in the study.

17.0 Data Management* and Confidentiality

17.1 All statistical analyses will be conducted via the Statistical Package for Social Science (SPSS) software for Windows version 21 (SPSS Inc., Chicago, IL). Data will be statistically analyzed using separate 1-way analysis of covariance (ANCOVA) for TTE, HRV-Slope Recovery, and Sweat Rate measures. The pretest and

the posttest values will be used as a covariate and dependent variable, respectively. If the ANCOVA is significant, Fisher's LSD Post-Hoc analysis will be used to determine group differences. For effect size (ES), the partial eta squared statistic will be reported, with 0.01, 0.06, and 0.14 representing small, medium, and large ES, respectively. The significance level will be set at $p \leq 0.05$.

17.2 The calculation of sample size (G*Power 3.1.9.4) was based on pilot data in low-to-moderately trained young men (22.1 + 3.2 years) collected in our lab using the same protocol (90% PPO TTE) as outlined in this study. The total sample size was determined to be a total of 18 (6 in each group) based on a one-way ANCOVA, power of 0.90, and an alpha of 0.05. However, due to potential dropout rates, a total of 30 participants (10 per group) will be recruited with the goal of completing 18.

17.3 Individual results will remain confidential and only be related to the participant upon request once the study is completed. All questionnaires and data collection sheets will be kept in a locked cabinet during and following the study. Data that is electronically stored will be kept on a password-protected computer in the office of the investigator who oversees data collection. All information will be destroyed six years from the end of the study. Participant folders will be marked with an I.D. number to protect against a breach of confidentiality. Participant names and I.D. numbers will be stored apart from the subject folders in a password protected electronic file.

17.4 Quality control of collected data will be performed by ensuring all data files remain in filing cabinets when not in use.

17.5 Participant folders and other data will be kept in a locked filing cabinet and stored on a computer whose files are encrypted with a password. Further, all identifiable information will be stored separately from any data collection sheets; Computer file containing participants' names will be discarded upon completion of data analysis. Only study personnel will have access to data. All signed consent forms will be stored separately from the rest of the data and will be held for a minimum of 5 years after completion of the study.

18.0 Provisions to Monitor the Data to Ensure the Safety of Subjects*

This section is required only when research involves more than Minimal Risk to subjects

18.1 Participants will be instructed to immediately stop and report any injury, discomfort, or concern associated with the

performance assessments. Any new information or adverse event will be reported to the IRB. Safety data will be reviewed by the study investigators at the time of any reported incidents. The investigator in charge of data collection will consult with the principal investigator in case of injury or concern raised by the participants.

19.0 Provisions to Protect the Privacy Interests of Subjects

19.1 Participant privacy will be protected at all times. All data collection processing pertaining to this study will be solely conducted by its own personnel. No other persons will have access to the previously mentioned facilities during data collection and processing. Participants will be provided locker room access to change into their attire if needed.

19.2 All consent forms will be completed privately in a small office at the University of Central Florida. All performance assessments and testing sessions will be conducted in the Kinesiology Laboratories. Only the primary investigators on this study will have access to any personal information which will be stored privately in a locked file cabinet. All assessments will take place in a laboratory that is not accessible to the general public.

19.3 Only the approved principal investigator and co-investigators will be permitted to access any sources of information about the subjects, which will be in locked cabinets and password-protected computers and files.

20.0 Compensation for Research-Related Injury

20.1 Participants are instructed to report any discomforts related to the study to the principal investigator. If immediate assistance is needed, it will be provided via the emergency medical system. For non-emergency discomforts, participants must seek their own physician for medical attention. Adverse events/side effects will be reported to the IRB immediately upon notification.

21.0 Economic Burden to Subjects

21.1 Participants will be responsible for providing their own means of transportation and parking to and from the Kinesiology Laboratories at the University of Central Florida for testing sessions.

22.0 Consent Process

This study will require a written form of consent for subject participation

- The consent process will take place in a private room at the University of Central Florida.
- All participants will be provided with the time necessary to read all documents, and an investigator will be available to explain the study protocol and answer any questions that each potential participant may have.
- Ongoing consent will be obtained by verbal agreement of the participant to come in for future testing sessions.
- This study will utilize "SOP: Informed Consent Process for Research (HRP-090)".

Include if there are Non-English Speaking Subjects, otherwise delete.

- N/A: Participants who only speak languages other than English will not be enrolled.

Include if you are requesting Waiver or Alteration of Consent Process (consent will not be obtained, required information will not be disclosed, or the research involves deception), otherwise delete.

- N/A: A waiver or alteration of consent process will not be used

Include if you are enrolling subjects who are not yet adults (infants, children, teenagers), otherwise delete.

- N/A: Subjects who have not attained the legal age for consent to treatments or procedures involved in research will not be enrolled.

Include if you are enrolling Cognitively Impaired Adults, otherwise delete.

- N/A: Adults who are cognitively-impaired will not be enrolled.

Include if you are enrolling Adults Unable to Consent, otherwise delete

- N/A: Adults unable to consent will not be enrolled.

23.0 Process to Document Consent in Writing

23.1 This study will be following the guidelines of "SOP: Written Documentation of Consent (HRP-091).

23.2 This research requires written documentation of consent.

23.3 Consent form attached using "CHECKLIST: Waiver of Written Documentation of Consent (HRP-411)."

24.0 Setting

24.1 All testing procedures will take place at the University of Central Florida in the Physiology of Work & Exercise Response Laboratory in the Education Complex.

- The research team will identify and recruit potential subjects at the University of Central Florida and in surrounding locations through Advertisements, flyers, posters, notices, internet postings, and/or media.
- All research procedures will be performed at the University of Central Florida in the Education Complex.
- There will not be any involvement of a community advisory board.
- No research will be conducted outside of the University of Central Florida.

25.0 Resources Available

25.1 All research personnel are currently certified in adult cardiopulmonary resuscitation (CPR) and automated external defibrillator (AED) use. An AED is located outside of the Kinesiology Laboratories within the Education Building. All the assessments will be monitored by a Certified Strength and Conditioning Specialist (National Strength and Conditioning Association).

25.2 Our research team is experienced and has strong expertise in similar types of research projects. As collaborating researchers in UCF's School of Kinesiology and Physical Therapy, our team has published dozens of peer-reviewed research articles covering areas of nutritional supplementation, exercise, and physical performance involving subjects of varying ages, training levels, and health statuses.

25.3 The Kinesiology laboratories are research spaces featuring some of the latest technologies and instrumentation to evaluate human performance on many levels, ranging from physical functioning of the whole body to molecular and signaling pathways underlying such functioning. Beyond our exercise equipment to assess muscular strength and endurance, our facility is also equipped with instruments designed to assess body composition and non-invasive, in vivo imaging of the

musculoskeletal system and vasculature. Additionally, our laboratory features devices used to evaluate the visual-motor function and motor skills, electrical activity of muscles, and energy expenditure. This facility is conducive to exercise training and supervision.

25.4 Prior to data collection and participant recruitment, all personnel involved in the investigation will be provided with copies of all study materials for review. All personnel will meet to discuss the study protocol, research procedures, and individual duties. Additionally, all persons assisting with the research will be appropriately trained in laboratory safety and the treatment of human subjects. Besides interacting with human subjects, data analysis and preparation will occur on equipment in one of the Kinesiology Laboratories and the study investigators' secured offices.

26.0 Multi-Site Research*

N/A.

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