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Low-intensity strength training with partial vascular occlusion (PVO) was reported to result in muscle hypertrophy and strength increases similar to high-intensity training without PVO. Resistance training has been reported to increase markers of oxidative stress and inflammation. A recent study reported that PVO by itself may result in elevated oxidative stress markers.

The purpose of this study was to examine the effects of PVO on oxidative stress and inflammatory markers.

Twelve resistance trained males (18-35yrs) completed three sets of elbow flexion at either moderate (70% 1RM) or low (30% 1RM) intensity with or without PVO. Two rest (R) conditions done with and without PVO were also included. All exercise conditions were done to failure with the exception of one condition done at 30%1RM and repetition matched to the 30%1RM condition with PVO. The seven conditions were completed at least72 hours apart in a counterbalanced fashion over 3-4 weeks. Blood was obtained before and immediately after each condition. Protein carbonyls (PC), glutathione ratio (GSSG/TGSH), xanthine oxidase (XO), oxygen radical absorbance capacity (ORAC), and interleukin-6 (IL-6) were analyzed in the plasma.

The addition of PVO impacted the number of repetitions done and time to completion in both the low and moderate intensity conditions. The analysis of PC levels revealed interaction effects which post hoc analysis revealed a time effect for exercise.

Glutathione ratio measures revealed a PVO main effect independent of intensity level or time. ORAC analysis revealed significant interaction effects which were intensity x PVO, intensity x time, and PVO x time interactions. XO activity analysis noted an intensity x time interaction resulting from decreases in XO activity over time in both the moderate and low intensity conditions that were not observed in the rest condition. Analysis of IL-6 levels revealed an intensity x time interaction with a significant increase over time for the moderate intensity condition when compared to the rest condition. A PVO x time interaction was also noted and subsequent post-hoc analysis revealed a significant increase over time for the conditions with PVO. This resulted in a greater IL-6 increase over time in conditions with PVO compared to without PVO. The effect of completing each set to failure as opposed to repetitions matched resulted in no differences between low intensity groups without PVO.

In summary, this study shows that partial vascular occlusion can increase oxidative stress and inflammation independent of exercise and that combined with low or moderate intensity exercise there was not a significant change in the variables determined using the elbow flexor muscle group.

THE EFFECT OF PARTIAL VASCULAR OCCLUSION ON OXIDATIVE STRESS AND INFLAMMATORY MARKERS IN YOUNG RESISTANCE TRAINED INDIVIDUALS

by

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APPROVAL PAGE

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CHAPTER I

INTRODUCTION

Resistance exercise can have a significant effect on muscle physiology. Resistance exercise training with an intensity exceeding 65% of an individual's one repetition maximum (1RM) is typically required for increasing muscle size and strength (McDonagh 1984). Resistance exercise with intensity lower than 65% 1RM can often result in significant improvements in the muscle's oxidative capacity without considerable effect on muscular size (Holloszy 1976).

Recent studies have shown that when low-intensity strength training associated with partial vascular occlusion (PVO) is utilized both muscle hypertrophy and strength increases are similar to high-intensity training without PVO (Takarada et al. 2000, Takarada et al. 2002, and Abe et al. 2006). This type of training utilizes modified blood pressure cuffs to alter blood flow to certain areas of the body. The modified blood pressure cuffs are placed proximal to the exercising muscles and result in partial occlusion of the vasculature. This decreases the amount of oxygenated blood getting to the working muscle and can result in blood pooling distal to the partial occlusion.

Takarada *et. al.*(2000, 2002) reported significant increases in muscle size (cross sectional area), strength(isometric and isokinetic) and endurance in trained and untrained individuals with resistance training using intensities of 20-50% 1RM with PVO compared to without PVO. These strength increases were similar to strength increases

reported with high intensity resistance exercise (80%1RM) without PVO. Abe *et. al.*(2006) examined muscle size and muscle strength following walk training with blood flow restriction and noted significantly greater post-training thigh muscle size (cross sectional area and muscle volume) and strength (increased knee extension 1-RM and isometric strength) in the occlusion group. No changes in thigh muscle size or strength changes were reported in the control group. Research has reported that resistance training can increase markers of oxidative stress (Bloomer et al. 2006, Hudson et al. 2008) and it was recently reported that PVO by itself may result in an increase in markers of oxidative stress (Goldfarb et al. 2008).

Oxidative stress occurs when the generation of reactive species, also known as free radicals, in a system exceeds the system's ability to neutralize and eliminate these molecules. Accumulation of excess free radicals can damage a cell's lipids, protein, or DNA. Acute oxidative stress can also activate signaling pathways leading to cellular adaptation and protection against future stressors. Chronic oxidative stress can result in adverse situations that may lead to cell damage or even cell death (Powers et. al. 2010).

Measurement of oxidative stress can be done directly and indirectly. Direct measurement of free radicals is difficult as free radicals are highly reactive, have short half-lives and require expensive equipment. Indirect measurements are therefore more common and involve the measurement of a decrease in antioxidants, the disturbance of cellular redox balance, and markers of oxidative damage to cellular components (i.e. lipids, proteins, and/or DNA) (Powers and Jackson, 2008).

High intensity resistance exercise has been shown to result in an increase in oxidative stress (Hudson et al. 2008, Alessio et al. 2000, Zembron-Lacny et al. 2008, and Bloomer et al. 2007). Protein carbonyls (PC) are commonly used as oxidative stress biomarkers and indicate the oxidation of proteins due to increased free radicals (Powers and Jackson, 2008). Hudson (2008) reported a significant increase in PC immediately and 60 minutes after an exercise bout of 11 sets of three repetitions of squats done at 90% 1RM; the same increase in PC was reported after an exercise bout of 4 sets of 10 repetitions of squats done at 75% 1RM. Bloomer (2007) reported that PC were significantly elevated immediately following a squat test consisting of a single set of 15 repetitions using a load equal to 70% 1RM.

Lipid hydroperoxides and lipid peroxides are also used to assess oxidative stress damage as they indicate lipid peroxidation resulting from increased free radicals (Powers and Jackson, 2008). Alessio and colleagues (2000) showed a significant increase in lipid hydroperoxides immediately following an isometric exercise performed at 50% maximal voluntary contraction. The measurement of antioxidants is another common way to measure oxidative stress. Glutathione peroxidase is an important antioxidant enzyme that helps maintain the antioxidant/pro-oxidant balance. Decreases in this enzyme are common with increases in free radicals and can be used as a biomarker for oxidative stress. Zembron-Lacny and colleagues (2008) showed a decrease in glutathione peroxidase activity immediately following an isometric exercise. These previous studies consistently reported that many different markers of reactive species were elevated for

lipids and proteins or that various antioxidant defense system enzymes (i.e. glutathione peroxidase) had decreased activity following different forms of resistance exercise.

Limited research is available examining the effect of PVO on oxidative stress markers. Takarada *et. al*. (2000) examined the concentration of lipid peroxides (LP) following low-intensity exercise (20%1RM) with and without partial vascular occlusion and noted no significant increase over time or difference between groups in lipid peroxides concentration immediately post exercise up to 24 hours post exercise. In contrast, Goldfarb and colleagues (2008) reported that PC and glutathione status were significantly elevated immediately after exercise and 15 minutes post-exercise in the moderate resistance without PVO group and partial vascular occlusion only group when compared to pre-exercise values. Unfortunately, Goldfarb *et. al.* did not examine the combined influence of moderate resistance exercise with PVO nor did they examine low intensity resistance exercise without PVO.

In summary, low intensity exercise combined with PVO has been shown to increase muscle size and strength in multiple populations. Few studies have examined the effect of PVO on oxidative stress.

Purpose

The purpose of this study was to examine the effects of PVO and exercise intensity on oxidative stress and inflammatory markers over time in young resistance trained individuals. In addition, resistance exercise done to failure without PVO was examined to gain further insight into PVO effect on muscle adaptation.

Specific Aims

Specific Aim #1: Test the effect of partial vascular occlusion (PVO) on oxidative stress and inflammatory markers over time in conditions done at rest (without exercise).

H1: Oxidative stress and inflammatory markers will be significantly higher with PVO alone when compared to without PVO.

Specific Aim #2: Test the effect of partial vascular occlusion (PVO) on oxidative stress and inflammatory markers over time in conditions done at low intensity (30% 1RM).

- H2: Oxidative stress and inflammatory markers will be significantly higher in low intensity resistance exercise with PVO when compared to low intensity resistance exercise without PVO.

Specific Aim #3: Test the effect of partial vascular occlusion (PVO) on oxidative stress and inflammatory markers over time in conditions done at moderate intensity (70% 1RM).

- H3: Oxidative stress and inflammatory markers will be significantly higher in moderate intensity resistance exercise with PVO when compared to moderate intensity resistance exercise without PVO.

Specific Aim #4: Test the effect of intensity on oxidative stress and inflammatory markers over time in conditions done with PVO.

- H4a: Low and moderate intensity resistance exercise with PVO will result in significantly higher oxidative stress and inflammatory markers when compared to PVO at rest (no exercise).
- H4b: Moderate intensity resistance exercise with PVO will result in significantly higher oxidative stress and inflammatory markers when compared to low intensity resistance exercise with PVO.

Specific Aim #5: Test the effect of intensity on oxidative stress and inflammatory markers over time in conditions done without PVO.

- H5a: Low and moderate intensity resistance exercise will result in significantly higher oxidative stress and inflammatory markers when compared to rest (no exercise).
- H5b: Moderate intensity resistance exercise will result in significantly higher oxidative stress and inflammatory markers when compared to low intensity resistance exercise.

Specific Aim #6: Test the effect of resistance exercise done to failure compared to repetitions matched on oxidative stress and inflammatory markers over time in conditions done at low-intensity (30%) without PVO.

- H6: Low intensity resistance exercise done to failure will result in significantly higher oxidative stress and inflammatory markers when compared to low intensity resistance exercise done with repetitions matched.

Limitations

One limitation to this study was the amount of blood flow reaching the exercising muscle during each condition. The amount of blood flow to an exercising muscle can alter numerous intramuscular variables such as level of oxygen content and circulating antioxidants. This factor was not controlled for in this study and may vary due to the addition of PVO and different intensities. The addition of PVO resulted in an increase in blood pooling compared to without PVO. This increased blood pooling may have resulted in dilution of the blood markers measured due to an increase in total blood volume in the PVO limb compared to pre-exercise values. The increased blood pooling could also have resulted in an accumulation of these blood markers due to the reduction in clearance caused by the PVO effect on venous outflow.

Another limitation is the use of blood markers to evaluate the resulting intramuscular conditions following each condition. Oxidative stress and inflammatory markers seen in the blood may not accurately represent what is occurring in the muscle.

The use of the blood pressure cuff and tourniquet before blood draws could also be a limitation in this study. The subject's all had their blood pressure taken before each PVO condition to calculate the appropriate pressure to be applied. This however was consistent independent of PVO. The blood draws in this study required a tourniquet above the antecubital vein prior to the blood draw to increase blood flow. This factor could have resulted in a transient blood flow restriction and was always taken on the subject's dominant or non-dominant arm both before and after the time allocation. This

occlusion could have resulted in a small increase in oxidative stress and inflammatory markers and therefore could have affected the pre-exercise blood measures.

Delimitations

Subject age (18-35), gender (only males), training status (resistance trained), and health status (apparently healthy) were all controlled for in this subject population to limit the variability of measures between and within subjects. In addition, diet was factored into this by monitoring the subjects self-reported diet and having them maintain similar dietary habits during the study.

Definition of Terms

- Catalase (CAT) – Antioxidant enzyme that functions to catalyze the breakdown of hydrogen peroxide into hydrogen and water.
- Cyclic-Dependent Kinase Inhibitor 1A – Marker for increased satellite cell activity.
- Glutathione (GSH) – Non-proteinthiol utilized in antioxidant defense system.
- Glutathione Peroxidase (GPx) – Antioxidant enzyme that helps maintain antioxidant/pro-oxidant balance via reduction of hydrogen peroxide (Equation A).

Equation A: $H_2O_2 + 2\ GSH \rightarrow GSSG + 2\ H_2O$

- Glutathione Reductase (GR) and S-transferase(GST) – Enzymes involved in recycling oxidized glutathione back to a non-oxidized state (Equation B).

Equation B: $GSSG + NADPH + H^+ \rightarrow 2\ GSH + NADP^+$

- Heat Shock Protein 72 (HSP72) – Chaperone molecule that protects cells against ischemia.
- Interleukin-6 (IL-6) – Pro-inflammatory cytokine involved in the acute phase inflammatory response and repeated muscle contractions.
- Ischemia-Reperfusion (I/R) – Prolonged periods of low oxygen levels in the tissue followed by reperfusion with oxygenated blood.
- Lipid hydroperoxides (LH) – By-product of the oxidation of lipids caused by free radicals.
- Lipid peroxides (LP) – By-product of the oxidation of lipids caused by free radicals.
- Malonaldehyde (MDA) – End by-product of the oxidation of lipids caused by free radicals.
- Mitogen-activated protein kinase (MAPK) pathway– Cellular signaling pathway related to growth and differentiation in skeletal muscle.
- Mammalian target of rapamycin (mTOR) pathway – Signaling pathway that stimulates muscle protein synthesis translation and transcription.
- Muscle-specific RING finger 1 (MuRF1) – Marker for muscle protein turnover.
- MyoD - Marker for increased satellite cell activity.
- Myostatin – Negative regulator of muscle growth via decreasing protein content.

- Nicotinamide adenine dinucleotide phosphate (NADPH) – Coenzyme used in anabolic reactions such as lipid and nucleic synthesis.
- NADPH oxidase – Enzyme the transfers electrons from NADPH to oxygen resulting in superoxide radical production.
- Nuclear factor kappa B (NF-kB) – Transcription factor associated with inflammation and muscle protein turnover.
- Nitric Oxide Synthase (NOS) – Enzyme involved in production of nitric oxide.
- Oxidative stress - Generation of free radicals in a system exceeds the system's ability to neutralize and eliminate these molecules which leads to damage to a cell's lipids, protein, and/or DNA.
- Oxygen Radical Absorbance Capacity (ORAC) – Measure of oxidative stress that evaluates the ability of blood/plasma to neutralize free radicals.
- Partial vascular occlusion (PVO) - Partial occlusion of the vasculature that results in decreases in the amount of oxygenated blood getting to the working muscle and can result in blood pooling distal to the partial occlusion.
- Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1a) – Transcription factor associated with enhanced mitochondrial biogenesis.
- Protein carbonyl (PC) – By-product of the oxidation of proteins caused by free radicals.
- Ribosomal protein S6 kinase 1 (S6K1) – Involved in regulation of mRNA translation initiation for muscle protein synthesis.
- Superoxide Dismutase (SOD) – Antioxidant enzyme that dismutates superoxide

radicals (Equation C)

Equation C: $2\ O_2\cdot + 2H+ \rightarrow O_2 + H_2O_2$

- Xanthine dehydrogenase (XDH) - Enzyme involved in converting xanthine to uric acid with use of NAD as an electron acceptor (Equation D).

Equation D: Xanthine + $NAD^+ + H_2O\rightarrow$ urate +NADH + H^+

- Xanthine oxidase (XO) – Enzyme involved in converting xanthine to uric acid with use of oxygen as an electron acceptor (Equation F).

Equation F: Hypoxanthine + $H_2O + O_2 \rightarrow H_2O_2$ + Xanthine

CHAPTER II

REVIEW OF LITERATURE

Oxidative Stress

Oxidative stress occurs when the generation of reactive species, also known as free radicals, in a system exceeds the system's ability to neutralize and eliminate these molecules. Excess free radicals can damage a cell's lipids, proteins, or DNA. The generation of these reactive oxygen and nitrogen species occurs regularly as a part of normal cellular metabolism and is increased under conditions of physical stress (Bloomer 2007, Bloomer and Goldfarb 2004).

Ischemia-reperfusion (I/R) and exercise can both induce oxidative stress by altering the balance of pro-oxidants and antioxidants. I/R is characterized by prolonged periods of tissue ischemia leading to hypoxemia (low oxygen) followed by reperfusion of the ischemic tissue with oxygenated blood. Ischemia by itself can induce skeletal muscle dysfunction through increased purine metabolism and accumulation of potentially toxic tissue metabolites. Although restoration of adequate blood flow is essential to salvage ischemic skeletal muscle, a growing body of evidence indicates that highly reactive metabolites of molecular oxygen are formed during reperfusion (Gute 1998). Oxidant formation during reperfusion may exacerbate tissue injury induced by ischemia since they can induce alterations in the cell's structure and function (Gute 1998).

Aerobic exercise increases mitochondrial free radical generation largely via increased oxygen consumption in the contracting muscle. Anaerobic exercise can also increase free radical production, although the mechanisms are entirely different. Similar to ischemia-reperfusion, anaerobic exercise can result in alternating hypoxia and ischemia, which is characterized by low partial pressures of oxygen, acidosis, and an accumulation of metabolites and reducing equivalents, all of which can increase the generation of reactive oxygen species (Rodriguez 2003).

Aerobic Exercise and Oxidative Stress

Bloomer and colleagues (2006) examined the plasma PC response to exercise at 70%VO_2max with varied exercise durations in aerobically trained individuals. Subjects exercised for 30, 60, and 120 minutes on a cycle ergometer on three separate days. Their results indicated that all groups had significantly greater plasma PC immediately and 30 minutes post-exercise, when compared to resting values. The 120 minute duration group was the only group that displayed significantly greater plasma PC concentrations at 60 minutes post exercise and overall had significantly greater plasma PC concentrations than the other two groups at the time points investigated. This study suggests that plasma PC can be elevated with aerobic exercise at 70%VO_2max and the response is greater following longer exercise duration.

Alessio *et. al.* (1988) examined malonaldehyde (MDA) and lipid hydroperoxide (LH) muscle content in exercising rats. The groups were randomly assigned into either a high intensity group (HI), a moderate intensity group (MI), or a control group. The HI

group ran at a speed of 45 m/min for 1 minute and the MI group ran at a speed of 20 m/min for 20 minutes. The control group did not run. The animals were killed immediately following exercise and muscle samples were excised. The MDA concentration following exercise was significantly greater in both exercise groups when compared to controls. MDA content increased in the HI group in the white (157%) and red (167%) vastus muscles of the rats. MDA content increased in the MI group in the white (90%) and red (96%) vastus muscles of the rats when compared to controls. LH values increased but were not significantly different than controls. This study reported that MDA content does increase in rat skeletal muscle following both HI and MI exercise and that the change in MDA content was greater in the red fast twitch muscle compared to the white fast twitch muscle. The authors suggested this may be related to fiber type recruitment.

Alessio *et. al.* (2000) examined biomarkers of oxidative stress after exhaustive aerobic exercise in humans. Subjects performed a VO_2max test and blood samples were obtained. The plasma was analyzed for MDA, PC, LH, and oxygen radical absorbance capacity (ORAC). The researchers reported that PC, LH, and ORAC were significantly greater immediately after exercise compared to resting values and LH continued to be significantly greater 1 hour post exercise compared to resting values. MDA displayed no significant change from resting values. This study reported that exhaustive aerobic exercise increased specific biomarkers of oxidative stress such as PC, LH, and ORAC immediately following exercise.

Resistance Exercise and Oxidative Stress

Rietjens and colleagues (2007) showed a significant increase in glutathione reductase and glutathione S-transferase in plasma 30 minutes after eight sets of 10 repetitions of leg press and leg extension done at 75% 1RM. Hudson (2008) reported a significant increase in plasma PC immediately and 60 minutes following an exercise bout of 11 sets of three repetitions of squats done at 90% 1RM; the same increases were also observed after an exercise bout of 4 sets of 10 repetitions of squats at 75% 1RM. Alessio and colleagues (2000) showed a significant increase in plasma LH immediately following an isometric exercise bout at 50% maximal voluntary contraction using a hand grip dynamometer. The protocol utilized by the Alessio *et al.* study involved a repeated pattern of 45 seconds contracting followed by 45 seconds relaxing until the accumulated time equaled a previously done maximal treadmill exercise time (times not reported). Bloomer and colleagues (2005) reported a significant increase in plasma PC 6 hours and 24 hours after an exercise bout of squats consisting of 5-12 repetitions done to failure at 70% 1RM with 90-120 second breaks for a total time of 30 minutes. Bloomer (2006) also showed that plasma PC could be significantly increased immediately following a squat test consisting of a single set of 15 repetitions using a load equal to 70% 1RM. Zembron-Lacny and colleagues (2008) reported a decrease in glutathione peroxidase immediately following an isometric exercise bout of three 10 second maximal voluntary contractions at both 30 and 75 degrees of knee flexion. These studies indicate that resistance exercise ranging from 50% to 90% of the 1RM using different protocols can induce measureable

changes in oxidative stress, as assessed by PC, lipid peroxidation markers and by changes in antioxidant enzyme activity.

Blood Flow Changes and Oxidative Stress

Uchiyama and colleagues (2006) examined a weight-lifting model in rats and its effect on blood volume, hemoglobin levels, and the production of reactive oxygen species (ROS) in the rat's hind limb muscles. The results displayed an ischemia-reperfusion state following each weight lifting set. There was an initial anoxic state when the set was finished, followed by a rapid blood reperfusion to the muscle. Increases in superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT) activity were displayed with significant peaks immediately after exercise and 24-72 hours after exercise. The first increase was accredited to a repeated ischemia-reperfusion state following the exercise and the second increase was attributed to accumulation of phagocytic cells in the damaged portions of the hind limb muscles. Pattwell (2003) used a 4 hour ischemia/1 hour reperfusion model on the hind limb of anesthetized rats to evaluate free radical and reactive oxygen species generation in skeletal muscle. Changes in hydroxyl radical activity increased during ischemia and reperfusion (210%). This was measured via 2,3-dihydrooxybenzoic acid (2,3-DHB) generated from the hydroxyl radical reaction with salicylate. Rodriguez (2003) reported a significant increase in malonaldyhyde (MDA) immediately and 1 minute following an ischemic forearm exercise consisting of repeated cycles of 9 seconds contraction/1 second relaxation for 60

seconds. A blood pressure cuff occluded blood flow to the forearm during exercise and was deflated upon completion of the exercise to allow for reperfusion.

Xanthine oxidase and neutrophil nicotinamide adenine dinucleotide phosphate (NADPH) oxidase have received the most attention with regard to skeletal muscle in response to I/R (Gute). One potential source of reactive oxygen-derived free radicals in reperfused tissue is the xanthine dehydrogenase (XDH)/xanthine oxidase (XO) system. The enzyme XDH/XO is synthesized as XDH and accounts for 90% of the enzyme in healthy tissues. XDH uses NAD as the electron acceptor for the oxidation of hypoxanthine and xanthine (equation 1 below). Alternatively, the enzyme can exist as an oxidase using molecular oxygen as an electron acceptor to produce superoxide radicals or hydrogen peroxide (equation 2 below). It has been established that the conversion of XDH to the oxidase form occurs as a result of tissue ischemia. This may be precipitated by the depletion of ATP during ischemia and consequent loss of control over the membrane Ca^{2+} gradient. Increased cytosolic Ca^{2+} concentration activates Ca^{2+}-dependent proteases which convert the dehydrogenase form to the oxidase form by selective proteolysis. Only when molecular oxygen is readmitted during reperfusion does a rapid production of superoxide radicals occur due to the presence of high concentrations of xanthine, hypoxanthine and active XO. The hydroxyl radicals interact with lipids, proteins, and nucleic acids, resulting in the loss of membrane integrity, structural and functional changes in proteins, and genetic mutations (Misra 2009).

1) $xanthine + NAD^+ + H_2O \rightleftharpoons urate + NADH + H^+$ Xanthine dehydrogenase reaction

2) $hypoxanthine + H_2O + O_2 \rightleftharpoons xanthine + H_2O_2$ Xanthine oxidase reaction

Delample (2008) demonstrated an increase in XO activity following a localized quadriceps endurance test in COPD patients. This increase was matched by significant increases in lipid peroxidation markers 30 minutes after exercise and plasma protein oxidation concentrations 6 hours after exercise. Vina (2000) observed increases in plasma XO in rats exercised to exhaustion. The rats showed significantly greater oxidized glutathione (GSSG) concentrations and GSSG/reduced glutathione (GSH) ratio in their livers and significantly greater oxidized glutathione (GSSG) and MDA concentrations in their hearts following exercise when compared to a non-exercising control group. Hellsten (1997) examined XO response in skeletal muscle following eccentric exercise. The results showed an increased plasma concentration of XO following exercise at 24, 48, and 96 hours which was attributed to a secondary inflammatory response as no immediate post-exercise measures were taken. This increase in XO was seen in conjunction with significantly increased plasma interleukin-6 (IL-6) concentration as soon as 45 minutes and up to 4 days after eccentric exercise. However, muscle MDA and plasma antioxidant capacity were unchanged.

In summary reactive oxygen species generation has been reported following aerobic exercise, anaerobic exercise, and ischemia-reperfusion states. The extent of the

increase depends on the length of time of the activity, the type of tissue examined and the type of oxidative stress marker examined.

ROS, Cell Signalling, and Mitochondrial Biogenesis

Exercise can cause an increase in the generation of free radicals by cells and can lead to cell damage. However, free radicals not only cause damage but they can have a role in cell signaling (Gomez-Cabrera et. al. 2009). MAPK and NF-kB are two major redox-sensitive signaling pathways in the cell that suggest that ROS may participate in the regulation of mitochondrial biogenesis (Kang et. al. 2009). The MAPK family has been implicated in growth and differentiation and was shown to be activated through transcriptional redox regulation in skeletal muscle. Alterations in free radical concentration are rapidly sensed, neutralized, and accompanied by adaptive increases in oxidant buffering capacity through key up regulation of antioxidant enzymes (Kramer and Goodyear 2007). ERKs, JNKs, and p38 MAPK signaling modules can be activated by hydrogen peroxide in skeletal myoblasts (Kefaloyianni et. al. 2006). NF-kB is a major stimulator of inflammation and muscle protein turnover and rapidly responds to ROS by increasing transcription of at least three important antioxidant genes, including manganese-superoxide dismutase, inducible nitric oxide synthase, and y-glutamylcysteinesynthetase, each of which buffer increases in oxidative stress (Kramer and Goodyear 2007). NF-kB binding to MnSOD genes has been shown to be enhanced after treadmill exercise in rats (Hollander et. al. 2001).

Gomez-Cabrera and colleagues (2005) examined cellular adaptations to exercise in rats exercised on a treadmill to exhaustion. Two groups of rats ran to exhaustion with

and without allopurinol treatment used to inhibit free radical production by inhibiting xanthine oxidase activity. Results showed that XO, GSSG, and muscle protein oxidation in the exercise group without allopurinol were significantly higher than the allopurinol-treated group. The exercise group without allopurinol showed significantly greater activation of MAP kinases, greater DNA binding of NF-kB, and exercise-induced expression of MnSOD, iNOS, and eNOS in the muscle. This study reported that RONS produced in exercise act as signals that regulate molecular events important in muscle cell adaptation to exercise in animals. The inhibition of these cellular signals could have a detrimental effect on improving redox muscle cell status, increasing blood flow and vasodilation of vessels, and increasing mitochondrial respiration.

PGC-1a has been shown to lead to enhanced mitochondrial biogenesis controlled by a variety of physiological conditions such as cold exposure, caloric loading, hyperthyroidism, and energy demand. Co-activation of PGC-1a induces nuclear respiratory factors which promote the expression of numerous nuclear encoding mitochondrial proteins as well as mitochondrial transcription factor A which directly stimulates mitochondrial DNA replication and transcription. This signaling sequence has been suggested to explain the molecular mechanisms for a wide range of biological functions (Kang et. al. 2009).

Kang and colleagues (2009) examined the effects of an intermittent sprinting exercise in rats on ROS production and its effects on PGC-1a expression and signaling in rat skeletal muscle. A control group, an exercise group, and an exercise group

administered allopurinol were used in this study. The results showed that xanthine oxidase and oxidant production was significantly higher in the exercise group compared to controls and the allopurinol group. Furthermore, this study demonstrated that the PGC-1a signaling pathway was activated. Associated with the elevated PGC-1a were significant increases in NRF-1 and Tfam protein contents, two important biomarkers of mitochondrial biogenesis. This study also showed that this signaling event was accompanied by activation of p38MAPK and cyclic AMP response element binding protein (CREB) phosphorylation. Phosphorylation of p38MAPK and CREB has been shown to be early events in the PGC-1a-mediated signaling process. The major finding from this study was that the PGC-1a pathway was redox sensitive and that oxidants were directly involved in the signaling process with the allopurinol group showing substantial decreases in PGC-1a, NFR-1, and Tfam protein contents compared to the exercise group despite similar exercise intensity and duration.

The previous section shows a significant link between ROS and adaptation to cellular stress through cell signaling and mitochondrial biogenesis. This further elucidates the positive correlation between moderate amounts of oxidative stress and cellular and muscular adaptation.

Partial Vascular Occlusion Effects on Muscle Size and Strength

Kubota and colleagues (2008) investigated the effects of PVO on muscle atrophy and muscle weakness induced by immobilization and non-weight bearing. Fifteen healthy males had their left ankles immobilized and were divided into three groups; 1) A control

group which was given no other intervention; 2) A partial vascular occlusion group that had an external compression force applied for 5 minutes followed by three minutes of rest 5 times each session, twice a day for fourteen days; 3) The isometric training group completed 20 exercises of 5 second isometric contractions followed by rest, twice a day for fourteen days. The results showed that the PVO treatment protected against decreases in muscle strength and leg/thigh circumference loss observed in the control group and the isometric training group. This study showed PVO to be an important factor in the prevention of muscle atrophy induced by immobilization.

Abe (2006) examined muscle size and muscle strength following exercise training with PVO. Nine men took part in 3 weeks of walk training done at 50 m/min for 6 days/wk. The subjects did 5 sets of 2 minute bouts with one minute rest with or without thigh occlusion (200 mmHg) throughout the entire session. Significantly greater thigh cross sectional area (CSA) and muscle volume was reported post-exercise in the PVO group when compared to pre-exercise. Significantly greater knee extension 1-RM and isometric strength was displayed post-exercise in the occlusion group when compared to pre-exercise. No CSA or strength changes were noted in the control group.

Takarada (2000) examined the effects of moderate vascular occlusion combined with low-intensity resistance exercise training on muscular size and strength in older women. Twenty four apparently healthy women aged 47-67 years underwent a 16 week resistance training program consisting of seated bicep curls. The subjects were divided into a low intensity (50% 1RM) with moderate vascular occlusion group, a low intensity

(50%1RM) without moderate vascular occlusion, and a high intensity (80%1RM) group without vascular occlusion. The low intensity group with occlusion showed significantly greater change in cross-sectional area in the biceps brachii and the brachialis when compared to the low intensity without occlusion group and significantly greater cross-sectional area in the triceps brachii when compared to the high intensity group. The low-intensity with occlusion group and the high intensity group without occlusion showed significantly greater strength increases then the low-intensity without occlusion with no significant difference between the two groups.

Takarada and colleagues (2000) later examined the long term effects of low-intensity resistance exercise combined with vascular occlusion on athletes. They used an isotonic leg extension machine to perform bilateral knee extensions on the subjects with or without pressure-occlusion (~214mmHg) at the proximal ends of the thigh. The exercise was performed twice a week for 8 weeks with each session comprised of four sets of exercise with 30 second rest intervals done at 50% 1RM until failure. A non-exercise group with the same vascular occlusion was also used to compare occlusion independent of exercise. An isokinetic dynamometer was used to measure isokinetic and isometric strength and magnetic resonance imaging (MRI) was used to measure thigh cross-sectional area (CSA).The results showed increased isometric and isokinetic strength as well as improvements in time to fatigue which indicated increased muscular endurance in the low-intensity with occlusion group. No changes were observed with the other two groups. The researchers suggested that individuals with much higher levels of physical activity than that of untrained individuals still showed significant increases in muscular

size and strength along with increases in muscular endurance with vascular occlusion and exercise.

Takarada and colleagues (2002) followed the last study up with an examination of the long term effects of low-intensity resistance exercise with vascular occlusion on muscular hypertrophy and strength. They used an isotonic leg extension machine to perform bilateral knee extensions on the subjects with or without pressure-occlusion of the proximal ends of the thigh. The exercise was performed twice a week for 8 weeks with each session comprised of five sets of exercise with 1 min rest intervals done at 20% 1RM until failure. A non-exercise group with vascular occlusion was also used to compare the influence of occlusion alone. There was a significant increase in total muscle CSA area as well as increases in isometric and isokinetic strength in the low-intensity with occlusion group. No changes were noted with the other two groups. The researchers concluded that low intensity resistance training combined with vascular occlusion done at a lower intensity than seen in previous research (Takarada et. al. 2000) can significantly increase muscular size and strength compared to low-intensity alone and vascular occlusion alone, suggesting a cooperative effect between the two stimuli.

Partial Vascular Occlusion Effects on Gene Expression

Kawada (2005) looked at cell signaling in rats following crush occlusion of veins in the rat's hindlimb. The rats were examined for 2 weeks after crush occlusion and compared to sham operated animals after being killed. The rats in the crush occlusion group showed significant increases in heat shock protein (HSP)-72 and nitric oxide

synthase (NOS)-1 and a significant decrease in myostatin compared to the sham group. HSP-72 has been shown to be a chaperone molecule to protect against ischemia in such cells as myoblast and myocardium. Myostatin is a potent negative regulator of muscle growth and has been shown to decrease protein content within muscle in response to mechanical stress. NOS-1 produces nitric oxide which may stimulate muscle growth through a series of phosphorylation cascade reactions. This study showed enhancement of muscular hypertrophy factors and regulators similar to what was seen with exercise; despite that crush occlusion at rest was the only stimulus.

Drummond and colleagues (2008) examined the effect of low intensity resistance exercise with blood flow occlusion on expression of several anabolic and catabolic genes typically reported to change with high intensity resistance exercise. They used a protocol of 20% 1RM bilateral knee extensions done for 4 sets for a total of 75 repetitions with and without 200mmHg of occlusion at the most proximal part of the leg. The results displayed no difference in the groups as they both had increased satellite cell activity markers [cyclic-dependent kinase inhibitor 1A (p21) and MyoD] and increased muscle protein turnover markers [muscle-specific RING-finger protein-1 (MuRF1) and myostatin]. The researchers concluded that low-intensity resistance exercise alone resulted in the increases seen in p21, MyoD, MuRF1, and myostatin as there was no significant differences between blood flow occlusion groups.

To examine exercise-induced muscle protein synthesis and training-induced muscle hypertrophy, Fujita and colleagues (2007) examined the mammalian target of

rapamycin (mTOR) signaling pathway through ribosomal protein S6 kinase (S6K1) phosphorylation during low intensity resistance exercise with partial vascular occlusion. The mTOR signaling pathway plays a significant role in stimulating translation and transcription of muscle protein synthesis while S6K1 is involved in the regulation of mRNA translation initiation and appears to be a critical regulator of exercised-induced muscle protein synthesis and training induced hypertrophy. A lower extremity cuff was inflated to 200mmHg around the proximal portion of each leg and the subjects performed a set of 30 repetitions of bilateral leg extensions at 20% 1RM followed by a 30 second rest period. The subjects then completed three more sets of 15 repetitions with 30 second rest intervals for a total of four sets and 75 repetitions. The researchers reported that S6K1 became phosphorylated and muscle protein synthesis was stimulated following the acute bout of low intensity resistance exercise with partial vascular occlusion when compared to the low intensity resistance exercise alone. The partial vascular occlusion group showed a significant increase in muscle protein synthesis in the mixed muscle fractional synthetic rate when compared to the low-intensity alone group.

Partial Vascular Occlusion Effects on Physiological Responses and Muscle Fatigue

Iida (2007) studied the physiological responses to an acute bout of PVO on the thighs in the supine position compared to standing without occlusion. Nine men had their thighs partially occluded (200 mmHg) and there was a significant increase in heart rate, total peripheral resistance, noradrenaline, and plasma renin activity during the occlusion when compared to without occlusion. Significantly decreased stroke volume was also

reported in the occlusion group when compared to without occlusion. The previous measures were similar to the values obtained in the standing condition without occlusion. This showed that partial vascular occlusion can stimulate an orthostatic response similar to that of standing and may have important implications in zero gravity environments.

Moore (2004) and colleagues reported significantly decreased resting twitch torque and increased post-activation potentiation in an occlusion group compared to a control group. Eight untrained male subjects resistance trained their elbow flexors for 8 weeks at a workload of 50% 1RM with or without occlusion of 100 mmHg. The results suggest that vascular occlusion combined with low intensity resistance exercise does have some effect on neuromuscular adaptations such as muscle activation and twitch contractile properties.

Cook (2007) studied muscle fatigue and maximal volitional contraction (MVC) decrement across nine different conditions of intensity, occlusion type, and occlusion time using fourteen male and seven female subjects that completed 8 acute bouts of knee extension done for 3 sets to failure. The subjects completed the bouts at either 20% or 40% 1RM, partial (~160mmHg) or complete (~300mmHg) occlusion, and with either continuous occlusion or intermittent occlusion that was released during rest periods. The subjects also completed an acute bout of 80%1RM with no occlusion and MVC decrement was compared across groups. No significant difference was noted between the groups with the exception of the 80%1RM group without occlusion (19% MVC

decrement) and 20%1RM group with continuous, partial occlusion(32% MVC decrement).

Partial Vascular Occlusion Effects on Hormones, Metabolites, and Oxidative Stress

Takano (2005) and colleagues examined hemodynamic and hormonal responses following an acute bout of knee extension with or without partial vascular occlusion. Eleven men completed four sets of knee extensions at 20%1RM with or without occlusion. The first set was 30 repetitions followed by three sets done to failure with 20 seconds rest between the sets. The occlusion was set at 1.3 times greater than systolic blood pressure (160-180mmHg). The results showed significant increases in growth hormone, insulin-like growth factor-1 (IGF-1), and vascular endothelial growth factor (VEGF) in the occlusion group compared to the control group. The increases in VEGF were similar to high intensity exercise that resulted in increases in angiogenesis and arteriogenesis (Yang et. al. 1990; Gute et. al 1996). The GH and IGF-1 increases were related to the muscle cell growth and hypertrophy, which has also been reported with high intensity exercise.

Abe (2006) examined growth hormone (GH) response following exercise training with partial vascular occlusion. Nine men took part in 3 weeks of walk training done at 50 m/min for 6 days/wk. The subjects did 5 sets of 2 minute bouts with one minute rest with or without thigh occlusion (200 mmHg) throughout the entire session. There was significantly greater GH concentration in the occlusion group compared to the control group after exercise. Fujita and colleagues (2007) examined training-induced muscle

hypertrophy during low intensity resistance exercise with partial vascular occlusion. A lower extremity cuff was inflated to 200mmHg around the proximal portion of each leg and the subjects performed a set of 30 repetitions of bilateral leg extensions at 20% 1RM followed by a 30 second rest period. The subjects then completed three more sets of 15 repetitions with 30 second rest intervals for a total of four sets and 75 repetitions. The partial vascular occlusion group showed significantly higher lactate, growth hormone, and cortisol concentrations immediately after exercise and up to 40-60 minutes post-exercise when compared to the low-intensity group without occlusion.

Takarada and colleagues (2004) examined the effects of low intensity exercise with vascular occlusion compared to low intensity alone on hormonal and inflammatory responses in the body. Subjects performed bilateral leg extensions at 20% 1RM until failure for 5 sets. Significant increases in growth hormone 15 minutes post exercise, lactate immediately post exercise and IL-6 at all-time points from 30 minutes to 24 hours, were reported in the experimental group compared to the control group. No significant changes in markers for muscle damage (creatine kinase) and oxidative stress (lipid peroxide) were noted between groups. Lactate increases were presumably caused by both local hypoxia and suppression of lactate clearance from the exercising muscle. Plasma IL-6 concentrations were elevated in the absence of muscle damage suggesting that micro-damage might have occurred within the vessel walls and/or muscle tissue or that the IL-6 was released from the muscle for reasons other than muscle damage. The researchers concluded that acute hypoxia and accumulation of metabolites were identified

as positive stimuli for the muscle hypertrophy caused by low intensity exercise with vascular occlusion.

Burgomaster and colleagues (2003) examined the differences in low intensity resistance exercise with and without vascular occlusion on resting energy metabolites and training-induced adaptations. Eight healthy men took part in 8 weeks of periodized resistance training with two sessions per week. The exercise consisted of unilateral elbow flexion using a load equivalent to 50% 1RM. The results showed an increase in muscle glycogen storage in the elbow flexors of the occlusion group, most likely due to alterations in glucose transport induced as a result of compromised oxygen delivery. Resting levels of ATP were decreased which led the researchers to hypothesize that vascular occlusion placed a greater stress on the adenine nucleotide pool compared to low-intensity exercise without occlusion.

Reeves and colleagues (2006) examined the hormonal responses of growth hormone, testosterone, free testosterone, cortisol, and lactate concentrations to low-intensity resistance training with partial vascular occlusion. The researchers compared the responses to moderate exercise without occlusion and partial occlusion alone. The subjects complete three different sessions of exercise (elbow flexion and calf extension) or occlusion alone. The three groups were light resistance (30% 1RM) with partial occlusion, moderate resistance (70% 1RM) without occlusion, and partial occlusion only. The light resistance with occlusion group showed similar increases in lactate post-exercise as the moderate resistance group. The authors suggested that this indicated that

the same metabolic stress was produced by both trials. Growth hormone was significantly greater in the light resistance with occlusion group when compared to the other groups, which provides evidence that the differences in growth hormone between the groups was not due to the increase in lactate production. No other hormones were significantly different between trials.

Goldfarb and colleagues (2008) examined PC and glutathione status in plasma samples obtained from the Reeves (2006) study. They reported that PC and glutathione status were significantly elevated immediately after exercise and 15 minutes post-exercise in the moderate resistance without occlusion group and partial occlusion only group with no resistance exercise when compared to pre-exercise values. Unfortunately the Reeves (2006) study lacked the conditions of moderate resistance exercise with partial occlusion, low intensity resistance exercise without occlusion, and a rest condition without occlusion. Changes in this design would have allowed Goldfarb *et al*. (2008) to make the comparisons necessary to make more definitive conclusions about oxidative stress when PVO was coupled with resistance exercise.

PVO combined with low-intensity resistance training has shown positive results related to increasing muscle size and strength in both trained and untrained subjects. In certain cases, these results are comparable to increases in muscle size and strength reported with high intensity resistance exercise alone. The research in the area of oxidative stress and PVO is limited. Despite the lack of certain comparison groups from the Reeves (2006) study, the results from Goldfarb et al. (2008), suggest that partial

vascular occlusion coupled with different exercise intensities may result in an intensity-dependent response in oxidative stress markers. Given this preliminary finding, the purpose of the current study was to examine the effects of PVO on oxidative stress markers across three different exercise intensities in young resistance trained individuals.

CHAPTER III

METHODS

The methods and procedures to conduct this research will be outlined below. All subjects read and signed a consent form prior to participation (see Appendix A). They were initially screened as indicated below.

Pilot Study

A pilot study was used to determine if partial vascular occlusion (PVO) affects the number of repetitions done to failure when intensity is held constant. Multiple studies have examined the effect of fatigue during resistance exercise with and without PVO. Previous research done with dynamic knee extensions at 30% one-repetition maximum (1RM) reported significant decreases in repetitions done per set and total repetitions completed with PVO compared to without PVO (Wernbom, Augustsson, and Thomee 2006). Wernbom *et. al.* (2009) reported significant decreases in repetitions done to failure during one set at20%, 30% and 40%1RM with PVO when compared to without PVO. The previous study also reported no difference in repetitions done to failure during one set at50% 1RM with PVO when compared to without.

Two resistance trained males (ages 25 and 30) were recruited and completed four exercise conditions with each condition separated by 72 hours. The four exercise conditions included a low (30% 1RM) and moderate (70% 1 RM) intensity resistance exercise condition with or without PVO. The subjects also participated in the research

study as well as the pilot study and followed all the appropriate methods listed below such as prescreening, 1-RM determination, and procedures for exercise conditions with and without PVO. All conditions were done to failure and repetitions were counted and recorded with the exception of the condition matched for repetitions.

The low intensity (30%1RM) condition with PVO resulted in a large decrease in total repetitions completed when compared to low intensity condition without PVO. The moderate intensity (70%1RM) condition with PVO resulted in a modest decrease in total repetitions completed. The pilot study data is further presented in Appendix B. After presenting the pilot study findings to the dissertation committee it was suggested that a seventh condition be added. This condition was done at low intensity without PVO and with repetitions matched to the low intensity condition done with PVO. This condition was added to give better insight into the effect of resistance exercise done to failure without PVO on the oxidative stress and inflammatory markers measured in this study.

Research Design

A prescreening session and seven different exercise or rest conditions (with or without partial vascular occlusion) were completed by each subject. The seven exercise/rest conditions are described in detail in Table 1. These conditions were counterbalanced. Blood samples were taken immediately before and immediately after the exercise/rest conditions. The study design used to analyze specific aims #1-5 is placed in Table 2 and the study design to analyze specific aim #6 is placed in Table 3.

Table 1

Study Conditions

Condition	Description
RPVO	Resting with partial vascular occlusion
R	Resting without partial vascular occlusion
30PVO	30% one repetition maximum done to failure with partial vascular occlusion
30F	30% one repetition maximum done to failure without partial vascular occlusion
30RM	30% one repetition maximum done with repetitions matched to 30% one repetition maximum with partial vascular occlusion
70PVO	70% one repetition maximum done to failure with partial vascular occlusion
70F	70% one repetition maximum done to failure without partial vascular occlusion

Table 2

Study Design Utilized for Specific Aims #1-5

Intensity	PVO		Without PVO	
	Pre-Exercise	**Post-Exercise**	**Pre-Exercise**	**Post-Exercise**
Rest (No Exercise)	R-PVO-Pre	R-PVO-Post	R-Pre	R-Post
30%1RM	30-PVO-Pre	30-PVO-Post	30-Pre	30-Post
70%1RM	70-PVO-Pre	70-PVO-Post	70-Pre	70-Post

Table 3

Study Design Utilized for Specific Aim #6

Intensity	Sets Done to Failure		Sets Repetition Matched to 30%1RM w/PVO	
	Pre	**Post**	**Pre**	**Post**
30%1RM	30-Failure-Pre	30-Failure-Post	30-Reps Matched-Pre	30-Reps Matched-Post

Subjects

Twelve resistance trained males (24.6 ± 3.0 yrs) were recruited for this study from the student population at the University of North Carolina-Greensboro. Flyers were placed at various locations across campus detailing subject requirements, inclusion criteria, and exclusion criteria. Research members also made announcements in classes taught in Kinesiology Department following previous approval from instructors. Inclusion Criteria were: 1) greater than one year of resistance training prior to this study, 2) apparently healthy, and 3) non-tobacco users. Exclusion criteria were: 1) current use of ergogenic or dietary aid that could affect results; e.g. cannot be taking any antioxidant vitamins or supplements, 2) any cardiac or circulatory ailment, any metabolic disease, any muscle abnormalities or a reduced calorie diet that could alter normal body responses, and 3) on any medicines or drugs that may alter blood flow or oxidative stress (e.g. non-steroidal anti-inflammatory drugs (NSAIDs)). All subjects signed the consent form prior to any measures were taken and all measures were obtained in the Exercise Physiology Laboratory. Subjects were instructed to maintain their resistance training programs and keep them as constant as possible to avoid any detrimental changes in

muscle function and oxidative stress blood markers due to delayed onset muscle soreness or training changes (Newton et. al. 2008, Bryer and Goldfarb 2006, Lee et. al. 2002). A three day diet recall was taken before each subject's first condition and analyzed using Diet Analysis Plus 8.0 (ESHA Research, Salem, OR). The average dietary intake of the subjects in this study is presented in Appendix C. Subjects were instructed to fast at least 6 hours before each condition. Research has reported significant increases in protein carbonyls, xanthine oxidase, hydrogen peroxide, and MDA up to 6 hours following ingestion of a high-fat meal (Bloomer et. al. 2009) believed to be mediated by the blood triglyceride response to feeding.

Training Status, Gender, and Age

Resistance trained individuals were important for this study since research has reported that trained individuals have smaller changes in muscle function, limb circumference, and plasma creatine kinase activity following unaccustomed exercise when compared to untrained individuals (Newton et. al. 2008). This protective effect in trained individuals reduced variation due to muscle damage and strength changes that could occur if the individuals were untrained. Significant differences between genders for markers of inflammation and oxidative stress have been reported (Kerksick et. al. 2008, Goldfarb et. al. 2007). In addition, variations due to menstrual cycle phase and oral contraceptive use have been suggested to effect oxidative stress markers. This difference is most likely due to the antioxidant effects of estrogen (Tiidus et al. 2000), which would have been difficult to control for in a within subjects design. Thus, only males were

utilized in this study in an effort to control for variability in both resting and exercise oxidative stress measures and inflammatory responses to acute exercise (Goldfarb et al. 2007). The specific age range was chosen to reduce variability due to age-related changes in biochemical alterations in cell structure, increased exercised-induced inflammation, and limits to antioxidant defense and repair capacity (Fulle et. al. 2004).

Safety, Risks, and Side Effects

The idea of combining exercise training with PVO has been around for about ten years and originated in Japan. The type of exercise training is called KAATSU (Japanese for "increased pressure") training and involves both aerobic and anaerobic training.

A survey of safety of KAATSU training was taken by 12,462 persons who had received the training (Nakajima et. al. 2006). The most noteworthy finding is that the number of severe side effects due to KAATSU training was very low based on these survey results. The results reported that 2205 individuals (17%) surveyed reported side effects. The most frequent side effect was subcutaneous hemorrhage. It has been observed in 13.1% of cases. However, subcutaneous hemorrhage was usually transient and should minimal since this is not a training study. Transient numbness has been observed in 1.3% of the time in persons who received KAATSU training, probably due to the compression of peripheral nerves in the extremities. The transient numbness was abolished immediately after the release of KAATSU pressure. The current study monitored oxygen saturation and heart rate by using a pulse oximeter on the pointer finger of the exercising arm. If a pulse was not detected or oxygen saturation dropped

below 95% then the occlusion pressure was dropped by 5 mm Hg increments until oxygen saturation increased above 95% and a pulse was detected. Several papers reported that KAATSU training combined with low-intensity exercise does not cause severe muscle injury, and there is no elevation of creatine phosphokinase (CPK) (Takarada et al. 2000, Abe et al. 2005). This eliminates the occurrence of any delayed onset of muscle soreness symptoms. The incidence of serious side effects was low and no fatal complications were reported with KAATSU training. However, venous thrombus was observed in 7 cases (0.055%) and pulmonary embolism was noted in 1 case (0.008%). Dizziness, fainting and cerebral anemia were observed in a few cases. These results were from subjects with age ranges up to 80 years old. It should be noted that only ~25.1% of the individuals surveyed fall into the subject population that was recruited for this study. The other severe side effects listed were reported by individuals who would be excluded from this study due to specific ailments.

Minor pain and bruising can be associated with the blood sampling. The bruising was minimized by placing direct pressure over the area where the blood was taken. Appropriate sterile techniques were used.

Prescreening

Subjects filled out a Health History Questionnaire (AHA) (see Appendix D) to ensure there were no existing health risks and an Activity Questionnaire (see Appendix E) to ensure the subject fell under the criteria of resistance trained (at least one year resistance training). Specific body measures were assessed such as height, weight, resting

heart rate, and blood pressure to help characterize the subjects. Height was measured to the nearest 0.5 cm and weight was measured to the nearest 0.1 kg using a Seca scale. Body Mass Index (BMI) was calculated using the height and weight data obtained. Resting heart rate was taken at the radial artery using a ten second count. Resting blood pressure was taken at the brachial artery with the subjects in a seated position using a sphygmomanometer and a stethoscope. The sphygmomanometer was inflated to 200mmHg and then released slowly until both systolic and diastolic blood pressures were obtained. Body fat percentage was assessed using a seven-site skinfold measurement and used the Siri equation (Jackson and Pollack 1985). A maximal isometric force (MIF) value was determined for elbow flexion in the dominant and non-dominant arms using a Biodex isokinetic dynamometer (Biodex Medical Systems Inc., Collierville, TN) and appropriate Biodex chair measures were recorded to keep distances constant for the subsequent post-study MIF measure.

Maximal Isometric Force Determination

Maximal isometric force (MIF) was determined before the first condition and again after the last condition was completed. This measure was utilized to identify if strength changes that may have occurred during the study in the dominant arm and any cross-transfer effects of strength to the non-dominant occurred due to possible neural factors (Madarame et. al. 2008).

Subjects were seated on the Biodex chair and were rotated to 0°and a seat back tilt set to 85°. The limb support pad was attached to the chair and positioned at the distal

humerus with the angle directed posteriorly. The seat cushion was adjusted for leg length, such that the subject was as far back in the seat as possible with relation to their distal femur. The dynamometer was adjusted so position and height placed the axis aligned with the lateral epicondyle of the humerus. The elbow attachment was then adjusted for forearm length. Shoulder, waist, and elbow stabilizing straps were then secured on the subject. The range of motion limits were determined by setting the away limit with the subject in full elbow extension and toward limit to full elbow flexion. The subject was then set to full elbow extension and the subject's anatomical position was calibrated to 0°. The dynamometer then automatically positioned the elbow to 45° and three MIF trials were completed with the subject being verbally instructed to contract at 50%, 75%, and 100% of their maximal isometric volitional contraction. The subject was then given a 60 second break. The test began with a five second countdown and then the subject completed three seconds of maximal volitional contraction with verbal encouragement. Three repetitions of this were completed with thirty seconds of rest between sets. Peak torque, average peak torque, and peak torque per kilogram of body weight was measured and recorded by Biodex. The test was then completed and all data was recorded manually from the Biodex data screen. The shoulder, waist, and elbow stabilizing straps were removed and the test was finished.

1-RM Determination

A free-weight dumbbell (Bowflex Selecttech 1090) was utilized for the elbow flexion to determine the 1-RM of the dominant arm. Determination of the dominant arm was obtained by the subject's self-reporting what they considered to be their dominant arm. The dominant arm was used in previous research utilizing acute resistance exercise and PVO (Reeves et. al. 2006, Goldfarb et. al. 2008) while the non-dominant arm was utilized in a 16 week training program (Takarada et. al. 2000). Two other studies used elbow flexors, but both studies used both arms and randomized one arm to an occlusion condition and the other to a non-occlusion condition (Moore et. al. 2004, Burgomaster et. al. 2003). Since no differences in arms have been noted in previous studies with PVO, this study utilized the procedures reported by Reeves *et. al.* and Goldfarb *et. al.* and utilized the dominant arm. Subjects stabilized their upper torso by grasping a stationary structure with their non-dominant hand to ensure proper form and minimize momentum. Three trials of submaximal weight approximately 40, 60, and 80% of the subject's estimated 1-RM served as warm-up sets and all rest intervals were 3 minutes. A 1-RM determination was considered legitimate when the subject successfully completed a repetition moving the heaviest dumbbell through the full range of motion with no other body manipulation to enhance momentum or swinging (Reeves et. al. 2006).

Partial Vascular Occlusion

Partial vascular occlusion was set to 20mmHg below the subjects' resting systolic blood pressure determined 10 minutes before exercise (Reeves et al. 2006). A cuff

pressure of 20mmHg below the systolic blood pressure was selected as this pressure has been suggested to restrict venous blood flow and cause blood pooling in the capacitance vessels distal to the cuff, and partially restricting arterial blood flow (Takarada et. al. 2000, Burgomaster et. al. 2003, Moore et. al. 2004). A cuff was placed on the dominant arm between the superior aspect of the biceps brachii muscle and the inferior aspect of the anterior deltoid muscle. Length of cuff occlusion in the low intensity condition was measured in a previous study to be 341 $\pm$ 4.5 seconds (Reeves et. al. 2006). Length of time for the moderate intensity condition with PVO was not measured previously but was noted to be less than the low intensity repetitions done with PVO (Wernbom et. al. 2009).

Conditions

The seven exercise or rest conditions (Table 1) were separated by at least 72 hours and no subject took more than 4 weeks to complete all seven conditions. Time between conditions was chosen to ensure no carry-over effect of the blood markers from visit to visit. Protein carbonyls and glutathione ratio have been reported in previous research to return to baseline levels within an hour of resistance exercise in trained individuals (Bloomer et. al. 2007, Goldfarb et. el. 2008) and interleukin-6 has been reported to return to baseline level six hours after accustomed exercise (Sorichter et. el. 2006). Subjects were instructed to sit and rest for 15 minutes after arriving to the lab for each condition to reduce any variation before blood was taken. The conditions were counterbalanced contingent on the specific condition. The low intensity condition done without PVO was matched to repetitions done in low intensity condition with PVO so it occurred after this

condition. The resting condition with and without PVO were matched to the amount of time needed to complete the low intensity condition without PVO so it occurred after this condition.

Resting Conditions

Subjects sat for the allotted amount of time that was matched for the time of the low intensity occlusion time. This was done with or without partial vascular occlusion and without any exercise intervention. Blood was taken both before and after the allotted amount of time.

Exercise Conditions

The resistance was set to 30% or 70% of the subject's 1-RM. Due to the set weight increments of the Bowflex Selecttech 1090 the subjects calculated 30% and 70% 1RM values that did not fall on an exact weight increment (5 lb increments) were rounded up or down to the closest weight increment. This appropriate adjustment is listed in Table 6. The subjects were encouraged to maintain a repetition cadence of 2 seconds of concentric phase and 2 seconds of eccentric phase. The subjects were instructed to perform all repetitions with cadence and through a full range of motion until failure. Failure was determined once the subject could not perform a repetition through their full range of motion in a smooth, timely fashion. Total work was calculated by multiplying the total number of repetitions done by the weight lifted in kilograms.

Low and Moderate Intensity Conditions with Partial Vascular Occlusion

Subjects completed 3 sets of 30% or 70% 1RM elbow flexion done to failure with partial vascular occlusion. Rest periods were set for 1 minute between sets and the blood pressure cuff remained inflated on the arm until all sets were completed. A pulse oximeter was used to monitor oxygen flow to the fingers of the arm and was placed on the subject immediately before each set to ensure that oxygen saturation did not decrease below 95% and blood flow was reaching the forearm during and after the exercise. If a pulse was not detected the pressure was lowered until a pulse was detected in the finger and adequate oxygen saturation (95%) was obtained. This only occurred in two subjects and both subjects regained oxygen saturation above 95% with only a small decrease in pressure. Blood samples were taken immediately before and immediately these exercise conditions. The total time to complete all sets was recorded.

Low and Moderate Intensity Conditions without Partial Vascular Occlusion

Subjects completed 3 sets of 30% or 70% 1RM elbow flexion done to failure without partial vascular occlusion. In addition, the subjects completed 3 sets of 30% 1RM elbow flexion with repetitions matched to the 30%1RM condition done with PVO. Multiple studies have examined differences in low intensity resistance exercise with PVO by equating total repetitions done to failure or specific workloads and utilizing a control group to complete the same amount of repetitions or workload (Takarada et. al. 2000 and 2002, Burgomaster et. al. 2003, Moore et. al. 2004, and Fujita et. al. 2007). This repetition matched condition was compared to another low intensity condition done to

failure without PVO to better compare the effect of failure on the specific blood markers measured.

The pulse oximeter was placed on the subject to assess blood oxygen saturation as previously stated. Blood was taken before and after these exercise conditions. The total time to complete all sets was recorded.

Blood Analysis

Protein Carbonyls

Whole blood (2 milliliters (ml)) mixed with EDTA was immediately centrifuged at 3,000 rpm at 4ºC for 15 min. Plasma was pipetted into 1.5 ml microcentrifuge tubes and stored at −80ºC until analyzed. Total plasma protein was determined in each sample by the Lowry technique. Five microliters (ul) of plasma was utilized in an immunosorbent assay via a commercially available ELISA Kit (Cayman Chemical Co., Ann Arbor, MI, USA). The plate was read at 450 nm wavelength on a microplate reader (BioTek Instruments, Winesski, VT). The data was then processed by a KC Junior software package. All samples were measured in duplicate and compared to standards.

Glutathione Ratio

Whole blood (2 ml for each GSH and GSSG) was pipetted into tubes containing 10% 5-sulfosalicylic acid containing bathophenantrolinedisulfonic acid (BPDS) at final concentration of 1 mM. Tubes were immediately mixed (Fisher Scientific Touch Mixer

Model 232) to ensure destruction of the red blood cells and destruction of enzymatic activity and were centrifuged at 3,000 rpm at 4ºC for 15 min. Supernatant from the tubes was then pipetted into 1.5 ml microcentrifuge tubes, centrifuged at 11, 000 rpm for 10 min to further separate the supernatant from any remaining cellular debris, and then placed into a −80ºC freezer until analyzed. Glutathione was determined spectrophotometrically (Shimadzu UV-1601, Baltimore, MD). This method enabled both the GSH and GSSG forms to be determined. All samples were measured in duplicate and compared to standards. GSSG was then recycled back to GSH by 2 vinyl pyridine and the total glutathione amount determined. The GSSG amount is equal to TGSH – GSH/2. The final ratio that was calculated is the GSSG/TGSH. The assay required monitoring the absorbance at 412 nm and comparing the change in absorbance over time with a kinetics package. Standards were utilized each time.

Oxygen Radical Absorbance Capacity

Whole blood (2ml) mixed with EDTA was immediately centrifuged at 3,000 rpm at 4ºC for 15 min. Plasma was pipetted into 1.5 ml microcentrifuge tubes and stored at −80ºC until analyzed. ORAC was measured using a modification of the methodology of Ou *et. al.* (2001). A serial dilution of a 50 uM Trolox solution (Wako Chemical) was made with phosphate buffer solution to produce 25, 12.5, 6.25 uM Trolox standards. Twenty ul of sample, blank and Trolox standard solutions were pipetted into appropriate wells. Then, 200 ul of fluoroscein working solution was added to each well. The plate was then incubated at 37 degrees Celsius for at least 20 minutes. Twenty ul of 2, 2'-

Azobis (2-methylpropionamidine) dihydrochloride (AAPH) (Sigma-Aldrich) was added as quickly as possible. The plate was read with a fluorometer using an excitation wavelength of 485nm and an emission wavelength of 520nm. All samples were measured in duplicate and compared to standards.

Xanthine Oxidase Activity

Whole blood (0.5ml) mixed with EDTA was immediately centrifuged at 700-1000 rpm at 4ºC for 10 minutes. Plasma was pipetted into 1.5 ml microcentrifuge tubes and stored at −80ºC until analyzed. The xanthine oxidase activity of plasma samples was measured using an assay kit (Cayman Chemical Co., Ann Arbor, MI, USA), according to the manufacturer's instructions. The plate was read with a fluorometer using an excitation wavelength of 525nm and an emission wavelength of 585nm. All samples were measured in duplicate and compared to standards.

Interluekin-6 Concentration

Whole blood(1.5ml) mixed with EDTA was immediately centrifuged for 15 minutes at approximately 1000 x g at 4ºC within 5 minutes of collection. Plasma was pipetted into 1.5 ml microcentrifuge tubes and stored at −80ºC until analyzed. IL-6 was analyzed by ELISA (Quantikine high-sensitivity IL-6; R&D System, Minneapolis, MN) and the plates were read at 450 nm on a microplate reader (BioTek Instruments, Winesski, VT). The data was then processed by a KC Junior software package. All samples were measured in duplicate and compared to standards.

Statistical Analysis

Strength Measures

Pre- and post-study values of isotonic (1RM) and isometric (MIF) strength measures were analyzed using a paired samples t-test.

Total Repetitions Completed Each Condition

The total number of repetitions needed to complete each condition was analyzed using a 2 (Intensity) x 2 (PVO/no PVO) repeated measures analysis of variance (RMANOVA).

Total Time to Complete Each Condition

The total time needed to complete each condition was analyzed using paired samples t-tests.

Total Work Completed Each Condition

The total work completed each condition was analyzed using a 2 (Intensity) x 2 (PVO/no PVO) repeated measures analysis of variance (RMANOVA).

Blood Markers

The oxidative stress and inflammatory blood markers were analyzed using a 3 (Intensity) x 2 (PVO/no PVO) x 2 (Time) RMANOVA.

Specific Aims #1-3

If a significant PVO x time interaction effect was noted for the 3 x 2 x 2 RMANOVA design then individual RMANOVAs were employed to further examine the effect of PVO on oxidative stress and inflammatory markers over time.

Specific Aim #4-5

If a significant intensity x time interaction effect was noted for the 3 x 2 x 2 RMANOVA design then a subsequent 2 (Intensity) x 2 (Time) RMANOVAs were employed to further examine the effect of intensity on oxidative stress and inflammatory markers over time. If a significant interaction was still present in the 2 x 2 RMANOVAs than individual RMANOVAs were utilized to see if significance still existed.

Specific Aim #6

To further examine the effect of failure compared to repetitions matched on oxidative stress and inflammatory markers over time in conditions done at low intensity (30%) without PVO a 2 (Failure/Repetitions Matched) x 2 (Time) RMANOVA was utilized.

Data was analyzed using an SPSS statistical package (version 15.1) with the level of significance set at an alpha level ≤ 0.05. The data is presented as means ± SD.

CHAPTER IV

RESULTS

The results section is organized as follows: Subject characteristics, strength measures, total repetitions completed during each condition, total time to complete each condition. A brief review of the statistical analysis of the blood markers (protein carbonyl level, glutathione ratio, oxygen radical absorbance capacity, xanthine oxidase activity and interleukin-6 concentration) will follow.

Subjects

Subjects reported to the lab at the same time for each condition with only a few exceptions (n=2) due to scheduling conflicts (see Appendix F). This resulted in these two subjects being tested at least 1.5 hours earlier than their normal scheduled testing time. Testing time varied between subjects and were spread throughout the day. Mean and standard deviation values of descriptive and anthropometric characteristics of the subjects are presented in Table 4. These anthropometric data were collected at rest at the start of the study prior to any lifting by the subjects.

Table 4

Subject Descriptive and Anthropometric Characteristics (N=12)

Variable	Mean±SD
Age (yrs)	24.67±2.96
Height (cm)	178.75±5.84
Weight (kg)	80.33±7.00
BMI (kg/m^2)	25.13±1.80
Body Fat (%)	11.21±5.10
RHR (bpm)	63.50±5.40
SBP (mmHg)	112.58±12.55
DBP (mmHg)	69.92±9.29

Elbow Flexion, Actual, and Adjusted Percent Workloads

Mean and standard deviation values for elbow flexion one repetition maximum (1RM) taken before and after the study, calculated 30% and 70%1RM loads, and loads and final percentages adjusted for the equipment used are presented in Tables 5 and 6. The statistical analysis of the 1RM measure revealed no significant difference between pre- and post-study values.

Table 5

Elbow Flexion 1RM

	Pre-Study (Mean±SD)	Post-Study (Mean±SD)	p-value*
1RM (kg)	19.89±3.23	20.45±3.36	0.08
* - Pre-study vs. Post-study analysis			

Table 6 **Actual and Adjusted Percent Workloads**	
Variable	**Mean±SD**
30%1RM (kg)	5.97±0.97
70%1RM (kg)	13.92±2.26
Adjusted 30% (kg)	5.87±1.52
Adjusted 70% (kg)	14.02±1.90
Adjusted % 1RM	29.21±3.58
Adjusted % 1RM	70.80±3.57

Maximal Isometric Force

Isometric strength measures were taken before the start of the study during the prescreening session and after the subjects completed all study conditions. There were no significant differences for peak torque, average peak torque, or peak torque per kg of body weight for either the dominant arm or the non-dominant arm. The means, standard deviations, and p-values are presented in Table 7.

Table 7

Maximal Isometric Force Pre- and Post-Study

	Dominant Arm			Non-Dominant Arm		
Variable	**Pre-Study (Mean±SD)**	**Post-Study (Mean±SD)**	**p-value***	**Pre-Study (Mean±SD)**	**Post-Study (Mean±SD)**	**p-value***
Peak Torque (N·m)	72.3±11.3	69.1±11.6	0.11	65.6±10.1	64.3±10.7	0.40
Average Peak Torque (N·m)	69.8±11.2	66.2±10.5	0.09	63.4±10.3	62.5±10.8	0.59
Peak Torque/kg (N·m)	87.2±12.8	82.9±12.2	0.09	79.6±13.2	78.35±14.1	0.61
* - Pre-study vs. Post-study analysis						

Total Repetitions Completed Each Condition

The total number of repetitions was significantly greater for all conditions done at 30%1RM compared to all conditions done at 70%1RM. When intensity was held constant, PVO significantly decreased the number of repetitions to failure. The means and standard deviations are presented in Table 8 and the RMANOVA analysis is presented in Table 9.

Table 8

Total Repetitions Completed Each Condition

Condition	Mean (repetitions)±SD
30PVO	53.5±13.8
30F	77.25±22.4
70PVO	15.3±3.0
70F	18.6±4.9

Table 9

RMANOVA [2 (Intensity) x 2 (PVO)] analysis of Total Repetitions Completed Each Condition

Effect	Df	F-value	p-value
Intensity	2	97.54	0.00*
PVO	1	50.04	0.00*
Intensity x PVO	2	23.21	0.00*
* - Significant at the $p \leq 0.05$ level.			

Total Time to Complete Each Condition

Paired t-tests reported significant time differences for all conditions done at 30%1RM when compared to conditions done at 70%1RM ($p = .001$). The conditions 30PVO and 30F were also significantly different from one another ($p = .001$). No significant differences were obtained between 30PVO and 30RM or between 70PVO and 70F. Means and standard deviations for the total time to completion for each condition are presented in Table 10.

Table 10

Total Time to Complete Each Condition

Condition	Mean (seconds) ± SD
RPVO	262.58±42.86
R	262.58±42.86
30PVO	262.58±42.86
30RM	228.92±40.29
30F	315.83±105.87*
70PVO	173.75±10.38†
70F	179.17±12.01†

* - Significantly different than 30PVO and 30RM conditions.
† - Significantly different from low intensity (30%) and rest conditions.

Total Work Completed Each Condition

The total work completed in each exercise condition was significantly increased in all conditions done at 30%1RM compared to all conditions done at 70%1RM. When intensity was held constant, PVO significantly decreased the total work completed for each condition. The means and standard deviations are presented in Table 11 and the RMANOVA analysis is presented in Table 11.

Table 11

Total Work (Weight(kg) x Total Repetitions) Completed Each Condition

Condition	Mean ± SD
30PVO	300.8±59.2
30F	427.8±61.8
70PVO	210.8±31.6
70F	256.8±55.3

Table 12

RMANOVA [2 (Intensity) x 2 (PVO)] analysis of Total Work Completed Each Condition

Effect	Df	F-value	p-value
Intensity	2	54.90	0.00*
PVO	1	125.88	0.00*
Intensity x PVO	2	13.03	0.00*
* - Significant at the $p \leq 0.05$ level.			

Protein Carbonyls

A 3 (Intensity) x 2 (PVO) x 2 (Time) RMANOVA analysis revealed significant intensity x PVO x time (p=0.00) and intensity x time interaction effects (p=0.01). A significant time main effect was also revealed with this analysis (p=0.00). Means and standard deviations for each condition are presented in Table 13. The specific main effects and interaction effects are presented in Table 14 along with F-values and p-values.

Table 13

Protein Carbonyls Levels (nmol/mg) Before and Immediately After Each Exercise/Rest Condition

Condition	Pre-Exercise (mean±SD)	Post-Exercise (mean±SD)
RPVO	0.08±0.04	0.11±0.04
R	0.11±0.04	0.11±0.04
30PVO	0.08±0.03	0.13±0.05
30F	0.09±0.04	0.13±0.03
70PVO	0.10±0.03	0.11±0.02
70F	0.09±0.03	0.15±0.05

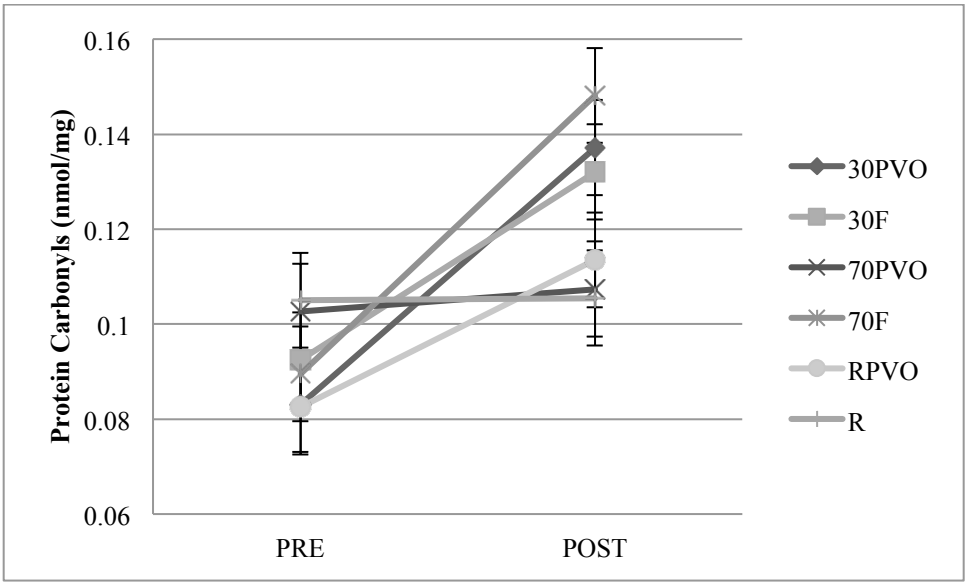

Figure 1. Protein Carbonyls Levels Before and Immediately After Each Exercise/Rest Condition.

Table 14

RMANOVA [3(Intensity) x 2 (PVO) x 2 (Time)] analysis of PC levels

Effect	df	F-value	p-value
Intensity	2	0.84	0.45
PVO	1	2.74	0.13
Time	1	17.18	0.00*
Intensity x PVO	2	0.30	0.75
Intensity x Time	2	5.54	0.01*
PVO x Time	1	0.15	0.70
Intensity x PVO x Time	2	6.83	0.01*
* - Significant at the $p \leq 0.05$ level.			

A 3 (Intensity) x 2 (Time) RMANOVA was utilized for conditions with and without PVO (Table 15) to further probe the significant 3-way (intensity x PVO x time) interaction observed in the 3 x 2 x 2 RMANOVA. Significant intensity x time interactions were revealed in conditions with and without PVO.

Table 15

RMANOVA [3 (Intensity) x 2 (Time)] analysis of PC levels with and without PVO

Effect	PVO			Without PVO		
	df	F-value	p-value	df	F-value	p-value
Intensity	2	0.75	0.48	2	0.51	0.61
Time	1	13.53	0.00*	1	14.00	0.00*
Intensity x Time	2	5.33	0.01*	2	6.14	0.02*
* - Significant at the $p \leq 0.05$ level.						

Six different 2 (Intensity) x 2 (Time) RMANOVAs were utilized to further identify which conditions were contributing to the interaction effects. Intensity x time interactions were reported when comparing 30PVO and 70PVO conditions, 30F and R conditions, and 70F and R conditions (Table 16 and 17). Time main effects were reported

for all comparisons.

Table 16

RMANOVA [2 (Intensity) x 2 (Time)] analysis of PC levels for conditions with PVO

Effect	30% and 70%			30% and Rest			70% and Rest		
	df	F-value	p-value	df	F-value	p-value	df	F-value	p-value
Intensity	2	0.47	0.51	2	1.12	0.31	2	0.46	0.51
Time	1	9.52	0.01*	1	14.48	0.00*	1	7.96	0.02*
Intensity x Time	2	6.99	0.02*	2	2.76	0.13	2	4.76	0.052
* - Significant at the $p \leq 0.05$ level.									

Table 17

RMANOVA [2 (Intensity) x 2 (Time)] analysis of PC levels for conditions without PVO

Effect	30% and 70%			30% and Rest			70% and Rest		
	df	F-value	p-value	df	F-value	p-value	df	F-value	p-value
Intensity	2	0.23	0.64	2	0.28	0.61	2	1.13	0.31
Time	1	15.28	0.00*	1	10.47	0.01*	1	9.10	0.01*
Intensity x Time	2	1.50	0.25	2	10.51	0.01*	2	10.20	0.01*
* - Significant at the $p \leq 0.05$ level.									

Individual RMANOVA revealed significant time main effects in RPVO, 30PVO, 30F, and 70F conditions (Table 18).

Table 18

RMANOVA [1 (Condition) x 2 (Time)] analysis of PC levels in conditions with and without PVO

Time Effect			
Condition	df	F-value	p-value
RPVO	1	13.39	0.00*
R	1	0.01	0.94
30PVO	1	10.64	0.01*
30F	1	13.71	0.00*
70PVO	1	0.27	0.61
70F	1	10.65	0.01*
* - Significant at the $p \leq 0.05$ level.			

Glutathione Ratio

A 3 (Intensity) x 2 (PVO) x 2 (Time) RMANOVA analysis revealed only a significant PVO main effect. Means and standard deviations for each condition are presented in Table 19. The specific main effects and interaction effects are presented in Table 20 along with F-values and p-values.

Table 19

Glutathione Ratio (GSSG/TGSH (%)) Before and Immediately After Each Exercise/Rest Condition.

Condition	**Pre-Exercise (mean±SD)**	**Post-Exercise (mean±SD)**
RPVO	18.03±6.41	17.18±8.01
R	21.01±7.82	21.02±7.89
30PVO	18.83±7.07	20.71±8.35
30F	17.47±6.54	17.11±5.72
70PVO	18.06±8.12	19.19±7.30
70F	22.16±7.39	20.14±6.97

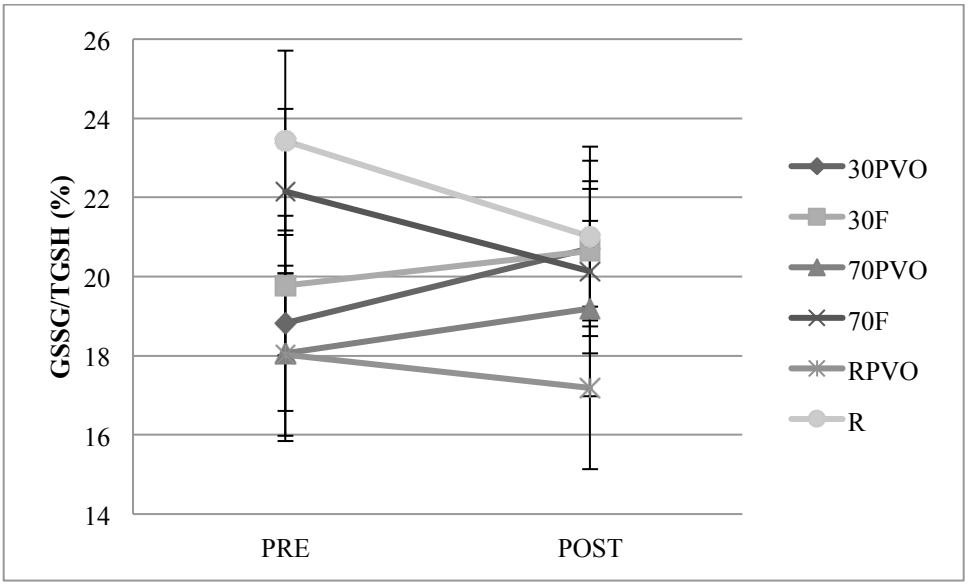

Figure 2. Glutathione Ratio Before and Immediately After Each Exercise/Rest Condition.

Table 20

RMANOVA [3 (Intensity) x 2 (PVO) x 2 (Time)] analysis of Glutathione Ratio

Effect	df	F-value	p-value
Intensity	2	0.00	0.99
PVO	1	8.44	0.01*
Time	1	0.10	0.75
Intensity x PVO	2	0.66	0.54
Intensity x Time	2	2.30	0.15
PVO x Time	1	3.04	0.11
Intensity x PVO x Time	2	0.27	0.77
* - Significant at the $p \leq 0.05$ level.			

Oxygen Radical Absorbance Capacity (ORAC)

A 3 (Intensity) x 2 (PVO) x 2 (Time) RMANOVA analysis revealed significant intensity x PVO, PVO x time, and intensity x time interaction effects. A significant intensity effect was also reported. Means and standard deviations for each condition are presented in Table 21. The specific main effects and interaction effects are presented in Table 22 along with F-values and p-values.

Table 21

ORAC (uM Trolox) Before and Immediately After Each Exercise/Rest Condition.

Condition	Pre-Exercise (mean±SD)	Post-Exercise (mean±SD)
RPVO	6.23±0.69	6.23±0.80
R	5.99±0.84	5.71±1.01
30PVO	5.92±0.77	5.93±0.82
30F	5.72±0.96	5.66±0.88
70PVO	5.76±0.88	6.01±0.69
70F	6.18±0.60	6.24±0.57

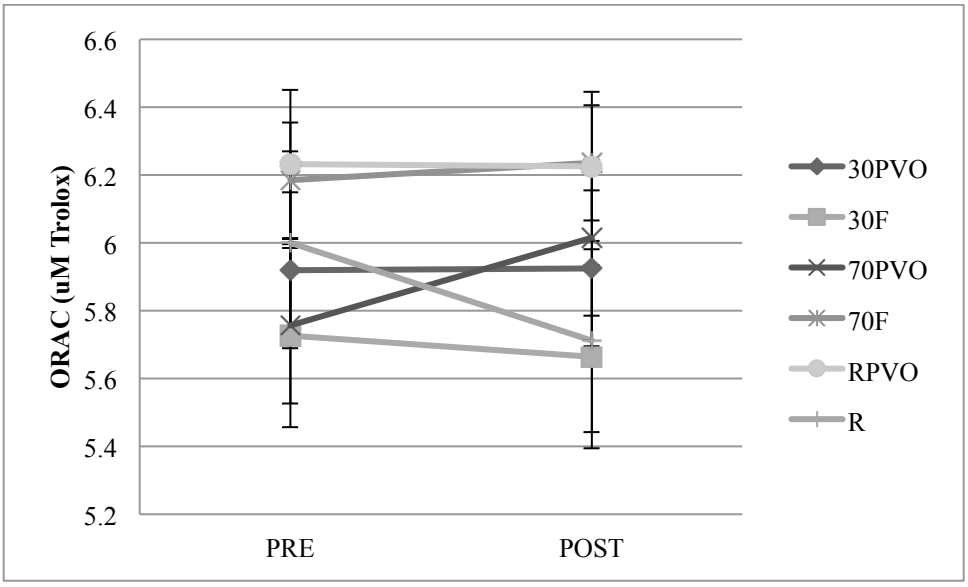

Figure 3. Oxygen Radical Absorbance Capacity Before and Immediately After Each Exercise/Rest Condition.

Table 22

RMANOVA [3 (Intensity) x 2 (PVO) x 2 (Time)] analysis of ORAC

Effect	df	F-value	p-value
Intensity	2	16.58	0.00*
PVO	1	1.99	0.19
Time	1	0.11	0.75
Intensity x PVO	2	20.36	0.00*
Intensity x Time	2	6.35	0.02*
PVO x Time	1	9.62	0.01*
Intensity x PVO x Time	2	0.44	0.66
* - Significant at the $p \leq 0.05$ level.			

2 (Intensity) x 2 (PVO) RMANOVAs (Table 23) were utilized to further examine the intensity x PVO interactions reported in Table 22 and identify interaction effects. This analysis revealed a significant intensity main effect as well as an intensity x PVO interaction when comparing the low and moderate intensities. An intensity and PVO main effect but no interaction effect was revealed when comparing the low intensity to rest. The comparison of moderate intensity and rest revealed an intensity x PVO interaction effect.

Table 23

RMANOVA [2 (Intensity) x 2 (PVO)] analysis of ORAC

Effect	30% and 70%			30% and Rest			70% and Rest		
	df	F-value	p-value	df	F-value	p-value	df	F-value	p-value
Intensity	2	6.92	0.02*	2	21.24	0.00*	2	0.00	0.95
PVO	1	0.63	0.44	1	29.45	0.00*	1	0.10	0.76
Intensity x PVO	2	15.76	0.00*	2	0.94	0.34	2	57.42	0.00*
* - Significant at the $p \leq 0.05$ level.									

Individual RMANOVAs were utilized to further examine the intensity x PVO interactions reported in Table 23 and identify PVO effects. The analysis revealed significant PVO main effects in all intensities (Table 24).

Table 24

RMANOVA [1 (Intensity) x 2 (PVO)] analysis of ORAC

Effect	Rest			30%			70%		
	df	F-value	p-value	df	F-value	p-value	df	F-value	p-value
PVO	1	17.19	0.00*	1	5.55	0.03*	1	13.35	0.00*
* - Significant at the $p \leq 0.05$ level.									

2 (Intensity) x 2 (Time) RMANOVAs were utilized to further examine the intensity x time interactions reported in Table 24 and identify interaction effects. A significant intensity x time interaction was reported when comparing low intensity to moderate intensity as well as when comparing moderate intensity to rest. This analysis also revealed a significant intensity and time main effect when comparing low intensity to rest (Table 25).

Table 25

RMANOVA [2 (Intensity) x 2 (Time)] analysis of ORAC

Effect	30% and 70%			30% and Rest			70% and Rest		
	df	F-value	p-value	df	F-value	p-value	df	F-value	p-value
Intensity	2	4.01	0.06	2	11.60	0.00*	2	0.00	0.96
Time	1	2.03	0.17	1	5.65	0.03*	1	0.01	0.92
Intensity x Time	2	4.78	0.04*	2	0.98	0.33	2	13.27	0.00*
* - Significant at the $p \leq 0.05$ level.									

Individual RMANOVAs were utilized to further examine the intensity x time interactions reported in Table23 and identify time effects. The analysis revealed significant time effects for moderate intensity and rest (Table 26).

Table 26

RMANOVA [1 (Intensity) x 2 (Time)] analysis of ORAC

Effect	Rest			30%			70%		
	df	F-value	p-value	df	F-value	p-value	df	F-value	p-value
Time	1	4.41	0.05*	1	0.16	0.69	1	9.48	0.01*
* - Significant at the $p \leq 0.05$ level.									

Individual RMANOVAs were utilized to further examine the PVO x time interactions reported in Table 23 and identify interaction effects. A significant time effect was reported in the conditions done with PVO, but was not seen in the conditions done without PVO (Table 27).

Table 27 RMANOVA [1 (PVO) x 2 (Time)] analysis of ORAC						
Effect	**PVO**			**Without PVO**		
	df	F-value	p-value	df	F-value	p-value
Time	1	4.18	0.05*	1	2.45	0.13
* - Significant at the $p \leq 0.05$ level.						

Xanthine Oxidase Activity

A 3 (Intensity) x 2 (PVO) x 2 (Time) RMANOVA analysis revealed a significant intensity x time interaction effect. In addition, a significant time main effect was obtained with this analysis. Means and standard deviations for each condition are presented in Table 28. The specific main effects and interaction effects are presented in Table 29 along with F-values and p-values.

Table 28:

Xanthine Oxidase Activity (uU/mL) Before and Immediately After Each Exercise/Rest Condition.

Condition	Pre-Exercise (mean±SD)	Post-Exercise (mean±SD)
RPVO	33.34±17.83	37.81±16.05
R	44.97±15.30	44.54±15.62
30PVO	45.62±16.86	35.69±16.97
30F	39.22±11.91	30.45±8.76
70PVO	41.76±27.85	36.91±20.83
70F	44.72±16.46	34.38±14.92

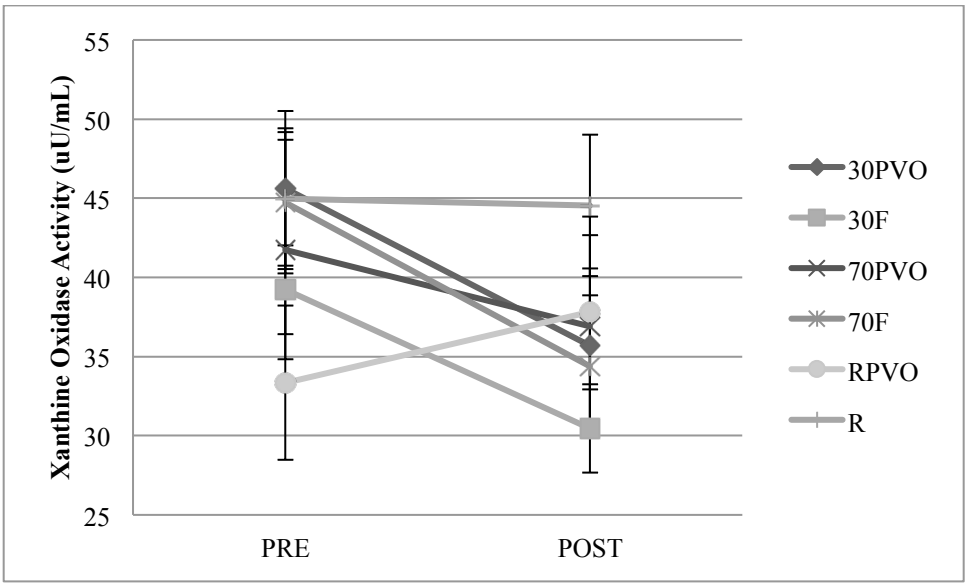

Figure 4. Xanthine Oxidase Activity Before and Immediately After Each Exercise/Rest Condition.

Table 29

RMANOVA [3 (Intensity) x 2 (PVO) x 2 (Time)] analysis of XO activity

Effect	df	F-value	p-value
Intensity	2	0.21	0.81
PVO	1	0.24	0.63
Time	1	16.45	0.00*
Intensity x PVO	2	2.30	0.15
Intensity x Time	2	6.46	0.02*
PVO x Time	1	1.39	0.26
Intensity x PVO x Time	2	1.97	0.19
* - Significant at the $p \leq 0.05$ level.			

2 (Intensity) x 2 (Time) RMANOVAs were utilized to further examine the intensity x time interactions reported in Table 29 and identify interaction effects. This analysis revealed a significant time main effect when comparing the low and moderate

intensities. A significant time main effect and intensity x time interaction was reported when comparing low intensity and rest. An intensity x time interaction was also reported when comparing moderate intensity and rest (Table 30).

Table 30

RMANOVA [2 (Intensity) x 2 (Time)] analysis of XO activity

Effect	30% and 70%			30% and Rest			70% and Rest		
	df	F-value	p-value	df	F-value	p-value	df	F-value	p-value
Intensity	2	0.16	0.69	2	0.35	0.56	2	0.02	0.90
Time	1	21.47	0.00*	1	9.16	0.01*	1	3.10	0.09
Intensity x Time	2	0.39	0.54	2	9.07	0.01*	2	13.40	0.00*
* - Significant at the $p \leq 0.05$ level.									

Individual RMANOVAs were utilized to further examine the intensity x time interactions reported in Table 30 and identify time effects. The analysis revealed significant time effects for moderate and low intensity (Table 31).

Table 31

RMANOVA [1 (Intensity) x 2 (Time)] analysis of XO activity

Effect	Rest			30%			70%		
	df	F-value	p-value	df	F-value	p-value	df	F-value	p-value
Time	1	1.04	0.32	1	14.30	0.00*	1	12.77	0.00*
* - Significant at the $p \leq 0.05$ level.									

Interleukin-6 Concentration

A 3 (Intensity) x 2 (PVO) x 2 (Time) RMANOVA analysis revealed significant intensity x time and PVO x time interaction effects. A time main effect of was also obtained with this analysis. Means and standard deviations for each condition are presented in Table 32. The specific main effects and interaction effects are presented in Table 33 along with F-values and p-values.

Table 32

IL-6 concentration (pg/mL) Before and Immediately After Each Exercise/Rest Condition.

Condition	Pre-Exercise (mean±SD)	Post-Exercise (mean±SD)
RPVO	0.57±0.23	0.68±0.29
R	0.68±0.49	0.67±0.52
30PVO	0.74±0.37	0.84±0.41
30F	0.58±0.24	0.65±0.29
70PVO	0.77±0.38	0.91±0.40
70F	0.64±0.27	0.72±0.30

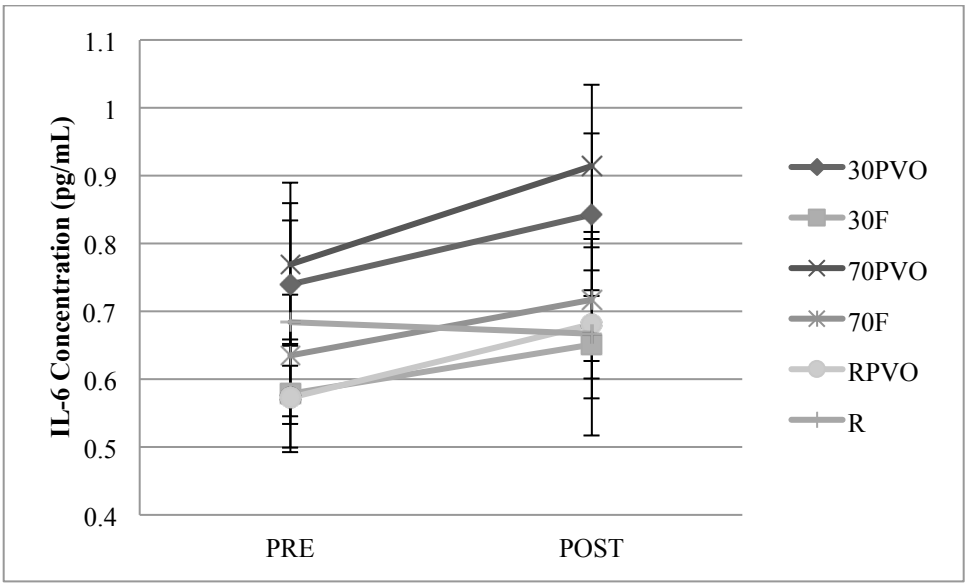

Figure 5. IL-6 Concentration Before and Immediately After Each Exercise/Rest Condition.

Table 33

RMANOVA [3 (Intensity) x 2 (PVO) x 2 (Time)] analysis of IL-6 concentration

Effect	df	F-value	p-value
Intensity	2	0.54	0.60
PVO	1	0.76	0.41
Time	1	29.97	0.00*
Intensity x PVO	2	2.46	0.14
Intensity x Time	2	10.42	0.01*
PVO x Time	1	10.26	0.01*
Intensity x PVO x Time	2	0.48	0.63
* - Significant at the $p \leq 0.05$ level.			

2 (Intensity) x 2 (Time) RMANOVAs were utilized to further examine the intensity x time interactions reported in Table 33 and identify interaction effects. This analysis revealed a time main effect in all comparison and an intensity x time interaction when comparing moderate intensity and rest (Table 34).

Table 34

RMANOVA [2 (Intensity) x 2 (Time)] analysis of IL-6 concentration

Effect	30% and 70%			30% and Rest			70% and Rest		
	df	F-value	p-value	df	F-value	p-value	df	F-value	p-value
Intensity	2	0.46	0.50	2	0.52	0.48	2	1.32	0.26
Time	1	43.39	0.00*	1	11.78	0.00*	1	15.85	0.00*
Intensity x Time	2	0.57	0.46	2	1.28	0.27	2	4.81	0.04*
* - Significant at the $p \leq 0.05$ level.									

Individual RMANOVAs were utilized to further examine the intensity x time interactions reported in Table 34 and identify time effects. The analysis revealed significant time effects for moderate and low intensity (Table 35).

Table 35

RMANOVA [1 (Intensity) x 2 (Time)] analysis of IL-6 concentration

Effect	Rest			30%			70%		
	df	F-value	p-value	df	F-value	p-value	df	F-value	p-value
Time	1	2.42	0.14	1	13.23	0.00*	1	31.44	0.00*
* - Significant at the $p \leq 0.05$ level.									

Individual RMANOVAs were utilized to further examine the PVO x time interactions reported in Table 32 and identify time effects. This analysis revealed a significant time main effect in conditions with and without PVO (Table 36).

Table 36

RMANOVA [1 (PVO) x 2 (Time)] analysis of IL-6 concentration

Effect	PVO			No PVO		
	df	F-value	p-value	df	F-value	p-value
Time	1	43.92	0.00*	1	4.61	0.04*
* - Significant at the $p \leq 0.05$ level.						

The effect of failure compared to repetitions matched on oxidative stress and inflammatory markers over time in conditions done at low intensity (30%) without PVO.

2 (Failure/Repetitions Matched) x 2 (Time) RMANOVAs (Table 38) were utilized to identify interaction and main effects between conditions (30F and 30RM) and over time. Means and standard deviations for each condition and blood marker are presented in Table 37.

Table 37

Blood marker values pre- and post-exercise in low intensity conditions (30%) without PVO

Blood Marker	30F		30RM	
	Pre-Exercise (mean ±SD)	Post-Exercise (mean ±SD)	Pre-Exercise (mean ±SD)	Post-Exercise (mean ±SD)
PC (nmol/mg)	0.08±0.03	0.13±0.05	0.10±0.04	0.11±0.04
GSSG/TGSH (%)	18.83±7.07	20.71±8.35	19.77±5.25	20.65±7.24
ORAC (uM Trolox)	5.92±0.77	5.93±0.82	5.52±0.80	5.52±0.86
XO (uU/mL)	45.62±16.86	35.69±16.97	27.97±15.76	22.57±11.43
IL-6 (pg/mL)	0.74±0.37	0.84±0.41	0.76±0.42	0.91±0.47

A significant time effect was reported for PC levels, XO activity, and IL-6 concentration and a significant main effect was reported for XO activity (Table 38 and 39). No interaction or main effects were reported for glutathione ratio or ORAC.

Table 38

RMANOVA [2 (Failure/Repetitions Matched) x 2 (Time)] analysis of Protein Carbonyls levels, Glutathione Ratio, and ORAC in low intensity conditions (30%) without PVO.

Effect	PC			GSSG/TGSH			ORAC		
	df	F-value	p-value	df	F-value	p-value	df	F-value	p-value
Failure/Repetitions Matched	1	0.36	0.56	1	2.27	0.16	1	1.00	0.34
Time	1	21.64	0.00*	1	0.06	0.82	1	0.09	0.78
F/RM x Time	1	1.81	0.21	1	0.24	0.63	1	0.17	0.69
* - Significant at the $p \leq 0.05$ level.									

Table 39

RMANOVA [2 (Failure/Repetitions Matched) x 2 (Time)] analysis of Xanthine Oxidase Activity and IL-6 in low intensity conditions (30%) without PVO.

Effect	XO			IL-6		
	df	F-value	p-value	df	F-value	p-value
Failure/Repetitions Matched	1	6.67	0.03*	1	2.94	0.12
Time	1	11.95	0.01*	1	8.78	0.01*
F/RM x Time	1	0.72	0.41	1	1.42	0.26
* - Significant at the $p \leq 0.05$ level.						

Individual RMANOVA revealed a significant time effect in PC levels and XO activity in the 30F condition and IL-6 concentration in the 30RM condition (Table 40).

Table 40

RMANOVA [1 (Condition) x 2 (Time)] analysis of low intensity conditions (30%) without PVO.

	Time Effect					
Blood Marker	30F			30RM		
	df	F-value	p-value	df	F-value	p-value
PC	1	13.71	0.00*	1	2.19	0.17
XO	1	8.05	0.02*	1	4.36	0.06
IL-6	1	3.80	0.08	1	6.31	0.03*
* - Significant at the $p \leq 0.05$ level.						

Summary

The addition of PVO impacted the number of repetitions done, time to completion, and total work completed in both the low and moderate intensity conditions.

The analysis of PC levels revealed an intensity x PVO x time interaction. Further analysis of this interaction revealed specific interactions between the 30F and R conditions, 70F and R conditions, and 30PVO and 70PVO conditions. The interactions were due to PC level increases seen in the 30F, 70F, and 30PVO while no change over time in the R and 70PVO conditions.

Glutathione ratio measures revealed a PVO main effect independent of intensity level or time.

ORAC analysis revealed intensity x PVO, intensity x time, and PVO x time interactions. The intensity x PVO interaction resulted from higher ORAC values in the moderate intensity condition without PVO compared to with PVO independent of time. This was different than the low intensity and rest conditions which reported higher

ORAC values in conditions with PVO compared to without PVO. Intensity x time interactions were further analyzed and revealed specific interactions between moderate and low intensity conditions as well as moderate intensity and rest conditions. This was due to increases over time seen in moderate intensity when compared to decreases over time during rest and no change over time during low intensity. The PVO x time interaction was due to increases over time in conditions with PVO that was not reported in conditions without PVO.

XO activity analysis reported an intensity x time interaction. This interaction resulted from decreases in XO activity over time in both the moderate and low intensity conditions that were not seen in the rest conditions.

Analysis of IL-6 levels revealed an intensity x time and PVO x time interaction. Further analysis of the intensity x time interaction revealed a significant increase over time in the moderate intensity conditions when compared to the rest conditions that reported no change over time. The PVO x time interaction was a result of significant increases of time in the conditions with PVO to a much greater extent than increases over time in conditions without PVO.

The effect of completing each set to failure as opposed to repetitions matched resulted in no differences between low intensity groups without PVO. The 30%1RM without PVO condition done to failure resulted in significant increases in PC and significant decreases in XO over time and the 30%1RM with PVO condition done with

repetitions matched to the 30PVO condition resulted in significant IL-6 increases over time.

CHAPTER V

DISCUSSION

The main focus of this study was to examine the effects of PVO and different intensities of resistance exercise on oxidative stress markers and inflammation over time.

This discussion will begin with the analysis of isometric force values taken before and after the study and then examine the differences in the conditions concerning total repetitions completed in each condition and total time to complete each condition. The effect of PVO in the absence of resistance exercise will then be discussed, followed by the effect of resistance exercise done at the different intensities with and without PVO.

The subjects demonstrated no significant differences in maximal isometric or isotonic force measures taken before and after completion of the study. This finding substantiates to some extent that the subject's resistance training status was stable and that the study had no substantial effect on their elbow flexion strength over time. The subjects included in the study were required to be resistance-trained to minimize the amount of muscle adaptation during the study conditions which could influence the oxidative stress and inflammatory blood markers over time. The subjects were instructed to maintain their resistance training programs and avoid any increases in training intensity or changes in training mode. These abrupt changes in training status could have affected the resting and exercise blood markers as well as the subject's performance on their testing days.

The total number of repetitions done and total time to completion was significantly different between low intensity conditions done with PVO and without PVO. Wernbom *et. al.* (2007) reported significantly lower repetitions completed at low intensities (20%, 30%, and 40%1RM) using the dynamic knee extensions done to failure with PVO when compared to without PVO. Central and peripheral fatigue may also play a role in the effect of PVO on total repetitions and time to completion. A recent study by Karabulut and colleagues (2010) showed significant decreases in potentiated twitch, EMG amplitude, and maximal voluntary activation after a leg extension exercise done at 20% 1RM with PVO compared to without PVO. Previous research reported increased lactate concentration immediately after resistance exercise at low-intensity with PVO compared to without PVO (Takarada et. al. 2004 and Fujita et. al. 2007). In addition, Reeves et. al. (2006) reported increases in lactate during low intensity exercise with PVO that were similar to a high intensity resistance exercise condition without PVO. These studies support the finding that PVO done at low intensity can result in an increase in metabolic stress not seen in low intensity conditions matched for repetitions. This increase in metabolic stress may manifest itself in decreased total repetitions and less total time to complete the exercise.

The significant difference in total repetitions was expected in the low intensity condition but not the moderate intensity condition based on the pilot study findings. The moderate intensity condition with PVO decreased the amount of repetitions done to failure by an average of 3.3±1.8 repetitions. The two moderate intensity conditions were not repetition matched as a pilot study (n=2) reported no difference in repetitions done

with and without PVO at moderate intensity. Wernbom *et. al.* (2007) reported no significant difference in repetitions done after completing one set of dynamic knee extensions performed at 50%1RM with and without PVO. It was thought that the 70% 1RM intensity in the present study which is greater than the 50% 1RM in the Wernbom *et. al.* study should not have influenced the number of repetitions. Clearly the results in this study are not congruent with the pilot study and the Wernbom *et. al.* study results. Differences in muscles utilized, cuff pressure, and number of sets may have had an effect on total repetitions completed. Wernbom *et. al.* utilized knee extensors and a higher cuff pressure (200mmHg) whereas the present study utilized elbow flexors and a lower cuff pressure (20mmHg below SBP). Wernbom *et. al.* also utilized only one set of knee extension. It is possible that multiple sets may decrease the total amount of repetitions completed due to increased metabolite accumulation or other local factors activated by an increasingly hypoxic state of the muscle. Though the moderate intensity conditions resulted in a significant difference in total repetitions when comparing with and without PVO there was no significant difference in total time to completion (5.42±1.63 seconds). To my knowledge, no studies have examined PVO and fatigue done at this particular moderate intensity level.

The first specific aim examined the effect of PVO on conditions done at rest. The main finding for this specific aim is that no significant differences were reported when comparing resting conditions with and without PVO. Significant increases were reported over time in PC levels in the RPVO condition and may be due to ischemia reperfusion (I/R) injury. I/R, as stated in the literature review, involves increased tissue hypoxia

followed by reperfusion of this tissue with oxygenated blood. I/R was shown to result in increases in free radicals and inflammatory markers in rat (Uchiyama et. al. 2006 and Pattwell et. al. 2003) and human models (Rodriguez et. al. 2003). Protein carbonyl levels have been shown to significantly increase in rat skeletal muscle following I/R injury (Ozyurt et. al. 2006). However, the magnitude of the increase in the present study, although it was significant over time, was modest and may be related to the total time of PVO and the small amount of muscle mass affected by the PVO.

The mechanism which induced the increases in PC levels may differ from the normal I/R model as the occlusion was moderate and blood oxygen saturation levels were not permitted to decrease below 95%. The excessive amount of blood pooling on the venous side upstream of the pressure cuff may have led to a significant elevation in venous pressure and endothelial stretch. This resulting stretch has been implicated in the release of reactive oxygen species. Reactive oxygen species have been shown to play an important role in signal transduction and physiologic regulation of vascular function (Touyz and Briones, 2011 and Birukov, 2009).A major source of reactive oxygen species in the vascular wall has been speculated to be generated from NADPH oxidase, xanthine oxidase, nitric oxide synthase, and/or mitochondrial respiratory strain (Birukov 2009). The extent to which this endothelial stretch affected the blood markers is not known as this study did not measure changes in vessel diameter due to the PVO. A future study would benefit from measuring the impact of endothelial stretch and its potential impact on oxidative stress markers.

Glutathione ratio was elevated in conditions with PVO compared to without PVO. These changes were independent of intensity and time suggesting that this difference was due to variation in glutathione ratio pre-exercise levels.

The second specific aim of this study was to examine the effect of PVO on resistance exercise done at low intensity exercise. The major finding reported with the low intensity condition was the lack of significance between the conditions with and without PVO. These findings suggest that the addition of PVO to low intensity resistance exercise had no additional impact on oxidative stress and inflammatory markers. Since there was an impact in total repetitions completed with the resistance exercise to failure at this intensity, this suggests that taking the muscles to failure may be an important aspect of the response. As previously noted, studies have reported an increase in lactate levels (Takarada et. al. 2004 and Fujita et. al. 2007) with low intensity resistance exercise with PVO when compared to repetition matched conditions done at the same intensity. This study employed a low intensity condition done to failure without PVO. This may have equated the muscular environment at the end of each condition resulting in similar hypoxic and metabolite conditions. A future measure of lactate from the blood collected in this study could be utilized to compare the metabolic stress that occurred during resistance exercise done to failure with and without PVO.

Takarada *et. al.* (2004) reported significant increases in IL-6 immediately and 30 minutes post-exercise following low-intensity (20%1RM) done to failure with PVO. This study implemented a larger muscle group (bilateral leg extensions) and a higher number

of sets (n=5). Pedersen and Febbraio (2008) noted that contracting muscle is an important source of IL-6 found in the plasma and that a greater muscle mass utilized during exercise would result in a greater IL-6 response. This suggests that the modest increases in IL-6 reported in the present study may be related to the smaller muscle mass used. A future study should examine the IL-6 response to PVO incorporating a larger muscle mass. IL-6 increases are also correlated highly with increases in intensity and duration of exercise and and have been postulated to mediate exercise-associated metabolic changes as well as exercise-related immune changes. This is important to note when comparing exercise conditions to resting conditions as the increases in IL-6 during exercise conditions are most likely due to repeated muscle contractions while IL-6 increases in the rest condition with PVO may be related to other signaling pathways. Tumor Necrosis Factor alpha (TNFα) is another cytokine related to systemic inflammation. If increases in TNFα were reported prior to IL-6 this would indicate that IL-6 is acting as a pro-inflammatory agent. However, if no increases in TNFα are observed then IL-6 is primarily acting as a myokine (Pederson and Febbraio, 2008). TNFα was not determined in this study.

The low intensity conditions also reported significant XO decreases over time. This study hypothesized that XO activity would be increased in the blood following resistance exercise with and without PVO and should have contributed to some increases in oxidative stress markers previously reported. XO activity has been reported to increase following repeated sprint exercise (Abbey and Rankin 2011) and resistance exercise in trained (Spiering et. al. 2007) and untrained individuals (Ho et. al. 2010). The resistance

exercise done in the Spiering *et. al.* and Ho *et. al.* studies differed from this study as more sets were completed (4-5 sets)and larger muscle groups were utilized (squats). These differences could possibly have increased the total amount of purine metabolism due to the increased metabolic stress occurring in the exercising muscles.

Diet can also effect XO activity as increases following resistance exercise have been attenuated in studies implementing supplementation with green tea (Panza et. al. 2008) and L-carnitine (Speiring et. al. 2007). It has been reported that catechins found in green tea can inhibit xanthine oxidase activity (Aucamp et. al. 1997) and L-carnitine supplementation may lessen metabolic stress during resistance training by reducing purine metabolism and XO production (Speiring et. al. 2007). This is an unlikely reason for the decrease in XO activity as the dietary analysis reported no extreme levels of major exogenous antioxidants (Vitamin C and E) in the subjects' diets.

It should be noted that overall the XO activity recorded was extremely low compared to previous research studies examining XO in young resistance trained males (Panza et. al. 2008) and active apparently-healthy older adults (Ho et. al. 2010) There has been some speculation in the literature that xanthine oxidase levels in the blood are extremely low in humans compared to rat models and may not play a significant role in superoxide production in the skeletal muscle (Powers et. al. 2010). The present study supports the premise that XO levels are extremely low in humans. However, to confirm this assumption a xanthine oxidase inhibitor could be used to determine if this enzyme influences the amount of oxidative stress observed with resistance exercise.

The third specific aim examined the effect of PVO on resistance exercise done at moderate intensity. This study's findings revealed that there was no significant difference between moderate intensity conditions with the addition of PVO. The lack of significant differences between the moderate intensity conditions may be due to the fact that both conditions were done to failure. While significant changes over time were reported in PC, XO, and IL-6 these changes were modest and may be due to the small muscle mass involved. PC levels changed differently over time as increases were reported in the 70F condition while the 70PVO condition reported no change from pre-exercise levels. Though both conditions were done to failure the 70PVO condition resulted in a significantly lower number of total repetitions (3.3±1.8 repetitions) and total work completed (46.0±23.7 kg). Intramuscular acidosis has been hypothesized to be the predominant reason for fatigue in high intensity resistance exercise done to failure with multiple sets (Lambert and Flynn, 2002). The combination of PVO to a resistance exercise of higher intensity (70%1RM) may have resulted in decreased total number of repetitions completed due to this higher intramuscular acidosis. This difference in total work in the 70PVO condition when compared to the 70F condition may explain the attenuation of PC levels.

Another explanation could be that PVO resulted in an increase in pooled blood in the exercising arm and a subsequent increase in circulating antioxidants. Vitamin C, Vitamin E, and uric acid have been shown to increase in previous research during shorter (Reitjens et. al. 2007) and longer bouts (Ramel et. al. 2004) of resistance exercise. The measurement of ORAC was utilized to identify changes due to oxidative stress following

each condition. However, changes seen over time in ORAC can be influenced by an increase in oxidative stress markers (decrease in ORAC) or increases in circulating antioxidants (increase in ORAC). The magnitude to which increases in oxidative stress and increased antioxidant capacity are affecting ORAC cannot be elucidated from this analysis. Future studies could benefit from more specific antioxidant measurements such as Vitamin E, Vitamin C, and uric acid levels. Also, the measurement of uric acid's oxidative product allantoin could be measured to reveal its impact as a free radical scavenger in resistance exercise done with PVO.

Due to no significant differences in oxidative stress and inflammatory markers with the addition of PVO the initial hypotheses for specific aims 1-3 were rejected.

The fourth specific aim was to examine the effect of PVO at different intensities. The major finding of this study was that there were no significant differences between intensities for the outcome measures. The changes due to the addition of PVO could be related to ischemia/reperfusion injury in the rest condition and/or blood flow changes altering the intramuscular environment of the exercising muscles in the exercise conditions. As previously stated, ischemia-reperfusion injury has been shown to be a potent contributor to oxidative stress (Uchiyama et. al. 2003 and Rodriguez et. al. 2003). Previous research has shown that PVO causes increased metabolite accumulation, increased motor unit recruitment, and an increased hormonal response not seen in non-PVO exercise matched for repetitions and load (Suga et. al. 2009 and Burgomaster et. al. 2003). Suga (2010) recently reported no significant difference between intramuscular

metabolite markers and pH in low intensity resistance exercise (30%1RM) with moderate PVO (130% of resting SBP) when compared to high intensity resistance exercise (70%1RM) done without PVO. This study employed a lower percentage of SBP (~82%) but may further the findings that the use of PVO during resistance exercise done at low intensity can result in an intramuscular metabolic stress equivalent to resistance exercise done at higher intensities. This may be related to the mechanisms needed to induce large gains in strength and muscle hypertrophy. Muscle biopsies (which were not done in the present investigation) with these subjects could reveal changes within the muscles that are not evident when examining the blood.

The fifth specific aim of this study was to examine the effect of intensity on resistance exercise done without PVO. Previous research (Hudson et. al. 2008, Bloomer et. al. 2007, and Rietjens et. al. 2007) reported significant increases in PC immediately following resistance exercise and this research study reports similar findings. The conditions done to failure (70F and 30F) showed a significant increase in PC over time and a significant decrease in XO but the modest changes were not enough to show significance when compared to the resting condition. Bloomer (2007) showed a significant increase in PC levels compared to resting controls in a one-set squat exercise done at 70% 1RM done for approximately 15 repetitions. This study utilized a similar 1RM percentage but the levels did not reach significance. This is most likely due to the differences in muscles used as the present study focused on a unilateral elbow flexion exercise compared to a bilateral squat exercise. The lack of significant difference between the 70F and 30F conditions in PC levels may further support the idea of

exercising to failure as an important factor in increasing oxidative stress. Further research needs to be conducted to support this hypothesis. Resting ORAC levels again varied at pre-exercise levels in the 30F and 70F condition and resulted in a significant difference in conditions without any change over time.

The fourth and fifth specific aim revealed no significant differences in the exercise conditions done at different intensities (30% and 70%1RM). As previously stated the lack of significance could be due to fact that all exercise conditions analyzed in specific aims four and five were done to failure. Another explanation could be related to the analysis of total work completed. The analysis of total work was utilized by multiplying the total repetitions completed to the specific weight lifted. This analysis revealed that significantly more work was completed in the low intensity conditions when compared to the moderate intensity conditions. This may further alter the intensity effect that would result if the repetitions per set were predetermined and matched independent of intensity. The matching of total work should be examined in future studies.

Due to no significant differences in oxidative stress and inflammatory markers with increasing intensity the initial hypotheses for specific aims 4 and 5 were rejected.

The effect of resistance exercise to failure on oxidative stress and inflammatory blood markers was examined further in the last specific aim of this study. The sixth specific aim then compared the 30F condition to another low intensity condition without PVO matched for the specific number of repetitions needed to complete the 30PVO condition. A significant increase over time in PC levels and a significant decrease over

time in XO activity were noted in the 30F condition but not the 30RM condition. IL-6 levels were significantly increased over time in the 30RM condition while increases seen in the 30F condition did not reach significance ($p = .080$). With that stated, no significant differences between conditions were revealed. The 30RM condition may have not resulted in a similar trend in PC due to the fact that it was not done to failure and was completed with a smaller number of repetitions compared to the 30F condition (53.5 ± 13.8 repetitions vs. 77.25 ± 22.42 repetitions). A future study utilizing a larger muscle mass would be beneficial in elucidating if a repetition-matched condition can impose a significant enough stress to the muscle compared to resistance training done to failure. The lack of significant differences also resulted in a rejection of the hypothesis for the sixth specific aim.

No significant increases over time were reported in glutathione ratio in any of the exercise conditions. This ratio has been shown in previous research to increase immediately following eccentric resistance exercise (Goldfarb 2005 and Zembron-Lacny 2009) and concentric resistance exercise (Goldfarb et. al. 2008 and Bloomer et. al. 2005) as well as high intensity aerobic exercise (Goldfarb et. al. 2007) and ischemia-reperfusion (Bloomer et. al. 2010). One possible explanation for the lack of change noted with this study could be related to the subject population utilized. The subjects in the present study were resistance trained as opposed to untrained. The subjects were also accustomed to this type of concentric exercise. This may have decreased the amount of oxidative stress and inflammatory response typically observed with untrained subjects.

This study, as stated previously, was a follow-up to further examine changes reported in oxidative stress markers (Goldfarb et. al. 2008) using a similar protocol (Reeves et. al. 2006). This study did not implement the calf extensions immediately following the elbow flexion exercise. This difference could explain the modest increases in oxidative stress and inflammatory markers as the addition of another muscle group could have led to the increase in systemic oxidative stress following both exercises.

This study employed as much control as possible to limit any outside factors affecting the blood markers being measured. However, many limitations arose as the study was implemented. One limitation would be the subject requirement. This study on average lasted almost a month from prescreening to post-measures and would most likely benefit from a decrease in conditions done and overall time obligations. The subjects were instructed to maintain a uniform resistance training schedule and diet to limit the effect of these factors on the blood markers. A decrease in study length would limit any changes in these factors along with any lifestyle changes that may have occurred such as increased stress, illness, injury, etc. over time. Another limitation would be the training style and status of each of the individuals. While all subjects were classified as resistance trained some subtle differences arose during the study. Anecdotal data obtained from the subjects noted increased soreness levels in two subjects who did not perform resistance exercises that isolated the biceps (i.e. bicep curls). These individuals trained either primarily with body weight exercises or multi-joint exercises that involved the biceps but did not specifically isolate this muscle group. Soreness levels were not measured in this study. The soreness reported occurred after the low intensity conditions done to failure

and did not affect the subject's testing schedule. A further analysis of the blood to examine creatine kinase, a marker of muscle damage, could be utilized to examine if muscle damage was present in the subjects and could have resulted in any blood marker variation. A future study would also potentially benefit from including only resistance trained subjects that include a specific form of biceps isolation into their resistance training. This could better control for any unaccustomed soreness responses following completion of bicep curls done to failure for the first time and limit any additional blood marker variation. This study did not use a metronome or other device to control for rate of the elbow flexion exercise. The subjects were just instructed to try and maintain a pace of two seconds in the concentric phase and two seconds in the eccentric phase of elbow flexion. The use of a metronome would have been beneficial in the citing exercise failure in the subjects as they would show the inability to maintain a predetermined rate. Exercise failure determination was employed using a subjective method of the researcher observing the subject's rate and form and stopping the subject when he felt the rate had become slower and less consistent and/or the form was altered.

Future research is needed to examine the effect of PVO on oxidative stress markers done with a larger muscle mass. This may increase the systemic oxidative stress in conditions done with and without PVO and help further examine any potential differences that may or may not exist. The implementation of muscle measures (i.e. muscle biopsies) along with blood measures could provide valuable insight into conditions in the muscle and the effect of PVO on the exercising muscle. These muscle measures could also help give a better understanding of the role, if any, of xanthine

oxidase in production of free radicals during resistance exercise. And finally, specific measures of circulating antioxidants should be utilized to examine the possible impact of these antioxidants during exercise with PVO.

In summary, this study showed that partial vascular occlusion can increase oxidative stress and inflammation independent of exercise intensity. In addition, the combination of low or moderate intensity exercise with PVO did not alter the findings compared to without PVO.

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APPENDIX A

INFORMED CONSENT

UNIVERSITY OF NORTH CAROLINA AT GREENSBORO

CONSENT TO ACT AS A HUMAN PARTICIPANT: LONG FORM

Project Title: The Effects of Partial Vascular Occlusion on Oxidative Stress Markers in Young Resistance Training Males

Project Director: Ryan Garten

Participant's Name: _____ Date:_____

Introduction

This is a research project. The purpose of this study is to determine if pressure applied above an exercising muscle which reduces blood flow to the muscles changes blood markers of oxidative stress done at two different intensities of exercise compared to resting condition.

Subject Requirements

This study requires resistance trained males between 18 and 35 years of age. Inclusion criteria include: 1) greater than one year of resistance training prior to this study, 2) apparently healthy, and 3) non-tobacco users. Exclusion criteria include: 1) current use of ergogenic or dietary aid that could affect results; e.g. cannot be taking any antioxidant vitamins or supplements, 2) any cardiac or circulatory ailment, any metabolic disease, any muscle abnormalities or a reduced calorie diet that could alter normal body responses, and 3) on any medicines or drugs that may alter blood flow or oxidative stress (e.g. anti-inflammatory drugs, antioxidants, vitamins).

Subject Participation

You will fill out a Health History Questionnaire (AHA) to ensure there are no existing health risks and an Activity Questionnaire to ensure you satisfy the criteria of resistance trained. Specific body measures will be assessed such as height, weight, resting heart rate, and blood pressure to help characterize you. Body fat percentage will be

assessed and your one repetition maximum value will be determined for the elbow flexors of the dominant arm. Three practice trials will be done with you being verbally instructed to contract at about 50%, 75%, and 100% of the hardest effort you can produce. You will be given a 60 second break between efforts. Then the test will begin with a five second countdown and you contract your elbow flexors as hard as they can for three seconds. During this time you will have verbal encouragement. Three repetitions of this will be completed with thirty seconds of rest in between sets. You will take part in six conditions. Four conditions will involve exercise while two will be done at rest. Conditions will be separated by at least 72 hours and no more than 3 weeks to complete all six conditions.

For the exercise conditions the resistance will be set to either 30% or 70% of the most force you produced. You will be encouraged to contract and relax while maintaining a repetition cadence of 2 seconds of contracting phase and 2 seconds of relaxing set to a metronome. You will be asked to perform all repetitions with cadence and through a full range of motion. You will do 3 sets to either failure or a predetermined number of repetitions. Rest periods will be set for 1 minute between sets. A pulse oximeter will be used to monitor oxygen flow to a finger below the exercising muscle. It will be placed on you immediately before each set to ensure that oxygen saturation does not fall below 95% and flow is reaching the forearm during and after the exercise. If a pulse is not detected the pressure will be lowered until a pulse is detected in the finger and adequate oxygen saturation (95%) is obtained. Blood samples will be taken immediately before, immediately after and 15 minutes after the exercise/rest conditions. Four teaspoons of blood will be taken at each of these times.

The applied pressure with exercise conditions will follow the exact same procedures as the exercise conditions without applied pressure. The applied pressure will be set to 20mmHg below your systolic blood pressure determined 10 minutes before exercise. A blood pressure cuff will be placed on your dominant arm between the top of the biceps and the bottom of the shoulder. The cuff will be inflated prior to exercise and will remain inflated on your arm until all sets are completed. Blood samples will be taken immediately before, immediately after and 15 minutes after the exercise/rest conditions using sterile techniques by a trained phlebotomist.

The resting conditions will require you to sit for a similar amount of time as the low intensity exercise condition with or without applied pressure and without doing any exercise. Blood samples will be taken immediately before, immediately after and 15 minutes after the exercise/rest conditions.

Four teaspoons of blood (10 mls) will be taken from an antecubital vein of the non-exercising arm pre-exercise. This will ensure no additional reopening of insertion site caused by exercise. The same amount of blood will be taken immediately post-exercise and 15 minutes post-exercise from your exercised arm. One condition will need a total blood volume requirement of 30mls and a total of 180mls to complete all parts of the study. You will receive 3 blood draws for each condition for a study total of 18 blood draws over the three weeks of your participation.

Risks and Discomforts

A survey of safety asking 12,462 persons with this type of applied pressure training reported that 2205 individuals (17%) reported some side effects. The most frequent side effect (13.1%) was slight bruising at the site of the blood pressure cuff. However, this bruising was short in duration and should be reduced in this study as this is not a training study. Some numbness was reported (1.3%) with training but this numbness was temporary and immediately gone after the release of the pressure. This study will minimize this effect as we will monitor oxygen saturation and blood flow by using a pulse oximeter on the pointer finger of the exercising arm. If a pulse is not detected or oxygen saturation drops below 95% then the applied pressure will be reduced until oxygen saturation increases above 95% and a pulse is detected. The incidence of serious side effects was very low and no fatal complications have occurred with this type of training. Blood clot in the veins was observed in 7 cases (0.055%). Pulmonary embolism was noted in only 1 case (0.008%). Dizziness, fainting and cerebral anemia were observed in some cases with older individuals. It should be noted that only ~25.1% of the individuals who have had this type of training fall into the subject population that will be recruited for this study. The severe side effects listed were reported by individuals who would be excluded from this study due to specific ailments. Therefore your risks will be reduced as you are an apparently healthy individual with no known cardiovascular problems and you have been resistance training for at least one year and are familiar with this type of activity. If you have any concerns about your rights, how you are being treated or if you have questions, want more information or have suggestions, please contact Eric Allen in the Office of Research Compliance at UNCG at (336) 256-1482. Questions, concerns or complaints about this project or benefits or risks associated with being in this study can be answered by Ryan Garten or Dr. Allan H. Goldfarb who may be contacted at 336-334-4062 or 336-334-3029. You can also e-mail them at rsgarten@uncg.edu or ahgoldfa@uncg.edu.

Potential Benefits

Through your participation in this study, you will learn your maximal biceps strength and your diet analysis. In addition, you will be given your percent body fat. Otherwise, the information gathered in this study may not directly benefit you; however it may advance the exercise physiology literature. Society may benefit from a better understanding of the mechanism of how partial pressure above a working muscle may influence its function.

Compensation

There will be no compensation for participating in this study and there are also no costs to you or payments made for participating in this study.

Confidentiality

All information concerning your records will be kept confidential and you will not be identified in any presentation or published work.

Study Withdrawal

You have the right to refuse to participate or to withdraw at any time, without penalty. If you do withdraw, it will not affect you in any way. If you choose to withdraw, you may request that any of your data which has been collected be destroyed unless it is in a de-identifiable state.

New Information/Changes in the Study

If significant new information relating to the study becomes available which may relate to your willingness to continue to participate, this information will be provided to you.

Voluntary Consent by Participant:

By signing this consent form you are agreeing that you have read it, or that it has been read to you and you fully understand the contents of this document and are openly willing to consent to take part in this study. All of your questions concerning this study have been answered. By signing this form, you are agreeing that you are 18 years of age or older and are agreeing to participate in this study described to you by Ryan Garten.

Signature: _____ Date: _____

APPENDIX B

PILOT STUDY RESULTS

The actual elbow flexion 1RM for each subject taken before the pilot study, calculated 30% and 70%1RM loads, and loads and final percentages adjusted for the equipment used are presented in Table X.

Table X: Pilot Study Subject's 1RM, Actual, and Adjusted Percent Workloads

	Subject 1	**Subject 2**
1RM (kg)	**20.45**	**25**
30%1RM (kg)	**6.14**	**7.5**
70%1RM (kg)	**14.32**	**17.5**
Adjusted 30%1RM (kg)	**6.82**	**6.82**
Adjusted 70%1RM (kg)	**13.63**	**18.18**

Table X: Repetitions Completed Each Set

Subject 1	1st set (reps)	2nd set (reps)	3rd set (reps)	Total Repetitions
30%1RM	32	11	9	52
30%1RM w/PVO	24	8	5	37
Difference	**8**	**3**	**4**	**15**
Subject 2	1st set (reps)	2nd set (reps)	3rd set (reps)	Total Repetitions
30%1RM	52	18	10	80
30%1RM w/PVO	21	11	9	41
Difference	**31**	**7**	**1**	**39**

Subject 1	1st set (reps)	2nd set (reps)	3rd set (reps)	Total Repetitions
70%1RM	9	5	3	17
70%1RM w/PVO	8	4	2	14
Difference	**1**	**1**	**1**	**3**

Subject 2	1st set (reps)	2nd set (reps)	3rd set (reps)	Total Repetitions
70%1RM	6	5	3	14
70%1RM w/PVO	8	2	2	12
Difference	**-2**	**3**	**1**	**2**

APPENDIX C

DIETARY INTAKE AVERAGES FROM 3 DAY FOOD RECORDS.

Variable	Mean	SD
Total Calories (kcals)	3008.2	707.5
Carbohydrate(%)	46.3	10.8
Fats(%)	33.9	7.7
Protein (%)	20.0	3.8
Saturated Fat (%)	10.4	3.2
Monounsaturated Fat (%)	11.1	4.3
Polyunsaturated Fat (%)	5.6	2.2
Omega 6 (g)	14.4	10.2
Omega 3 (g)	0.8	0.6
Vitamin C (mg)	107.7	68.3
Vitamin D (ug)	5.7	2.9
Vitamin A (ug)	1873.0	881.3
Vitamin E (mg)	13.4	5.6

APPENDIX D

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

Symptoms

__ You experienced 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.

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.

_____

_____

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 post menopausal.

__ 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 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.

_____

_____

Please print, complete and bring this questionnaire to your physician if you have any further questions.

APPENDIX E

HEALTH HISTORY, DRUG USAGE, AND FITNESS ACTIVITY QUESTIONNAIRE

Subject: _____

ID: _____

Please circle Yes or No for each question. If Yes please explain.

Health History

Do you have any musculoskeletal illnesses or injuries that would restrict your participation in a submaximal exercise bout (as performed on a treadmill)?

YES NO

If yes, please describe._____

_____

Have you ever been diagnosed with cardiovascular disorders (heart problems, high blood pressure, high cholesterol, abnormal heart rhythms, etc.)?

Family History	YES	NO
Cigarette smoking	YES	NO
Hypertension	YES	NO
Hypercholesterolemia	YES	NO
Impaired fasting glucose	YES	NO
Obesity	YES	NO
Sedentary lifestyle	YES	NO
High serum LDL cholesterol	YES	NO

If yes, please describe._____

_____

Have you ever been diagnosed with any metabolic disorders (diabetes, etc.)?

YES NO

If yes, please describe._____

_____

Could you currently be pregnant? YES NO

Please list any major illnesses, hospitalizations, or surgical procedures within the last two years.

_____

_____

Drug/Supplement Usage

Are you a *current* smoker or user of tobacco? YES NO

Have you ever smoked in the past? YES NO

If yes, please describe history._____

_____

Are you currently taking any medication(s)? YES NO

If yes, please list name of medication(s), reason for usage and length of administration.

_____

_____

_____

Are you currently taking any nutritional/vitamin supplements?

YES NO

If yes, please list name of supplement, reason for usage and length of administration.

Any pains in joints? YES NO

Any shortness of breath at rest or during exercise? YES NO

Have you had a large change in weight over the past 3 months? YES NO

Have you seen a change in your health status in the past 3 months? YES NO

Fitness Activity

Please describe your current participation in the following types of exercise:

1. Aerobic (aerobic classes, walking, jogging, stair climbing, hiking, cycling, etc.)

Frequency (# of days per week): _____

Duration (time spent per session on avg.): _____minutes

Intensity (difficulty level): light somewhat hard hard very hard

If you know your HR responses please list it here. _____

_____

How long have you been participating in aerobic activity as described above?

Less than three months 3-6 months 6-12 months greater than 12 months

Type of activity:_____

Additional comments:_____

_____

2. Anaerobic (weight training, sprinting, etc. situps,)

Frequency (# of days per week): _____

Duration (time spent per session): _____minutes

Intensity (difficulty level): light somewhat hard hard very hard

How long have you been participating in anaerobic activity as described above?

Less than three months 3-6 months 6-12 months greater than 12 months

Type of activity: _____

Additional comments:_____

_____

3. Organized or Recreational sports or activities (Yoga, Tai –chi)

Type of sport(s):_____

Frequency (# of days per week): _____

Duration (time spent per session): _____minutes

Intensity (difficulty level): light somewhat hard hard very hard

How long have you been participating in sports activity as described above?

Less than three months 3-6 months 6-12 months greater than 12 months

Additional comments:_____

_____

Classification (to be assessed by researchers—leave blank)

Trained ***Untrained***

APPENDIX F

SUBJECT VISIT TIMES

Subject	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
1	9:00am	9:00am	9:00am	9:00am	9:00am	9:00am	9:00am
2	7:00am	8:00am	8:00am	7:00am	8:00am	7:00am	8:00am
3	1:15pm	1:15pm	1:15pm	1:15pm	1:15pm	1:15pm	9:00am*
4	9:00am	9:30am	9:30am	9:00am	9:00am	10:00am	10:00am
5	12:00pm	12:00pm	12:15pm	12:00pm	1:00pm	12:00pm	1:00pm
6	11:30am	12:00pm	11:00am	11:00am	9:30am*	11:30am	11:30am
7	10:15am	10:00am	10:00am	10:00am	10:00am	10:00am	8:45am
8	12:15pm	1:00pm	12:15pm	1:00pm	1:00pm	1:00pm	1:00pm
9	11:00am	11:00am	10:30am	11:00am	11:00am	11:00am	11:00am
10	1:30pm	1:00pm	1:00pm	1:15pm	1:15pm	1:15pm	1:00pm
11	7:00am	7:00am	7:00am	7:00am	7:00am	7:00am	7:00am
12	10:00am	10:00am	10:00am	10:00am	10:00am	10:00am	10:00am

***** - Indicates this time was more than 1.5 hrs different than all other times for that subject.