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The Impact of Intensity on Exercise-Induced Oxidative Stress in Trained Men

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THE IMPACT OF INTENSITY ON EXERCISE-INDUCED OXIDATIVE STRESS IN TRAINED MEN

by

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ABSTRACT

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Exercise has been noted in some, but not all studies, to elicit an oxidative stress response. The discrepancy in findings may be related to differences in exercise intensity across study protocols. Biomarkers of oxidative stress were compared for aerobic and anaerobic exercise bouts of different intensities and durations. On different days, exercised-trained men (n=12; 21-35 yrs) performed aerobic cycle exercise (60 min at 70% HR reserve) and anaerobic cycle sprints (five, 60 sec sprints at 100% max GXT watts; and ten, 15 sec sprints at 200% max GXT watts). Blood was collected before and 0, 30, and 60 min post-exercise and analyzed for malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and Trolox Equivalent Antioxidant Capacity (TEAC). No differences were noted in MDA or H_2O_2. TEAC was significantly higher at 30 and 60 min post-exercise. In exercise-trained men, no increase was noted in post-exercise oxidative stress, possibly due to the increase in antioxidant defense.

Key words: malondialdehyde, hydrogen peroxide, reactive oxygen species, free radicals, exercise, high-intensity sprints

PREFACE

This thesis was written in article format for submission to the journal, *Medicine & Science in Sports & Exercise* following defense. The content and organization of this thesis represent and fulfill the requirements for submission to this journal.

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INTRODUCTION

Over the past three decades the investigation of oxidative stress has continued to gain popularity in the medical and exercise science community (19). A state of oxidative stress occurs when the body's antioxidant defenses are unable to adequately combat reactive oxygen and nitrogen species (RONS), ultimately leading to the oxidation of lipids, proteins, DNA, and other molecules (5). While the production of RONS is a normal biological process and can occur with a variety of stressors (e.g., nutrient consumption, ozone exposure, acute exercise), when produced in excess, RONS can be damaging to tissues within the body (42). In fact, extensive cellular oxidation can severely compromise cell viability and contribute to ill health and disease (15).

It is well documented that acute exercise of sufficient intensity (31, 32, 40) and duration (6) can lead to an increase in RONS and induce an acute state of oxidative stress (19). However, participating in a regular exercise program is also associated with a chronic increase in endogenous antioxidant enzyme activity (35, 36, 45, 49), which may serve to provide protection against the exercise-induced increase in RONS. Moreover, a decrease in RONS production may be apparent after repeated exposure to the same stressor, as well as an increased activity of specific repair enzymes (42). Such adaptations are in accordance with the principle of hormesis, which states that in response to repeated exposure to various stressors, the body undergoes favorable adaptations that result in enhanced physiological performance and improved physical health (28, 41). It has been noted that exercise-induced RONS production leads to the activation of the redox sensitive transcription factor nuclear factor (NF)-kappa (κ)B, which upon activation leads to the expression of antioxidant enzymes (21). Combined with dietary

antioxidants, protection is providing against exercise-induced RONS, which can potentially provide support against the development and progression of certain diseases (e.g., cardiovascular diseases, diabetes) (23, 24).

Exercise-induced oxidative stress has been observed for all types of exercise, including aerobic (18, 35, 45) and anaerobic (9), with different pathways of RONS generation for each (26). In terms of aerobic exercise, which clearly represents the most well-studied form of exercise related to oxidative stress, the majority of laboratory based studies have used treadmill walking/running or stationary cycling performed for 30-60 minutes at 60-80% VO_{2max} (19). In terms of anaerobic exercise, in which a relatively low number of studies have been conducted, protocols have involved dynamic resistance exercise (7, 8, 10, 25), eccentric exercise (8, 37), jumping (39), and sprinting (8, 22). Related to the latter form, very few studies have compared sprint intensity and duration within the same design. This is true despite the widespread use of sprint protocols within the training regimen of various athletes.

It is believed that the oxidative stress response to acute exercise is intensity dependent. In an early study on exercise-induced oxidative stress, Lovlin and colleagues reported an increase in the lipid peroxidation marker malondialdehyde (MDA) in subjects immediately following a graded exercise test performed to exhaustion, while exercise at 70% of VO_{2max} and lower percentages resulted in no increase in MDA (32). Support for this finding was provided by Leaf and colleagues who reported an intensity dependent increase in expired ethane and pentane when subjects performed a graded exercise test to exhaustion (31). Additional support is provided by Quindry and coworkers (40) who measured blood markers of oxidative stress in response to different intensities of aerobic

exercise and concluded that "Exercise intensity plays a major role in post-exercise blood oxidative stress, whereas total exercise energy expenditure does not."

However, in opposition to the above findings, an increase in MDA occurred independent of exercise intensity of one repetition maximum squats (25). It is likely that the oxidative stress response to exercise is both intensity and mode dependent (10), as well as impacted by exercise duration (6). To our knowledge, no study to date has compared both aerobic and anaerobic exercise bouts of different intensities and/or durations on biomarkers of oxidative stress—in particular within a sample of exercise-trained men. Therefore, the purpose of this study was to compare the changes in oxidative stress biomarkers following 1) moderate-intensity and moderate-duration steady state aerobic exercise, 2) high-intensity and moderate-duration interval sprints, and 3) maximal-intensity and short duration interval sprints. We hypothesized that the oxidative stress response would be intensity dependent (i.e., highest for the sprints compared to the aerobic exercise bout).

METHODS

Subjects

Twelve healthy, exercise-trained men between the ages of 21 and 35 years were recruited to participate. All subjects completed a health history and physical activity questionnaire prior to enrollment, including training status classification. To be classified as "exercise-trained" for the purposes of the study, subjects had to be participating in a structured exercise training program (including both aerobic and anaerobic) for the past 12 months with each session lasting no less than 45 minutes per session, as well as no less than three sessions per week. Subjects' exercise sessions had to be performed at a

minimum average rating of 15 (i.e., hard) on the Borg rating of perceived exertion (RPE) scale. This training classification was documented with each subject by completing a detailed exercise training history in conjunction with personal interviews.

All experimental procedures were performed in accordance with the Helsinki Declaration and approved by the University Human Subjects Review Board. Subjects provided both verbal and written consent. Along with completing a health history and physical activity questionnaire, subjects underwent a physical examination, including anthropometric testing. Body fat was estimated via 7-site skinfold determination and use of the Siri equation. Resting heart rate (HR) and blood pressure were recorded after a 10-minute quiet rest period. Subjects were not obese (body mass index [BMI] $\leq 30 kg \cdot m^{-2}$) and were free of any diagnosed cardiovascular, metabolic, or pulmonary disease as defined by the American College of Sports Medicine (51). In addition, subjects were non-smokers and did not use medications (e.g., anti-inflammatory or cardiovascular drugs), or nutritional supplements (e.g., antioxidants) during the course of the study as they may have impacted our outcome measures.

Following all screening procedures (including the graded exercise test [GXT] as described below), subjects were scheduled for testing and given detailed instructions and data forms related to the recording of both dietary and physical activity data during the 48 hours before the testing days. Participant characteristics are presented in Table 1.

Exercise Protocol

Graded Exercise Testing

A maximal graded exercise test (GXT) was conducted to determine aerobic capacity (VO_{2max}) and maximal aerobic power output (W_{max}) using a Lode Excalibur

Sport™ cycle ergometer. Expired gases were collected via facemask and analyzed using a SensorMedics Vmax 229™ metabolic system for determination of maximal oxygen consumption (VO_{2max}). This test was necessary for prescribing the intensity for the acute exercise sessions (moderate-intensity and moderate-duration steady state aerobic exercise, high-intensity and moderate-duration interval sprints, and maximal-intensity and short-duration interval sprints, as described below). After warming up at 50W for 3 minutes, the test began at 100W and increased 25W·min^{-1}. The test was terminated once the participant was no longer able to continue due to fatigue (RPMs drop below 50). The maximal wattage obtained during testing was used to calculate the workloads to be used during sprint intervals. During the final stage, subjects had to continue for a minimum of 30 sec in order for the wattage to be considered their peak wattage, as has been done previously (2, 11). Before and during the GXT, HR was continuously monitored via electrocardiograph (ECG) tracings using a SensorMedics Max-1™ ECG unit. Expired oxygen and respiratory exchange ratio data were continuously monitored via breath-by-breath samples. Participant effort was monitored using the Borg scale of exertion. Subjects were allowed an active cool-down period (e.g., slow-speed cycling) for several minutes until their HR fell below 120 beats per minutes or stabilized. Subjects were instructed not to perform any strenuous physical tasks during the 48 hour period prior to the GXT.

Acute Exercise Sessions

Approximately one week after the GXT, subjects participated in four additional sessions, each separated by one week. Sessions were counterbalanced and included either a no-exercise condition or each of the three exercise conditions. For the no-

exercise rest bout, subjects reported to the lab and simply rested for the entire period. As with the exercise bouts, four blood samples were obtained. The first was taken following a 10-minute rest period (as was the case for all conditions). Subjects then rested for 20 minutes (the same duration as the sprint exercise bouts). A blood sample was collected at the end of the 20-minutes rest period (corresponding to the immediate post-exercise blood samples), and 30 and 60 minutes following the 20-minutes rest period (corresponding to the post-exercise blood samples).

All exercise bouts were performed on the same cycle ergometer used for the GXT, and subjects reported to the laboratory in the morning (0600-0900 hours) following a minimum 10-hour overnight fast. Heart rate was continuously monitored via Polar™ heart rate monitors, and subjects received similar verbal encouragement by research assistants during all three exercise bouts. The exercise included one steady state aerobic bout and two different intermittent anaerobic sprint bouts. The rationale for our use of the specific intensity and duration of the exercise bouts was based on recent literature demonstrating that low volume, high intensity "sprint" type exercise elicits similar acute responses and chronic adaptations as compared to the more traditional high volume aerobic exercise (12-14, 46, 47). It should be noted that the denotation of exercise as "moderate" or "short" duration was related to the specific type of exercise (i.e., 60 minutes was considered moderate duration for aerobic exercise, while 60 seconds was considered moderate duration for anaerobic sprint exercise). Pilot testing confirmed that the three bouts were challenging, yet subjects were able to complete all protocols. The exercise bouts were as follows:

1. The moderate-intensity and moderate-duration steady state aerobic exercise bout was performed at 70% of HR reserve ({(220-age)-resting HR} x 0.70 + {resting HR}), for a duration of 60 minutes. This intensity corresponds to approximately 70% VO_{2max}. Heart rate and RPE were monitored continuously during the protocol, and the workload (wattage) was adjusted every five minutes as necessary in order to maintain 70% of HR reserve. Heart rate, RPE, and workload (wattage) were recorded every five minutes. The mean values for all variables were calculated and reported. A similar intensity and duration of exercise have been used in many other studies focused on exercise-induced oxidative stress (6, 20, 35).
2. The high-intensity and moderate-duration interval sprints consisted of five, 60-second sprints performed at a wattage equal to 100% of that obtained during the GXT (i.e., wattage at 100% of VO_{2max}), followed by 225 seconds of recovery (1:3.75 work to rest ratio). Within each interval, subjects were instructed to pedal between 80-100 RPMs for the first 45 seconds, and then for the final 15 seconds, subjects were instructed to pedal as fast as possible. RPE was recorded twice during each interval, once at 45 seconds, and again after the final 15 seconds of sprinting. The average of the two RPE values was used in the analysis (for comparison of RPE during the three different exercise bouts). Heart rate was recorded at the cessation of each sprint. During the recovery periods, the subjects were encouraged to get off of the cycle and walk around, in an attempt to reduce venous pooling in the lower extremities and to minimize feelings of light-headedness or nausea. This protocol equated to a total exercise bout duration of

20 minutes; however, only 300 seconds of actual work was performed, but done at half the intensity of the short intervals.

3. The maximal-intensity and short-duration interval sprints consisted of ten, 15-second sprints performed at a wattage equal to 200% of that obtained during the GXT (i.e., wattage at 200% of VO_{2max}), followed by 116 seconds of recovery (1:7.7 work to rest ratio). Following each sprint, HR and RPE were recorded. During the recovery periods, the subjects were allowed to get off of the cycle and walk around, in an attempt to reduce venous pooling in the lower extremities and to minimize feelings of light-headedness or nausea. This protocol equated to a total exercise bout duration of 20 minutes; however, only 150 seconds of actual work was performed, but done at double the intensity of the moderate duration intervals.

For all exercise conditions, blood was collected from subjects pre-exercise (following 10-minutes of quiet rest), immediately concluding exercise (0 minutes), and at 30 minutes and 60 minutes post-exercise. The collection times for the no-exercise rest condition matched those of the sprint sessions. Subjects remained in the laboratory during this period and expend little energy (i.e., watch movies, work on the computer). No meals or calorie-containing beverages were allowed during this period. Water was allowed ad libitum.

Blood Collection and Biochemistry

Blood samples were collected into vacuum tubes via venipuncture by a trained phlebotomist. Approximately 15mL of blood was taken from subjects for all exercise modes (and for the no-exercise rest condition), at the following time points: before

exercise, immediately post-exercise, and 30 and 60 minutes post-exercise. Samples were analyzed for plasma MDA, plasma hydrogen peroxide (H_2O_2), and serum Trolox Equivalent Antioxidant Capacity (TEAC). Blood lactate was also measured as an indicator of anaerobic metabolism.

Single samples were immediately analyzed (from EDTA containing tubes) for whole-blood lactate using an Lactate Plus™ portable lactate analyzer (Nova Biomedical,Waltham, Massachusetts, USA). The remainder of whole blood collected into EDTA tubes was centrifuged at 1500 rpm for 15 minutes at 4°C, and then processed for plasma and stored at -70°C until analyzed. Whole blood collected into tubes containing no additive was allowed to clot for 30 minutes at room temperature, centrifuged at 1500 rpm for 15 minutes at 4°C, and then processed for serum and stored at -70°C until analyzed.

Malondialdehyde was analyzed in plasma following the procedures of Jentzsch et al. (27) using reagents purchased from Northwest Life Science Specialties (Vancouver, WA). The reaction mixture was transferred to respective microplate wells and the absorbance read using a spectrophotometer at both 535 and 572nm to correct for baseline absorption. Malondialdehyde equivalents were calculated using the difference in absorption at the two wavelengths. Quantification was performed with a calibration curve using tetramethoxypropane in a stabilizing buffer; (coefficient of variation [CV] = 6.5%).

Hydrogen peroxide was measured in plasma using the Amplex Red reagent method as described by the manufacturer (Molecular Probes, Invitrogen Detection Technologies, Eugene, OR). In the reaction mixture, H_2O_2, in the presence of

horseradish peroxidase, reacts with Amplex Red reagent to generate the red-fluorescence oxidation product, resorufin. Quantification was performed with a H_2O_2 calibration curve; (CV = 7.9%).

Antioxidant capacity was analyzed in serum using the Trolox-Equivalent Antioxidant Capacity (TEAC) assay using procedures outlined by the reagent provider (Sigma Chemical, St. Louis, MO). Quantification was performed with a Trolox calibration curve; (CV = 2.7%).

Finally, hematocrit and hemoglobin were measured as part of a complete blood count using an automated cell counter (Coulter LH750). Plasma volume was then corrected using the guidelines provided by Dill and Costill (17).

Dietary Records and Physical Activity

All subjects were instructed to maintain their normal diet throughout the study period. Food logs during the day before each test day were maintained by subjects, analyzed by research staff, and returned to subjects so that they were able to mimic this intake during all subsequent days preceding meal test days. Nutritional records were analyzed for total calories, protein, carbohydrate, fat, and a variety of micronutrients (Food Processor SQL, version 9.9, ESHA Research, Salem, OR).

Subjects were instructed to maintain their normal physical activity habits. Subjects were given specific instructions regarding abstinence of alcohol consumption, in addition to the avoidance of strenuous exercise during the 48 hours immediately preceding the test days to control for any acute effects of physical activity on oxidative stress, and associated variables (e.g. inflammation). In addition, we wanted to make

certain that subjects did not encounter any undue fatigue by performing strenuous exercise during the hours prior to the actual test days.

Statistical Analysis

The data obtained were analyzed using a 4 (condition) by 4 (time) repeated measures analysis of variance (RMANOVA). Significant interactions and main effects were further analyzed using Tukey's *post hoc* tests. Dietary variables and all other data collected in relation to the exercise tests (e.g., mean HR and RPE) were compared using a one-way ANOVA. All analyses were performed using JMP statistical software (SAS Institute, Cary, NC). Statistical significance was set at $P \leq 0.05$. The data are presented as mean ± SEM.

RESULTS

All 12 subjects completed all aspects of this study. Subject's characteristics are presented in Table 1. As expected, a condition effect was noted for heart rate ($P < 0.0001$) and perceived exertion ($P < 0.0001$), with heart rate and perceived exertion values higher for all exercise conditions as compared to rest ($P < 0.05$). For heart rate, the 60 sec sprint was greater than the 60 min aerobic and 15 sec sprint ($P < 0.05$). For perceived exertion, the 60 sec sprint and the 15 sec sprint were greater than the 60 min aerobic and the 15 sec sprint was greater than the 60 sec sprint ($P < 0.05$). Also as expected, a condition effect was noted for power (watts) and total work ($P < 0.0001$), with values higher for all exercise conditions as compared to rest ($P < 0.05$). For power, values for the 60 sec sprint and 15 sec sprint were greater than those for the 60 min aerobic, while values for the 15 sec sprint were greater than those for the 60 sec sprint ($P < 0.05$). For total work, values for the 60 sec sprint and 15 sec sprint were less than those

for 60 min aerobic ($P < 0.05$). Exercise test related data are presented in Table 2. Dietary data during the 24 hours prior to each test day were not different ($P > 0.05$). Dietary data are presented in Table 3.

Biochemical Data

With regards to lactate, an interaction was noted ($P < 0.0001$). Values were higher at 0 min and 30 min post-exercise as compared to pre exercise for the 60 sec sprint and the 15 sec sprint ($P < 0.05$). Values were different between the two sprint exercise bouts and the rest and 60 min aerobic exercise conditions at the 0 min and 30 min post-exercise times ($P < 0.05$). A condition effect was noted ($P < 0.0001$), with values for the two sprint exercise bouts higher than those for the rest and 60 min aerobic exercise conditions ($P < 0.05$). A time effect was noted ($P < 0.0001$), with values higher at all times post-exercise as compared to pre exercise, at 0 min post-exercise as compared to 30 and 60 min post-exercise, and at 30 min post-exercise as compared to 60 min post-exercise ($P < 0.05$). Data for lactate are presented in Figure 1.

With regards to MDA, no interaction ($P = 0.97$), condition ($P = 0.28$), or time ($P = 0.79$) effect was noted. Data for MDA are presented in Figure 2. With regards to H_2O_2, no interaction ($P = 0.49$) or condition ($P = 0.58$) effect was noted. A trend for a time effect was noted ($P = 0.06$). Data for H_2O_2 are presented in Figure 3.

With regards to TEAC, an interaction was noted ($P = 0.0006$), with values higher at 60 min post-exercise as compared to pre exercise for the 15 sec sprint ($P < 0.05$). A condition effect was noted ($P = 0.004$), with values higher for 60 sec sprint and 15 sec sprint as compared to 60 min aerobic ($P < 0.05$). A time effect was noted ($P < 0.0001$),

with values higher at 30 and 60 min post-exercise as compared to pre exercise and 0 min post-exercise ($P < 0.05$). Data for TEAC are presented in Figure 4.

DISCUSSION

The present study compared oxidative modification of blood lipids, hydrogen peroxide, and antioxidant capacity following both aerobic and anaerobic exercise bouts performed by exercise-trained men. To our knowledge, no study to date has compared both aerobic and anaerobic exercise bouts of different intensities and/or durations on biomarkers of oxidative stress—in particular within a sample of exercise-trained men. The main findings from this investigation were (a) lipid peroxidation as assessed using MDA was similar for pre-exercise and post-exercise time points for both aerobic and anaerobic cycling bouts; (b) hydrogen peroxide was similar for pre-exercise and post-exercise time points for both aerobic and anaerobic cycling bouts; (c) antioxidant capacity as assessed using TEAC increased ($P \leq 0.05$) from pre- to post-exercise for both sprint bouts, in particular at the 30 and 60 min post-exercise time points; and (d) lactate increased ($P \leq 0.05$) from pre- to post-exercise, in particular for the sprint bouts immediately post-exercise. Our findings regarding no significant increases in oxidative stress biomarkers are specific to blood measured up to 60 min post-exercise. It is possible that increases in oxidative stress may have occurred in other tissues, as well as at times distant to the 60 min post-exercise time period.

Subjects in this study were young (23.7±1.1 yrs) in apparently-good health (body fat: 12.8±1.3%; body weight: 80.7±2.6kg; VO_{2max}: 40.0±2.1ml·kg^{-1}·min^{-1}). In addition, all subjects were exercise-trained (as can be viewed in Table 1). Of the exercise protocols, the 60 sec sprints produced the greatest increase in HR, while the 15 sec

sprints produced the greatest increase in RPE (Table 2). The 60 min aerobic bout produced the lowest HR and RPE, which was expected due to the moderate intensity, steady state nature of the aerobic exercise. Power (watts) was highest in the15 sec sprint bout and lowest in the 60 min aerobic bout (Table 2). However, total work kJ was highest in the 60 min aerobic exercise bout, which was expected based on higher duration of this bout. All subjects appeared to consume adequate dietary energy, macronutrients, and vitamins (C, E, and A) during the days surrounding all exercise protocols, with no significant differences were noted for dietary energy, macronutrients, and vitamins (Table 3).

Our findings of no significant increase in oxidative stress biomarkers is interesting, despite the very high intensity nature of the exercise protocols—yielding a significant post-exercise increase in blood lactate. These results are in accordance with other investigations that have found no significant rise in oxidative stress biomarkers despite large increases in lactate (4, 43). However, they are contradictory to the work of Lovlin and colleagues who noted an association between MDA and lactate at maximal exertion (32). The current investigation used a more specific MDA analysis technique than that of Lovlin and colleagues. (32), which utilized thiobarbituric acid (TBA), a reagent that reacts with other molecules not associated with oxidative stress and has been criticized for its lack of specificity (38). Additionally, blood was drawn for the current investigation within one minute of exercise completion, whereas samples were collected at the midpoint of a 5 min rest period between each stage by Lovlin et al. (32). The current investigation found no significant rise in oxidative stress biomarkers despite exercise being performed at an intensity corresponding to approximately 100-

200%VO_{2max}, whereas Lovlin and colleagues (32) selected a range of exercise intensity from 40-100% VO_{2max} and noted a significant increase in MDA. It is likely that difference in the assay techniques, the timing of sample collection, as well as the fact that our subjects were well-trained men and the subjects in the Lovlin et al. study consisted of only "six male subjects" (with no indication of training status), may be responsible for the discrepancies in findings.

In addition to the above, it is important to note that in the present study all post-exercise blood samples were corrected for changes in plasma volume. This is something that has not been done in many studies of exercise-induced oxidative stress, and may be one additional reason for conflicting findings. For example, in the present study plasma volume during the immediate post-exercise period was decreased approximately 11% following aerobic exercise, and 19% following both sprint exercise bouts. Prior to correcting for plasma volume, there was an increase noted for both MDA and H_2O_2 at the immediate post-exercise time. However, once values were corrected, the increased was abolished. These findings call into question prior reported results, including our own, in which investigators noted an exercise-induced increase in oxidative stress but failed to correct for such potential changes in plasma volume.

Contrary to what we hypothesized, and possibly based on factors discussed above (e.g., plasma volume correction, use of exercise-trained men), our investigation failed to report a significant increase in either MDA or H_2O_2. Results from sprinting protocols have been mixed amongst the different biomarkers used to assess oxidative stress, and increases have been noted for lipid peroxidation (3, 22, 33). Marzatico et al. investigated two different types of exercise involving either running a half marathon or a sprint

exercise session of 6x150 m sprints (subjects were either marathon trained or sprint trained) (33). The authors noted significant post-exercise increases in MDA for both protocols—in opposition to the present findings. Modality may be one discrepancy between our investigation and that of Marzatico and colleagues. Running was the mode used in the Marzatico et al. investigation, which when compared to cycling, uses more muscle groups and incorporates eccentric muscle actions. This may elicit greater muscular damage, potentially leading to increased RONS production. When comparing the individual exercise bouts, we have no firm explanation for the differences between our study and that of Marzatico et al. with regards to the sprint protocol. On the other hand, the significant findings regarding the aerobic bout may be due to intensity. For example, while the duration used in our investigation was 60 min—compared to the 68 min taken to run the half marathon in the Marzatico et al. investigation (33), subjects likely were exercising at a higher intensity during the half marathon, in order to complete a 13.1 mile run in such a short time. On the other hand, our findings are in accordance with other investigations that have failed to find significance regarding MDA after sprint exercise (7, 8, 22, 48).

Hydrogen peroxide is not a radical itself due to the fact that it does not have any unpaired electrons. It is actually a relatively stable compound and is not very reactive, and acts as either a mild oxidizing or weak reducing agent (34). Even though H_2O_2 itself is not very reactive, it can be converted into the hydroxyl radical, thus, becoming a reactive species. Hydrogen peroxide is reduced via the enzyme glutathione peroxidase (GPX) by using the reduced glutathione (GSH) as the electron donor (44). Reduced glutahione is then oxidized to glutathione disulfide (GSSG), and finally, GSSG is reduced

by glutathione reductase (GR). Although we did not measure GPX or GSSG, it is likely that since there was no significant increase in H_2O_2, GSH was available in abundant and/or GPX was efficient at reducing what H_2O_2 was actually produced. Our findings for an increase in TEAC during the post-exercise period lend some support to this hypothesis.

Laaksonen et al. investigated the relationship between glutathione and selected antioxidant enzymes and determinants of lipid peroxidation at rest and in response to exercise in men (30). Subjects cycled for 40 min at 60% VO_{2max}, and the authors reported an increase of about 50% in GSSG, with the exercise also leading to an increase of about 50% in lipid peroxidation (30). Some discrepancies amongst the Laaksonen et al. investigation and the current investigation may be due to the training status of subjects. Laaksonen et al.(30) reported that their subjects exercised an average of 1.2 times per week, while our subjects exercised an average of 3.4 times per week (primary in the form of anaerobic training).

The young age of our subjects may also have played a part in our null findings for oxidative stress markers. For example, it has been reported that younger individuals possess a greater antioxidant capacity as opposed to older individuals (29). It is plausible to believe that our study failed to report any significance in regards to H_2O_2 due to the relatively young age of subjects—in particular as compared to prior studies (18, 30).

As noted above, we noted an increase in TEAC following exercise (in particular sprint exercise), which may have counteracted the acute increase. Our findings demonstrate an acute decrease in TEAC, followed by an increase above basal levels at both the 30 and 60 min recovery period. These findings are in accordance with the

literature regarding the initial drop during and post-exercise (16, 45), as well as with the increase above basal levels at times distant to the immediate post-exercise period (35, 45). Others have failed to demonstrate an increase in TEAC following exercise (1, 50); however, these investigations may have missed any potential increase in TEAC due to the failure to include sample measurement beyond the immediate post-exercise period. Michailidis and colleagues have shown that the collection schedule for blood samples analyzed for oxidative stress biomarkers may extend to 4 hrs post-exercise (35). Our cessation of measurement at 60 mins post-exercise may be partly responsible for our lack of observed effect for all measures. However, our relatively stable findings for MDA and H_2O_2 through the 60 min post-exercise period dampen our enthusiasm for such a suggestion. Clearly, not all studies demonstrate an exercise-induced oxidative stress. For those that do (9, 18, 35, 45), this often raises a concern due to the fact that elevated oxidative stress is associated with human disease (15).

However, as stated earlier, engaging in a regular exercise program is also associated with an increase in endogenous antioxidant enzyme activity (35, 36, 45, 49), which may serve the cell with protection against the exercise-induced increase in RONS. Exercise-induced RONS production leads to the activation of NF-κB, with its activation leading to the expression of antioxidant enzymes (21). All of these up-regulations are in accordance with the principle of hormesis (28, 41); therefore, any concerns regarding the transient rise in RONS, and potentially in oxidative stress, should be appeased.

It is important to note that despite the fact that we did not observe an increase in MDA or H_2O_2 in response to exercise, this does not necessarily indicate that RONS were not produced. It is possible that RONS production simply did not overwhelm the

antioxidant defense. In much the same way as blood lactate may not *accumulate* despite a significant increase in lactate production, the oxidation of molecules may not occur despite the rise in RONS.

CONCLUSION

Exercise performed at maximal-intensity, yielding a high lactate response, does not result in an increase in oxidative stress as measured by blood MDA and H_2O_2. This may be partly explained by an increase in TEAC during the post-exercise period. These findings are specific to a sample of exercise-trained men. Future work is necessary to investigate the impact high intensity training on cellular protection against RONS—in particular as related to the antioxidant defense system.

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Table 1. Descriptive characteristics of 12 exercise-trained men

Variable	Value
Age (years)	23.7±1.1
Height (cm)	179.6±2.0
Body Weight (kg)	80.7±2.6
Body Mass Index ($kg \cdot m^{-2}$)	25.0±0.7
Waist (cm)	83.6±1.5
Hip (cm)	98.4±2.5
Waist:Hip	0.85±0.02
Body Fat (%)	12.8±1.3
Heart Rate (bpm)	62.2±2.0
Systolic Blood Pressure (mmHg)	116.7±3.9
Diastolic Blood Pressure (mmHg)	70.7±3.3
Anaerobic Exercise (years)	4.9±1.4
Anaerobic Exercise ($hours \cdot week^{-1}$)	4.0±0.6
Aerobic Exercise (years)	2.8±0.6
Aerobic Exercise ($hours \cdot week^{-1}$)	2.6±0.7
VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	40.0±2.1
Max Watts on GXT	322.9±12.9
Max Heart Rate on GXT	190.5±2.0

Values are mean±SEM.

Table 2. Heart rate, perceived exertion, power, and total work data of 12 exercise-trained men during a rest or exercise condition

Variable*	Rest	60 min aerobic	60 sec sprint	15 sec sprint
Heart Rate (bpm)	64.5±3.0	155.2±3.4	171.7±4.0	157.6±3.2
Perceived Exertion (6-20 scale)	6.0±0.0	13.5±0.4	15.6±0.3	16.7±0.2
Power (watts)	0.0±0.0	128.1±6.5	322.9±12.9	645.8±25.7
Total Work (kJ)	0.0±0.0	461.1±23.4	96.9±3.9	96.9±3.9

Values are mean±SEM.

*Values are averages taken every 5 min during the 60 min aerobic exercise bout and at the conclusion of the 60 sec and 15 sec sprints.

Heart Rate: $P < 0.0001$; All conditions > Rest; 60 sec sprint > 60 min aerobic and 15 sec sprint ($P < 0.05$).

Perceived Exertion: $P < 0.0001$; All conditions > Rest; 60 sec sprint and 15 sec sprint > 60 min aerobic; 15 sec sprint > 60 sec sprint ($P < 0.05$).

Watts: $P < 0.0001$; All conditions > Rest; 60 sec sprint and 15 sec sprint > 60 min aerobic; 15 sec sprint > 60 sec sprint ($P < 0.05$).

Total Work: $P < 0.0001$; All conditions > Rest; 60 min aerobic > 15 sec sprint and 60 sec sprint ($P < 0.05$).

Table 3. Dietary intake of 12 exercise-trained men during the 24 hours prior to a rest or exercise condition

Variable	Rest	60 min aerobic	60 sec sprint	15 sec sprint
Kilocalories	2540.0±200.3	2550.9±191.3	2265.8±185.4	2344.5±154.6
Protein (g)	118.4±8.8	123.5±14.5	116.7±10.7	110.3±9.0
Protein (%)	19.3±1.4	19.2±1.4	21.3±2.0	19.0±1.1
Carbohydrate (g)	347.7±34.7	347.9±27.9	310.6±37.1	310.4±31.5
Carbohydrate (%)	55.3±4.0	55.7±3.3	53.6±4.5	53.2±4.2
Fat (g)	79.0±14.9	81.0±12.4	67.0±10.8	80.2±14.1
Fat (%)	27.0±4.3	27.8±3.3	26.8±3.6	30.0±4.5
Vitamin C (mg)	96.5±21.6	110.5±20.9	71.7±17.6	124.8±28.2
Vitamin E (mg)	9.0±2.8	7.1±2.8	7.1±2.4	8.4±3.3
Vitamin A (RE)	734.8±211.5	761.0±222.2	686.6±216.6	713.8±221.0
Selenium (µg)	69.1±19.1	61.6±12.7	88.0±16.9	70.0±23.1

Values are mean±SEM.

No significant differences noted for kilocalories ($P = 0.64$), protein grams ($P = 0.79$), protein % ($P = 0.71$), carbohydrate grams ($P = 0.77$), carbohydrate % ($P = 0.97$), fat grams ($P = 0.86$), fat % ($P = 0.93$), vitamin C ($P = 0.37$), vitamin E ($P = 0.95$), vitamin A ($P = 0.99$), or selenium ($P = 0.67$).

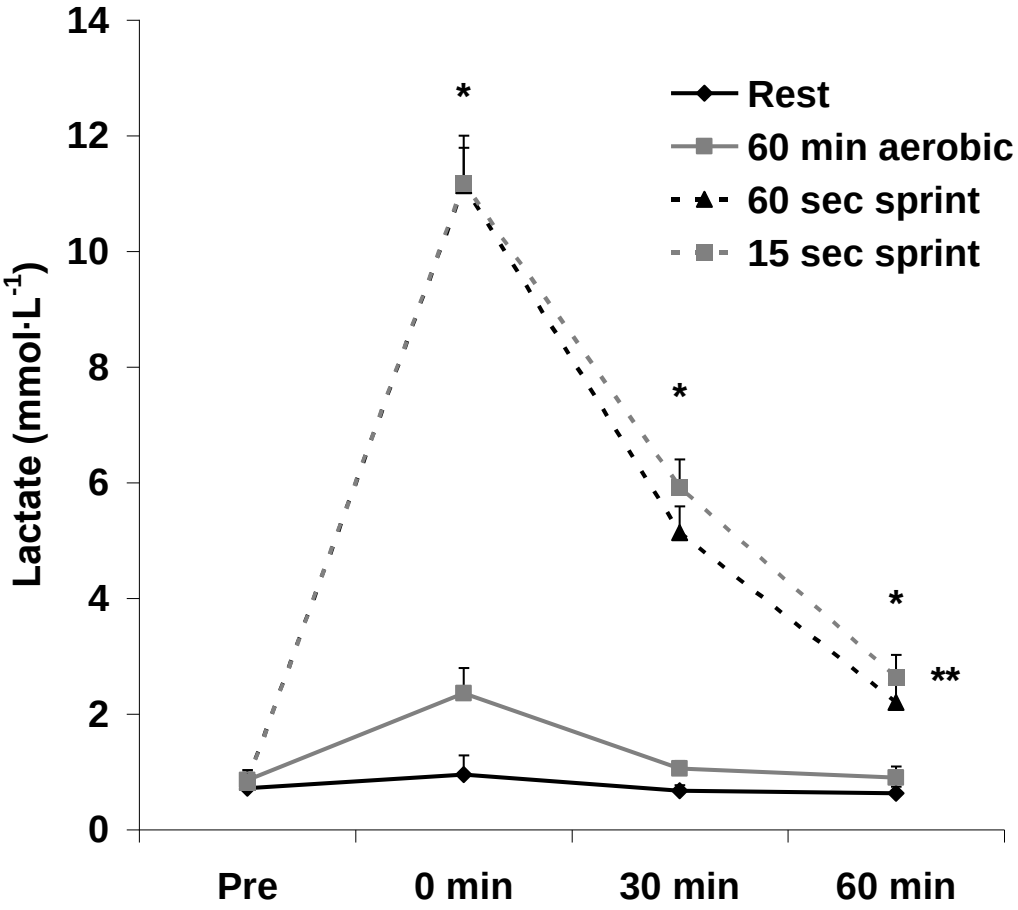

Figure 1. Blood lactate data of 12 exercise-trained men before and after a rest or exercise condition

Values are mean±SEM.

Interaction ($P < 0.0001$); Values higher at 0 min and 30 min post-exercise as compared to pre exercise for the 60 sec sprint and the 15 sec sprint ($P < 0.05$); Values different between the two sprint exercise bouts and the Rest and 60 min aerobic exercise conditions at 0 min and 30 min post-exercise ($P < 0.05$).

**Condition effect ($P < 0.0001$); values for the two sprint exercise bouts higher than those for the Rest and 60 min aerobic exercise conditions ($P < 0.05$).

*Time effect ($P < 0.0001$); values higher at all times post-exercise as compared to pre exercise, at 0 min post-exercise as compared to 30 and 60 min post-exercise, and at 30 min post-exercise as compared to 60 min post-exercise ($P < 0.05$).

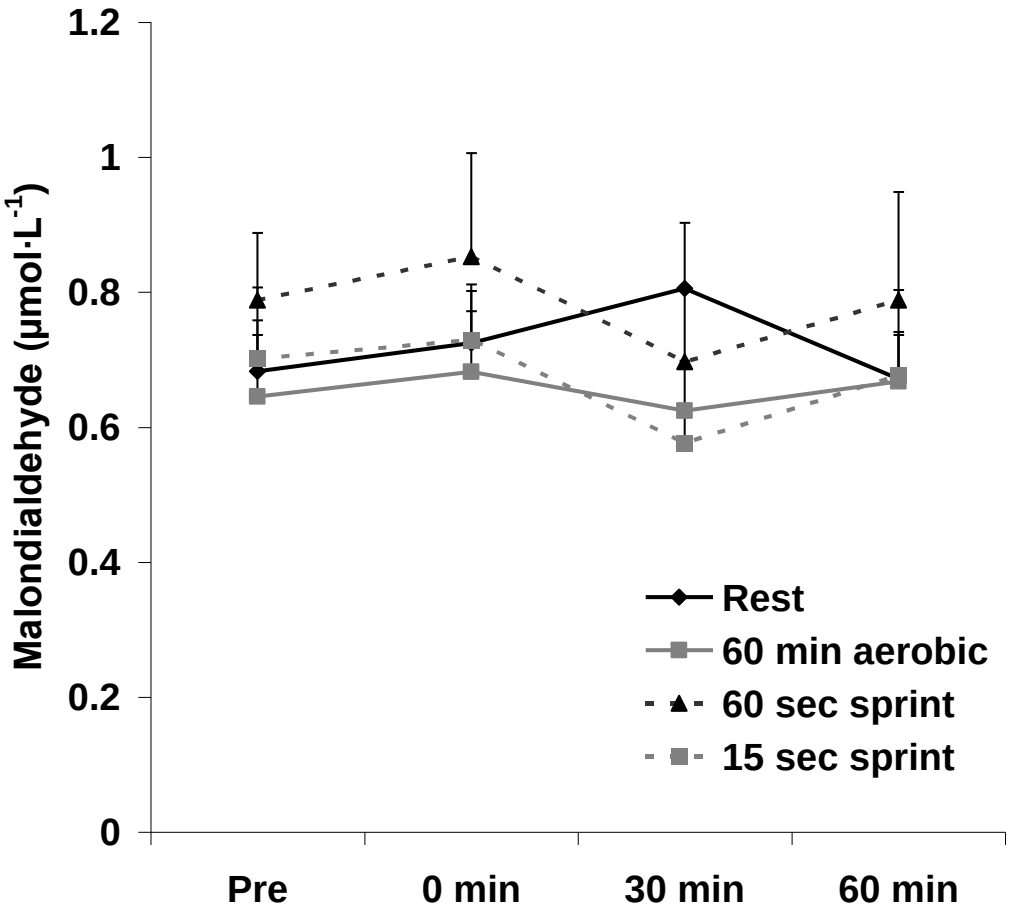

Figure 2. Blood malondialdehyde data of 12 exercise-trained men before and after a rest or exercise condition

Values are mean±SEM.

No interaction (P = 0.97).

No condition effect (P = 0.28).

No time effect (P = 0.79).

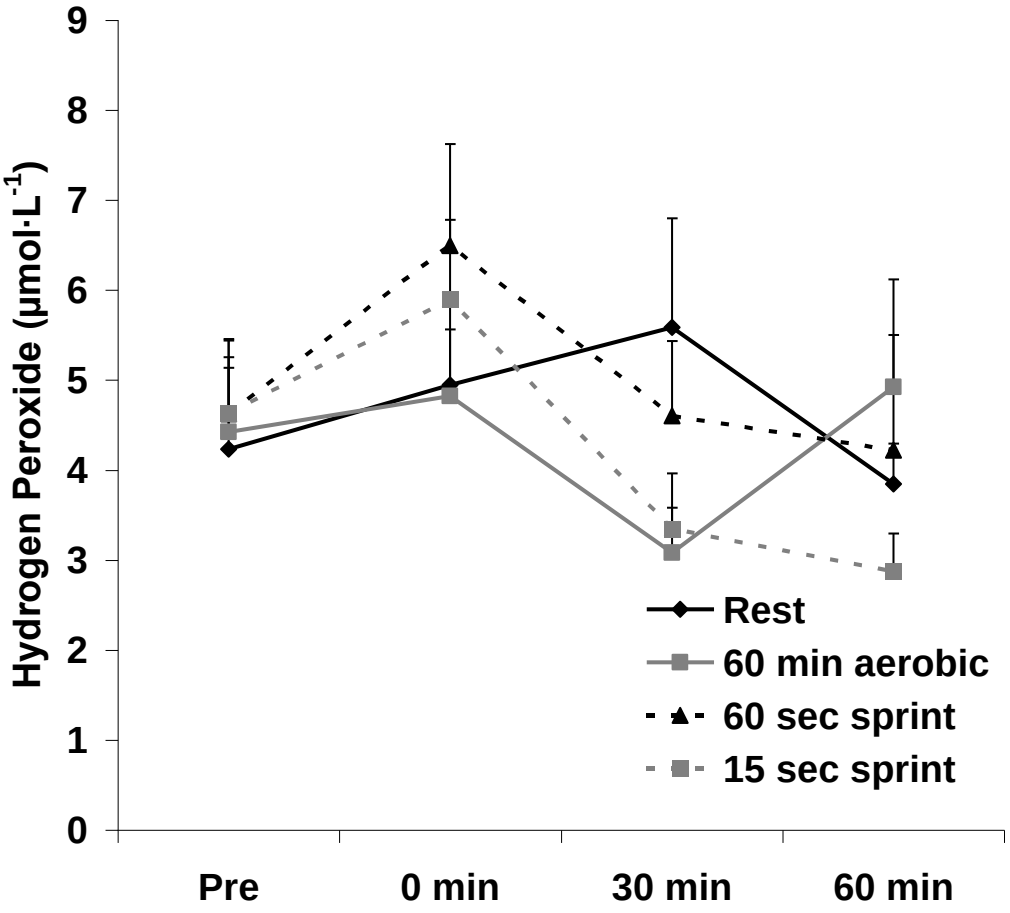

Figure 3. Blood hydrogen peroxide data of 12 exercise-trained men before and after a rest or exercise condition

Values are mean±SEM.

No interaction (P = 0.49).

No condition effect (P = 0.58).

A trend for time effect (P = 0.06).

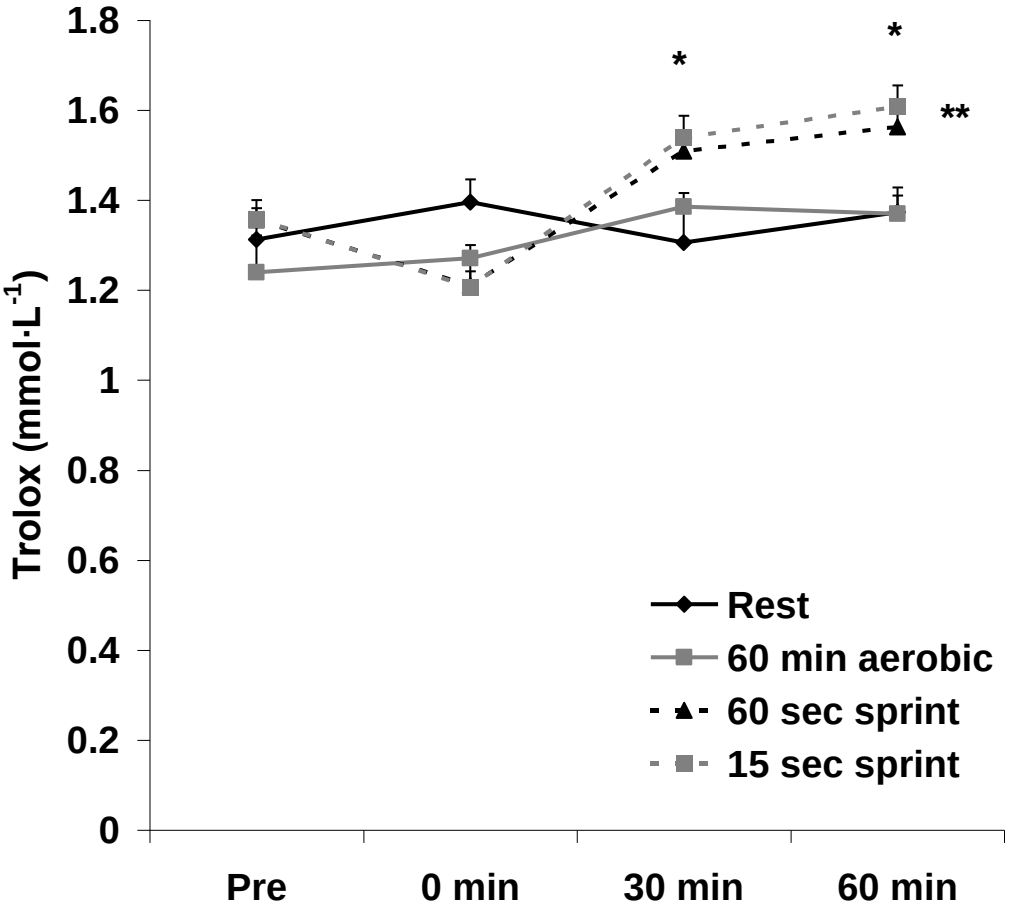

Figure 4. Blood Trolox Equivalent Antioxidant Capacity data of 12 exercise-trained men before and after a rest or exercise condition

Values are mean±SEM.

Interaction ($P = 0.0006$); values higher at 60 min post-exercise as compared to pre exercise for 15 sec sprint ($P < 0.05$).

**Condition effect ($P = 0.004$); values higher for 60 sec sprint and 15 sec sprint as compared to 60 min aerobic ($P < 0.05$).

*Time effect ($P < 0.0001$); values higher at 30 and 60 min post-exercise as compared to pre exercise and 0 min post-exercise ($P < 0.05$).

APPENDIX A – EXTENDED LITERATURE REVIEW

1. Introduction

Oxidative stress is a condition in which the body's antioxidant defenses are overcome by the production of reactive oxygen and nitrogen species (RONS), sometimes referred to as pro-oxidants (6). The imbalance between pro-oxidants and antioxidants in favor of the former, may contribute to the advanced oxidation of macromolecules leading to the initiation and progression of disease (36, 37). While the production of RONS is a normal biological process and can occur with a variety of stressors (e.g., nutrient consumption, ozone exposure, acute exercise), when produced in excess, RONS can be damaging to tissue within the body (77). A major harm associated with increased RONS production is the damage they may cause to nucleic acid bases, lipids, and proteins, which can severely compromise cell health and viability (23)In fact, extensive cellular oxidation can severely compromise cell viability and contribute to ill-health and disease (23). This brief literature review will provide an introduction to oxidative stress, with a specific focus on exercise-induced oxidative stress.

2. Free Radicals

What are Reactive Oxygen and Nitrogen Species?

In chemistry, molecules are bonded to one another through the sharing of electrons, forming pairs (37). Surrounding the atom is the orbital region that contains the electrons, with a maximum of two orbitals. When a bond is broken, a molecule then has an unpaired electron, from which becomes "free." A free radical is defined as any molecule species capable of independent existence (36), with one or more unpaired

electrons. Most molecules in biology are non-radicals; that is, they have only paired electrons. However, once they have a single unpaired electron, then they are said to be "free." Free radicals can exist as either hydrogen atoms, transition metal ions, carbon centered radicals or sulfur centered radicals (36). Primary examples of RONS, or more specifically ROS, include singlet oxygen (•O), superoxide radical (O_2•$^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH). The most damaging radicals are those derived from oxygen and/or nitrogen.

Singlet Oxygen

Although essential for life, oxygen itself is a radical, due to its two unpaired electrons located in different antibonding π*orbitals* (55). This radical is referred to as a diradical. These two electrons have the same spin in a triplet state, and are considered the most stable state of oxygen, or the so called ground state (55). If oxygen should be electronically excited, then a reactive form of oxygen will form called singlet oxygen (•O). Singlet oxygen may be generated in some biological systems, and even though it has a very short half-life, it is capable of diffusion and is permeable to membranes (70). Singlet oxygen exists in one of two states, the first excited state ($^1\Delta gO_2$) or the more reactive second state ($^2\sum gO_2$) (70). Even though •O has no unpaired electrons and is not a radical, it has no spin restriction. This enhances the potential for it to react with other biomolecules.

Superoxide

When there is a one electron reduction of oxygen, a superoxide radical is formed (O_2•$^-$). Some characteristics of this anion are that it is negatively charged and is relatively membrane permeable. When compared to other radicals, O_2•$^-$ has a relatively long half-

life that enables diffusion within the cell, therefore, increases the potential number of potential targets (70). A major source of the $O_2\bullet^-$ formation is through the leakage of electrons from the electron transport chain within the mitochondria. Another main source of $O_2\bullet^-$ formation is from the respiratory burst of phagocytic cells, such as neutrophils, macrophages, and lymphocytes (55). One thing to note is that $O_2\bullet^-$ is generally considered relatively nonreactive compared with other radical species (70), but is dangerous to biological systems because it is easily converted to other RONS (55).

Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is not a radical itself due to the fact that it does not have any unpaired electrons. It is actually a relatively stable compound and is not very reactive, and acts as either a mild oxidizing or weak reducing agent (55). Even though H_2O_2 itself is not very reactive, it can be converted into the hydroxyl radical, thus, becoming a reactive species. With the production of $O_2\bullet^-$, most biological systems will produce H_2O_2 as well, mainly through mitochondria and phagocytic cells (70).

Hydroxyl Radical

One highly reactive radical that has strong oxidizing potential is the hydroxyl radical (•OH). Hydroxyl radicals are potentially the most damaging RONS, with molecules in close proximity to this radical being the primary target (70). Due to their high reactivity, •OH are not membrane permeable. Hydroxyl radicals are produced by radiolysis of water under high-energy ionizing radiation, and thought to be produced mainly in biological systems by reductive cleavage of H_2O_2 via the Fenton reaction (55). The Fenton reaction involved the decomposition of H_2O_2 with reduced transition metal ions. Due to the spin of ground state oxygen and H_2O_2, they cannot directly react with

biomolecules at significant rates. On the other hand, transition metals do exist in several spin states. Therefore, these can relieve the spin restriction of ground state oxygen and H_2O_2, thus enhancing the rate of biomolecule oxidation, therefore, leaving transition metals as efficient catalysts of redox reactions (60).

Mechanisms of RONS Actions

The unpairing and formation of free radicals can occur by three different mechanisms (45). The first is through the homolysis of covalent bonds. This is the dissociation of a molecule into two separate species: $A–B \rightarrow A^{\bullet} + B^{\bullet}$. The second mechanism is through the addition of a single electron (e) to a neutral atom: $A + e \rightarrow A^{\bullet-}$. The last mechanism is through the loss of a single electron (e) to form a neutral atom: $A \rightarrow A^{+\bullet} + e$. Free radicals are reactive due to the desire to exist in a state of homeostasis; that is, in a stable state. Once these free radicals accept electrons from another molecule, they become stable. In turn, they force the other molecules to become unstable. These other molecules then look to become stable themselves, promoting a cascade of events that are redox reactions.

Mitochondrial

One major pathway for the generation of RONS is through the reduction of molecular oxygen in mitochondria (41), and is thought to be the result of electron leakage within the electron transport chain. With exercise come high amounts of oxygen being consumed for energy. About 95-98% of total oxygen consumption is accounted for as it undergoes four electron reduction catalyzed by cytochrome oxidize, but for the remaining 2-5%, it may undergo one electron reduction with the production of the $O_2\bullet^-$ (41). If $O_2\bullet^-$ undergoes one more electron reduction, it produces H_2O_2 radical. The electron leakage

may stem from the one electron reduction of ubiquinone (coenzyme Q), which in turn generates ubisemiquinone (65). This generation is what leaks the electron to oxygen to form the $O_2\bullet^-$ radical.

Xanthine Oxidase and NADPH Oxidase

Free radical formation may come from the conversion of xanthine dehydrogenase to xanthine oxidase, which is a $O_2\bullet^-$ radical generator (41). This process comes from acute states of ischemia (followed by reperfusion) when ATP stores have been temporarily exhausted and there are high levels of intracellular ADP. These high levels are what start ADP degradation and conversion of xanthine dehydrogenase to xanthine oxidase (41). In order for this process to generate a large amount of free radicals, there are certain criteria that must be met (41): First, the enzyme xanthine dehydrogenase/oxidase must be present within the muscle. Second, calcium-activated proteases must be stimulated via failure of calcium homeostasis. Third and finally, the substrate hypoxanthine must be produced in substantial amounts by exercising muscle (41). Assuming the above conditions are met, xanthine oxidase production may increase.

Aside from xanthine oxidase, present within neutrophils and other cell types is the enzyme NADPH oxidase (41). Although this is thought to be a mechanism for RONS production, it is unclear as to whether or not the enzyme is present within the skeletal muscle or if it is influenced by contractile activity (41). Therefore, NADPH oxidase activation of RONS remains speculative at the present time.

Prostanoid Metabolism

Another possible site of RONS formation comes from prostaglandins being released from various cell types in response to a stimuli (41). Prostaglandins are lipid

compounds derived enzymatically from free fatty acids. They are from the prostanoid class of fatty acid derivatives, which is a subclass of eicosanoids. Through prostaglandin metabolism, many intermediates are produces that are free radical species. Arachidonic acid is a prostaglandin precursor that becomes an active metabolite when it reacts with lipoxygenase enzymes. This ultimately produces additional free radicals. The exact role of prostaglandin as a source of oxidative stress in exercise is unclear and further research is needed in this area (41).

Catecholamines

Catecholamines are released during exercise and serve many physiological purposes, i.e. increased heart rate and blood flow. They can, on the other hand, auto-oxidize leading to RONS production. Catecholamines contain the catechol moiety as an intergral part of their structure, which is a phenolic compound (5). As a group, phenols are susceptible to oxidation with oxygen being the most important due to being virtually ubiquitous and present in relatively high concentrations (5). With exercise, there generally is an increase in heart rate due to the increase release of catecholamines, therefore, potentially leading to an increase in free radical formation.

Secondary Pathways

While the above discussion focused on primary pathways of RONS generation, other "indirect" generators are known and referred to as secondary pathways. These include tissue injury, which is associated with acute inflammation and the generation of high amounts of RONS (41). Specifically, neutrophils (neutrophilic polymorphonuclear leukocytes) are activated via immune responses, and act to contain and kill invading microbial pathogens (29). As the neutrophils act on the invading pathogens, they release

substantial amounts of oxygen radicals, which in turn may damage surrounding viable tissue (41).

The disruption of iron-containing proteins such as erythrocytes and myoglobin can lead to an increase in free iron, which is known to catalyze radical reactions and form RONS. Exercise may create a significant degree of trauma (e.g., high-force eccentric muscle actions) and may lead to destruction of these proteins. Also, anaerobic exercise promotes acidosis and excess lactate accumulation leading to iron release from transferrin and the potential for the conversion of $O_2\bullet^-$ to •OH. Relevant to iron containing proteins, the increased oxidation of hemoglobin and myoglobin that occurs with intense exercise can cause RONS formation. The oxidation of hemoglobin may produce methaemoglobin and $O_2\bullet^-$ (102), while the oxidation of myoglobin generates H_2O_2 (16). Such changes may manifest in increase macromolecule oxidation.

3. Biomarkers of Oxidative Stress

Lipid Peroxidation

Lipids have received a great deal of attention due to their susceptibility to oxidative damage, especially with acute bouts of exercise (6, 66). Lipid peroxidation involves the reaction of oxidative deterioration of polyunsaturated lipids (66, 77). The common biomarkers used to measure lipid peroxidation are F_2-isoprostanes, lipid hydroperoxides (LOOH), maldondialdehyde (MDA), thiobarbituric acid reactive substances (TBARS), and conjugated dienes (often specific to oxidized low-density lipoprotein). The most commonly used method includes MDA, with thiobarbituric acid being used in the assay to measure aldehyde products (primarily MDA) formed from the deterioration of polyunsaturated lipids.

Lipids are oxidized when RONS absorb a hydrogen atom from an esterified polyunsaturated fatty acid (66, 77). Lipid peroxidation happens in part because through exercise, there is an increase in mitochondrial respiration due to increases in oxygen uptake (26). This also has an effect on electron transport disturbances, which produces a chain reaction. This is where a hydrogen atom is removed from a methyl group (CH_2) in the carbon chain, forming a free radical on the carbon due to it having an unpaired electron. A conjugated diene is then formed by the carbon undergoing molecular rearrangement, and is free to combine with another molecule. Usually, oxygen combines with the carbon, then forming a peroxyl radical. This new radical is then capable of reacting with other molecules and abstracting more hydrogen atoms. This then gives rise to other free radicals.

Protein Oxidation

Proteins are also directly targeted by RONS and can be affected during oxidation in four ways: 1) Oxidation of the protein backbone, leading to protein fragmentation, 2) formation of protein-protein cross linkages, 3) oxidation of amino acid side chains, or 4) generation of carbonyl derivatives (18). RONS attack proteins because they are abundant in biological systems and are responsible for many functional processes within cells. Oxidation susceptibility may be protein-specific, such as certain proteins are more easily oxidized compared to others. Reactions may involve specific alterations where phenylalanine residues are converted to *o*-tyrosine or of tyrosine to dityrosine. Techniques to measure advanced oxidation protein products have been developed in recent years to study RONS related modifications. The formation of carbonyl derivatives

is the most widely used biomarker of oxidative modifications to proteins for exercise-induced oxidative stress (77).

DNA Oxidation

Oxidation of DNA usually involves damage to single bases, with different modifications observed depending on the RONS interacting with the DNA (72). For exercise-induced oxidative stress, the most commonly used biomarker is the measurement of 8-hydroxy-2´-deoxyguanosine (8-OHdG). This formation is assessed in muscle and organ tissue, urine, serum, and isolated leukocytes. Mitochondrial and nuclear DNA can be both affected by RONS, and may have strand breaks or single base modifications. 8-OHdG is not a normal intermediate in nucleotide metabolism, but its presence is used to indicate oxidative DNA damage (72). Only about ~10% of total damage to DNA is represented by 8-OHdG, but it has a very high mutagenic potential. The damage is frequently found specific to tumor-related genes, and has elevated concentrations with various physiological diseases and disorders, such as aging and cancer (72).

Assessing RONS Formation

Free radicals have a very short life span (e.g., 10^{-6}, 10^{-5}, 10^{-9} seconds for •O, O_2•$^-$, and •OH, respectively), but at the same time a very highly reactive life span (6). Assessing RONS formation directly is very difficult, especially within plasma and other bodily fluids. One direct method of assessing RONS formation is by the use of electron spin resonance (ESR) spectroscopy. This method involves spin traps, which allows for a more stable product. Indirect methods of assessing RONS formation is the most commonly used techniques. The typical indirect measurements include assays of high

performance liquid chromatography (HPLC), gas chromatography-mass spectroscopy (GC-MS), fluorometric and colorometric assays, and gel electrophoresis (80). These techniques are used for assessing the oxidation of lipids, proteins, and DNA—as discussed above.

Markers of Antioxidant Status

In addition to the above, individual antioxidants and antioxidant enzymes can be measured as indicators of oxidative stress. Moreover, global makers of antioxidant status, which provide an overall measure of "total" antioxidant capacity, are widely utilized as indicators of oxidative stress. These are discussed below.

4. Defense Mechanisms against RONS

RONS are constantly being produced within the body from normal physiological processes, such as meal consumption and exercise. The main purpose behind defense mechanisms is to prevent RONS-induced oxidation or to scavenge radical species and convert them to lesser active molecules (46). Within the body reside numerous defenses mechanisms that will help either minimize RONS formation or neutralize their damaging effects once formed. The main defense mechanisms include antioxidant enzymes and non-antioxidant enzymes scavengers (6).

Antioxidant Enzymes

Superoxide Dismutase

One such antioxidant enzyme family is superoxide dismutase (SOD). Superoxide dismutase acts to catalyze the conversion of $O_2\bullet^-$ to H_2O_2, and three primary forms are known to exist: a cytosolic-zinc enzyme (Cu-ZnSOD), a mitochondrial enzyme requiring manganese (MnSOD), and an extracellular SOD (EC-SOD) (42). Superoxide dismutase

acts as the first line of enzymatic defense against free radical formation by removing $O_2\bullet^-$, reducing it to H_2O_2. Even though $O_2\bullet^-$ is not highly reactive, if left alone, the potential increases to extract electrons from biomolecules, thus causing a free radical chain reaction (42). It is important to minimize $O_2\bullet^-$ anions as much as possible.

Glutathione Peroxidase

The main purpose of glutathione peroxidase (GPX) is to catalyze the reduction of both organic peroxides and H_2O_2 to water and alchohol, respectively, while using reduced glutathione (GSH) as the electron donor (87). Reduced glutahione is then oxidized to glutathione disulfide (GSSG). Finally, GSSG is reduced by glutathione reductase (GR), with the help of NADPH acting as the reducing power. This reaction takes place simultaneously with GPX, allowing for the regeneration of GSH from GSSG (42). Glutathione plays an important part in antioxidant defenses, and the ratio of reduced GSH versus GSSG may exceed 100:1, but experimental data suggest a value of 10 to 50:1 (43).

Catalase

Catalase (CAT) shares the same function of GPX in that it catalyzes the decomposition of H_2O_2 to water (42). The catalytic function of CAT is high when H_2O_2 levels are high, as it is decomposed to O_2 and water. Catalase targets specific H_2O_2 that is produced in the peroxisomes and this is due to enzymes such as flavoprotein dehydrogenase in the β-oxidation of fatty acid, urate oxidase, and the metabolism of D-amino acids (42). Catalase utilizes peroxide to oxidize a range of H donors (AH_2) such as methanol, ethanol, and formate (87).

Non-enzymatic Antioxidants

Non-enzymatic antioxidants are represented by ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), glutathione (GSH), carotenoids, flavonoids, and other antioxidants such as thiols and metal binding proteins (97). Certain antioxidants such as vitamin E, vitamin C, and β-carotene cannot be synthesized by humans and intake must come exclusively from the diet. The main purpose of non-enzymatic antioxidants is to help transfer electrons from radical species. The main role of vitamin E main is to quench an electron from a free radical species, but in doing so, it becomes a vitamin E radical. It is then reduced back to vitamin E by ascorbate (vitamin C), which in turn becomes a radical—ultimately reduced back to the active state by GSH (42).

5. Exercise-Induced Oxidative Stress

Over the past 30 years, there have been over 300 original investigations pertaining to exercise-induced oxidative stress (30). The consensus of evidence indicates that increasing exercise intensity and duration results in greater oxidative stress. While not all studies demonstrate an exercise-induced oxidative stress, many do; which is somewhat concerning considering that elevated oxidative stress is associated with human disease (23). However, it should be understood that participating in a regular exercise program is also associated with an increase in endogenous antioxidant enzyme activity (59, 63, 91, 99), which may serve to provide protection against the exercise-induced increase in RONS. Moreover, a decrease in RONS production may be apparent after repeated exposure to the same stressor, as well as an increased activity of DNA specific repair enzymes (77). Such adaptations are specific to the principle of hormesis, which states that in response to repeated exposure to various stressors, the body undergoes favorable

adaptations that result in enhanced physiological performance and improved physical health (44, 74). It has been noted that exercise-induced RONS production leads to the activation of the redox sensitive transcription factor nuclear factor (NF)-kappa (κ)B, which upon activation leads to the expression of antioxidant enzymes (34). Combined with dietary antioxidants, protection is providing against exercise-induced RONS, which can potentially provide support against the development and progression of certain diseases (e.g., cardiovascular diseases, diabetes (36, 37)).

Exercise-induced oxidative stress has been observed for all types of exercise, including both aerobic (28, 59, 91) and anaerobic (12), with different pathways of RONS generation for each (41). In terms of aerobic exercise, which clearly represent the most well-studied form of exercise related to oxidative stress, the majority of laboratory based studies use treadmill walking/running or stationary cycling performed for 30-60 minutes at 60-80% VO_{2max} (30). In terms of anaerobic exercise, in which a relatively low number of studies have been conducted, protocols have involved dynamic resistance exercise (9, 11, 15, 39), eccentric exercise (11, 64), jumping (68), and sprinting (11, 35). Related to the latter form, very few studies have compared sprint duration within the same design. This is true despite the widespread use of sprint protocols within the training regimen of many athletes.

As stated above, it is believed that the oxidative stress response to acute exercise is intensity dependent. In an early study on exercise-induced oxidative stress, Lovlin and colleagues reported an increase in the lipid peroxidation marker malondialdehyde (MDA) in subjects immediately following a graded exercise test performed to exhaustion, while exercise at 70% of VO_{2max} and lower percentages resulted in no increase in MDA (52).

Support for this finding was provided by Leaf and colleagues who reported an intensity dependent increase in expired ethane and pentane when subjects performed a graded exercise test to exhaustion (48). Additional support is provided by Quindry and coworkers (71) who measured blood markers of oxidative stress to different intensities of aerobic exercise and concluded that "Exercise intensity plays a major role in post-exercise blood oxidative stress, whereas total exercise energy expenditure does not." However, in opposition to the above findings, one more recent study employing resistance exercise (i.e., squats) noted an increase in MDA which occurred independent of exercise intensity (39). It is likely that the oxidative stress response to exercise is both intensity and mode dependent (15), as well as impacted by exercise duration (8). To our knowledge, no study to date has compared both aerobic and anaerobic exercise bouts of different intensities and durations on biomarkers of oxidative stress—in particular within a sample of exercise-trained men.

Aerobic Exercise

The generation of RONS during aerobic exercise is thought to be due mostly to the leakage of electrons through the respiratory chain, due in part to the increase in oxygen consumption through sustained exercise (22, 66). The majority of research focusing on oxidative stress and acute exercise utilizes aerobic exercise as the mode of choice, with protocols typically involving exercise performed at >60% VO_{2max} and lasting longer 30 minutes or longer (except for studies involving a GXT, for which exercise duration may be far less than 30 minutes). This intensity is usually considered submaximal (8, 13, 15, 47, 53, 59, 63, 101), although may be maximal is some cases (2,

48, 71, 90, 91). In laboratory studies, the mode of exercise typically consists of cycle ergometry (8, 15, 47, 53, 59, 90-92) and treadmill walking/running (2, 13, 71, 101).

Lipid Peroxidation

The most common method used to access exercise-induced oxidative stress with aerobic exercise is the assessment of lipid peroxidation. This is often done via MDA and TBARS, with F_2-isoprostanes increasing in popularity in recent years. There is over three decades of evidence indicating that lipid peroxidation is increased with acute aerobic exercise (30). Several studies have shown that steady state exercise results in an increase of TBARS at both the submaximal (47, 63, 101) and maximal (59, 61, 90, 91) workloads. Biomarkers of lipid peroxidation for most investigations peaked either immediately post-exercise or within one hour post-exercise, and returned to baseline values shortly afterwards. Contrary to the above findings, some investigations have reported no increase in TBARS following maximal (78, 86) or submaximal (33, 98) exercise.

When focusing on MDA measurements alone, investigations have reported mixed results. Some have shown no increases while exercising at maximal (2, 7, 48, 71) or submaximal (13, 15, 104) workloads. Opposing these data, others have found an increase in MDA levels (19, 28, 32, 96). The differing results may relate to differences in exercise modes, durations, and intensities across studies, as the majority of studies involving maximal or near maximal intensity protocols (>70%VO_{2max}) performed for moderate durations (>30 minutes) have noted an increase in MDA. This may give some insight as to the role that intensity plays in exercise-induced oxidative stress. Conflicting findings may also relate to the time course of sample collection following exercise. Specifically,

many studies have only obtained a single sample immediately post-exercise. It is certainly possible that an increase in MDA (or other oxidative stress biomarkers) could have been elevated at times distant to this. Therefore, the possibility remains that erroneous conclusions may have been made related to the exercise-induced elevation in MDA.

Protein Oxidation

As stated earlier, RONS attack proteins because they are abundant in biological systems and are responsible for many functional processes within cells. An increase in protein oxidation has been reported by many investigators (7, 8, 13, 32, 59, 63), with evidence that protein carbonyl concentrations are dependent on exercise duration (8). There is also evidence that concentrations stay elevated up to eight hours post-exercise (59). Null findings have also been reported when focusing on protein oxidation (15, 61, 62, 78). The difference in finding may be attributed to sampling times, training status of subjects, and/or exercise protocols.

DNA Oxidation

As stated earlier, oxidation of DNA usually involves damage to single bases. The most common method of assessing DNA oxidation is through the product 8-hydroxy-2-deoxyguanosine (8-OHdG). Besides two investigations finding significant increases in 8-OHdG (62, 67), the majority of studies reported no increases in DNA oxidation (7, 13, 15, 32). The potential reason as to why investigations had no significant findings may be attributed to the fact that moderated-duration and intensity aerobic exercise does not increase DNA oxidation to an extent that may be measured in blood samples (15), possibly due to the ability of DNA to be rapidly repaired following oxidation (73).

Antioxidant Capacity

Antioxidant capacity may be depleted during strenuous exercise due to combating RONS formation. Besides including more global markers of total antioxidant status, such as TEAC, performing assays for individual antioxidant enzymes is another method to assess oxidative stress (e.g., SOD, GPx, CAT, or GR), as these enzyme may be up-regulated by an acute bout of exercise or may be somewhat depleted owing to their combating nature against RONS. Many investigations have been performed to confirm the change in antioxidant capacity, which has been noted to decrease in some studies (25, 91, 96, 103). In such work, though antioxidant defenses decreased transiently after exercise, they either returned to or exceeded their pre-exercise levels within one hour after exercise (2, 28, 59, 63, 91, 99, 103). This finding confirms the short-term activity of antioxidants to assist in handling RONS production, as well as the stimulatory effect of acute exercise on antioxidant status. However, despite the increase in antioxidant status noted in some studies, other authors have reported no increase (88, 101), which may be attributed to sampling times related to the acute session. When focusing specifically on individual antioxidant enzymes, investigations have reported increases in SOD (17, 19, 27), GPx (17, 27, 28, 47), and CAT (17, 59, 63, 99) following exercise. Contrary to the above findings, only a few studies have reported null findings for SOD (96), GPx (1), and GR (27).

Anaerobic Exercise

There are fewer investigations pertaining to anaerobic exercise and oxidative stress as compared to aerobic exercise. Since anaerobic exercise (e.g., resistance training, isometric exercise, jumping, sprinting) does not require the same amount of oxygen to

fuel muscular work as does aerobic exercise, other pathways of RONS generation likely contribute to oxidative stress (e.g., xanthine and NADPH oxidase, prostanoid metabolism, ischemia/reperfusion, phagocytic respiratory burst activity, and disruption of iron-containing proteins) (12, 41). During resistance training, there is greater stress placed on the body from the increased amounts of eccentric muscle actions, which causes greater muscle damage (58), which may be associated with increased RONS production (14)

Dynamic Resistance Exercise

Investigators have utilized dynamic resistance exercise to study exercise-induced oxidative stress. The majority of studies have utilized protocols that consisted of two or more compound lifts, usually performed for multiple sets at an intensity equal to 60-95% 1 RM (51, 56, 57, 79, 100, 101). Other investigations used only one movement, such as the squat (9, 11, 15, 39, 93) or knee extension (3).

The oxidative stress response after dynamic resistance exercise is similar to that following aerobic exercise, with the majority of studies reporting an increase in lipid peroxidation (3, 39, 51, 57, 79, 100), protein oxidation (15, 84, 100), and changes in glutathione status (15, 93). Contrary to these findings, other investigation have reported null findings for each of the biomarkers, including lipid peroxidation (9, 11, 15, 56, 93), protein oxidation (9, 93), and glutathione status (51). When focusing on DNA oxidation, no human study to our knowledge has reported a significant increase (11, 15).

Eccentric Resistance Exercise

The majority of protocols focused on eccentric resistance exercise have utilized untrained subjects and involved eccentric actions of either the knee extensors (21, 64, 69,

76, 83) or elbow flexors (21, 31, 49, 50, 83). Eccentric exercise is utilized in these protocols due to the increased muscle damage that is associated with the high force that is placed on the muscles while lengthening during contractions. Through this high force comes an increase in RONS production, as evidenced by increased creatine kinase activity and impaired muscle force following eccentric exercise (10, 20, 21, 38, 50, 64, 83).

Results have been mixed following eccentric exercise, with studies noting an increase in lipid peroxidation (21, 31, 64, 69), protein oxidation (31, 50, 64, 69), DNA oxidation (75), and changes in glutathione status (31, 49, 64, 69). Other investigations have reported null findings, even with similar protocols, for lipid peroxidation (10, 20, 38, 83), protein oxidation (83), and glutathione status (50). Unlike aerobic exercise and non-damaging anaerobic exercise, due to the fact that muscle injury and associated signs and symptoms may persist for days following the protocol, many oxidative stress biomarkers peak between 1-3 days post-exercise.

Isometric Exercise

Another form of resistance exercise utilized to induce a state of oxidative stress is isometric exercise. Some protocols have involved thumb adduction (24, 91) while others have involved maximal voluntary contractions (MVC) (2, 81, 82, 89). Those that utilized thumb adduction performed exercise until exhaustion (24, 91), while those that tested handgrip did so by performing MVC with the dominant hand (2, 89). Static knee extension has also been used at an intensity of 30% (81) or 66% of MVC (82). Relative to other exercise forms, there are far fewer investigations utilizing isometric exercise. However, of those studies performed, increases in lipid peroxidation (2, 24, 89, 91),

changes in glutathione status (89, 91), and decreases in antioxidant capacity (89) have been reported. Although such changes have been noted, biomarkers returned to pre-exercise values within 20 to 60 minutes post-exercise.

Sprinting/Jumping Protocols

Interval sprint exercise is growing in popularity as a form of regular exercise for both athletes and non-athletes. Related to the oxidative stress literature, protocols generally include sprints performed at maximal intensities while subjects pedal on a cycle ergometer (4, 9, 11, 35, 94), run (54, 85, 95), or swim (40). Although not sprint exercise, one study involved interval jumping (68).

Results from sprinting protocols have been mixed amongst the different biomarkers used to assess oxidative stress. Increases have been noted for lipid peroxidation (4, 35, 54), protein oxidation (11), and DNA oxidation (85). Opposing these findings, some investigators observed no changes in lipid peroxidation (9, 11, 94), protein oxidation (9), or DNA damage (11). Aside from pure sprint protocols, MDA levels increased when utilizing intermittent shuttle running (95), but were unchanged while jumping (68) or swimming (40).

The mixed finding may be attributed to many different factors, such as the test subjects, the exercise protocols, and the timing of blood collections relative to the end of exercise. Cycling protocols have consisted of subjects performing either a maximal test via a Wingate test (4, 11, 35) or repeated cycle sprints (9). Running protocols have consisted of two sprints performed until exhaustion (85) or 90 minutes of variable intensity shuttle running over a 20 meter distance, including walking, jogging, and sprinting (95). Swimming protocols have involved either a 800 or 100 meter swim (40).

Clearly, exercise protocols denoted as "sprints" have varied considerably. Although all exercise protocols likely involved a relatively high intensity and perceived level of work, performing only one maximal sprint (e.g., Wingate test), as opposed to repeated sprints, could impose a drastically different burden on the body leading to different degrees of RONS generation. In exercise-trained individuals in particular, a single sprint may not impose a significant enough demand on the body to impose a state of oxidative stress. Therefore, although the intensity may have been at a significant level during all such sprint protocols, the duration of exercise may have been too short. Future studies, in particular those involving exercise-trained subjects (who are better protected against exercise-induced oxidative stress than their untrained counterparts), should seek to include a multiple-set sprint protocol (as is typical in the training regimens of many individuals), in an attempt to induce an oxidative stress.

Besides differences in the actual exercise protocols, discrepancies in findings may also be attributed to the different blood sampling times across studies. For example, blood collections times have included immediately post-exercise (4, 11, 40, 85), 24 hours post-exercise (68), 48 hours post-exercise (9, 54), and 72 hours post-exercise (95). However, while some studies have extended the time frame of collection several hours or days post-exercise, others have only included samples immediately post-exercise (4, 11, 40, 85). This is obviously a limitation of this work, as elevations in oxidative stress may have been potentially missed at times distant to the immediate post-exercise period.

The subjects' population may also influence results. The subjects utilized in the majority of prior investigations have been men (4, 9, 11, 35, 54, 68, 95), with one study utilizing both men and women (40). With regards to training status, studies have

included apparently healthy (4, 35, 85, 95), resistance trained (11), or anaerobically trained men (9). A few investigations have utilized athletes, including sprinters/marathoner runners (54), volleyball players (68), and swimmers (40). The mixed results across studies may be attributed to the different training programs of the subjects, their familiarity to the exercise stressor, as well as their overall training status. From a practical standpoint, the majority of prior studies that have involved sprinting do not mimic what might actually be done during a typical exercise training session. To our knowledge, no study has investigated the oxidative stress response to two different sprint protocols of varying intensity and duration within the same design.

Summary of Anaerobic Exercise

The text above outlines the evidence for anaerobic exercise-induced oxidative stress. As with aerobic exercise, the degree of oxidative stress is likely dependent on exercise mode, duration, and intensity. Aside from eccentric exercise which often causes muscle injury and inflammation, many anaerobic exercise bouts performed by healthy subjects (in particular those who are resistance trained) do not lead to prolonged oxidative stress; rather, values return to pre-exercise levels within one to two hours following exercise (4, 35, 85, 95). Anaerobic exercise has been shown to increase lipid and protein oxidation, with minimal impact on DNA oxidation. The studies also show that the fitness level of subjects plays a major role on oxidative stress levels post-exercise. Many studies include well-trained individuals who typically experience a blunted oxidative stress response to acute exercise as compared to untrained individuals, providing evidence that exercise increases antioxidant defense, which aids against RONS formation during exercise. While it may seem reasonable to consider the inclusion of

untrained individuals within a design to measure exercise-induced oxidative stress (in an attempt to observe more robust effects in the chosen biomarkers), such work has little practical value, as untrained individuals seldom perform strenuous exercise.

6. Conclusion

There is substantial evidence that acute exercise of sufficient intensity and duration can lead to an increase in RONS and induce an acute state of oxidative stress. What remains unknown is what specific intensity is required to induce an oxidative stress, in particular in well-trained subjects. Some individuals express concern due to the fact that exercise is known to induce an oxidative stress. However, considering that many studies have been conducted in untrained subjects (or have involved trained subjects undergoing extreme duration exercise), it is possible that in trained individuals, moderate to high intensities of exercise are inadequate to induce an oxidative stress. That is, perhaps only super-high intensities and durations of exercise may induce such a condition (this is particularly true when considering the potential exercise-induced increase in antioxidant defense which may counteract any acute increase in RONS). To date, little work has been done to investigate this question. Future studies should seek to do so. Regardless of whether the concern of an individual is to determine the threshold needed in order to induce an oxidative stress 1) with the objective of engaging in such exercise with the goal of activating a hormetic response to allow for up-regulation in antioxidant defense or 2) to avoid such exercise due to concern over potential ill-effects of the elevated RONS, such future studies should provide information pertaining to the impact of intensity on exercise-induced oxidative stress.

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THE UNIVERSITY OF MEMPHIS

Institutional Review Board

To: Richard Bloomer, R. Canale, T. Farney, M. Kabir, R. Alleman, C. McCarthy, J. Trepanowski, & M. Oliver
Health & Sport Sciences

From: Chair, Institutional Review Board
for the Protection of Human Subjects

Subject: Impact of exercise intensity on postprandial oxidative stress (H11-23)

Approval Date: 9/23/2010

This is to notify you of the board approval of the above referenced protocol. This project was reviewed in accordance with all applicable statutes and regulations as well as ethical principles.

Approval of this project is given with the following obligations:

1. At the end of one year from the approval date an approved renewal must be in effect to continue the project. If approval is not obtained, the human consent form is no longer valid and accrual of new subjects must stop.
2. When the project is finished or terminated, the attached form must be completed and sent to the board.
3. No change may be made in the approved protocol without board approval, except where necessary to eliminate apparent immediate hazards or threats to subjects. Such changes must be reported promptly to the board to obtain approval.
4. The stamped, approved human subjects consent form must be used. Photocopies of the form may be made.

This approval expires one year from the date above, and must be renewed prior to that date if the study is ongoing.