Low volume-high intensity interval exercise elicits antioxidant and anti-inflammatory effects in humans

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Low volume-high intensity interval exercise elicits antioxidant and anti-inflammatory effects in humans

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Abstract

The purpose of the present study was to compare acute changes in oxidative stress and inflammation in response to **steady state and low volume, high intensity interval exercise (LV-HIIE)**. Untrained healthy males (n=10, mean ± SD: age 22 ± 3 yrs; VO_{2MAX} 42.7 ± 5.0 ml/kg/min) undertook three exercise bouts: a bout of LV-HIIE (10*1 minute 90% VO_{2MAX} intervals) and two energy-matched steady-state cycling bouts at a moderate (60% VO_{2MAX}; 27 min, MOD) and high (80% VO_{2MAX}; 20 min, HIGH) intensity on separate days. Markers of oxidative stress, inflammation and physiological stress were assessed before, **at the end of exercise** and 30 minutes post-exercise (post+30). **At the end of all exercise bouts**, significant changes in lipid hydroperoxides (LOOH) and protein carbonyls (PC) (LOOH (nM): MOD +0.36; HIGH +3.09; LV-HIIE +5.51 and PC (nmol/mg protein): MOD -0.24; HIGH -0.11; LV-HIIE -0.37) were observed. Total antioxidant capacity (TAC) increased post+30, relative **to the end of** all exercise bouts (TAC (µM): MOD +189; HIGH +135; LV-HIIE +102). Interleukin (IL)-6 and IL-10 increased post+30 in HIGH and LV-HIIE only ($p<.05$). HIGH caused the greatest lymphocytosis, adrenaline and cardiovascular response ($p<.05$). **At a reduced energy cost and physiological stress, LV-HIIE elicited similar cytokine and oxidative stress responses to HIGH.**

Keywords: Reactive oxygen species, Cytokine, Lipid Oxidation, Protein Oxidation, Antioxidant

Abbreviations:

ANOVA: Analysis of Variance, AUC: Area under the curve, CV: Coefficient of variance, DNA: deoxyribonucleic acid, DNPH: Dinitrophenylhydrazine, ELISA: Enzyme Linked Immunosorbent Assay, FRAP: Ferric Reducing Ability of Plasma, HCl: Hydrochloric acid, HIGH: high intensity steady state bout, HIIT: High intensity interval training, HPLC: High Performance Liquid Chromatography, HRP: Horseradish Peroxidase, HS: High Sensitivity, IL: Interleukin, LOOH: Lipid Hydroperoxides, LV-HIIE: Low volume high intensity interval exercise, MOD: moderate intensity steady state bout, NaCl: Sodium Chloride, PC: Protein Carbonyl, ROS: Reactive oxygen and nitrogen species, SD: Standard deviation, TAC: Total antioxidant capacity, TBS: Tris-Buffered Saline, and

VCO_2: Carbon dioxide consumption, VO_2: Oxygen Consumption and VO_{2MAX}: Maximum oxygen consumption.

Introduction

Reactive oxygen species (ROS) are by-products of cellular respiration that regulate signalling and homeostasis. Oxidative stress is a state whereby ROS exceed endogenous and exogenous antioxidants systems, resulting in the progressive oxidation of macromolecules. It is now widely accepted that the increase in ROS that follows an acute bout of exercise can facilitate a host of beneficial whole body adaptations (Gomez-Cabrera, Domenech, & Vina, 2008; Ristow et al., 2009). Markers of ROS mediated protein oxidation (protein carbonyls), lipid oxidation (lipid hydroperoxides) and exogenous antioxidant utilisation (total antioxidant capacity) are commonly measured in blood plasma following steady state exercise (Berzosa et al., 2011; Bloomer, Davis, Consitt, & Wideman, 2007; Bloomer, Goldfarb, Wideman, McKenzie, & Consitt, 2005; Wadley et al., 2014) with some evidence that the magnitude of change in these markers reflects perturbations within exercising skeletal muscle (Goldfarb, Bloomer, & Mckenzie, 2005; Samjoo, Safdar, Hamadeh, Raha, & Tarnopolsky, 2013; Veskoukisa, Nikolaidisb, Kyparosa, & Kouretas, 2009).

Steady state exercise is also accompanied by an increase in pro and anti-inflammatory cytokine production (Fischer, 2006); proteins known to be associated with oxidative stress (Wadley, Veldhuijzen van Zanten, & Aldred, 2012). Particular attention has been paid to interleukin (IL)-6, a cytokine with a role in regulating the anti-inflammatory response to exercise (Fischer, 2006; Gleeson et al., 2011). The acute anti-oxidant and anti-inflammatory response commonly observed in response to exercise, indicates in part, the rapid nature of the adaptive response. Whilst there is some evidence to suggest that the magnitude of increase in ROS and cytokines in response to exercise is intensity (Bailey et al., 2004; Ostrowski, Schjerling, & Pedersen, 2000) and duration (Bloomer et al., 2007; Fischer, 2006) dependent, the influence of exercise modality remains incompletely understood.

High intensity interval exercise (HIIE) is a recently developed exercise regimen, working at a higher exercise intensity, but with a reduced time commitment and energy cost relative to traditional steady state exercise (Gibala, Little, MacDonald, & Hawley, 2012; Wisløff et al., 2007). Low volume HIIE (LV-HIIE) is a form of HIIT exercise that has been applied in a range of populations (Gibala et al., 2012; Hood, Little, Tarnopolsky, Myslik, & Gibala, 2011; Little et al., 2012). LV-HIIE has been

shown to increase markers of muscle metabolism (Hood et al., 2011) induce increases in VO_2 peak, (Rognmo, Hetland, Helgerud, Hoff, & Slørdahl, 2004) and improve endothelial function (Wisløff et al., 2007) to the same, or greater degree as steady state exercise. Recent evidence has indicated that LV-HIIE can induce an increase in plasma oxidative stress (G. Fisher et al., 2011) and inflammation (Zwetsloot, John, Lawerence, Battista, & Shanely, 2014) following exercise. To our knowledge, there are no studies to date that directly compare oxidative stress and inflammation in response to LV-HIIE vs. steady state exercise. One previous study showed that the increase in interleukin (IL)-6 following HIIT was greater than moderate intensity steady state exercise, however the protocols used were matched for total workload (Leggate, Nowell, Jones, & Nimmo, 2010), therefore not incorporating the energy saving nature of 'classical' HIIT exercise. The aim of the present study was to compare changes in oxidative stress and inflammation in response to a bout of LV-HIIE and two energy matched steady state exercise bouts of high and moderate intensity.

Methods

Participants

Ten healthy, untrained (defined as VO_{2MAX} < 50ml/kg/min) males (mean ± SD: age 22 ± 3 yrs; body mass index 24.0 ± 3.1 kg/m^2; VO_{2MAX} 42.7 ± 5.0 ml/kg/min) took part in three separate exercise bouts. All participants gave their informed written consent and the investigation was approved by the Science and Technology ethical review committee at the University of Birmingham. Participants were non-smokers and excluded if they had ingested vitamin supplements or anti-inflammatory drugs in the two weeks prior to the first laboratory visit. In addition, participants were required to refrain from any strenuous physical activity or consumption of alcoholic beverages in the 48 hours prior to testing sessions.

Preliminary Assessments

Participants undertook all bouts of exercise in the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham. All experimental procedures conformed to the Declaration of Helsinki. Exercise bouts took place on an electromagnetically braked cycle ergometer (*Lode Excalibur Sport*, Groningen, *Netherlands*). Height and weight was recorded (*Seca Alpha, Hamburg, Germany*) and questionnaires administered for demographic and health screening

purposes (W. J. Fisher & White, 1999). Cardiorespiratory fitness was assessed by determining the maximum oxygen consumption (VO_{2MAX}) of the participant and expressed relative to body weight ($ml.kg^{-1}min^{-1}$). A breath-by-breath system (*Oxygon Prx*, Jaeger, Wuerzberg, Germany) was used for continuous measurement of oxygen uptake and heart rate was monitored using a Polar Vantage heart rate monitor (Polar Vantage, Kempele, Finland). After a 3 minute warm up at 30 Watts, participants undertook an incremental exercise test to exhaustion, whereby workload increased 30 Watts every minute until volitional exhaustion. Participants were asked to maintain a constant pedal rate and encouragement was given by an experimenter. A respiratory exchange ratio (VCO_2/VO_2) >1.10-1.15, plateau in participant oxygen consumption or a maximal heart rate >220 beats min^{-1} - age were all factors used to indicate VO_{2MAX} and thus the termination of the test (Howley, Bassett Jr, & Welch, 1995).

Exercise Bouts

One week after the first visit, participants returned to the laboratory following an overnight fast to undertake one of three randomised exercise bouts, each separated by at least one week (Figure 1). All exercise bouts took place at the same time in the morning (8:00-10.00 AM) and under similar environmental conditions (21°C and 35% relative humidity). Following a 30 minute period of rest, a catheter (*Becton, Dickson & Company, Oxford, UK*) was inserted into the antecubital vein of the forearm and a baseline blood sample taken (baseline). The catheter was kept patent through regular flushes with saline (0.9% NaCl). Participants then undertook a 5 minute warm up at a workload that elicited 40% of their maximum aerobic capacity, followed by the exercise bout at 60% VO_{2MAX} for 27 minutes (Moderate, MOD), 80% VO_{2MAX} for 20 minutes (HIGH) and ten 1 minute stages at 90% VO_{2MAX}, interspersed with nine 1 minute intervals at 40 % VO_{2MAX} (LV-HIIE) (Figure 1). Total workloads for MOD and HIGH were energy-matched (kcal) and based on pilot testing in our laboratory that determined 20 minutes cycling at 80% VO_{2MAX} to be sufficiently exhaustive exercise (as assessed by maximal rate of perceived exertion). A second blood sample (exercise) was taken **at the end of exercise** and then 30 minutes following exercise (post+30). At each time point, 7 ml of blood was drawn into two vacutainer tubes containing potassium ethylene diaminetetraacetic acid (*Becton, Dickson & Company, Oxford, UK*). One vacuatiner (2ml) was used for coulter analysis of

haemoglobin, haematocrit and leukocyte differential and another (5ml) centrifuged at 2800 rpm for 15 minutes at 4 °C. Plasma was then extracted and stored at -80 °C until further analysis.

[insert Figure 1 here]

Blood Assessments

Blood samples were assessed for blood cell composition, specifically total peripheral blood lymphocytes. In addition, haemoglobin (g/dL) and haematocrit (%) assessed to calculate plasma volume changes as a result of exercise (Bacon, Ring, Lip, & Carroll, 2004). All of these variables were assessed using a coulter analyser (*Coulter Analyser, Beckman-Coulter, High Wycombe, UK*).

Lipid Hydroperoxides

LOOH concentrations were assessed using a spectrophotometric assay (Görög, Kotak, & Kovacs, 1991). Samples and a blank standard (10 µl) were added in triplicate to a 96 well microtitre plate. Reagent mix (100 µl, 0.2 M Potassium phosphate (pH=6.2), 0.12 M potassium iodide, 0.15 mM 0.15mM sodium azide, **polyethylene glycol** mono p-(1,1',3,3'-tetramethylbutyl)-phenyl]ether (Triton X, 2 g/l), alkylbenzyldimethylammonium chloride (0.1 g/l), 10 µM ammonium molybdate in HPLC-grade water was added for 30 minutes at room temperature. The plate was read at 365nm (Multiscan MS, Labsystems), concentration of lipid peroxides (µM) determined using the Beer-Lambert Law (extinction co-efficient ε_{340} = 24600 $M^{-1}cm^{-1}$) and adjusted for changes in plasma volume. The inter-assay coefficient of variation (CV) was 8.9%.

Total Antioxidant Capacity

TAC was assessed using the Ferric Reducing Ability of Plasma (FRAP) assay (Benzie & Strain, 1996). Plasma samples (10 µl per well) and standards (10 µl per well, ascorbic acid, 0-1000 µM) were added in triplicate to a flat bottomed 96 well plate. FRAP reagent (300 mM sodium acetate (pH=3.6), 160 mM 2, 4, 6- tripyridyltriazin and 20mM ferric chloride; 300 µl) was added to each well and left to incubate for 8 minutes at room temperature, then absorbance's read at 650 nm. TAC values were obtained using absorbance values of known ascorbic acid concentrations, expressed as

µM of antioxidant power relative to ascorbic acid (McAnulty et al., 2005) and adjusted for changes in plasma volume. The inter and intra-assay CV's were <3% and <1% respectively.

Protein Carbonylation

PC was assessed by ELISA (Buss, Chan, Sluis, Domigan, & Winterbourn, 1997; Carty et al., 2000). The protein concentration of all plasma samples was obtained using the bicinchoninic assay method (Smith et al., 1985). Samples and standards (50µl) were then diluted accordingly in coating buffer to a concentration of 0.05mg/ml (50mM sodium carbonate, pH=9.2) and added in triplicate to a 96 well NUNC maxisorb microtitre plate for 1 hour at room temperature. Bound protein was incubated with 2, 4-dinitrophenylhydrazine (DNPH) (1mM, in 2M HCl) for 1 hour and then all wells blocked with TBS Tween (0.1%, 200µl) overnight at 4°C. Wells were incubated with monoclonal mouse anti-DNP antibody (50µl, 1:1000) for 2 hours at room temperature, followed by peroxidase conjugated rat anti-mouse IgE conjugated HRP (50µl, 1:5000) for 1 hour at room temperature. All steps were followed by three washes using TBS Tween (0.05%). Substrate (0.5M **citrate** phosphate buffer (10mls, pH=5), hydrogen peroxide (8µl) and Ortho-Phenylenediamine tablet (2mg); 50µl) was added to each well and the reaction stopped after 45 minutes with 2M sulphuric acid (50µl). Well absorbance was measured at 490nm (Multiscan MS, Labsystems) and quantified using absorbance values of known PC standards (1.28-5.20 nmol/mg protein). The inter and intra-assay CV's were 8.8% and 1.4 % respectively.

Interleukins

Plasma concentrations of IL-6 and IL-10 were determined using a commercially available high-sensitivity (HS) ELISA kits according to manufacturer instructions (*R&D Systems*, assay sensitivity, IL-6: 0.11 pg/ml and IL-10: 0.17 pg/ml). **All samples** were analysed in triplicate and values were obtained from a linear standard curve of known IL-6 and IL-10 concentrations (IL-6: 0.156-10 pg/ml and IL-10: 0.78-50 pg/ml) and adjusted for changes in plasma volume. The inter and intra-assay CVs for the HS IL-6 and HS IL-10 kits are reported as 6.5% and 6.9% respectively.

Adrenaline

Plasma concentrations of adrenaline were determined using a commercially available High-Sensitivity ELISA kit (assay sensitivity: 3 pg/ml), according to manufacturer instructions (*Rocky Mountain Diagnostics Inc. USA*). Values were obtained from a standard curve of known adrenaline concentrations (0-1500 pg/ml) and adjusted for changes in plasma volume. The intra-assay CV is reported as 9.3%.

Sample size calculation and Statistical Analysis

Power analyses using Gpower3 (Faul, Erdfelder, Lang, & Buchner, 2007), with significance at .05 and power at .90, were conducted based upon results from previous studies and preliminary pilot work. Primary outcome measures of protein oxidation, IL-6 concentration changes (2-fold) were used. A sample size of 10 participants was required to detect differences with an effect size of .24 (medium effect size).

Statistical analyses were performed using SPSS (PASW Statistics, 21.0). Kolmogorov–Smirnov tests were used to investigate normal distribution and differences between variables at baseline were assessed using one-way analyses of variance (ANOVA). The physiological response to exercise was assessed by an exercise bout (MOD, HIGH, LV-HIIE) by time (baseline, exercise, post+30) repeated-measures ANOVA, with Bonferroni correction. Post hoc analysis of the interaction effects was performed by a test of simple effects by pairwise comparisons (with Bonferroni correction). Primary outcome measures (TAC, LogLOOH, PC, IL-6 and IL-10) were further probed using one-way ANOVAs to assess responses in response to each exercise bout. Assessments of heart rate and blood pressure over time by area under the curve (AUC) were undertaken using one-way repeated measures ANOVA. Data which was not normally distributed was log transformed prior to statistical analyses. Statistical significance was accepted at the $p<0.05$ level.

Results

Total Workload and Energy Expenditure

The average workload (watts) for the three bouts of exercise were 110 ± 18 (MOD), 169 ± 32 (HIGH) and 211 ± 38 (LV-HIIE). Total energy expenditure for the LV-HIIE (190 ± 30) bout was significantly lower ($p<.0001$) than both MOD (264 ± 39) and HIGH (266 ± 39).

Oxidative Stress

Figure 2(a-c) shows the response of plasma LogLOOH, TAC and PC to the three different exercise bouts. LogLOOH and TAC significantly increased **at the end of exercise** (p=0.033) and post+30 (p=0.004), relative to baseline and **the end of exercise** respectively (pairwise comparisons). Thirty minutes following exercise, LogLOOH returned to baseline concentrations (p=0.023). PC significantly decreased **at the end of all exercise** bouts (p<.0001) and returned to baseline levels post+30 (p=.013). Further analysis of the individual exercise bouts using one-way ANOVAs revealed that a significant increase in TAC occurred following exercise (post+30 relative **to the end of exercise**) in MOD (P=.0001). Increases in LOOH were detected **at the end of** HIGH (p=.047) and LV-HIIT (p=0.041) **bouts** only. PC decreased significantly **at the end of exercise**, relative to baseline in LV-HIIT (p=.003).

[insert Figure 2 here]

Cytokines

The effect of exercise on IL-6 and IL-10 concentrations can be seen in Figure 3(a-b). IL-6 concentrations increased at the end of exercise in HIGH and LV-HIIE (p<0.05), with elevations post+30, relative to baseline in all bouts (p<0.016). The concentration of IL-6 post+30 was significantly higher in HIGH compared to MOD (group x time interaction effect; p=0.037). IL-10 concentrations were unchanged at the end of exercise, however increases were observed post+30, relative to baseline (p=0.05) and the end of exercise (p=0.05) in LV-HIIE and HIGH respectively (group x time interaction effect; p=0.015). IL-10 concentrations post+30 were higher in HIGH than MOD (group x time interaction effect; p=0.05). No statistical differences in IL-6 and IL-10 responses were observed between LV-HIIE and HIGH. Further analysis of the individual exercise bouts using one-way ANOVAs revealed that IL-10 concentration decreased post+30 relative to baseline in MOD (p=0.01).

[insert Figure 3 here]

Other physiological measures

Total Peripheral Blood Lymphocytes and plasma adrenaline

Table 1 shows the response of total peripheral blood lymphocytes and plasma adrenaline to the different exercise bouts. There was a significant lymphocytosis **at the end of all exercise** bouts ($p<.0001$), which returned to baseline values post+30 ($p<.0001$). Adrenaline significantly increased **at the end of all exercise** bouts ($p=0.04$) and returned to baseline values post+30 ($p=0.10$). Significant group x time interaction effects were found ($p's<.0001$) and pairwise comparisons indicated that HIGH elicited a significantly greater lymphocytosis and plasma adrenaline response than both LV-HIIE ($p's<.049$) and MOD ($p's<0.037$) **at the end of exercise**. There were no statistical differences in the response of both variables between LV-HIIE and MOD.

[insert Table 1 here]

Heart Rate and Blood Pressure

Figure 4 indicates the changes in heart rate and systolic blood pressure over time as assessed by the area under the curve (AUC). Total AUC for heart rate was significantly lower in MOD and LV-HIIE when compared to HIGH (MOD -31.8% ($p<0.001$) and LV-HIIE -31.1% ($p=0.001$)). A similar trend was observed for SBP, however only LV-HIIE was significantly lower than HIGH (MOD -33.5% ($p=0.098$) and LV-HIIE -29.1% ($p=0.034$)) (Figure 4).

[insert Figure 4 here]

Discussion

To our knowledge this is the first study to compare plasma markers of oxidative stress and inflammation in response to LV-HIIE and steady state exercise bouts. All bouts stimulated a transient change in markers of oxidative stress, irrespective of exercise intensity or mode. An anti-inflammatory cytokine response **(i.e. IL-10)** was observed thirty minutes following cessation of LV-HIIE and HIGH bouts only.

297 The significant increases in LOOH concentrations observed in all bouts indicate the presence
298 of ROS following exercise. With compelling evidence that exercise-induced increases in oxidative
299 stress are adaptive (Mari Carmen. Gomez-Cabrera et al., 2008; Ristow et al., 2009), the peroxidation
300 of lipids may stimulate adaptations such as structural remodelling of external cellular membranes and
301 lipoproteins (Aldred, 2007). Further probing of the data (one-way ANOVAs) suggested that the
302 magnitude of increase in LOOH was greatest **at the end of exercise in** HIGH and LV-HIIE. This
303 supports previous data reporting intensity-dependent increases in markers of oxidative stress in
304 response to acute exercise (Lamprecht, Greilberger, Schwaberger, Hofmann, & Oettl, 2008). The
305 observed antioxidant response following MOD and HIGH supports previous studies assessing
306 responses to steady state exercise (Berzosa et al., 2011; Turner, Bosch, Drayson, & Aldred, 2011).
307 Importantly, this study is the first to provide evidence that a bout of LV-HIIE elicits a plasma
308 antioxidant response, confirming previous data in lymphocytes (G. Fisher et al., 2011). Further
309 exploration of the data suggested that the greatest antioxidant response was observed following MOD,
310 possibly a reflection of lower LOOH **at the end of exercise**. The decrease observed in PC **at the end**
311 **of all exercise bouts**, and return to baseline levels thirty minutes post-exercise is perhaps more
312 unexpected, however previous studies have reported no change, or indeed a decrease in protein
313 carbonylation following exercise of varying intensities in blood (Goldfarb et al., 2005), muscle
314 (Saxton, Donnelly, & Roper, 1994) and brain (Ogonovszky et al., 2005). Moderately carbonylated
315 proteins are degraded by proteasomes, and it has been noted that proteasome activity does increase
316 during exercise (Ogonovszky et al., 2005). This may also explain the delayed increases in PC reported
317 in some previous studies (R.J. Bloomer et al., 2005; Michailidis et al., 2007). Of note, no significant
318 differences were observed in the magnitude of oxidative stress response (LOOH, PC and TAC)
319 between exercise bouts (Figure 2). There are limited similar studies (within-subject design) that
320 compare whole body (i.e. plasma, serum) oxidative stress responses to varying exercise intensities,
321 and thus it is difficult to assess whether this finding is due to the duration of the bouts, or other factors
322 related to experimental design or analysis. This finding warrants further investigation. Collectively,
323 these data are the first to indicate the LV-HIIE elicits a comparable oxidative stress response to short
324 duration high intensity steady state exercise.

325 IL-6 and IL-10 significantly increased in response to a single bout of LV-HIIE as previously
326 demonstrated (Zwetsloot et al., 2014). **When comparing the IL-10 responses, LV-HIIE was**

comparable to HIGH, indicating that these exercise bouts were more effective than MOD in stimulating an anti-inflammatory response to exercise (Figure 3). Aside from the classical role of IL-6 to signal and facilitate the inflammatory response, there is evidence to suggest that its release during exercise can inhibit pro-inflammatory cytokine production (Nimmo, Leggate, Viana, & King, 2013) and up-regulate the transcription of anti-inflammatory cytokines such as IL-10 (Fischer, 2006). The present results are in support of these studies, with IL-10 elevation seen thirty minutes following the increase in IL-6 in both LV-HIIE and HIGH. Previous evidence has highlighted that exercise intensity may be the key factor governing IL-6 release when considering shorter bouts of steady state exercise under an hour (Fischer, 2006; Ostrowski et al., 2000). Despite the higher peak intensity of LV-HIIE, the intermittent nature of the stimulus may have aided the clearance of IL-6 by the liver and kidneys (Febbraio et al., 2003) during the active rest intervals. Nevertheless, these data indicate that LV-HIIE and short duration high intensity, steady state exercise can elicit comparable **IL-6 and IL-10** responses in untrained participants.

In the current study, changes in heart rate and SBP were assessed over the course of the exercise bouts, as previously demonstrated (W. J. Fisher & White, 1999). Cardiovascular responses during LV-HIIE were comparable to MOD (Figure 4) and significantly lower than HIGH. When considering other physiological markers, HIGH caused a significantly greater lymphocytosis and adrenaline response than both MOD and LV-HIIE bouts (Table 1). The physiological parameters studied suggest that LV-HIIE provides a lower physiological stress compared to short duration high intensity steady state exercise.

Conclusions

In conclusion, this study presents evidence to suggest that a bout of LV-HIIE does not stimulate a significantly different response in plasma markers of oxidative stress to the responses seen following short duration high or moderate intensity steady state exercise. When considering other physiological markers (i.e. heart rate, adrenaline and lymphocytosis), HIGH was the greatest stressor, with LV-HIIE being more comparable to MOD. Importantly, a single bout of LV-HIIE elicited a comparable anti-oxidant, **IL-6 and IL-10** response to HIGH, in terms of magnitude and timecourse. Given the reduced energy cost compared to steady state exercise, LV-HIIE may be an attractive exercise modality, for a variety of populations.

References

Aldred, S. (2007). Oxidative and nitrative changes seen in lipoproteins following exercise. *Atherosclerosis*, *192*(1), 1–8. doi:S0021-9150(07)00104-9 [pii] 10.1016/j.atherosclerosis.2007.02.001

Bacon, S. L., Ring, C., Lip, G. Y. H., & Carroll, D. (2004). Increases in lipids and immune cells in response to exercise and mental stress in patients with suspected coronary artery disease: effects of adjustment for shifts in plasma volume. *Biological Psychology*, *65*(3), 237–250. doi:10.1016/S0301-0511(03)00113-3

Bailey, D. M., Young, I. S., McEneny, J., Lawrenson, L., Kim, J., Barden, J., & Richardson, R. S. (2004). Regulation of free radical outflow from an isolated muscle bed in exercising humans. *American Journal of Physiology-Heart and Circulatory Physiology*, *287*(4), H1689–H1699. doi:DOI 10.1152/ajpheart.00148.2004

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, *239*(1), 70–76. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8660627

Berzosa, C., Cebrian, I., Fuentes-Broto, L., Gomez-Trullen, E., Piedrafita, E., Martinez-Ballarin, E., … Garcia, J. J. (2011). Acute exercise increases plasma total antioxidant status and antioxidant enzyme activities in untrained men. *J Biomed Biotechnol*, 1–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21436993

Bloomer, R. J., Davis, P. G., Consitt, L. A., & Wideman, L. (2007). Plasma protein carbonyl response to increasing exercise duration in aerobically trained men and women. *International Journal of Sports Medicine*, *28*(1), 21–25. Retrieved from <Go to ISI>://000243359800004

Bloomer, R. J., Goldfarb, A. H., Wideman, L., McKenzie, M. J., & Consitt, L. a. (2005). Effects of acute aerobic and anaerobic exercise on blood markers of oxidative stress. *Journal of Strength and Conditioning Research / National Strength & Conditioning Association*, *19*(2), 276–85. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15903362

Buss, H., Chan, T. P., Sluis, K. B., Domigan, N. M., & Winterbourn, C. C. (1997). Protein carbonyl measurement by a sensitive ELISA method. *Free Radical Biology and Medicine*, *23*(3), 361–366. Retrieved from <Go to ISI>://A1997XG67400002

Carty, J. L., Bevan, R., Waller, H., Mistry, N., Cooke, M., Lunec, J., & Griffiths, H. R. (2000). The effects of vitamin C supplementation on protein oxidation in healthy volunteers. *Biochem Biophys Res Commun*, *273*(2), 729–735. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10873672

Faul, F., Erdfelder, E., Lang, A. G., & Buchner, A. (2007). G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, *39*(2), 175–91. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17695343

Febbraio, M. A., Ott, P., Nielsen, H. B., Steensberg, A., Keller, C., Krustrup, P., … Pedersen, B. K. (2003). Hepatosplanchnic clearance of interleukin-6 in humans during exercise. *American Journal of Physiology Endocrinology and Metabolism*, *285*(2), E397–402. doi:10.1152/ajpendo.00134.2003

Fischer, C. P. (2006). Interleukin-6 in acute exercise and training: what is the biological relevance? *Exercise Immunology Review*, *12*(115), 6–33. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17201070

400 Fisher, G., Pascoe, D. D., Schwartz, D. D., Quindry, J., Barberio, M. D., Foster, E. B., & Jones, K. W.
401 (2011). Lymphocyte enzymatic antioxidant responses to oxidative stress following high-
402 intensity interval exercise Lymphocyte enzymatic antioxidant responses to oxidative stress
403 following high-intensity interval exercise. *Journal of Applied Physiology*, *110*, 730–737.
404 doi:10.1152/japplphysiol.00575.2010

405 Fisher, W. J., & White, M. J. (1999). Training-induced adaptations in the central command and
406 peripheral reflex components of the pressor response to isometric exercise of the human triceps
407 surae. *The Journal of Physiology*, *520 Pt 2*, 621–8. Retrieved from
408 http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2269582&tool=pmcentrez&rendert
409 ype=abstract

410 Gibala, M. J., Little, J. P., MacDonald, M. J., & Hawley, J. A. (2012). Physiological adaptations to
411 low-volume, high-intensity interval training in health and disease. *Journal of Physiology*, *590.5*,
412 1077–1084.

413 Gleeson, M., Bishop, N. C., Stensel, D. J., Lindley, M. R., Mastana, S. S., & Nimmo, M. A. (2011).
414 The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and
415 treatment of disease, *11*, 607–615.

416 Goldfarb, A. H., Bloomer, R. J., & Mckenzie, M. J. (2005). Combined Antioxidant Treatment Effects
417 on Blood Oxidative Stress after Eccentric Exercise. *Medicine & Science in Sports & Exercise*,
418 *37*(2), 234–239. doi:10.1249/01.MSS.0000152887.87785.BE

419 Gomez-Cabrera, M. C., Domenech, E., Romagnoli, M., Arduini, A., Borras, C., Pallardo, F. V., …
420 Viña, J. (2008). Oral administration of vitamin C decreases muscle mitochondrial biogenesis
421 and hampers training-induced adaptations in endurance performance. *The American Journal of*
422 *Clinical Nutrition*, *87*(1), 142–9. Retrieved from
423 http://www.ncbi.nlm.nih.gov/pubmed/18175748

424 Gomez-Cabrera, M. C., Domenech, E., & Vina, J. (2008). Moderate exercise is an antioxidant:
425 upregulation of antioxidant genes by training. *Free Radic Biol Med*, *44*(2), 126–131. doi:S0891-
426 5849(07)00108-6 [pii] 10.1016/j.freeradbiomed.2007.02.001

427 Görög, P., Kotak, D. C., & Kovacs, I. B. (1991). Simple and specific test for measuring lipid
428 peroxides in plasma. *Journal of Clinical Pathology*, *44*(9), 765–7. Retrieved from
429 http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=496727&tool=pmcentrez&renderty
430 pe=abstract

431 Hood, M. S., Little, J. P., Tarnopolsky, M. A., Myslik, F., & Gibala, M. J. (2011). Low-volume
432 interval training improves muscle oxidative capacity in sedentary adults. *Medicine and Science*
433 *in Sports and Exercise*, *43*(10), 1849–56. doi:10.1249/MSS.0b013e3182199834

434 Howley, E. T., Bassett Jr, D. R., & Welch, H. G. (1995). Criteria for maximal oxygen uptake: a
435 review and commentary. *Medicine & Science in Sports & Exercise*, *27*(9), 1292–1301.

436 Lamprecht, M., Greilberger, J. F., Schwaberger, G., Hofmann, P., & Oettl, K. (2008). Single bouts of
437 exercise affect albumin redox state and carbonyl groups on plasma protein of trained men in a
438 workload-dependent manner. *J Appl Physiol*, *104*(6), 1611–1617. doi:01325.2007 [pii]
439 10.1152/japplphysiol.01325.2007

440 Leggate, M., Nowell, M. a, Jones, S. a, & Nimmo, M. a. (2010). The response of interleukin-6 and
441 soluble interleukin-6 receptor isoforms following intermittent high intensity and continuous
442 moderate intensity cycling. *Cell Stress & Chaperones*, *15*(6), 827–33. doi:10.1007/s12192-010-
443 0192-z

Little, J. P., Gillen, J. B., Percival, M. E., Safdar, A., Mark, A., Punthakee, Z., … Tarnopolsky, M. A. (2012). Low-volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes. *Journal of Applied Physiology*, *111*, 1554–1560. doi:10.1152/japplphysiol.00921.2011

McAnulty, S. R., McAnulty, L. S., Morrow, J. D., Khardouni, D., Shooter, L., Monk, J., … Brown, V. (2005). Effect of daily fruit ingestion on angiotensin converting enzyme activity, blood pressure, and oxidative stress in chronic smokers. *Free Radic Res*, *39*(11), 1241–1248. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16298751

Michailidis, Y., Jamurtas, A. Z., Nikolaidis, M. G., Fatouros, I. G., Koutedakis, Y., Papassotiriou, I., & Kouretas, D. (2007). Sampling time is crucial for measurement of aerobic exercise-induced oxidative stress. *Medicine and Science in Sports and Exercise*, *39*(7), 1107–13. doi:10.1249/01.mss.0b013e318053e7ba

Nimmo, M. A., Leggate, M., Viana, J. L., & King, J. A. (2013). The effect of physical activity on mediators of inflammation. *Diabetes, Obesity and Metabolism*, *15*, 51–60.

Ogonovszky, H., Berkes, I., Kumagai, S., Kaneko, T., Tahara, S., Goto, S., & Radák, Z. (2005). The effects of moderate-, strenuous- and over-training on oxidative stress markers, DNA repair, and memory, in rat brain. *Neurochemistry International*, *46*(8), 635–640. doi:10.1016/j.neuint.2005.02.009

Ostrowski, K., Schjerling, P., & Pedersen, B. K. (2000). Physical activity and plasma interleukin-6 in humans effect of intensity of exercise. *European Journal of Applied Physiology*, *83*(6), 512–5. doi:10.1007/s004210000312

Ristow, M., Birringer, M., Kiehntopf, M., Zarse, K., Oberbach, A., Klo, N., … Blu, M. (2009). Antioxidants prevent health-promoting effects. *PNAS*, *106*(21), 8865–8670.

Rognmo, Ø., Hetland, E., Helgerud, J., Hoff, J., & Slørdahl, S. A. (2004). High intensity aerobic interval exercise is superior to moderate intensity exercise for increasing aerobic capacity in patients with coronary artery disease. *European Journal of Cardiovascular Prevention & Rehabilitation*, *11*, 216–222. doi:10.1097/01.hjr.0000131677.96762.0c

Samjoo, I. A., Safdar, A., Hamadeh, M. J., Raha, S., & Tarnopolsky, M. A. (2013). The effect of endurance exercise on both skeletal muscle and systemic oxidative stress in previously sedentary obese men. *Nutrition & Diabetes*, *3*(9), e88. doi:10.1038/nutd.2013.30

Saxton, J. M., Donnelly, A. E., & Roper, H. P. (1994). Indices of free-radical-mediated damage following maximum volunatry eccentric and concentric muscular work. *European Journal of Applied Physiology*, *68*, 189–193.

Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., … Klenk, D. C. (1985). Measurement of protein using bicinchoninic acid. *Analytical Biochemistry*, *150*(1), 76–85. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3843705

Turner, J. E., Bosch, J. A., Drayson, M. T., & Aldred, S. (2011). Assessment of oxidative stress in lymphocytes with exercise. *Journal of Applied Physiology (Bethesda, Md. : 1985)*, *111*(1), 206–11. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21493722

Veskoukisa, A. S., Nikolaidisb, M. G., Kyparosa, A., & Kouretas, D. (2009). Blood reflects tissue oxidative stress depending on biomarker and tissue studied. *Free Radical Biology and Medicine*, *47*(10), 1371–1374.

Wadley, A. J., van Zanten, J. J. C. S. V., Stavropoulos-Kalinoglou, A., Metsios, G. S., Smith, J. P., Kitas, G. D., & Aldred, S. (2014). Three months of moderate-intensity exercise reduced plasma 3-nitrotyrosine in rheumatoid arthritis patients. *European Journal of Applied Physiology*. doi:10.1007/s00421-014-2877-y

Wadley, A. J., Veldhuijzen van Zanten, J. J. C. S., & Aldred, S. (2012). The interactions of oxidative stress and inflammation with vascular dysfunction in ageing : the vascular health triad. *AGE*, *35*(3), 705–718.

Wisløff, U., Støylen, A., Loennechen, J. P., Bruvold, M., Rognmo, Ø., Haram, P. M., … Ellingsen, Ø. (2007). Superior Cardiovascular Effect of Aerobic Interval Training Versus Moderate Continuous Training in Heart. *Circulation*, *115*, 3086–3094. doi:10.1161/CIRCULATIONAHA.106.675041

Zwetsloot, K. A., John, C. ., Lawerence, M. ., Battista, R. A., & Shanely, R. A. (2014). High-intensity interval training induces a modest systemic inflammatory response in active , young men. *Journal of Inflammation Research*, *7*, 9–17.

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519 **Tables**

520

521 **Table 1:** Mean (SD) lymphocyte number and adrenaline concentrations before, **at the end of**

522 **exercise** and thirty following the three exercise bouts.

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	Moderate			High			LV-HIIE		
	Baseline	Exercise	Post+30	Baseline	Exercise	Post+30	Baseline	Exercise	Post+30
Adrenaline (pg/ml)	7.39 (±2.94)	97.57 (±21.64)*	20.61 (±9.14)**	15.11 (±7.36)	360.29 (±91.53)*#	52.81 (±16.96)**	15.82 (±7.82)	145.73 (±25.27)*	66.67 (±18.77)**
Lymphocyte Number ($\times 10^9$/cells/L)	1.89 (±0.47)	2.67 (±0.75)*	1.67 (±0.35)**	1.86 (±0.42)	4.63 (±1.41)*#	1.78 (±0.39)**	1.79 (±0.47)	3.57 (±1.40)*	1.62 (±0.38)**

524 * indicates significant differences **at the end of** exercise, relative to baseline ($p<0.05$). ** indicates

525 significant differences relative to **the end of** exercise in all bouts. # indicates significant differences in

526 HIGH compared to MOD and LV-HIIE.

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538 **Figure Legends**

539 **Figure 1: Schematic representation of the study protocol.** Participants completed an exercise test
540 to exhaustion and then participated in 3 subsequent exercise bouts. LV-HIIE was ten 1 minute stages
541 at 90% VO_{2MAX}, interspersed with nine 1 minute intervals at 40% VO_{2MAX}.
542 ↓ Blood samples (baseline, exercise & post+30); R, Rest; W, Warm up
543

544 **Figure 2: Acute oxidative stress responses to the three exercise bouts.** Bars represent mean values
545 of (a) LOOH (b) TAC and (c) PC to the different exercise bouts, ± standard error. Only 3×3 ANOVA
546 outputs are reported. **All markers report a main effect for time ($p<0.05$). Pairwise comparisons:** *
547 indicates significant differences **at the end of** exercise, relative to baseline in all bouts ($p<0.05$). **
548 indicates significant differences relative to **the end of** exercise in all bouts.

549 **Figure 3: Acute cytokine responses to the three exercise bouts**. Bars represent mean plasma
550 concentrations of (a) IL-6 and (b) IL-10 before (Base), **at the end of exercise** (Exercise) and thirty
551 minutes following exercise (Post+30), ± standard error. Only 3×3 ANOVA outputs are reported. *
552 indicates significant differences **at the end of** exercise, relative to baseline in all bouts ($p<0.05$). **
553 indicates significant differences relative to **the end of** exercise in all bouts. # indicates significant
554 differences between HIGH and MOD. Figure 3B: + indicates a significant difference between
555 baseline and post+30 in LV-HIIE. ++ indicates a significant differences between exercise and post+30
556 in HIGH.

557 **Figure 4: Acute cardiovascular responses to the three exercise bouts.** Lines represent the change
558 in (a) heart rate and (b) systolic blood pressure relative to baseline in all participants in the different
559 exercise bouts, assessed by area under the curve. Data are means ± standard error. * indicates
560 significant differences relative to HIGH ($p<0.05$).
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