Effect of a non-ketogenic carbohydrate restricted diet on oxidative stress response to high intensity resistance exercise.

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Abstract

Ketogenic diets consisting of >70% of total caloric intake from lipids have been shown to induce favorable shifts in the redox environment such as mitochondrial biogenesis and reduced markers of oxidative stress (OS). However, a diet with such low dietary carbohydrate intake is likely difficult to sustain for long periods of time. The purpose of the study was to investigate the effects of a non-ketogenic, low carbohydrate, high fat (LCHF) diet compared to a western diet (WD) diet on markers of OS before and after high intensity resistance exercise (HIRE). The HIRE protocol involved five rounds of back squat, deadlift, and bench press completed in respective order and in a descending ladder (10, 8, 6, 4, 2 repetitions) until completion with minimal to no rest. The LCHF diet was 15-days of >50% of calories from fat, ≥ 25% of calories from protein, and ≤ 25% of calories from carbohydrates. Blood was sampled before exercise, immediately after exercise, as well as 60-minutes post-exercise. Plasma was analyzed for total antioxidant capacity (TAC), tumor necrosis factor-α (TNF-α), malondialdehyde (MDA), superoxide dismutase (SOD) and glucose. The main findings demonstrate the the exercise protocol increased ($p<0.05$) plasma levels of glucose, TNF-α, and MDA. There was no effect ($p>0.05$) on TAC or SOD. The 15-day LCHF diet did not significantly impact pre- or post-exercise blood markers of OS. However, these findings suggest HIRE is effective at eliciting a small but significant increase in blood markers of OS.

Keywords: Antioxidant, Inflammation, Training, Lipid oxidation.

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Introduction

Oxidative stress (OS) is associated with an imbalance between the accumulation of pro-oxidant molecules and endogenous antioxidant defenses [1]. OS is associated with excessive accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [2]. Sources of OS may include-but are not limited to-acute exercise, excessive caloric intake, cigarette smoke, and psychological stress [1]. Yet, exposure to moderate amounts of ROS and RNS may also serve as a trigger for up-regulation of endogenous antioxidant status. This likely occurs via activation of the Kelch-like ECH associated protein 1 (Keap1) nuclear factor erythroid-2 related factor-2 (Nrf2) pathway [2].

Ketogenic diets (KD) consisting of >70% of total caloric intake from exogenous lipids have been shown to induce favorable shifts in the redox environment. KD may increase uncoupling protein activity and reduced ROS accumulation [3], mitochondrial biogenesis [4], and increased glutathione levels [5]. It is also important to note significant improvements in mitochondrial function have been found from a KD, even compared to isocaloric western diets (WD) [6]. One study has shown elevations in blood antioxidant capacity following 14-days of a KD [7]. However, human trials are scarce; therefore further research is needed. In addition, the KD involves extreme restrictions in dietary carbohydrate intake (less than 10% of total calories) which makes the diet less practical. It is possible that less extreme carbohydrate restricted diets which allow for more flexibility in carbohydrate intake (less than 25% total calories) may induce similar physiological adaptations without such rigorous dietary restrictions [8]. Previous findings from our lab demonstrated positive hormonal and metabolic responses from a non-ketogenic carbohydrate restricted diet [8]. Therefore, further examination on OS markers is warranted.

Some studies have reported potential health related benefits of a non-ketogenic, low carbohydrate, high fat (LCHF) diet, including improvements in blood lipids [9], body composition [10], cellular signaling, cognitive functioning, and hormonal profiles [10]. To our knowledge, no study has investigated effects of a non-ketogenic LCHF diet on markers of OS. In addition to dietary modifications, a range of exercise modalities and intensities-including high-intensity steady-state aerobic exercise and resistance training-have been shown to induce acute OS which may serve as a trigger to up-regulate endogenous antioxidants [1]. Although the literature shows favor to an increase in ROS production when eccentric muscle actions are involved [1], more information is needed regarding to the impact of intensity (i.e., low, moderate, or high) in relation to the resulting magnitude of OS [11]. To date, only one study has investigated the OS response of a very high intensity CrossFit style resistance training (HIRE) protocol [12]. Therefore, the

purpose of the current study was two-fold: 1) to determine if short duration HIRE would elicit significant increases in markers of OS and inflammation, and 2) to investigate if a LCHF diet would significantly impact biomarkers of antioxidant status and OS after following either a WD or LCHF diet.

Methods

Subjects

Thirteen (n=13) apparently healthy, moderately trained males (21.6 ± 1.8 yrs.; 178.52 ± 4.95 cm) participated in this study. Inclusion criteria included: (I) meet the American College of Sports Medicine aerobic activity guidelines: at least 150 minutes of moderate-intensity aerobic activity or 60 minutes of vigorous intensity aerobic activity per week [13], (II) meet the National Strength and Conditioning Association resistance training status for an intermediate training age: currently training for 2-6 months ≥ 3 days per week, [14] (III) be between the ages of 18-35, (IV) no history of cardiometabolic, neurological, or musculoskeletal disorders, (V) currently following a WD diet consisting of ≤ 30% of total caloric intake from dietary fat, and (VI) not currently smoking or taking antoxidant or multivitamin supplements. Each subject provided written informed consent, completed a health questionnaire, a training history form, a lifestyle evaluation, and a physical activity readiness questionnaire to assess risk stratification. All procedures of this study were approved by the University's Institutional Review Board. Subjects were made aware of potential risks that could occur during all trials, including the diet phase, and acknowledged that their participation may be terminated at any point upon their request. Following completion of paperwork, age (yrs), height (cm), body mass (kg), resting metabolic rate (kcal), fat free mass (kg), and fat mass (kg) were collected.

Dietary manipulation

Subjects submitted a written 3-day ad libitum food journal consisting of two weekdays and one weekend day. A free online food diary, MyFitness Pal, was used during the duration of the study to record dietary composition. MyFitnessPal (MyFitnessPal, Calorie Counter, 2017, Baltimore, MD.), is an online food tracker used by registered dieticians and has been successfully used to track diets >7 days [10]. These dietary food logs were subsequently analyzed for caloric intake and macronutrient composition through food analysis software Nutrionist-Pro (version 2.2, 2005, Axxya Systems-Nutrionist Pro, Stafford, TX. USA), developed from the USDA National Nutrient Database for Standard Reference. Following preliminary dietary analysis, an extensive overview was explained before implementation of the LCHF diet. Each subject was prescribed a specific caloric range (total kcals ± 250 kcals) with macronutrients from fat, protein, and carbohydrates (> 50%, ≥ 25%, and ≤ 25% respectively) based off each subject's preliminary food log. Further, each subject was provided a LCHF weekend and weekday sample menu with kcals and macronutrient grams per food item. Due to the lifestyle of our subjects, college aged males, convenient LCHF fast food options and a grocery list with LCHF foods, snacks, and desserts were also made available. Daily correspondence was maintained between all subjects and the primary investigator for any questions that might arise regarding the dietary protocol. Reminders were sent out each night for subjects to review the current day's macronutrient consumption and to make sure all ingestion of liquids and food were recorded. Food logs from the previous day were asked at random by the investigators to further ensure adherence to the diet. Macronutrient percentages, and overall kcals following the WD and LCHF diet, can be viewed in Table 1. Calories were <500 kcal difference between diets to ensure participants were not in a hypo- or hypercaloric state. Subjects were asked to continue normal training routines. A registered dietician located within the university's nutrition department oversaw the entire process.

Table 1. *Macronutrient Composition during 15-days LCHF.*

Diet Variable	Western Diet	LCHF Diet
K/cals	2357 ± 649	2593 ± 836
Protein (E%) Protein (g) Protein (g/kg)	19% 106 ± 32 1.17 ± 0.55	29% 167 ± 68 2.07 ± 0.68
CHO (E%) CHO (g) CHO (g/kg)	52% 295 ± 40 3.2 ± 0.70	15% 89 ± 39 1.14 ± 0.50
Fat (E%) Fat (g) Fat (g/kg)	29% 79 ± 65 0.87 ± 0.87	56% 147 ± 53 1.90 ± 0.47
Data are presented and mean ± SD. %E=% energy consumed as percentage of total kilocalories. CHO=carbohydrate.		

Experimental design

The present investigation used a within, repeated measures design to examine the effects of a WD diet compared to a 15-day LCHF diet on OS and inflammatory responses to exercise. Our laboratory has shown a two week dietary intervention to be sufficient to induce metabolic and hormonal adaptations in trained individuals [8], concequently a 15-day period was chosen for this study. All subjects reported to the departmental laboratory for two testing sessions (one before and one after the diet protocol) following a 10-h fast between the hours of 0500 and 0800. Participants were reminded 48-h in advance to refrain from strenuous exercise, alcohol, and caffeine consumption before testing. The preceding 24-h food log from each subject was recorded and analyzed before each trial. A preliminary trial was used to explain the exercise protocol, assess participants' height (235D; QuickMedical, Issaquah, WA, USA), body mass (Defender 5000, Ohaus Corporation, Parsippany, NJ, USA), resting metabolic rates, and body density (shown as % fat) with the BOD POD version 1.69 (Body Composition System; Life Measurement Instruments, Concord, CA).

Exercise protocol

The prescribed exercise protocol was adopted based on the HIRE methodology incorporated into common CrossFit workouts. Intensity by definition is force multiplied by distance and divided by time, or the ability to do more work at a faster rate. Therefore, rather than prescribe intensity based on barbell weight alone, the current study used a time to completion protocol in addition to a prescribed barbell weight. Subjects were instructed through a standardized 10-min warm-up consisting of full body, dynamic exercises. Following the warm-up, three stations were set up, and the same load of 75% of the subject's body weight (lbs) was used for each exercise. This is a common method for CrossFit style resistance training and was chosen to allow each participant to work at an intensity

and an external load relative to their body mass. Exercises included the main common powerlifts including the back squat, deadlift, and bench press and were completed in respective order in a descending ladder (10-8-6-4-2 repetitions) circuit. More specifically, each subject completed 10 repetitions in the back squat, then deadlift, then bench press before moving to the set of 8 repetitions for each exercise. Subjects performed two repetitions of each exercise as the last round of each exercise. Due to the metabolic requirements of HIRE and potential changes in the primary dependent variables, the protocol was completed as fast as feasible for each individual with rest not included in the workout and finishing time recorded. Proper execution (i.e., form and repetitions) for each exercise was monitored by a NSCA certified strength and conditioning specialist and terminated if improper form put the participant at risk for skeletal or muscular injury.

Blood sampling and analysis

Blood samples were obtained just prior to the start of exercise, immediately post-exercise, and 60 min post-exercise. Blood draws were performed with the participants in a supine position. A total of 14 mL of blood were sampled at each draw from the antecubital vein. Blood was drawn into an EDTA anticoagulant sealed vacutainer and centrifuged for 10 min at 1000 x g at 4°C and stored at -80°C. Plasma samples were analyzed per assay instructions for glucose (Pointe Scientific, Canton, MI, USA), as previously performed by our lab [15], tumor necrosis factor-α (TNF-α) (R and D systems, Minneapolis, MN, USA), thiobarbituric acid reactive substances (TBARS), which is expressed in units of malondialdehyde (MDA) equivalents (Cayman Chemical, Ann Arbor, MI, USA) [16], total antioxidant capacity (TAC) which is expressed as trolox equivalents (Cayman Chemical, Ann Arbor, MI, USA) [17] and superoxide dismutase (SOD) Cayman Chemical, Ann Arbor, MI, USA) [18]. Absorbance was read using an iMark Bio-Rad microplate absorbance reader (Life Science Research, Hercules, California, USA).

Statistical analysis

Data are presented as mean ± standard deviation (SD) unless otherwise specified. Data were analyzed using SAS version 9.4 (SAS Institute, Cary, NC, USA). Since inclusion criteria required participants currently practice a WD, the initial exposure to the exercise protocol (before the LCHF intervention) was analyzed as a response following the habitual diet of the participants. Thus, after the 15-day period, the same exercise protocol was performed following the LCHF treatment. A 2 X 3 (Diet X Time) RMANOVA was conducted for plasma levels of TNF-α, TAC, MDA, SOD, and glucose. Fisher's least significant different (LSD) post-hoc tests were used in the instance of a significant main effect ($p<0.05$).

Results

Anthropometric characteristics

Thirteen subjects completed the testing procedures as well as the LCHF dietary protocol. There was a decrease in absolute mass from baseline to post LCHF diet (89.6 ± 10.9 vs. 88.5 ± 10.5 kg). Descriptive characteristics including body mass (kg), body fat percentage, fat mass (kg), fat free mass (kg), and resting metabolic rate are shown in Table 2.

Table 2. *Subject descriptive characteristics.*

Characteristics	Baseline	Post-LCHF
Mass (kg)	89.6 ± 10.9	88.5 ± 10.5
Body fat (%)	19.7 ± 7.5	19.2 ± 7.2
RMR	1918.8 ± 176.4	1904.1 ± 183.6
Fat Free Mass (kg)	71.5 ± 6.6	71.1 ± 6.9
Fat Mass (kg)	18.1 ± 8.1	17.37 ± 7.5

Data are presented and mean ± SD. T-value. *p<0.05. RMR=Resting metabolic rate expressed in units of kilocalories. LCHF=Low carbohydrate non-ketogenic carbohydrate restricted diet.

Total antioxidant capacity

There was no significant Diet X Time interaction for TAC ($F=0.14$, $p=0.86$). There was no significant main effect for diet ($F= 1.37$, $p=0.24$) or time ($F=0.12$, $p=0.88$) indicating no change in TAC from the exercise or dietary intervention. Plasma TAC results are shown in Figure 1.

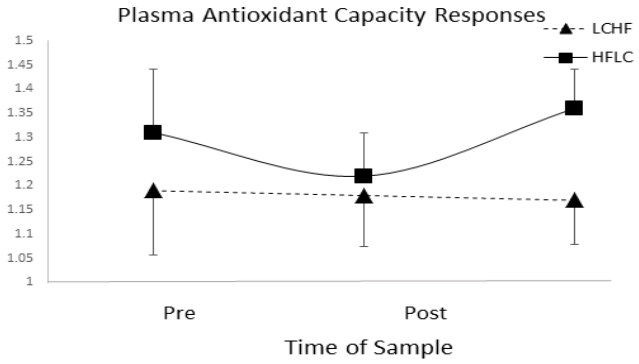

Figure 1. *Changes in plasma total antioxidant capacity before and after exercise. LFHC=low fat high carbohydrate western diet. LCHF=low carbohydrate non-ketogenic carbohydrate restricted diet. Data are shown as mean ± SE.*

Glucose

Regarding plasma glucose levels, there was no significant Diet X Time interaction ($F=0.63$, $p=0.53$). There was no significant main effect from the diet ($F=0.41$, $p=0.52$); nonetheless, there was a main effect for time ($F=12.01$, $p < 0.01$) indicating a significant change from pre- to post-exercise. Changes in plasma glucose concentrations over time for both dietary treatments are shown in Figure 2.

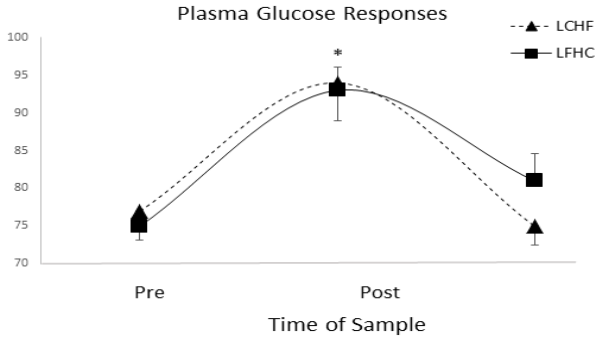

Figure 2. *Changes in plasma glucose before and after exercise. HCLF=high carbohydrate low fat western diet. LCHF=low carbohydrate non-ketogenic carbohydrate restricted diet. * denotes significant ($p<0.05$) increase compared to all other time-points. Data are shown as mean ± SE.*

MDA

There was no significant Diet X Time interaction (F=3.40, p=0.70) and no significant main effect for diet (F = 0.69, p=0.41). However, there was a significant main effect for time (F=3.97, p=0.02) indicating a significant change in plasma MDA from pre- to post-exercise. Further post-hoc analysis reveals that, when averaging both treatments, there was a significant increase in MDA from pre- to immediately post-exercise (p=0.01) and a significant decrease from immediately post-exercise to 60-minutes post-exercise (p=0.02). Despite these findings, the significant increase in MDA from pre- to post-exercise was only found following the WD (p=0.02) and did not occur following the LCHF intervention (p=0.24). Data for plasma MDA are shown in Figure 3.

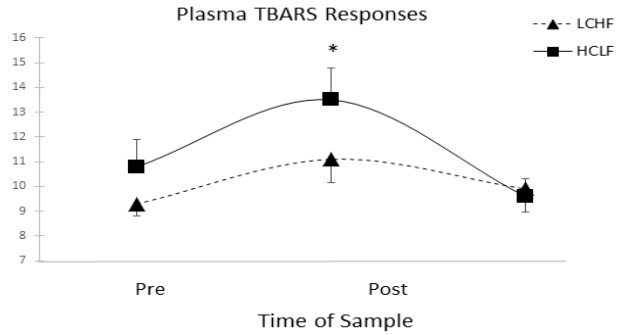

Figure 3. *Changes in MDA before and after exercise. HCLF=high carbohydrate western diet. LCHF=low carbohydrate non-ketogenic carbohydrate restricted diet. MDA=malondialdehyde equivalents. * denotes significant (p<0.05) increase compared to all other time-points. Data are shown as mean ± SE.*

TNF-α

Regarding plasma TNF-α concentrations, there was no significant Diet X Time interaction (F=0.54, p=0.58) nor a significant main effect for diet (F=0.11, p=0.74). However, there was a main effect for time (F=13.48, p<0.01), indicating a significant change from pre- to post-exercise. Data showing mean TNF-α concentrations are shown in Figure 4.

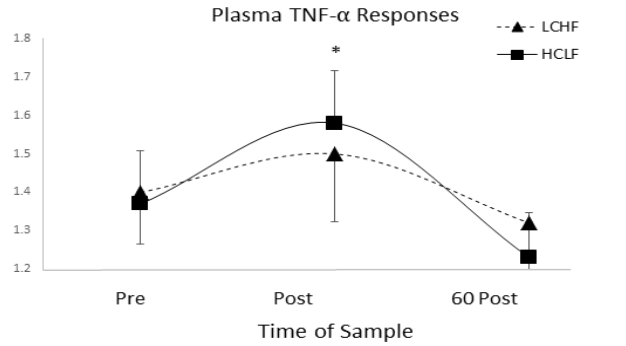

Figure 4. *Changes in plasma tumor necrosis factor alpha (TNF-α) before and after exercise. HCLF=high carbohydrate western diet. LCHF=low carbohydrate non-ketogenic carbohydrate restricted diet. * denotes significant (p<0.05) increase compared to all other time-points. Data are shown as mean ± SE.*

SOD

Regarding SOD, there was no significant Diet X Time interaction (F=0.27, p=0.76) and no significant main effect for time (F=1.34, p=0.27) or diet (F=0.35, p=0.55). SOD results are shown in Figure 5.

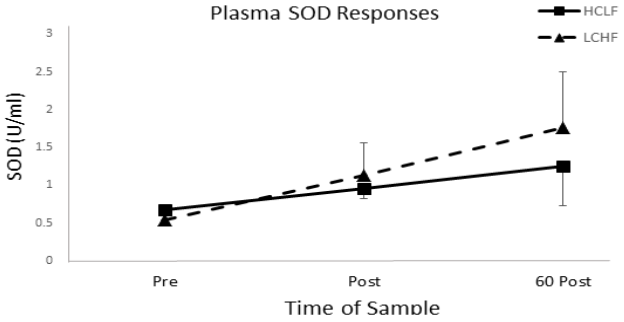

Figure 5. *Changes in plasma superoxide dismutase (SOD) levels before and after exercise. HCLF=high carbohydrate western diet. LCHF=low carbohydrate non-ketogenic carbohydrate restricted diet. Data are shown as mean ± SE.*

Discussion

Main findings of this study are that the LCHF did not significantly impact markers of OS before or in response to the resistance exercise. Yet, the short duration HIRE protocol was effective in eliciting a small, but significant, increase in plasma glucose, MDA, and TNF-α. This is not surprising since exercise induced ROS accumulation is known to produce markers of OS and inflammation [11,19]. This is an especially meaningful finding since the exercise protocol only lasted on average between 5.4 and 6.6 minutes. This is also in agreement with our study design suggesting that intensity, as by its definition, is an appropriate tool for mediating ROS production. Previous studies have also reported correlations between plasma glucose levels and OS [20]. These data suggest short duration, HIRE/CrossFit style resistance exercise is challenging to the redox environment; thus, chronic HIRE training may in turn be effective at up-regulating beneficial ROS induced antioxidant adaptations [2].

In terms of the magnitude of inflammatory response to the protocol, it is important to note the elevation in blood levels of MDA and TNF-α in response to this exercise protocol are relatively small compared to other sources-such as cigarette smoke, air pollution, obesity, etc. Previous findings have also reported ingestion of lipid rich meals are an acute source of OS [21]. Despite these results, we did not find a 15-day LCHF diet resulted in significant alterations of resting or exercise induced OS or inflammation. Several studies have reported detrimental effects of chronic high fat diets [22-24]. In these cases, it is difficult to speculate whether or not the composition of the diet was responsible for the changes, or simply caloric surplus alone. In regards to the current findings, it is possible significant changes occurred in some OS markers soon after the initiation of the diet (i.e., within the first few days); which may have been followed by an adaptation which appears to result in no change in a 15-day period. However, the current study is limited by not measuring these biomarkers more frequently within a 2-wk window. While exercise did not cause a significant increase in MDA after the 15-day LCHF diet (but did cause a significant increase following the WD), strong implications should not be made from these findings since there were no significant treatment effects.

Our findings are similar to those reported from Kliszczewicz et al. that acute HIRE/CrossFit exercise is effective at challenging the redox environment. The current findings also support the

speculation that intensity of exercise is a stronger predictor to OS responses as opposed to modality alone [12]. Azizbeigi et al. reported both moderate and high intensity resistance training interventions are similarly effective at reducing markers of OS and inflammation for a period of 8 wks [11]. Although, it is important to note this intervention allowed one to four-min of rest between sets; therefore, the suggestion of exercise intensity alone not being a significant predictor of antioxidant effects of chronic resistance exercise [11] cannot be applied to a HIRE or CrossFit style protocol, such as the one currently utilized. The current protocol involved five rounds of three exercises with minimal-to-no rest between sets, and the entire exercise protocol being completed in less than 10-min. It is also important to note the term "intensity," when discussing resistance exercise, typically refers to training load (normally expressed as a percentage of 1RM). While the training load for the current study was likely low for this resistance trained population (75% of body mass), an important finding is that we did find significant elevations of markers of OS, which is not necessarily due to training load per se, but rather due to elevated intensity in terms of metabolic demand. This study is limited since metabolic demand (i.e., volume of oxygen consumption, etc.) was not measured. However, the purpose of the study was not to investigate cardiorespiratory and metabolic responses, but to investigate OS and inflammatory responses as potentially effected from the dietary intervention.

Several studies have reported physiological benefits of a KD [5-7]. Albeit, most of these studies have involved testing in rodent models. Thus, additional human trials are needed. 3-wks of a KD in humans has been shown to reduce OS resulting from caloric restriction and exercise as previously found in Taekwondo athletes [25]. Rhyu and Cho also reported attenuated elevations in TNF-α during a weight loss program associated with a KD [25]; though, these data are not entirely comparable since a change in body mass and/or body composition likely effects circulating cytokine levels. Further, 14-days of a KD has also been shown to increase blood antioxidant status in active, healthy women in response to a caloric restricted intervention [7]. However, these studies were conducted in a state of nutritional ketosis, and therefore elevated blood ketones are perhaps necessary to induce anti-oxidative effects reported in previous literature [7,26]. It is also possible that changes in blood glucose and ketone levels from caloric restriction may potentially be responsible for some of the documented benefits. Thus, future studies should seek to investigate the effects of elevated ketone levels (perhaps via exogenous ingestion) and anti-oxidative effects. Alternatively, the eNOS, cAMP and/or AMP activated protein kinase (AMPK)/NAD dependent deacetylase sirtuin 1 (SIRT1) pathway may also be activated during a period of caloric restriction which may enhance mitochondrial capacity and decrease susceptibility to OS.

The participants involved in this study were chronically exercise trained, therefore they likely had training induced increases in endogenous antioxidant status [1] which possibly explains the finding of no significant change in pre- or post-exercise OS markers. Training status has specifically been shown to effect OS responses to acute exercise [27]. While the majority of literature has investigated and reported chronic anti-oxidative responses of aerobic exercise [28,29], some have reported improved antioxidant status from resistance and anaerobic training as well [30]. Resistance exercise has also been shown to reduce OS associated with chronic diseases, including Parkinson's disease [31] and obesity [32]. Nonetheless, regarding anaerobic exercise, data are extremely limited and-to our knowledge-no study has investigated the effects of chronic HIRE training. In conclusion, the present data are unique in demonstrating that an acute session of resistance exercise does significantly increase plasma glucose, MDA, and TNF-α. Since reoccurring exposure to moderate amounts of OS in the form of exercise is known to induce favorable redox adaptations, future studies should investigate potential chronic anti-oxidative effects of HIRE training.

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