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The Effects of Acute Exercise of Plasma Volume Expansion in Men – Variations of the Intensity/Duration Relationship

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THE EFFECTS OF ACUTE EXERCISE ON PLASMA VOLUME EXPANSION IN MEN – VARIATIONS OF THE INTENSITY/DURATION RELATIONSHIP

by

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THE EFFECTS OF ACUTE EXERCISE ON PLASMA VOLUME EXPANSION IN MEN – VARIATIONS OF THE INTENSITY/DURATION RELATIONSHIP

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The purpose of this study was to examine the exercise variables of intensity and duration in relation to PV expansion during an acute bout of exercise, when the total amount of work performed was kept constant. Six male subjects completed a maximal graded exercise test and three two-day experimental trials. During the experimental trials, each subject performed one of three exercise protocols on a cycle ergometer: 50% VO_{2peak} (60 minutes), 65% VO_{2peak} (45 minutes), and 80% VO_{2peak} (30 minutes). Measurements of hematocrit, hemoglobin, and serum albumin and total protein concentrations were obtained before exercise (BASE), immediately post-exercise (Post-EX), 2-hours post-exercise (2-hr REC), and 24-hours post-exercise (24-hr REC). Although none of the protocols resulted in PV expansion 24-hr post-exercise, the highest intensity/shortest duration protocol (80% VO_{2peak}) resulted in the greatest recovery in PV after the post-exercise decline and the 65% and 80% VO_{2peak} protocols resulted in the greatest increase in serum albumin concentration from Post-EX to 24-hr REC. This data suggests that higher intensity exercise may result in a more beneficial PV response compared to lower intensity exercise even when the total amount of work is kept constant.

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CHAPTER I

INTRODUCTION

Chronic endurance training (3,4,6,12,23,27) and acute exercise sessions (11,13,19) have been shown to result in the expansion of plasma volume (PV). PV expansion typically occurs within 24 hours of a given acute exercise bout (2,10,11,13,19) and becomes a chronic adaptation during progressive endurance training exposure (3,4,6,9,16,20). The magnitude of PV expansion depends on the duration, intensity, frequency, and mode of exercise performed and has been reported to range from 9 to 25% (2,4,6,7,10,11,12,20,23,27).

During exercise, there is an acute decrease in PV (7,8) and this decrease is primarily caused by an increase in water loss due to sweating and an increase in plasma leakage from the blood vessels due to an increase in hydrostatic pressure (15,16,17). The body attempts to correct this acute decrease in PV through a variety of mechanisms. The body initiates a physiological response almost immediately upon finishing exercise via an auto-restoration mechanism (2,10,13). Post-exercise, sodium retention by the body may increase resulting in a decrease in water loss from the body (3,5). The body also increases synthesis of the plasma protein albumin, which contributes to the retention of water in the vascular space (3,11,25,30). A resulting fluid shift of body water from the interstitial space and other fluid compartments to the vascular space in part occurs due to elevations in plasma albumin content, in turn causing the subsequent fluid-flux (3,11,25,30). Thus, the PV expansion and shift of body water accompany the translocation and mobilization of plasma albumin from the interstitial space into the intravascular compartment. The end result of these mechanisms is an increase in water

retention by the body and the restoration or increase of PV approximately 24 hours post-exercise (3,4,6,11,12,13,19,23,27). The exact signal which prompts the body to begin to restore the PV post-exercise is not entirely understood, but it has been suggested that post-exercise hypotension may be an important signal (15,16,17). This post-exercise hypotension has been shown to exist anywhere from 2 to 24 hours (16). The post-exercise hypotension may act as a signal to the body to increase water retention, and thus PV, in hopes of restoring blood pressure to normal values. It has also been suggested that the observed increases in albumin content and synthesis after intense exercise (11,25,30) can help contribute to a retention of fluid and reduction of free water clearance within the body.

PV expansion may be one of the most beneficial adaptations to exercise. PV expansion is advantageous for thermoregulation during endurance exercise and the proper flow of blood throughout the circulatory system (2,10,20,26). Less viscous blood, resulting from a greater PV, may reduce stress on the heart and arterial walls during moderate to high intensity exercise. During exercise, the cardiovascular system is responsible for the delivery of oxygen and substrates to working muscle and the delivery of blood to the skin for thermoregulation. An increase in plasma volume may better serve to optimize blood flow for these competing mechanisms (2,10,20,26). In addition, a greater PV may result in a more efficient sweating response thus allowing greater evaporation and cooling of the body in an attempt to maintain body temperature homeostasis during exercise (2,26). A decline in blood volume via water loss may also be detrimental to performance due to a decline in central venous pressure, stroke volume, and potentially cardiac output. A greater level of PV may assist in maintaining heart rate

and stroke volume during prolonged exercise, thus delaying the onset of cardiovascular drift while more adequately maintaining cardiac output and mean arterial blood pressure.

Previous studies have utilized a variety of exercise protocols to elicit PV expansion. Many initial studies that examined training adaptations and PV expansion utilized protocols that had subjects ride on a cycle ergometer for 2 hours per day for three to eight consecutive days (3,4,6,15,22,27) or an even more extended length of five to six sessions per week for eight weeks (12). These investigations studied the physiological responses involved with acute and chronic endurance protocols and training. Other studies reviewed adaptations to shorter six minute exercise periods at various intensities (5) and supramaximal exercise performance with high intensity bouts alternated with recovery intervals (13). An example of a protocol commonly used by researchers in more recent and subsequent studies included sessions in which subjects would exercise on a cycle ergometer for four minutes at approximately 75–85% of maximal oxygen consumption, and then pedal at a recovery cadence for five minutes (11,19,25,30). This cycle is then repeated eight times. Although this protocol is usually successful in eliciting an expansion of PV, it is a difficult protocol that the average individual would typically not use in his or her training for general health and fitness purposes. Other protocols have used more continuous exercise workloads for fairly extended durations at a moderately high intensity (~65%) to elicit an expansion of PV (3,4,6,15,17,22,27).

Various researchers have alluded to exercise intensity appearing to be the most important exercise factor that influences the expansion of PV (2,3,5,6,13). However, researchers have suggested that different stimuli variations and factors including intensity, frequency, duration, and mode of exercise may also influence the magnitude of

PV expansion (2,10,20). The conclusions from these studies have alluded to the suggestion for further investigations in order to determine which combinations of exercise intensity, duration, and frequency would likely produce the maximal and most beneficial PV expansion (2,3). The critical factor would be to take into account total work performed while keeping the exercise sessions appropriate and applicable for the average individual desiring health and fitness benefits.

The rationale for intensity being the most important exercise variable stimulus for PV expansion enhancements is twofold: the physiological response and actual data comparisons from that of previous studies. A higher exercise intensity more effectively changes osmolality, thus causing a greater elevation of plasma renin activity and arginine vasopressin concentration within the blood. Such a response in turn facilitates the necessary sodium and water retention during and following exercise (3,5,6,13). In conjunction, a chronic increase in plasma total protein content, specifically albumin synthesis (a "carrier" protein) provides an increased water binding capacity for the blood (3,11). One study in particular by Green et al. reported a larger PV expansion with shorter duration, high intensity exercise in comparison to previous investigations with longer duration and lower intensity exercise bouts (13). Based upon the data observed by the researchers, this theory appeared to be well supported in regards to situations where total work output remained constant in a given exercise session. Their protocol required subjects to cycle during sessions of intermittent work performed as 1-min work to 4-min rest intervals until fatigue or until a maximum of 24 repetitions were completed. The 1-min intense repetition was training at supramaximal training performed at 120% maximal oxygen consumption. The authors indicated that "intensity may be the single most

important factor in inducing chronic hypervolemia" and "that exercise intensity appears to be a potent stimulus for accelerating the rate at which a chronic expansion of PV occurs" (13).

To our knowledge, no previous studies have examined the effects of varying both the intensity and duration of exercise, while keeping the total work performed constant, and how these factors might affect PV expansion after exercise. In addition, the exercise protocols that were utilized in the present study are similar to exercise recommendations made by the American College of Sports Medicine (ACSM) (1) and may be more applicable to the average individual during his or her exercise training for health and fitness purposes.

Thus, the purpose of this investigation was to examine the exercise variables of intensity and duration in relation to PV expansion during an acute bout of exercise, when the total amount of work performed was kept constant for each subject during the given protocols. It was hypothesized that, of the three protocols utilized, the protocol employing the highest intensity and shortest duration of exercise would result in the greatest level of PV expansion and the greatest increases in albumin and total protein concentrations.

CHAPTER II

RESEARCH DESIGN AND METHODS

Subjects

Six male subjects were recruited for this study. Subject characteristics are listed in Table 1. All of the subjects were healthy and free of disease, recreationally active, free from supplements and ergogenic aid use, and were placed within the "Low Risk" category for exercise based upon the ACSM guidelines (1). The subjects were required to visit the laboratory eight times during the study. The first visit to the laboratory was an orientation session, the second visit was a maximal graded exercise test, and the final six visits were the three two-day experimental trials. Subjects were instructed to arrive at the laboratory dressed in a T-shirt, shorts, and athletic shoes. The behavioral guidelines for the study required subjects to abstain from exercise and to not ingest alcohol and/or caffeine on the day before and the day of their visits to the laboratory for the experimental trials. In addition, each subject was instructed to ingest a minimum fluid intake of: 15 mL·kg bodyweight^{-1} the night before Day 1 and Day 2, 8 mL·kg bodyweight^{-1} the morning of Day 1 and Day 2, and 10 mL·kg bodyweight^{-1} during the afternoon of Day 1 of the experimental trials (11,30). The order of the experimental trials was counter-balanced and at least 7 days separated each of the three two-day trials. All testing was conducted in the Exercise Physiology Laboratory at Western Michigan University at a temperature of 23° C (71° F). The research study was approved by the Human Subjects Institutional Review Board at Western Michigan University.

Table 1. Subject Characteristics (M ± SD)

Variable	N = 6
Age (yrs)	24.6 ± 4.8
Body Weight (kg)	78.9 ± 10.1
Height (cm)	177.9 ± 7.3
Body Fat (%)	13.2 ± 7.6
VO_{2peak} ($L \cdot min^{-1}$)	2.894 ± 0.246
VO_{2peak} ($mL \cdot kg^{-1} \cdot min^{-1}$)	37.1 ± 4.98
HR_{max} ($b \cdot min^{-1}$)	184 ± 7

Research Procedures

Orientation Procedures

The first visit to the laboratory was for an orientation session at which time each subject became familiarized with the study procedures, a health history questionnaire was completed, and informed consent was obtained. The subject underwent an assessment of anthropometrical measurements including height and weight (Health-o-meter, Perspective Enterprises Inc., Kalamazoo, MI), and skinfold thickness (Lange Skinfold Caliper, Cambridge Scientific Industries Inc., Cambridge, MD). The Jackson & Pollock 7-site (chest, abdomen, thigh, subscapular, suprailliac, triceps, midaxillary) equation was used to calculate body density from the skinfold assessment (18) and the Siri equation was used to calculate percent body fat from body density (28).

Maximal Graded Exercise Test

Upon arrival to the laboratory for the maximal graded exercise test, each subject's body weight was measured. The subject was then be fitted for seat height on the cycle ergometer so that the subject's knee was within 5–10° of flexion at the pedal's lowest point.

Each subject was then fitted with a noseclip and a mouthpiece for the collection of expired respiratory gases using the metabolic measurement cart (Vmax 229 Series, Sensor Medics Corporation, Yorba Linda, CA). Prior to all tests, the metabolic cart was calibrated using a 3-L syringe and gases of known concentration.

Once instrumentation of the subject was completed, the exercise test began. Each subject performed the exercise test on a magnetically-braked cycle ergometer (Bosch ERG 551, Robert Bosch GmbH, Berlin, Germany). The exercise test protocol consisted

of a 2-min warm-up stage at 60 Watts after which exercise intensity was increased 20 Watts every minute. The exercise test continued until the subject reached volitional fatigue or was unable to maintain a pedaling frequency of 50 revolutions per minute (RPM). Once the exercise test protocol was terminated, each subject continued to cycle at a very low intensity for approximately five minutes as an active cool-down.

During the exercise test protocol, expired respiratory gases were measured continuously using the metabolic measurement cart in mixing chamber mode with values being reported as 30-sec averages, heart rate was measured continuously using a telemetry heart rate monitor (GO Heart Rate Monitor, Cardiosport/Sports Beat, Deer Par, NY), and rating of perceived exertion (RPE) was assessed during the last 10 seconds of each exercise stage. Peak oxygen consumption (VO_{2peak}) was defined as the greatest 30-sec average during the test.

Experimental Trials

Each subject visited the laboratory for three two-day experimental trials. The order of the experimental trials was counter-balanced and at least 7 days separated each of the three two-day trials.

Upon arrival to the laboratory on Day 1 of the experimental trials, each subject had his body weight measured post-void. The subject also had a heart rate monitor secured around his chest. A catheter (20G 1 ¼" ProtectIV Plus, Medex, Inc., Carlsbad, CA) was then inserted into one of the antecubital veins for subsequent blood sampling. The subject then began the baseline period consisting of 60 minutes of seated rest (BASE). Heart rate and blood pressure measurements were obtained at the end of BASE. Blood pressure (Standby Baumanometer, W. A. Baum, Co. Inc., Copiague, NY) was

taken in duplicate with a cuff that was secured on the arm opposite the catheter. In addition, a 5-mL blood sample was obtained for subsequent analysis (Details stated in the *Blood Analysis Section*). Immediately following BASE measurements, the subject was outfitted with a noseclip and mouthpiece for the measurement of oxygen consumption (VO_2) during exercise performed on the cycle ergometer.

After BASE and after instrumentation of the subject was complete, the exercise session began. During each individual experimental trial, the subject performed one of three different exercise protocols: 50% VO_{2peak} for a duration of 60 minutes, 65% VO_{2peak} for a duration of 45 minutes, or 80% VO_{2peak} for a duration of 30 minutes. For each experimental trial, the workload was determined via a regression equation with subject data from the maximal graded exercise test in conjunction with the ACSM metabolic prediction equation for cycle ergometry. The duration of the first experimental trial was performed for the exact amount of time (i.e. 30, 45, or 60 min) for the assigned protocol. Total work in kilojoules (kJ) was then calculated as the exercise intensity (1 Watt = 1 Joule·sec^{-1}) multiplied by the duration of the exercise session. After the first trial, the duration of the final two trials/protocols was adjusted so that the total amount of work performed was equivalent to the total work performed during the first exercise protocol. During each exercise session, heart rate and VO_2 were measured continuously via the heart rate monitor and metabolic measurement cart, respectively. At the eight minute mark of the exercise session, the subject's VO_2 was compared to the goal VO_2. If the subject's VO_2 was not within five percent of the goal VO_2, the workload was adjusted accordingly. Following the exercise session, body weight was measured and the subject returned to a sitting position, at which time a second 5-mL blood sample was obtained

(Post-EX). After the blood sample, the subject began a 2 hour seated recovery period (2-hr REC). Following the 2 hour recovery period, a third 5-mL blood sample was obtained and the subject was allowed to leave the laboratory.

On Day 2 of each experimental trial, the subject returned to the laboratory (24 hours post-exercise). Upon arrival to the laboratory, each subject had another post-void body weight measurement. The subject also had a heart rate monitor secured around his chest. The subject once again began a seated 60 minute seated-rest baseline period (24-hr REC). Heart rate and blood pressure measurements were obtained at the end of the 60 minute baseline period. In addition, a 5-mL blood sample was obtained from an antecubital vein via needle injection (Eclipse Blood Collection Needles, BD Vacuatainer, Franklin Lakes, NJ).

Blood Analysis

For each blood sample, 5-mL of whole blood was collected from an antecubital vein. Two milliliters of blood was aliquotted into a potassium-EDTA vacutainer for the whole blood determinations of hematocrit and hemoglobin. Three milliliters was aliquotted into a serum vacutainer and centrifuged for 10 minutes, after which the serum was separated and stored in microcentrifuge tubes for later analysis of albumin and total protein concentrations. To measure hematocrit values, three 30uL capillary tubes were filled with whole blood and centrifuged for 4 minutes, afterwhich the ratio of the red blood cell column to the total blood column length was measured. Hemoglobin was measured in triplicate using the cyanmethemoglobin method (29). Hematocrit and hemoglobin values were then used to calculate the percent changes in PV (7,8). Serum albumin concentration was determined via the bromcresol green method (9) and serum

total protein concentration was determined using a modification of the biuert method (21). For the hemoglobin, albumin, and total protein assays, absorbencies were determined using a GENESYS 10 UV Spectrophotometer (Thermo Electron Corporation, Madison, WI).

Statistical Analysis

A two-way analysis of variance (ANOVA) with repeated measures was used to determine statistical differences in the dependent variables. The factors used in the analysis were protocol (50% vs. 65% vs. 80% VO_{2peak}) and time with both factors being repeated measures factors. Significance was established *a priori* at $P<0.05$. In the case of a significant interaction, a simple effects analysis was used to examine specific contrasts and statistical significance was determined using the Bonferroni adjustment. All values are expressed as mean (M) ± standard deviation (SD).

CHAPTER III

RESULTS

Protocol Variables

Total Work, Goal Total Work, and Percent of Goal Total Work

The total work performed kilojoules (kJ), the goal total work (kJ), and the percent of the goal total work performed for the three exercise protocols is depicted in Table 2. There were no significant differences in total work performed among each of the three exercise protocols. In addition, there was no significant difference between the total work performed and the goal total work for each of the three exercise protocols, respectively. Lastly, there was no significant difference in the percent of the goal total work performed between any of the three exercise protocols. These findings confirm that the subjects were performing the appropriate amount of total work for the three exercise protocols.

Actual VO_2, Goal VO_2, and Percent of Goal VO_2

The actual VO_2 ($L \cdot min^{-1}$), goal VO_2 ($L \cdot min^{-1}$), and percent of goal VO_2 for the exercise protocols is depicted in Table 2. There was no significant difference between the actual VO_2 and the goal VO_2 for any of the three exercise protocols. In addition, there was no difference in % of goal VO_2 among any of the three exercise protocols. Again, these finding confirm that the subjects were exercising at the appropriate intensities to elicit the desired VO_2 for the three exercise protocols.

Table 2. Exercise protocol characteristics (M ± SD).

Variable / Protocol	50% VO_{2peak}	65% VO_{2peak}	80% VO_{2peak}
Actual Exercise Time (min)*	61 ± 4	44 ± 3	35 ± 4
Workload (Watts; $J \cdot sec^{-1}$)§	100 ± 14	140 ± 16	175 ± 18
Work			
Total Work Performed (kJ)	364.67 ± 63.62	367.20 ± 64.34	360.83 ± 68.71
Goal Total Work (kJ)	362.30 ± 69.54	362.30 ± 69.54	362.30 ± 69.54
% of Goal Total Work Performed	101.0 ± 3.6	101.7 ± 3.2	99.6 ± 0.9
VO_2			
Actual VO_2 ($L \cdot min^{-1}$) §	1.483 ± 0.155	1.917 ± 0.194	2.385 ± 0.217
Goal VO_2 ($L \cdot min^{-1}$) §	1.447 ± 0.123	1.881 ± 0.160	2.316 ± 0.217
% of Goal VO_2	102.4 ± 4.5	101.8 ± 3.4	103.0 ± 3.4

* denotes 50% VO_{2peak} > 65% VO_{2peak} > 80% VO_{2peak}; $P < 0.05$
§ denotes 50% VO_{2peak} < 65% VO_{2peak} < 80% VO_{2peak}; $P < 0.05$

Body Weight

The values for body weight (kg) for the three exercise protocols is depicted in Table 3. There was a significant decrease in body weight from BASE to Post-EX for the 50%, 65%, and 80% VO_{2peak} protocols. The magnitude of this decrease in body weight was not different among the three protocols.

For the 50%, 65%, and 80% VO_{2peak} protocols there was no significant difference between the Post-EX and 24-hr REC values, but the 24-hr REC value was still significantly lower compared to BASE for each respective protocol. At the 24-hr REC timepoint, there was no significant difference in body weight between any of the three exercise protocols.

At each timepoint, there was no significant difference in body weight between any of the three exercise protocols.

Blood Variables

Change in Plasma Volume

The change in PV (%) relative to baseline for the three exercise protocols is depicted in Figure 1. There was a significant decrease in PV Post-EX for the 50% and 80% VO_{2peak} protocols while the 65% VO_{2peak} protocol approached significance ($P = 0.077$). The magnitude of the decrease was different among the three protocols, with the 80% VO_{2peak} protocol being significantly different from the 50% VO_{2peak} protocol. There was no significant difference between the 50% and 65% VO_{2peak} protocols and the 65% and 80% VO_{2peak} protocols.

Table 3. The response in body weight (kg) during the three exercise protocols (M ± SD).

Protocol / Time	BASE	Post-EX	24-hr REC
50% VO_{2peak}	79.1 ± 10.7	78.5 ± 10.7*	78.7 ± 11.1*
65% VO_{2peak}	79.7 ± 10.4	78.8 ± 10.6*	79.0 ± 10.4*
80% VO_{2peak}	79.5 ± 10.6	78.7 ± 10.6*	78.8 ± 10.3*

* denotes significant difference ($P < 0.05$) from BASE within respective protocol.

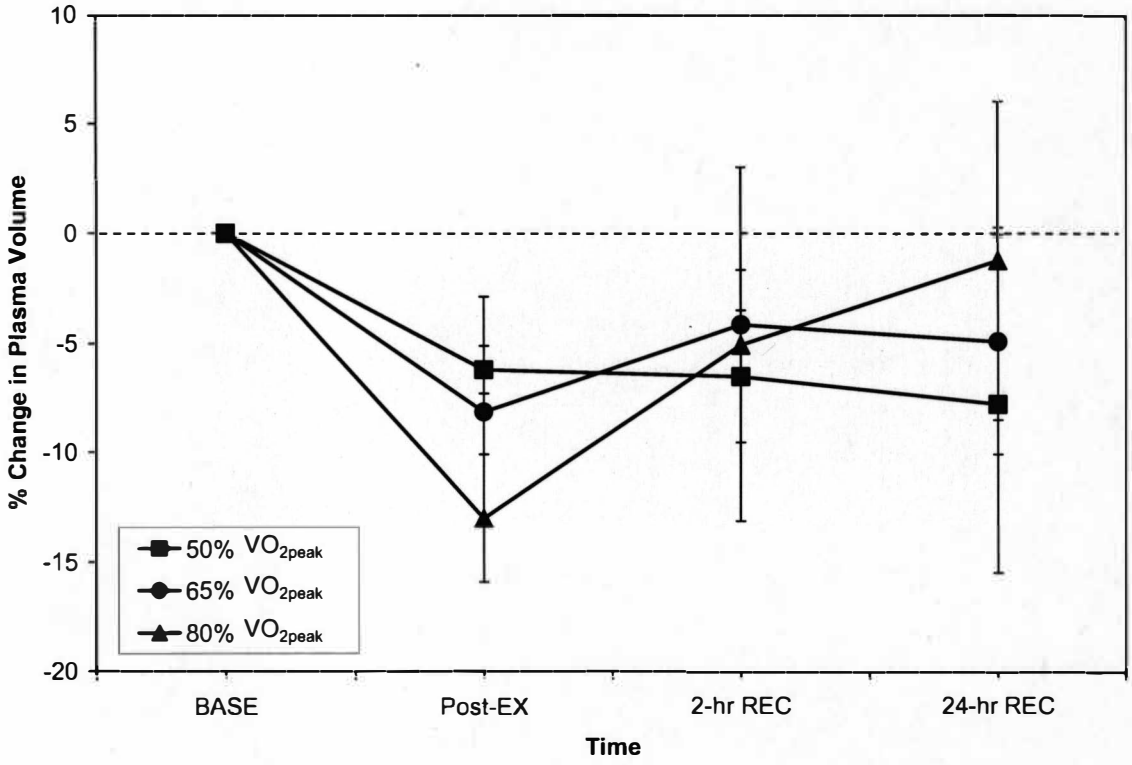

Figure 1. % change in plasma volume relative to BASE during the exercise protocols (M ± SD) (Protocol × Time; $P < 0.05$).

For the 50% VO_{2peak} protocol, there was no significant difference between the 2-hr REC and the Post-EX values but the 2-hr REC value remained significantly lower compared to BASE. For the 65% VO_{2peak} protocol, there was no significant difference between the 2-hr REC and the Post-EX values and, although not reaching statistical significance, the 2-hr REC value tended to remain lower compared to BASE ($P = 0.056$). For the 80% VO_{2peak} protocol, although the mean increase in PV from Post-EX to 2-hr REC was the greatest among the three protocols, the difference was not statistically significant. However, this increase in PV resulted in there being no significant difference between the 2-hr REC value and the BASE value. At the 2-hr REC timepoint, there was no significant difference in the change in PV between any of the three exercise protocols.

For the 50% and 65% VO_{2peak} protocols, there was no significant difference in the change in PV between the 24-hr REC value and any of the other timepoints for each respective protocol. For the 80% VO_{2peak} protocol, there was no significant difference between the 24-hr REC value and the BASE value or the 2-hr REC value. However, there was a significant increase in PV compared to the Post-EX value. At the 24-hr REC timepoint, there was no significant difference in PV between any of the three exercise protocols.

Serum Albumin Concentration (No Plasma Volume Correction)

The response in serum albumin concentration ($g \cdot dL^{-1}$) for the three exercise protocols is depicted in Figure 2A. At BASE, there was no significant difference between any of the three exercise protocols. There was a significant decrease in albumin concentration from BASE to Post-EX for the 50%, 65%, and 80% VO_{2peak} protocols. At

Post-EX, serum albumin concentration was not different between any of the three exercise protocols.

For the 50% VO_{2peak} protocol, there was no significant difference between the 2-hr REC and the Post-EX values but the 2-hr REC value was still significantly lower compared to BASE. For the 65% VO_{2peak} protocol, serum albumin was significantly greater at the 2-hr REC compared to the Post-EX value, but there was no significant difference between the 2-hr REC value when compared to BASE. For the 80% VO_{2peak} protocol, there was no significant difference between 2-hr REC and Post-EX however, the 2-hr REC value tended to be lower compared to BASE ($P = 0.070$). At the 2-hr REC timepoint, there was no significant difference in serum albumin concentration between any of the three exercise protocols.

For the 50% VO_{2peak} protocol, there was no significant difference in serum albumin concentration between the 24-hr REC value and any of the other timepoints. For the 65% and 80% VO_{2peak} protocol, there was no significant difference between the 24-hr REC value and the BASE value or the 2-hr REC value for each respective protocol. However, there was a significant increase in serum albumin compared to the Post-EX value for both the 65% and 80% protocols. At the 24-hr REC timepoint, serum albumin concentration was different among the three protocols, with the 80% VO_{2peak} protocol being significantly greater than the 50% and 65% VO_{2peak} protocols. However, there was no significant difference between the 50% and 65% VO_{2peak} protocols.

Serum Albumin Concentration (With Plasma Volume Correction)

The response in serum albumin concentration ($g \cdot dL^{-1}$) corrected for change in PV for the three exercise protocols is depicted in Figure 2B. At BASE, there was no

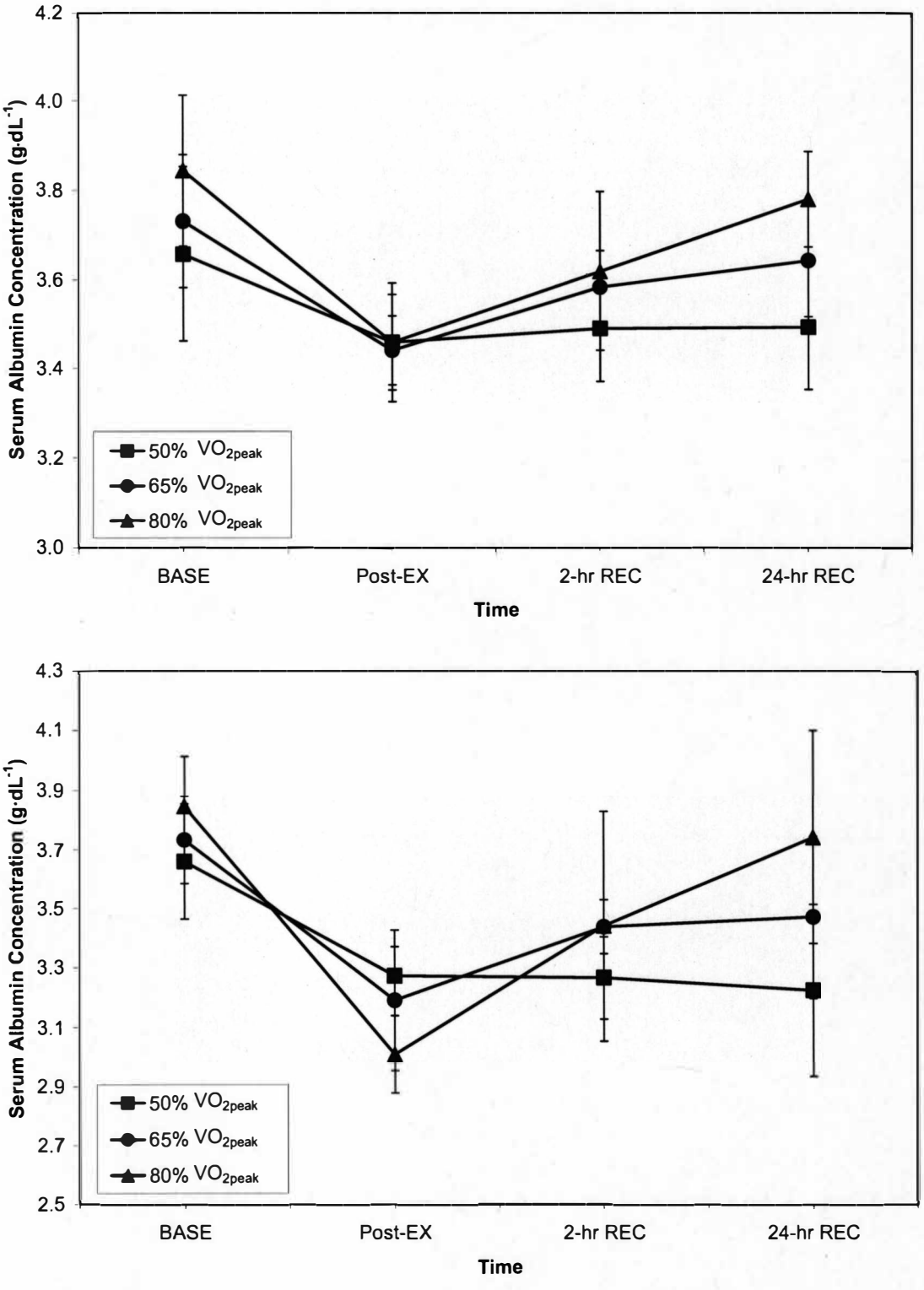

Figure 2. Serum albumin concentration during the exercise protocols both uncorrected (A) and corrected (B) for plasma volume change (M ± SD) (Protocol × Time; $P < 0.05$).

significant difference between any of the three exercise protocols. There was a significant decrease in serum albumin concentration from BASE to Post-EX for the 50%, 65%, and 80% VO_{2peak} protocols. At Post-EX, serum albumin concentration was different among the three protocols, with the 80% VO_{2peak} protocol being significantly lower than the 50% VO_{2peak} protocol. However, there was no significant difference between the 50% and 65% VO_{2peak} protocols and the 65% and 80% VO_{2peak} protocols.

For the 50% and 65% VO_{2peak} protocols, there was no significant difference between the 2-hr REC and the Post-EX values but the 2-hr REC value was still significantly lower compared to BASE. For the 80% VO_{2peak} protocol, there was no significant difference between the 2-hr REC and the Post-EX values and there was no significant difference between the 2-hr REC values when compared to BASE. At the 2-hr REC timepoint, serum albumin concentration corrected for PV was different among the three protocols, with the 80% VO_{2peak} protocol being significantly different from the 50% VO_{2peak} protocol. There was no significant difference between the 50% and 65% VO_{2peak} protocols and the 65% and 80% VO_{2peak} protocols.

For the 50% VO_{2peak} protocol, there was no significant difference in serum albumin concentration between the 24-hr REC value and any of the other timepoints. For the 65% and 80% VO_{2peak} protocol, there was no significant difference between the 24-hr REC value and the 2-hr REC value. However, serum albumin concentration was significantly greater at the 24-hr REC value compared to the Post-EX value for both the 65% and 80% protocols. For the 65% VO_{2peak} protocol, although not reaching statistical significance, the 24-hr REC value tended to be lower compared to BASE ($P = 0.080$). For the 80% VO_{2peak} protocol, there was no significant difference between the 24-hr REC

value and the BASE value. At the 24-hr REC timepoint, serum albumin concentration was different among the three protocols, with the 80% VO_{2peak} protocol being significantly greater than the 50% VO_{2peak} protocol. However, there was no significant difference between the 50% and 65% VO_{2peak} protocols and the 65% and 80% VO_{2peak} protocols.

Serum Total Protein (No Plasma Volume Correction)

The response in serum total protein concentration ($g \cdot dL^{-1}$) for the three exercise protocols is depicted in Figure 3A. At BASE, there was no significant difference between any of the three exercise protocols. There was a significant decrease in serum total protein concentration from BASE to Post-EX for the 65% and 80% VO_{2peak} protocols; however there was no significant difference for the 50% VO_{2peak} protocol between the Post-EX and BASE values. At Post-EX, serum total protein concentration was not different between any of the three exercise protocols.

For the 50% VO_{2peak} protocol, the Post-EX value was significantly lower compared the 2-hr REC but there was no significant difference between the 2-hr REC and BASE values. For the 65% VO_{2peak} protocol, although not reaching statistical significance, the 2-hr REC value for serum total protein concentration tended to be lower compared to Post-EX ($P = 0.066$); however, there was no significant difference between the 2-hr REC values when compared to BASE. For the 80% VO_{2peak} protocol, there was no significant difference between the 2-hr REC and the Post-EX values and there was no significant difference between the 2-hr REC values when compared to BASE. At the 2-hr REC timepoint, there was no significant difference in serum total protein concentration between any of the three exercise protocols.

For the 50% VO_{2peak} protocol, there was no significant difference in serum total protein concentration between the 24-hr REC value and any of the other timepoints. For the 65% and 80% VO_{2peak} protocol, there was no significant difference between the 24-hr REC value and the BASE value or the 2-hr REC value for each respective protocol. However, at the 24-hr REC timepoint, there was a significant increase in serum albumin compared to the Post-EX value for both the 65% and 80% protocols. At the 24-hr REC timepoint, serum total protein concentration was different among the three protocols, with the 80% VO_{2peak} protocol being significantly greater than the 50% VO_{2peak} protocol. However, there was no significant difference between the 50% and 65% VO_{2peak} protocols and the 65% and 80% VO_{2peak} protocols.

Serum Total Protein (With Plasma Volume Correction)

The response in serum total protein concentration ($g \cdot dL^{-1}$) corrected for change in PV for the three exercise protocols is depicted in Figure 3B. At BASE, there was no significant difference between any of the three exercise protocols. There was a significant decrease in serum total protein concentration from BASE to Post-EX for the 50%, 65%, and 80% VO_{2peak} protocols. At Post-EX, serum total protein concentration was different among the three protocols, with the 80% VO_{2peak} protocol being significantly greater than the 50% VO_{2peak} protocol. However, there was no significant difference between the 50% and 65% VO_{2peak} protocols and the 65% and 80% VO_{2peak} protocols.

For the 50% VO_{2peak} protocol, there was no significant difference between the 2-hr REC and the Post-EX values and there was no significant difference between the 2-hr REC values when compared to BASE. For the 65% VO_{2peak} protocol, there was a

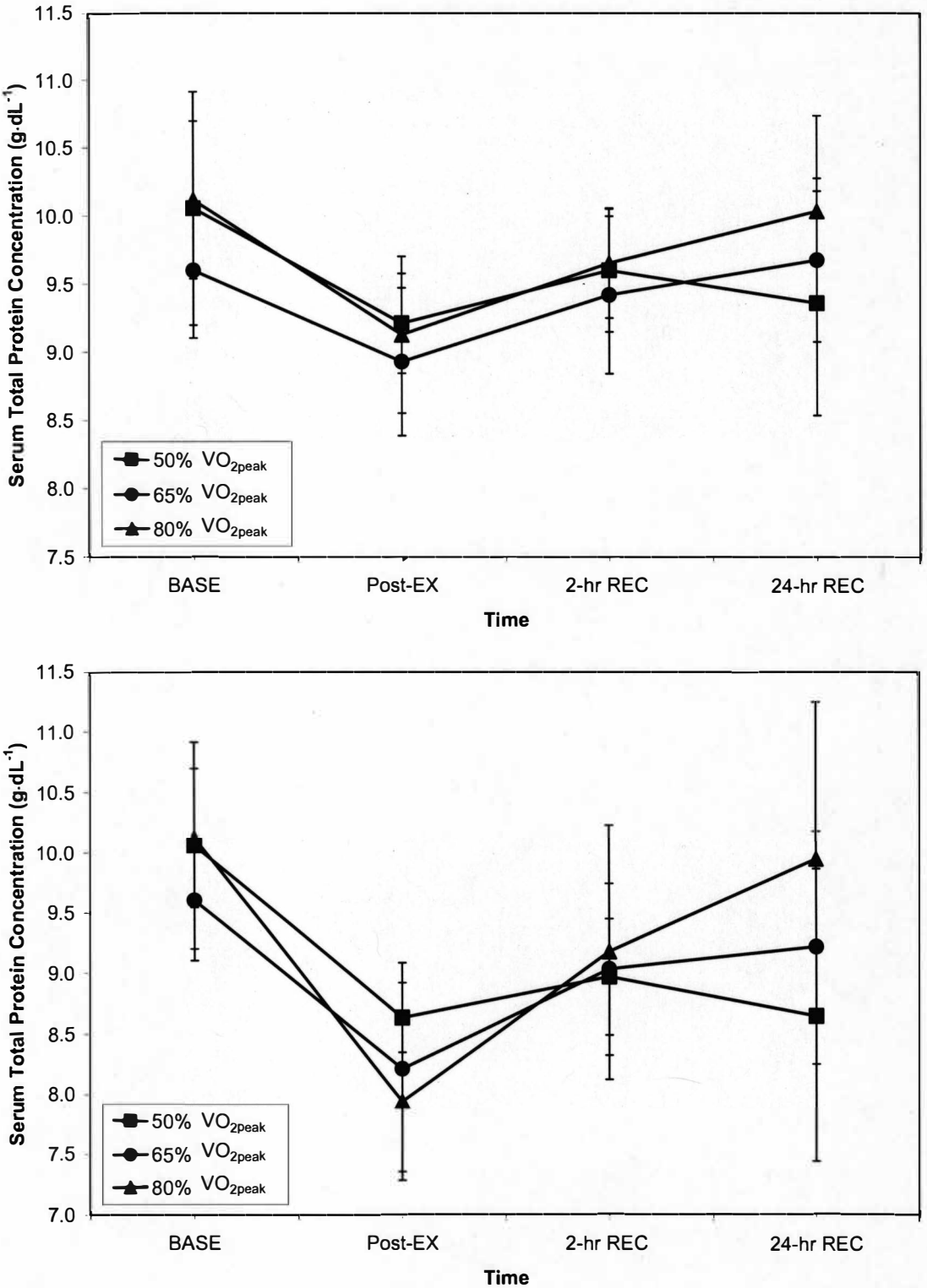

Figure 3. Serum total protein concentration during the exercise protocols both uncorrected (A) and corrected (B) for plasma volume change (M ± SD) (Protocol × Time; $P < 0.05$).

significant difference between the 2-hr REC and the Post-EX values and there was a significant difference between the 2-hr REC values when compared to BASE. For the 80% VO_{2peak} protocol, there was no significant difference between 2-hr REC and Post-EX values and there was no significant difference between 2-hr REC values when compared to BASE. At the 2-hr REC timepoint, there was no significant difference in the serum total protein concentration corrected for PV between any of the three exercise protocols.

For the 50% VO_{2peak} protocol, there was no significant difference in serum total protein concentration between the 24-hr REC value and any of the other timepoints. For the 65% and 80% VO_{2peak} protocol, there was no significant difference between the 24-hr REC value and the BASE value or the 2-hr REC value. However, serum total protein concentration was significantly greater at 24-hr REC compared to Post-EX for both the 65% and 80% protocols. At the 24-hr REC timepoint, serum total protein concentration was different among the three protocols, with the 80% VO_{2pcak} protocol being significantly greater than the 50% VO_{2peak} protocol. However, there was no significant difference between the 50% and 65% VO_{2peak} protocols and the 65% and 80% VO_{2peak} protocols.

Cardiovascular Variables

Heart Rate

The response in heart rate ($b \cdot min^{-1}$) for the three exercise protocols is depicted in Table 4. At BASE, there was no significant difference between any of the three exercise protocols. From BASE to exercise, there was a significant increase in heart rate for all three exercise protocols. The magnitude of this increase was different between the three

exercise protocols, with the 80% VO_{2peak} protocol being significantly different from the 50% VO_{2peak} protocol. However, there was no significant difference between the 50% and 65% VO_{2peak} protocols and the 65% and 80% VO_{2peak} protocols.

For the 50%, 65%, and 80% VO_{2peak} protocols there was a significant decrease in heart rate between the exercise and 2-hr REC values, but the 2-hr REC value was not significantly different compared to BASE for each respective protocol. At the 2-hr REC timepoint, there was no significant difference in heart rate between any of the three exercise protocols.

For the 50%, 65%, and 80% VO_{2peak} protocols there was no significant difference between the 2-hr REC and 24-hr REC values and the 24-hr REC value was still not significantly different compared to BASE for each respective protocol. At the 24-hr REC timepoint, there was no significant difference in heart rate between any of the three exercise protocols.

Mean Arterial Blood Pressure

The response in MABP (mmHg) for the three exercise protocols is depicted in Table 4. At BASE, there was no significant difference between any of the three exercise protocols. There was no significant difference in MABP between the 2-hr REC and BASE values for any of the three exercise protocols. At 2-hr REC, there was no difference in MABP between any of the three exercise protocols.

For the 50%, 65%, and 80% VO_{2peak} protocols, there was no significant difference in MABP between the 24-hr REC and 2-hr REC timepoints or between the 24-hr REC

Table 4. The response in heart rate and mean arterial blood pressure during the exercise protocols (M ± SD).

Variable / Protocol / Time	BASE	Exercise	2-hr REC	24-hr REC
Heart Rate ($b \cdot min^{-1}$)				
50% VO_{2peak}	62 ± 7	123 ± 7	61 ± 7	64 ± 8
65% VO_{2peak}	64 ± 9	148 ± 11	66 ± 11	61 ± 8
80% VO_{2peak}	62 ± 5	168 ± 12	64 ± 8	62 ± 6
MABP (mmHg)				
50% VO_{2peak}	84 ± 3	N/M	84 ± 5	83 ± 4
65% VO_{2peak}	85 ± 2	N/M	85 ± 4	81 ± 4
80% VO_{2peak}	84 ± 5	N/M	87 ± 5	83 ± 2

N/M, Not Measured

and BASE timepoints. At the 24-hr REC timepoint, there was no significant difference in MABP between any of the three exercise protocols.

CHAPTER IV

DISCUSSION

The purpose of the current investigation was to study the effects of acute exercise on PV while examining variations of the intensity and duration relationship of the exercise protocols while keeping the total amount of work performed in each session the same for each subject. It was hypothesized that the protocol consisting of the highest intensity and the shortest duration would yield the greatest level of PV expansion and the largest increases in total protein and albumin concentrations.

The mechanisms for the adaptation of PV expansion result from a variety of physiological responses to counteract the acute decrease in PV post-exercise. Upon the completion of exercise, auto-restoration of lost PV is initiated (2,10,13). It has been suggested that post-exercise hypotension may be an important signal for prompting the body to begin PV restoration post-exercise (15,16,17). There are a variety of mechanisms involved in this post-exercise restoration of PV. Sodium retention by the kidneys increases, resulting in a decrease in water loss from the body (3,5). Increased synthesis of the plasma protein albumin and the subsequent increase in albumin content of the blood also contributes to a fluid shift from the interstitial space to the vascular space and the retention of water within the vascular space (3,11,25,30). As a consequence of these mechanisms, the increase in water retention by the body results in the restoration or increase of PV approximately 24 hours post-exercise (6,11,12,23,27).

Previous researchers have reported that exercise intensity may be the most important exercise variable that influences PV expansion (2,3,5,6,13). Higher intensity exercise is associated with greater increases in osmolality, plasma renin activity, and

arginine vasopressin concentrations (3,5,11). Due to these responses, sodium and water retention during and following exercise are facilitated (3,5,6,13). In conjunction with the aforementioned mechanisms, a chronic increase in plasma total protein content (i.e. albumin synthesis or decrease in albumin degradation) provides an increased water binding capacity for the blood (3,11). Green et al. reported a larger PV expansion with shorter duration, higher intensity exercise in comparison to previous studies with longer duration and lower intensity exercise bouts (13). The observation that higher intensity exercise may provide a greater level of PV expansion appears well supported in regards to their data. This is based upon situations in which total work output remained constant for each individual subject during a multi-day, repeated exercise session and total work performed per session on these consecutive days. The authors indicated that intensity may be the single most important factor as a potent stimulus for inducing PV expansion and accelerating the rate at which PV expansion occurs (13).

The major finding of this study was that among the exercise protocols, none of the three intensity/duration combinations resulted in an actual PV expansion which exceeded the initial BASE measurement. However, the 80% VO_{2peak} protocol yielded the greatest PV increase measured at the 24-hr REC time point in relation to the Post-EX timepoint. This notable PV increase likely occurred, in part, due to the fact that the 80% VO_{2peak} protocol also resulted in the largest initial decrease in PV measured at the Post-EX timepoint. In conjunction, the data revealed a similar pattern for responses in both serum albumin and serum total protein concentrations (corrected for PV change) in relation to the measurements for the 80% VO_{2peak} protocol.

Changes in Plasma Volume during Exercise and Recovery

As previously noted, no expansion of PV was observed 24 hours post-exercise for any of the three exercise protocols used in this study. In fact, from BASE to 24-hr REC, a decrease in PV was observed for all three protocols. However, the mean decline in PV was the lowest for the 80% VO_{2peak} protocol (– 1.2%) compared to the 50% and 65% VO_{2peak} protocols (– 7.8 and – 4.9%, respectively).

From BASE to Post-EX, the 80% VO_{2peak} protocol caused the greatest decrease in PV (– 13.0%) compared to the 50% and 65% VO_{2peak} protocols (– 6.2 and – 8.1%, respectively). The initial decrease in PV from BASE to Post-EX for the 80% VO_{2peak} protocol is probably attributed, in part, to this protocol being the highest intensity exercise bout of the three protocols, which is consistent with data from previous studies (2,3,5,6,13) that revealed a larger PV decrease following higher intensity exercise bouts. Interestingly, when examining the change in PV from Post-EX to 24-hr REC, the 80% VO_{2peak} protocol exhibited the greatest recovery in PV (11.8%) compared to the 50% and 65% VO_{2peak} protocols (– 1.6 and 3.2%, respectively). This current finding coincides closely with suggestions of previous researchers that exercise intensity appears to be the most important exercise factor in influencing PV expansion (2,3,5,6,13). Hence, the significant decrease in PV at the Post-EX timepoint relative to BASE is probably one of the mechanisms (along with greater responses in albumin concentration) for such a large increase in PV at the 24-hr REC timepoint for the 80% VO_{2peak} protocol. Thus, by comparison, there was a significant difference with the PV increase for the 80% VO_{2peak} protocol, whereas the PV values for the 50% VO_{2peak} protocol and 65% VO_{2peak} protocol

revealed very little deviation from the Post-EX to 24-hr REC timepoints and remained lower than the BASE measurements.

In consideration to total work output being performed, the current results are similar to a study by Green et al., which reported a larger PV expansion with shorter duration, high intensity exercise in comparison to previous investigations with longer duration and lower intensity exercise bouts (13). The subjects in their study were asked to complete an exercise protocol consisting of 1 minute of exercise at 120% VO_{2max} followed by 4 minutes of rest. This exercise/rest cycle was repeated until the subject fatigued or completed 24 cycles and each subject completed this protocol for three consecutive days. Of the four subject participating in the study, three were able to complete between 22-24 cycles of the exercise, while one subject was only able to complete 12 cycles. Even though this subject completed only 50% of the total work compared to the other three subjects, he exhibited the greatest PV expansion (477 vs. 372 mL). Therefore, the authors concluded that the intensity of the exercise influenced the PV expansion more so than the total amount of work performed. Our study would appear to indicate a high capacity for PV recovery from Post-EX to 24-hr REC following exercise at a higher intensity; similar to Green et al (13). Data from both studies support the concept of the intensity/duration relationship, which indicated a greater increase in PV during higher intensity exercise for shorter durations under the scenario of keeping total work output constant.

Though the mean data from the present study is inconsistent with previous studies that report a PV expansion 24-hr after exercise (2,3,4,6,10,11,12,13,19,23,27), it is interesting to note that the current data did uncover PV expansion within some of the

protocols. In relation to individual subjects, when comparing the change in PV from BASE to 24-hr REC for all the trials, seven of 18 instances revealed a PV expansion (mean = 3.1%). Despite the percent increase not being as high as previous investigations (2,4,6,10,11,12,13,23,27) that have traditionally resulted in PV expansion within the range of 9 to 25%, the current data is similar to past studies, especially in regards to the influence of exercise intensity. The 50% VO_{2peak} protocol caused only one PV expansion occurrence, whereas the 65% and 80% VO_{2peak} protocols both resulted in three instances of PV expansion. Hence, this most recent data delivers continuing support for the importance of higher intensity exercise to initiate this PV expansion response. These data do support the hypothesis that the highest intensity exercise protocol would yield the greatest increase in PV; however, we were expecting an actual PV expansion, which unfortunately did not occur, hence not entirely supporting our hypothesis.

Inconsistency of the current data with previous studies could potentially be due to variations in methods of blood volume and PV measurements based upon equipment and monetary insufficiencies. Many researchers utilized the Evan's blue dye dilution method to measure actual total blood volume values (3,4,6,11,14,15,17), which is a more effective method of determining PV expansion since absolute values can be calculated. Other investigations (5,12,13,14) employed multiple calculations for measurements of hematological variables including more complex calculations of PV. These researchers also made determinations of red blood cell mass, hematocrit, hemoglobin, osmolality, plasma arginine vasopressin, and plasma renin activity, thus providing more data information. Another possibility for our data being inconsistent with previous studies is that subjects may not have adhered to the fluid ingestion guidelines put forth by the

researchers, thus resulting in a non-euhydrated state prior to exercise and/or failing to consume the required amount of fluid post-exercise to elicit additional enhancement of the PV expansion response. Subjects may also have possibly been in a chronically dehydrated state prior to entering the study, consequently producing unfavorable mathematical results for the percent change in PV by altering any typically expected responses.

From the standpoint of hydration, and fluid loss and replacement, a recent study by Kay et al. examined the effects of ingesting different post-exercise fluid volumes on PV expansion. Different volumes of fluid consumption will also have implications on PV expansion similar to varying levels of exercise intensity. Data from their investigation (19) revealed that when subjects consumed 1.5 L of fluid post-exercise, it induced a PV expansion of 2.3% (± 2.0%), whereas the 3.0 L of fluid after exercise caused a 5.0% (± 2.0%) increase in PV. Variations in fluid volume would appear almost synonymous to different exercise intensities at a constant workload, when considering that greater volumes of fluid replacement and higher intensity exercise both cause a greater increase in PV. In contrast, smaller volumes of fluid replacement and lower intensity exercise result in a smaller increase in percent PV. By integrating the data of the present study and the results of Kay et al., it would appear that PV could most effectively be induced through combining a higher intensity exercise protocol with a larger amount of fluid volume for replacement post-exercise. Investigating various combinations of fluid ingestion both pre- and post-exercise and various exercise intensities to determine the effects on overall percent change in PV and which

combinations result in the greatest PV expansion would be research possibilities and questions to answer for future studies.

Response in Serum Albumin Concentrations

The response in serum albumin concentration was very consistent with the data for the percent change in PV. There was a decrease in serum albumin concentration observed for all three protocols from BASE to 24-hr REC. Similar to PV, the mean decline in albumin concentration was the lowest for the 80% VO_{2peak} protocol (– 5.1%) in comparison to the 50% and 65% VO_{2peak} protocols (– 13.5% and – 5.4%, respectively).

Likewise, from BASE to Post-EX, the 80% VO_{2peak} protocol caused the greatest decrease in albumin concentration (– 21.1%) compared to the 50% and 65% VO_{2peak} protocols (– 10.8% and – 13.5%, respectively). Similarly, when observing the change in albumin response from Post-EX to 24-hr REC, the 80% VO_{2peak} protocol displayed the greatest recovery in albumin concentration (24.7%) in comparison to the 50% and 65% VO_{2peak} protocols (– 3.0% and 9.4%, respectively). The response in albumin during the 80% VO_{2peak} protocol in which there was the greatest decrease in concentration for the Post-EX measures are likely attributed to being the highest intensity exercise bout of the three protocols. Previous investigations by Convertino et al. and Gillen et al. have reported a correlation between PV expansion and an increase in albumin content during high intensity exercise (3,11). The researchers indicated that 1 g of albumin binds anywhere from 14–18 mL of water (3,4,11), thus allowing for an increase in PV ranging from 390–465 mL. Our data is inconsistent with these previous studies in the fact that the current results revealed no mean PV expansion. It has also been reported by Yang et al. (30) that fluid retention will occur within the circulation after intense exercise due to

the contributions of both increased albumin content and free water clearance. A subsequent study by Nagashima et al. (25) observed increased albumin synthesis after intense exercise, which contributes a greater albumin content. Once again, these observations were made while utilizing a high-intensity exercise protocol. Our current data remains consistent with those conclusions of previous researchers through interpretation our results via correlation to these studies considering they have also indicated that exercise intensity causes an increase in albumin content and synthesis (3,11,25,30). This conclusion can be made when considering the connection of the response in serum albumin concentrations being corrected for PV that was indicated by our data. Thus, the significant response in albumin decrease at the Post-EX timepoint relative to BASE is probably one of the mechanisms for such a large increase in albumin concentration at the 24-hr REC timepoint for the 80% VO_{2peak} protocol. By comparison, there was a significant difference for albumin concentration increase for the 80% VO_{2peak} protocol (24.7%), whereas the albumin values for the 50% VO_{2peak} protocol (– 3.0%) remained about the same and the 65% VO_{2peak} protocol (9.4%) only slightly increased from the Post-EX to 24-hr REC timepoints; both protocols also remained lower than the BASE measurements. Our current data exposes continuing support for the necessity of utilizing higher intensity exercise to cause the greatest increase of the response in albumin concentration. Yang et al. (30) reported albumin and total protein content elevations during recovery from intense exercise. Total protein content was elevated at 1 hr post-exercise in association with elevated PV, whereas albumin content reached a significant level of increase by 5 and 6 h of recovery. The researchers indicated that their data confirmed the findings of previous studies by Gillen et al. (11) and Convertino et al.

(3), though Gillen et al. reported an elevated albumin content by 1 hr of recovery. Nagashima et al. (25) reported an increase in albumin content within 1 hr after intense exercise, similar to Gillen et al. The researchers (25) also indicated a reduction of albumin synthesis at 1–5 hr of recovery, which later elevated at 21–22 hr of recovery. Our findings for albumin concentration measures from Post-EX to 24-hr REC for the recovery of albumin for the higher intensity 65% and 80% VO_{2peak} protocols are similar to the findings of these studies, especially in consideration to those researchers who observed a "delayed" response for increase in albumin content (3,30). We can only theoretically conclude that this increased response in albumin concentration allowed for an increased albumin synthesis and elevated albumin content during the 2-hr REC and 24-hr REC timepoints when utilizing the higher intensity 80% VO_{2peak} protocol based on results of the aforementioned investigations.

Another interesting observation is that the initial mean BASE measurement of albumin concentration was the highest for the 80% VO_{2peak} protocol (3.8 $g \cdot dL^{-1}$). Although true, this protocol revealed the lowest Post-EX value (3.0 $g \cdot dL^{-1}$) while still resulting in the greatest measure at the 24-hr REC timepoint (3.7 $g \cdot dL^{-1}$) of the three protocols. Our finding is consistent with Gillen et al. (11) as they reported a return of albumin concentration to the control measure 24-hr after intense exercise, though albumin content was significantly increased. This further supports our theoretical conclusion that our intense protocol caused an elevation in albumin content, considering our data indicated a return in albumin concentration within 0.1 $g \cdot dL^{-1}$ from the BASE to 24-hr REC timepoint. In contrast, the BASE measure of albumin concentration for the 50% VO_{2peak} protocol (3.7 $g \cdot dL^{-1}$) was the lowest of the three protocols and the resulting

24-hr REC value (3.2 $g \cdot dL^{-1}$) resulted in the lowest value for albumin concentration, which was not only a decrease from the BASE value (– 13.5%) but also lower than the Post-EX measure (– 3.0%). The 65% VO_{2peak} protocol resulted in mean values for the BASE (3.7 $g \cdot dL^{-1}$), Post-EX (3.2 $g \cdot dL^{-1}$), and 24-hr REC (3.2 $g \cdot dL^{-1}$) timepoints that measured within the range in between the 50% VO_{2pcak} and 80% VO_{2peak} protocols. The measurements for serum albumin concentrations within the current study unfortunately were only that: the response in serum albumin concentration for each of the various timepoints and exercise protocols. From a results perspective, it is reassuring to note that this data was consistent with that of results from previous studies despite the fact that we were unable to analyze actual albumin content and synthesis, due to equipment restrictions. Considering the data and conclusions of previous researchers concerning albumin in which actual content and synthesis of albumin were measured (3,11,25,30), our similarities for the findings of the response in albumin concentrations to the various exercise protocols also gives continuing support to the conclusion that higher intensity exercise is more effective for eliciting an increased albumin response and synthesis within the vascular space.

Response in Serum Total Protein Concentrations

The results for the response in serum total protein concentrations were very consistent to the data for responses in serum albumin concentrations (Figures 2B and 3B). As indicated by previous researchers (3,11), albumin accounts for ~ 90% of the increase in total protein concentration and content, thus verifying the very similar results observed within the current study between these two blood measures. Convertino et al. reported that 86% of the increase in total protein content after exercise was due to albumin,

verifying the importance of albumin as the main factor for causing a total protein increase within the vascular space (3). In another study by Gillen et al., the researchers indicated that the increase in albumin content at 1 hr of recovery of intense exercise accounted for the entire increase in total protein content within the vascular region (11). Understanding this correlation between total blood protein and albumin and the fact that albumin comprises a large majority of total protein content within the vascular space helps to verify our results and the similarities of the responses between both variables. In conjunction, based upon our data being consistent to the results of previous studies, it is not pertinent to discuss in any further detail our findings concerning the responses in serum total protein after exercise.

Response in Mean Arterial Blood Pressure

Previous studies have indicated that post-exercise hypotension may have implications on long-term adaptations such as plasma volume expansion that are associated with exercise training. Post-exercise hypotension is usually demonstrated after a single bout of exercise due to profound changes in the mechanisms that regulate arterial blood pressure; the changes usually last for about 2 h following exercise (15,16). Post-exercise hypotension has been shown to facilitate a net gain of intravascular albumin during recovery from exercise via albumin translocation from the interstitial fluid space to the intravascular space, which may contribute to PV restoration (17). Our data was not consistent with previous findings as we did not observe post-exercise hypotension at the 2-hr REC or the 24-hr REC timepoints. Potential causes for the lack of post-hypotension response within our study may have been that our subjects were not in a euhydrated state or they may have been chronically dehydrated coming into the study (see *Limitations of*

the Study). Data from the current study revealed neither PV expansion nor any post-exercise hypotension as indicated by the MABP measures during the 2-hr REC and 24-hr REC timepoints. This is inconsistent with the study by Hayes et al. as their data revealed that the exercise-induced decrease in PV was completely restored during the 90 min recovery period. The measurements of blood pressure also indicated a post-exercise hypotension throughout the 90 min recovery period (17).

Changes in Body Weight

As expected, there was a significant decrease in body weight for all three of the protocols from the BASE to Post-EX timepoints, which is consistent with a number of investigations (3,4,7,8,11,12,13,19,30). However, our data revealed that the 24-hr REC values were significantly lower compared to BASE for all three exercise protocols. These results for changes in bodyweight coincide with the fact that no PV expansion was observed. Previous studies have revealed the connection between an increase in PV expansion and body weight at the measurement 24-hr post-exercise (11,19). Potential causes for a decrease in body weight at the 24-hr REC timepoint within our study may have been that our subjects did not adhere to the fluid ingestion requirements of the investigators or they may have been chronically dehydrated coming into the study (see *Limitations of the Study*). The change in body weight indicating a decrease at the 24-hr REC timepoint was consistent with the decrease in percent chance for PV for our results.

Limitations of the Study

There are many speculations as to why the percent changes in PV for all three exercise protocols did not surpass the initial value at the BASE time point, aside from the intensity variable. Various explanations could include but are not limited to reasons such

as: subjects not ingesting the required amounts of water prescribed for the experimental trials; some subjects may have been chronically dehydrated, thus not in an optimal condition to allow for typical physiological responses to water consumption and PV response during and following the exercise protocols; and subjects may not have followed the dietary recommendations of the researchers for appropriate body water regulation for the experimental trials.

One consideration is that the subjects might not have ingested the indicated amount of water as prescribed by the researchers. This would have included the water that should have been consumed the night before Day 1 and Day 2 of the experimental trials, the morning of the experimental trials on Day 1 and Day 2, and the time period during the afternoon just following the experimental trial on Day 1. The necessary amount of water would need to be consumed prior to, immediately following, and within the subsequent 24-hr period following the experimental trial exercise protocol to help illicit the most beneficial PV expansion. The importance of fluid ingestion for enhancing PV expansion was supported in a recent study by Kay et al. as the researchers examined the effects of ingesting different post-exercise fluid volumes and how they affect fluid replacement (see *Changes in Plasma Volume during Exercise and Recovery*). This data indicates the importance of fluid ingestion following exercise for enabling the most beneficial PV expansion post-exercise. One can inductively conclude that fluid consumption within the 24-hr time period prior to exercise would be just as critical for allowing the most effective PV response. Thus, maintaining adequate water and fluid consumption on a regular basis to keep a bodily state of euhydration would likely be most beneficial as well. We can only make the supposition that some of the subjects might

have been in a chronically dehydrated state and possibly preventing an optimal PV response. Even if a chronically dehydrated subject would have consumed the required amount of fluid throughout the two-day experimental trials, it would have appeared to be of little consequence based upon their condition prior to the study, hence resulting in an unfavorable PV response. All of the measures for PV throughout a given experimental trial would have been affected due to having a reduced amount of PV within the vascular space. Any dietary miscues on the part of a subject may have caused water retention within the body, hence affecting the physiological response of fluid flow within the body. Our dietary suggestions required subjects to write down food intake on a dietary record sheet and attempt to be consistent during the day before and the two actual days of all three of the experimental trials. Such recommendations would have included avoiding any consumption of foods high in sodium or saturated fats or drinking alcohol. We did not control diet during the experimental trials by monitoring calorie, sodium, protein, and water intake as some studies have done previously (11,25,30) to enhance PV expansion, albumin synthesis, and ensure an adequate level of euhydration. Hence, we may have limited our results of any physiological responses after subjects performed the exercise protocols. There also may have been times when the subject was simply tired from daily activities of life, including work and school, thus not allowing them to give their best exercise effort during an experimental trial.

All of these things are dependent upon the subject and their behavior during the few days of our two-day experimental trials. Control of fluid intake could have been done at three timepoints: during the morning of Day 1, prior to the exercise protocol; immediately following the 2-hr REC period on Day 1; and during the morning of Day 2,

prior to taking the final blood samples. By monitoring fluid ingestion at these three timepoints, we could have ensured that the subject consumed at least the minimum required amount of fluid for optimizing a potential positive PV response. However, these results do also help confirm the importance of consuming fluid within the 2-hr "window" period post-exercise (possibly up to 4 hours) for eliciting PV expansion. Our subjects went at least two hours and potentially almost three hours post-exercise, before having the opportunity to ingest fluid. None of the three exercise protocols, under these given circumstances, produced any PV expansion. These data indicate the importance of fluid ingestion within the immediate and extended post-exercise time period to assist with body water replacement and PV expansion as the Kay et al. study indicated. Their study required subjects to consume one of two volumes (1.5 L or 3.0 L) for the rehydration beverage in five equal aliquots structured out at five set times equally spread during the following 24-hr post-exercise period (19). Total fluid volume and timing of fluid replacement are critical factors for post-exercise fluid consumption and enhancing the response in PV.

Our initial research plans were to implement the Evan's blue dye dilution method to measure actual total blood volume values as a more effective method of determining PV expansion since absolute values can be calculated. Unfortunately, due to equipment restrictions, we were unable to utilize this method for PV expansion for our measures. Similarly, we were then unable to measure the actual content of serum albumin and total protein, which would have also been more beneficial for data collection. By not being able to measure these variables, there were definitely limitations for our data and our initial research expectations for the study. It probably would have been beneficial to

measure MABP during the exercise protocols, since we measured heart rate during exercise as well. Even though the response in MABP during exercise is well documented and traditionally understood (16,17), remaining consistent with our timing and measures of our cardiovascular variables would have given more data. Thus, it may potentially have revealed more understanding into the mechanism of post-exercise hypotension and how it plays a role in the change and response in PV.

Summary

In conclusion, our data revealed that none of the three exercise protocols resulted in an actual PV expansion 24-hr post-exercise. However, our findings provide evidence that the 80% VO_{2peak} protocol, which implemented the highest intensity and shortest duration, resulted in the greatest recovery in PV after the post-exercise decline. The data also indicated that the 65% and 80% VO_{2peak} protocols resulted in the greatest increase in serum albumin concentration from immediately post-exercise to 24-hr post-exercise. Therefore, this data suggests that even when the total amount of work is kept constant, higher intensity exercise may result in a more beneficial PV and serum albumin concentration response compared to lower intensity exercise.

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APPENDIX A

HUMAN SUBJECTS INSTITUTIONAL REVIEW BOARD APPLICATION

THE EFFECTS OF ACUTE EXERCISE ON PLASMA VOLUME IN MEN – VARIATIONS OF INTENSITY/DURATION RELATIONSHIP

HSRIB APPLICATION

Principal Investigator/Faculty Advisor: Christopher C. Cheatham, Ph.D.
Student Investigator: Christopher Gregory, BS, CSCS

PROJECT DESCRIPTION

Purpose

The purpose of this study is to determine if variations of exercise intensity and duration, while keeping the total amount of exercise work similar, will influence plasma volume expansion after an acute exercise session.

Background

Chronic endurance training (3,4,6,9,16,20) and acute exercise sessions (8,10) have been shown to result in the expansion of plasma volume. Plasma volume expansion occurs almost immediately following acute exercise and becomes progressively larger during chronic endurance training exposure. Plasma volume expansion can range anywhere from 9 to 25%, depending on duration, intensity, frequency, and mode of exercise performed (2,4,6,7,8,9,10, 14,16,20). Plasma volume is the amount of water content within the blood stream, which is the clear substance aside from the hematocrit, hemoglobin, and red blood cells. The typical average for plasma volume expansion is around 12 to 15%. Investigations commonly utilize cycling or running as the method of exercise. Plasma volume increases occur within 24 hours of given exercise bouts (2,7,8,10).

During exercise, there is an acute decrease in plasma volume. This decrease is primarily caused by an increase in water loss due to sweating and an increase in plasma leakage from the blood vessels due to an increase in blood pressure and the forces pushing fluid out of the blood vessel (12,13). The body attempts to correct this acute decrease in plasma volume through a variety of mechanisms. Post-exercise, sodium retention by the body may increase resulting in a decrease in water loss from the body. The body also increases synthesis of the protein albumin, which contributes to the retention of water in the body (3,18,21). The end result of these mechanisms is an increase in water retention by the body and the restoration or increase of plasma volume approximately 24 hours post-exercise. The exact signal which prompts the body to begin to restore the plasma volume post-exercise is not entirely understood, but it has been suggested that post-exercise hypotension may be an important signal (12,13). After exercise, blood pressure is actually lower than pre-exercise levels. This post-exercise hypotension has been shown to remain anywhere from 2 to 24 hours. The post-exercise

hypotension may act as a signal to the body to increase water retention, and thus plasma volume, in hopes of restoring blood pressure to normal values.

Plasma volume expansion is one of the most beneficial adaptations to exercise. Plasma volume expansion is advantageous for thermal regulation during endurance exercise and the smoother flow of blood throughout the circulatory system (2,19). An enhanced perfusion rate causes greater evaporation and cooling of the body to maintain body temperature homeostasis during exercise, and less viscous blood resulting from higher plasma content would reduce stress on the heart and arterial walls during moderate to high intensity exercise.

Previous studies have utilized a variety of exercise protocols to elicit plasma volume expansion. An example of a protocol commonly used is to have the subject exercise on a cycle ergometer for four minutes at approximately 85% of maximal exercise capacity, and then to rest for five minutes (8,18,21). This cycle is repeated eight times. Although, this protocol is usually successful in eliciting an expansion of plasma volume, it is a difficult protocol that the average individual would not use in his or her training. Other protocols have used more continuous exercise at a relatively high intensity to elicit an expansion of plasma volume. However, to our knowledge, no previous studies have examined how varying the intensity and duration of exercise, while keeping the total amount of exercise performed constant, affects plasma volume expansion after exercise. In addition, the exercise protocols that we will use will be similar to exercise recommendations made by the American College of Sports Medicine (1). Therefore, the protocols we will use will be more applicable to the average individual during his or her exercise training.

Research Procedures

Six male participants will be recruited for the study. The participants must meet the inclusion requirements for study. Once they have met the requirements they will be asked to not to alter their workout regimen. The participants will be asked to meet with the student investigator in the Exercise Physiology Laboratory in the Student Recreation Center on the campus of Western Michigan University. The participants will visit the laboratory eight times during the study. The first visit to the laboratory will be an Orientation visit, the second visit will be a maximal graded exercise test, and the final six visits will be the Experimental trials.

In addition, we will request that participants refrain from drinking any alcohol or take any caffeine the day before and the day of the visits to the laboratory for the experimental trials. Caffeine can increase heart rate and increase nervous system activity. Caffeine and alcohol can cause a person to become dehydrated which may also affect the responses to exercise. Therefore, to obtain accurate/representative responses to the exercise, we ask that the participants refrain from these compounds.

Orientation Procedures

The first visit to the laboratory will serve as an Orientation Visit at which time each participant will be familiarized with the study procedures, a health history questionnaire will be completed, informed consent will be obtained, and anthropometrical measurements will be assessed (height, weight, percent body fat).

Each participant will read the informed consent form and the research protocols will be explained by the student investigator. Each participant will then be given the opportunity to ask any questions he might have concerning the research study. Once the participant understands the study and the procedures and all questions have been answered, informed consent will be obtained.

After informed consent is obtained, the participant will complete the health history questionnaire. The participant will then have his/her anthropometric measurements assessed. Height and weight will be measured using standardized techniques. Percent body fat will be measured using skinfold calipers. This procedure requires that the thickness of the skin and the subcutaneous fat layer be measured at seven sites on the body (tricep, abdomen, thigh, calf, suprailiac, chest, subscapular) using skinfold calipers

Maximal Graded Exercise Test

Upon arrival to the laboratory for the maximal graded exercise test, each participant's body weight will be measured. The participant will then be fitted for seat height on the cycle ergometer. Seat height will be determined by having the participant's knee within 5–10° of flexion at the pedal's lowest point.

Each participant will then be fitted with a noseclip and a mouthpiece for the collection of expired respiratory gases using the metabolic measurement cart. The metabolic measurement cart measures ventilation and the oxygen and carbon dioxide concentrations of the expired respiratory gases to determine oxygen consumption (VO_2) and carbon dioxide production (VCO_2). The mouthpieces and noseclips will be disinfected after each trial with Cidex disinfectant. Each participant will then be fitted with a Polar heart rate monitor which is a strap that goes around the chest. Lastly, each participant will be instructed on using the Borg Ratings of Perceived Exertion (RPE) chart (appendix A). This procedure requires that the participant point to a number on a chart representing the level of fatigue that he/she feels.

Once instrumentation of the participant is complete, the exercise test will begin. Each participant will exercise on a Bosch cycle ergometer. The exercise test protocol will consist of a 2-min warm-up stage at a light intensity (60 watts) after which exercise intensity will be increased 20 watts every minute. The exercise test will continue until the participant reaches volitional fatigue or is unable to maintain a pedaling frequency of 50 revolutions per minute (RPM). Volitional fatigue is the point during exercise when the participant feels like they can exercise no longer. Although there are no specific criteria for this concept, it is analogous to the exercise being of a sufficient intensity that the participant feels like he or she has reached his or her maximal potential. In other words,

the exercise is just too hard to continue. This feeling might occur due to leg fatigue, overall fatigue, hyperventilation, or other factors relating to maximal exercise. Once the exercise test protocol is terminated, each participant will continue to cycle at a very low intensity for approximately five minutes as an active cool-down.

During the exercise test protocol, expired respiratory gases will be measured continuously using the metabolic measurement cart, heart rate will be measured continuously using the Polar heart rate monitor, and RPE will be assessed during the last 10 seconds of each exercise stage.

Experimental Trials

Each participant will visit the laboratory for three experimental trials consisting of two consecutive days in order to complete each of the three exercise protocols for the assessment of plasma volume expansion. The order of the experimental trials will be counter-balanced and at least 7 days will separate each of the three 2-day trials. During each visit to the laboratory, subjects will be asked questions about alcohol and caffeine consumption within the past 24 to 48 hours. These questions will include the following:

- Have you consumed alcohol or caffeine within the past 24 to 48 hours?

We will be certain to inform those who normally consume caffeine that abstention from caffeine for the 24 hours prior to each experimental trial may produce some symptoms of caffeine withdrawal such as mild headache. Caffeine can increase heart rate and increase nervous system activity. Caffeine and alcohol can cause a person to become dehydrated which may also affect the responses to exercise. After asking the questions, the investigators will then insure that, based upon the answers, each subject will be eligible for participation and that no additional risks are involved. These precautions will be taken in an effort to ensure that subjects are comfortable and fully capable of completing the exercise protocols.

During the experimental trials of "Day 1", similar to the procedures described for the maximal graded exercise test, each participant will have his weight measured. A catheter will then be inserted into one of the antecubital arm veins to allow for subsequent blood samples that will be obtained. Procedures for drawing blood samples will be outlined in a following subsection of this application. The subject will then have a blood pressure cuff placed on the arm opposite the catheter and a heart rate monitor will be secured around his midsection. A 60 minute rest period will ensue to allow the subject to rest and enable the baseline measures of the body to be established. During rest periods, the subject will be permitted to utilize study materials and complete homework or assignments for classes. They will also have the choice of bringing materials for leisure reading and we will also have a television and VCR set up if they chose to watch a movie or other program.

Immediately following the 60 minute rest period a baseline blood sample will then be taken. The subject will be outfitted with a noseclip and mouthpiece as the final instrumentation preparations leading into the exercise test.

Once instrumentation of the participant is complete, the exercise test will begin. Each participant will exercise on a Bosch electronically-braked cycle ergometer. The exercise test protocols will consist of three different exercise sessions with various intensity/duration combinations assigned to each trial. The three different intensity/duration combinations for the exercise protocols will be as follows:

- Protocol #1 will consist of pedaling at an intensity of 50% VO_2max for a duration of 60 minutes;
- Protocol #2 will consist of pedaling at an intensity of 65% VO_2max for a duration of 45 minutes;
- Protocol #3 will consist of pedaling at an intensity of 80% VO_2max for a duration of 30 minutes.

During each exercise protocol, heart rate and VO_2 will be measured continuously via the heart rate monitor and metabolic cart analyzer. Blood pressure and RPE will be taken every five minutes throughout the cycle ergometer test. Following the exercise session, the subject will be returned to a sitting position with the noseclip, mouthpiece, heart rate monitor, and blood pressure cuff all being removed. The subject's body weight will also be measured again. In addition, a second blood sample will immediately be obtained post-exercise. After the blood sample, the subject will undergo a 2 hour rest period. The subject will once again have the opportunity to review study materials and complete homework for classes, read other materials, or watch a movie or TV program with the television and VCR provided.

Directly following the 2 hour rest period, a third blood sample will be obtained. Upon this completion of the experimental trial, the final step is for the catheter to be removed from the arm of the subject.

During the procedures of "Day 2" for each exercise protocol, subjects will then return the following morning – 24 hours later – and have their weight measured. A catheter will once again be placed into an antecubital arm vein. Measurements of blood pressure and heart rate will be determined. The subject will then stay in a seated position for a 60 minute rest period to allow baseline measures to be established within the body. Immediately following the 60 minute rest period, another blood sample will be taken. After this second blood sample, Evans' blue dye will then be injected into the blood stream followed by a 30 minute adjustment period to allow the solution to circulate throughout the blood stream. Three measurements via blood samples will then be taken to determine the actual absolute amount of plasma volume expansion in milliliters. The procedures for Evans' blue dye analysis will be described within a subsequent subsection. Immediately after the 30 minute period of Evans' blue dye analysis and blood samples, the catheter will then be removed from the subject's arm. Each subject will complete this

2-day cycle for each of the three separate exercise protocols, with a minimum of a one-week wash-out period (7 to 10 days) in between two given sessions.

During the exercise test protocol, expired respiratory gases will be measured continuously using the metabolic measurement cart, heart rate will be measured continuously using the Polar heart rate monitor, and blood pressure and RPE will be assessed every five minutes during the exercise test. In addition, catheter blood samples will be obtained to determine percentage of plasma volume concentrations of resting values, immediately post-exercise, and 2-hours post-exercise. Details of the blood sampling procedures will be described in the next section.

Blood Sampling Procedures

As previously mentioned, during the experimental trials, blood samples will be obtained at baseline (after the 60 minute resting period, Day 1), immediately post-exercise (Day 1), 2-hrs post-exercise (Day 1), 24-hrs post-exercise (after the 60 minute resting period, Day 2), and at 10, 20, and 30 minutes of the Evans Blue Dye assessment (end of experimental trial, Day 2).

Blood samples will be analyzed for hemoglobin, hematocrit, albumin, and total protein concentrations using commercially available reagent kits for use with a spectrophotometer. Blood samples obtained during the Evans Blue Dye procedure will be analyzed for dye concentration with the results being used to calculate absolute plasma volume.

During each blood sample, a total of 5 mL of blood will be obtained. The total amount of blood obtained during the two-day Experimental Trial is 35 mL.

All blood samples will be obtained using a 21-gauge ProtectivPlus intravenous catheter. Catheters will be new, sterile, and only used once. This catheter is designed to pull the needle into a protective, plastic sheath to protect the investigator from accidental needle pricks. The catheter will remain in the arm for all of the sessions. However, it is important to emphasize that the catheter is removed at the end of each day of the Experimental Trials. In other words, the participant never leaves the laboratory with the catheter still in the arm. During exercise, the catheter is secured with tape and offers no impedance to performing the exercise session.

The catheter will be inserted by Dr. Cheatham. Dr. Cheatham was trained in the insertion and use of catheters at the John B. Pierce Laboratory at the Yale University School of Medicine while serving as a post-doctoral associate under Dr. Gary Mack. Dr. Cheatham was the investigator on the study "Manipulation of exercise stimulated hormone release and plasma volume expansion." (NIH R01 Funded Project, Dr. Gary Mack Principal Investigator). Dr. Cheatham has performed over 150 catheter insertions. In addition, Dr. Cheatham has completed a phlebotomy safety course.

Prior to the insertion of the catheter, a suitable antecubital vein will be located using a tourniquet. The area will then be cleaned with an alcohol pad and allowed to dry. Once the catheter is inserted, a small amount of blood will be obtained to insure that the blood is free-flowing through the catheter. The catheter will then be flushed with a sterile heparinized saline (0.2 mL heparin/10 mL saline). Prior to each blood sample, a small amount of blood will be collected in a syringe and discarded. This will insure that the subsequent blood sample obtained is not diluted with saline. After the blood sample is obtained, the catheter will again be flushed with the sterile heparinized saline.

At the end of Day 1 of the Experimental Trials, the catheter will be removed and a band-aid will be applied over the collection site.

A new catheter will be inserted into an antecubital vein at the beginning of Day 2 of the Experimental Trials using the same procedures listed above. Again, at the end of Day 2 of the Experimental Trials, the catheter will be removed and a band-aid will be applied to the collection site.

Assessment of Plasma Volume using Evans' Blue Dye

Absolute blood volume will be measured by dilution of a known amount of Evans' blue dye. Evans Blue Dye is an azo dye used in blood volume and cardiac output measurement by the dye dilution method. It is very soluble, strongly bound to plasma albumin, and disappears slowly from the body. This technique involves injection of an accurately determined volume/weight of dye (specific gravity of dye is 1.0) into an arm vein and sampling blood for determination of dye dilution after complete mixing has occurred (at 10, 20, and 30 minutes). The dye itself attaches to plasma albumin and is evenly distributed. Evans Blue Dye has no effect on the body and only a small amount (1.5%) actually leaves the circulation. It has been used extensively in research settings over the past 20-30 years with no reported ill effects. Plasma volume will be determined from the product of the concentration and volume of dye injected divided by the concentration in plasma after mixing, taking into account 1.5% lost from the circulation within the 10 minutes. Blood volume will be calculated from plasma volume and hematocrit concentration and corrected for peripheral sampling. This will occur 24 hours on the morning immediately following the day in which each exercise protocol will be performed. We have included an appendix containing abstracts from previous studies utilizing Evans Blue Dye to serve as a representative sample of the types of studies that the procedure is used for. We also offer these abstracts to demonstrate that the use of Evans Blue Dye is a common procedure used in the study of exercise and plasma volume.

Research Design

This research will employ a repeated measures design. Each participant will complete an experimental trial for each of the three exercise protocols. Due to the use of a repeated measures design, testing of a large number of participants is not necessary and statistical power can be achieved with only six participants. A schematic of the protocol is depicted in Appendix F.

Location of Data Collection

All testing and data collection will be performed in the Exercise Science Laboratory located on the first floor of Western Michigan University's Student Recreation Center.

Duration of the Study

Each participant will visit the laboratory on eight occasions. The initial orientation visit will last approximately 30 minutes. The visit for the maximal graded exercise test will last approximately 60 minutes. There will be three exercise treatments consisting of two consecutive days in order to complete each of the three exercise protocols. The three "first-day" experimental trials will last approximately four to four and a half hours. The three "second-day" experimental trials will last approximately 120 minutes. The two washout periods, in between the three exercise sessions, are both seven to ten days in length. The minimum amount of time for each participant from the beginning of the study to the end of their commitment will be four weeks, however, due to scheduling; completion of the study for each participant may take upwards of six weeks. The requested length of approval for the entire study is one year although data collection should take considerably less time than the requested approval period.

Dissemination of Results

Once the research study is completed, a manuscript will be submitted to an appropriate exercise science related journal for publication.

METHODS OF ANALYSIS

Sample size was determined using a power analysis. An alpha level of 0.05 and a beta level of 0.2 were established a priori. Mean values for plasma volume expansion and the variability of the plasma volume measurement were obtained from previous research utilizing a similar sample population. To achieve a power level of 0.81 and to detect a treatment difference of 6% in the plasma volume expansion, a sample size of six is necessary. Data on the skinfold measurements of participants will be used to calculate percentage body fat. This information will be used as a participant/population descriptor.

Data on the blood measurements, plasma volume expansion, heart rate, and blood pressure will be analyzed using a two-way ANOVA with repeated measures. The two factors will be treatment (i.e. protocol number) and time with both factors being repeated measures factors.

If necessary, post-hoc analysis of the data will be performed using a simple effects analysis with a Bonferroni adjustment.

The level of statistical significance will be established *a priori* as $P < 0.05$. The SPSS statistical package will be used for data analysis.

BENEFITS OF RESEARCH

The benefits of the study to the participants of the research study include an increase in knowledge about their blood plasma volume expansion levels for various exercise bouts as well as their perfusion capabilities and aerobic fitness level. Following the data collection and analysis, each subject will be informed of their total absolute plasma volume expansion, as well as their percentage increase in plasma volume. We will also inform them of the benefits to this training adaptation and how the body has been acclimated to respond to exercise with better performance. Because all three experimental trials involve exercise intensities at a given relative percentage of VO_2max, a measurement of the maximal oxygen uptake of each participant is obtained. Maximal oxygen uptake is the single, best indicator of aerobic fitness. Therefore, that information will be provided to the participant and we will educate that person as to how his or her value compared to population norms. In addition, the participants will gain exposure to the procedures involved in scientific research.

The benefits to the investigators as well as the scientific community include an increase in knowledge with regards to the effects of the intensity and duration relationship on plasma volume expansion during exercise and the possible implication of these results on the ability to perform aerobic exercise activity.

SUBJECT SELECTION

Participants will be solicited from the Student Recreation Center at Western Michigan University. The exact procedures to recruit participants will be to personally inquire for potential volunteers, while also having HPER Exercise Science professors make announcements to their students for volunteer interest within their classes at the Student Recreation Center. If a prospective participant calls inquiring about the study, he will be told the general purpose of the study and the requirements for the participants as listed in the consent form.

When potential participants call to inquire about the study, they will be informed of the basics of the investigation and the requirements of being involved as a subject. "We are going to be performing a study involving plasma volume expansion. The purpose is to see how various acute sessions of exercise affect the water content (plasma volume) within the bloodstream, and to see which protocol causes the greatest plasma volume expansion. Three various exercise protocols of 30, 45, and 60 minutes will need to be completed. The time requirement for the study is about four to six weeks. You will be required to visit the laboratory on eight separate occasions. The first and second visits will last about 45 minutes. The remaining six days will be three two-day trials, in which the first day visits will last about 4 to 4.5 hours, and the second day visits will last about 2 hours. This study includes blood sampling, which means that you will have a catheter inserted during the six experimental trials. The location is in the Exercise Physiology Laboratory on the first floor of the SRC on the campus of WMU. If you are interested, then you will have an orientation visit in which more details of the study will be reviewed

through the informed consent document and you will have the opportunity to ask any other questions. Thank you for your interest and for the call."

Participants must be between the ages of 18 and 35 years. Any male over the age of 45 years and any female over the age of 55 years are automatically classified as "moderate-risk" according to the ACSM Guidelines for Exercise Testing and Prescription. Because we will only test persons classified as "low-risk" we have chosen the upper age range of 35 years to allow for an even greater safety margin.

Six recreationally active male subjects will be used for the study. Recreationally active subjects will be any person that exercises or is physically active for less than 10 hours a week and that is not involved in organized competitive sports. The minimum time of activity per week needs to be 1.5 total hours. Participants cannot be taking any type of ergogenic aids or supplementation including vitamin and minerals. In order to assess their level of activity and if they are recreationally active, we will ask the subject how often they exercise or participate in physical activity or recreational sports. We will then ensure that they are not trained for athletic or competitive sports and competition. We will also ask the subject if they are currently taking, or have recently taken any supplements or ergogenic aids for performance or physique enhancement, making certain that they may not participate if they have been taking anything that falls within this description for the requirements of the study.

The reason for choosing to include only males for this investigation and not females is due to the fact that this is the first research study to examine this specific topic in regards to plasma volume and the intensity/duration relationship. Females are known to have various hormonal responses throughout the menstrual cycle, hence changing physiological responses to exercise. Thus, in order to control the data as much as possible, keeping this initial study limited to investigating male subjects will prevent any confounding results that may potentially come about from female participation. Subsequent studies in the future that may also examine this topic would have the opportunity to include female subjects and determine if the physiological response is similar to males and how it varies throughout the menstrual cycle encountered by females.

Each participant will also complete a health history questionnaire to determine each participant's level of risk stratification as outlined in the American College of Sports Medicine's (ACSM) Guidelines for Exercise Testing and Prescription (appendix C). Only participants with the ACSM Risk Stratification Level of Low Risk will be eligible to participate in the study. ACSM Guidelines have established that it is not necessary for participants who are classified at the level of Low Risk to have a physician's exam prior to maximal exercise testing. Each individual not meeting the inclusion criteria will be read the exclusion script below:

> "I regret to inform you that based on your answers to the inclusion and exclusion questionnaire you do not meet the inclusion criteria for this study and will not be permitted to participate. Although you are not permitted to participate in this study at this time you will assume no prejudice, penalty, or

risk of loss of service you would otherwise receive. Thank you for your time and consideration in participating in this process."

RISK TO SUBJECTS

- Intense, near-maximal exercise and/or long duration exercise can cause fatigue, weakness, dizziness, and/or disorientation.
- Risks associated with blood sampling can include infection, soreness, and possible hematoma.
- By following proper testing procedures, these risks will be minimized. The procedures to minimize these risks are outlined in the "Protection for Subjects" section of this document.
- The abstention from caffeine for the 24 hours prior to each experimental trial may produce some symptoms of caffeine withdrawal such as a mild headache.
- There is a risk for an allergic reaction to Evans Blue Dye. However, the likelihood of an allergic reaction is extremely minimal. The principal investigator has previously worked in the laboratory of Dr. Gary Mack at the Yale School of Medicine. Dr. Mack is one of the leading researchers on plasma volume. During his 20 years of research and use of Evans Blue Dye, his laboratory has never observed an allergic reaction.
- There is a significant time commitment by participating in this study, which may be an inconvenience. In order to minimize the inconvenience, the investigators will be willing to adjust meeting times for any of the visits, so that they avoid conflict with class/work schedule. These meeting times must also work around the schedules of the investigators and be adequate for the purpose of the study.

PROTECTION FOR SUBJECTS

The risks associated with intense, near-maximal exercise will be minimized by the inclusion of participants who only meet the level of ACSM Risk Stratification of Low Risk. The risk of a major cardiac event during exercise testing in this participant population (Low Risk) is less than 0.1 incidences per 10,000 tests (1). In addition, heart rate and blood pressure will be monitored continuously throughout the exercise testing in order to ensure that each participant is having a normal response to the exercise testing. Lastly, the investigators are trained in CPR techniques and emergency procedures. A spotter will be standing next to the cycle ergometer throughout the entire test if the participant becomes dizzy or disoriented.

The risks associated with blood sampling will be minimized by using universal precautions. The area of blood sampling (antecubital forearm) will be cleaned prior to blood sampling using an alcohol swab. All supplies utilized for the blood collection will be new, sterile and only used once. All investigators will wear lab coats and latex gloves throughout the testing. All blood sampling supplies will be properly disposed of in biohazard bags and a sharps container after use. If complications arise during the blood collection, the participant will be referred to Sindecuse health center for further evaluation.

It will be explained to each participant that he/she is allowed to terminate testing at anytime for any reason during the study. It will be explained that the participant will suffer no penalty or prejudice from any investigator if they chooses to terminate their participation in the study.

Prior to the beginning of the study, each participant will be explained the details of the research protocol and all risks associated with the research. Each participant will be given the opportunity to ask any questions he/she might have about the research and the protocols employed.

The faculty advisor (principal investigator), Dr. Christopher Cheatham, is experienced in administering maximal exercise tests as well as blood collection using intravenous catheters. The principal investigator has been trained in the insertion and collection of blood using intravenous catheters during his position as a post-doctoral fellow at the John B. Pierce Laboratory at the Yale University School of Medicine. In addition, the principal investigator has completed a safety course as the proper procedure for collecting and processing blood samples. The student investigator, Chris Gregory, will be trained on the proper testing procedures prior to any interaction with any participant, however, only the principal investigator will be involved with inserting the catheters and obtaining the blood samples

In addition, both investigators are trained in CPR and Chris Gregory is a certified CPR and First Aid Instructor.

During testing, there will be no other individuals in the immediate testing area except for those involved in data collections.

Lastly, in the unlikely event of an emergency, campus police (7-5555) will be immediately called which will initiate the process for the arrival of emergency personnel. During safety training provided by the University, it was stated to call campus police instead of immediately dialing 911.

CONFIDENTIALITY OF DATA

All participants will have their privacy protected. At the start of the study each participant will be assigned a case number for data recording purposes. The principal and student investigators will have a list of participant names, contact information and case numbers that will be used for contact with the participants during the course of the study. The data and results from this study will be stored and locked in a file cabinet in the Exercise Science Laboratory of Western Michigan University with only the principal investigator having access to the data. Data will be kept on file for a minimum of three years at which time it may be destroyed.

No names or pictures of the participant will be used in any subsequent publication of the research data.

INSTRUMENTATION

A copy of the Borg RPE scale to be used (Appendix A) is included. The health history questionnaires (Appendix B, C) to be used are included with this document. The flyer (Appendix D) that will be posted around campus for the recruitment of participants is included. Collection of blood questionnaire is included (Appendix E). Appendix F contains a schematic overview of the research protocols for the Experimental Trials.

INFORMED CONSENT PROCESS

When a prospective participant first contacts the student investigator and expresses interest in participating in the study, the student investigator will briefly go over the purpose of the study and a summary of the major research protocols (i.e. exercise test, antecubital forearm blood draws, blood plasma measurements/expansion). If the prospective participant is still interested, the student investigator will make an appointment with the individual and explain that at that time all of the research protocols, risks, benefits, etc. will be explained and all questions will be answered. The student investigator will also explain that the prospective participant should come to that meeting with the appropriate attire (i.e. t-shirt, shorts) so that if the person does choose to participate then skinfold and anthropometrical measurements can be made.

During the initial contact over the phone when a potential participant expresses interest in being involved in the study, the student investigator will review the script (under "Subject Selection" p. 9) over the phone with that particular student. Following the phone contact, there will then be a subsequent in-person meeting between the student investigator, project advisor, and potential participant in order to review the documents (informed consent) pertaining to this study. If the student is still interested and would like to be involved as a subject in the study, then a meeting time for an individual orientation will be determined in an effort to begin the experimental trials and exercise protocols.

As previously discussed, each participant will read the informed consent form and the research protocols will be explained by the student investigator. Each participant will then be given the opportunity to ask any questions he might have concerning the research study. Once all questions have been answered, those who still want to participate will be invited to sign the consent document. A copy of the informed consent form signed by the participant and the investigators will be given to each participant to take with them.

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APPENDIX B

HUMAN SUBJECTS INSTITUTIONAL REVIEW BOARD INFORMED CONSENT

Western Michigan University
Department of Health, Physical Education, and Recreation

Principal Investigator: Christopher C. Cheatham, Ph.D.
Student Investigator: Christopher Gregory, BS, CSCS

You have been invited to participate in a research project entitled ***"The Effects of Acute Exercise on Plasma Volume In Men – Variations of Intensity/Duration Relationship."*** This study is Christopher Gregory's thesis project. This consent document will explain the purpose of this research project and will go over all of the time commitments, the procedures used in the study, and the risks and benefits of participating in this research project.

Study Purpose & Overview

The purpose of this study is to determine if variations of exercise intensity and duration will influence plasma volume expansion after an acute exercise session on a cycle ergometer. Plasma volume expansion occurs within 24 hours after an moderate to intense exercise session and can be a beneficial adaptation for training and exercise. Plasma volume is the amount of water content within the blood, which is the clear substance aside from the red blood cells. Plasma volume expansion is the increase of the water content within the blood. Many research studies have shown plasma volume expansion to take place after chronic endurance exercise training, as well as acute, high-intensity exercise sessions. We are interested in looking at which exercise variable, intensity or duration, plays a more influential role in regards to plasma volume expansion. Also, we are interested in being able to understand to what extent each variable causes changes in plasma volume concentrations. Because increased total plasma volume can help allow for more efficient sweat rates and smoother flowing blood throughout the body, thus placing less stress on the heart and vessels, athletic performance can likely be improved through training and greater plasma volume. Ultimately, the goal is to then relate the results to exercise recommendations for the average individual.

Requirements for this study include a significant time commitment, a specific range of physical activity, and the insertion of a catheter into an arm vein during the experimental trials. Participation in the study will be over a 4 to 6 week period. The first orientation visit and the maximal graded exercise test will each last approximately 45 minutes. There will also be six days of experimental trials (three 2-day trials): three of those would last about 4 to 4.5 hours, while the other three would last about 2 hours. You must be recreationally active, which means exercise participation within a range of 1.5 to 10

hours per week. This study involves blood sampling; thus a catheter, or IV, will be inserted in an arm vein during the six days of experimental trials.

Qualifications to Participate in this Research

To be able to participate in this research project, you must meet the following criteria:

- You must be between the ages of 18 and 35 years old.
- You must be of the male gender.
- You must be recreationally active. This means that you exercise for no more than **10** hours per week and that you are not involved in any competitive sports.
- You must fill out a health history questionnaire and only if the results from this questionnaire place you in a category of "Low Risk" for exercise will you be allowed to participate.
- You must not be currently taking any supplements such as sports supplement, vitamins, or minerals.

Duration of the Study

You will be asked to come to the Exercise Physiology Laboratory of Western Michigan University located on the first floor of the Student Recreation Center three times. The first visit will be an "Orientation" visit and the second visit will be for the "Maximal Graded Exercise Test". The final six visits will be for the "Experimental Trials/Exercise Tests".

The "Orientation" visit will take approximately 30 to 45 minutes. The "Maximal Graded Exercise Test" will take approximately 60 minutes. The "Experimental Trials/Exercise Tests" will consist of three two-day trials thus resulting in 6 total days. The three "first-day" experimental trials will last approximately 4 to 4 1/2 hours. The three "second-day" experimental trials will last approximately 2 hours.

You will be asked to complete an exercise session of Day 1 of each of the three Experimental Trials. It is also necessary to have a 7 – 10 break period between each of the "Experimental Trials/Exercise Tests". The break period is so the effects of one "Experimental Trials/Exercise Tests" don't effect the next trial.

Your participation in this study should last about four total weeks, but no more than six weeks.

Study Procedures

You will be asked to attend eight sessions with Chris Gregory and Dr. Cheatham in the Exercise Physiology Laboratory located in the Student Recreational Center on the campus of Western Michigan University. The first session will be an "Orientation" visit and the second one will be the "Maximal Graded Exercise Test". The final six visits will be for the "Experimental Trials/Exercise Tests". You will not have to change your exercise program or diet during the study. However, you will be asked to not exercise for the 24 hours before you come in for the Experimental Trials/Exercise Tests. We will also ask that you not drink any alcohol or take any caffeine the day before and the day of your visits to the laboratory.

The student investigator will call you 48 hours prior to your appointments to remind you about the time and location of the study.

Orientation Visit

When you arrive to the laboratory for the "Orientation" visit, one of the investigators will go over this consent form with you and explain the study and all of its procedures, risks, and benefits to you. You will be encouraged to ask any questions that you may have. If you decide to participate, one of the investigators will ask you to sign this consent form. We will ask you to wear a t-shirt and shorts for this visit.

You will also complete a health history questionnaire. The investigators will use this information to classify your "risk level" for exercise based on guidelines established by the American College of Sports Medicine. You can participate in this study only if your "risk level" is "Low-Risk". This risk-level procedure will be explained to you.

You will also have your anthropometric measurements assessed. This means that we will measure your height, weight, and percentage body fat. Body fat percentage will be measured using skinfold calipers. We will measure the thickness of your skin and the layer of fat underneath it at seven different places on your body. We will then enter these numbers into a math equation to determine your percentage body fat.

Graded Exercise Test

The purpose of this part of the experiment is to find out how well your body is able to use oxygen during exercise. To do this, you will exercise for about 15 to 20 minutes on a cycle ergometer. You will need to bring with you a set of exercise clothes (t-shirt, shorts, athletic/ running shoes). During the exercise period, you will be asked to breathe through a plastic mouthpiece so that we can measure how much air you breathe and how much

oxygen is in the air you breathe out. The exercise period will begin at a very low intensity and then gradually increase every 2 minutes. You will pedal until you are tired and can exercise no longer.

Experimental Trials/Exercise Tests

For the "Experimental" trials, we ask that you come to the laboratory wearing a t-shirt, shorts, and running/gym shoes. We will then measure your weight using a scale. After we measure your weight, we will strap a heart rate monitor around your chest. We will also place a blood pressure cuff around your arm. We will explain how to use the Borg Ratings of Perceived Exertion Scale. This is a scale with numbers and words describing how hard the exercise is. To use the scale, you simply point to a number.

You will then sit on the stationary cycle ergometer and we will adjust the height of the seat so you are comfortable. We will then hook you up to the "Metabolic Measurement Cart" so that we can measure how much oxygen your body uses during exercise and how much carbon dioxide your body produces. To do this, you will breathe through a clean, sanitized mouthpiece (similar to a snorkel mouthpiece) and you will wear a pair of noseclips so that you can only breathe through your mouth. The air you blow out during exercise goes into the "Metabolic Measurement Cart" and the amount of oxygen and carbon dioxide is measured.

We will then begin the exercise test. There are three different exercise protocols. Each protocol will be performed once and only one protocol will be performed during each specific experimental trial. The exercise test protocols will consist of three different exercise sessions with various intensity/duration combinations assigned to each trial. The three different intensity/duration combinations for the exercise protocols will be as follows:

- Protocol #1 will consist of pedaling at an intensity of 50% VO_2max for a duration of 60 minutes;
- Protocol #2 will consist of pedaling at an intensity of 65% VO_2max for a duration of 45 minutes;
- Protocol #3 will consist of pedaling at an intensity of 80% VO_2max for a duration of 30 minutes.

During the exercise test, we will measure your heart rate every minute using the heart rate monitor and we will ask you how you feel using the Borg scale. We will also measure blood pressure every 5 minutes with a standard blood pressure cuff and stethoscope.

When you are finished with the exercise test, we will take the mouthpiece and noseclips off and you will ride the bike for a few minutes at a very low level for a cool-down period. Once you finish on the cycle ergometer, we will remove the heart rate monitor and blood pressure cuff.

Blood Sampling Procedures

Upon arrival to the laboratory for the "Exercise Test/Experimental Trial" days (after taking a weight measurement), you will have an intravenous catheter inserted in one of your arm veins. This catheter will stay in place for the whole time that you are in the laboratory. During the
"first-day" experimental trials, blood samples will be obtained from these catheters on three separate occasions, one time before the exercise protocol and twice post-exercise. During the "second-day" experimental trials, four blood samples will be obtained. The samples are obtained by attaching a syringe to the catheter and pulling the plunger. The use of a catheter means we don't have to repeatedly insert a needle into your vein. Once the catheter is inserted, blood samples are obtained simply by attached a syringe to the catheter and pulling the plunger of the syringe. The total amount of blood we are taking during the two-day Experimental Trial is approximately 35 mL (about 2-3 tablespoons). The removal of this amount of blood has no negative impact on your body. Universal precautions will be taken during these procedures to ensure safety and sterilization, and the investigators are qualified to perform the procedures.

Measurement of Plasma Volume

Plasma volume will be measured at the end of the second morning for each of the three experimental trials. This procedure requires that a small amount of dye (Evans' Blue Dye) be infused through the catheter in the arm. Then, small blood samples are taken 10, 20, and 30 minutes after the injection of the dye. Evans Blue Dye has no effect on your body. It is simply a way for us to measure how much plasma you have in your body.

Possible Risks of Your Participation In This Study

Risks and inconveniences associated with intense exercise include muscular fatigue and possibly muscle soreness on the following day. The exercise will be stressful but is generally easily tolerated by individuals and is not dangerous for healthy individuals. The investigators are trained in performing exercise tests and are familiar with emergency procedures.

The risks associated with the drawing of intravenous blood samples include soreness, bruising, and infection. These risks are minimized by observing proper sterile

techniques. Also, the investigators are properly trained and have much experience in taking blood samples. Universal precautions will be taken during these procedures to ensure safety and sterilization, and the investigators are qualified to perform the procedures. Intravenous catheter insertion will be performed by an experienced professional, thereby reducing the risk of infection. The total volume of blood drawn during any portion of the protocol will not exceed 35 mL (about 2-3 tablespoons), or about one-tenth of normal blood donation. The removal of this amount of blood poses no risk to a healthy subject.

The overall time commitment to be a subject for this study is significant, thus the time factor may be an inconvenience.

As in all research, there may be unforeseen risks to the participant. If an accidental injury occurs, appropriate emergency measures will be taken; however, no compensation or additional treatment will be made available to you except as otherwise stated in this consent form. You will NOT be compensated for participating in this study.

Benefits of Your Participation in this Study

From your participation in this study, you will learn about your body composition, or in other words, your percent body fat. You will also learn about your fitness level. The investigators will explain all your results to you.

You will also benefit by learning about research and some of the laboratory procedures used in collecting research data.

Conditions of Participation in the Study

There are conditions that must be met in order for you to participate in this study. One is being physically active for less than 10 hours a week in recreational activities and at least a minimum of 1.5 hours. This does not include organized competitive sports. You must be a male between the ages of 18-35 years. You cannot be a competitive athlete. You must not be taking any ergogenic aids or supplements, including vitamins and minerals for the past 14 days.

We also ask that you follow all of the study guidelines, such as not exercising, not drinking alcohol, and not taking any caffeine for the day before and the day of your visits to the laboratory for the "Experimental Trials/Exercise Tests."

Because the data collected could be affected if you do not follow these guidelines, we ask that you tell us if you did not follow any of the guidelines. You will not suffer any

penalties from the investigators if you do not follow the guidelines, but it is important for us to know. In this case, we can reschedule one of your appointments or you can choose not to participate in the study any longer.

Confidentiality of Your Results

In order to maintain confidentiality the study will be focused on group data and an identification number (rather than the subject's name) will be used to record data. Following the study, the primary investigator and the research committee will have access to the original data. The original data will be retained in a locked cabinet for a minimum of three years after the completion of the study in the department of Health, Physical Education, and Recreation at Western Michigan University and then destroyed.

If the results of the study are published in a journal or presented at a conference, no names will ever be used.

Withdrawal from the Study

You can choose to stop participating in the study at anytime for any reason. You will not suffer any prejudice or penalty by your decision to stop your participation. You will experience NO consequences either academically or personally if you choose to withdraw from this study.

The study investigators can also decide to stop your participation in the study without your consent.

Should you have any questions prior to or during the study, you can contact the student investigator, Chris Gregory, at 269-321-8497 or 269-387-2689 (campus GA office), or the primary investigator, Dr. Christopher Cheatham at 269-387-2542. You may also contact the Chair, Human Subjects Institutional Review Board at 269-387-8293 or the Vice President for Research at 269-387-8298 if questions arise during the course of the study.

This consent document has been approved for use for one year by the Human Subjects Institutional Review Board (HSIRB) as indicated by the stamped date and signature of the board chair in the upper right corner. Do not participate in this study if the stamped date is older than one year.

"I have read this informed consent. The risks and benefits have been explained to me. I agree to take part in this study."

_____
Please Print Your Name

_____ _____
Participant's Signature Date

Permission obtained by: _____ _____
Signature of Investigator Date

APPENDIX C

HUMAN SUBJECTS INSTITUTIONAL REVIEW BOARD APPROVAL LETTER

WESTERN MICHIGAN UNIVERSITY

Centennial 1903-2003 Celebration

Human Subjects Institutional Review Board

Date: August 26, 2004

To: Christopher Cheatham, Principal Investigator
Christopher Gregory, Student Investigator for thesis

From: Amy Naugle, Ph.D., Interim Chair

Re: HSIRB Project Number: 04-07-08

This letter will serve as confirmation that your research project entitled "The Effects of Acute Exercise on Plasma Volume in Men – Variations of Intensity/Duration Relationship" has been **approved** under the **full** category of review by the Human Subjects Institutional Review Board. The conditions and duration of this approval are specified in the Policies of Western Michigan University. You may now begin to implement the research as described in the application.

Please note that you may **only** conduct this research exactly in the form it was approved. You must seek specific board approval for any changes in this project. You must also seek reapproval if the project extends beyond the termination date noted below. In addition if there are any unanticipated adverse reactions or unanticipated events associated with the conduct of this research, you should immediately suspend the project and contact the Chair of the HSIRB for consultation.

The Board wishes you success in the pursuit of your research goals.

Approval Termination: July 21, 2005

Walwood Hall, Kalamazoo, MI 49008-5456
PHONE: (269) 387-8293 FAX: (269) 387-8276

APPENDIX D

HEALTH HISTORY QUESTIONNAIRES

TABLE 2. AHA/ACSM Health/Fitness Facility Preparticipation Screening Questionnaire

Assess your health needs by marking all *true* statements.

History

You have had:

_____ a heart attack
_____ heart surgery
_____ cardiac catheterization
_____ coronary angioplasty (PTCA)
_____ pacemaker/implantable cardiac defibrillator/rhythm disturbance
_____ heart valve disease
_____ heart failure
_____ heart transplantation
_____ congenital heart disease

If you marked any of the statements in this section, consult your healthcare provider before engaging in exercise. You may need to use a facility with a medically qualified staff.

Symptoms

_____ You experience chest discomfort with exertion.
_____ You experience unreasonable breathlessness.
_____ You experience dizziness, fainting, blackouts.
_____ You take heart medications.

Other health issues:

_____ You have musculoskeletal problems.
_____ You have concerns about the safety of exercise.
_____ You take prescription medication(s).
_____ You are pregnant.

Cardiovascular risk factors

_____ You are a man older than 45 years.
_____ You are a woman older than 55 years or you have had a hysterectomy or you are postmenopausal.
_____ You smoke.
_____ Your blood pressure is >140/90.
_____ You don't know your blood pressure.
_____ You take blood pressure medication.
_____ Your blood cholesterol level is >240 mg/dL.
_____ You don't know your cholesterol level.
_____ You have a close blood relative who had a heart attack before age 55 (father or brother) or age 65 (mother or sister).
_____ You are diabetic or take medicine to control your blood sugar.
_____ You are physically inactive (ie, you get <30 minutes of physical activity on at least 3 days per week).
_____ You are >20 pounds overweight.

If you marked 2 or more of the statements in this section, consult your healthcare provider before engaging in exercise. You might benefit by using a facility with a professionally qualified exercise staff to guide your exercise program.

_____ None of the above is true.

You should be able to exercise safely without consulting your healthcare provider in almost any facility that meets your exercise program needs.

AHA/ACSM indicates American Heart Association/American College of Sports Medicine.

These forms are taken from the following reference:

Gary J. Balady, MD, Chair; Bernard Chaitman, MD; David Driscoll, MD; Carl Foster, PhD; Erika Froelicher, PhD; Neil Gordon, MD; Russell Pate, PhD; James Rippe, MD; ; Terry Bazzarre, PhD. Recommendations for Cardiovascular Screening, Staffing, and Emergency Policies at Health/Fitness Facilities. Circulation. 97:2283-2293, 1998.

Par-Q

Please read the questions carefully and answer each one honestly. Circle YES or NO.

1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor.	YES	NO
2. Do you feel pain in your chest when you engage in physical activity?	YES	NO
3. In the past month, have you had chest pain when you were not doing physical activity?	YES	NO
4. Do you lose your balance because of dizziness or do you every lose consciousness?	YES	NO
5. Do you have a bone or joint problem that could be made worse by a change in your physical activity?	YES	NO
6. Is your doctor currently prescribing drugs for a blood pressure or heart condition?	YES	NO
7. Do you know of any other reason you should not participate in physical activity engage in physical activity?	YES	NO

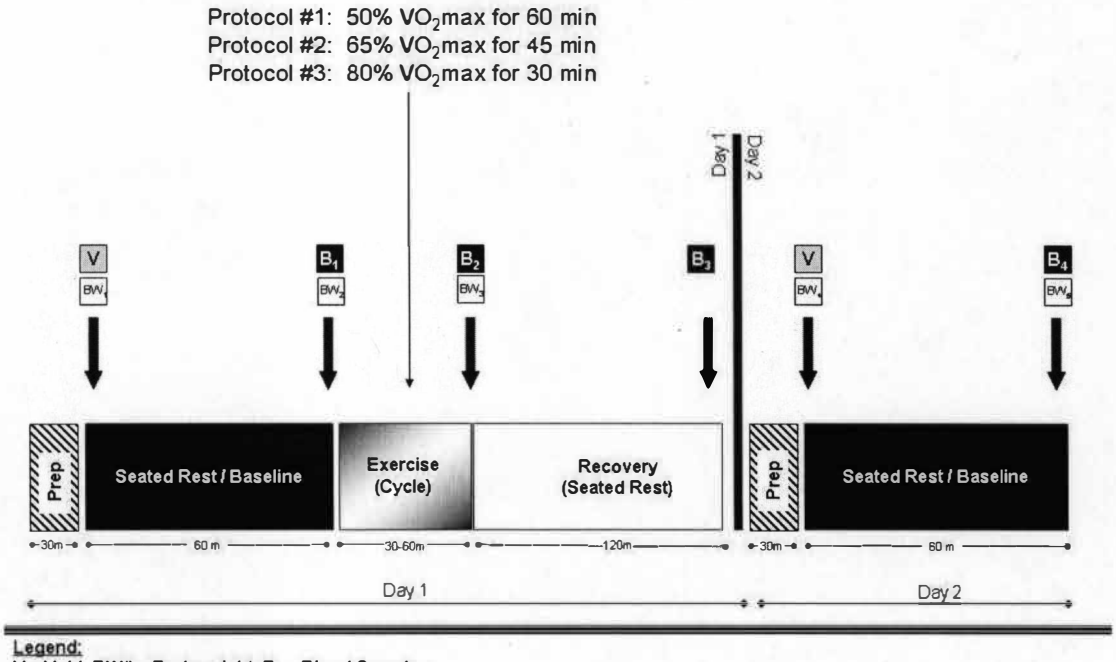
I have read, understood, and completed this questionnaire. Any questions I had were answered to my full satisfaction.

Name _____ Date _____

Signature _____ Witness _____

APPENDIX E

EXPERIMENTAL TRIALS PROTOCOL SCHEMATIC



Legend:
V = Void, BW# = Bodyweight, $B_{\#}$ = Blood Sample

APPENDIX F

ABSTRACT FOR MEDICINE AND SCIENCE IN SPORTS AND EXERCISE

ABSTRACT

MEDICINE AND SCIENCE IN SPORTS AND EXERCISE

PURPOSE: To examine the exercise variables of intensity and duration in relation to PV expansion during an acute bout of exercise, when the total amount of work performed was kept constant. **METHODS:** Six male subjects completed a maximal graded exercise test and three two-day experimental trials: 50% VO_{2peak} (60 minutes), 65% VO_{2peak} (45 minutes), and 80% VO_{2peak} (30 minutes). Measurements of hematocrit, hemoglobin, and serum albumin and total protein concentrations were obtained before exercise (BASE), immediately post-exercise (Post-EX), 2-hours post-exercise (2-hr REC), and 24-hours post-exercise (24-hr REC). **RESULTS:** Relative to BASE, no PV expansion was observed at 24-hr REC in any of the three exercise protocols (50%: -7.8 ± 7.7%; 65%: -4.9 ± 5.2%; 80%: -1.2 ± 7.3%; $P > 0.05$). However, the recovery in PV from Post-EX to 24-hr REC was greatest for the 80% VO_{2peak} protocol (50%: -1.6 ± 7.1%; 65%: 3.2 ± 3.0%; 80%: 11.8 ± 4.7%; $P < 0.05$). There was no difference in serum albumin concentration between BASE and 24-hr REC for any of the three protocols. However, there was a significant increase in serum albumin concentration from Post-EX to 24-hr REC for both the 65% and 80% VO_{2peak} protocols. Although none of the protocols resulted in PV expansion 24-hr post-exercise, the highest intensity/shortest duration protocol (80% VO_{2peak}) resulted in the greatest recovery in PV after the post-exercise decline and the 65% and 80% VO_{2peak} protocols resulted in the greatest increase in serum albumin concentration from Post-EX to 24-hr REC. **CONCLUSION:** This data suggests that higher

intensity exercise may result in a more beneficial PV response compared to lower intensity exercise even when the total amount of work is kept constant.