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The Role of High-fat Feeding on the Up-regulation in Antioxidant Enzymes: A Potential

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THE ROLE OF HIGH-FAT FEEDING ON THE UP-REGULATION IN ANTIOXIDANT ENZYMES: A POTENTIAL "REPEATED BOUT EFFECT" OF HIGH FAT MEAL INGESTION

by

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DEDICATION

Mom and Dad,

Without your love and support, none of this would have been possible.
I love and appreciate both of you more than you know.

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I would also like to pay gratitude to the other members of my thesis committee, Dr. Randal Buddington and Dr. Zsolt Murlasits, as well as Dr. Brian Schilling. They have provided great support during my time here and helped me improve in my studies.

ABSTRACT

Harvey, Innocence Christianna. M.S. The University of Memphis. May, 2013. The Role of High-fat Feeding on the Up-regulation in Antioxidant Enzymes: A Potential "Repeated Bout Effect" of High Fat Meal Ingestion. Major Professor: Richard J. Bloomer, PhD.

While RONS are produced as part of normal metabolism, excess production can occur in response to stressors such as physical exercise and ingestion of excess nutrients (e.g., saturated fat). Over time this may lead to an up-regulation in antioxidant enzyme activity, in an attempt to protect cells. The purpose of this investigation was to determine if an increase in antioxidant enzyme activity is observed following repeated ingestion of high fat meals. Sixteen healthy men consumed 10 high fat milkshakes over a 3+ week period. Blood was taken from subjects on the first day of each week (before and for 4 hours after milkshake ingestion) and analyzed for oxidative stress biomarkers. Repeated high-fat feeding did not result in any up-regulation in antioxidant enzymatic activity or attenuation in oxidative stress. These data indicate that the RONS production associated with repeated consumption of high-fat meals does not induce an adaptive response within the blood antioxidant defense system.

Key words: Free radicals, oxidative stress, postprandial, antioxidant defense.

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BACKGROUND

Oxidative stress occurs when the production of reactive oxygen and nitrogen species (RONS) overwhelms antioxidant defenses—and involves oxidative damage to lipids, proteins, DNA, and other macromolecules [1] in ways that impair cell function [2]. As a result, oxidative stress is known to be a major contributor to many chronic diseases, as well as to the aging process [3]. The production of RONS is a component of normal cell metabolism [2,4] and serves multiple vital roles in human physiology (e.g., cell signaling, apoptosis, and immune function) and only becomes problematic when in excess. Determining the amount or type of RONS can be done using the direct measurement of electron spin resonance spectroscopy [5]; however, byproducts of RONS formation measured in the blood are the usual outcome measures of most exercise- and feeding-induced oxidative stress studies [6]. Likely the most prevalent factor in Western society known to increase RONS production is the consumption of food [7,8], typically high in saturated fat and simple sugar. Strenuous exercise is also associated with RONS production; the degree depending on the type, intensity, and duration of exercise [9-11].

Postprandial oxidative stress is a state in which RONS production surpasses the body's antioxidant defenses after meal consumption (generally lipid meals which are high in total kilocalories). RONS production has been shown to be greater after lipid-rich meals as compared to protein- or carbohydrate-rich meals, with protein causing the least oxidative insult [8]. It has also been noted that as the lipid or carbohydrate load is increased, the oxidative stress response is increased [7], suggesting either increased RONS generation and/or decreased antioxidant defense. High fat meals and the

associated oxidative stress have also been reported to impair endothelium-dependent vasodilation, which may contribute to the development of atherosclerosis [12].

Exercise has also been shown to increase RONS production acutely; in particular when performed at extremely high intensities and for long durations (e.g., marathons) [9-11]. Increased oxygen consumption is thought to be a primary cause of exercise-induced RONS formation; however, other factors such as muscle injury and inflammation can lead to an increase in RONS [6]. Despite findings of increased oxidative stress in response to acute exercise, a careful review of literature indicates that the extent of oxidative stress experienced following exercise is less than that experienced following ingestion of high fat meals. We have recently completed a direct comparison study in which subjects consumed a high fat meal and performed three different bouts of exercise (at intensities ranging from moderate [~70% of VO_{2max}] to extreme [200% of VO_{2max}]) on four different days [13]. We noted a significantly greater oxidative insult with feeding as compared to any bout of exercise. This was evident using the oxidative stress blood biomarkers hydrogen peroxide, malondialdehyde, and advanced oxidation protein products. These data indicate that RONS production is likely greater in response to feeding as compared to very strenuous exercise.

In relation to the above, it has been noted that routine exposure to RONS leads to an up-regulation in antioxidant defense. Through the process of hormesis, the increase in RONS serves as a stimulus for the up-regulation in antioxidant defense, which minimizes future cases of oxidative insult. While this is well-described for chronic exercise, we are unaware of studies focused on the role of routine ingestion of high fat meals to improve antioxidant defense. Considering findings from our recent study, it is possible that high

fat feeding, which promotes a significant rise in RONS, also promotes an up-regulation in vital antioxidant enzymes—which
may reduce oxidative stress observed following feeding, as well as following acute bouts of exercise.

This improvement in antioxidant defense is not merely important for purposes of attenuating postprandial oxidative stress occurring in response to future ingestion of high fat meals. This adaptation may also serve to combat the exercise-induced rise in RONS, as once the antioxidant defense system is heightened, it is available to counteract RONS produced via any source (e.g., feeding, exercise, environmental toxins). Antioxidant supplementation has been shown to be a source of protection against exercise-induced oxidative stress [1,11], as well as other forms of RONS production. As indicated above, results have shown that moderate to high intensity exercise training can reduce exercise-induced oxidative stress by up-regulating antioxidant enzyme activity, along with enhancing other protective mechanisms [9,13]. These findings are in support of the principle of hormesis, which states that if the body is repeatedly exposed to a stressor, it will develop favorable adaptations which will serve to enhance physiological performance and overall health [14].

However, it should be noted that if there is not adequate stress to induce an increase in RONS, as seen with low intensity exercise [9], the body will not develop a means of protection against that stressor. This intensity-dependent RONS generation is the basis for our rationale surrounding this proposal; where "intensity" is not restricted to a physical definition, but rather, a "magnitude of effect." That is, 1) RONS generation is the stimulus for improved antioxidant defense; 2) exercise is known to increase RONS

and to thus result in an improved antioxidant defense; 3) high fat feeding causes a much more robust increase in RONS as compared to exercise; 4) high fat feeding should result in an improvement in antioxidant defense—one that may be greater than what is typically observed as an adaptation to chronic exercise. For example, the improved antioxidant defense resulting from exercise training is inadequate to counteract the RONS production observed following high fat feeding [15,16]. In much the same way as with specificity of training, it is possible that "specificity of adaptation" in terms of the degree of antioxidant protection may apply to minimizing oxidative stress resulting from future intake of high fat meals. Of course, if the antioxidant defense system is then heightened, it should more easily counteract the lesser RONS production occurring in response to acute exercise bouts. Finding ways to reduce oxidative stress in response to both feeding and exercise may prove beneficial to an individual's 1) overall health and 2) overall physical performance and recovery—as some authors have suggested a relationship between increased oxidative stress and impaired muscle performance.

METHODS

Design

Pre-post assessment was made within the same group of subjects (comparisons made across meals and across time). Subjects were assigned to either a moderate fat meal or a high fat meal; to be consumed a total of 10 times over the period of 3+ weeks.

Subjects

Ten healthy men between the ages of 19-43 years were recruited for this study. Subjects were physically active and consuming a "healthy", low-moderate fat diet prior to enrolling. Specifically, their diet did not include routine (i.e., daily) ingestion of high fat

foods, in particular those rich in saturated fat. A detailed interview was conducted with potential subjects to determine eligibility, in a similar manner as with using a food frequency questionnaire. All participants were non-smokers with no known cardiovascular or metabolic diseases. Subjects were advised to refrain from antioxidant supplementation at least two weeks prior to the start of the study, as well as during the study. Women were prohibited from participation in this study to allow for a more homogeneous sample—due to the potential influence of estradiol on antioxidant activity and oxidative stress. Recruitment of subjects took place at the University of Memphis via flyers, e-mail, and word of mouth.

Initial Lab Visit

Eligibility was determined via interview and the completion of questionnaires pertaining to health history, physical activity, and medication and dietary supplement use. Each subject was informed of all procedures, potential risks, and benefits associated with the study in both verbal and written form in accordance with the approved procedures of the University Institutional Review Board for Human Subjects Research. Subjects were asked to provide written informed consent prior to being enrolled in the study.

After consent was provided and eligibility determined, subjects were asked to void bladder and rest for 10 minutes in a seated position. Following the rest period, two technicians assessed heart rate (via 60 second palpation of radial artery) and blood pressure (via auscultation). These measurements were used for descriptive characteristics, along with height, weight, body composition, BMI, circumference measurements, and dietary intake (described below). Subjects' height was measured using a stadiometer, and their body weight was measured using a calibrated scale. Body mass index was calculated

as bodyweight (kg) divided by height squared (m^2). Waist and hip circumferences were measured using a tension-regulated tape. Body composition was determined via dual energy x-ray absorptiometry (Hologic QDR-4500W) using a 4-minute fan array. Dietary intake was assessed from diet records completed during the three days prior to each test day. All above information was used for descriptive purposes.

Test Days

Within two weeks of the initial lab visit, subjects returned to the lab for the first of 10 meal (milkshake) visits. Of these 10 meal visits, only four were actual "test" visits in which blood was collected. Specifically, the "test" visits were meals 1, 4, 7, and 10 (the Monday of each week). Additional meals (Wednesday and Friday of each week) were consumed in the lab during the morning, but no blood was collected on these days. All 10 meals were identical for each subject. The information below outlines the timeline of the study:

Monday-Day 1: Milkshake and 3 blood draws (4 hour lab visit)
Wednesday-Day 2: Milkshake only
Friday-Day 3: Milkshake only
Monday-Day 4: Milkshake and 3 blood draws (4 hour lab visit)
Wednesday-Day 5: Milkshake only
Friday-Day 6: Milkshake only
Monday-Day 7: Milkshake and 3 blood draws (4 hour lab visit)
Wednesday-Day 8: Milkshake only
Friday-Day 9: Milkshake only
Monday-Day 10: Milkshake and 3 blood draws (4 hour lab visit)

The meal consisted of a milkshake made with whole milk, ice cream, and heavy whipping cream adjusted for body mass. One half of subjects were randomly assigned to consume a high calorie/ high fat milkshake (1.0g carbohydrate/kg; 0.8g fat/kg; 0.25g protein/kg), while the remaining subjects were assigned to consume a moderate calorie/

moderate fat milkshake (one half of the amount noted above). This was done in an attempt to provide two different "intensities" of treatment, in much the same was as has been done when studying exercise intensity and the impact on antioxidant enzyme levels. The composition of the milkshake is the same as we have used in our recent studies of postprandial oxidative stress and similar to the meal provided in many of our other studies of postprandial oxidative stress. Moreover, both milkshakes deliver kilocalorie loads similar to what are provided within milkshakes in many commercial establishments and are well-tolerated by subjects.

Subjects reported to the lab in a 10 hour fasted state on the morning of each test meal day (again, a total of 4 of the total 10 meal days). Blood was collected prior to meal ingestion (following a 20 minute rest period), and at 2 and 4 hours post meal ingestion. This time frame has been verified as ideal in our prior work, in particular when using healthy men as subjects. No other food was allowed during the 4 hour postprandial period; however, water was provided ad libitum.

Blood Collection and Biochemistry

Venous blood samples were taken from the subjects via needle and Vacutainer™. Blood samples that were collected in tubes containing EDTA were immediately separated via centrifugation at 1500g for 15 minutes at 4°C for collection of plasma. Blood samples that were collected in tubes containing no additives were allowed to clot at room temperature for 30 minutes and then separated by centrifugation at 1500g for 15 minutes at 4°C for serum collection. Blood samples were immediately stored in multiple aliquots at -70°C until analyzed for the variables indicated below.

Triglycerides (TAG) were analyzed in serum following standard enzymatic procedures as described by the reagent manufacturer (Thermo Electron Clinical Chemistry). Malondialdehyde (MDA) was analyzed in plasma using reagents purchased from Northwest Life Science Specialties (Vancouver, WA). Hydrogen peroxide (H_2O_2) and catalase (CAT) activity were analyzed in plasma using the Amplex Red reagent method as described by the manufacturer (Molecular Probes, Invitrogen Detection Technologies, Eugene, OR). Trolox Equivalent Antioxidant Capacity (TEAC) was analyzed in serum according to the procedures outlined by the reagent provider (Sigma Chemical, St. Louis, MO). Serum superoxide dismutase (SOD) activity was measured using enzymatic procedures as described by the reagent provider (Cayman Chemical, Ann Arbor, MI), where 1 unit of SOD is the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. Plasma glutathione peroxidase (GPx) activity will be measured using enzymatic procedures as described by the reagent provider (Cayman Chemical). Values for GPx were calculated using the nicotinamide adenine dinucleotidephosphate (NADPH) extinction coefficient and are presented in nanomoles per minute per milliliter where 1 unit is defined as the amount of enzyme needed to oxidize 1.0 nmol of NADPH to NADP+.

Samples were analyzed in duplicate. All variables were analyzed on each test day (meals 1, 4, 7, and 10) before and at 2 and 4 hours following meal ingestion.

Dietary Records and Physical Activity

All subjects were instructed to maintain their normal diet during the study and were asked to record all food and drink consumed during the three days prior to each test meal (meals 1, 4, 7, and 10). Records were analyzed for total kilocalorie, macro- and

micro-nutrient composition using Food Processor SQL (version 9.9; ESHA Research, Salem, OR). Subjects were asked to maintain their usual activities of daily living throughout the study, but not to perform any strenuous activity during the 48 hours prior to each test meal (meals 1, 4, 7, and 10).

Statistical Analysis

All biomarker data obtained before and following milkshake ingestion were analyzed using a 4 (milkshake) x 3 (time) repeated measures analysis of variance (ANOVA). Significant effects were analyzed further using Tukey's post hoc tests. A one-way ANOVA was used to analyze dietary intake prior to each test meal. All analyses were performed using JMP statistical software (version 4.0.3, SAS Institute, Cary, NC). Statistical significance was set at $p \leq 0.05$.

RESULTS

A total of 16 subjects completed all aspects of this study. Subject characteristics are presented in Table 1. Three of the subjects reported taking nutritional supplements prior to enrolling in the study—in the form of multivitamin and fish oil. The milkshakes were tolerated by all subjects without significant adverse effects. One subject noted GI distress (e.g., bloating and gas) after consuming the milkshake. Dietary data during 72 hours prior to each test day were not different ($p>0.05$). Data for dietary data are presented in Table 2.

Although subjects were assigned to two separate groups, either a high- or moderate-fat milkshake group, there were no interaction effects noted for any outcome ($p>0.05$). Therefore, the data were pooled and presented as such.

No interaction (p=1.00) or day effect (p=0.99) was noted for TAG. A trend was noted for a time effect (p=0.09), with values highest at four hours post-meal ingestion. Data for TAG are presented in Figure 1.

No interaction (p=0.96) or day effect (p=0.89) was noted for MDA. A time effect (p<0.0001) was noted with values greater at two and four hours post consumption compared to pre (p<0.05). Data are presented in Figure 2.

No interaction (p=0.94) or day effect (p=0.94) was noted for H_2O_2. A time effect (p<0.0001) was noted with values greater at two and four hours post consumption compared to pre (p<0.05). In addition, a condition effect (p=.0008) was noted with higher values in the high-fat group compared to the moderate-fat group (p<0.05). Data are presented in Figure 3.

No interaction (p=0.38) or day effect (p=0.18) was noted for TEAC. A time effect (p=0.02) was noted with values greater at pre compared to two hours post (p<0.05). Data are presented in Figure 4.

No interaction (p=0.72) or day effect (p=0.61) was noted for SOD. A time effect (p=0.00) was noted with values greater at pre and two hours compared to four hours (p<0.05). Data are presented in Figure 5.

No interaction (p=0.95), day (p=0.92), or time effect (p=0.81) was noted for GPx. Data are presented in Figure 6.

[17] No interaction (p=0.98) or day effect (p=0.94) was noted for CAT. A time effect (p=0.00) was noted with values lower at two and four hours compared to pre (p<0.05). In addition, a condition effect (p=0.00) was noted with lower values of CAT in the high-fat group compared to the moderate-fat group (p<0.05). Data are presented in Figure 7.

DISCUSSION

This was the first investigation to our knowledge to study the possible adaptations within the antioxidant defense system to repeated exposure to high-fat loads. Our main findings from this study are as follows: 1) systemic antioxidant capacity does not improve in response to the oxidative stress caused by high-fat feeding over time (3 weeks), despite a significant increase in oxidative stress in response to feeding, and 2) H_2O_2 and catalase were the only variables that were sensitive to differing meal sizes, with high-fat meals inducing a greater oxidative stress than moderate-fat meals (as evidenced by higher H_2O_2 and lower catalase).

As we have reported in several prior studies, oxidative stress is observed in response to high fat feeding [7,8,16,18-22], with values elevated for hours following meal ingestion. These findings may have implications for overall vascular and metabolic health over time, as increased postprandial oxidative stress is associated with endothelial dysfunction [23,24] and metabolic dysfunction [17,25]. We hypothesized based on the principle of hormesis that repeated exposure to the same high fat meal would resulted in a blunted oxidative stress response to feeding, inclusive of increased antioxidant enzyme activity.

The present results lead us to reject our initial hypothesis, which proposed that the increased RONS generated by high fat meal ingestion would lead to an up-regulation in antioxidant defense. Our hypothesis was derived from the principle of hormesis, which states that if an adequate degree of stress is placed on a system, the system will adapt accordingly to that stress. As mentioned previously, this is what is typically observed in response to chronic exercise-induced stress [26,27].

While we are uncertain of the exact explanation for our lack of findings, we propose the following for consideration. First, our time frame of repeated RONS exposure may have been too short to provide for adaptation within the antioxidant defense system. Indeed, prolonged exposure RONS may induce greater adaptation, although prior exercise studies support our timeframe to some extent. For example, while it is noted that even acute exercise [28,29] can result in increases in antioxidant activity, relatively short duration training protocols have allowed for an adaptation in antioxidant defense [28-30]. Berzosa and colleagues studied the acute effects of two strenuous VO_2 max tests, as well as a submaximal test (70% VO_2 max) on various antioxidant enzymes in untrained men [28]. They found that CAT, GPx, Glutathione reductase (GR), and SOD were significantly elevated following the exercise tests. Additionally, a single bout of maximal effort exercise has been shown to increase SOD activity in sedentary men and women [29]. Three weeks of aerobics training for 30 minutes a day, 5 days a week has been shown to increase SOD expression, as well as activity in mouse aorta [31]. However, human training studies have incorporated longer time intervals. For example, Rabinovich and colleagues noted enhanced GSH activity in healthy subjects following 8 weeks of interval training [27]. Miyazaki et. al observed the chronic effects of a 12 week endurance training program on antioxidant status in sedentary men [14]. Subjects ran at 80% of their maximum heart rate for 60 minutes per day, five days per week and performed a graded exercise test (GXT) pre- and post-training. It was noted that SOD and GPX activities were enhanced following the post-training GXT compared to pre-training GXT. Likewise, Fatouros and co-workers studied the changes in antioxidant defense in elderly men following 16 weeks of endurance training [30]. Participants completed a

GXT before and after the training program, which consisted of walking/jogging at 50-80% of their maximum heart rate 3 times a week for up to 42 minutes each session. It was reported that TEAC and GPx activities were significantly increased following training. Additionally, Elousa et. al found that 16 weeks of aerobic training at 65-80% VO_2 max increased SOD, GPX, and GR activities in young, healthy men and women [29]. Due to the fact that our potential for RONS generation was greater with our high fat meals as compared to what is observed with routine exercise training [13], we thought that a shorter exposure period may lead to similar outcomes. This was not the case in the present study and perhaps an exposure period of longer than three weeks of high fat meal ingestion would be needed to promote an increase in antioxidant defense. However, our data do not support this hypothesis, as there is no indication that adaptive responses are beginning to occur at the three week time period.

Second, the frequency at which we provided the meals may have been too low. We attempted to mimic what most individuals may do in terms of an exercise prescription aimed at improving antioxidant defenses. That is, three days per week of "stress." However, it is possible that a more frequent protocol of high fat feeding may be necessary to signal for an up-regulation in antioxidant enzyme activity.

Third, and taking a conflicting approach to the two ideas presented above, it is possible that the size of the meals may not have been appropriate to promote the desired degree of RONS production necessary for optimal adaptations to the antioxidant defense system. That is, the fat load provided at each individual feeding may have been too high and caused such a severe oxidative burden that the system was overloaded, thus possibly causing a depletion in antioxidants potentially offsetting any increase in antioxidant

capacity. This phenomenon is thought to occur in cigarette smokers. For example, it has been noted that cigarette smoking induces a significant oxidative stress [32,33], as evidenced by increased levels of lipid peroxidation products in smokers compared to non-smokers, coupled with lower antioxidant capacity [32]. Because cigarette smoking promotes such a massive oxidative insult, it has been suggested that antioxidant enzymes systems may actually be heightened in response to the increase in RONS [34]. However, with continuous RONS exposure of high magnitude, antioxidant systems are depleted [32,35]. It is possible that a similar phenomenon may have occurred in the present study. Alternatively and in line with our rationale for points one and two above, the meals may have been too small to promote the degree of RONS production needed for optimal adaptation. While this is possible, we do not believe it is probable, as lower degrees of oxidative stress experienced at any given one point in time (e.g., in response to acute exercise) have been reported to increase antioxidant enzyme activity. Therefore, we believe that the oxidative insult provided by our meals was likely adequate; though, it is possible the volume of feeding was too low. That is, we only provided a high-fat meal once per day, three days per week. In contrast, animal studies that have noted an increase in antioxidant enzyme activity in response to high-fat feeding have provided increases in fat intake in small doses at each meal [36,37].

Fourth and of main importance, in the present study oxidative stress biomarkers and antioxidant enzyme activity was only assessed in blood samples. It is possible that differing results may have been present in tissues unrelated to blood, such as skeletal muscle, liver, and heart. In support of this statement, Webster and colleagues studied oxidative stress in male rats exposed to hyperoxia over the course of a five day period

[38]. The investigators measured SOD, CAT, and GPx activity in the liver, lung, and blood. SOD and CAT were significantly increased in both liver and lung tissue, but not in blood; whereas, GPx was significantly increased in lung only. Similar findings for increased CAT expression have recently been reported within mouse cardiac mitochondria in response to high fat feeding [36]. Conversely, Das and colleagues studied oxidative stress levels in the placental tissue and serum of pre-eclamptic women [39]. Significant increases in TBARS and SOD were noted in both tissue and serum, whereas GSH levels remained constant at both sites. These authors stated that that the stress levels measured in serum were a good indicator of what is occurring at the tissue level. While this may be the case in the specific design used by Das et al (26), this may not hold true in designs such as ours in which healthy individuals are consuming high fat loads with the expectation of increased oxidative stress. Clearly, it is possible that findings for oxidative stress and antioxidant biomarkers may differ across tissues being investigated and further research will be needed to more fully elucidate the potential changes in antioxidant activity in various tissues in response to high fat feeding and other generators of RONS. Our failure to measure antioxidant activity in tissues other than blood should be considered a limitation of this work.

CONCLUSION

To our knowledge, this is the first study to determine the influence of repeated high fat feedings on blood antioxidant enzyme activity, in addition to postprandial oxidative stress. Our results indicate that repeated moderate-to-high fat meals ingested over the course of a three week period by healthy men do not induce an adaptive response to the antioxidant enzyme system, such as is observed with exercise training. There are

several possible explanations for our noted lack of effect (e.g., duration, frequency, volume of fat intake; tissue investigated) that should be considered. With this understanding, our findings indicate that the RONS production associated with repeated consumption of high-fat meals does not induce an adaptive response within the blood antioxidant defense system. Future studies involving a variety of tissues may be needed to more fully elucidate the potential impact of high fat feeding on the up-regulation in endogenous antioxidant enzymes, as well as oxidative stress biomarkers.

APPENDIX A- Tables and Figures

Table 1. Subject characteristics of 16 healthy men consuming high-fat meals over the course of a 4 week period

Variable	
Age (years)	24.9±1.6
Height (cm)	176.3±1.6
Body Weight (kg)	80.3±2.5
Body Mass Index ($kg \cdot m^{-2}$)	25.9±0.7
Total Body Fat (%)	17.2±6.7
Trunk Body Fat (%)	17.9±1.8
Fat Mass (kg)	14.1±1.5
Fat Free Mass (kg)	66.2±1.6
Waist Circumference (cm)	85.7±1.9
Hip Circumference (cm)	101.6±1.5
Waist:hip	0.8±0.0
Resting Heart Rate (bpm)	71.9±3.0
Resting Systolic Blood Pressure (mmHg)	120.8±2.7
Resting Diastolic Blood Pressure (mmHg)	76±2.8
Total Fasting Cholesterol ($mg \cdot dL^{-1}$)	159.9±3.9
Fasting HDL Cholesterol ($mg \cdot dL^{-1}$)	51.4±3.9
Fasting VLDL Cholesterol ($mg \cdot dL^{-1}$)	23.2±3.4
Fasting LDL Cholesterol ($mg \cdot dL^{-1}$)	85.3±2.8
LDL:HDL	1.8±0.1
Total:HDL	3.3±0.2
Weekly Aerobic Training (hrs)	3.0±0.6
Aerobic Training History (yrs)	6.2±1.8
Weekly Anaerobic Training (hrs)	3.8±0.7
Anaerobic Training History (yrs)	6.1±1.5

Values are mean±SEM.

Table 2. Dietary data of 16 healthy men consuming high-fat meals recorded three days prior to each test day.

Variable	Day 1	Day 2	Day 3	Day 4	P value
Kilocalories	1930±132	1728±118	1678±121	1814±107	p>0.47
Protein (g)	111±7	92±9	89±9	97±8	p>0.26
Carbohydrate (g)	227±18	202±14	212±19	205±14	p>0.71
Fat (g)	58±5	58±5	49±4	65±5	p>0.17
Saturated Fat	20±2	19±2	17±2	21±2	p>0.43
Cholesterol	333±41	318±35	207±34	321±43	p>0.08
Vitamin C (mg)	69±14	65±11	85±23	44±7	p>0.31
Vitamin E (mg)	7±1	6±1	5±1	7±2	p>0.80
Vitamin A (RE)	621±214	329±75	269±52	284±92	p>0.17
Selenium (µg)	66±9	53±8	47±8	67±13	p>0.38

Values are mean±SEM.

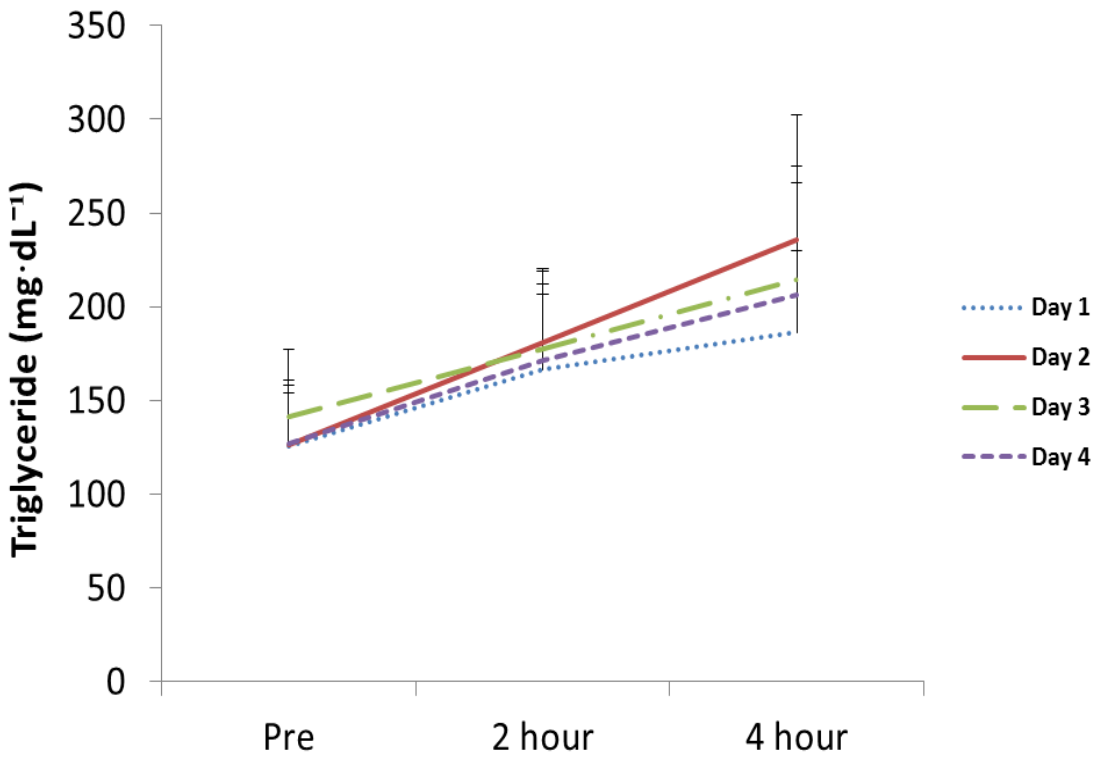

Figure 1. Blood triglyceride data of 16 healthy men before (Pre) and after (2 and 4 hours) consumption of a high-fat meal on four different occasions, separated by one week.
Values are mean±SEM
No interaction (p=1.00) or day effect (p=0.99).
Trend for time effect (p=0.09).

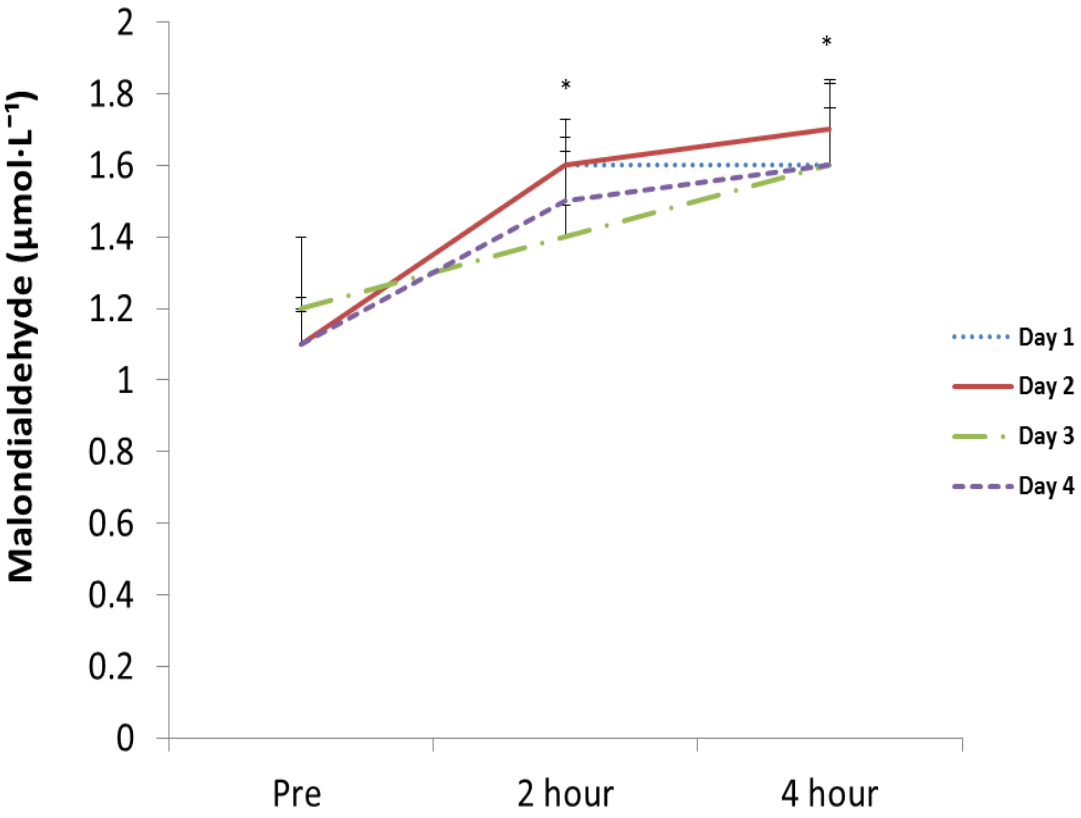

Figure 2. Blood MDA data of 16 healthy men before (Pre) and after (2 and 4 hours) consumption of a high-fat meal on four different occasions, separated by one week. Values are mean±SEM
No interaction (p=0.96) or day effect (p=0.89).
*Time effect (p=0.00); 2 hour and 4 hour > Pre

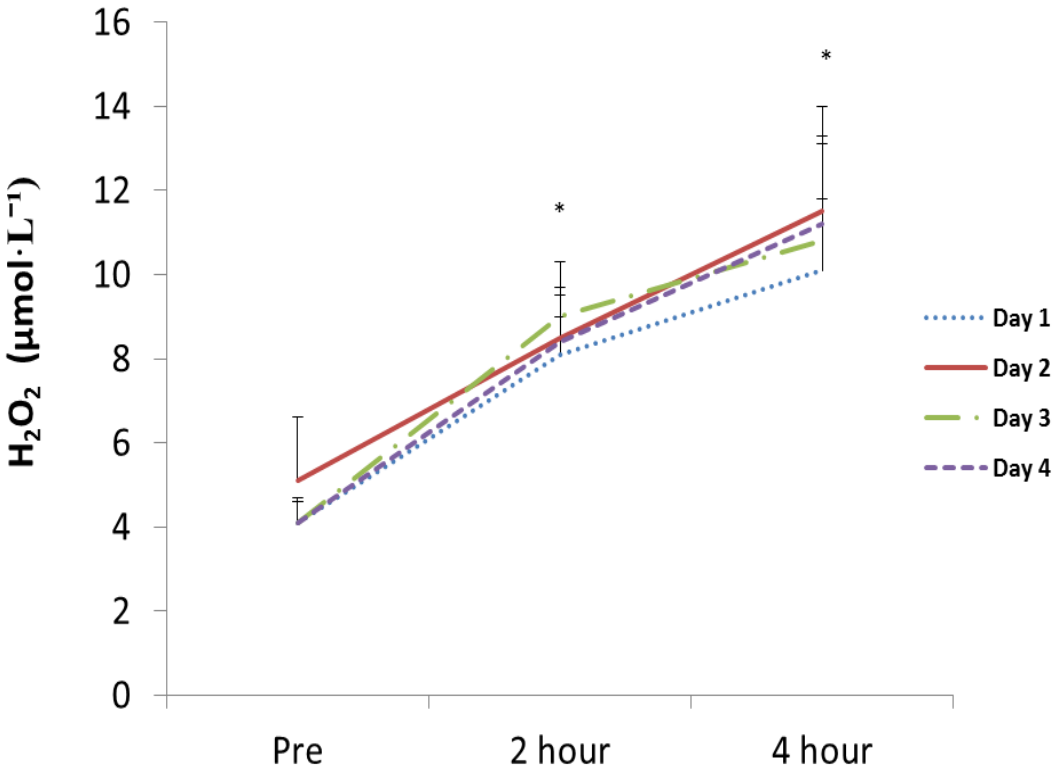

Figure 3. Blood H_2O_2 data of 16 healthy men before (Pre) and after (2 and 4 hours) consumption of a high-fat meal on four different occasions, separated by one week. Values are mean±SEM
No interaction (p=0.94) or day effect (p=0.94).
*Time effect (p=0.00); 2 hour and 4 hour > Pre

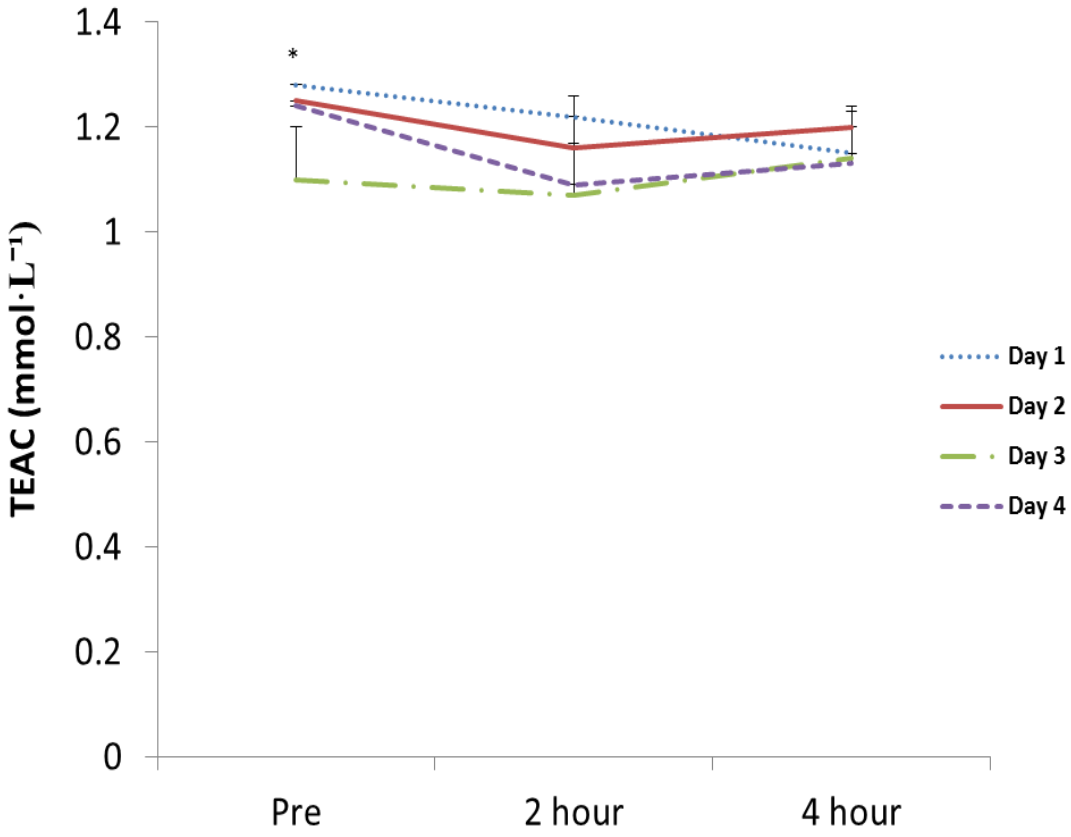

Figure 4. Blood TEAC data of 16 healthy men before (Pre) and after (2 and 4 hours) consumption of a high-fat meal on four different occasions, separated by one week. Values are mean±SEM
No interaction (p=0.38) or day effect (p=0.18).
*Time effect (p=0.02); Pre > 2 hour

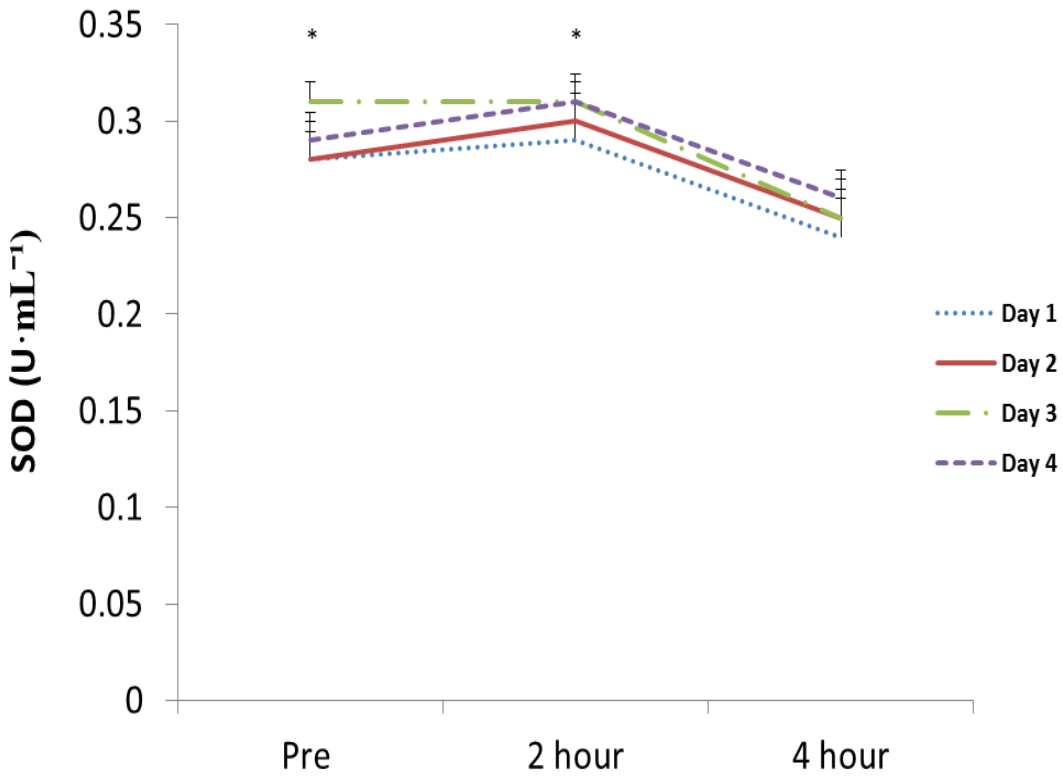

Figure 5. Blood SOD data of 16 healthy men before (Pre) and after (2 and 4 hours) consumption of a high-fat meal on four different occasions, separated by one week. Values are mean±SEM
No interaction (p=0.72) or day effect (p=0.61).
*Time effect (p=0.00); Pre and 2 hour > 4 hour

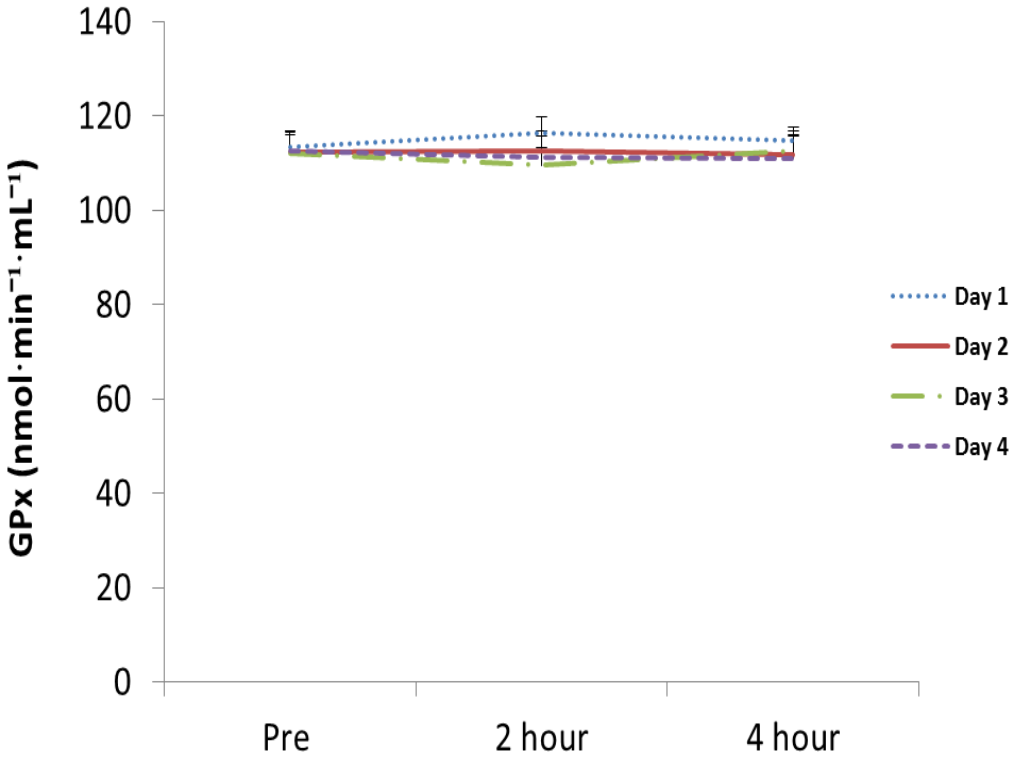

Figure 6. Blood GPx data of 16 healthy men before (Pre) and after (2 and 4 hours) consumption of a high-fat meal on four different occasions, separated by one week. Values are mean±SEM
No interaction (p=0.95), day (p=0.92), or time effect (p=0.81).

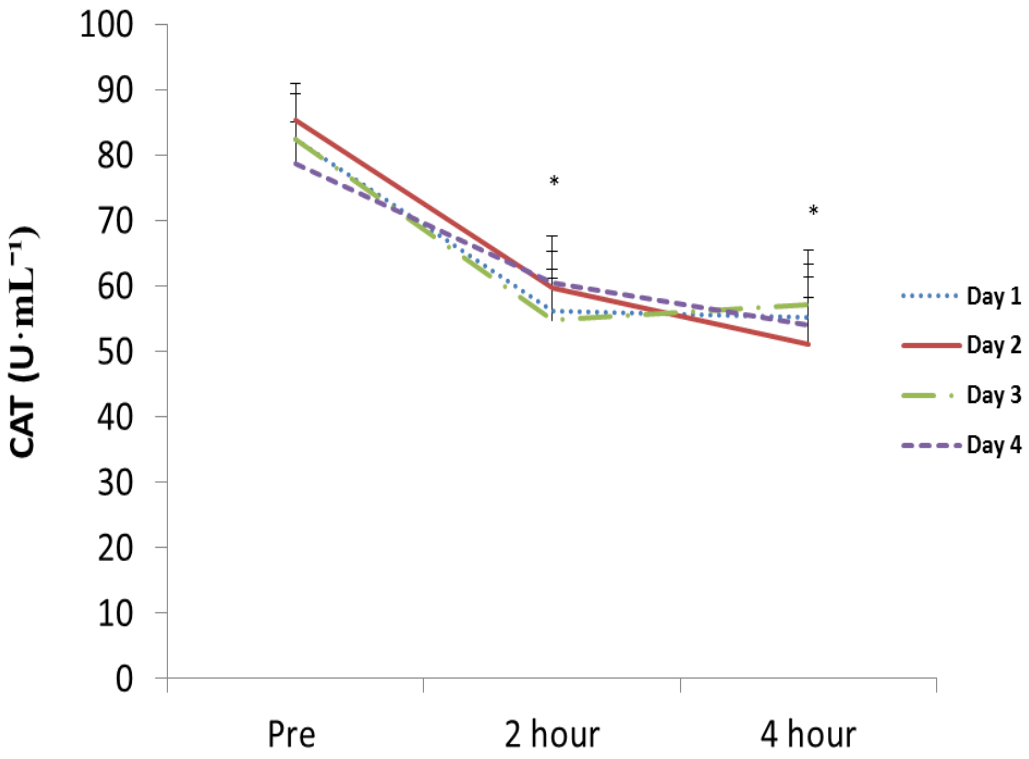

Figure 7. Blood CAT data of 16 healthy men before (Pre) and after (2 and 4 hours) consumption of a high-fat meal on four different occasions, separated by one week. Values are mean±SEM
No interaction (p=0.98) or day effect (p=0.94).
*Time effect (p=0.00); 2 hour and 4 hour < Pre

Appendix B – IRB APPROVAL

THE UNIVERSITY OF MEMPHIS

Institutional Review Board

To: Richard Bloomer, Innocence Harvey, Sang-Rok Lee, Trint Gunnels,
John Henry Schriefer, and Spencer Pope
Health and Sport Sciences

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

Subject: Influence of high fat feeding on up-regulation in antioxidant defense
(#2348)

Approval Date: August 31, 2012

This is to notify you of the board approval of the above referenced protocol. This project was reviewed in accordance with all applicable statuses 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 unless your consent is electronic. Electronic consents may not be used after the approval expires. 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.

Chair, Institutional Review Board
The University of Memphis

APPENDIX C- EXTENDED LITERATURE REVIEW

What is oxidative stress?

Oxidative stress is a condition in which the amount of pro-oxidants produced in the body exceeds what the antioxidant system can defend against. The production of these pro-oxidants, also referred to as "free radicals" and reactive oxygen and nitrogen species (RONS), is a normal cellular process that occurs on a daily basis and is necessary for cell signaling and other actions [2,4]; however, high levels of RONS can pose a problem as this may cause damage to macronutrients, DNA, and other molecules [40], possibly interfering with normal cellular function [2]. Therefore, it is ideal to keep the system in balance (e.g., production and antioxidant defense)

Promoters of RONS are multiple and include strenuous physical exercise, macronutrient consumption, and environmental factors such as cigarette smoke and other pollutants [41]. Here we will focus only on exercise- and feeding-induced RONS production and oxidative stress. Exercise has the ability to cause oxidative stress when performed at extremely high intensities or long durations, such as sprinting and marathon running [9-11]. Increases in oxidative stress can also result from the consumption of food—known as postprandial oxidative stress—often observed after ingesting meals that are high in total kilocalorie content and/or saturated fat [7,8]. These topics will be discussed in further detail later in this review.

Free Radicals

The excess RONS formed by activities such as eating and exercise are commonly referred to as free radicals, reactive oxygen species (ROS), or simply reactive species (RS). A free radical is any molecule having one or more unpaired electrons that is able to

subsist [42]. The most common of these radicals include singlet oxygen (O·), superoxide radical (O_2·-), nitric oxide (NO·), and hydroxyl radical (OH·). Hydrogen peroxide (H_2O_2) is not technically a free radical, as it has no unpaired electrons; however, it can be converted into OH· by partial reduction via dismutation of O_2·$^-$ [43]. Most molecules found in living substances are non-radicals; however, there are many mechanisms in the body by which free radicals can develop [42]. The most common mechanism involved in the endogenous production of RONS is the electron transport chain of the mitochondria [44], which is known to be responsible for the majority of our energy production. Though RONS have the potential be damaging, many of the reactions known to produce these free radicals are necessary for life [44].

Assessing RONS production

RONS can be measured in a variety of different ways. The optimal form of measure would be *directly*, through electron spin resonance (ESR) spectroscopy. This method has been compared to nuclear magnetic resonance and actually detects unpaired electrons [45]; however, this technique is quite costly and time consuming. Therefore, the more common measures are *indirect* and use the detection of multiple biomarkers in blood (particularly in the plasma and serum components) and other tissues (e.g. muscle) [46]. Biomarkers serve to provide an indication of oxidative damage; however, they cannot provide a direct measure of the actual RONS that are causing these changes. Indirect measure is the primary technique used when studying feeding-induced and exercise-induced oxidative stress in human and animal investigations. Biomarkers are also used in the detection of antioxidant activity.

Biomarkers Involved in Oxidative Stress

There is an array of biomarkers to choose from when measuring oxidative stress, whether actual oxidative stress biomarkers or antioxidant activity. Common biomarkers for oxidation include, but are not limited to, malondialdehyde (MDA), 8-hydroxy-2'-deoxyguanosine (8OHdG), and protein carbonyls via the 2,4-dinitrophenylhydrazine (DNPH) method; which are used to test for lipid, DNA, and carbonyl protein oxidation respectively [46]. Additionally, lipid peroxidation is often times measured with the thiobarbituric acid reactive substances (TBARS) biomarker and isoprostanes (perhaps most specifically).

There are two forms of antioxidants, enzymatic and non-enzymatic. Enzymatic antioxidants are produced endogenously and include, but are not limited to, superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). These antioxidants are commonly used as biomarkers when testing antioxidant activity. Glutathione (two forms: oxidized [GSSG], and reduced [GSH), vitamins E and C, Beta-carotene, and coenzyme Q_{10} (CoQ_{10}) (two forms: oxidized [ubiquinone], and reduced [ubiquinol]) are components of the non-enzymatic antioxidant family [46]. In addition, the trolox equivalent antioxidant capacity (TEAC) assay, the oxygen radical absorbance capacity (ORAC) assay, the ferric reducing ability of plasma (FRAP) assay, and the total radical-trapping antioxidant parameter (TRAP) assay can be used to measure "global" antioxidant capacity. Biomarkers are used to determine the redox status when observing the effects of exercise, meal consumption, and various treatments (i.e. antioxidants, statins, etc.), as well as in the study of diseases.

Oxidative Stress and Disease

Oxidative stress has been found to be involved in the onset, as well as the progression of many prominent diseases, including cardiovascular disease (CVD), cancer, diabetes (primarily Type II), ischemia/reperfusion, atherosclerosis, rheumatoid arthritis, and neurological disorders [47]. In addition, oxidative stress has been implicated in the process of aging and age-related diseases [3]. As previously mentioned, uncontrolled elevations in RONS can cause damage to DNA and other macromolecules, which could potentially lead to or exacerbate these diseases [48]. Moreover, inflammation, which is strongly associated with oxidative stress [49,50], is often involved in disease [51,52]. For example, obesity is generally accompanied by chronic low-grade inflammation [53] and increased oxidative stress [54], and is often strongly associated with multiple diseases mentioned above, such as cardiovascular disease (CVD), type II diabetes, and atherosclerosis. Inflammation is known to cause further production of RONS [55] and RONS, in turn, induces further inflammation [56]. Therefore, it is obvious that the process of RONS production is quite cyclical in nature and can become problematic when left uncontrolled.

Ways to Control Excess RONS Production

Controlling for excess RONS can be accomplished by taking certain medications/drugs, consuming specific nutrients, and through proper exercise (in an attempt to both decrease RONS production and up-regulate antioxidant defenses). Drugs such as statins and angiotensin-converting enzyme (ACE) inhibitors are used in the treatment of hypercholesterolemia and high blood pressure, respectively, and have been shown to lower oxidative stress [57,58]. The lowering of oxidative stress with the use of

ACE inhibitors is, in part, due to the reduced susceptibility of low-density lipoprotein (LDL) cholesterol to be oxidized and decreased NO· synthesis [57], as well as increased antioxidant enzyme activity [59]. Likewise, the use of statins has been shown to decrease oxidative stress by the up-regulation of antioxidant enzymes [60,61], in addition it has been shown to decrease the production of RONS [58]. Though these drugs have shown to be beneficial in reducing oxidative stress, their primary use is for the treatment of the specific diseases stated, with the noted impact related to oxidative stress considered to be a pleiotropic effect.

Antioxidants—Overview

Antioxidants are also seen as beneficial in reducing oxidative stress. The previously discussed enzymatic antioxidants react with radicals to form less reactive species; whereas, vitamins A, C, and E, carotenoids (beta-carotene, lycopene, etc.), thiols, and flavonoids (quercetin, catechin, etc.) actually scavenge RONS, and are referred to as antioxidant scavengers. Vitamins A, C, and E are exogenous antioxidants, meaning they cannot be synthesized in the body and must be provided from dietary sources. Vitamin E (primarily the tocopherols—and also the tocotrienols), as well as many of the flavonoids, has been shown to enhance antioxidant activity in response to lipid peroxidation and aid in the protection of red blood cells against hemolysis *in vitro* [62,63]. Studies show that vitamin C (ascorbic acid) can prevent the occurrence of lipid peroxidation when detectable, as well as inhibit reproduction following initial RONS generation [64,65]; though, α-tocopherol and other scavengers can only slow the rate of progression [64]. Resveratrol, a less traditional antioxidant found in red wine, is extremely potent and is

thought to bring about cardioprotective qualities [66,67]; however, limited *in vivo* work has been conducted using isolated resveratrol in human subjects.

Antioxidant Supplementation

Many investigations have been carried out to study the use of antioxidant supplementation with exercise and feeding in attempt to blunt the oxidative stress response that would normally occur following activities such as these. Ashton and colleagues performed an investigation focused on acute supplementation with 1000 mg of ascorbic acid (2 hours prior to exercise) and found a significant decrease of oxidative stress following VO_{2max} testing, along with significant increases in TEAC (pre exercise) compared to controls [5]. Fourteen days of supplementation with 1200 mg vitamin E alone has been shown to protect against DNA damage due to RONS, as well as attenuate lipid oxidation following an exhaustive exercise bout [68]. Additionally, Vincent et al. performed an 8-week supplementation study focused on the effects of antioxidants on oxidative stress following acute exercise [69]. They found that the supplement, which included a mixture of vitamin C and E and β-carotene, decreased exercise-induced oxidative stress and inflammatory markers, as well as lowered triglyceride and cholesterol levels in overweight adults; however, resting levels were unchanged from baseline values.

The use of antioxidants to reduce postprandial oxidative stress has been studied as well. Ghanim and associates found that 100 mg of resveratrol combined with 75 mg of polyphenols from grape extract taken 10 minutes prior to consumption of a high fat, high carbohydrate meal significantly reduced many measures of oxidative stress and inflammation [70]. A similar study noted attenuation in plasma lipid hydroperoxides, as

well as significant increases in other antioxidants (α-tochoperhol and ascorbic acid) following a mixed meal supplemented with a mixture of polyphenols (300 mg) when compared to controls [71]. A decrease in response for plasma TBARS in response to meal consumption was noted after four weeks of supplementation with vitamin E, as compared to pre-supplementation values, in patients with controlled diabetes [72]. Additionally, Neri and colleagues investigated the effects of 15 days of supplementation with a mixture of antioxidants (N-acetylcysteine at 600 g/d, α-tochopherol at 300 g/d, and ascorbic acid at 250 g/d) on markers of oxidative stress following a high-fat meal in subjects with type 2 diabetes mellitus (T2D), impaired glucose tolerance (IGT), and healthy controls [73]. Significant decreases in markers of oxidative stress were noted in all three groups, as compared to pre-supplementation values; however, healthy subjects exhibited the lowest degree of oxidative stress. A similar study investigated the effect of 8 days of supplementation with a mixture of antioxidants (including vitamins C and E, and thiols) following a high fat meal in subjects with T2D, IGT, and healthy controls [74]. It was found that the antioxidants decrease levels of oxidative stress in healthy controls and IGT subjects, but did not have an effect on subjects with T2D. As previously mentioned, diseased individuals present higher levels of oxidative stress than do healthy individuals; therefore, it is possible that the potency of these antioxidants was not strong enough to combat the augmented stress.

Adaptations to Exercise Training

As with many physiological systems, imposed stress results in adaptations that allow the system to better cope with such stress. This principle of hormesis has been well-described in relation to the generation of RONS and the subsequent decrease in

oxidative stress, either due to a decrease in RONS production or an increase in endogenous antioxidant defense [12,75,76]. This topic is further discussed in the following section.

It is evident that acute exercise can increase RONS production [1,10], observed in untrained individuals [77] as well as trained individuals exposed to exercise of different type [78], duration [79], and intensity [80]. However, reductions in oxidation levels have been noted following training [14,26]. This is thought to be partially due to an up-regulation of antioxidant activity, which has been noted in numerous animal [40,81] and human [82] studies following chronic exercise training. This enhanced antioxidant defense has been observed at rest, as well as following exhaustive exercise. Elosua et al. reported significant increases in GPx and glutathione reductase (GR) at rest following 16 weeks of aerobic training [29]. Additionally, significant increases in TEAC and GPx, as well as decreases in MDA were noted following 16 weeks of endurance training in older adult males [30]. Likewise, six months of resistance training elicited significant increases in plasma thiols, and decreases in TBARS in elderly subjects [26]. Rabinovich and colleagues investigated antioxidant enzyme activity following 8 weeks of interval training in patients with chronic obstructive pulmonary disease (COPD) and healthy subjects [27]. A significant increase in resting levels of GR, as well as total glutathione status was noted in healthy controls, but not in subjects with COPD. Therefore, it is possible that adaptations to exercise training in regards to oxidative stress are less prominent in the diseased populations. It is important to note that increases in antioxidant activity in sedentary subjects in response to acute exercise bouts have been seen as well [28,40]. However, these increases are typically transient and will return to basal levels

shortly following the cessation of exercise [83]; whereas with chronic exercise training, antioxidant levels tend to remain elevated. This suggests that subsequent bouts of exercise are required for an adaptation to occur.

A decrease resting levels of RONS has also been seen following exercise training, which leads to decreased oxidative stress. Attenuation of DNA damage (measured by 8-OHdG in muscle) was noted at rest in aged and young rats exposed to 9 weeks of endurance exercise compared to age matched sedentary controls [84]. Lower resting levels of oxidative stress biomarkers have been reported for exercise trained compared to untrained individuals, as well as for women compared to men [85]. Specifically, trained individuals exhibited lower levels of plasma MDA and PC as compared to untrained individuals; and MDA was significantly lower in trained women compared to trained men. Finally, when compared to sedentary controls of similar ages, soccer players have been noted to have lower levels of MDA at rest [86]. Taken together, these results provide evidence of decreased oxidative stress as an adaptive response to exercise training, which may be at least partially explained by the process of hormesis.

What is Hormesis?

The principle of hormesis suggests that there exists a relationship between the amount of stress applied and the adaptation that follows; therefore, it is a dose-response relationship [87]. This phenomenon can be generalized to many different biological systems, including the body's physical, biochemical, and molecular adaptations to exercise. Hormesis is closely associated with the commonly known SAID principle, which states that there are Specific Adaptations to Imposed Demands. Hormesis, as it

relates to exercise, states that if the body is repeatedly exposed to a stressor, it will develop favorable adaptations, which will serve to enhance physiological performance and overall health [12]; however, the adaptations appear both intensity and volume specific. Therefore, if not enough stress is applied to the system, the system will not adapt most favorably. Likewise, if the stress is too excessive, negative adaptations may occur [12]. Of course, what constitutes "too excessive" remains to be determined and is likely highly individualized.

What is Required for Hormesis to Occur?

The process of hormesis has been extensively studied in relation to exercise and oxidative stress [12]. Acute exercise, in particular in untrained individuals, is known to produce excess RONS production [88]; however, many studies have noted a decrease in oxidative stress following chronic training [82]. Therefore, it appears that increased RONS production is the stimulus needed to produce an adaptive response. Results from various studies have shown reductions in RONS biomarkers, as well as increases in antioxidants with adequate exercise training [82]. Miyazaki et al. reported increases in antioxidants and a decrease in oxidative stress following endurance training [14]. Subjects were exposed to an exhaustive exercise test pre and post 12 weeks of running 5 days/week at 80% of maximum heart rate for 1 hour each day. Post testing revealed increases in plasma biomarkers of SOD (17.1%) and GPX (11.5%) at rest, along with significant reductions in TBARS following exhaustive exercise when compared to pre-testing measures. Goto et al. investigated the effect of various training intensities on blood markers of oxidative stress in previously sedentary subjects [9]. Subjects participated in 12 weeks of training on a bicycle ergometer 5-7 times/week for 30

minutes each time. Prescribed intensities were set at 25, 50, and 75% VO_{2max}, and labeled mild-, moderate-, and high-intensity respectively. Mild-intensity training indicated no change in oxidative stress, while moderate-intensity training, though insignificant, tended decrease measures of oxidative stress. Significant increases in plasma 8-OHdG and serum MDA-modified LDL concentrations were found following high-intensity training. These results serve as an indication of the importance of intensity on the body's adaptive response to exercise-induced oxidative stress. Based on this study, and what hormesis entails, it appears that moderate to high intensity exercise (that which is capable of sufficiently increasing RONS) is required for the process of hormesis to occur.

Frequency and volume of training also play a role in the adaption process. Margonis and associates investigated the effect of overtraining on oxidative stress and found that 4-6 days of resistance training 4-6 sets/day, 1-10 reps/set at 75-100% of 1RM dramatically increased indices of lipid and protein oxidation, as well as decreased glutathione status and TEAC in trained men [89]; however, when volume, duration, and intensity were brought down for the 4 weeks following overtraining, levels returned to normal. Therefore, it appears that too much stress (and associated RONS production) will not bring forth positive adaptations. To the contrary, when the body is not taxed enough, there is a lack of adaptation, as reported by Tiidus and colleagues [90]. This study consisted of untrained men and women who participated in 8 weeks of aerobic training 3 days/week for 35 minutes/day at 70% of their VO_{2max}. Antioxidant activities of SOD, CAT, and GPX were assessed via muscle biopsy and were reported to have been unchanged following training. In summary, it appears that frequency, volume, duration, and intensity all influence adaptations to regular exercise training in relation to

antioxidant defense and oxidative stress. The ideal stimulus for antioxidant enzyme up-regulation remains to be determined.

Exercise-induced Oxidative Stress

Exercise-induced oxidative stress is the process that occurs when the amount of RONS being produced exceeds what the antioxidant system can defend against during or following a bout of exercise. It is well documented that this increase in RONS production can be brought on by both acute aerobic and acute anaerobic exercise [83]. The exercise bout must be at a high enough intensity and/or long enough duration to overwhelm the antioxidant system for it to cause oxidative stress. Lipid peroxidation, via MDA and TBARS, is the most common measure of oxidative stress in regards to aerobic exercise. However, the results are mixed as to whether aerobic exercise causes a significant increase in lipid peroxidation [83]. Many studies have noted an increase in lipid peroxidation incorporating intensities at or above 70% of one's VO_{2max} [83]; however, in the case of trained subjects, it has been suggested that even higher intensities may be required [1,91].

It is more difficult to determine a set level of intensity to produce oxidative stress in terms of anaerobic exercise, since this type of exercise encompasses both sprinting type exercises, as well as resistance activities. In the case of sprints, whether cycling, swimming, or running, most studies have shown an increase in oxidation, since they are naturally performed at a high level of intensity; however, some have reported no change [82]. For example, in our most recent work using very high intensity bouts of cycle sprints, we noted no increase in oxidative stress in a sample of 12 well-trained men in response to repeated cycle sprints [92]. In regards to resistance exercise, Bloomer et al.

reported an increase in protein oxidation following 15 squats at 70% of 1 RM in trained subjects; however, there were no changes in MDA or 8-OHdG [93]. Conversely, Ramel and associates found increases in MDA in both trained and untrained subjects after performing a circuit of 10 exercises at 75% of RM [94]. Likewise, Childs and co-workers also noted increased lipid peroxidation in untrained men submitted to 3 sets of 10 eccentric arm flexion repetitions at 80% of RM [49]. With the protocols being so varied, it is hard to determine what level of intensity induces oxidative stress in resistance exercise. However, given these data, it appears that type of exercise (eccentric vs. concentric), biomarker chosen, training status, and volume of exercise, along with level of intensity, should be considered when observing exercise-induced oxidative stress.

Magnitude of RONS Increase with Exercise

It is clear that given sufficient intensity and duration, oxidative stress may occur with exercise. The extent to which it occurs will depend on both of these aspects. Lovlin and co-workers investigated the effect of exercise intensity on RONS production [91]. MDA was measured at rest and following exercise at 40, 70, ad 100% VO_{2max}. A significant increase from 2.26+/-10 to 2.88 +/-.25 mmol/l was noted following the exhaustive exercise bout; however, MDA levels remained below resting up to 70% of VO_{2max}, indicating increased antioxidant activity. Likewise, Fatouros et al. noted a significant increase in MDA from 1.01+/-.12 to 1.39+/-.17 following a graded exercise test [30]. In addition, protein carbonyls have been shown to increase by 161%, 197%, and 245% following 30-, 60-, and 120-minutes of cycling at 70% VO_{2max} respectively [95]. Therefore, it appears that both duration and intensity can affect the magnitude of increase in exercise-induced oxidative stress.

Time Course of Increase—Oxidative Stress and Exercise

The increase in RONS is usually transient and varies depending on which biomarker is studied. Michailidis and colleagues conducted a study solely based on the appropriate times of measure for different indices of oxidative stress [96]. Eleven untrained men participated in 45 minute of treadmill running at 70-75% of their VO_{2max}, after 45 minutes the intensity was increase to 90% of VO_{2max} and subjects continued until volitional exhaustion. It was concluded that optimal measurement times were immediately post exercise for CAT, 1 h for TBARS, 2 h for TEAC, GSH, and GSSG, and 4 h for protein carbonyls. Bloomer et al. investigated the effects of cycling at 70% of VO_{2max} for 30 minutes and squatting at 70% of 1 RM for 30 minutes on blood markers of oxidative stress in trained subjects [1]. Conversely, the findings from this study indicated peak times for measuring protein carbonyls to be immediately after for aerobic exercise and 24 hours post for anaerobic exercise (though levels failed to reach significance even at peak for submaximal aerobic exercise). Therefore, it is likely that exercise training status, as well as type of exercise can affect the time course of increase in blood biomarkers of oxidative stress.

Mechanisms of RONS Production with Exercise

It is clear that exercise can cause an increase in RONS production; however, there are many different mechanisms through which this can occur. Furthermore, there are primary and secondary mechanisms. Primary mechanisms are responsible for directly causing an increase in RONS generation; whereas, secondary mechanisms can increase RONS production following oxidative damage from other mechanisms [97]. Here we will discuss only the primary forms of RONS production.

Mitochondrial RONS Generation

Mitochondrial generation is one primary mechanism known to increase RONS production during exercise [97]. The mitochondria are responsible for energy production (via ATP) and electrons are constantly being shuttled across the inner mitochondrial membrane. The demand for energy is dramatically increased when exercising; therefore, there is a greater influx of electrons to produce ATP. Additionally, it is well known that oxygen consumption increases with exercise. It is possible for some (2-5%) of the oxygen consumed to sustain one electron reduction, via electron 'leakage' from the mitochondria; thereby, becoming a superoxide radical [$O_2^{\bullet -}$] [97]. Therefore, it is highly likely that aerobic exercise will lead to the generation of superoxide radicals [97]. However, if an adequate amount of specific mitochondrial SOD is present, this production is inhibited [97].

Xanthine Oxidase

Another mechanism thought to be primarily responsible for RONS production during exercise is known as the xanthine oxidase system [98]. Xanthine oxidase is also involved in the generation of superoxides by the following reaction [98]:

$$Hx + O_2 + H_2O \rightarrow X + O_2^{\bullet -} + H^+$$

In addition, aerobic action of xanthine oxidase leads to the generation of singlet oxygen as a byproduct of the synergistic actions of O_2^- and H_2O_2 [99] and is a known contributor of lipid peroxidation [98]. However, SOD, in addition to CAT, can prevent production of RONS from this particular mechanism.

NAD(P)H Oxidase

NADPH oxidases are involved in the generation RONS during exercise as well [100]. These enzymes are associated with the plasma membrane and are located in many different cells, including the endothelium [101]. NADPH oxidase is known to catalyze the production of O_2^-, H_2O_2, and many other RONS [101]. It generates superoxide by way of donating an electron to oxygen, causing it to become reduced [101]:

$$2\ O_2 + NADPH \rightarrow 2\ O_2^- + NADP + H^+$$

Then the production of H_2O_2 comes from dismutation of the superoxide radical:

$$2\ O_2^- + 2\ H^+ \rightarrow O_2 + H_2O_2$$

As previously discussed, H_2O_2 can then be reduced to OH•, which is one of the most potent oxidants known to man [43]. However, the generation of these species through exercise may not be problematic, as they has been shown to elicit cardioprotection [102].

Summary of Exercise-induced Oxidative stress

The information presented here, as well as within other reviews [40,76], strongly suggests that exercise training provides the stimulus necessary for a lasting adaptive response of the antioxidant system to oxidative damage. This has been seen through increases in antioxidant activity and reductions in oxidant activity at rest, as well in response to exercise. Therefore, it is possible that any system that generates significant RONS will lead to an adaptation resulting in improved antioxidant defense, as is suggested by the principle of hormesis.

Postprandial Oxidative stress

Postprandial oxidative stress occurs when the redox system becomes imbalanced, in favor of RONS, after a meal and has been noted to occur in multiple studies. Tsai et al. reported an increase in urine oxidative stress biomarkers, as well as an increase in blood

triglycerides following a high fat meal [103]. High carbohydrate loads (75g of glucose or fructose) have also been shown to increase oxidative stress (measured by plasma MDA) in healthy subjects [104].

Though, not all meals induce the same amount of oxidative stress; the response is macronutrient and size dependent. Typically meals high in total kilocalorie and saturated fat bring about the highest oxidative stress response; whereas, meals that are high in protein and carbohydrate are not as likely to overwhelm the antioxidant system. A recent investigation tested the effects of different macronutrients on oxidative stress in healthy subjects [8]. The meals consisted of either heavy whipping cream (100% fat kcal; 60% saturated fat, 30% monounsaturated fat, 10% polyunsaturated fat), dextrose powder (100% carbohydrate kcal; 100% sugar), whey/casein protein powder (100% protein kcal), or a mixed meal with 33% percent of each. Significant increases in H_2O_2 were noted following consumption of the lipid meal compared to all other meals; in addition, significant increases in MDA, nitric oxide (NOx), and blood triglycerides were found in the area under the curve (AUC) in response to the lipid meal. Furthermore, a similar study compared postprandial responses to high lipid and high carbohydrate meals of differing portions (carbohydrate: dextrose at 75 and 150 grams; lipid at 33 and 66 grams) [7]. Significant meal effects were seen in triglycerides, H_2O_2, and MDA with lipid at 66 grams being higher than both dextrose meals. Furthermore, H_2O_2 levels were significantly higher in lipid at 66 grams compared to lipid at 33 grams, indicating that smaller lipid loads are not as likely to be problematic acutely in terms of oxidative stress; therefore, there is seemingly a dose-response relationship.

Triglycerides and Oxidative Stress

Enhanced levels of oxidative stress have been highly correlated with increased levels of triglycerides (TAGs) [7,8]. Therefore, it is possible that increased TAGs contribute to the increase in RONS and subsequent oxidative stress. Additionally, hypertriglyceridemia, a term for high levels of triglycerides in the blood, has been found to be a major risk factor for CVD [105-108], a disease that embraces multiple problematic conditions such as coronary heart disease, atherosclerosis, stroke, and many others. A meta-analysis run by Austin and co-workers documented that a 1 mmol/L increase in plasma triglycerides (fasted state) was analogous to a 14% and 37% increased of risk of CVD in men and women respectively, even when looked at independently of HDL and other risk factors [108]. Furthermore, it is estimated that 31% of Americans have hypertriglyceridemia [107], and over 20% of youths reported to have abnormal lipid levels [109].

One possible mechanism by which postprandial hypertriglyceridemia leads to the production of RONS and subsequent oxidative stress is by way of nitrotyrosine (NT). Nitrotyrosine is involved in the production of peroxynitrite, which is a highly reactive oxidant and nitrate formed by the reaction of O_2^- and NO• [110]. Elevated levels of NT have been noted following a high fat meal in healthy and diabetic subjects [111]. For this process to occur, O_2^- must already be present. Possible mechanisms involved in superoxide generation have previously been discussed. Therefore, it is likely that more than one mechanism is responsible for the production of RONS with postprandial hypertriglyceridemia.

A recent study conducted in our lab illustrates that high fat feeding induces a much greater oxidative stress response than acute exercise [13].Twelve exercise trained men completed three exercise protocols, as well as consumed a high fat meal, with one week between each of the tests. The three exercise tests consisted of moderate intensity and moderate duration aerobic cycling (60 minutes at 70% of VO_{2max}), high intensity and moderate duration interval sprints (5 sets of sprints for 60 seconds each at 100% of VO_{2max}), and maximal intensity and short duration exercise (10 sets of sprints for 15 seconds each at 200% of VO_{2max}) all performed on cycle ergometer. The meal consisted of whole milk, ice cream, and heavy whipping cream and portion received was based off of the participants' body mass (0.8 grams of fat, 1.0 grams of carbohydrate, and 0.25 grams of protein per kilogram of body weight). Oxidative stress, as measured by biomarkers MDA and H_2O_2, was significantly higher in response to feeding compared to exercise. In addition, a substantial increase in plasma TAG levels was noted following the test meal. However, it should be noted that the subjects studied were exercise trained and multiple studies have shown that oxidative stress is attenuated in trained subjects, as has been previously discussed. Although, this blunted response to exercise-induced oxidative stress that is observed following acute and strenuous exercise in trained men, possibly via enhanced antioxidant activity and decreased RONS production, is *not* observed in the postprandial state within the same individuals—as reported here and in other work [6,16]. Since high fat feeding has been shown to elicit greater oxidative stress than exercise of sufficient intensity and duration, it can be hypothesized that RONS production is significantly greater in response to high fat feeding—and may be a stimulus for greater adaptations in antioxidant defense if such high fat feedings were consumed on

a regular basis—in accordance with the principle of hormesis. If so, there exists the possibility that adaptations to repeated high fat loads will be adequate to protect against future insults of a similar magnitude.

Conclusion

Based on the hormesis findings linked to exercise and improved antioxidant defense, it is possible that repeated ingestion of high fat meals (which will cause a much greater increase in RONS) will lead to improved antioxidant defense. If this is the case, this improved defense may attenuate the rise in oxidative stress seen with high fat feeding. However, it is also possible that the system will be overwhelmed by routine high fat feeding, with no observed improvement in antioxidant defense and an actual increase in oxidative stress biomarkers. If so, it remains possible for oxidative stress to be lessened if TAG clearance is improved following repeated bouts of high fat loads. This line of questioning would require that an additional investigation be designed to determine methods of lowering the TAG response to feeding.

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