Oxidative Stress and Heart Rate Variability Following an Acute Bout of CrossFit

by

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Abstract

The purpose of this Dissertation was to provide a better understanding of the physiological stress involved in the newly popular style of exercise named CrossFit, and compare these findings to a more 'traditional' mode of exercise, treadmill running. Heart Rate Variability (HRV), which is a marker of parasympathetic activity and cardiac autonomic control, and oxidative stress, which are biomarkers of cellular damage are both independent markers of physiological stress. This dissertation covers two studies. The first study examined the affects of CrossFit and an intensity-matched bout of treadmill running on markers of HRV in ten CrossFit trained participants. The results of this study showed that both markers of HRV (LnRMSSD, LnHF) significantly decreased following both CrossFit and treadmill running. Furthermore, both markers of HRV were significantly lower following the trial when compared to treadmill running. The second study examined biomarkers of oxidative-stress following a bout of CrossFit and intensity-matched bout of treadmill running. Ten CrossFit Trained male athletes participated in this study. Markers of oxidative stress can be divided into two categories: oxidative-damage (LOOH, PC), and antioxidant capacity (FRAP, TEAC). The results of this study were mixed, with biomarkers of oxidative-stress LOOH and FRAP becoming significantly elevated following both trials and an unexpected significant decrease in PC and TEAC. No biomarker showed a trial dependent difference.

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List of Abbreviations

CF	CrossFit
ECG	Electrocardiogram
FRAP	Ferric-reducing antioxidant potential
HF	High frequency domain
HIIT	High-intensity interval training
HIPT	High-intensity power training
HR	Heart rate
HR_{max}	Heart rate max
HRV	Heart rate variability
LF	Low frequency domain
LF:HF	High frequency to low frequency ratio
LnHF	Log transformed high frequency domain
LnRMSSD	Log transformed square root of the mean sum of the squares of differences between adjacent R to R (normal to normal) intervals
LOOH	Lipid hydroperoxides
PC	Protein Carbonyl
RMSSD	Square root of the mean sum of the squares of differences between adjacent R to R (normal to normal) intervals
TEAC	Trolox equivalent antioxidant capacity

TM	Treadmill
VLF	Very low frequency domain

CHAPTER I

INTRODUCTION

High intensity power training (HIPT) has become a popular form of exercise. The relatively new style of training involves multiple joint resistance exercises while attempting to maintain high power outputs (63). The best-known form of HIPT, CrossFit, is globally popular and has garnered a strong faction. The growth in popularity has occurred for several reasons: easy access to programming, a short time commitment, and anecdotal results in body composition and aerobic fitness. Despite these claims, there is only one scientific study currently available on CrossFit (63). Therefore, it is evident that much of the claims behind the effectiveness of CrossFit are unsubstantiated.

Although there is very little information specifically about CrossFit within exercise science literature, it is clear that the approach to exercise training is physically demanding. Exercise poses a challenge to the maintenance of homeostasis. However, the disruption of homeostasis becomes greater as the intensity increases (55, 65). Heart rate variability (HRV) and oxidative stress are two independent physiological markers that are often used to evaluate alterations of cardiovascular activity and physiological stress, respectively. Research determining post exercise HRV and oxidative stress following a bout of CrossFit is warranted. The following paragraphs examine these markers as they relate to exercise and risks to health.

Heart rate variability (HRV) is commonly used in research and clinical settings to noninvasively assess autonomic nervous system modulation of the cardiovascular system (20–24, 51–53, 66). HRV involves the measurement of the oscillations that occur between consecutive R-R intervals derived from an electrocardiograph (ECG). During exercise a shift of autonomic control from parasympathetic to sympathetic dominance occurs, resulting in acute drop in HRV (7, 11, 31, 49, 50). Depression of HRV continues for a prolonged period of time following the cession of an acute bout of exercise. The magnitude and duration of depression is dependent on the intensity or modality of exercise (31, 62). Post exercise depression of HRV is associated with dyshomeostasis and decreases in performance (18, 65). Interestingly, the rate of HRV return mimics that of physical recovery (18, 65), which makes HRV an important marker for post high-intensity exercise.

Oxidative stress results from free radical damage. Free radicals are atoms or molecules that contain one or more unpaired electrons, which make them highly reactive (4, 19). Oxidative stress is the bi-product of normal cellular activity and is commonly referred to in literature as reactive oxygen species (ROS). Increased levels of ROS are central to dyshomeostasis during exercise by means of protein damage and lipid damage (4, 16, 17, 47). Acute exercise leads to increased ROS production and consequent oxidative stress (2, 33, 46, 56, 57). The duration and magnitude of ROS production is proportional to the oxidative stress that is experienced. Low to moderate levels of ROS and subsequent levels of oxidative stress are considered healthy and fundamental to cellular adaptive responses to exercise training (4, 43). Although chronically elevated levels of ROS can be indicative of pathology (48, 64, 67), the response to acute physical

activity is proportional to the stress experience (2, 3, 8, 34), and therefore indicative of physiological stress.

In summary, due to the high intensity nature of CrossFit it is reasonable to expect a greater shift in cardiac-autonomic modulation and higher oxidative stress response compared to traditional exercise. The primary purpose of this study is to determine the independent responses of HRV and oxidative stress following an acute bout of CrossFit compared to a high-intensity intensity (90% of HR max) bout of treadmill exercise. The treadmill protocol will be included in the study for comparative purposes.

SIGNIFICANCE OF STUDY

In terms of widespread popularity, CrossFit is a relatively new style of training. CrossFit is perhaps best described as high-intensity power training (HIPT) due to the physically demanding and time component-based workouts. Very little research is currently available on CrossFit despite its popularity and unsubstantiated claims.

The occurrence of physiological alterations following an acute bout of high-intensity exercise is well established (2, 15, 29, 45, 55). Studies show that post exercise alterations result in increased susceptibility to untoward cardiac events as well as damage to cellular components (1, 4, 20, 31, 57). Heart rate variability (HRV) and oxidative stress are two independent physiological markers commonly used to evaluate alterations of cardiovascular activity and cellular stress following acute bouts of exercise. Because of the lack of knowledge surrounding the high-intensity programming of CrossFit, further research and investigation of the physiological responses that occur following an acute bout is warranted. Therefore, it is the primary purpose of this study to investigate the

alterations of HRV and oxidative stress following an acute bout of CrossFit and compare the alterations to that of a traditional high-intensity treadmill bout.

ASSUMPTIONS

The following assumptions were made: that all subjects are truthful in regards to inclusion criteria; that training state will allow subjects to perform the bouts of exercise at the necessary intensity to elicit a physiological response; that HRV and oxidative stress markers are sensitive to this type of physiological stress. Furthermore, the use of VO_{2max} will be used as a tool to predict CrossFit performance and therefore assist in the selection of subjects to better homogenize the population. The assumption being that VO_{2max} will accurately predict performance, such that lower VO_{2max} would predict lower number of rounds completed in CF and higher VO_{2max} would predict a greater number of rounds completed in CF.

LIMITATIONS

There are several limitations with the following studies. The first limitation is the training status is not controlled in this study. Increased tolerance to exercise stimulus has been well established in participants who engage in regular physical activity (55). Secondly, outside caffeine and dietary supplementation (i.e., vitamins) restriction prior to the exercise bouts, diet is not controlled. Thirdly, this study will only involve male subjects and will not allow a determination of gender specific alterations or differences in physiological responses in regards to a CrossFit workout. Lastly, the bout of CrossFit selected will not fully represent CrossFit training as a whole due to the continuously varied nature of the programming. Therefore, the findings of this study will be limited and only relate to CrossFit style workouts that are similar in duration and intensity.

STATEMENT OF RESEARCH OBJECTIVES

The overall objective of this research will be to provide a better understanding of HRV and oxidative stress following an acute bout of CrossFit. Furthermore these responses will be compared to a traditional modality of exercise (treadmill running). This bout will be a traditional high-intensity bout of treadmill exercise used as a comparative control.

The specific objective of the first study is to determine the effect of a single bout of CrossFit and the high-intensity treadmill bout on the following markers of cardiac-autonomic modulations:

Cardiac-Autonomic Modulations (HRV)

a. The time domain measure of root mean square of the standard deviation of N-N intervals (RMSSD)
b. The frequency measure High Frequency measures within the recorded power spectral density (HF)

The specific objective of the second study is to determine the effect of a single bout of CrossFit and high-intensity treadmill bout on the following markers of oxidative-stress responses within blood serum samples obtained:

Oxidative-stress:

c. Oxidation of Protein: Protein Carbonyls (PC)
d. Oxidation of Lipids: Lipid Hydroperoxide (LOOH)
e. Antioxidant Capacity: Trolox Equivalent Antioxidant Capacity (TEAC)
f. Antioxidant Potential: Ferric-reducing Antioxidant Potential (FRAP)

CHAPTER II

LITERATURE REVIEW

OVERVIEW

CrossFit is a relatively new form of "high intensity power training" (HIPT) that has been growing in popularity within the mainstream. However, very few published scientific studies have examined this form of exercise empirically. As such, the current study will measure blood markers of oxidative stress and cardiac-autonomic modulation before and after an acute bout of CrossFit. The remaining sections contained within this chapter will provide a detailed description of CrossFit, cardiac-autonomic control, and oxidative stress.

CROSSFIT

High-intensity interval training or "HIIT" has become a popular form of exercise within mainstream. HIIT exercise combines multiple short bouts of high intensity exertion (i.e. above anaerobic threshold) with brief periods of complete rest or active recovery repeatedly for a prescribed period of time (e.g., 2-minute work intervals with 1-minute recovery intervals for 30 minutes) (39). Numerous studies have examined the effectiveness of HIIT and have demonstrated significant gains in aerobic capacity (25, 32, 39, 68).

Recently, a form of HIIT training has emerged that removes scheduled rest periods and incorporates "power" training within its format and has been termed "high-intensity power training" (HIPT) (63). The goal of HIPT is to complete a prescribed amount of work as fast as possible. In other words, participants perform high-intensity, multiple joint resistance-based movements while maintaining a high power output (63). The lack of prescribed rest periods, the maintenance of a high power output, and the time-oriented goals (i.e., completing the overall workout as quickly as possible) are the major components that distinguish HIPT from HIIT (63).

CrossFit is a form of HIPT that attempts to train a wide spectrum of physical fitness components within one exercise program. Even though CrossFit is a newer approach to exercise, it is growing rapidly in popularity around the world (Smith et al), but has yet to gain footing within the scientific literature. For example, a recent (1/29/2014) Google search of "Crossfit exercise" yielded over 14.2 million results. However, a PubMed search of the same phrase yielded only 5 scientific studies. Therefore, it is obvious that much of the growth of CrossFit is based on unsubstantiated claims.

The following five paragraphs provide an overview of the CrossFit standards and methods of programming. Please note that the information is referenced directly from CrossFit material (i.e., references 25 and 26), which is not validated by empirical evidence. Therefore, it is within this chapter only to discuss the principles behind their methodology.

Discussion from CrossFit material:

Theoretically, the foundation of CrossFit training began with the objective to best prepare an athlete for any physical contingency and to prepare them for the "unknown and unknowable" (71). The programming is based on enhancing an individual's competency across 10 general physical skills (70) that CrossFit state as the following: Cardio Respiratory Endurance, Stamina, Strength, Flexibility, Power, Speed, Coordination, Agility, Balance, and Accuracy. CrossFit training purportedly achieves fitness acquisition across these skills by utilizing continuously varied, high-intensity, functional movements during each workout (71). The program combines multi-joint resistance exercises (e.g., deadlift, squat, power clean, and shoulder press), gymnastic-based exercises (e.g., Olympic ring work, hand stand, and parallel bars), and traditional endurance-based modalities (e.g., running, rowing and biking). There are several variations and differences between CrossFit workouts. The only consistent variable is the goal of moving weight for the heaviest load or as fast as possible in the given period of time (i.e., HIPT training).

The daily CrossFit workout, as presented by CrossFit Head Quarters, is commonly referred to as the, "workout of the day", or "WOD" (63). The WOD varies from day to day and typically includes a combination of the exercise listed above. However, the primary focus of a particular CrossFit workout may be categorized as mainly gymnastics based, Olympic lifting based, or "metabolic conditioning" based (e.g., endurance). Each workout requires different physical demands and therefore varying the workout intensities. Some WODs are used as

performance benchmarks and are typically named after a girl: e.g. “Fran” and “Cindy”.

Two popular styles of CrossFit WODs are the “classic triplet” and as many rounds as possible (or AMRAP). The classic triplet is a three round workout with the repetition scheme of 21-15-9. An example would be “Fran”, which consists of a “thruster” (i.e., an exercise that involves the front squat and press) followed by pull-ups. All repetitions of the first exercise must be completed before beginning the next exercise. In other words, 21 thrusters and pull-ups must be completed before moving on to the round of 15, and so on. The overall goal of this type of WOD is to complete the prescribed exercises and reps as fast as possible; no rests are scheduled or required.

The goal of an AMRAP is exactly as the name implies, to complete as many rounds of a given exercise(s) as possible within a given time period. An example of an AMRAP would be “CINDY”, which involves completing as many rounds of 5 pull-ups, 10 push-ups, and 15 air squats in 20 minutes. The goal is to perform as much work as possible in a giving time period.

Most CrossFit facilities create and post their own WODs. However, CrossFit headquarters posts a new WOD everyday on the Internet (www.crossfit.com) for anyone to attempt. Further examples of WODs and their potential variations can be examined in Table 1.

Proponents of CrossFit claim that the rationale of program design is based off empirical data and is “science-driven” (70), which is partially true, in that CF parallels

well quantified forms of exercise such as HIIT and Circuit training (5, 6, 30, 37–40, 44, 68). However, as previously stated only one published study has been performed on CrossFit. Smith et al. determined the effects of a CrossFit-based 10-week program on aerobic fitness and body composition in apparently healthy adult subjects. The results of the study revealed a 12% and 10% improvement in VO_{2max} and a 4.2% and 3.4% reduction in body fat % in male and female participants, respectively. Obviously, the study demonstrated a positive adaptation in aerobic fitness and body composition after 10 weeks of CrossFit training. Smith et al. (63) provides a glimpse into the possible benefits of chronic exposure to CrossFit. However, for being the first of its kind, this study did not demonstrate the physiological changes of an acute bout. Furthermore, there is no comparison to a traditional bout of exercise, e.g. treadmill running. Even though Smith et al. provides a novel first step, the overall lack of scientific data along with the drastic increases in the global popularity of this publicly accessible program warrants further investigation.

CARDIAC ACTIVATION

The heart is a complex organ that requires the synchronization of several tissues and physiological processes to properly function. The cardiac muscle fibers are responsible for the contraction of the heart. These fibers share many similar properties as skeletal muscle fibers in that they are striated, contain contractile proteins, require calcium for muscular contraction, and can control the amount of force production (29, 55). Despite these similarities, cardiac muscle fibers have several important and distinctive differences. In many respects cardiac tissue is expressed as a biochemically and physiologically unique fiber type as compared to skeletal muscle fibers.

Furthermore, unlike skeletal muscle fibers, cardiac fibers are more homogeneous in composition. Cardiac fibers contain a large number of mitochondria and are more oxidative than the highly endured type I skeletal muscle fiber. Another physical distinction is the short length and tight series formation of the fibers, connecting each other through intercalated discs (55).

The intercalated discs allow the transmission of an electrical impulse from fiber to fiber, which is important when considering the depolarization of the cell. The intercalated discs create a leaky membrane, allowing ions to cross from one another. The importance of this characteristic is when one cell contracts, all interconnected cells contract. The unique functional arrangement of the discs can be referred to as syncytium, the firing of all cells as a single unit (29). For proper contraction of the heart it is vital that syncytium be under some form of control or cadence.

It is the function of the sinoatrial node (SA node) to set the cadence for myocardial depolarization. The SA node is comprised of highly excitable tissue that possess a lower resting membrane potential then the other myocardium tissue, which allows it to depolarize first. Heart rate is controlled by both parasympathetic and sympathetic stimulation. The rate of depolarization of the SA node of the heart (pace maker) is determined by the fluctuations and dominance of parasympathetic/sympathetic activity (21, 29). The dynamics of this interplay becomes a pivotal role on resting heart rate and are sensitive to changes in the internal environment.

Parasympathetic fibers (vagus nerve) are supplied by the cardiovascular control center in the medulla oblongata. The vagus nerve innervates the SA and atrioventricular (AV) nodes of the right atrium. When activated the nerves release acetylcholine on to

muscarinic-cholinergic receptors of the SA and AV nodes, reducing their depolarization and decreasing heart rate (55). Vagal activity is the typical stimulation at rest and is referred to as parasympathetic tone (29). Regulation of HR during resting periods is accomplished through increased or decreased parasympathetic activity allowing HR to increase or decrease respectively.

Sympathetic fibers (cardiac accelerator nerves, T-1 through T-4) are also supplied by the cardiovascular control center (29). The cardiac accelerator nerve innervates the beta-receptors of the SA and AV nodes as well as the beta-receptors on the ventricular myocardium. When the sympathetic nerves are stimulated they release epinephrine (E) or norepinephrine (NE) on to the receptors of the SA and AV nodes causing them to depolarize more rapidly, resulting in a more rapid HR. Furthermore, stimulated beta-receptors on the ventricular myocardium cause an increase in contractility (or strength of contraction), which increases the stroke volume. The combination of beta-receptor activation results in an increased cardiac output. Any increase in HR below 100bpm is primarily responsible to a withdrawal of parasympathetic activity, while anything above 100bpm is typically due to increase in sympathetic activity (55). The continuous changing of the parasympathetic/sympathetic ratio in response to physiological stimuli is known as sympathovagal balance and can be examined through the measure of heart rate variability. Heart rate variability is a commonly used measure of the autonomic nervous systems control of the heart.

HEART RATE VARIABILITY

The autonomic nervous system (ANS) plays a key role in regulating the cardiovascular system (21, 29, 55). The sympathetic and parasympathetic branches of

the ANS harmoniously increase and decrease, respectively, the heart's rate and activity. The degree of influence of these systems is continuously changing and is often described as sympathetic-to-parasympathetic ratio or sympathovagal balance, which leads to oscillations in heart rate. Therefore, heart rate variability (HRV) is commonly used in research to noninvasively assess ANS modulation of the cardiovascular system (12, 21, 22, 27, 69).

Measurement of HRV is accomplished through the quantification of the variations that occur between successive R-R intervals on an electrocardiogram (ECG). The "R" of the R-R interval is the peak of the QRS complex during ventricular depolarization. The R-R interval itself is the distance or time between QRS complexes (21). In other words, HRV measures the variation of time in between consecutive heartbeats.

MEASUREMENT TECHNIQUES

Time domain analysis of HRV involves transforming the ECG segment in which HRV is analyzed to a graph called a tachogram (21). The time domain is a statistical calculation of adjacent R-R intervals. The y-axis of the tachogram represents the time (usually in milliseconds) between each consecutive N-N interval, while the number of total beats is represented on the x-axis (21). There are a large number of time domain measures used in the literature (21). However, the root mean square of the successive differences (RMSSD) is one of the more common time domain parameters, as it appears to be an accepted marker for parasympathetic modulation (21, 51–53, 65). Traditionally, time domain measures were reported to require long-term recordings, such as a 24-hour period, and relatively stationary conditions (21). However, Tulppo (69) demonstrated that time domain measures in short recording periods (e.g., around 5-minutes)

demonstrate a higher sensitivity to cardiac-autonomic modulations. Therefore, this dissertation will use RMSSD as the time domain measure of HRV for various 5-minute segments recorded during the pre- and post-exercise conditions.

The frequency domain is another method to analyze HRV that is often coupled with time domain measures in research (15, 21, 54). However, this method of HRV is more complex, yet has been suggested appropriate to use in short-term (i.e., ~5-minute) recordings (21). The frequency domain utilized complex algorithms such as the fast Fourier transformation to create a power spectral density. From the power spectrum, the power of the entire waveform (0.00 to 0.40 Hz) is evaluated as Total Power (TP). The power spectrum may also be characterized at different frequencies, such as very low frequency (VLF, <0.04Hz), low frequency (LF, 0.04-0.15Hz), and high frequency (HF, 0.15-0.40Hz) power. What the power spectrums represent are often debated (7). There appears to be little evidence to explain what VLF represents (21). Though LF is often reported in research, its meaning is still unknown as it has been shown to be influenced by parasympathetic and sympathetic modulation, as well as baroreflex sensitivity (7). However, HF is widely accepted as the representative marker of parasympathetic activity and controlled almost solely by the vagus nerve (31), as atropine and vagal nerve removal abolishes this marker (7, 27). Furthermore, the ratio between LF and HF (LF:HF) is commonly used to represent sympathovagal balance (21, 31). However, Billman discusses several factors that demonstrate the unreliability of the LF:HF ratio as an accurate marker of symapthovagal-balance (7).

In order for the LF:HF ratio to be considered an accurate measure of the balance of activity between sympathetic and parasympathetic nervous systems the following three

assumptions need to be made. First, SNS and PNS must be the major/exclusive factors involved in LF and HF frequency, respectively. Second, changes in one branch of the ANS must be met with corresponding changes in the other. Third, a linear relationship must exist between cardiac and ANS activity. According to Billman, the SNS in not the major contributor to LF frequency (7). Furthermore an alteration in one branch does not directly correspond to changes in the other as the relationship between ANS and cardiac activity is complex and non-linear (7). Therefore, the assumptions necessary for LF:HF ratio to be considered accurate do not remain consistent, decreasing the reliability of that marker (7).

The measurement of LF and HF components are often made in absolute values of power spectral density (i.e., in milliseconds squared), which represents the total amount of time the heart spent in each power domain. Each frequency domain is also normalized to account for the total power and VLF weight by dividing either LF (LFnu) or HF (HFnu) by (total power-VLF) and multiplying by 100. Furthermore, HRV data within research studies often violate the assumption of normality and is naturally logged transformed (ln) (65).

PHYSIOLOGIC MECHANISMS OF HRV

The direct relationship between HRV and parasympathetic and sympathetic activity is known. Furthermore, it is known that exercise increases the sympathetic response in the body (29, 31, 55, 69). Exercise is related to a decrease in RMSSD and HF, a phenomena typically observed during exercise (11, 27, 31, 49). Increasing sympathetic activity and parasympathetic withdrawal results in a maximum loss of variance and no further alteration can be examined (e.g., total abolishment of HF) (13,

14). However, there are reports where HF levels increased during exercise despite sympathetic activity, which is believed to be due to ventilation frequency and excitation of the vagal nerve (50, 66).

The rate of which HRV returns to normal is also an indication of parasympathetic control of the cardiovascular system (11). There are several physiological reasons why exercise alters HRV and its recovery such as: parasympathetic withdrawal, circulating sympathetic nervous activity increases, hormones increase, breathing rate, and exercise induced increases in blood pressure (29, 55). Of the aforementioned, the most obvious components that are responsible for alterations of HRV post exercise are changes in parasympathetic and sympathetic activity (21, 66).

Previous sections detail how parasympathetic and sympathetic control work to increase or decrease heart rate. Parasympathetic-mediated changes in heart rate occur more rapidly than sympathetic-mediated effects (29, 66). Meaning that when control is parasympathetic dominant heart rate will fluctuate more readily due to its increased sensitivity, creating more variability between beats (21, 66). Sympathetic dominance results in a rapid, but more consistent/less varied heart rate, and subsequently a decreased HRV (21, 66). The differences in sensitivity of parasympathetic vs. sympathetic reaction may be caused by several factors: kinetics of β_1-adrenergic vs. muscarinic receptors, different kinetics of adenylate cyclase vs. phosphodiesterase, or the fast opening parasympathetic K_{ach} channels (66).

As the duration of a submaximal exercise increases or the intensity of a maximal bout of exercise increase so does the level of circulating hormones of epinephrine (E) and norepinephrine (NE), aldosterone, and angiotensin II (55). Increased adrenal hormones

such as E and NE are well known to increase heart rate and contractility and subsequently decrease HRV. Decreases in blood pressure and glucose may also contribute to increased levels during exercise, through the increased activation of the sympathetic nervous system (55). Circulating levels of E and NE remain elevated following removal of sympathetic activity following bouts of exercise. Aldosterone and angiotensin II increase linearly with exercise intensity and exert their effect on blood pressure resulting in retaining water from the kidney and vasoconstriction respectively (66).

Respiration plays a role in parasympathetic activity because the frequency is driven by the vagus nerve (50, 66). Respiration has been shown to cause fluctuations in the heart rate in a process called respiratory sinus arrhythmia (66). It is believed that exacerbation of respiratory sinus arrhythmia during exercise may be the cause increases in HF (50, 66). Further research is needed in regard to this relationship.

HRV AND EXERCISE

The measurement and interpretation of HRV originated clinically as a pragmatic instrument to predict untoward cardiac events (1, 21, 31, 69). Depressed HRV; that is, less variation of time between consecutive heartbeats, has been related to disease and increase risk for untoward cardiac events in clinical and non-clinical populations (21, 31, 69). However, recent applications involved the measure and analysis of HRV prior to or following bouts of exercise. The rebound of vagal activity, as measured through HRV, is believed to be an indirect measure of the bodies return to homeostasis (65). Furthermore, the recovery of HRV mirrors that of physical recovery and performance, becoming a viable tool in gauging physiological stress and the prescription of exercise (18, 36, 51–53, 65).

The parasympathetic nervous system plays a large role in recovery of HRV after physical activity. Goldberger et al. (27) demonstrated this relationship during exercise recovery through the use of the parasympathetic blocking drug atropine. Subjects participated in a maximal effort cycling bout and recovered in a seated position for 5min to record HRV. When the subjects were given the drug there was no recovery of time domain measures (SDNN, Root Mean Square of the residual (RMS), RMSSD). In contrast, there was a significant recovery of RMS and RMSSD within the 5min recovery when the subjects were not provided with atropine.

These findings demonstrate the role of the parasympathetic system in recovery. Furthermore, initial recordings of HRV post exercise were approximately the same as the parasympathetic blunted group, demonstrating the extent of parasympathetic depression following exercise (27). Several other studies confirmed these findings of decreased HRV post acute exercise, revealing that the extent and duration of HRV depression is related to the type, range, and duration of activity (15, 31, 69).

Exercise intensity plays a role in alterations of cardiac autonomic modulations. In a study by Parekh and Lee, subjects performed isocaloric treadmill bouts at 50% and 80% of $VO_{2reserve}$. The findings indicate a reduction of HRV occurs following both bouts of exercise. However, HRV was significantly lower following the bout of 80% $VO_{2reserve}$. SDNN showed a similar trend with overall reduction post exercise, but the greater reduction was observed in the 80% intensity session. Frequency domain measures showed a reduction of TP, HF and LF in the 80% intensity session. HF measures were significantly reduced only during the first time point of the 50% intensity session.

Normalized LF (LF/[TP-VLF]x100) and LF/HF ratio increase was only observed in the 80% intensity session.

The findings of a study performed by Buchheit et al. supports Parehk and Lee in that they observed similar decreases in HF markers for high intensity exercise (~105% of final running speed at VO_2max (V_{IFT})) and maintenance of HF markers following moderate intensity exercise (65% of V_{IFT}) (15). However, the time domain RMSSD demonstrated no depression in the moderate intensity trial, unlike what was observed in the Parekh and Lee study. This may be in part due to differences in protocol or differences in time domain measurement techniques.

Despite the findings of the aforementioned studies that have observed an overall reduction of HRV markers post acute exercise, Pober et al. demonstrated that markers of HRV might increase following a bout of submaximal exercise. Eleven healthy college-aged males participated in a 60min cycling bout at 65% VO_{2peak}. Measurements of HRV were obtained while subjects were in a supine position and breathing at a rate of 12bpm; time points taken were pre, 1, 3, 6, and 22hours post exercise. The time domain measures of SDNN and RMSSD yielded no significant changes post exercise. However, a positive numerical, but statistically insignificant trend was observed in pNN50 with increases at time points 1, 3, and 6. Frequency measure showed increases in HFNU at all time points following exercise. Similarly, there was a decrease in LFNU and LF:HF values. The review of the aforementioned studies suggests the overall mechanisms of control/recovery of cardiac autonomic modulation differ between maximal and submaximal exercise (15, 49, 54).

A large amount of the literature on exercise and HRV is focused on endurance exercise. However, resistance based exercise has been shown to affect cardiac autonomic modulation as well (18, 31, 62). For instance, Heffernan et al. (31) examined HRV recovery following aerobic and resistance training modalities. Fourteen moderately active males performed a resistance bout of exercise of 3x10 (10RM) and a 30 minute aerobic cycling bout at 65% VO_{2peak}, in random order. These findings suggest that HRV was depressed in both groups but the resistance bout had a more dramatic effect. Furthermore, Rezk et al. (62) where resistance exercise bouts were examined by intensity. Two exercise sessions (40%[E40%], and 80% [E80%]) performed a series of resistance exercises (E40%=3x20, E80%=3x10) and rested in the seated position to record ECG. The results of this study revealed a significant decrease in HF as well as a significant increase in both LF and LF:HF ratio for both exercise intensity sessions. Interestingly, there was no significant difference between E40% and E80%. Though further research is needed, this demonstrates that within the mode of resistance exercise, intensity may not be the factor in alterations in HRV.

As shown in the previously discussed studies, exercise intensity within aerobic/endurance exercises plays a major role in the magnitude of HRV depression. Findings from several studies demonstrate the relationship between high intensity and a greater drop in cardiac-autonomic modulation (31, 35, 62). Modality plays a role in the degree of HRV depression as well. Resistance training depresses HRV to a greater extent than endurance training (31). However, when examining intensity between resistance bouts, the differences appear to be negligible (62).

In conclusion, the application of HRV as a gauge of physiological stress and recovery has been demonstrated. Furthermore, the effects of exercise intensities and modalities on HRV differ and warrants consideration. The lack of empirical information concerning the exercise programming of CrossFit is evident. Therefore, it becomes imperative to examine the markers of HRV during recovery from a bout of CrossFit exercise.

OXIDATIVE STRESS

Oxidative stress results from free radical over production. Free radicals are atoms or molecules that contain one or more unpaired electrons, which makes them highly reactive (19, 56). Reactive Oxygen Species (ROS) is a term generally used to describe a family of oxygen based radical species. Most pertinent to physiologic application are the ROS superoxide and hydrogen peroxide, respectively (56). ROS are a natural bi-product of numerous cellular reactions and are routinely produced at different sites within exercised skeletal muscle tissue. Superoxide is the most common form of ROS and is produced at multiple enzymatic and non-enzymatic sub-cellular sites both at rest and during exercise (56).

Mitochondria: The mitochondria are the primary producer of aerobic generated ATP and thus a major source of muscular energy. The electronic transport chain within the mitochondria possesses protein channels that transport H^+ ions across the membrane in order to continue the electron further down the chain. As this occurs superoxide is produced in a stoichiometric, specifically at complex I and III, relative to total metabolism (4, 56).

Sarcoplasmic Reticulum: Cardiac and skeletal muscle possess NAD(P)H oxidase enzymes within the sarcoplasmic reticulum. Using NADH as substrate, NAD(P)H oxidase will produce superoxide as a bi-product (56).

Transverse Tubules: Possessing a similar enzyme to NAD(P)H found in the sarcoplasmic reticulum, transverse tubules possesses NADP(H) oxidase. As depolarization occurs NADP(H) oxidase activity is increased and release superoxide into the cytosol as a bi-product (56).

Other Sites: Quantified to a lesser degree, ROS production also occurs from the plasma membrane, phospholipase A2-dependant processes, and xanthine oxidase (56). The growing influence of these ROS in exercise scenarios is only now being revealed (60).

There are several sites within the skeletal muscle capable of producing ROS. At rest production is minimal, while periods of exercise will result in significant increases in production (43, 57). Major alterations of the internal environment due extreme exercise or physiological stress can result in severe levels of intracellular ROS and cell tissue damage or death (4, 43).

Increased levels of ROS are responsible for the disruption of homeostasis by means of protein damage (contractile, transport, channel, structural, enzyme), lipid damage (damage to membrane structure and integrity), and damage to DNA (4, 43). The duration and magnitude of the increased levels of ROS are proportional to the oxidative damage incurred. In extreme cases this stress may lead to cellular apoptosis/necrosis, while other cases may increase the likelihood of chronic disease. However, within the context of healthful exercise, ROS and the consequent oxidative stress are not considered

negative. The idea that exercise and disease are both associated with oxidative stress is paradoxical at first glance. Resolving the matter, the oxidative stress associated with exercise is transient, while disease causing oxidative stress is chronic (60).

Recent evidence indicates that not only are ROS produced during exercise not detrimental to cellular health, they're essential to exercise induce adaptations (57). Moderate levels of exercise induced ROS production provide regulatory roles in the cells such as control of gene expression, regulation of cell signaling and modulation of skeletal muscle contraction force (56, 57). In regards to gene expression and cell signaling, lower levels of oxidative induced stress results in immediate activation of endogenous antioxidants coupled with a delayed expression and up regulation of antioxidant enzymes (58). These adaptations result in physiological protection against oxidative stress. Exercise induced oxidative stress is directly related to the duration and intensity of the exercise that is taking place (43). As previously mentioned low to moderate levels of exercise induced oxidative stress provides positive adaptations, but extreme exercise may result in excessive oxidative stress levels are sometimes interpreted as acutely negative in terms of contractile performance (43, 56).

OXIDATIVE STRESS AND EXERCISE

Exercise elicits a transient oxidative stress response after an acute bout (10, 26, 33, 34, 41, 42, 46, 59, 61). Accordingly, both aerobic (8, 43) and strength (28, 34) exercise stimuli produce increases in identifiable oxidative stress biomarkers. The post exercise increases are often quantified in blood plasma/serum due to the availability of sample and the modern ability to quantify oxidized end products and antioxidant potential (17, 47). Bloomer et al. examined the change in protein carbonyl concentration in trained

men and women after three separate time trials on a cycle ergometer. Subjects performed cycling bouts at 70% of their VO_{2peak} for 30, 60, or 120 minutes, each trial occurring on a separate day. In order to assess plasma protein carbonyl levels 4 separate blood draws were taken; pre, 0post, 30min post, and 60min post. For both the 30 and 60min sessions protein carbonyl levels significantly increased at 0post and 30post with a peak change of 167% and 197%, respectively. Both returned to non-significant levels at 60post. In the 120min session all post exercise time points were significant with a peak change of 245% (8). Alessio et al. examined acute treadmill exercise at 80% of VO_{2max} and the effect of Vitamin C on oxidative stress. Oxidative stress was assessed by measuring the level of lipid peroxidation through TBARS. Subjects performed three bouts of exercise, the first a placebo trial, the second a one day vitamin C supplementation, and the third was a two week vitamin C supplementation. The only group to elicit an oxidative response was the placebo exercise group with a 46% increase in TBARS (33). As discussed above, exercise induced increases in oxidative stress may seem to be counter intuitive without recent revelations that a wide variety of exercise derived benefits are due to ROS production (55). The up regulation of endogenous antioxidants post exercise makes this paradoxical relationship possible; however, too high of oxidative stress levels may lead to serious tissue damage (4, 56, 57).

When examining the literature on oxidative stress and exercise a relatively small amount exists on its relationship with resistance training. The existing research on resistance training and oxidative stress is equivocal with some studies having shown no oxidative response (9, 41), while others show significant increases after the bout (28, 34, 42).

Bloomer et al. investigated the oxidative stress response following sprints and repeated squats in anaerobically trained men. Each subjected performed repetitions of the squat until fatigue at 70% of their 1RM with 3min of rest in between sets. The number of sets was determined by the total amount of worked performed on a cycle ergometer 2 weeks earlier, the average number of sets were 6.4± 0.28 (mean ± SED) while the number of repetitions averaged 40 ± 2. Bloomer measured oxidative stress through protein carbonyl and took blood measures at 5 different times points: Pre, Post, 30min, 24 hr, and 48 hr. Post-exercise measures of protein carbonyl did not significantly differ from pre measures (p=0.447), demonstrating that resistance training did not elicit an oxidative stress response (9). Similarly, McAnulty et al. showed no change in oxidative stress biomeasures in their study of resistance training and carbohydrate ingestion and its relation to oxidative stress. Oxidative stress was quantified through a gas chromatography mass spectrometry approach to measureusing F_2-isoprstanes as the marker of stress. Subjects performed 10 different resistance training exercises consisting of 4 sets of 10 with the first set at 40% of 1RM and the following at 60% with 2-3minutes of rest between sets and exercises. Mean F_2-isoprstane values exhibited no change after exercise compared to pre exercise measures, (p=0.193).

In contrast to the findings of Bloomer and McAnulty, several studies have demonstrate a significant rise in oxidative stress after an acute bout of resistance exercise (10, 28, 34, 42). Hudson et al. compared the oxidative responses of acute strength based resistance exercise and acute hypertrophic based resistance exercise. In this latter study, subjects performed two different back squat protocols, a hypertrophy and strength, separated by 1 week. The hypertrophy protocol consisted of 4 sets of 10 at 75% of 1 RM

with 90sec of rest between sets. The strength program consisted of 11 sets of 3 at 90% of 1RM with 5min of rest between sets. Blood draws were collected pre, immediately post and 60min post for the measure of plasma oxidative stress markers protein carbonyl and lipid hydroperoxides. The hypertrophic and strength measures of lipid peroxidation showed no significant changes despite the upward drift in the 60min post hypertrophy measure, ($p=0.156$). Hypertrophic and strength measure of protein carbonyls showed significant increases only in the immediately post strength measure and in both 60min post measures (34). Goldfarb et al. examined resistance exercise and blood flow occlusion and its effects on glutathione and protein carbonyls. The non-occluded moderate exercise group performed bicep curls and calf extensions at 70% of their 1RM. Subjects completed all repetitions smoothly and through full rang of motion at a cadence of 0.67hz. All sets progressed to failure with 1min of rest between each set. Blood draws were taken pre, post and 15min post to determine oxidative stress through protein carbonyl. The non-occluded group showed significant increases of protein carbonyls post and 15min post exercise (28).

In summary, although some studies demonstrate mixed results on the oxidative response, an overall review of the literature reveals that intensity and modality of exercise will determine the magnitude of oxidative stress experienced (2, 8, 9, 34, 41, 45, 46). Such that the greater intensity of exercise, the greater the oxidative stress response.

MECHANISMS OF OXIDATIVE STRESS

Like most reactions in the cell, ROS act on molecules by virtue of availability, and because all cell membranes are composed of proteins and phospholipids, they are

readily available for interaction. Oxidative modifications to proteins and lipids disrupt membrane stability and cellular function. ROS induced free radical damage to proteins occur primarily in amino acids with exposed thiol groups (4). The damage to these amino acids results in decrease protein function, which can result in a variety of problems in that proteins serve several cellular functions such as transport, receptor, enzyme activity, structural integrity or contraction (4). Oxidative damage of lipids is commonly referred to as lipid peroxidation. ROS will react with polyunsaturated fatty acids in membranes, resulting in propagation reactions creating more ROS as well as continued lipid peroxidation (2). Oxidative damage to the lipid membrane results in altered membrane fluidity, increased permeability, and altered membrane function (2). Because cell membranes are composed of both lipids and proteins several sites for oxidative stress exist and membrane integrity will be at risk. As the cellular membrane loses integrity ionic dyshomeostasis occurs as evidenced by a rise in the concentration of intracellular levels of Na^{+}, Ca^{++}, and H^{+} rise (2, 57, 58). These constituents will eventually lead to a noxious environment in the cell causing the release of mitochondrial cytochrome C, activating apoptosis (4).

Quantification of the process of oxidative stress includes biomarkers for protein modifications. Specific quantification of protein oxidation is commonly achieved through the measure of protein carbonyls found in the plasma (17). Exposed thiol groups in the amino acid are sensitive to ROS damage and serve as the location of carbonyl formation (4). Protein carbonyls are formed by a variety of oxidative stress mechanisms such as fragmentation and amine oxidation due to metal catalysis or by hydropochlorous acid (17). As the level of oxidants or ROS increase so does the likelihood of protein

damage. The most common and accurate lab technique to measure protein carbonylation is through enzyme-linked immunosorbent assay (ELISA). The protein carbonyl (PC) ELISA is an involved assay that requires several binding steps, however less plasma is required. The first step of this assay uses dinitrophenylhydrazine (DNP) as a primary binding agent to carbonyl groups (17). DNP specifically binds to proteins that contain carbonyls, which flags oxidative damage. DNP alone is difficult to read and measure so streptavidin-linked horseradish peroxidase (hrp) is used as a marker that can be read through absorbance; however, Anti-DNP-biotin-antibody is required to attach to both DNP and hrp (17). In 1997 Buss et al. measured the sensitivity of the PC measurement for oxidative stress and found the PC ELISA assay to be both accurate and reliable (17). The protein carbonyl assay has been used successfully in my previous work and is an established measure of protein oxidative stress in exercise applications (43).

The measure of lipidperoxidation is performed through a variety of assays such as thiobarbituric acid reactive substances (TBARS), malondialdehyde (MDA), and ferrous oxidation-xylenol orange (FOX) (19, 47). This project will use the FOX assay to measure oxidative stress damage to lipids. The process of unsaturated lipid oxidation involves an allylic hydrogen removal, replacement by molecular oxygen, immediately followed by its reduction. The end product of this reaction is hydroperoxide (47). A simple and sensitive test for hydroperoxides is the method based on the oxidation of ferrous to ferric ions under acidic conditions (47). The molecular indicator of ferric ion is xylenol orange, which when bound to ferric ion produces a colored complex with an extinction coefficient of 1.5×10^4 M^{-1} cm^{-1} at 560nm (47). The assay is commonly referred to as the ferrous oxidation-xylenol

orange assay or FOX. The FOX assay is a well-established approach for determination of hydroperoxide levels in whole blood plasma and has the distinct advantage in that total available plasma hydroperoxides can be accessed and measured (47).

SUMMARY

1) The HIPT known as CrossFit is growing in popularity worldwide. Many claims of physiological benefits have been unsubstantiated; this is evident due to the existence of only five published study performed on CrossFit.
2) The physiological markers that will be used in this study are derived from examination of Heart Rate Variability (HRV) and oxidative stress. Both markers are associated with the acute physiologic stress associated with exercise and have been studied extensively in high-intensity exercise research applications.

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WOD	Exercises and Repetitions	Prescribed Weights male/female	Type
Angie:	100 Pull-ups 100 Push-ups 100 Sit-ups 100 Squats	Body Weight	For Time
Annie:	Double-unders and Sit-ups: 50/40/30/20/10	Body Weight	For Time
Cindy:	5 pull-ups 10-push-ups 15 squats	Body Weight	(AMRAP)20 min
Diane:	Deadlift and Handstand Push-ups: 21-15-9	Men (255lbs) Female (155lbs)	For Time
Elizabeth:	Clean and Ring Dips: 21-15-9	Men (135lbs) Female (85lbs)	For Time
Eva:	800 meter run 30 Kettlebell Swings 30 Pull-ups	Male (70lbs) Female (53lbs)	5 rounds
Fran:	Pull-up and Thruster: 21-15-9	Male (95lbs) Female (65lbs)	For Time
Grace:	30 Clean and Jerks	Male (135lbs) Female (85lbs)	For Time

TABLE 1. Example CrossFit Workouts of the Day (WODs). For Time= complete as fast as possible, AMRAP= As Many Rounds As Possible. Prescribed weights are only for movements that require weight.

CHAPTER 3

ACUTE EXERCISE AND HEAR RATE VARIABILITY: CROSSFIT VS. CONTINUOUS RUNNING

ABSTRACT

CrossFit (CF) has become a popular form of exercise training throughout the world. Despite this, basic empirical evidence to describe physiological responses from CF, such as autonomic modulation of the heart, is lacking. The purpose of this study was to compare post-exercise HRV between CF and treadmill (TM) exercise. Ten men (age = 26.4 yrs ±2.7yrs) with at least three months of CF experience participated in the study. Aerobic power was determined with a maximal graded exercise test on a treadmill (GXT). On two separate occasions, each participant completed either the CF workout "Cindy" (20min of as many rounds of 5 pull-ups, 10 push-ups, and 15 squats as possible) or a 20 min bout of TM exercise at 90% of GXT determined maximal heart rate. The exercise bouts were performed in a randomized crossover fashion. HRV was quantified through changes in the log-transformed square root of the successive R-R differences (lnRMSSD) and high frequency power (lnHF), which was analyzed 5-min segments at 5-10 min pre-exercise period (PRE), and during the post-exercise period at 15-20min (POST1), 20-25min (POST2), 25-30min (POST3), and 1hour (POST4). Significant time dependent decreases occurred following both CF and TM in lnRMSSD and lnHF ($p = 0.003$) ($p = 0.001$) respectively. Trial dependent differences were also observed in post-

exercise lnRMSSD and lnHF measures, CF being significantly lower than TM (p= 0.002)(p= 0.000). Though a significant decrease in lnRMSSD occurred after both exercise trials, lnRMSSD at 1-hour post was not significantly different compared to pre following the TM bout (p= 0.172). However, lnRMSSD at 1-hour post-CF remained significantly lower compared to PRE ($p < 0.05$). lnHF returned to baseline in CF and TM (p= 0.081)(p= 0.065). The results of this investigation demonstrated a depression of HRV following CF compared to TM exercise.

KEY WORDS: Heart Rate Variability, RMSSD, HF, CrossFit, High-intensity

INTRODUCTION

Heart rate variability (HRV) is a noninvasive measure of the autonomic nervous (ANS) control of the cardiovascular system (8, 10, 19) and is basically defined as the oscillations that occur between consecutive R-R intervals that are derived from an electrocardiogram (ECG) (7, 10, 19). HRV is believed to be an indirect measure of parasympathetic activity (vagal tone) when assessed as the square root of the successive R-R differences (RMSSD) and the high frequency (HF) of a power spectrum (10, 31). HRV application originated clinically in order to indirectly predict untoward cardiac events in both clinical and nonclinical patients, via chronic depression (1, 2, 9, 11, 15, 31). Importantly, HRV is also depressed during periods of physical or emotional stress outside the presence of disease, and therefore can broadly be described as an indirect marker of physiological stress (2, 9, 13). Homeostatic recovery following physiological stress has become a focus in the applied sciences in an attempt to improve performance

and safer prescription of exercise (8, 17, 23, 29). The recovery of HRV (i.e., vagal rebound) post-exercise is used as a crude indicator of homeostatic recovery (4, 8, 17, 21–23, 29).

Exercise provides acute physiological stresses to the body. For example, within seconds of dynamic muscle contraction, a withdrawal of vagal tone occurs, resulting in an acute period of depressed HRV that may last up to 72 hours post-exercise (8, 14, 20, 25). The magnitude and duration of post-exercise parasympathetic depression depends on the intensity of the exercise, in that higher intensity exercise results in greater HRV depression when compared to lower exercise intensities (8, 15, 16, 19). The recovery of HRV following acute bouts of exercise becomes important when considering future performance and prescription. Chen et al., found that recovery status mirrored vagal rebound following bouts of heavy resistance exercises (8). Furthermore, Kiviniemi et al., found that endurance programs guided by daily HRV measured upon waking used to determine the exercise intensity to be more successful than standardized programming (17). A review by Stanely et al., examined the application of HRV as a tool to gauge the recovery status of an athlete for exercise prescription on basic cardiovascular exercise (29). Understanding the effect of exercise type and intensity on recovery is an important concept in HRV mediated programming (29). However, information on post-exercise HRV outside of endurance type exercise is lacking (29), and currently no empirical data is available on the high-intensity power training style known as CrossFit.

CrossFit (CF) is a relatively new form of training that drastically warrants scientific study (27). The basic structure of a CrossFit workout is completing a combination of resistance and endurance exercise modalities as fast as possible, creating

a short duration and high-intensity bout. The popularity of CF has grown significantly over the past few years due to the short time commitment of each workout and frequent anecdotal reports of success on the Internet. A Google search of "CrossFit" and "exercise" offers 14.2 million results (1/29/14), while a scientific search on PubMed of the same key words offers only 5 results. This demonstrates the overall popularity of CF as well as the lack of empirical evidence surrounding this form of training. The magnitudes of physiological stress caused by CF's multi-modal and high-intensity exercise bouts are unknown. Due to the increasing popularity across the world, it becomes important to investigate this topic.

It is the purpose of this study to evaluate exercise induced stress through the magnitude of parasympathetic depression following a 20-minute bout of CF compared to a 20-minute bout of high-intensity treadmill (TM) running. A comparative analysis of post-exercise HRV will provide a look at any existing differences in modality. Modality has been shown to affect HRV differently, most notability in Heffernan et al., where an acute bout of resistance exercise demonstrated a greater drop in HRV following resistance based exercise (15). This current study is novel in that it is the first to examine HRV following a bout of CF; furthermore, it is the first study to compare CF to a time and intensity matched bout of TM. It is hypothesized that the CF trial will elicit a greater drop in parasympathetic activity as well as a greater duration of parasympathetic depression when compared to time-matched treadmill running.

Methods

All data were collected in the Human Performance Laboratory (HPL), Auburn University at Montgomery (AUM). Each participant was required to arrive at the HPL

on three separate occasions; the first for familiarization of protocols and body composition measurements, followed by two separate occasions for data collection between 7am and 11am.

PARTICIPANTS

Auburn University and Auburn University at Montgomery institutional review boards approved this study. Participants were recruited through flyers and emails sent to local CrossFit establishments. Prior to participation, written informed consent was obtained from each participant. Ten apparently healthy males participated in this study, descriptive data expressed as means ± SD can be seen in Table 1. Each participant followed CrossFit training for a minimum of three months. All participants were of low risk for cardiovascular, metabolic, and/or pulmonary diseases as determined by PAR-Q and Health History Questionnaire. Participants reported not taking any prescribed medication during the time of the study. An inability to properly perform required movements, maximal oxygen consumption (VO_{2Max}) under $40ml \bullet kg^{-1} \bullet min^{-1}$, or any symptom or contraindication of health resulted in exclusion from the study. Participants were informed that they could withdraw from the study at anytime. Participants were instructed to wear exercise clothing for testing, abstain from exercise 24 hours prior, and abstain from caffeine 12 hours prior to exercise testing sessions.

MAXIMAL EXERCISE CAPACITY AND BODY COMPOSITION VARIABLES

Maximal oxygen consumption (VO_{2max}) and maximal heart rate (HR_{max}) was assessed during the first session through a graded exercise test (GXT) on a treadmill (Trackmaster, Newton, KS). Using Bruce Protocol, the workload during the GXT was increased incrementally every 3 minutes until a maximal value was reached. Expired gas

(oxygen and carbon dioxide) fractions were continuously sampled at the mouth using a pneumotach, mixing chamber, and gas analyzers from a metabolic cart (ParvoMedics, Sandy, UT). During the test, heart rate was assessed continuously using a heart rate monitor (Polar Electro Oy, Oulu, Finland). Maximal heart rate obtained during this test was used to determine the target HR of the TM bout. Test termination required two of the following to occur: a plateau in VO_2 with increasing workload; respiratory exchange ratio (RER) of > 1.10; heart rate within 10 beats of age predicted maximum.

Body fat percentage was assessed through Dual Energy X-Ray Absorptiometry (DXA) scan (GE Lunar Prodigy, Software version 10.50.086: GE Lunar, Corp., Madison, WI, USA). Prior to scanning, the DXA was calibrated using a standard calibration block. Participants were measured for height and weight then instructed to remove any metallic objects prior to scanning. Subjects were placed in a supine position and remained motionless during the scan.

EXERCISE PROTOCOLS

Exercise trial visits consisted of a 20-minute bout of either CF or TM, with pre and post period measurements of HRV through ECG. Exercise trials were randomized between CF and TM sessions. After resting ECG was acquired participants performed a standardized 5-minute warm up on the treadmill, rest for 1-minute then perform the exercise bout of CF or TM. Participants were not allowed to eat during the 1-hour recovery period. The study design can be seen in Figure 1. HR intensity ($\%HR_{max}$) and rate of perceived exertion (RPE) scale numbered 1-10, with 1 being no exertion and 10 being maximal effort, were examined during this study to ensure similar effort between trials; results can be seen in Table 2.

CrossFit Protocol

The CrossFit named workout "Cindy" consists of as many rounds possible of 5 pull-ups, 10 push-ups, and 15 air squats in 20-minutes. The workout required that one complete all prescribed repetitions for the movement before moving on the next and to do so as fast as possible. Each movement was standardized for all participants. Pull-up form standards required that participant to start with arms locked out, pull their chin just above the bar, and return to the locked position. Push-up form standards required that participant to start with the arms locked out, lower the body to the ground until the chest touched, then return to the starting position. Air-squat form standards required that participant to start in the upright standing position, squat until hips past the knee, and return to starting position. Failure to achieve these standards resulted in the participant correctly repeating that movement. Upon completion of the workout participants were placed in a supine position for the designated periods of time, where post-exercise ECG was obtained to analyze HRV.

High-Intensity Treadmill Protocol

To perform the TM trial, participants performed a minimum of 85% maximal HR obtained during the GXT. The target HR was determined via pilot study data not published. Participants ran for 20-minutes at a rate and incline that yielded a HR response within the target zone. Upon completion of the workout participants were placed in a supine position for the designated periods of time, where post-exercise ECG was obtained to analyze HRV.

Analysis of Heart Rate Variability

For HRV assessment, participants were placed in a quiet, dimly lit room, and instructed to lie in a supine position on a comfortable examination table. ECG assessment was performed with a modified Lead II configuration using three Ag/AgCl electrodes and was interfaced with a Biopac MP100 data acquisition system (Goletta, GA). Three separate ECG recordings were obtained throughout each trial; Two, 10-minute segments with one at PRE and one at POST-60-minute, One 30-minute ECG segment immediately following the exercise bouts. The ECG recordings obtained in this study were divided into five 5-minute segments for analysis of HRV: the PRE time point measuring the last 5-minutes of the 10-minutes recording; the POST time points from the 30-minute post exercise recording, which was broken down into 3 POST 5-minute segments at 15-20, 20-25, and 25-30 minutes; and the 60-minute post-exercise ECG recordings examining the last 5-minutes of the 10-minute recording. All ECG segments were visually inspected for ectopic/non-sinus beats, which were replaced by the adjacent R-R interval when observed. Any segment containing three or more ectopic beats was excluded from analysis. Vagal activity was quantified through changes in the heart rate variability index of log-transformed square root of the successive R-R differences (lnRMSSD) and log-transformed high frequency (lnHF) power, which was analyzed through 5-minute ECG segments.

The markers chosen for HRV in this study were the time domain as the root mean square of the standard deviation of consecutive N-N intervals (RMSSD), and frequency domain as High Frequency (HF) power (0.15-0.40 Hz). The transformation of ECG into time domain and Frequency domain components was done through specialized HRV software (Nevrokard version 11.0.2, Izola, Slovenia). In order to assess RMSSD, the

ECG recordings were converted into a tachogram, which plots the successive R-R intervals (y-axis) against the number of beats within the ECG (x-axis). From the tachogram the 5-minute segments were calculated for RMSSD. Analysis of the frequency domain was performed through a power spectral analysis, which was completed by applying a fast Fourier transformation to the R-R intervals of the sampled ECG. RMSSD and HF are sensitive markers of parasympathetic activity and have been used in several studies (3, 18, 21–23). RMSSD is not significantly influenced by breathing frequency and is capable of measuring parasympathetic activity in a short period of time (23), making it a suitable marker for this study. The frequency domain has several components (i.e., VLF, LF, HF, LF:HF); however HF Is the only frequency marker that is widely accepted to accurately reflect vagal activity (2, 3, 6, 10, 12, 16).

STATISTICAL ANALYSIS

Subject HRV data was entered into SPSS for statistical analysis. A Shapiro-Wilk test was used to determine normal distribution of HRV data. Because data were skewed, a natural logarithmic transformation was performed on the RMSSD and HF data prior to further statistical analysis (raw data can be observed in appendix A). A 2 (Trial) x 5 (Time) repeated measures analysis of variance (ANOVA) was used to assess differences from resting HRV to post exercise HRV in and between both CrossFit and Treadmill trials. A Paired Samples T-Test was used to further assess differences between trial-to-trial time points, and pre to 1-hour post same trial time points. Statistical analysis was performed on SPSS 19.0 (Chicago, IL). Statistical significance was set to $\alpha \leq 0.05$. Data presented as means ± standard deviations.

RESULTS

All 10 participants of the study completed pre and post-exercise supine HRV analysis for the CrossFit and Treadmill trials. The markers of HRV are presented in lnRMSSD (Figure 2a.) and lnHF (figure 2b.). The repeated measures ANOVA revealed that both CF and TM trails experienced a time dependant change in lnRMSSD. For instance, lnRMSSD dropped significantly in all time points when compared to PRE values following the bout of CF ($p < 0.05$). A Paired Samples T-test revealed POST 60-minute lnRMSSD following CF remained significantly lower than PRE lnRMSSD values ($p < 0.05$). Following the TM bout a significant, yet lesser time dependant change in lnRMSSD was observed ($p < 0.05$), which returned to near baseline values by POST 60-minute with no statistical difference from PRE ($p = 0.17$). A significant trial-to-trial difference was observed between CF and TM. Following a bout of CF a significantly greater depression of lnRMSSD occurred in each POST time point when compared to TM values ($p < 0.05$).

A repeated measures ANOVA of the frequency domain revealed a significant time dependant decrease in lnHF following both CF and TM trials ($p < 0.05$). In both CF and TM POST 15-20, 20-25, and 25-30-minutes lnHF were significantly lower compared to PRE values ($p < 0.05$); however, both CF and TM returned to non-significant lnHF by POST 60-minute ($p = 0.81$, $p = 0.65$, respectively). A trial-to-trial comparison revealed a significant difference between the first three recovery time points with CF lnHF being significantly lower ($p < 0.05$). No significant difference was observed between CF or TM at POST 60-minute ($p = 0.90$).

DISCUSSION

CrossFit is a relatively new style of training that has recently increased in popularity. To date, little is known about the effects of a single bout of CF on HRV or how these results may compare to traditional exercise, such as continuous running. The magnitude and duration of HRV depression may be related to cardiovascular strain following an acute bout of exercise (9, 10, 15, 29, 31). The magnitude of cardiovascular strain (i.e., dyshomeostasis) relates to the duration of recovery (29). Research in this area could have important implications related to the safe and effective prescription of exercise. Therefore, this investigation will contribute to the current body of knowledge involving HRV following acute bouts of high-intensity exercise and short-term recovery. The purpose of this study to examine the affects of a CF bout versus a bout of time and intensity matched TM running on post-exercise HRV. We hypothesized that each trial would elicit an acute drop in both markers of HRV (lnRMSSD and lnHF), but that CF would elicit a greater drop in the HRV parameters.

The major findings of this study were that both CF and TM bouts resulted in a time and trial dependent depression of HRV (lnRMSSD and lnHF). Both trials resulted in post-exercise depression of HRV markers were significantly different from resting measures. However, the CF trial demonstrated a greater depression of HRV when compared to the bout of TM exercise. The POST 60-minute recovery time point for lnRMSSD following CF remained significantly depressed compared to PRE lnRMSSD parameters. In contrast, lnRMSSD following TM and lnHF following CF and TM returned close to baseline levels by POST 60-minute.

Control of cardiac function is accomplished though both branches of the autonomic nervous system: parasympathetic (vagus nerve) and sympathetic (cardiac accelerator

nerves) branches. The vagus nerve innervates the sinoatrial and atrioventricular nodes (14, 25). During the onset of exercise, vagal activity is removed resulting in the initial increase of HR up to 100bpm (14, 25). At the same time, working tissue activates muscle afferents through muscle metabolites and or mechano/baro/chemoreceptors of the heart and skeletal muscle (25). Muscle afferents signal the cardiac command center (CCC) to increase HR via vagal withdrawal and cardiac sympathetic stimulation (14, 25). At the completion of exercise, cardiac activity progressively returns to a resting state, which is majorly contributed by sympathetic withdrawal coupled with increased vagal outflow (7). Despite the return to resting HR, vagal activity remains blunted which can be observed through post exercise HRV (7, 12). The continued vagal depression is a result of complex autonomic nervous system function and dynamics not yet fully understood (7, 30). Other researchers have found decreased lnRMSSD and lnHF following acute bouts of exercise, which supports the findings of the current study (6, 13, 15, 19, 24, 26).

The intensity of exercise influences the magnitude and duration of depressed HRV, and therefore becomes an important factor in sympathovagal balance (6, 19, 29). Parekh & Lee examined differences in intensity between 50% and 80% $VO_{2Reserve}$ in treadmill running, finding that 80% produced a greater drop in lnHF and HFnu (19). Similarly, Buchhiet et al., observed a greater drop in RMSSD and lnHF following high-intensity running versus sub-maximal running (6). Despite the observed influence of intensity within the current literature, this study found a greater depression of lnHF and lnRMSSD following CF compared to TM running even though HR intensities were similar.

The Trial dependant differences observed in this study are in agreement with the hypothesis despite a similarity in intensity, which suggests a relationship between the exercise modality and the magnitude of depression of HRV. Heffernan et al. (15) examined this relationship between a bout resistance exercises versus a bout of cycling. Interestingly, both bouts resulted in significant depression of HF and HFnu. However, a greater depression was observed following resistance exercise (15). Although there is some evidence to support differences in modalities and their affects on HRV recovery (15), a lack of information regarding this relationship exists and further investigation is required.

Though it is outside the scope of this study to find physiological reasons for the alterations of HRV, we can postulate on a few physiological responses related to the differences between the trials. The CF workout, "CINDY", consisted of pull-ups, push-ups, and body weight squats, which by nature of the resistance-based exercise and the loads experienced by the muscle groups, would recruit more muscle mass than treadmill running (28). Increase muscle recruitment is associated with raises in circulating epinephrine (28), which has been shown to decrease HRV (25, 30). Furthermore, the alterations in posture and redistribution of blood to active muscle groups from transition from one exercise to another throughout the trial likely presented a challenge to hemodynamics and consequently increased the catecholamine response (5, 25). Although this may offer an explanation for the observed differences found within this study, further investigation is necessary to provide a better understanding.

LIMITATIONS

Although this study was a novel step towards the better understanding of HRV following a multimodal high-intensity exercise bout such as CF, it was not without limitations. The marker used to control for intensity (%HR_{max}) was similar between trials, but statistically different. The observed marker of intensity (RPE) also significantly differed between trials; however, psychological perception and influences may be responsible for these observed differences. Future studies should examine differences in muscle recruitment, blood lactate, and plasma catecholamine levels in conjunction to HRV in order to provide a comprehensive view of vagal activity and potential constituents of its alteration. Furthermore, the participants of this study were trained and experienced with high-intensity exercise, and may respond to this type of stimulus differently than an untrained participant. Therefore, future studies should examine the affects of CF on HRV in untrained participants.

CONCLUISON

In summary, results show that the depression and recovery of parasympathetic markers (e.g., lnRMSSD and lnHF) were significantly greater and of longer duration (CF lnRMSSD only) than treadmill running when compared to baseline values. Therefore, CF workouts such as the type examined in this study may provide a greater degree of physiological stress compared to high-intensity treadmill running at a continuous pace within trained participants. Understanding the recovery status and the return to homeostasis becomes important when safely prescribing future bouts of exercise.

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TABLE 1. Participant Characteristics

Characteristic	Values ± SD
Age (yr)	26.4 ± 2.7
Height (in)	69.8 ± 3.0
Weight (lb)	181.7 ± 8.2
Body Fat(%)	11.2 ± 7.6
VO_{2max} ($ml \bullet kg^{-1} \bullet min^{-1}$)	44.4 ± 16.2
Max HR (bpm)	183.6 ± 6.4

N=10 Means +/- SD

TABLE 2. Exercise Intensity (Perceived and $\%HR_{max}$)

	6minute	10minute	16minute	20minute	p value
RPE					
CF	5.4(±1.7)	6.4(±1.5)	7.6(±0.8)	9.0(±1.0)	(p= 0.005) time
TM	4.6(±1.4)	5.6(±1.2)	7.2(±1.2)	7.2(±1.5)	(p= 0.007) trial
$\%HR_{max}$					
CF	93.3(±3.9)	94.6(±2.9)	95.9(±3.0)	97.7(±5.9)	(p= 0.005) time
TM	89.3(±3.9)	92.9(±2.4)	94.2(±2.7)	93.6(±3.3)	(p= 0.000) trial

N=10 Means +/- SD. RPE scale 1-10 lowest to highest exertion. CF= CrossFit, TM= Treadmill.

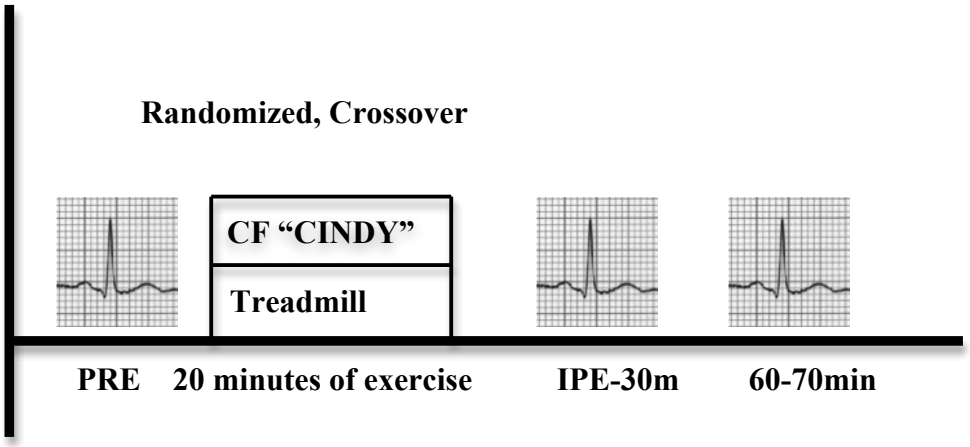

Figure 1. Study design. ECG samples were taken at designated time points, represented ECG icons. Samples taken before and after (randomized, crossover design) 20 minutes CF and TM bouts.

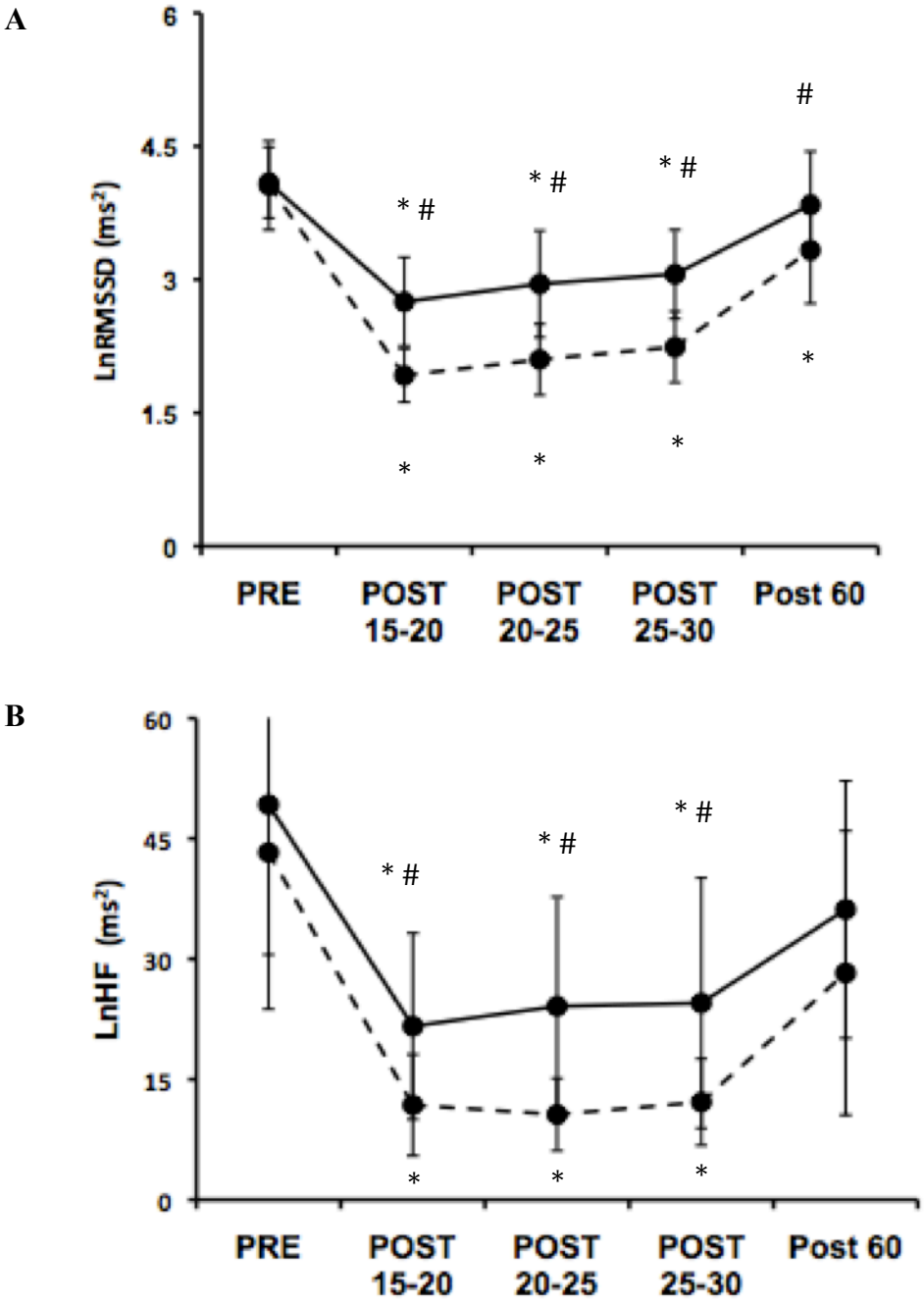

FIGURE 2. HRV markers before and after exercise, Means ± SD, n=10. A) LnRMSSD CF and TM B) LnHF CF and TM. * significant from pre, # significant CF vs TM. Dashed line = CF, Solid line = TM

Appendix A. Raw RMSSD & HF PRE and POST Trials (ms^2)

		PRE	15-20	20-25	25-30	60-65
RMSSD						
	CF	**64.73 (±33.22)**	**7.21 (±2.60)**	**8.92 (±4.10)**	**10.26 (±4.5)**	**33.24 (±22.10)**
	TM	**65.24 (±27.40)**	**18.39 (±12.75)**	**22.83 (±16.14)**	**24.09 (±12.66)**	**55.72 (±37.90)**
HF						
	CF	**1670.35 (±1567.57)**	**25.67 (±44.23)**	**32.29 (±41.49)**	**36.12 (±37.38)**	**551.89 (±897.65)**
	TM	**1794.53 (±1674.76)**	**301.57 (±422.30)**	**264.19 (±292.98)**	**1532.38 (±2468.20)**	**36.16(±16.03)**

CHAPTER 4

ACUTE EXERCISE AND OXIDATIVE STRESS: CROSSFIT VS. TREADMILL BOUT

ABSTRACT

Purpose: High intensity power training (HIPT), including CrossFit, is a global fitness phenomenon. Despite increasing popularity, basic empirical evidence to describe physiologic responses including acute oxidative stress to CrossFit are lacking. Therefore, the purpose of this study was to examine the acute effects CrossFit on blood redox changes. Methods: Oxidative stress responses were compared to a traditionally prescribed bout of high-intensity treadmill exercise in 10 male 26.4 yrs (+/- 2.7yrs) with at least three months of CrossFit experience. Participant VO_{2max} was determined prior to completion of the CrossFit workout "Cindy" (20min of as many pull-ups, push-ups, and squats as possible) or a 20 min 90% HR_{max} Treadmill bout as determined by graded exercise test. Treadmill and CrossFit works were performed in a randomized crossover fashion. Blood plasma was collected at 4 different time points for each trial: pre, post, 1hr post and 2hr post, to examine lipidperoxidation (LOOH), Protein Carbonyl (PC) and antioxidant capacity. Results: CrossFit and Treadmill elicited a time dependent increase of LOOH in 1hr post (143% and 115%) and 2hr post (256% and 167%) respectively. PC demonstrated a slight increase IPI (5% and 0%) then a time dependent decrease 1hr post (-16% and -8%) and 2hr post (-16% and -1%) compared to IPE. Ferric Reducing

Antioxidant Power (FRAP) also demonstrated a time dependent increase within CrossFit and Treadmill bouts; post (25%, 17%), 1hr post (26%, 4.8%), 2hr (20%, 12%) respectively. Total Enzymatic Antioxidant Capacity (TEAC) showed a time dependent decrease in post (-10%, -12%), 1hr post (-12%, -6%), 2hr post (-7%, -11%) respectively. No trial dependant differences were observed in any biomarker of oxidative-stress. Conclusion: CrossFit elicited an acute blood oxidative stress response that is comparable to a traditional bout of high intensity treadmill running; however, the blood plasma antioxidant response to Cindy exhibited differences during recovery.

INTRODUCTION

Reactive oxygen species (ROS) are oxygen centered radical compounds that modify molecules within a cell, or tissue resulting in damage (3, 23, 27). ROS encompasses free radicals, which are atoms or molecules that contain one or more unpaired electrons in their outer shell, which results in high reactivity. Oxidative stress is a physiological phenomenon that occurs when oxidative damage surpasses antioxidant defense (8). The production of ROS occurs during numerous physiological processes, including physical exercise, and thus is a naturally occurring event in cells throughout the body (3, 10). A prolonged increase of ROS is central to dyshomeostasis through the modification of protein and lipids and is influenced by exercise (5, 7, 14, 23, 25). Moderate increases of ROS are beneficial if not essential for optimal physiological performance (27), while excessive amounts hinder performance, or in rare and extreme cases cause cellular death (3, 27). As applied to exercise, oxidative stress is commonly quantified using blood biomarkers of oxidative damage and antioxidant defense capacity (18, 21, 23, 31).

The production of ROS is proportional to the intensity of the exercise or the fatigue inducing duration of the bout (1, 2, 5, 31). In this regard, the majority of prior exercise induced oxidative stress findings are derived from studies that utilize 'traditional' modalities of exercise such as running, cycling, and resistance training. However, the blood oxidative stress response to power-based exercise bouts that employ low duration and high intensity have yet to be fully examined.

Among the novel low duration-high intensity exercise modalities that are yet to be understood relative to blood oxidative stress is high-intensity power training (HIPT). The increase in popularity of HIPT has skyrocketed over recent years due to the brief time requirement and purported success in weight loss (33). The most popular form of HIPT is CrossFit, a program that trains a wide spectrum of physical fitness components within one exercise program. CrossFit incorporates both resistance and endurance modalities within a single bout. In addition, participants complete a prescribed workout as fast as possible, creating a short duration and high-intensity bout. Despite the increasing popularity of CrossFit, basic empirical evidence is ultimately lacking in terms of various physiologic outcomes including oxidative stress responses. Therefore, it was the purpose of this study to examine the blood oxidative stress response following a single bout of CrossFit. To undertake this study, a comprehensive biomarker panel of blood oxidative damage and antioxidant defense measures was performed before and after an acute bout of CrossFit exercise. Furthermore, these findings were compared to more traditionally prescribe high-intensity treadmill exercise matched for time in order to assess any differences between modalities.

METHODS

PARTICIPANTS

Auburn University and Auburn University of Montgomery institutional review boards approved this study. Prior to participation, signed informed consent was obtained from each participant. Ten healthy males, age 26.4 yrs (± 2.7) weight 181.7lbs (± 8.2), height 69.8in (± 3.0), and body fat 11.2 % (± 7.6%) participated in this study. Each participant partook in CrossFit training for a minimum of three months. All participants were of low risk for cardiovascular, metabolic, and/or pulmonary diseases as determined by PAR-Q and Health History Questionnaire. Participants reported not taking any prescribed or over the counter medication during the time of the study. Subjects were instructed to abstain from exercise 24 hours prior to each trial, as well as caffeine, alcohol, and vitamin supplementation 12 hours prior.

MAXIMAL EXERCISE CAPACITY AND ANTHROPOMORPHIC MEASUREMENTS

Maximal exercise capacity (VO_{2max}) and maximal heart rate (HR_{max}) were assessed during the first session through a graded exercise test (GXT) on a treadmill (Trackmaster, Newton, KS). Using Bruce Protocol, the workload during the GXT was increased incrementally every 3 minutes until a maximal value was reached. Expired gas (oxygen and carbon dioxide) fractions were sampled continuously using a pneumotach, mixing chamber, and gas analyzers through a Parvo Medics cart (Sandy, UT). During the test, heart rate was assessed continuously using heart rate monitor (Polar Electro Oy, Oulu, Finland). Test termination required achievement of two of the following criteria: a plateau in VO_2 occurs with increasing workload; respiratory exchange ratio (RER) of > 1.10; heart beat within 10 beats of age predicted maximum.

Body fat percentage was assessed through total body Dual Energy X-Ray Absorptiometry (DXA) scan (GE Lunar Prodigy, Software version 10.50.086: GE Lunar, Corp., Madison, WI, USA). Prior to scanning, the DXA was calibrated using a standard calibration block, and followed manufacturer's procedures. Participants were measured for height and weight then instructed to remove any metallic objects prior to scanning. Subjects were placed in a supine position and remained motionless during the scan. Subject characteristics are presented in Table 1.

OXIDATIVE STRESS EXPERIMENTAL DESIGN

All data were collected in the Human Performance Laboratory (HPL), Auburn University of Montgomery (AUM). Each participant arrived at the HPL on three separate occasions for data collection between the 7am and 11am. The study design is presented in Figure 1. Exercise trials were randomized between CrossFit and High-intensity treadmill sessions. Blood was sampled before and after each trial. After the pre blood draw participants performed a standardized 5-minute warm up on the treadmill, rest for 1-minute then perform the exercise bout of "Cindy" or High-intensity Treadmill. Participants were not allowed to eat during the 2-hour recovery period.

BLOOD SAMPLES AND STORAGE

Blood samples were collected and assayed for biomarkers of oxidative damage and antioxidant potential at the following time points; before exercise (PRE), immediately post exercise (IPE), 1-hour post exercise (1-HP), and 2-hours post exercise (2-HP). Participants were in a seated position while the 10 mL blood samples were taken. Draws were taken via venipuncture through the antecubital vein and were collected in EDTA tubes (2 mL) and heparinized tubes (8mL). Heparinized tubes were

immediately centrifuged at 3000 rpm for 15 minutes, aliquotted, and stored in ultra-low freezer -80°C until subsequent assay. Hematocrit and hemoglobin were determined using EDTA tube whole-blood aliquots using a hematology analyzer (CellDyn 1800, Abbott Park, IL). Participants were instructed to abstain from food or beverage other than water during the post exercise period.

CROSSFIT PROTOCOL

The CrossFit named workout "Cindy" consists of as many rounds possible of 5 pull-ups, 10 push-ups, and 15 air squats in 20-minutes. The workout required that one complete all prescribed repetitions for the movement before moving on the next and to do so as fast as possible. Each movement was standardized for all participants. Pull-up form standards require the participant to start with arms locked out, pull their chin just above the bar, and return to the locked position. Push-up form standards require the participant to start with the arms locked out, lower the body to the ground until the chest touched, then return to the starting position. Air-squat form standards require the participant to start in the upright standing position, squat until hips past the knee, and return to starting position. Failure to achieve these standards resulted in a repetition of that movement. Upon completion of the workout participants were placed in a seated position for subsequent blood draws.

HIGH-INTENSITY TREADMILL PROTOCOL

To perform the HITP trial, participants performed a minimum of 85% maximal HR obtained during the GXT. The target HR was determined via pilot study data not published. Participants ran for 20-minutes at a rate and incline that yielded a HR

response within the target zone. Upon completion of the trial participants were placed in a seated position for subsequent blood draws.

OXIDATIVE STRESS BIOMARKERS

Blood plasma samples were assayed for oxidative stress biomarkers, which are used previously (18, 23, 30). Two biomarkers for oxidative damage were chosen, Plasma lipid hydroperoxides (LOOH) and protein carbonyls (PC). Two additional biomarkers were selected to examine antioxidant capacity, Ferric-reducing antioxidant potential (FRAP) and Trolox-equivalent antioxidant capacity (TEAC).

Lipid peroxidation was determined through modified ferrous oxidation-xylenol orange assay (FOX) and reported as μmol/L (26). FOX measures the oxidation of ferrous ions to ferric ions by lipid hydroperoxide, which reacts with the ferrous-sensitive dye (xylenol orange). In the presence of lipid hydroperoxides this assay creates a complex that can be quantified spectrophotometrically (26). Protein carbonyls were assayed using a commercially available ELISA kit (Biocell, Papatoetoe New Zealand), protocols followed assay kit instructions and reported as μM/mg protein. Prior to analysis, individual plasma protein content was normalized using the methods of Bradford (1976). Antioxidant capacity was measured through the Trolox equivalent antioxidant capacity (TEAC) assay with modified methods form Re et al. (1999) and Villano, Fernandez-Pachon, Troncosco, and Garcia-Parrilla (2004) (32, 35). The principle of the TEAC assay is that oxidized 2,2'-azinobis-(3-ethylbenzo-thiazoline-6-sulfonic acid) is reduced via antioxidants in the blood plasma (9, 32, 35). Total antioxidant potential was measured using ferric-reducing antioxidant potential (FRAP) assay, which methods were adopted and modified from Benzie and Strain (1996)(4). TEAC and FRAP are both assessed

through colorimetric solutions spectrophotometrically (4, 35). The biomarkers of oxidative stress selected for this study are sensitive to exercise application and have been used on several occasions by our lab and others (7, 18, 23–25, 30). The rationale for this comprehensive examination of the selected biomarkers of blood oxidative stress comes from the concept of the 'oxidative stress pecking order'. According the pecking order, oxidative stress related damage would not occur until after endogenous antioxidant defense has failed (i.e., water-soluble antioxidants and lipid-soluble antioxidants, respectively) (8). Due to this current understanding, the oxidative stress biomarker panel used in this study is sensitive to protein and non-protein water-soluble antioxidants as well as lipid-phase antioxidants (9). All biomarkers were normalized for plasma-volume changes that occurred following the exercise trials using the formulas based on the established protocols of Dill & Costill, normalizing plasma through hemoglobin and hematocrit levels compared to preexisting levels (11).

STATISTICAL ANALYSIS

Participant oxidative stress data was entered into SPSS for statistical analysis. A 2 (Trial) x 4 (Time) repeated measures analysis of variance (ANOVA) was used to assess differences from resting oxidative stress to post exercise values in and between both CrossFit and Treadmill trials. For the key dependent variables, a Mauchly's Test was run to determine there was no violation of sphericity. Statistical analysis was performed on SPSS 19.0 (Chicago, IL). A Tukey post hoc was used to determine significant mean differences when indicated. Statistical significance was set to $\alpha \leq 0.05$. Data presented as means ± standard deviations.

RESULTS

All participants completed CrossFit and Treadmill protocols. Mean anthropomorphic values can be seen in Table 1. In addition to monitoring HR for intensity, a rate of perceived exertion (RPE) scale numbered 1-10, with 1 being no effort and 10 being maximal effort, was used. Percent HR_{max} and RPE were used to ensure a similar effort of intensity between trials; results are presented in Table 2.

Oxidative damage biomarkers are presented for blood plasma lipid hydroperoxides (Figure 2a) and blood plasma Protein Carbonyl content (Figure 2b). Statistical analysis of lipid damage (LOOH) demonstrated a significant time dependant increase in both CrossFit and Treadmill trials ($p< 0.001$), while no trial-dependent difference where observed ($p= 0.623$). Plasma LOOH did not exhibited a significant increase after 1-HP for CrossFit and Treadmill, 143% and 115% respectively ($p< 0.001$). At 2-HP values increased further to 256% and 167% compared to PRE values ($p< 0.001$). A significant difference was also observed between 1-HP and 2-HP ($p= 0.025$). Analysis of mean plasma protein carbonyl, demonstrated a time dependent decrease ($p= 0.001$), but no trial dependant differences ($p= 0.245$). A non-significant increase was observed IPE ($p= 0.187$) followed by significant decreases in both 1-HP ($p= 0.001$) and 2-HP ($p< 0.001$) in both trials, only when compared to IPE.

Biomarkers for antioxidant capacity are presented as FRAP (Figure 3a) and TEAC (Figure 3b). Analysis of FRAP demonstrated a significant time dependant increase in both trials ($p< 0.001$), but not between trials ($p= 0.080$). FRAP concentration increased significantly in all post exercise time points IPE ($p< 0.001$), 1-HP ($p=0.001$), and 2-HP ($p=0.004$). TEAC unexpectedly showed a negative time dependant reaction in both trials ($p= 0.00$), while no trial dependant differences were observed ($p= 0.134$).

TEAC significantly decreased in all post measurements IPE ($p < 0.001$), 1-HP ($p = 0.001$), 2-HP ($p = 0.001$).

DISCUSSION

The purpose of this investigation was to examine the blood oxidative stress response to an acute bout of CrossFit exercise and compare them to a more traditional Treadmill bout normalized for time and HR intensity. The marker used to control for intensity ($\%HR_{max}$) was similar between trials, but statistically different when examining area under the curve; however, this difference is not believed to be of physiological significance. The observed marker of intensity (RPE) also significantly differed between trials despite the similarities; psychological perception and influences may be responsible for the observed differences. The key finding of this study was that markers of oxidative stress did not significantly differ between trials; however, a time dependent increase in oxidative stress markers occurred in each. These findings suggest the oxidative stress response to CrossFit is proportional to the exercise intensity performed and as such the time-frame for the observed oxidative stress responses parallel prior observations using different exercise modalities (14, 17, 18, 21, 22, 30). Additional points of consideration are provided below.

MARKERS OF OXIDATIVE STRESS

While not measured directly in this investigation, the sources of oxidative stress in this study are multi-faceted. One of the most physiological abundant oxidants, superoxide, is widely produced in the body and is rapidly converted to other ROS (3). Based the on current literature, there are several known sites for superoxide production, which include mitochondrial respiration through complex I and III, catecholamine auto-

oxidation, and various oxidases (3, 10, 27). Exercise is a well-documented facilitator of ROS production throughout the body (10, 27, 28). This study examined two types of high-intensity exercise bouts in the form of CrossFit and time-matched Treadmill running. These trials were matched by time and $\%HR_{max}$ to produce a common level of exercise intensity. Despite differences in modality and muscle recruitment, no differences in redox reaction were observed between trials. These findings support recent works that suggests the oxidative stress response is more sensitive to exercise intensity than modality (31).

The marker for lipid damage, lipid hydroperoxide (LOOH), increased in a trial-independent fashion following each bout and confirmed that exercise induced oxidative-stress occurred. Previous works supports these findings in that increasing measures of LOOH occur following several different exercise modalities such as hiking, cycling, and weight lifting (18, 23, 30). Quindry et. al., observed that rises in LOOH occur independently of aerobic metabolism, and significantly rise following exercise that surpasses lactate threshold (31). Therefore, it is more likely that the exercise intensity of the bouts, approximately 90% of HR_{max}, was the driving force behind the lipid hydroperoxide production. Given that CF and TM were closely matched for intensity, the similar rise in plasma LOOH is not surprising.

The time dependent decrease in Protein Carbonyl (PC) was an unexpected result of the study. PC is a sensitive marker of ROS mediated protein damage and has been shown to increase in plasma after bouts of acute exercise of varying intensities (5, 14, 18, 24, 29, 30). However, the current observation is not the first of such instances where exercise does not result in an increase in PC (Quindry in review, (6). Bloomer et al.

observed no change in PC following an acute bout of squats or sprints, which interestingly trended towards a decrease (6). Similarly, Quindry et al. observed no change immediately post eccentric exercise; however, 24 hours following the session, a significant rise in PC was observed (29).

The oxidative damage response of this study yielded mixed results, both expected and unexpected. LOOH increased following the trials only after the first hour of recovery, while PC unexpectedly decreased following the trials. The lack of response in PC suggested that ROS mediated protein damage may not have occurred; however, the delayed PC response in the Quindry et al., study suggests that oxidative stress mediated protein damage may occur through a larger window of time, and therefore the magnitude of response in this study may not yet have occurred (29). These findings of discordant biomarker results also reinforce the need for a panel approach to quantify wide biological processes such as oxidative stress.

MARKERS OF ANTIOXIDANT DEFENSE

In order to gain a comprehensive look at redox balance, both oxidative damage and antioxidant defense were examined. Findings from the current study included antioxidant defense through the antioxidant potential (FRAP), which is sensitive to aqueous- and lipid-phase anti-oxidants, and antioxidant capacity (TEAC), which is sensitive to protein and nonprotein sulfhydryl antioxidants (9). The importance of redox balance is illustrated in a classic concept referred to as the "oxidative stress pecking order". According the pecking order, oxidative stress related damage would not occur until after endogenous antioxidant defense has failed (8). Therefore, oxidative damage will occur when redox balance is lost.

The antioxidant potential, ascorbate equivalent (FRAP), measures the antioxidant reducing ability in plasma and is commonly elevated in response to rising levels of ROS (4). The blood plasma biomarker FRAP increased significantly IPE and remained elevated throughout the time points following CrossFit and Treadmill protocols, which is an expected response due to the presence of oxidative-stress in both trials. A similar elevation of ascorbate equivalent was observed in a study performed by Quindry et al., where cyclists performed three separate climate conditioned (cold, warm, neutral) 60min protocols (30). Hudson et al. experienced mix results when examining FRAP levels following bouts of strength and hypertrophy exercises, only observing a significant rise 60min following hypertrophy (18). However, MaCnulty reported elevated levels of FRAP following ten separate resistance exercises in a interval scheme (21). The response to increased repetition scheme suggests that duration and intensity play a role in antioxidant potential. Uric acid, a by-product of Purine metabolism, has been shown to increase with elevated exercise intensity (34), which is important to note because the FRAP assay is primarily driven by uric acid (9), and therefore a potential explanation for the observed increase in post exercise FRAP.

Antioxidant capacity measured through TEAC decreased significantly following both trials. A decrease in antioxidant capacity is an unusual occurrence in the presence of oxidative damage as well as increased antioxidant potential. Several studies have demonstrated a concurrent increase of TEAC and oxidative stress (21, 23, 30), while only a few studies have observed a decrease in TEAC following exercise(12, 18). Hudson et al. observed a decline of TEAC 60 minute following strength-based exercise, while hypertrophy based resistance exercise resulted in an increase IPE (18). In untrained

participants Falone et al. observed a decrease in TEAC following a maximal treadmill test, in contrast they observed an increase in TEAC in trained participants (12). There was no physiological evidence or explanation for the decline in TEAC in these studies.

The observed decrease of plasma TEAC and PC in this study may reflect a shift in plasma volume and density known as Haemoconcentration (16). In a postural study by Szalky et al., plasma density was measured through plasma albumin and globulin, which decreased during periods of haemoconcentration, resulting in a net loss of plasma protein (16). Haemoconcentration following bouts of high-intensity exercise results in no change in plasma protein concentration or in a few instances a decrease in plasma protein (13, 15, 19); however, shortly after haemoconcentration (times may vary up to 24-hours) plasma shifts rebound and result in a period of haemodilution, which increases plasma protein concentration (19). An additional phenomenon that may be responsible for decreased plasma protein concentration is post exercise proteinuria. During heavy exercise filtration fraction in the kidneys increase allowing proteins into the ultrafiltate or maximal tubular reabsorption resulting in consequential protein loss in urine (proteinuria) (20). The possibility of haemoconcentration and/or post exercise proteinuria are both potential factors for the observed decreases in PC levels following the trials and subsequently decreased TEAC. The TEAC assay is driven primarily through an albumin-based reaction, which contributes approximately 28% to the assay (9). Because both PC and TEAC are albumin-based assays, a decrease in plasma protein (i.e., albumin) may be related to the decreases observed both PC and TEAC assays. Further investigations of these relationships are needed.

Summary and Conclusions

CrossFit is a relatively new style of training that has become widely popular. To date, little is known about the affects of a single bout of CrossFit on Redox reaction or how these results may compare to a time-matched bout of Treadmill running. High levels of free-radicals are known to cause cellular damage, however moderate increases are responsible for several beneficial cellular functions (27). The oxidative stress response to CrossFit was significant enough to illicit lipid peroxidation and increase antioxidant potential, although this was not significantly different than a time and intensity matched bout of treadmill running. This indicates that CrossFit may be no more stressful than high-intensity treadmill running, within trained subjects. The amount of oxidative stress is directly related to the level of exercise intensity rather than the exercise modality, and therefore should be taken into consideration during the prescription of exercise. The lack of PC and TEAC in this study may be more of an indication of timing rather than a lack of oxidative stress and therefore, further research is necessary to determine the full extent of acute oxidative stress on CrossFit bouts.

In summary, the findings of this study indicate that there were no statistical differences in oxidative damage or redox balance between CrossFit and Treadmill running. Results also confirm that exercise intensity is a major contributing factor in the nature of the oxidative response as well as the time course. These tests were performed on CrossFit trained individuals familiar to the stress involved with this type of exercise. Future research should examine untrained participants in order to gain a better understanding of the physiological stresses involved in CrossFit. Furthermore, because the redox time course was not evaluated beyond two hours into recovery, future studies should examine high-intensity bouts with a more extensive timeline. The implications of

these findings suggest that complex redox reactions involved in high-intensity exercise are not fully known, and that further research is needed.

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TABLE 1. Participant Characteristics

Characteristic	Values ± SD
Age (yr)	26.4 ± 0.9
Height (in)	69.8 ± 0.9
Weight (lb)	181.7 ± 2.5
Body Fat(%)	11.2 ± 2.4
VO_{2max} ($ml \bullet kg^{-1} \bullet min^{-1}$)	44.4 ± 5.1
Max HR (bpm)	183.6 ± 2.0

N=10 Means +/- SEM

TABLE 2. Exercise Intensity (Perceived and %HRmax)

	6minute	10minute	16minute	20minute	p value
RPE					
CF	5.4(±0.5)	6.4(±0.5)	7.6(±0.2)	9.0(±0.3)	(p= 0.005) time
TM	4.6(±0.4)	5.6(±0.4)	7.2(±0.4)	7.2(±0.5)	(p= 0.007) trial
%HRmax					
CF	93.3(±1.2)	94.6(±0.9)	95.9(±1.0)	97.7(±1.9)	(p= 0.005) time
TM	89.3(±1.1)	92.9(±0.8)	94.2(±0.9)	93.6(±1.0)	(p= 0.000) trial

N=10 Means +/- SEM. *RPE scale 1-10 lowest to highest exertion.*

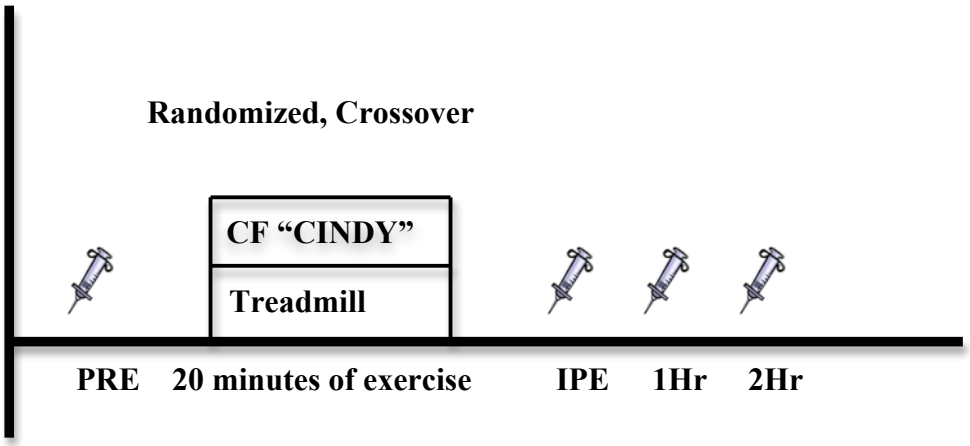

Figure 1. Study design. Biomarkers of oxidative-stress (LOOH, PC, FRAP, TEAC) were taken at designated time points, represented by blood draw (syringe) icons. Samples taken before and after (randomized, crossover design) 20 minutes CF and TM bouts.

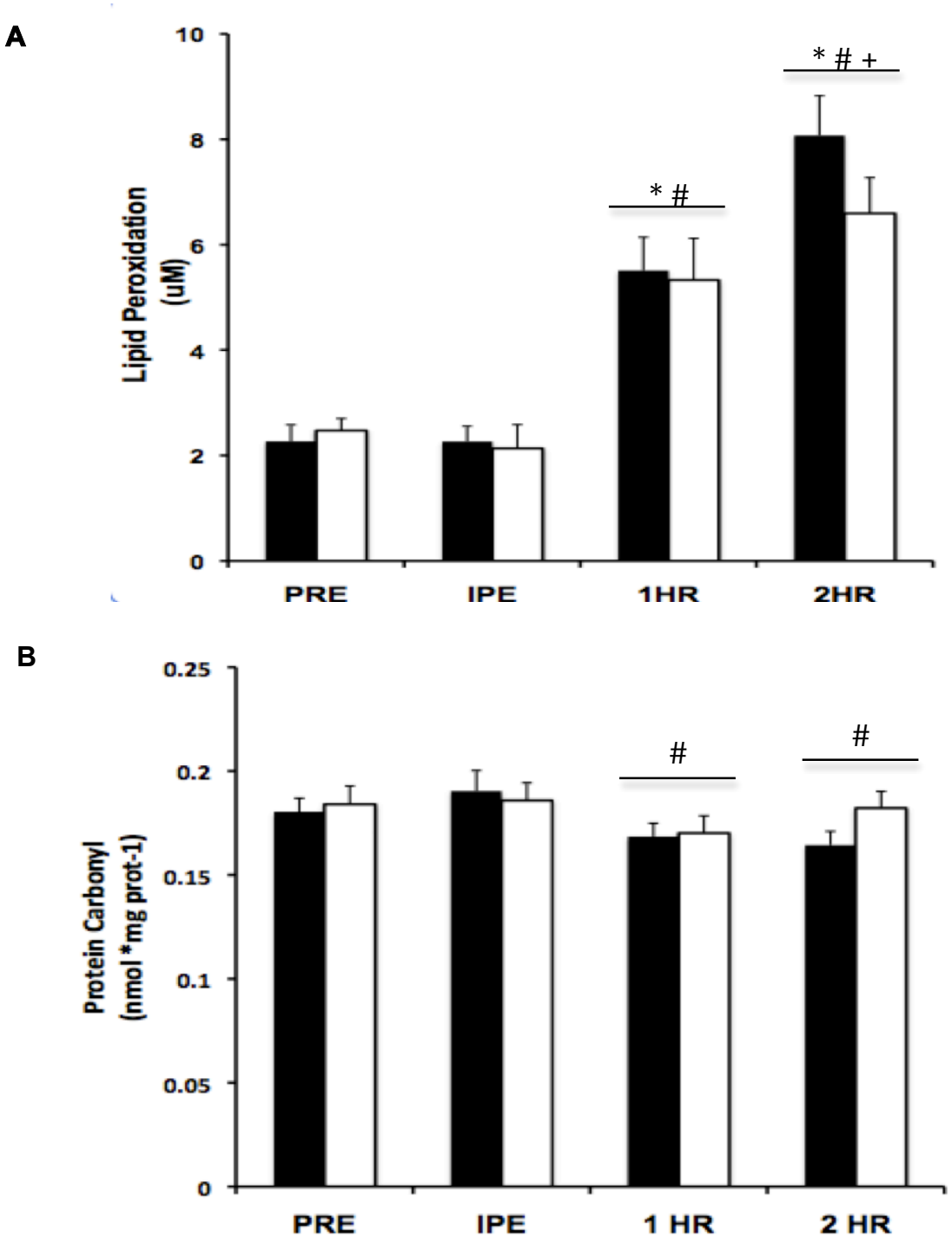

FIGURE 2. Biomarkers of Oxidative Damage, before and after exercise, Means ± SEM, n=10. A) lipid peroxidation of blood plasma B) Protein Carbonyl blood plasma. * significant from PRE, # significant from IPE, + significant from 1-HP. Black bars = CF, Open bars = TM

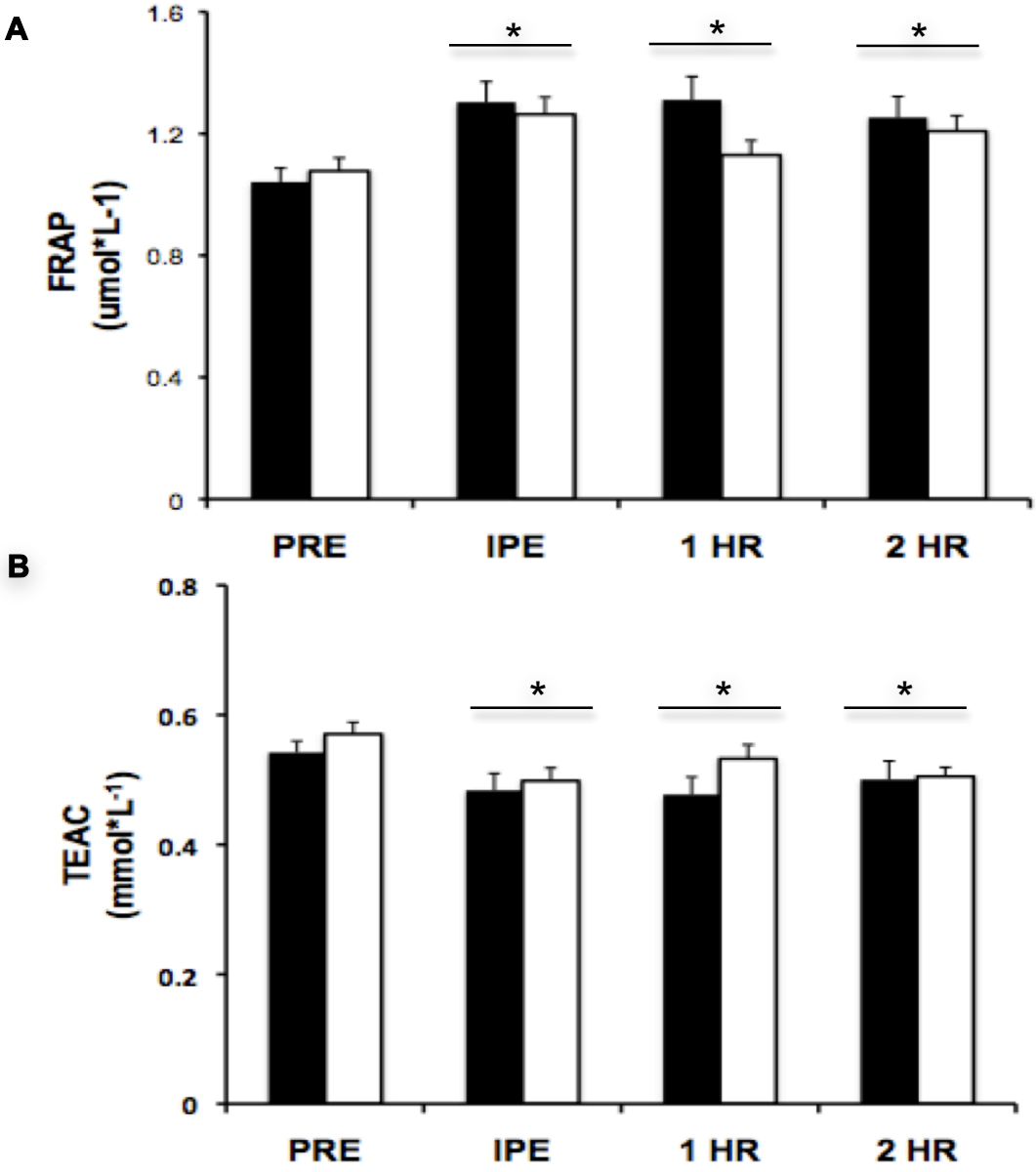

FIGURE 3. Biomarkers of Antioxidant Capacity. Means ± SEM, n=10. A) Blood plasma antioxidant potential FRAP B) Blood plasma antioxidant capacity TEAC. * Significantly different from PRE. Black bars = CF, Open bars = TM.