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COMPARATIVE ANALYSIS OF THE MAGNITUDE OF OXIDATIVE STRESS
FOLLOWING ACUTE HIGH FAT FEEDING AND ACUTE STRENUOUS
EXERCISE

by

Cameron G. T. McCarthy, BS, CSCS

A Thesis

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

Major: Health and Sport Sciences

The University of Memphis

August 2011

Mum and Dad,

As long as I have made you proud, then it has all been worth it.

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Firstly, my utmost gratitude and appreciation has to be extended to my advisor, thesis chair, and mentor, Dr. Richard Bloomer. His standards of excellence in the pursuit of knowledge have helped garner the tools I need to succeed at wherever my career and life take me.

I would also like to thank the other members of my thesis committee, Dr. Brian Schilling and Dr. Zsolt Murlasits. Their support and feedback throughout my graduate career at the University of Memphis was invaluable to my learning and development.

ABSTRACT

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Two prevalent origins of oxidative stress in Western society are the ingestion of certain nutrients and exercise. The purpose of this investigation was to compare the magnitude of increase in oxidative stress following acute feeding and acute exercise. Twelve exercise-trained men consumed a high-fat meal or performed one of three exercise bouts, in a random order, cross-over design. Blood samples were assayed for malondialdehyde (MDA), hydrogen peroxide (H_2O_2), trolox equivalent antioxidant capacity (TEAC), and triglycerides (TAG). A significant condition effect was noted for MDA ($p=0.01$) and H_2O_2 ($p<0.0001$), with values highest for the meal condition. A trend was also noted for TAG ($p=0.07$), with values highest for the meal condition. These results illustrate that the magnitude of oxidative stress following a meal is significantly greater than the magnitude of oxidative stress elicited after an acute bout of strenuous exercise, in a sample of healthy, exercise-trained men.

Key words: Free radicals, oxidative stress, exercise, postprandial.

PREFACE

This thesis was submitted in partial fulfillment of the requirements for the degree of Master of Science for the University of Memphis Graduate School. The thesis is formatted according to the style of *Medicine & Science in Sports & Exercise*®, and will be submitted for publication following the defense, in an attempt to help advance the exercise-science and nutrition literature.

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INTRODUCTION

Reactive oxygen and nitrogen species (RONS) are very small, short-lived molecules which are highly reactive due to their unpaired valence shell electron (50). As RONS seek to accept electrons, they often react with other molecules promoting either positive or negative effects. While RONS generation occurs in part as a consequence of normal cellular metabolism, they may also be generated through exposure to environmental pollutants (52), cigarette smoke (1), excess nutrient intake (92), and physical exercise (40).

The formation of RONS and the subsequent amelioration via the antioxidant defense system is a delicately balanced and continual process *in vivo* that serves several key roles in human physiology (39). Under optimal conditions, RONS regulate vital processes such as cellular signaling, immune function, apoptosis, and gene transcription (99). However, in response to a variety of stressors, as mentioned above, RONS production increases and may cause damage to cellular constituents.

The precise cellular damage resulting from RONS generation is specifically related to which macromolecules (lipids, proteins, and nucleic acids) are being targeted by the oxidants, the frequency and duration of the attack, as well as the tissue-specific antioxidant defenses present. Excessive RONS formation that overwhelms antioxidant defenses leads to a state of oxidative stress. Such a condition has been suggested to lead to mutagenic adaptations, perhaps promoting ill-health (36), physiological dysfunction (99), and impaired physical performance (78). Hence, an ideal balance between RONS production and antioxidant defenses is paramount.

Two of the most prevalent RONS generators in Western society include excess nutrient intake (in particular saturated fat) (92) and strenuous physical exercise (40), both of which have been studied extensively. While a review of extant literature indicates that the overall magnitude and time course of oxidative stress appears to be greater following feeding than following typical bouts of acute exercise (e.g., 30-60 minutes of moderate-to-high intensity exercise), to our knowledge, no study to date has directly compared the oxidative stress response between these two RONS generators.

Postprandial oxidative stress, which appears contingent upon the magnitude and rate of postprandial glycemia and triglyceridemia (76), has been shown to peak in healthy, non-obese subjects (regardless of training and cigarette smoking status), between two and four hours post feeding in most studies (7, 13-16, 18, 42, 70, 98), with at least one exception noting peak values between four and six hours post feeding (34). The specific composition (23, 42, 42) and size (23) of the meal plays a role in the overall magnitude and time course of response, with pure lipid feedings leading to the greatest increase, when compared to isocaloric carbohydrate, protein, and mixed macronutrient feedings (42). However, from a practical point of view, most individuals do not consume pure lipid feedings. Hence, a mixed macronutrient meal, inclusive of a high amount of dietary fat, seems to be the logical choice for inclusion within a research design aiming to provide practical application of findings.

Moreover, while the specific biomarker studied may influence the degree of change in oxidative stress, similar results have been noted for various measures of lipid peroxidation. For example, the percent change in malondialdehyde (MDA) following a lipid meal was estimated to be between 60% (14) and 95% (13), while estimates of 60%

(34) and 105% (70) have been noted for lipid hydroperoxides (LOOH). Collectively, the literature is clear that lipid-rich meals induce an acute state of oxidative stress (41).

On the other hand, an exhaustive review of the literature in regards to exercise-induced oxidative stress indicates that while many studies do demonstrate an increase post-exercise oxidative stress, many others do not (40). This lack of an increase in oxidative stress biomarkers is particularly noted in exercise-trained individuals, who may be protected from such an increase due to increased antioxidant defense (17). Unlike the postprandial literature for which oxidative stress markers generally peak between 2-6 hours following feeding (7, 13-16, 18, 34, 42, 70, 98), exercise-induced oxidative stress is usually transient, with most biomarkers usually peaking between 0 and 60 minutes following exercise, at least in regard to steady-state aerobic exercise (11, 21, 22, 43, 56, 80, 89, 100), exhaustive aerobic exercise (3, 43, 80, 89, 93), and certain single (6, 20, 35, 48, 49, 55) and multiple bout (12, 35, 69, 86) sprint exercise protocols. However, with exercise that results in muscle injury (26, 30, 44, 54, 65-67, 77, 82, 85) or that is extreme in duration (e.g., ultra-marathon running) (53), values for oxidative stress biomarkers may peak from hours to days following strenuous exercise.

The time course and increase in oxidative stress variables are biomarker dependent (72), exercise prescription dependent (modality (22), intensity (80), and duration (11)), and subject population dependent (10). Collectively, although some studies have reported a magnitude of increase in selected oxidative stress biomarkers that parallels or exceeds the typical increase in the same biomarkers noted following feeding, the bulk of studies indicate a lesser degree of exercise-induced oxidative stress as compared to feeding. However, difficulty exists in making such direct comparisons, as

no study has included both a feeding and exercise stress within the same design in an attempt to characterize the oxidative stress response.

Therefore, based on the current state of knowledge, the purpose of this investigation was to compare the magnitude of increase in oxidative stress biomarkers following acute feeding and acute exercise. We hypothesized that a greater oxidative stress would be observed following feeding as compared to exercise. Data collected from this experiment may direct future recommendations regarding the need for antioxidant supplementation in relation to both acute feeding and acute strenuous exercise.

MATERIALS AND METHODS

Participants

Twelve healthy, exercise-trained men between the ages of 21 and 35 years were recruited to participate. All participants 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, participants had to be participating in a structured exercise training program (a combination of both aerobic and anaerobic), for no less than 45 minutes per session, at least three times per week, for the past 12 months. Specifically, the subjects' exercise sessions had 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.

Along with completing a health history and physical activity questionnaire, participates underwent a physical examination, including anthropometric testing. Body fat was estimated via 7-site skinfold determination and the use of the Siri equation.

Resting heart rate and blood pressure was recorded after a 10-minute quiet rest period. Subjects were not obese (body mass index [BMI] $\leq 30 kg \cdot m^{-2}$) and did not have any diagnosed cardiovascular, metabolic, or pulmonary disease as defined by the American College of Sports Medicine (101). In addition, participants 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 influenced our outcome measures.

Following all screening procedures (including the graded exercise test (GXT) as described below), participants 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. All experimental procedures were performed in accordance with the Helsinki Declaration and approved by the University Institutional Review Board for Human Subjects Research. Participants provided both verbal and written consent.

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 (steady-state, moderate interval, and short interval, as described below). After warming up at 50W for three minutes, the test began at 100W and increased $25W \cdot min^{-1}$. The test was terminated once the participant could no longer continue due to

fatigue (revolutions per minute (rpm) dropped below 50). The maximal wattage obtained

during testing was used to calculate the workloads used during sprint interval the exercise

trials. The maximal heart rate (HR) obtained during testing was used to calculate the

workload to be used during the steady state exercise trial. It was required of the

participants to continue within the final stage for a minimum of 30 seconds in order for

the wattage to be considered their peak wattage, as has been done previously (27).

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 was continuously monitored via breath-by-breath samples.

Participant effort was monitored and the Borg RPE scale. Participants were allowed an

active cool-down period (e.g., slow speed cycling) for several minutes until their HR fell

below 120 beats per minute or stabilized. Participants 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, participants were assigned in random

order, separated by one week, to either the feeding condition (as outlined below), or to

perform one of three bouts of exercise. All exercise bouts were performed on the same

cycle ergometer used for the GXT, and participants 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 participants were similarly

encouraged by research assistants during all three exercise bouts. The exercise protocols

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 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 (31-33, 68). Moreover, individuals engaged in

exercise programs often perform acute bouts of exercise that vary in mode and intensity

from session to session.

It should be noted that the denotation of exercise as "moderate" or "short"

duration is related to the specific type of exercise (i.e., 60 minutes is considered moderate

duration for aerobic exercise, while 60 seconds is considered moderate duration for

anaerobic sprint exercise). Pilot testing confirmed that the three bouts were challenging,

yet subjects were able to complete all protocols (unpublished findings). 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 has been used in many other studies focused on exercise-induced

   oxidative stress (40).

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}). The total duration of the exercise bout was 20 minutes, and the four rest periods between the five sprints were spread evenly, as is outlined in the Table 1. Within each interval, participants were instructed to pedal between 80-100 RPMs for the first 45 seconds, and then for the final 15 seconds, participants were instructed to pedal as fast as possible. Rating of perceived exertion 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 participants were allowed to get off of the cycle and walk around/stretch, in an attempt to reduce venous pooling in the lower extremities and to minimize feelings of light-headedness or nausea. This protocol equated to only 300 seconds of actual work, however, it was performed 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}). The total duration of the exercise bout was 20 minutes, and the nine rest periods between the ten sprints were spread evenly, as is outlined in the Table 2. Following each sprint, HR and RPE were recorded. During the recovery periods, the participants were allowed to get off of the cycle and walk around/stretch, in an attempt to reduce venous pooling in the lower extremities and to minimize feelings of light-headedness or nausea. This

protocol equated to only 150 seconds of actual work, however, it was performed

at double the intensity of the moderate duration intervals.

For all exercise conditions, the total work performed was calculated. Blood was

collected from participants pre-exercise (following a 10 minute quiet rest), immediately

concluding exercise (0 hour), and at 30 minutes and 60 minutes post-exercise.

Participants remained in the laboratory during this period and expended little energy (i.e.,

watched movies, worked on the computer). No meals or calorie containing beverages

were allowed during this period. Water was allowed ad libitum.

Test Meal

As with the exercise sessions, the participants reported to the laboratory in the

morning (0600-0900 hours) following a minimum 10-hour overnight fast. A pre-meal

blood sample was collected following a 10 minute rest period, and participants then

consumed the test meal (maximum ingestion time equal to 10 minutes). The meal

consisted of a lipid, carbohydrate, and protein milkshake, typical of that found in many

commercial establishments. Although it has been noted that meals that are comprised

exclusively of lipid produce a more robust increase in circulating oxidative stress

biomarkers than do mixed meals (42), such a pure fat load may not represent typical

dietary intake. Therefore, for the sake of applicability, we believed that it was more

practical to examine postprandial oxidative stress after consumption of a lipid-rich, but

mixed macronutrient meal. Specifically, the test meal consisted of a milkshake made

with a combination of whole milk, ice cream (Breyers® "all natural" vanilla), and heavy

whipping cream. The size (dietary energy) of the milkshake was determined based on

participants' body mass, and equal to 0.8 grams of fat, 1.0 grams of carbohydrate, and

0.25 grams of protein per kilogram, totaling approximately 12.2 kcal per kilogram of body mass. This is the same amount of lipid used in a prior study of postprandial oxidative stress (23), and less than that used in some of our prior work (9, 13, 15, 16, 19, 24, 71).

Blood was collected from participant's pre-meal, and at 2 and 4 hours post-meal. Participants remained in the laboratory during this period and expended little energy (i.e., watched movies, worked on the computer). No additional meals or calorie containing beverages were allowed during this period. Water was allowed ad libitum.

Blood Sampling and Biochemistry

Venous blood samples (~15mL per draw) were taken from participants' antecubital vein via needle and collection tube, by a trained phlebotomist. Blood samples were collected pre-meal/pre-exercise and at the specified time periods defined above, depending on whether the participant consumed the test meal or undertook an exercise protocol. Blood collected in tubes containing EDTA was centrifuged at 1500 rpm for 15 minutes at 4°C immediately and the plasma was stored in multiple aliquots at -70°C until analyzed. Blood collected in tubes with no additive was allowed to clot for 30 minutes at room temperature and then centrifuged at 1500 rpm for 15 minutes at 4°C. The serum was then stored in multiple aliquots at -70°C until analyzed. Samples were analyzed for plasma MDA, plasma hydrogen peroxide (H_2O_2), serum trolox equivalent antioxidant capacity (TEAC), and serum triglycerides (TAG). Blood lactate was also measured as an indicator of anaerobic metabolism during the exercise bouts. All assays were performed in duplicate upon first thaw.

Malondiadehyde, a major 3-carbon chain aldehyde produced during the decomposition of a lipid hydroperoxide, was measured in plasma using the method described by Jentzsch et al. (58); 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%).

Although technically not a free radical because it has no unpaired electron, H_2O_2 is still considered a marker of oxidative stress because its capacity to cause RONS formation. The danger that H_2O_2 possesses, largely comes from its conversion to the indiscriminately reactive hydroxyl radical ($OH^\bullet$) (51). 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, reacted 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 measured in plasma using the 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%).

Assays for TAG was performed using serum following standard enzymatic procedures as described by the reagent manufacturer (Thermo Electron Clinical Chemistry); (CV = 6.2%).

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 (38).

Dietary Records and Physical Activity

All participants were instructed to maintain their normal diet throughout the study period. Food logs during the day before each test day were maintained by participants, analyzed by research staff, and returned to participants so that they could mimic this intake during all subsequent days preceding 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).

Participants were instructed to maintain their normal physical activity habits. Participants 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 participants did not encounter any undue fatigue by performing strenuous exercise during the hours prior to the actual test days.

Statistical Analysis

For each of the three exercise conditions, the average for the three post-exercise times (0 min, 30 min, 60 min) was computed and used as the "post" measure. For the

meal condition, the average for the two post-meal times (2 hour, 4 hour) was computed and used as the "post" measure. The data were then compared between conditions using a 4 (condition) x 2 (time) analysis of variance (ANOVA). Post hoc comparisons were made using the method of Tukey. Dietary variables and exercise related data were analyzed using an ANOVA. All analyses were performed using JMP statistical software (version 4.0.3, SAS Institute, Cary, NC). Statistical significance was set at $P \leq 0.05$. The data are presented as mean $\pm$ SEM.

RESULTS

All 12 subjects completed all aspects of this study. Subject characteristics are presented in Table 3. The exercise tests were challenging for all subjects, as indicated by the heart rate, RPE, and blood lactate response. This was particularly true for the sprint exercise tests. Exercise test data are presented in Table 4. Dietary data during the 24 hours prior to each test day were not different ($p > 0.05$; Table 5).

No interaction ($p = 0.13$) or time effect ($p = 0.19$) was noted for TAG. A trend was noted for a condition effect ($p = 0.07$), with values highest for the meal condition. Data for TAG are presented in Figure 1.

An interaction was noted for MDA ($p = 0.008$), with values higher post-meal compared to pre-meal ($p < 0.05$). No differences were noted from pre- to post-exercise for any of the exercise conditions ($p > 0.05$). A condition effect was noted ($p = 0.01$), with values higher for the meal condition as compared to the 60 minute aerobic and 15 second sprint conditions ($p < 0.05$). A trend was noted for a time effect ($p = 0.06$). Data for MDA are presented in Figure 2.

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An interaction was noted for H_2O_2 (p<0.0001), with values higher post-meal compared to pre-meal (p<0.05). No differences were noted from pre- to post-exercise for any of the exercise conditions (p>0.05). A condition effect was noted (p<0.0001), with values higher for the meal condition as compared to all exercise conditions (p<0.05). A time effect was also noted (p=0.003), with values higher post-meal/exercise as compared to pre-meal/exercise. Data for H_2O_2 are presented in Figure 3.

No interaction was noted (p=0.72) for TEAC. A condition effect was noted (p=0.04), with values higher for the 60 second sprint and the 15 second sprint as compared to the 60 minute aerobic exercise (p<0.05). A time effect was noted (p=0.05), with values higher post-meal/exercise as compared to pre-meal/exercise. Data for TEAC are presented in Figure 4.

DISCUSSION

This was the first investigation to our knowledge that directly compared the magnitude of oxidative stress between acute feeding and acute exercise. The main findings from this study were that feeding elicits a significantly higher oxidative insult compared to exercise, as measured by MDA and H_2O_2. Secondly, it was illustrated that the approximately two-fold increase in TAG post-meal mimics the greater than two-fold increase in oxidative stress biomarkers post-meal. Thirdly, despite the strenuous nature of the acute exercise bouts (in particular the sprint bouts), exercise does not induce a significant oxidative stress in exercise-trained men. Finally, sprint exercise may be considered a more potent stimulus for an increase in endogenous antioxidant defense, as significantly higher TEAC values were noted for these conditions, as compared to the 60 minute aerobic condition. Of course, a longer time course of study for this and other

antioxidant markers is needed to more fully elucidate the impact of high-intensity sprint exercise on antioxidant defense.

These results supported our main hypothesis, as the meal condition elicited a magnitude of oxidative stress that was far greater than any of the exercise conditions. However, none of the exercise conditions elicited a significant increase in oxidative stress.

The analysis of data for this investigation was slightly different than that which we have previously used in studies of postprandial oxidative stress—using an area under the curve approach (13, 13, 14, 23), calculated using the trapezoidal method (79). However, due to the comparative objective in the present design, as well as the fact that the timing of post-meal and post-exercise blood collection differed (due to our attempt to capture the peak oxidative stress response for both of these challenges), we computed the mean response following both feeding and exercise and used these figures as the "post" values. After carefully reviewing the individual data, we believed that the "averaging" of the time points post-meal and post-exercise and then comparing these values using a 4 (condition) x 2 (time) ANOVA was the best way to represent the magnitude of oxidative stress, in order to objectively compare each condition. It should be noted that before the exercise data was averaged, a 3 (condition) x 4 (time) ANOVA was conducted and no statistically significant findings were noted ($p > 0.05$).

In this investigation, hematocrit and hemoglobin were measured as part of a complete blood count in order for us to correct for plasma volume using the guidelines provided by Dill and Costill (38). This is important because plasma volume shifts may occur during longer duration exercise, as well as during short duration, high-intensity

exercise. In the present investigation, 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 increases were abolished. These findings call into question prior reported results in which investigators noted an exercise-induced increase in oxidative stress but failed to correct for such potential changes in plasma volume.

Although we failed to note any significant increase in exercise-induced oxidative stress in the present study, it should be noted that many other reports exist to refute our findings (62). There are several possible mechanisms by which RONS are increased in response to acute exercise. When oxygen is consumed, the cytochrome oxidase complex housed within the mitochondria of the cell works to reduce the oxygen to water, through a series of one-electron reductions. However, a portion of this oxygen passing through the electron transport chain may give rise to superoxide formation (8). Therefore, when oxygen consumption is increased for ATP generation during aerobic exercise, superoxide is produced at a greater rate and consequently "leaked" from the electron transport chain. Subsequently, this leakage may give rise to other RONS species. In addition to superoxide formation through enhanced oxygen uptake, it has been suggested that oxidative stress specific to anaerobic exercise may be mediated through various other pathways (57); xanthine and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase production, prostanoid metabolism, ischemia/reperfusion, phagocytic respiratory burst activity, disruption of iron-containing proteins, and alteration of calcium

homeostasis. These mechanisms may act synergistically, and it is possible that different types of exercise involve different mechanisms of RONS production.

Our noted lack of increase in the measured oxidative stress biomarkers may be due to our use of exercise-trained men, who may have had increased endogenous antioxidant defense, which allowed them to cope with the increase in RONS imposed by our three bouts of exercise. In much the same way that lactate is produced in response to exercise but is quickly buffered in such a way that "accumulation" is not observed, it is possible that a low grade production of RONS occurred in response to the exercise bouts but was quickly rendered inactive by endogenous antioxidant defense—hence, the presence of increased oxidative stress biomarkers was not seen. Clearly, both the lactate and RPE data illustrate that the bouts of exercise were challenging, and we do not believe that our lack of effect for an increase in MDA or H_2O_2 was due to a low level stressor.

In support of our non-significant oxidative stress results related to the exercise bouts, in healthy subjects and with regards to multiple biomarkers, some investigations have reported non-existent (<10%) (12, 21, 94) and small (10-29%) (35, 90, 96, 100) increases. However, other have noted medium (30-49%) (2, 6, 55, 61, 88, 89) and large (>50%) (11, 20, 22, 37, 43, 45, 47, 49, 64, 95) increases. Of course, many of these investigations may not have accounted for plasma volume changes (often not indicated within manuscripts). Regardless, in the present study it is clear that even strenuous exercise does not result in an oxidative stress in exercise-trained men.

A review of the postprandial oxidative stress literature clearly illustrates an elevation in RONS and associated biomarkers as a result of acute feeding. However these investigations, especially those pertaining to healthy subjects, have illustrated the

percent rise in oxidative stress to be variable, with some studies demonstrating the change to be relatively non-existent (<10%) (5), small (10-29%) (84, 87), medium (30-49%) (4, 73), and large (>50%) (7, 13, 14, 16, 18, 24, 34, 70). In the present study, we noted a greater than 50% rise in both MDA and H_2O_2 as a result of feeding, and these paralleled the almost 50% rise in TAG post-feeding. As a result, this bolus of substrate overwhelmed the endogenous antioxidant defenses of the exercise-trained men.

Therefore, our data clearly indicate that despite the protection afforded by the antioxidant defense system following a bout of strenuous exercise in which RONS may or may not be produced, such antioxidant defense is inadequate to handle the abundance in RONS produced in response to a high-fat feeding. Due to the complexity of the cellular signaling cascade, the exact mechanisms involved in the postprandial production of RONS have yet to be fully elucidated. However, it is believed that substrates such as saturated fats and simple carbohydrates cause exaggerated supraphysiological surges in blood glucose and lipids (41). Subsequently, the metabolic capabilities of the mitochondria within overnourished muscle and adipose tissue are then overwhelmed with glucose and free fatty acids. The Krebs cycle attempts to compensate for this disruption in homeostasis by stimulating excess production of the hydrogen carrier molecules, nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD), which outpaces the capacity for oxidative phosphorylation. However, this in turn creates a buildup of NADH and FADH within the electron transport chain and increases the mitochondrial proton gradient which drives the transfer of single electrons to oxygen, thereby creating RONS such as superoxide anion, at an accelerated rate (25, 63, 75). This ensuing postprandial oxidative stress likely triggers a harmful biochemical cascade

throughout the circulation, including inflammation, endothelial dysfunction, hypercoaguability, and sympathetic hyperactivity, all of which may promote further RONS generation and oxidative damage (41). Moreover, the severity of the oxidative load imposed appears contingent upon the magnitude and rate of glycemia and hypertriglyceridemia experienced post-feeding (76).

Although beyond the scope of the present data set, based on our findings of increased oxidative stress following the high-fat meal, antioxidant supplementation would perhaps be most beneficial if taken concomitantly with feeding, as opposed to with exercise—as is traditionally done. This is especially pertinent given the recent investigations by Gomez-Cabrera (46) and Ristow (83) who have suggested that antioxidant supplementation taken in conjunction with exercise training, may in fact blunt the endogenous production of certain antioxidant enzymes, in accordance with the law of hormesis (60, 81).

The implications of repeated exposure of this exacerbated oxidative stress following feeding have lead some to speculate that postprandial oxidative stress is a common environmental antecedent that underlies the development of chronic ailments, such cardiovascular disease and diabetes (i.e., the *"common soil hypothesis"* (28, 41)). Therefore, individuals who frequently consume high-fat, high-calorie meals (which are well-documented to cause a significant and prolonged oxidative stress) may be at increased risk for developing oxidative stress related disease. On the other hand, repeated exposure to exercise-induced oxidative stress, which is generally mild and transient, is believed to be the stimulus for upregulation of antioxidant defenses (59), potentially being associated with improved health. Clearly, the magnitude and duration

of RONS production and subsequent oxidative stress is important when considering health and disease.

Considering our findings, some limitations to our investigation exist. For example, we only investigated a specific subset of the population, i.e., exercise-trained men. It is possible that we would have noted different results had we undertaken the same protocol with women, because of the potential protection afforded by endogenous estradiol (14, 62, 97). Individuals who are obese or have metabolic or cardiovascular disease likely would have elicited slightly different results due to the fact that they typically experience more robust and prolonged periods of hyperglycemia (73, 87, 91) and hypertriglyceridemia (29, 74) post-feeding. Finally, the inclusion of a population of untrained individuals, those who were older, or those who routinely consume high fat meals (in which case they may have developed a tolerance to such feeding), may have yielded differing results.

Our findings only relate to exercise performed on a cycle ergometer. There are limitless manipulations that we could have considered in order to increase or decrease the oxidative insult, the most commonly seen being intensity (80), duration (11), and type of exercise (22). We designed, pilot-tested, and implemented the exercise protocols with the understanding that we would include a continuum of exercise intensity, similar to that used by exercise-trained individuals. It is possible that our use of different forms (e.g., running) and types (e.g., resistance exercise) of exercise may have yielded different results.

Finally, our failure to include other biomarkers of oxidative stress, in addition to more frequent sampling of blood, may be considered a limitation of this work. These

data are only pertinent for the blood biomarkers investigated in this study, and for the associated time course of sampling. Our results do not exclude the possibility of elevated oxidative stress in other body fluids (e.g., urine, saliva), as well as muscle and organ tissue at times included in the present investigation, as well as at times other than those measured. If additional biomarkers had been included we could have provided a more comprehensive assessment of the overall oxidative stress response to both feeding and exercise. The inclusion of DNA (8-hydroxydeoxyguanosine; 8-OHdG) and protein (protein carbonyls) markers may have strengthened our design.

In summary, acute strenuous exercise in exercise-trained men does not induce an oxidative stress. On the other hand, acute high-fat feeding does indeed induce an oxidative stress, evidenced by an approximate two-fold increase from baseline during the post-feeding period. Therefore, we can conclude that the magnitude of oxidative stress following an acute mixed macronutrient meal is significantly greater than the magnitude of oxidative stress elicited after an acute bout of exercise, in a sample of young, exercise-trained men. These findings allow us to postulate that antioxidant supplementation, if considered for use, may be prescribed as a prophylactic against postprandial oxidative stress, rather than against exercise-induced oxidative stress. Additional work is needed to support this hypothesis.

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APPENDIX A – TABLES & FIGURES

Table 1. Time Schedule for 60 Second Sprint Protocol

Start Time	Sprint 1	Rest	Sprint 2	Rest	Sprint 3	Rest	Sprint 4	Rest	Sprint 5
Sec	0	60	285	345	570	630	855	915	1140
Min	0:00	1:00	4:45	5:45	9:30	10:30	14:15	15:15	19:00

Table 2. Time Schedule for 15 Second Sprint Protocol

Start	Sprint 1	Rest	Sprint 2	Rest	Sprint 3	Rest	Sprint 4	Rest	Sprint 5	Rest	Sprint 6	Rest	Sprint 7	Rest	Sprint 8	Rest	Sprint 9	Rest	Sprint 10
Time	1		2		3		4		5		6		7		8		9		10
Sec	0	15	132	147	264	279	396	411	528	543	659	674	790	805	921	935	1052	1067	1185
Min	0:00	0:15	2:12	2:27	4:24	4:39	6:36	6:51	8:48	9:03	11:00	11:15	13:12	13:27	15:24	15:39	17:36	17:51	19:45

Table 3. 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·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·week^{-1})	4.0±0.6
Aerobic Exercise (years)	2.8±0.6
Aerobic Exercise (hours·week^{-1})	2.6±0.7
VO_{2max} (mL·kg^{-1}·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 4. Heart rate, perceived exertion, and blood lactate data of 12 exercise-trained men related to a rest (meal) or exercise condition

Variable*	Rest (Meal)	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
Blood Lactate (mmol·L^{-1})	0.9±0.3	2.4±0.4	11.1±0.7	11.2±0.8

Values are mean±SEM.

*Values for heart rate and perceived exertion 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; Values for blood lactate are those obtained immediately post-exercise (values for the Rest condition were matched to the exercise conditions for time of collection).

Table 5. Dietary intake of 12 exercise-trained men during the 24 hours prior to a rest (meal) or exercise condition

Variable	Rest (Meal)	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 triglyceride data of 12 exercise-trained men before and after consumption of a high-fat meal or the performance of an exercise test

Values are mean±SEM.

No interaction (p=0.13) or time effect (p=0.19).
Trend for condition effect (p=0.07).

Note: For the meal condition, post values are the average of values obtained at 2 and 4 hours post-meal ingestion; for the exercise conditions, post values are the average of values obtained at 0, 30, and 60 minutes after the completion of the exercise tests.
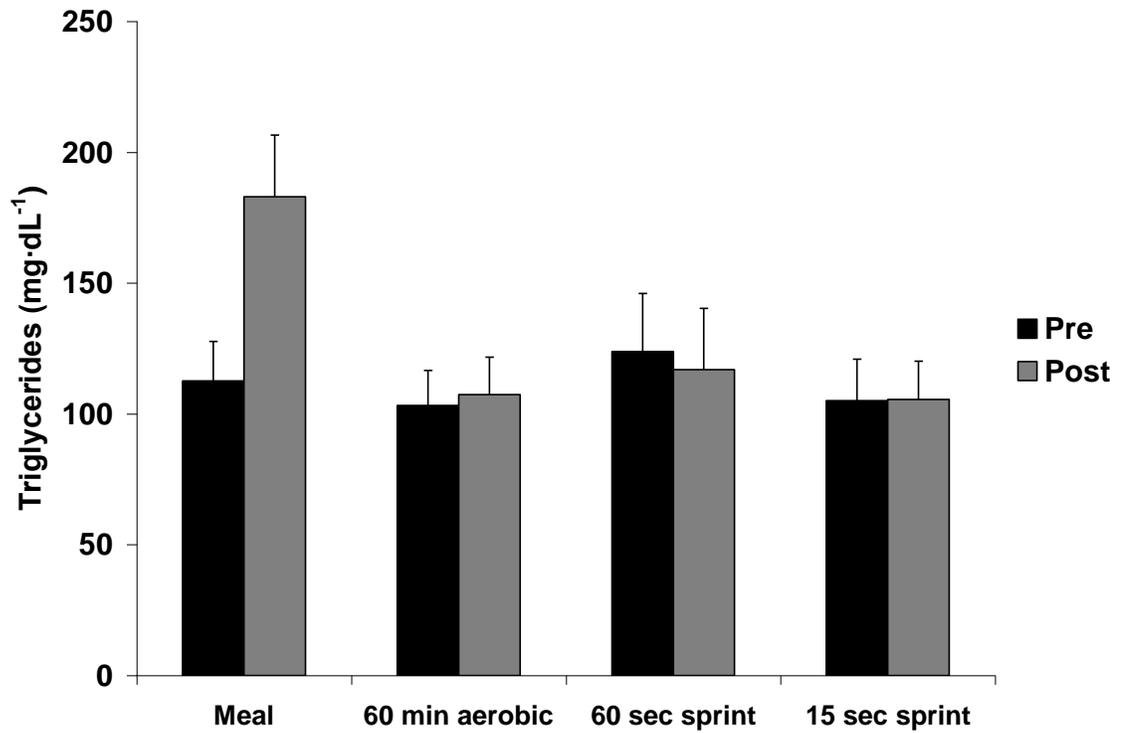
Figure 2. Blood malondialdehyde data of 12 exercise-trained men before and after consumption of a high-fat meal or the performance of an exercise test

Values are mean±SEM.

**Interaction (p=0.008); values higher post-meal compared to pre-meal (p<0.05).
*Condition effect (p=0.01); values higher for the meal condition as compared to the 60 min aerobic and 15 sec sprint conditions (p<0.05).
Trend for time effect (p=0.06).

Note: For the meal condition, post values are the average of values obtained at 2 and 4 hours post-meal ingestion; for the exercise conditions, post values are the average of values obtained at 0, 30, and 60 minutes after the completion of the exercise tests.
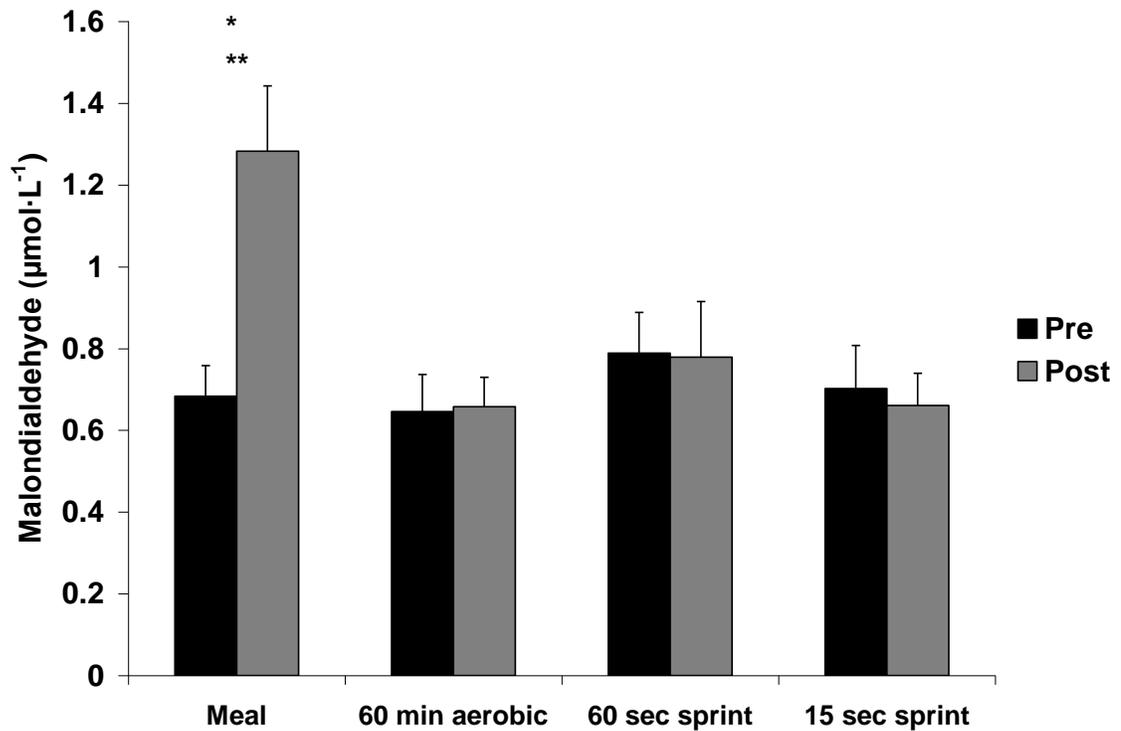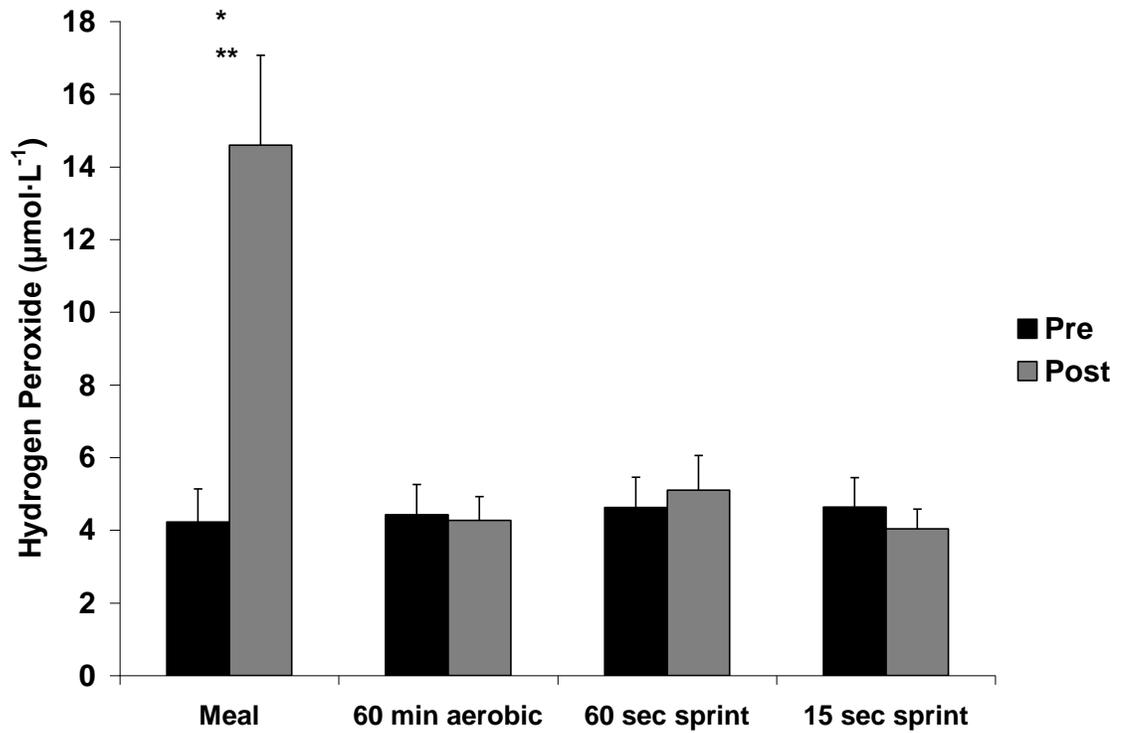
Figure 3. Blood hydrogen peroxide data of 12 exercise-trained men before and after consumption of a high-fat meal or the performance of an exercise test

Values are mean±SEM.

**Interaction ($p<0.0001$); values higher post-meal compared to pre-meal ($p<0.05$).
*Condition effect ($p<0.0001$); values higher for the meal condition as compared to all exercise conditions ($p<0.05$).
Time effect ($p=0.003$); values higher post-meal/exercise as compared to pre-meal/exercise.

Note: For the meal condition, post values are the average of values obtained at 2 and 4 hours post-meal ingestion; for the exercise conditions, post values are the average of values obtained at 0, 30, and 60 minutes after the completion of the exercise tests.
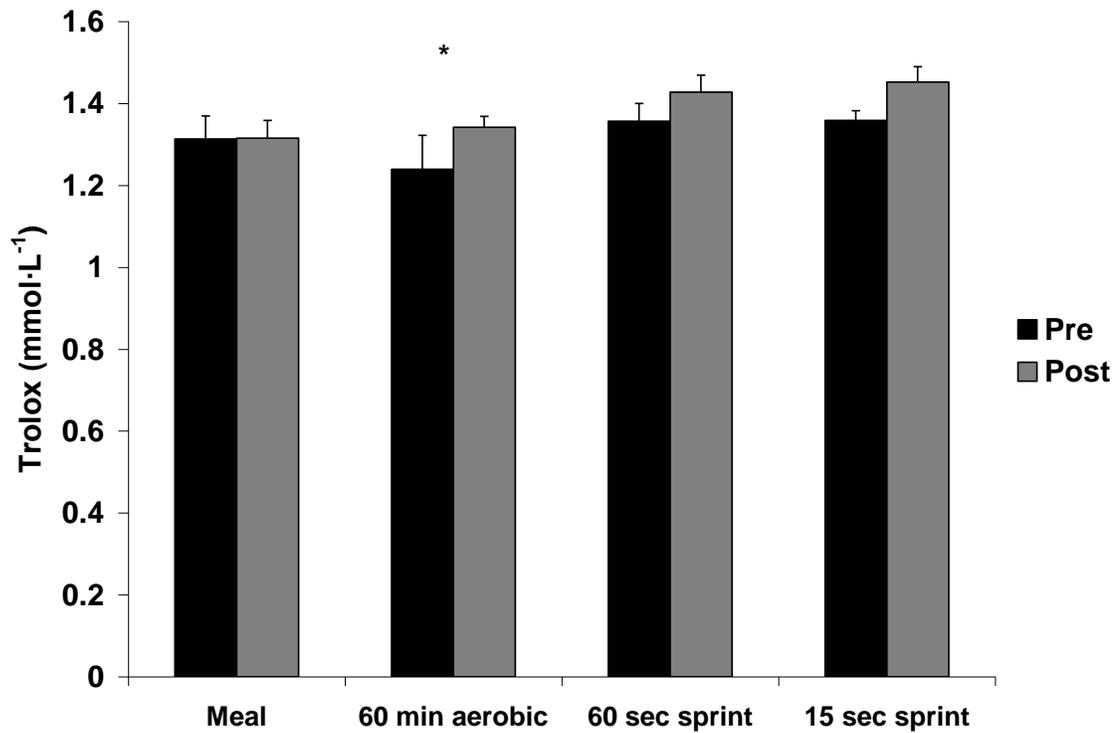
Figure 4. Blood Trolox Equivalent Antioxidant Capacity data of 12 exercise-trained men before and after consumption of a high-fat meal or the performance of an exercise test

Values are mean±SEM.

No Interaction (p=0.72).
*Condition effect (p=0.04); values higher for 60 sec sprint and 15 sec sprint as compared to 60 min aerobic (p<0.05).
Time effect (p=0.05); values higher post-meal/exercise as compared to pre-meal/exercise.

Note: For the meal condition, post values are the average of values obtained at 2 and 4 hours post-meal ingestion; for the exercise conditions, post values are the average of values obtained at 0, 30, and 60 minutes after the completion of the exercise tests.

APPENDIX B – EXTENDED LITERATURE REVIEW

Introduction

Oxidative stress and the increased production of reactive oxygen and nitrogen species (RONS) is an underlying feature of many acute and chronic diseases that can be induced by a number of physiological and environmental stressors. Aside from activities such as cigarette smoking, two of the more widespread stressors in Western society are acute strenuous exercise and the ingestion of excess nutrients (in particular, large amounts of saturated fat). Although *postprandial* oxidative stress has been suggested to be the unifying mechanism in the connection between pathological abnormalities such as insulin resistance, Type II diabetes, endothelial dysfunction, atherosclerosis, and cardiovascular disease, it remains unclear whether exercise-induced oxidative modifications have little significance, induce harmful oxidative damage, or are an integral part of redox regulation. The following text is intended to provide an overview of literature pertaining to the role of RONS production following exercise and feeding, and the subsequent effects.

What is Oxidative Stress?

   i.   **Reactive Oxygen and Nitrogen Species and Oxidative Stress**

Reactive oxygen and nitrogen species (RONS) are very small, short-lived molecules which are highly reactive due to their unpaired valence shell electron (78). As RONS seek to accept electrons, they often react with other molecules promoting either positive or negative effects. While RONS generation occurs in part as a consequence of normal cellular metabolism, they are also generated through exposure to a wide variety of stressors such as exposure to environmental pollutants (80), cigarette smoke (1), excess nutrient intake (154), and physical exercise (62).

The formation of RONS and the subsequent amelioration via the antioxidant defense system is delicately balanced and continual process *in vivo* that serves several key roles in human physiology (61). Under optimal conditions, RONS regulate vital processes such as cellular signaling, immune function, apoptosis, and gene transcription (165). However, as stated above, in response to a variety of stressors, RONS production increases. When in conjunction with impaired antioxidant defense, a state of oxidative stress may occur, which can ultimately lead to oxidative damage to cellular components, such as lipids, proteins, and nucleic acids. However, under ordinary physiological conditions, the body's endogenous antioxidant defense system, in combination with exogenous antioxidants consumed through dietary sources, acts to protect macromolecules from modification and destruction via RONS.

ii. Associations with Health, Disease, and Aging

RONS are not inherently harmful; however, during and following stressful conditions in which RONS production is increased, adequate protection may not be available, and the capacity of the antioxidant defense system may be overwhelmed by the oxidant attack. Such conditions have been implicated in the pathophysiology of multiple acute and chronic human diseases, as well as in the aging process (165).

While numerous studies have found that an association exists between elevated oxidative stress and disease (as reviewed previously (49)), specific cause-and-effect data are more scarce. Regardless, this area of study continues to be an important focal point regarding health-related research (15). This is because multiple diseases appear to have strong correlations to increased concentrations of RONS in both tissue and blood. Nonetheless, it is understood that the complexity of most disease processes certainly does

not exclude the possibility that factors outside of RONS also play a key role, as most pathologies are multi-factorial. Additional study is needed before firm conclusions can be made pertaining to whether RONS are a major cause of disease or merely a consequence of the disease process.

With this understanding, at least some direct evidence exists linking oxidative stress to both the initiation and progression of cardiovascular disease (122), diabetes (41), atherogenesis (166), cancer (172), and neurogenerative disorders (13). Moreover, oxidative stress appears to expedite the aging process (65). The precise cellular damage resulting from RONS is specifically related to which macromolecules are being targeted by the oxidants, the frequency and duration of the attack, as well as the tissue-specific antioxidant defenses present. This can potentially lead to mutagenic adaptations, perhaps promoting ill-health (49), physiological dysfunction (165), and impairing physical performance (126).

iii.   Gender Differences in Oxidative Stress

Collectively, both men and women are susceptible to oxidative stress. Women however, possess a larger concentration of estrogen and other hormones compared to men, and consequently, they may be less susceptible to oxidative damage (25, 93, 162). Moreover, estrogen is known to possess antilipidemic properties (7, 141), allowing for a greater uptake of triglycerides into tissue from circulation, which appears important in terms of attenuating postprandial oxidative stress (8, 24, 25), in addition to playing a role as an anti-inflammatory agent (175). Vina et al. (169) hypothesizes that the increased longevity experienced by females compared to males in many species, including humans, has been traced to the beneficial action of estrogens. Estrogen, 17β-estrodiol in

particular, binds to estrogen receptors and increases the expression of longevity-associated genes, including those encoding the antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx). As a result, mitochondria from females produce fewer RONS than those from males.

iv.   Methods of Assessing RONS Formation

In a comprehensive analysis of the effects of exercise on oxidative stress biomarkers, Bloomer summarizes the methods for assessing RONS formation (15). Since RONS are highly reactive and short lived (e.g., 10^{-6}, 10^{-5}, 10^{-9} seconds for singlet oxygen, superoxide radical and hydroxyl radical, respectively) they are extremely difficult to measure in biological systems, in particular, plasma and other body fluids. Nonetheless, there do exist direct procedures for measuring RONS activity, the most common being electron spin resonance (ESR) spectroscopy involving spin traps (which allows for a more stable product), in addition to less common techniques such as pulse radiolysis and laser flash photolysis. The equipment needed for analysis of samples using these techniques is costly, and the procedures are complex and labor intensive, making the analysis of large batches of samples difficult.

The majority of investigations focused on exercise-induced and postprandial oxidative stress have used indirect methods as a way to determine changes in tissue oxidation of lipids, proteins, and DNA resulting from exposure to RONS. In addition, alterations in components of endogenous antioxidant defense system, in particular glutathione status (e.g., increased oxidized and decreased reduced glutathione) and water- and lipid-soluble vitamins, have been used as markers of oxidative stress. Using this

approach, RONS formation is inferred based on the nature of oxidation caused to biological molecules, as well as the decrease in antioxidant capacity.

A variety of analysis procedures have been used (137) ranging from simple spectrophotometric assays, to more complex and time-consuming assays using gas chromatography-mass spectroscopy (GC-MS) and high performance liquid chromatography (HPLC) coupled with electrochemical or chemiluminescence detection. Procedures are available for analysis of several body fluids (e.g., blood, urine, salvia), as well as muscle and organ tissue (15).

Common biomarkers as indices of oxidative damage include malondialdehyde (MDA) and F_2-isoprostanes for lipids. In addition, thiobarbituric acid reactive substances (TBARS) is frequently used as a biomarker of lipid peroxidation, especially in the exercise-induced oxidative stress literature (48, 76, 151, 156). However, the efficacy of TBARS has been brought into question as it has been criticized due to reliability problems (88). Carbonyl derivatives for proteins and 8-hydroxydeoxyguanosine (8-OHdG) for DNA, as well as antioxidant vitamins (ascorbate and α-tocopherol) and enzymes, such as catalase (CAT), SOD and various peroxidases are also used. Moreover, total antioxidant status can be measured using more global assays such as Trolox Equivalent Antioxidant Capacity (TEAC), Oxygen Radical Absorbance Capacity (ORAC), Ferric Reducing Ability of Plasma (FRAP), and Total Radical-Trapping Antioxidant Parameter (TRAP). Finally, another common marker of oxidative stress is hydrogen peroxide (H_2O_2), despite technically not being a radical specie because it has no unpaired valence electron. Hydrogen peroxide is still considered a marker of oxidative stress because of its capacity to lead to RONS formation. The danger of H_2O_2 largely

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comes from its conversion to the reactive hydroxyl radical (OH$^\bullet$), either by exposure to ultraviolet light or by interaction with a range of transition metal ions, of which the most important *in vivo* is probably iron via the Fenton reaction (79).

In relation to assessing and evaluating biological systems in terms of RONS, it should be remembered that any particular assay procedure is merely capturing a "snapshot" of what is occurring at that particular time. That is, it is quite possible that when taking a single sample, the generation of RONS and associated macromolecules could be missed, either by taking the sample too late or by not waiting long enough for secondary generation of RONS and associated oxidation. Therefore, it is best to take repeated samples following a stressor, in order to best represent the oxidative status of the system.

v. Protective Mechanisms Against RONS

While RONS are constantly generated in cells and increase with environmental and physiological challenges, their production does not necessarily lead to cellular modification and degradation. This is because there exist numerous defenses either to minimize RONS formation or to neutralize their damaging effects once formed. These may be broadly categorized as endogenous antioxidants (those naturally produced within human body) and exogenous antioxidants (those obtained from dietary intake).

The ability of exogenous antioxidants to attenuate oxidative stress has been met with equivocal results. In the exercise setting, some reports suggest a potential beneficial role of exogenous antioxidant supplementation (33, 38, 112, 116), while others indicate no benefit (17, 22, 91, 161). The results supporting antioxidant supplementation in relation to feeding has been more successful in attenuating oxidative stress than was

illustrated in the exercise setting. Antioxidant supplementation (30, 103, 118, 119, 125) and dietary modification (to include a more antioxidant-rich diet) (173) have been shown to consistently assuage the rise in oxidative stress biomarkers and atherogenic indicators acutely. Although caution has to be observed when interpreting these results because of the pre-diabetic (30) or diabetic (103, 118, 119) populations that were primarily recruited (i.e., these populations are known to experience an exacerbated oxidative stress response post-feeding, therefore making the purported benefits of antioxidant supplementation that much more exaggerated), the efficacious nature the antioxidant modifications was also demonstrated to occur in healthy controls (118, 119).

In addition to the specific population being studied, discrepancies in findings may be due to the type (46), dosage (139), and timing (40) of administration of the antioxidants, in addition to the stress. Furthermore, exogenous antioxidants have only been found to reduce values of macromolecule peroxidation rather than eliminate it (33). Some well-described health benefits of regular exercise stem from the upregulation of endogenous antioxidant defense, likely coupled with decreased RONS formation. For adaptations to occur, exercise needs to be of sufficient volume and intensity to cause acute elevations in RONS production. In other words, a "sublethal" amount of oxidative stress is needed for such positive adaptations (15). Seemingly, the adaptations result from the cumulative effect of repeated exercise bouts and exposure to RONS. As with exogenous antioxidant supplementation, it should be mentioned that although antioxidant defenses may be increased as a result of chronic exercise training, oxidative stress following acute bouts of submaximal exercise and postprandial feeding is typically not eliminated, but rather reduced. It should be noted that individuals who regularly exercise,

likely have heightened endogenous antioxidant defense (28). Because of such adaptations these individuals may not benefit greatly from exogenous antioxidant intake for purposes of attenuating signs and symptoms of cellular and muscle damage.

The supposed benefit of antioxidant supplementation to attenuate exercise-induced oxidative stress has been brought into question in recent investigations. This is related to the principle of hormesis, which states that in response to repeated exposure to various toxins and/or stressors, the body undergoes favorable adaptations that result in enhanced physiological performance and improved physical health (89, 129). 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 certain antioxidant enzymes (71). Therefore, it has been suggested, based on data pertaining to vitamin C supplementation in conjunction with exercise training, that an attempt to minimize the post-exercise increase in RONS production (via exogenous antioxidant supplementation) may actually blunt the adaptive increase in endogenous antioxidant defenses. This may increase an individual's susceptibility to prooxidant attack both at rest, as well as following subsequent exercise bouts (72, 138). More recent evidence refutes these data, indicating that antioxidant supplementation in the form of vitamins C and E does not impair the typically observed improvement in antioxidant activity as an adaptation to regular exercise (176). Clearly, more investigation is needed to determine the potential benefit and possible harm of routine antioxidant supplementation.

Exercise-Induced Oxidative Stress

    i.   **Association with Physical Performance**

Although RONS are essential for normal physiological operations, their reactivity make it relatively easy for the intracellular redox status to slip into a state less compatible with optimal functioning. Thus, it is possible that RONS-mediated oxidative damage to cellular constituents can indeed impact muscle performance in a negative way. This is because physical exercise is associated with increased adenosine triphosphate (ATP) demand and an enhanced aerobic and anaerobic metabolism, which results in an increased formation of RONS (132).

Little direct evidence is available in humans to confirm the negative impact of RONS on exercise performance (15). Alternatively, animal studies have illustrated altered contractile function, reductions in muscle force output, and greater fatigue rates as a result of increased oxidative stress in isolated skeletal muscle (134). Specifically, RONS mediated impairment can diminish performance by oxidizing both contractile and enzymatic proteins (81) and thus interfering with excitation-contraction coupling (70), impairing mitochondrial enzymes required for energy production (e.g., succinate dehydrogenase, cytochrome oxidase) (81), and affecting calcium reuptake by the sarcoplasmic recticulum through RONS mediated damage to ATP pumps, leading to an imbalance in calcium homeostasis (147) and reduced muscle contractility. Moreover, oxidation of ryanodine receptors regulating calcium release channels in the sarcoplasmic recticulum leads to excess calcium availability and promotes abnormal muscle contractures (144).

There are several possible mechanisms by which RONS are increased in response to acute exercise. Under normal physiological conditions, most of the oxygen consumed by cells is reduced to water in the mitochondrial electron transport chain through a series of one-electron reductions via the action of the cytochrome oxidase complex. However, a small amount (1-5%) of the oxygen passing through the electron transport chain may give rise to superoxide (15). Therefore, production of RONS during and following aerobic exercise is logically associated with the increased oxygen uptake needed for greater ATP production. As a result, superoxide is produced at a greater rate and consequently "leaked" from the electron transport chain. This may give rise to other nonradical (e.g., H_2O_2) and radical species (e.g., $OH^{\bullet}$).

While RONS generation aerobically is fairly well-accepted, information pertaining to the production of RONS as a result of acute anaerobic exercise is lacking (32). In addition to superoxide leakage through enhanced oxygen uptake aerobically, it has been suggested that oxidative stress specific to anaerobic exercise may be mediated through various other pathways (87); xanthine and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase production, prostanoid metabolism, ischemia/reperfusion, phagocytic respiratory burst activity, disruption of iron-containing proteins, and alteration of calcium homeostasis. These mechanisms may act synergistically, and it is possible that different types of exercise involve different mechanisms of RONS production.

Contrary to a sometimes negative view associated with RONS, as mentioned above, repeated exposure of the system to increased RONS production from chronic exercise could potentially lead to upregulation in the body's antioxidant defense system. This is associated with a shift in redox balance in favor of a more reducing environment,

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thus providing adaptive possible protection from RONS during subsequent exercise sessions, as well as when exposed to non-exercise related conditions.

ii. Aerobic Exercise

While participating in regular aerobic exercise has been shown to be beneficial at reducing both morbidity and mortality rates, it is also known that aerobic exercise of sufficient intensity (128) and duration (20) can impose a state of oxidative stress through superoxide leakage from the electron transport chain, as described above.

iii. Steady-state/continuous

RONS generation following steady-state aerobic exercise has been comprehensively studied since the first investigation by Dillard et al. (56) showed that lipid peroxidation was increased following 60 minutes of cycling exercise. Specifically, it has been shown, that steady-state aerobic exercise performed at a moderate intensity (e.g., $\leq 70\%$ VO_{2max}) of sufficient duration (e.g., 30-60 minutes), can lead to an oxidative stress response in animals (3), as well as in humans (2, 20, 33, 34, 50, 66, 67, 69, 73, 86, 90, 91, 96, 123, 150-152, 158, 160, 167). However, only a limited number of studies have included a time course analysis, illustrating the transient nature of oxidative stress, as a result of steady-state aerobic exercise. The transient nature of oxidative stress means that most biomarkers peak shortly after the exercise bout and subsequently return toward basal levels thereafter (20, 33, 34, 66, 86, 128, 151, 167). This short-term elevation in selected oxidative stress biomarkers may be due to the relatively light physical burden (particularly in studies involving exercise-trained subjects), the increased catabolism, excretion, or redistribution of oxidized molecules throughout the body, or finally because

of the acute activation of redox reactions, i.e., the conversion of oxidized molecules to their reduced form (15).

iv. Incremental/graded

Not surprisingly, incremental exercise, which is primarily continuous in nature but involves progressively increasing intensity until volitional exhaustion, is associated with marked increases oxidative stress (4, 19, 66, 97, 101, 113, 128, 156, 158). As with steady-state moderate intensity and duration exercise, many studies have noted a transient elevation in oxidative stress biomarkers (4, 66, 97, 101, 113, 128, 151, 156), although some biomarkers may remain elevated for several hours post exercise (113).

Such graded exercise is exhausting to individuals and typically leads to a significant increase in anaerobic metabolism and lactate production. As exercise intensity increases beyond the lactate threshold, cytosolic concentrations of NADH (43) and/or NADPH are compromised . This decrease in NADH not only influences lactate accumulation in the working muscle (through NAD being unable to keep up with shuttling H^+ to mitochondrial electron transport chain and pyruvate accepting the subsequent H^+), but NADPH is also regarded as an indirect antioxidant, that works in conjunction with more traditional antioxidant enzymes (94). Consequently, as substrate depletion occurs due to increasing exercise intensity, and the pool of NADH and NADPH diminishes, peroxidation and oxidative stress have been shown to increase with lactate, in an intensity-dependent manner (101). Specifically, Lovin et al. (101) demonstrated that as the intensity of exercise increased up to 70% VO_{2max} and blood lactate accumulated, there was a significant increase in lipid peroxidation. The concomitant increase in lactate and lipid peroxidation elicited a Pearson product-moment correlation coefficient (r) =

0.51. Furthermore, Lovin et al. (101) postulated based off the findings of McLellan and

Skinner (110), that the reason why there was a drop in plasma MDA at 40% VO_{2max}

compared to pre-exercise was because of an enhanced lactate uptake by the working

muscles during low-intensity submaximal exercise. This consequently suggests that

during such submaximal efforts, capillaries may open, enhancing blood flow and lactate

uptake. With lactate utilization at submaximal efforts, the generation of cytosolic

NADH/NADPH would increase and the activity of the antioxidant enzymes is likely

enhanced, thus more effectively handling the RONS which are generated. Similar

findings were noted by Leaf et al. (97) with lipid peroxidation (serum MDA, and expired

ethane and pentane) increasing as subjects completed the modified Bruce protocol until

exhaustion. Lipid peroxidation was greater at lactate threshold (respiratory quotient >

1.0) and at VO_{2max} compared to pre-exercise. This study also illustrated the transient

nature of oxidative stress, as lipid peroxidation values were declining by five minutes

post-exercise.

Despite the exhaustive intensity of graded/incremental exercise, it has been found

that the duration of exercise still does play a role. Revan et al. (135) found with

untrained men, undertaking a modified Bruce Test Protocol (exercise slope and speed

were determined according to the maximum they could attain in regular Bruce Test

Protocol), that lipid hydroperoxides (LOOH) did not change, GPx decreased minimally

and catalase (CAT) increased non-significantly post-exercise. Based on these findings, it

may be concluded that a short duration exhaustive exercise test (03:50 +/- 00:06 min)

fails to induce an oxidative stress, while many prior studies involving exhaustive exercise

for longer durations have noted increases in oxidative stress biomarkers (4, 19, 66, 97, 101, 113, 128, 156, 158).

v. Anaerobic Exercise

Oxidative stress and subsequent damage to cellular proteins, lipids, and nucleic acids, as well as changes to the glutathione system, are well-documented in response to aerobic exercise (32). However, in addition to RONS production related to superoxide leakage from the electron transport chain, as may be the case for aerobic exercise bouts, anaerobic exercise may elicit oxidative stress through alternative mechanisms (87). As mentioned above, these other pathways may include xanthine and NADPH oxidase production, prostanoid metabolism, ischemia/reperfusion, phagocytic respiratory burst activity, disruption of iron-containing proteins, and alterations of calcium homeostasis (32). The extent of RONS generation arising from these sources may vary depending on the type of anaerobic stress, the most common being resistance (3, 4, 6, 11, 21-23, 31, 34, 39, 45, 52-54, 57, 68, 83, 84, 92, 98-100, 104, 107, 120, 124, 131, 133, 140, 142, 143, 146, 155, 156, 168, 174) and sprint (12, 21, 31, 48, 75, 76, 82, 85, 102, 130, 148, 159, 161) exercise. Additionally, it is likely that production of RONS during and after anaerobic exercise involves several pathways, which collectively lead to their presence in biological samples analyzed.

vi. Single Bout Anaerobic Exercise

Single bouts of exercise provide an adaptation, albeit to a limited extent, that reduces oxidative stress. During acute exercise, the antioxidant enzyme activity and protein content might be increased, but the levels of antioxidant vitamins are decreased

and thus oxidative damage is often observed. This acute damage seems to be necessary in order to cause adaptation to regular exercise longitudinally (132).

A single-sprint, 30-second Wingate protocol was used by Groussard et al. (76) to induce an acute state oxidative stress, evidenced by the direct detection of serum lipid radical production and by changes in the erythrocyte antioxidant system. Furthermore, the transient nature of oxidative stress was demonstrated to be evident in this type of anaerobic exercise, with radical levels returning to approximately pre-exercise levels by 40 minutes post-exercise. Paradoxically however, MDA levels, detected by the TBARS method, demonstrated a decrease which was strongly correlated with the peak power developed during the Wingate test. Groussard et al. (75) further demonstrated the detrimental effect of a Wingate sprint with acute decreases in plasma alpha-tocopherol (vitamin E) and beta-carotene, while Cuevas et al. (48) found that a Wingate test influenced blood glutathione status negatively. Bloomer et al. (31) indicated that a single bout of strenuous squatting and sprinting performed by resistance trained men results in elevated protein carbonyls (PC), while having little impact on 8-OHdG or MDA during the immediate post-exercise period. A limitation to this study could be the fact that there was no time course analysis of biomarkers post-exercise. Therefore, it is plausible that MDA and/or 8-OHdG could have been elevated at times beyond the immediate post-exercise period. Baker et al. (12) elicited a marked increase oxidative stress and loss of muscle integrity with 30 seconds of high-intensity cycling, at two different resistive force protocols–total-body mass or fat-free mass. Concentrations of LOOH, MDA, creatine kinase, and myoglobin measured immediately post-exercise were significantly greater compared to pre-exercise in both protocols. However, the total-body protocol was

illustrated to be more detrimental than the fat-free mass protocol despite the fat-free mass protocol recording a greater peak power output. Finally, Inal et al. (85) noted a decrease in reduced glutathione (GSH) in performance swimmers aged 15 to 21 years, following a single 100m swim sprint.

vii. Intermittent/interval

Anaerobic exercise, particularly *repeated* interval sprint exercise, is commonly used by athletes and other individuals who regularly train in an attempt to improve their metabolic conditioning, as well as their speed, agility, and sport-specific performance. Although this form of exercise is useful for these goals, it often results in an acute state of oxidative stress, which may be exaggerated in individuals who are untrained (28). The type of exercise that is typically used in intermittent sprint studies usually exhausts individuals anaerobically, and requires supraphysiological levels of oxygen consumption during the recovery periods. Consequently, a maximal rate of energy production via dephosphorylation of ATP and creatine phosphate (CP), as well as glycolytic sources, is observed. Conversely, the oxidative energy system is mainly required in the recovery phase between sprints. The high rate of energy turnover that is required in the active muscle during intermittent sprint exercise may induce a marked increase in oxidative stress based on the intensity of the exercise and the synergist (aerobic and anaerobic) mechanisms involved in RONS generation. Despite aerobic exercise being used as the modality, the significance of intensity on the oxidative stress was illustrated by Qunidry et al. (128). Specifically, this investigation found that a graded exercise bout until exhaustion elicited a more dramatic neutrophil demargination and blood oxidative stress

response, when compared with the other submaximal-intensity exercise sessions, including a submaximal bout set to precise exercise energy expenditure.

Specifically pertaining to sprint exercise, Thompson et al. (161) found that 90 minutes of intermittent shuttle-running, involving walking, jogging, and running, resulted in an increase in MDA in trained athletes. Marzatico et al. (102) investigated sprint athletes following the performance of six sprints (150m) on a track (weight bearing as opposed to non-weight bearing cycle ergometer sprints) and noted elevated MDA at six to 48 hours post-exercise and plasma conjugated dienes at six hours post-exercise. Finally, Schiffl et al. (148) illustrated DNA oxidation resulting from sprint exercise, performed by six healthy volunteers.

Contrary to the results from the sprint studies cited above (single set and intermittent), Groussard et al. (76) elicited paradoxical results, at least with regards to MDA measured by TBARS and ESR. Similarly, Bloomer et al. (31) found that 8-OHdG and MDA were not significantly elevated in a single set of strenuous cycle sprinting, despite eliciting significant results with PC. Finally, following strenuous, repeated bouts of intermittent cycling (and squatting), Bloomer et al. (21) did not note an oxidative stress or muscle injury response in aerobically trained men. Explanations for these conflicting findings may be due to the protocols employed in the studies, the biomarkers used for comparison, the timing of blood sample collection, and the subjects' training status. Bloomer et al. (21) acknowledges the importance of training status in eliciting a significant oxidative stress response. Variable and vague descriptions of subjects' past or current training status, make it difficult to know their potential for adaptations that may allow protection against oxidative stress (127) and muscle injury (109).

viii.   Concentric and Eccentric Muscle Actions

Although the term anaerobic means "without oxygen", dynamic resistance training does result in increased oxygen consumption during and following acute exercise. However, the magnitude of increase in VO_2 is far less than what is observed following acute aerobic exercise (16). Resistance exercise involving both concentric and eccentric muscle actions is the most commonly performed type of anaerobic exercise by the population at large. While concentric muscle actions require a greater energy cost compared to eccentric actions (58), eccentric actions are principally responsible for inducing muscle injury (60). In fact, both damage to the involved muscle tissue and concomitant soreness associated with such damage have been shown to be greatest following eccentric compared to concentric exercise (108).

Eccentric actions are characterized by a loading profile that combines high force and low fiber recruitment (i.e., high force per fiber ratio), which places a substantial mechanical stress on the associated structures (59). It is widely believed that exercise-induced muscle injury occurs as a result of these mechanical factors and that this injury is a function of the magnitude of strain and length of the muscle. The mechanical stress that causes disruption of the sarcomeres and failure within the excitation-contraction coupling system are theoretically believed to be the primary factors initiating injury, with a host of biochemical changes within the affected area such as increased inflammatory cytokines and RONS that may exacerbate the injury (60). A number of studies have measured the oxidative stress response to eccentric exercise, noting an increase during the hours to days following the exercise stress, likely due to the degree of muscle damage and inflammation (39, 45, 68, 83, 98-100, 124, 131, 146).

In contrast to these findings, Bloomer et al. (22) failed to illustrate any significant increase in oxidative stress biomarkers when resistance trained men performed a bout of eccentric bench press exercise. Furthermore, Nikolaidis et al. (120) noted that a repeated bout of lengthening actions attenuated muscle damage and blood oxidative stress compared with the initial bout. Finally, despite clear evidence of inflammation, Child et al. (44) found that muscle antioxidant status was not compromised, and MDA was unaltered in muscle biopsies and plasma. This was the first study to report inflammation without evidence of peroxidation in human exercise myopathy. Consequently, it was suggested that that the sampling time course utilized may have missed neutrophil-mediated oxidative stress.

The increase in oxidative stress as a result of both concentric and eccentric muscle actions (collectively described as dynamic resistance exercise) has also been comprehensively shown to exacerbate the production of RONS. McBride et al. (107) were the first to demonstrate that a full-body protocol of dynamic resistance exercise increases oxidative stress in the post-exercise period, as measured by blood MDA. While others have supported this claim that dynamic resistance exercise increases oxidative stress (6, 10, 11, 23, 53, 54, 77, 84, 106, 133, 136, 168, 170, 171, 174), others have elicited mixed, biomarker dependent results (21, 31, 34, 157).

ix. Isometric Muscle Actions

Isometric protocols have typically consisted of handgrip exercises (4, 52, 57, 104, 140, 155, 156) or have utilized static knee extension actions (142, 143). Despite the isolated musculature exercised, isometric protocols have also elicited an acute state of oxidative stress. It is believed that the acute ischemia and rapid reperfusion observed

during and following prolonged isometric exercise gives rise to increased RONS formation, perhaps via the radical generating enzyme xanthine oxidase (XO) (32). The majority of the studies incorporating isometric exercise have noted an increase in oxidative stress following exercise (4, 52, 57, 104, 140, 142, 155, 155, 156, 156) and a decreased antioxidant capacity (57, 155). As expected however, these changes appear to be transient, rapidly returning to pre-exercise levels within minutes following exercise (140, 156). The highly transient nature of changes in biomarkers may potentially, along with the previously discussed factors, explain some of the null findings (4, 142, 143, 156) with this type of exercise.

  x. Typical time course and magnitude of elevation in biomarkers

   The majority of exercise-induced oxidative stress literature, which has included a time course analysis post-exercise, has illustrated a transient increase in macromolecule oxidation or transient decrease in endogenous antioxidant capacity. This is illustrated across all modes exercise including steady-state aerobic (20, 33, 34, 66, 86, 128, 151, 167), exhaustive aerobic exercise (4, 66, 113, 128, 151, 156), sprint exercise (12, 31, 48, 76, 85, 161), dynamic resistance exercise (21, 34, 84, 107, 174), eccentric biased resistance exercise (45, 68, 99, 120, 124), and isometric exercise (140, 156). The relative increase in RONS formation is very much related to the biomarker studied (113), the type of exercise performed (34), exercise intensity (128), exercise duration (20), and subject population (trained or untrained) (28).

   Pertaining to steady-state aerobic exercise, Bloomer et al. (34) illustrated a transient elevation in PC, MDA, and glutathione status following 30 minutes of continuous cycling at 70% VO_{2max}, in cross-trained men. The increase was highest

immediately following exercise and fell to baseline values at one hour post-exercise for MDA and GSSG. A similar finding for PC was again found by Bloomer et al. (20) in aerobically trained men and women. Both men and women illustrated a transient increase in PC across all exercise durations (30 min, 60min, and 120min), with the 30 and 60 minute durations being closer to baseline at 60 minutes post-exercise. Unfortunately, because sampling was only taken for up to 60 minutes post-exercise, no data are available pertaining to when PC returned to baseline. Gohil et al. (66) established, with healthy male volunteers cycling at 65% VO_{2peak} for 90 minutes, that GSSG increases throughout the duration of the bout of exercise, peaking at the conclusion, and returning to baseline approximately 70 minutes post-exercise. Simultaneously, reduced glutathione (GSH) was depressed throughout the exercise duration time period, descending the furthermost immediately post-exercise, and returning to baseline approximately 70 minutes after the conclusion of the exercise. Viguie et al. (167) showed a similar glutathione response after one day of cycling at 65% VO_{2peak} for 90 minutes, by 11 moderately trained men. Time course analysis post-exercise was only extended to 15 minutes; however, even in this short time period it was evident that GSH and GSSG were returning to baseline levels. Quindry et al. (128) demonstrated that exercise of a sufficient intensity is required to elicit a significant transient increase in oxidative stress markers. Nine "physically prepared" male subjects illustrated that steady aerobic exercise at 10% above their lactate threshold elicited a transient increase in LOOH that returned approximately to baseline at one hour post-exercise. Inayama et al. (86) found that 30 minutes of treadmill running at ventilatory threshold, performed by untrained healthy females, caused a significant decrease in erythrocyte levels of protein-bound sulfydryl groups (p-SHs)–mostly

hemoglobin cysteine residues–and low molecular weight (LMW) thiols post-exercise, that returned to baseline values after two hours. Additionally, plasma levels of p-SHs significantly decreased after running; however, these remained unchanged after 24 hours. Finally, Sen et al. (151) reported a transient increase in oxidative stress biomarkers across varying levels of intensity (maximal oxygen uptake, aerobic threshold, and anaerobic threshold). Male volunteers were used for this study, and each of the aerobic and anaerobic workloads lasted 30 minutes. Although, some increases may have not been significant, and the time course analysis was not comprehensive (five minutes before exercise, two minutes post-exercise, and 24 hours post-exercise), it was illustrated that there was a transient increase in total glutathione (TGSH), GSSG, TBARS, and blood GSSG/TGSH ratio. All variables were greater two minutes post-exercise compared to pre-exercise, and had returned approximately to baseline values by 24 hours post-exercise. Further emphasizing this transient increase in oxidative stress was the decrease in GSH and net peroxyl scavenging capacity (PSC) at two minutes post-exercise compared to pre-exercise, and the subsequent return to baseline values at 24 hours post-exercise. These relative increases and decreases were evident across all intensities of exercise.

In addition to steady-state aerobic exercise, incremental (graded) exercise to exhaustion has also elicited a transient rise in oxidative stress; however because of the maximal intensity of the exercise, the time course above baseline typically extends for a greater period of time than steady state. In an investigation mentioned earlier, Quindry et al. (128) not only showed the transient increase in oxidative stress biomarkers with higher intensity steady-state bouts of exercise, but also illustrated it to a greater extent in the

graded exercise bout. Lipid hydroperoxides were still increasing at one hour post-exercise, compared to immediately post-exercise in the GXT; however they were on the decline by two hours. Similarly, Sen et al. (151) found that their biomarkers for oxidative stress following a VO_{2max} test were no different (i.e. transient) to the results cited above for the aerobic and anaerobic threshold intensities. Gohil et al. (66) elicited a similar, albeit non-significant, rise in GSSG and reduction in GSH following their GXT. Although the findings were non-significant, the data illustrated the transient nature of the glutathione markers. The non-significant findings were perhaps due to the fact that the GXT was probably less than 20 minutes in duration, as compared to 90 minutes for the regular steady-state protocol, and exercise duration for a greater period of time is need to elicit a significant change in oxidative stress biomarkers (135). Alessio et al. (4) elicited a transient rise in MDA after a maximum aerobic exercise test to exhaustion on a treadmill, with post-exercise values returning to baseline values after one hour. Lipid hydroperoxides and PC were only minimally reduced after one hour post-exercise compared to immediately post-exercise. Nonetheless, they were still decreasing at this time point. Their extended elevation is likely due to the exhaustive nature of the exercise undertaken. Steinberg et al. (156) illustrated the transient nature of exercise-induced oxidative stress, even after exhaustive cycling exercise, in healthy, untrained men and women. It was shown that oxidative stress, measured via TBARS, GSH, reduced ascorbic acid, and total antioxidant status (TAS), was gradually heighted from rest, through the subjects' ventilatory threshold, and climaxing at VO_{2max} (with the exception of TAS, which peaked 5 minutes post-exercise). Values for these biomarkers were clearly on their way back towards baseline 20 minutes after exercise, with TAS actually

exceeding baseline values at this time point. Finally, Michailidis et al. (113) specifically

sought to investigate the time-course changes of several commonly used markers of

oxidative stress following a bout of exhaustive aerobic exercise, with untrained men. The

results clearly indicate that sampling time after exercise may lead to different conclusions

regarding exercise-induced oxidative stress responses. In fact, the findings depict a non-

uniform changes in different markers because both transient (i.e., CAT) and prolonged

(i.e., PC) changes. Michailidis et al. concluded that there appears *no best time point*

applying to all markers for collecting blood samples after exhaustive aerobic exercise.

Intermittent and single sprint exercise has also illustrated an elevated increase in

oxidative stress transiently. Groussard et al. (76) illustrated that a Wingate test elicits a

transient increase in oxidative stress. Electron spin resonance, detecting lipid radical

level, illustrated an initial increase after the 30 second bout, which reached its maximum

approximately after 20 minutes of recovery. Oxidized lipid levels were returning close to

baseline at the last time measurement of 40 minute post-exercise. Lipid peroxidation,

measured by TBARS, paradoxically decreased immediately following the Wingate test,

increasing momentarily at five minutes post-exercise, and then dramatically falling by 40

minutes. Superoxide dismutase was initially decreased post-exercise, returning close to

baseline during the recovery period, and erythrocyte GSH level was initially decreased,

but returning to baseline by 40 minutes. Overall, these results by Groussard et al.

illustrate the transient nature of oxidative stress via the Wingate protocol; however,

variability is clearly evident with this type of exercise, and therefore more investigation is

warranted. Cuevas et al. (48) demonstrated a more uniform and transient increase in

oxidative stress following a single Wingate test. It was shown that a significant decrease

in GSH levels was observed immediately and at 15, 60 and 120 minutes time points post-exercise, followed by a return to basal value after 24 hours. This decrease was parallel to a significant increase of the GSSG/GSH ratio. Plasma TBARS concentration was not significantly modified following a single Wingate, although the values tended to increase at 60 and 120 minutes, returning close to baseline by 24 hours post. With 15-21 year old male and female swimmers, Inal et al. (85) demonstrated in the transient rise in oxidative stress markers in 100m and 800m distances. Specifically pertaining to the 100m distance (because of its sprint-like nature), it was found that CAT was significantly elevated at one, 20, and 40 minutes post-exercise compared to pre-swimming and it was clear that these values were declining towards pre-swimming levels throughout this time course. Glutathione peroxidase demonstrated a similar trend, with the one, 20, and 40 minute values all above the pre-swimming value, but clearly returning to pre-swimming levels throughout the post-exercise time course. Finally, as expected, GSH levels were decreased at the one, 20, and 40-minute intervals compared to the pre-swimming value. The GSH levels subsequently increased progressively at the 20 minute and 40 minute intervals, as compared with the one minute level. Despite non-uniform results for both biomarkers (LOOH & MDA), Baker et al. (12) illustrated a transient rise in oxidative stress across a single 30 second cycle ergometer test, with values generally elevated immediately post-exercise and then returning close to baseline 24 hours post-exercise. Thompson et al. (161) illustrated that plasma MDA was greatest immediately post exercise and had returned to baseline values 24 hours post-exercise, in both a vitamin C and placebo group, after intermittent shuttle running. Limitations to these last two

studies are that only two post-exercise measurements were taken, therefore, it is speculated that the oxidative stress was transient across this 24 hour period.

Interestingly, an increase in PC or MDA was not noted by Bloomer et al. (21) with aerobically trained men performing six repeated, 10-second cycle sprints. In fact, insignificant and inconsistent findings results were elicited. The reasons for these inconsistent findings have been postulated about earlier and are probably due to the protocols employed, biomarkers used, the timing of blood sample collection, and the degree of subjects' training status. Additionally, Marzatico et al. (102) failed to illustrate a transient increase in RONS over the 48 hours post-exercise in their sprint protocol. Interestingly, it was found that MDA continued to increase over this time course. The high elevation in lactate, and its subsequent interference with NADH and NADPH, was postulated as the possible reason for this obscure result. Furthermore, conjugate dienes were not found to be elevated significantly 30 minutes post-exercise; however, at the six hour measurement a significant increase in this lipid peroxidation marker was found.

Overall, the time course analysis for sprint protocols has been rather unsatisfactory in illustrating the transient rise in RONS generation post-exercise. It is obvious that RONS overwhelms the endogenous antioxidant protection, eliciting a state of oxidative stress, that returns to baseline within hours post-exercise (12, 21, 161). However, it has only been Groussard et al. (76), Inal et al. (85), Cuevas et al. (48), Marzatico et al. (102) and Bloomer et al. (21) that have attempted to illustrated this rise over multiple time periods post-exercise, and some of these studies have elicited ambiguous results. Therefore, further investigation into the oxidative stress response to this type of exercise is certainly warranted.

In summary, a review of the exercise-induced oxidative stress literature clearly illustrates the elevation in RONS and associated biomarkers as a result of exercise. However, with the wide variety of protocols employed, biomarkers used, and subject populations recruited, the results of studies have been variable. Consequently, it is difficult to determine whether intermittent anaerobic sprint exercise or steady-state aerobic exercise, is more detrimental in generating an oxidative stress. With specific regard to steady-state aerobic and sprint exercise involving healthy subjects, across multiple biomarkers, some investigations have illustrated the oxidative stress response due to exercise to be relatively non-existent (<10%) (21, 33, 158), small (10-29%) (48, 152, 161, 167), medium (30-49%) (2, 12, 85, 91, 150, 151), and large (>50%) (20, 31, 34, 50, 66, 69, 73, 76, 96, 160). Nonetheless, it remains unclear whether these exercise-induced oxidative modifications have little significance, induce harmful oxidative damage, or are an integral part of normal redox regulation.

Postprandial Oxidative Stress

i. What is Postprandial Oxidative Stress?

As outlined above, overproduction of RONS can indeed result from exposure to a variety of stimuli, including environmental pollutants such as ozone and cigarette smoke, physical stress such as acute aerobic or anaerobic exercise, and consumption and processing of dietary energy (165). In relation to the later, several reports indicate an increase in oxidative stress biomarkers following both high-fat and high carbohydrate feedings. This postprandial state appears ubiquitous regardless of health status, although has been shown to be exacerbated in diseased individuals, as compared to healthy controls (55, 145), including cigarette smokers (36)

ii. Mechanisms of Macronutrient Mediated Postprandial Oxidative Stress

Excessive intake of high-calorie and/or quickly digestible food (saturated fat and processed carbohydrate in particular) often results in exaggerated supraphysiological surges in blood glucose and lipids. This bolus of substrate essentially overwhelms the metabolic capabilities of the mitochondria within overnourished muscle and adipose tissue. Glucose and free fatty acids overwhelm the Krebs cycle, stimulating excess production of the hydrogen carrier molecules, nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD), which outpaces the capacity for oxidative phosphorylation. This buildup of NADH and FADH increases the mitochondrial proton gradient which drives the transfer of single electrons to oxygen, thereby creating RONS such as superoxide anion, at an accelerated rate (37, 95, 121). The ensuing postprandial oxidative stress triggers a harmful biochemical cascade throughout the circulation, including inflammation, endothelial dysfunction, hypercoaguability, and sympathetic

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hyperactivity, all of which may promote further RONS generation and oxidative damage (63). Moreover, the severity of the oxidative load imposed appears contingent upon the magnitude and rate of glycemia (i.e., increase in blood glucose) and hypertriglyceridemia (i.e., increase in blood triglycerides) experienced post-feeding (122). Indeed, moderate to strong correlations exist for both glucose (115) and triglycerides (8, 24, 25, 64, 145) and oxidative stress biomarkers post-feeding.

Due to the complexity of the cellular signaling cascade, the exact mechanisms involved in the production of RONS have yet to be fully elucidated. What is known is that the postprandial oxidative stress response *in vivo* acts in a cyclical fashion by which existing RONS activate additional pathways (i.e., gene transcription, inflammation, cellular adhesion), which in turn promote further RONS production and cellular damage (63). Whether RONS are the initial primary activators of the overall stress response or are recruited secondarily in response to some other stimulus remains unclear (63). However, observations and conclusions can be still inferred based on the current extensive body of literature related to postprandial oxidative stress. Moreover, while the mechanisms and consequences involved in the production of RONS either in the tissues or throughout the vasculature may differ, they all do appear to share a common thread. That is, they all appear dependent on the initial overwhelming influx of substrate (primarily fat and carbohydrate) oxidation products into the mitochondria, and subsequent increased formation of superoxide from the electron transport chain (5, 8).

iii. The Relationship Between Hypertriglyceridemia/Hyperglycemia and Disease

It is been associated that increased oxidative stress is associated with disease states, thus conditions which result in increased RONS production via postprandial

hypertriglyceridemia/hyperglycemia may stimulate pathogenesis. Individuals inhabiting the Western world may be subject to these deleterious postprandial conditions as much as 16 hours a day (51) due to the frequent intake of high calorie meals rich in processed carbohydrates and saturated fat. Intake of such meals would be expected to promote an acute period of hyperglycemia (114, 149, 153) and/or hypertriglycerdemia (42, 117), evident by elevated blood levels of glucose, triglycerides, and free fatty acids. Consequently, both postprandial hyperglycemia and hypertriglyceridemia have been suggested to be independent risk factors for the development of diabetes mellitus (145, 164) and cardiovascular disease (74, 122), including atherosclerosis (166). In accordance with the *"common soil hypothesis"* (41, 63) (postulating that Type II diabetes and cardiovascular disease share common genetic and environmental antecedents), this known association between postprandial hyperglycemia/hypertriglyceridemia and disease appears mediated by RONS (8, 145), as postprandial oxidative stress has been suggested as the link in the connection between insulin resistance, diabetes, and cardiovascular disease.

iv. Test Meal Composition

A common feature in many postprandial oxidative stress studies is the use of a pure lipid meal, often in the form of heavy whipping cream. While such a meal reliably induces a state of oxidative stress, it may be considered unrealistic as a meal choice outside of a laboratory environment. Fisher-Wellman and Bloomer (64) investigated this oxidative response to different isocaloric meals, and found that a lipid meal results in the greatest increase in postprandial oxidative stress in a sample of young, healthy men, as compared to a carbohydrate, protein, and mixed meal. More recently, Bloomer at al. (35)

replicated these findings in terms of comparing lipid with carbohydrate, and extended these findings by showing that meal size (in addition to composition) impacts postprandial oxidative stress. Specifically, minimal oxidative stress was observed following ingestion of dextrose loads of either 75g or 150g, or a lipid load of 33g; however, lipid ingestion at 66g lead to greater oxidative stress than lipid at 33g or dextrose at either 75g or 150g.

With regards to the findings described above, while pure lipid induces an oxidative stress in a dose dependent manner, such a meal may not be realistic in a free-living environment. Challengers to the use of high-fat test meals to elicit hypertriglyceridemia and oxidative stress cite that such meals greatly exceed the usual fat intake of a single feeding and may exaggerate the observed differences between rest and post-feeding (35, 64). Therefore, for the sake of applicability, it appears more practical to examine the magnitude of oxidative stress after a high-fat, high carbohydrate meal (e.g., a milkshake), more typically consumed as part of a Western diet and offered at many commercial establishments.

v.   **Typical time course and magnitude of elevation in biomarkers**

The time course elevation for oxidative stress biomarkers postprandially are extended well beyond the typical time course for exercise-induced oxidative stress. According to the literature, postprandial oxidative stress, which appears contingent upon the magnitude and rate of glycemia and hypertriglyceridemia experienced post-feeding (122), has been shown to peak in healthy, non-obese subjects (regardless of training and cigarette smoking status), between two and four hours (14, 24-27, 29, 64, 105, 163) and four and six hours (47), after which biomarkers start to decline towards pre-feeding

values. Individuals that are obese or have metabolic or cardiovascular disease appear to be more susceptible to postprandial oxidative stress, as they typically experience more robust and prolonged periods of hyperglycemia (114, 149, 153) and hypertriglycerdemia (42, 117) post-feeding. Elevations in blood glucose and triglycerides are directly associated with superoxide production (8), and consequently exacerbated oxidative stress is noted in patients with Type II diabetes (145) and coronary artery disease (74). As a result, time course analyses for these types of populations are generally extended towards six hours, with peak values normally occurring closer to 4-6 hours post-feeding (18, 27, 30, 111, 145).

Although it was found that men experience an exacerbated postprandial MDA and H_2O_2 response compared to women after a lipid meal, Bloomer and Fisher-Wellman (25) found that all oxidative stress biomarkers (nitrate/nitrite, MDA, H_2O_2, including triglycerides) peaked between 2-4 hours post-feeding after a lipid meal. Similarly, Bell and Bloomer (14), demonstrated in two subgroups of young, healthy women of differing menstrual cycle patterns (early follicular and pre-ovulatory), that MDA, H_2O_2, and triglycerides peaked between 2-4 hours and TEAC declined to its lowest values during the same time period. When physically stressful tasks were suspended for 24 hours preceding a lipid meal, Bloomer et al. (29) found that aerobic training status did not influence the postprandial oxidative stress or hypertriglyceridemia response in a sample of healthy trained and untrained men and women. Specifically, in agreement with the other studies conducted by our laboratory with healthy subjects, triglycerides, MDA, H_2O_2, and nitrite/nitrate were all found to peak (non-significantly) between 2-4 hours. In a comprehensive analysis of the role of sex and exercise training status on postprandial

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oxidative stress in healthy subjects, Bloomer et al. (24) found that in all oxidative stress biomarkers TEAC, MDA, H_2O_2, XO, PC, and triglycerides all peaked between 2-4 hours, despite women having lower overall values than men. Finally, the postprandial oxidative stress response peaked between 2-4 hours for TEAC, XO, MDA, H_2O_2, and triglycerides in a sample of cigarette smokers and non-cigarette smokers, after a lipid rich meal (36). Although the smokers experienced a heightened response to the feeding across all biomarkers, the relative time course was similar in both groups of subjects. When exercise was controlled for, McClean et al. (105) illustrated that the peak response in LOOH and nitrate/nitrite occurred between 3-4 hours post-exercise and triglycerides were still rising at four hours in a sample of healthy, recreationally trained men, after high-fat mixed meal. Additionally, it was found that mean pulse wave velocity (suggesting impaired vascular function) was greatest at four hours post-ingestion, further emphasizing the abundance of LOOH and decline in antioxidant protection (namely, SOD). In another sample of healthy, recreationally trained men, when exercise was controlled for, Clegg et al. (47) demonstrated that the peak LOOH and triglyceride result was between 4-6 hours post high-fat mixed meal. However, the mean pulse wave velocity was significantly increased above pre-ingestion between 2-4 hours post-ingestion which suggests an augmented oxidative stress response probably manifested by the increase in LOOH levels. The reason for this slightly extended postprandial peak in LOOH was unexplained.

In summary, a review of the postprandial oxidative stress literature clearly illustrates an elevation in RONS and associated biomarkers as a result of acute feeding. However these investigations, especially those pertaining to healthy subjects (or healthy

control subjects), have illustrated the rise in oxidative stress to be variable, with some

demonstrating the change to be relatively non-existent (<10%) (9), small (10-29%) (145,

149) medium (30-49%) (8, 114), and large (>50%) (14, 24, 25, 27, 29, 36, 47, 105).

Differences in results are most likely associated with differing methodologies between

investigations, in particular, the amount of saturated fat contained within the meal. In

those studies noting only minimal or small degrees of oxidative stress, the saturated fat

content was relatively low. The magnitude of the increased oxidative stress response due

to feeding is very much influenced by the size and the composition of the test meal

administered (35, 64), and the population of subjects used (24, 25, 29).

This unfavorable rise in postprandial RONS and associated oxidative stress

biomarkers, in conjunction with the extended time course of elevation, has lead

researchers to believe that chronic exposure to postprandial oxidative stress is the

unifying mechanism in the connection between pathological abnormalities such as insulin

resistance, Type II diabetes, endothelial dysfunction, atherosclerosis, and cardiovascular

disease (41, 63).

Conclusion

As illustrated above, oxidative stress and RONS generation occurs in response to

a variety of environmental and physiological stressors. Two prevalent origins of

oxidative stress in Western society are the ingestion of certain nutrients (saturated fat in

particular) and strenuous physical exercise, both for which antioxidant micronutrient

supplementation has been recommended in an attempt to combat the increased

production of RONS. While the overall magnitude and time course of oxidative stress

appears to be greater following feeding than typical bouts of acute exercise (in particular

when the feedings include a moderate-to-high amount of saturated fat), to our knowledge, no study to date has compared the absolute oxidative stress response between these two RONS generators.

Therefore, the purpose of the present investigation is to compare the magnitude of increase in selected oxidative stress biomarkers in a sample of healthy, exercise-trained men. It is our aim that data collected from this experiment, may direct future recommendations regarding the need for antioxidant supplementation in relation to both acute feeding and acute strenuous exercise. It should be noted that the present investigation will be the first to our knowledge to compare the oxidative stress response to feeding and exercise in a sample of men, using a cross-over design. It is important to note that the feeding test (mixed macronutrient milkshake) will be similar in composition to many commonly consumed milkshakes available worldwide; while the different exercise tests are commonly utilized by thousands of individuals who exercise regularly. Therefore, the protocol employed may be considered physiologically relevant, with data capable of being generalized to a large segment of the population.

Of course, limitations of the present work include the inclusion of only exercise-trained men, the use of only one type of meal, the use of only three different exercise challenges (although representing a continuum of exercise intensities), and exclusive use of the selected times of measurement and assays. Despite the above limitations, this work should provide interesting data pertaining to the oxidative stress response to feeding and exercise, which may help guide the decision to use or not to use antioxidant supplementation for the purpose of attenuating such oxidative stress. That is, currently, antioxidant supplementation is commonly used to combat exercise-induced oxidative

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stress, but is generally not considered by many individuals in an attempt to combat

feeding-induced oxidative stress (with the possible exception of "life extensionists").

However, if the present study provides evidence that feeding induces a greater oxidative

stress response than does exercise (even when exercise is performed at high intensities),

the current recommendations and protocol for antioxidant use may be challenged.

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APPENDIX C – IRB APPROVAL

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.

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Approved

Cc: