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ORIGINAL ARTICLE

Resistance exercise effects on blood glutathione status and plasma protein carbonyls: influence of partial vascular occlusion

A. H. Goldfarb · R. S. Garten · P. D. M. Chee · C. Cho · G. V. Reeves · D. B. Hollander · C. Thomas · K. S. Aboudehen · M. Francois · R. R. Kraemer

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Abstract Seven weight-trained males performed both light resistance with partial occlusion (LRO: 30% 1 RM) and moderate resistance (MR: 70% 1 RM) to failure to ascertain whether blood protein carbonyls (PC) and glutathione status was altered compared to partial occlusion (PO) in a counterbalanced fashion. PO was identical in duration to the LRO session and all sessions were on separate days. PC did not differ for the three conditions at PRE (0.05 nM mg protein^{-1}). PC significantly increased for PO and MR over time and was greater than the LRO treatment at POST (0.13 nM mg protein^{-1}). The GSSG/TGSH ratio at PRE did not differ across treatments (8%) whereas the ratio at POST was significantly elevated for PO and MR treatments (17%). In contrast, no change occurred for the LRO session at any time. These results indicate that MR to failure and PO can significantly increase blood oxidative stress but LRO did not elicit oxidative stress.

Keywords Oxidative stress · Ischemia · Reperfusion injury · Growth hormone

A. H. Goldfarb (✉) · R. S. Garten · P. D. M. Chee · C. Cho
Department of Exercise and Sport Science,
University of North Carolina at Greensboro,
260 Health and Human Performance Building,
Greensboro, NC 27412, USA
e-mail: ahgoldfa@uncg.edu

G. V. Reeves · D. B. Hollander · C. Thomas · K. S. Aboudehen · M. Francois · R. R. Kraemer
Department of Kinesiology and Health Studies,
Southeastern Louisiana University, Hammond, LA, USA

Introduction

Resistance exercise elicits hormonal responses that affect muscle tissue growth and remodeling (Kraemer and Ratamess 2005). The loading of muscle is an important factor for endocrine responses and muscle adaptation (Kraemer et al. 1990; McDonagh and Davies 1984); however, increases in muscular mass have been documented with as little as 3 weeks of low resistance training with partial vascular occlusion using intensities less than or equal to 50% of 1 RM (Abe et al. 2006; Burgomaster et al. 2003; Reeves et al. 2006; Takarada et al. 2000a, b). The possible metabolic adaptations from partial vascular occlusion point to increases in stored glycogen and decreases in stored ATP in partially occluded groups. The changes in ATP and glycogen concentrations suggest a fiber-type shift from type IIx to type IIa to enable more sustained contraction and capacity for more sustained work (Burgomaster et al. 2003). Recently, it was demonstrated that light resistance exercise with partial vascular occlusion significantly increased the growth hormone response compared to moderate resistance exercise without occlusion (Reeves et al. 2006). Moreover, Takarada et al. (2000b) reported a rapid increase in growth hormone after low-intensity resistance exercise with vascular occlusion. In the same study they also found that lipid peroxides in the blood were unaltered by low-resistance exercise with or without vascular occlusion. However, the technique used to determine lipid peroxidation was the thiobarbituric acid reactive substances method which is a non-specific and insensitive measure of lipid peroxidation. In contrast, Alessio et al. (2000) reported that isometric exercise increased lipid peroxides but not protein carbonyls in the blood directly after exercise. Usually intense acute resistance exercise elevates blood oxidative stress markers (Bloomer et al. 2007; Hudson et al. 2008; McBride et al.

1998; Uchiyama et al. 2006). In contrast, a study by Dousset et al. (2002) found that acute hypoxemia did not influence oxidative stress at rest or during muscle contraction.

It has been shown that partial occlusion which increases ischemia results in increases in reactive oxygen species (ROS) (Tsutsumi et al. 2007). Ischemia/reperfusion (IR) in muscle results in increased vascular permeability that has been attributed to an increase of xanthine oxidase activity during the hypoxic conditions to produce ROS (Korthuis et al. 1985). A single session of weight-lifting in rats resulted in ischemia which elevated ROS within the muscle (Uchiyama et al. 2006). IR in skeletal muscle activates inflammatory responses which can enhance oxidants and vascular permeability changes (Gute et al. 1998). Two sensitive indicators of oxidative stress (protein carbonyls and glutathione status) have been shown to increase with aerobic exercise and with eccentric muscle contractions (Goldfarb et al. 2005). Furthermore, changes in blood glutathione status were reported to be altered with ischemia (Tsutsumi et al. 2007), and high intensity resistance exercise was shown to increase protein carbonyls (Bloomer et al. 2007).

Although IR results in oxidative stress, this methodology probably does not reflect the magnitude of change in ischemia that occurs with resistance exercise. Resistance exercise produces transient decreases and increases in blood flow to the muscle during the contraction and relaxation phases of the exercise respectively; whereas the IR model uses sustained ischemia followed by reperfusion over several hours. Therefore, although the IR model has consistently demonstrated an oxidative stress response, it is unclear if partial occlusion in conjunction with light resistance exercise would demonstrate enhanced markers of oxidative stress compared to partial occlusion only. It is also unclear how moderate resistance exercise alone would influence these same markers of oxidative stress within the vasculature.

Using the same protocol and a subset of the subject sample from the previously reported effects of partial vascular occlusion on hormone responses (Reeves et al. 2006), we investigated whether partial vascular occlusion would alter the oxidative stress response with or without low resistance exercise and compared the response to moderate resistance exercise without partial occlusion on blood protein carbonyls (PC) and glutathione status. In addition, we compared the oxidative stress response to the growth hormone response.

Methods

Research design

A preliminary session and three different exercise and/or partial occlusion sessions that allowed different effects of resistance load and partial vascular occlusion to be compared were completed by the same subjects on four separate days at 1100 h to avoid diurnal variations. A preliminary screening was used to record descriptive characteristics, familiarize subjects with the equipment, and determine 1-RM resistances. That session was followed by either a light resistance partial occlusion (LRO) session or a moderate resistance (MR) without occlusion session, in a counterbalanced fashion. The final session was always the partial occlusion-only (PO) session in order to utilize the same duration of partial occlusion that was used in the LRO session. The duration of partial occlusion varied for each individual and depended on the number of repetitions performed to failure in the LRO session.

Subject

Seven resistance-trained men (18–30 years) were recruited from the student population at Southeastern Louisiana University. The investigation was approved by the IRB at Southeastern Louisiana University and all volunteers signed consent forms prior to any data collection and were made aware of the potential risks of the study prior to participation. Inclusion criteria were: (1) past experience in weight training (>1 year) to reduce the learning effect associated with first engaging in resistance exercise; (2) apparently healthy; and (3) non-tobacco user. Exclusion criteria for the study were: (1) current use of any ergogenic/dietary aid such as creatine monohydrate, herbal stimulants, antioxidants, or any anabolic agents such as steroids; (2) cardiac or circulatory ailments, including high blood pressure and/or hypertension (to avoid complications due to the hemodynamic alterations caused by partial vascular occlusion); (3) presence of metabolic diseases that could affect oxidative responses, e.g., diabetes; and (4) following a reduced-caloric-intake diet that could alter metabolism.

Volunteers completed a medical history questionnaire before acceptance to ensure that there were no existing health risks. The subjects were asked to refrain from resistance and cardiovascular exercise on the day prior to each session and abstained from caffeine and alcohol use for 12 h before each trial. Additionally, a standardized caloric beverage (Naturite, Jacksonville, FL) was given to each subject to consume 3 h before each experimental session (total calories per serving: 250; carbohydrates: 40 g; protein: 9 g; fat: 6 g) with no other food or caloric drink until after the session was completed.

Preliminary session

Each subject's anthropometric data were assessed. For the seven subjects in the study height, (180.53 ± 5.77 cm), age, (21.3 ± 4.8 years), weight (86.21 ± 6.88 kg), and % body

fat, (8.54 ± 2.1%) was determined. A three-site (chest, abdomen, thigh) skinfold measurement (Lange Instruments, Santa Cruz, CA) was used to calculate body fat percent (Jackson and Pollock 1985). A 1-RM value was determined (Durand et al. 2003) for the single-arm biceps curl and the single-leg calf extension, and performance was closely monitored for acceptable form and complete range of motion. These two specific resistance-exercises were chosen for several reasons: (1) the biceps was used in previous occlusive studies (Burgomaster et al. 2003; Reeves et al. 2006; Takarada et al. 2000a) due to its ease of occlusive application and exercise familiarity and has been utilized with oxidative stress resistance exercises (Bloomer et al. 2007, Takarada et al. 2000b); (2) the gastrocnemius represents a muscle of the lower limbs that has similar cross-sectional area to the biceps, simple plantar flexion action, and a convenient occlusive application area proximal to the muscle; and (3) use of two muscle groups (though proportionately small to moderate size) represents a protocol closer to typical exercise sessions than utilizing just one muscle group.

The subjects performed the biceps curl with their dominant arm, utilizing a free-weight dumbbell. Each subject's upper torso was stabilized to ensure proper form and minimize momentum. The warm-up consisted of three trials at 40, 60, and 80% of the subject's estimated 1-RM with 3 min rest intervals between sets. Determination of the 1-RM was made when the subject curled successfully the heaviest dumbbell through the full range of motion. The calf extension was performed with the dominant leg on the Body Masters (Rayne, LA) leg press device, using the dominant side of the body. The 1-RM was recorded as the highest resistance plantar flexed without any assistance from the quadriceps muscles after three warm-up sets. Workloads in the experimental sessions were then calculated as 30% 1-RM for the LRO session and 70% 1-RM for the MR session.

LRO session

A blood pressure reading was taken from the arm after 15 min of semi-recumbent resting. Fifteen minutes prior to testing, a resting blood sample was collected from an antecubital vein from the contralateral arm (nondominant side). A custom-designed narrow inflatable cuff (Hokanson SC-10 Segmental Pressure Cuff, Hokanson Vascular Instruments and Accessories, Bellevue, WA) was affixed to the dominant arm, centered in the space between the superior aspect of the biceps brachii and the inferior aspect of the anterior deltoid muscles. The cuff was inflated to 20 mmHg below the acute systolic pressure determined ~15 min prior to the exercise session, and the cuff remained in place for the duration of three sets of single arm biceps curls (30% 1 RM). The subject was told to complete all repetitions with smooth, timed, full range-of-motion contractions to failure following the cadence of an audible metronome set to 0.67 Hz. Rest periods between sets lasted 1 min each, with maintenance of cuff occlusion throughout and for 1 min following completion of the third and final set. A pulse oximeter (model 3301, Smiths Medical PM, Waukesha, WI) was used immediately after each set to failure to ensure that blood flow was not completely halted by tissue edema past the vascular cuff pressure. If a pulse was not detected, cuff pressure was reduced 5–10 mmHg until blood flow was detected at the finger. All of the O_2 saturation readings using the pulse oximeter were 97% or greater except on two occasions. On these two occasions the cuff pressure was reduced 5–10 mmHg and the pulse oximeter readings returned to >97%. The complete duration of occlusion was recorded as the time length of cuff maintenance for the PO condition (mean arm occlusion time was 341 ± 4.5 s). After cuff removal, the subject moved to the leg press machine, and a customized inflatable leg cuff was then applied to the proximal portion of the lower leg on the dominant side, centered in the space between the superior aspect of the gastrocnemius and the inferior edge of the patella. The cuff was inflated exactly 5 min after its removal from the arm, to a pressure of 40 mmHg above the arm occlusive pressure to account for the larger vasculature and muscle mass. Cuff pressure was maintained for the duration of three sets of calf extensions to failure with 1 min rest periods between sets. A pulse oximeter was affixed to the second toe on the exercised side of the subject to monitor blood flow between sets. Cuff pressure was lowered as previously described if a pulse was not detected. After the final set was completed, there was an additional minute of recovery while the inflated cuff remained in place (mean leg occlusion time was 387 ± 13.1 s). After deflation and removal of the cuff, a second blood draw was obtained within 1 min of completion of testing (collected on dominant side) and the subject remained seated for a 15-min period. The final blood draw was obtained at 15-min post-exercise.

MR session

This session was conducted in identical fashion to the LRO session except no partial vascular occlusion was applied. The session mimicked traditional weightlifting for strength and muscle mass gains by employing loads of ~70% 1 RM for both biceps curls and calf extensions. A blood draw occurred 15 min before beginning any lifting as was the case in the LRO session. The subjects performed three sets of single-arm biceps curls interspersed with 1-min rest intervals, followed by 1 min of recovery. Three sets of single-leg calf extensions were then completed with 1-min resting intervals. All sets progressed to failure (determined

by the first incomplete repetition), and good lifting form was maintained throughout. A blood draw was taken immediately after exercise and 15-min post-exercise, as in the LRO session.

PO session

The protocol of the PO session utilized the same procedures and time intervals of the LRO session but without any lifting. A blood draw occurred prior to the session, and then an inflatable cuff was affixed to the same upper arm location as in the LRO session with a pressure of 20 mmHg below the systolic pressure. Each subject stood for the duration of time recorded for the biceps curl portion of the LRO session, including the 1-min rest periods and recovery minute. The cuff was then deflated and removed, and the subject was seated with legs in the supine position. This simulated the posture during the calf extension portion of experimental session. The cuff was applied the lower leg in the exact position used in the LRO session and inflated to 40 mmHg above the PO arm occlusion pressure for the same time period recorded in the LRO session. The cuff was deflated and removed and a blood draw was obtained at this time with the subject seated in a normal position. After 15 min of relaxed sitting, the final blood draw was taken.

Blood collection and analysis procedures

Blood was immediately processed for the two oxidative markers glutathione status and protein carbonyls. For glutathione status, whole blood (1 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[3]. 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 then 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) by the procedure of Andersen (1985). This method enables both the GSH and GSSG forms to be determined. All samples were measured in duplicate and compared to standards.

For protein carbonyls, whole blood mixed with EDTA was immediately centrifuged at 3,000 rpm at 4ºC for 15 min. Plasma (1 ml) was pipetted into 1.5-ml microcentrifuge tubes and stored at −80ºC until analyzed. Plasma protein was determined in each sample by the Lowry technique (1951). Appropriate plasma volume was then utilized (Stadtman and Levine 2000) in an immunosorbent assay via an Elisa Kit (Zen Tech, New Zealand) following the procedures of Winterbourn and Buss (1999). The plate was read at 450 nm wavelength on a microplate reader (BioTek Instruments, Winesski, VT). The data was processed by a KC Junior software package. All samples were measured in duplicate and compared to standards.

Statistical analysis

The data on the blood measures were analyzed using a 3 (trial) × 3 (time) repeated-measures ANOVA. Data were analyzed by an SPSS statistical package (version 14.1) with the level of significance set at an alpha level ≤ 0.05. Post-hoc analysis was performed using the Tukey HSD analysis where appropriate. Correlational analysis of raw and change values comparing PC to growth hormone changes were calculated using SPSS statistical package. The data are presented as means ± SE.

Results

Plasma volume changes

Plasma volume changes were determined for the three trials. Plasma volume changes comparing pre to immediately post-exercise were -0.7 ± 2.9; 1.4 ± 2.24 and $7.0 \pm 2.6\%$ for the LRO, MR, and PO trials, respectively. The changes in plasma volume from pre to 15-min post exercise were 2.5 ± 3.5; 5.73 ± 2.6; and $7.80 \pm 2.1\%$ for the LRO, MR, and PO trials, respectively. The values for the blood oxidative stress markers were not adjusted for the changes in plasma volume.

Protein carbonyls

Resting levels of protein carbonyls taken 15 min prior to exercise were not different for the three conditions (0.05 nM mg protein^{-1}) (Fig. 1). Both the PO and MR sessions demonstrated significant increases in PC over time. PC concentration was significantly greater for both PO and MR sessions compared to the LRO session immediately after exercise (0.13 nM mg protein^{-1}). PC levels remained significantly higher than the PRE value 15 min after the exercise in both the MR and PO groups (0.10 nM mg protein^{-1}). In contrast, the LRO values did not significantly change over time.

Glutathione

Resting levels of the GSSG/TGSH ratio taken 15 min prior to exercise were not different for the three conditions at 8% (Fig. 2). The GSSG/TGSH ratio immediately after exercise

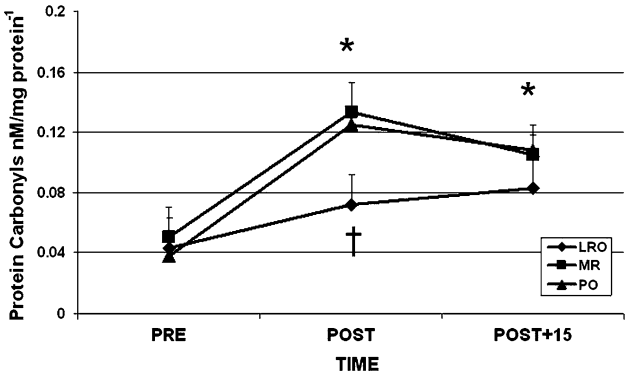

Fig. 1 Plasma protein carbonyls: response to resistance exercise and/or partial occlusion plasma protein carbonyl concentration from 7 trained-resistance males before (PRE), immediately after (POST) and 15 min after (POST + 15) treatment. *LRO* light resistance exercise (30% 1-RM) with partial occlusion; *MR* moderate resistance exercise (70% 1-RM) with no occlusion; *PO* partial occlusion only. Values are means ± SE. * Significant difference from PRE. † Significant difference from both MR and PO

for both PO and MR sessions was significantly elevated (17%) compared to rest but was not significantly changed by the LRO session (11%). The GSSG/TGSH ratio returned towards resting levels 15 min after exercise for both PO and MR sessions (14.9%) but was still significantly higher than the resting ratio. The LRO session did not demonstrate any significant change over time.

Relationship of the change in the oxidative stress markers to growth hormone change

The correlational analysis either with absolute or change values did not indicate that there was any significant relationship between the oxidative stress markers and the growth hormone change associated with any session.

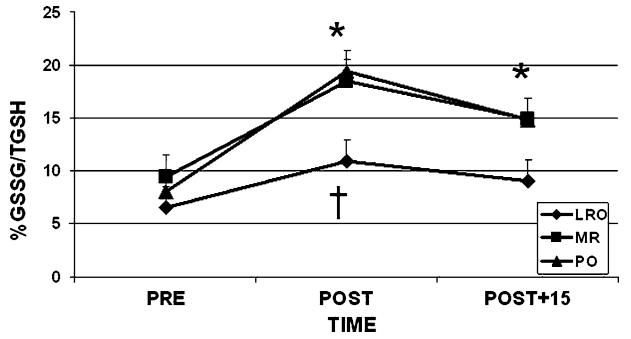

Fig. 2 Glutathione status: response to resistance exercise and/or partial occlusion glutathione status from whole blood [ratio of oxidized gluathione (GSSG) to total glutathione (TGSH)] from 7 trained-resistance males before (PRE), immediately after (POST) and 15 min after (POST + 15) treatment. *LRO* light resistance exercise (30% 1-RM) with partial occlusion; *MR* moderate resistance exercise (70% 1-RM) with no occlusion; *PO* partial occlusion only. Values are means ± SE. * Significant difference from PRE. † Significant difference from both MR and PO

Discussion

This is the first investigation to demonstrate that the blood glutathione ratio (GSSG/TGSH) and plasma protein carbonyls (PC) are elevated by partial vascular occlusion alone and that moderate resistance exercise at 70% 1-RM to failure demonstrated a similar extent of oxidative stress. In contrast, light resistance exercise in conjunction with partial vascular occlusion did not demonstrate significant changes in these oxidative stress markers over time. The addition of low intensity resistance exercise to the partial vascular occlusion attenuated the oxidative stress response. Previous studies using intense resistance exercise have reported oxidative stress using several different markers in response to an acute bout (Bloomer et al. 2007; Hudson et al. 2008; McBride et al. 1998; Rietjens et al. 2007). Two studies have included multiple set resistance bouts of the legs at 70% 1-RM (Bloomer et al. 2007; Rietjens et al. 2007). Another study utilized untrained subjects and employed leg exercise at 75% 1-RM until failure (McDonagh et al. 1984). In contrast, Hudson et al. (2008) examined two back squat protocols at 75% 1-RM and 90% 1-RM. They reported elevated protein carbonyls immediately post exercise for the 90% 1-RM and elevated protein carbonyls at 60 min for both protocols. However, there was no significant change in lipid hydroperoxides with either protocol. The present study incorporated a protocol that used a 70% 1-RM load for both the arm and the legs (smaller muscle groups) and reports an increase in both the PC and glutathione ratio markers of oxidative stress. The PO trail for a similar length of time without muscle contraction activity resulted in a similar extent of oxidative stress. This seems to suggest that a major contributor to the observed oxidative stress within the blood might have been a result of processes within the blood such as the xanthine oxidase response. Further study is needed to ascertain the origin of the oxidative stress within the blood and how low-resistance exercise attenuated the oxidative stress with partial vascular occlusion.

Previous studies with ischemia have reported increased ROS (Korthuis et al. 1985; Tsutsumi et al. 2007). Most IR studies have interrupted blood flow to a tissue for an extended period of time and then reperfused the tissue which resulted in increased ROS. Partial occlusion, depending on the severity of the occlusion and the length of time it is applied, has been reported to increase oxidative stress (Tsutsumi et al. 2007). Clearly the mechanisms for observed ROS following extended periods of ischemia followed by reperfusion are not physiological and are significantly different than the altered blood flow that occurs with resistance exercise. Depending upon muscle size and muscle load, during resistance exercise there can be an inherent interruption of blood flow during the contraction of the

muscle (partial occlusion of blood flow) followed by increased blood flow during the relaxation phase of the contraction. Often this results in an enhanced blood flow to the muscle (reactive hyperemia) which may contribute to the observed increase in ROS that we have reported in this study. Invariably, high intensity resistance exercise with multiple sets of exercise to failure (Bloomer et al. 2007; Hudson et al. 2008; McBride et al. 1998; Rietjens et al. 2007) demonstrates oxidative stress within the circulation. Even isometric exercise was reported to result in oxidative stress (Alessio et al. 2000).

In contrast, low intensity resistance exercise in conjunction with mild vascular occlusion was reported not to result in changes in TBARS in humans (Takarada et al. 2000b). Our data support their findings and show that both protein carbonyls and glutathione ratio are not significantly altered with this combined treatment. We postulated that the low-intensity resistance-exercise in conjunction with the partial occlusion would mimic the higher resistance exercise response; however, the LRO condition did not demonstrate any significant change in either oxidative stress marker over time. In addition, the combination of the low-intensity resistance-exercise with the partial vascular occlusion seemed to attenuate the extent of oxidative stress as denoted by both markers being lower in the LRO session compared to the PO session. We previously reported considerably greater growth hormone responses to the LRO trial than the MR and PO trials (Reeves et al. 2005). We compared the GH and PC changes from pre- to immediately post-exercise, immediately post- to 15 min post-exercise, and pre- to 15 min post-exercise for individual trials and no significant correlations were found. This result suggests that the oxidative stress response does not relate to the GH response.

It is unclear why there was less oxidative stress with the LRO session. We think it could be either one of two scenarios: (1) the muscle contractions during the partial vascular occlusion were able to overcome the resistance to venous outflow and thus help remove the oxidative stress markers from the circulation or; (2) the pressures during the contractions were sufficient to enhance blood flow during the muscle contractions to enable adequate blood flow delivery to overcome the partial vascular restriction. Further research is needed to ascertain the mechanism of this reduction in oxidative stress during light resistance exercise with partial vascular resistance.

Conclusion

The results indicate that moderate resistance exercise at 70% of 1 RM to failure and partial vascular occlusion for the same length of time increased blood oxidative stress markers to a similar extent, but light-resistance exercise at 30% of 1 RM to failure with partial occlusion attenuated the oxidative stress response within the blood. Further research is needed to ascertain the mechanism for this difference in response.

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