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

Alex J. Wadley\textsuperscript{ab}, Yu-Wen Chen\textsuperscript{c}, Gregory Y.H. Lip\textsuperscript{c}, James P. Fisher\textsuperscript{a} & Sarah Aldred\textsuperscript{a}

\textsuperscript{a} School of Sport, Exercise & Rehabilitation Sciences, The University of Birmingham, Birmingham, UK

\textsuperscript{b} Institute of Science and the Environment, University of Worcester, Worcester, UK

\textsuperscript{c} Centre for Cardiovascular Science, University of Birmingham, Birmingham, UK

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PLEASE SCROLL DOWN FOR ARTICLE
Low volume–high intensity interval exercise elicits antioxidant and anti-inflammatory effects in humans

ALEX J. WADLEY¹,², YU-WEN CHEN³, GREGORY Y.H. LIP³, JAMES P. FISHER¹ & SARAH ALDRED¹

¹School of Sport, Exercise & Rehabilitation Sciences, The University of Birmingham, Birmingham, UK, ²Institute of Science and the Environment, University of Worcester, Worcester, UK and ³Centre for Cardiovascular Science, University of Birmingham, Birmingham, UK

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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 ± s: age 22 ± 3 years; VO₂MAX 42.7 ± 5.0 ml · kg⁻¹ · min⁻¹) undertook three exercise bouts: a bout of LV-HIIE (10 × 1 min 90% VO₂MAX intervals) and two energy-matched steady-state cycling bouts at a moderate (60% VO₂MAX; 27 min, MOD) and high (80% VO₂MAX; 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 min post-exercise (post+30). At the end of all exercise bouts, significant changes in lipid hydroperoxides (LOOH) and protein carbonyls (PCs) (LOOH (nM): MOD +0.36; HIGH +3.09; LV-HIIE +5.51 and PC (nmol · mg⁻¹ 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 < 0.05). HIGH caused the greatest lymphocytosis, adrenaline and cardiovascular response (P < 0.05). 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

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 antioxidant systems, resulting in the progressive oxidation of macromolecules. It is now widely accepted that the increase in ROS, which follows an acute bout of exercise can facilitate a host of beneficial whole body adaptation (Gomez-Cabrera, Domenech, & Viña, 2008; Ristow et al., 2009). Markers of ROS mediated protein oxidation (protein carbonyls, PCs), lipid oxidation (lipid hydroperoxides, LOOH) and exogenous antioxidant utilisation (total antioxidant capacity, TAC) 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, Van Zanten, & Aldred, 2013). Particular attention has been paid to interleukin (IL)-6, a cytokine with a role in regulating the anti-inflammatory response to exercises (Fischer, 2006; Gleeson et al., 2011). The acute antioxidant 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 an 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; Wisloff et al., 2007). Low volume HIIE (LV-HIIE) is a form of high intensity interval training (HIIT) exercise that has been applied in a range of populations (Gibala et al., 2012; Hood, Little, Tarnopolsky, Myslik, & Gibala, 2011; Little et al., 2011). LV-HIIE has been shown to increase markers of muscle metabolism (Hood et al., 2011), induce increases in the VO$_2$ peak (Rognmo, Hetland, Helgerud, Hoff, & Størdahl, 2004) and improve endothelial function (Wisloff 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 (Fisher et al., 2011) and inflammation (Zwetsloot, John, Lawrence, Battista, & Shanely, 2014) following exercise. To our knowledge, to date there have been no studies 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 the response to 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 the “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 maximum oxygen consumption (VO$_{2\text{MAX}}$) < 50 ml · kg$^{-1}$ · min$^{-1}$) males (mean ± s: age 22 ± 3 years; body mass index 24.0 ± 3.1 kg · m$^{-2}$; VO$_{2\text{MAX}}$ 42.7 ± 5.0 ml · kg$^{-1}$ · min$^{-1}$) 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 2 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 48 h 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 were recorded (Seca Alpha, Hamburg, Germany) and questionnaires administered for demographic and health screening purposes (Fisher & White, 1999). Cardiorespiratory fitness was assessed by determining the VO$_{2\text{MAX}}$ of the participant and expressed relative to body weight (ml · kg$^{-1}$ · min$^{-1}$). A breath-by-breath system (Oxygon Prx, Jaeger, Wuerzburg, Germany) was used for continuous measurement of oxygen uptake and the heart rate was monitored using a Polar Vantage heart rate monitor (Polar Vantage, Kempele, Finland). After a 3 min 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 (carbon dioxide consumption (VCO$_2$)/oxygen consumption (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$_{2\text{MAX}}$ and thus the termination of the test (Howley, Bassett, & 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 min 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 min warm up at a workload that elicited 40% of their maximum aerobic capacity, followed by the exercise bout at 60% VO$_{2\text{MAX}}$ for 27 min (moderate intensity steady-state bout, MOD), 80% VO$_{2\text{MAX}}$ for 20 min (high intensity steady-state bout, HIGH) and ten 1 min stages at 90% VO$_{2\text{MAX}}$. 

...
interspersed with nine 1 min intervals at 40% VO₂max (LV-HIIE) (Figure 1). The total workloads for MOD and HIGH were energy-matched (kcal) and based on pilot testing in our laboratory that determined 20 min cycling at 80% VO₂max interspersed with nine 1 min intervals at 40% VO₂max. Blood samples (baseline, exercise and post+30); R, rest; W, warm up.

**Blood assessments**

Blood samples were assessed for blood cell composition, specifically total peripheral blood lymphocytes. In addition, haemoglobin (g · dl⁻¹) and haematocrit (%)) were assessed to calculate plasma volume changes as a result of the 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. The reagent mix [100 µl, 0.2 M potassium phosphate (pH = 6.2), 0.12 M potassium iodide, 0.15 mM sodium azide, polyethylene glycol mono p-(1,1’,3,3’-tetramethylbutyl)-phenyl ether (Triton X, 2 g · l⁻¹), alkyldimethylammonium chloride (0.1 g · l⁻¹), 10 µM ammonium molybdate in high performance liquid chromatography (HPLC)-grade water] was added for 30 min at room temperature. The plate was read at 365 nm (Multiscan MS, Labsystems), concentration of lipid hydroperoxides (µM) determined using the Beer–Lambert law (extinction coefficient ε₃₄₀ = 24,600 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-tripyridyltriazine and 20 mM ferric chloride; 300 µl) was added to each well and left to incubate for 8 min at room temperature, then absorbance was 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 CVs were <3% and <1%, respectively.

**Protein carbonylation.** PC was assessed by enzyme linked immunosorbent assay (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 (50 µl) were then diluted accordingly in coating buffer to a concentration of 0.05 mg · ml⁻¹ (50 mM sodium carbonate, pH = 9.2) and added in triplicate to a 96-well maxisorb microtitre plate for 1 h at room temperature. Bound protein was incubated with 2,4-dinitrophenylhydrazine (DNPH) (1 mM, in 2 M HCl) for 1 h and then all wells blocked with tris-buffered saline (TBS) Tween (0.1%, 200 µl) overnight at 4°C. Wells were determined 20 min cycling at 80% VO₂max, interspersed with nine 1 min intervals at 40% VO₂max. Blood samples (baseline, exercise and post+30); R, rest; W, warm up.

**Blood assessments**

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incubated with monoclonal mouse anti-DNP antibody (50 µl, 1:1000) for 2 h at room temperature, followed by peroxidase conjugated rat anti-mouse IgE conjugated horseradish peroxidase (50 µl, 1:5000) for 1 h at room temperature. All steps were followed by three washes using TBS Tween (0.05%). Substrate (0.5 M citrate phosphate buffer (10 ml, pH = 5), hydrogen peroxide (8 µl) and ortho-phenylenediamine tablet (2 mg); 50 µl) were added to each well and the reaction stopped after 45 min with 2 M sulphuric acid (50 µl). Well absorbance was measured at 490 nm (Multiscan MS, Labsystems) and quantified using absorbance values of known PC standards (1.28–5.20 nmol · mg⁻¹ protein). The inter- and intra-assay CVs 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 kit according to manufacturer instructions (R&D Systems, assay sensitivity, IL-6: 0.11 pg · ml⁻¹ and IL-10: 0.17 pg · ml⁻¹). 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⁻¹ and IL-10: 0.78–50 pg · ml⁻¹) 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 to be 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⁻¹), according to manufacturer instructions (Rocky Mountain Diagnostics Inc. USA). Values were obtained from a standard curve of known adrenaline concentrations (0–1500 pg · ml⁻¹) and adjusted for changes in plasma volume. The intra-assay CV is reported to be 9.3%.

Sample size calculation and statistical analysis

Power analyses using Gpower3 (Faul, Erdfelder, Lang, & Buchner, 2007), with significance at 0.05 and power at 0.90, were conducted based on results obtained 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 0.24 (medium effect size).

Statistical analyses were performed using statistical package for the social sciences (predictive analytics software 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 to each exercise bout. Assessment of the heart rate and blood pressure over time by area under the curve (AUC) was undertaken using one-way repeated measures ANOVA. Data which were not normally distributed were log transformed prior to statistical analyses. The 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). The total energy expenditure for the LV-HIIE (190 ± 30) bout was significantly lower (P < 0.0001) 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 the 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 < 0.0001) and returned to baseline levels post+30 (P = 0.013). 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 = 0.0001). Increases in LOOH were detected at the end of HIGH (P = 0.047) and LV-HIIT (P = 0.041) bouts only. PC decreased significantly at the end of exercise, relative to the baseline in LV-HIIT (P = 0.003).

Cytokines

The effect of exercise on IL-6 and IL-10 concentrations can be seen in Figure 3(a) and 3(b). IL-6 concentrations increased at the end of exercise in HIGH and LV-HIIE (P < 0.05), with elevations post±30, relative to the baseline in all bouts
The concentration of IL-6 post±0 was significantly higher in HIGH compared to MOD (group × time interaction effect; \( P = 0.037 \)). IL-10 concentrations were unchanged at the end of exercise, however increases were observed post±30, relative to the baseline (\( P = 0.05 \)) and the end of exercise (\( P = 0.05 \)) in LV-HIIE and HIGH, respectively (group × time interaction effect; \( P = 0.015 \)). IL-10 concentrations post±30 were higher in HIGH than MOD (group × 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 the baseline in MOD (\( P = 0.01 \)).

Other physiological measures

Total peripheral blood lymphocytes and plasma adrenaline. Table I 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 (\( P < 0.0001 \)), which returned to baseline values post+30 (\( P < 0.0001 \)). 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 × time interaction effects were found (\( P < 0.0001 \)) and pairwise comparisons indicated that HIGH elicited a significantly greater lymphocytosis and plasma adrenaline response than both LV-HIIE (\( P < 0.049 \)) and MOD (\( P < 0.037 \)) at the end of the exercise. There were no statistical differences in the response of both variables between LV-HIIE and MOD.

Heart rate and blood pressure. Figure 4 indicates the changes in heart rate and systolic blood pressure (SBP) over time as assessed by the area under the curve (AUC). The 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).

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 for 30 min following cessation of LV-HIIE and HIGH bouts only.

The significant increases in LOOH concentrations observed in all bouts indicate the presence of ROS following exercise. With compelling evidence that exercise-induced increases in oxidative stress are adaptive (Mari Carmen. Gomez-Cabrera et al., 2008; Ristow et al., 2009), the peroxidation of lipids may stimulate adaptation such as structural remodeling of external cellular membranes and lipoproteins (Aldred, 2007). Further probing of the data (one-way ANOVAs) suggested that the magnitude of the increase in LOOH was greatest at the end of exercise in HIGH and LV-HIIE. This supports previous data reporting intensity-dependent increases in markers of oxidative stress in response to acute exercise (Lamprecht, Greilberger, Schwaberger, Hofmann, & Oettl, 2008). The observed antioxidant response following MOD and HIGH supports previous studies assessing responses to steady-state exercise (Berzosa et al., 2011; Turner, Bosch, Drayson, & Aldred, 2011). Importantly, this study is the first to provide evidence that a bout of LV-HIIE elicits a plasma antioxidant response, confirming previous data in lymphocytes (G. Fisher et al., 2011). Further exploration of the data suggested that the greatest antioxidant response was observed following MOD, possibly a reflection of lower LOOH at the end of exercise. The decrease observed in PC at the end of all exercise bouts, and return to baseline levels 30 min post-exercise is perhaps more unexpected, however previous studies have reported no change, or indeed a decrease in protein carbonylation following exercise of varying intensities in blood (Goldfarb et al., 2005), muscle (Saxton, Donnelly, & Roper, 1994) and brain (Ogonovszky et al., 2005). Moderately carbonylated proteins are degraded by proteasomes, and it has been noted that proteasome activity does increase during exercise (Ogonovszky...
et al., 2005). This may also explain the delayed increases in PC reported in some previous studies (Bloomer et al., 2005; Michailidis et al., 2007). Of note, no significant differences were observed in the magnitude of oxidative stress response (LOOH, PC and TAC) between exercise bouts (Figure 2). There are limited similar studies (within-subject design) that compare whole body (i.e. plasma, serum) oxidative stress responses to varying exercise intensities, and thus it is difficult to assess whether this finding is due to the duration of the bouts, or other factors related to experimental design or analysis. This finding warrants further investigation. Collectively, these data are the first to indicate that LV-HIIE elicits a comparable oxidative stress response to a short duration high intensity steady-state exercise.

IL-6 and IL-10 significantly increased in response to a single bout of LV-HIIE as previously 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 in signalling and facilitating 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 for 30 min 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 in 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 (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 I). The physiological parameters studied suggest that LV-HIIE provides a lower physiological stress compared to a short duration high intensity steady-state exercise.

Conclusions

In conclusion, this study presents evidence to show 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 antioxidant, 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.

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All authors contributed to the following aspects of this study: conception and design of experiments, collection analysis and interpretation of data, and drafting the article and revising it critically for important intellectual content.

Disclosure statement

None of the authors declare a conflict of interest and have no financial interest in the study.

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Oxidative stress responses to LV-HIIT


