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DOI:

[10.1080/02640414.2015.1035666](https://doi.org/10.1080/02640414.2015.1035666)

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Document Version

Peer reviewed version

Citation for published version (Harvard):

Wadley, AJ, Chen, Y-W, Lip, GYH, Fisher, JP & Aldred, S 2015, 'Low volume-high intensity interval exercise elicits antioxidant and anti-inflammatory effects in humans', *Journal of Sports Sciences*.
<https://doi.org/10.1080/02640414.2015.1035666>

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This is an Accepted Manuscript of an article published by Taylor & Francis in *Journal of Sports Sciences* on 27/04/2015, available online: <http://www.tandfonline.com/10.1080/02640414.2015.1035666>

Checked September 2015

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

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11 **Running Title:** Oxidative stress responses to LV-HIIE

12 **Word Count:** 2924

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

28 **Funding:** University of Birmingham, UK

29

30

31 **Abstract**

32 The purpose of the present study was to compare acute changes in oxidative stress and inflammation
33 in response to **steady state and low volume, high intensity interval exercise (LV-HIIE)**. Untrained
34 healthy males (n=10, mean \pm SD: age 22 ± 3 yrs; VO_{2MAX} 42.7 ± 5.0 ml/kg/min) undertook three
35 exercise bouts: a bout of LV-HIIE (10*1 minute 90% VO_{2MAX} intervals) and two energy-matched
36 steady-state cycling bouts at a moderate (60% VO_{2MAX} ; 27 min, MOD) and high (80% VO_{2MAX} ; 20
37 min, HIGH) intensity on separate days. Markers of oxidative stress, inflammation and physiological
38 stress were assessed before, **at the end of exercise** and 30 minutes post-exercise (post+30). **At the**
39 **end of all exercise bouts**, significant changes in lipid hydroperoxides (LOOH) and protein carbonyls
40 (PC) (LOOH (nM): MOD +0.36; HIGH +3.09; LV-HIIE +5.51 and PC (nmol/mg protein): MOD -
41 0.24; HIGH -0.11; LV-HIIE -0.37) were observed. Total antioxidant capacity (TAC) increased
42 post+30, relative **to the end of** all exercise bouts (TAC (μ M): MOD +189; HIGH +135; LV-HIIE
43 +102). Interleukin (IL)-6 and IL-10 increased post+30 in HIGH and LV-HIIE only (p<.05). HIGH
44 caused the greatest lymphocytosis, adrenaline and cardiovascular response (p<.05). **At a reduced**
45 **energy cost and physiological stress, LV-HIIE elicited similar cytokine and oxidative stress**
46 **responses to HIGH.**

47
48 **Keywords:** Reactive oxygen species, Cytokine, Lipid Oxidation, Protein Oxidation,
49 Antioxidant

50
51
52 **Abbreviations:**

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

61 $\dot{V}CO_2$: Carbon dioxide consumption, $\dot{V}O_2$: Oxygen Consumption and $\dot{V}O_{2MAX}$: Maximum oxygen
62 consumption.

63

64 **Introduction**

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

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

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

91 shown to increase markers of muscle metabolism (Hood et al., 2011) induce increases in VO₂ peak,
92 (Rognmo, Hetland, Helgerud, Hoff, & Slørdahl, 2004) and improve endothelial function (Wisløff et
93 al., 2007) to the same, or greater degree as steady state exercise. Recent evidence has indicated that
94 LV-HIIE can induce an increase in plasma oxidative stress (G. Fisher et al., 2011) and inflammation
95 (Zwetsloot, John, Lawrence, Battista, & Shanely, 2014) following exercise. To our knowledge, there
96 are no studies to date that directly compare oxidative stress and inflammation in response to LV-HIIE
97 vs. steady state exercise. One previous study showed that the increase in interleukin (IL)-6 following
98 HIIT was greater than moderate intensity steady state exercise, however the protocols used were
99 matched for total workload (Leggate, Nowell, Jones, & Nimmo, 2010), therefore not incorporating the
100 energy saving nature of 'classical' HIIT exercise. The aim of the present study was to compare
101 changes in oxidative stress and inflammation in response to a bout of LV-HIIE and two energy
102 matched steady state exercise bouts of high and moderate intensity.

103

104 **Methods**

105 Participants

106 Ten healthy, untrained (defined as VO_{2MAX} < 50ml/kg/min) males (mean ± SD: age 22 ± 3
107 yrs; body mass index 24.0 ± 3.1 kg/m²; VO_{2MAX} 42.7 ± 5.0 ml/kg/min) took part in three separate
108 exercise bouts. All participants gave their informed written consent and the investigation was
109 approved by the Science and Technology ethical review committee at the University of Birmingham.
110 Participants were non-smokers and excluded if they had ingested vitamin supplements or anti-
111 inflammatory drugs in the two weeks prior to the first laboratory visit. In addition, participants were
112 required to refrain from any strenuous physical activity or consumption of alcoholic beverages in the
113 48 hours prior to testing sessions.

114

115 Preliminary Assessments

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

121 purposes (W. J. Fisher & White, 1999). Cardiorespiratory fitness was assessed by determining the
122 maximum oxygen consumption (VO_{2MAX}) of the participant and expressed relative to body weight
123 ($ml.kg^{-1}.min^{-1}$). A breath-by-breath system (*Oxygon Prx*, Jaeger, Wuerzberg, Germany) was used for
124 continuous measurement of oxygen uptake and heart rate was monitored using a Polar Vantage heart
125 rate monitor (Polar Vantage, Kempele, Finland). After a 3 minute warm up at 30 Watts, participants
126 undertook an incremental exercise test to exhaustion, whereby workload increased 30 Watts every
127 minute until volitional exhaustion. Participants were asked to maintain a constant pedal rate and
128 encouragement was given by an experimenter. A respiratory exchange ratio (VCO_2/VO_2) >1.10-1.15,
129 plateau in participant oxygen consumption or a maximal heart rate >220 beats min^{-1} - age were all
130 factors used to indicate VO_{2MAX} and thus the termination of the test (Howley, Bassett Jr, & Welch,
131 1995).

132

133 Exercise Bouts

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

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

152

153 *[insert Figure 1 here]*

154

155 Blood Assessments

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

160

161 *Lipid Hydroperoxides*

162 LOOH concentrations were assessed using a spectrophotometric assay (Görög, Kotak, &
163 Kovacs, 1991). Samples and a blank standard (10 µl) were added in triplicate to a 96 well microtitre
164 plate. Reagent mix (100 µl, 0.2 M Potassium phosphate (pH=6.2), 0.12 M potassium iodide, 0.15 mM
165 0.15mM sodium azide, **polyethylene glycol** mono p-(1,1',3,3'-tetramethylbutyl)-phenyl]ether (Triton
166 X, 2 g/l), alkylbenzyltrimethylammonium chloride (0.1 g/l), 10 µM ammonium molybdate in HPLC-
167 grade water was added for 30 minutes at room temperature. The plate was read at 365nm (Multiscan
168 MS, Labsystems), concentration of lipid peroxides (µM) determined using the Beer-Lambert Law
169 (extinction co-efficient $\epsilon_{340} = 24600 \text{ M}^{-1}\text{cm}^{-1}$) and adjusted for changes in plasma volume. The inter-
170 assay coefficient of variation (CV) was 8.9%.

171

172 *Total Antioxidant Capacity*

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

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

181

182 *Protein Carbonylation*

183 PC was assessed by ELISA (Buss, Chan, Sluis, Domigan, & Winterbourn, 1997; Carty et al.,
184 2000). The protein concentration of all plasma samples was obtained using the bicinchoninic assay
185 method (Smith et al., 1985). Samples and standards (50 μ l) were then diluted accordingly in coating
186 buffer to a concentration of 0.05mg/ml (50mM sodium carbonate, pH=9.2) and added in triplicate to a
187 96 well NUNC maxisorb microtitre plate for 1 hour at room temperature. Bound protein was
188 incubated with 2, 4-dinitrophenylhydrazine (DNPH) (1mM, in 2M HCl) for 1 hour and then all wells
189 blocked with TBS Tween (0.1%, 200 μ l) overnight at 4°C. Wells were incubated with monoclonal
190 mouse anti-DNP antibody (50 μ l, 1:1000) for 2 hours at room temperature, followed by peroxidase
191 conjugated rat anti-mouse IgE conjugated HRP (50 μ l, 1:5000) for 1 hour at room temperature. All
192 steps were followed by three washes using TBS Tween (0.05%). Substrate (0.5M **citrate** phosphate
193 buffer (10mls, pH=5), hydrogen peroxide (8 μ l) and Ortho-Phenylenediamine tablet (2mg); 50 μ l) was
194 added to each well and the reaction stopped after 45 minutes with 2M sulphuric acid (50 μ l). Well
195 absorbance was measured at 490nm (Multiscan MS, Labsystems) and quantified using absorbance
196 values of known PC standards (1.28-5.20 nmol/mg protein). The inter and intra-assay CV's were
197 8.8% and 1.4 % respectively.

198

199 *Interleukins*

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

206

207

208 *Adrenaline*

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

214

215 Sample size calculation and Statistical Analysis

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

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

232

233 **Results**

234

235 Total Workload and Energy Expenditure

236 The average workload (watts) for the three bouts of exercise were 110 ± 18 (MOD), $169 \pm$
237 32 (HIGH) and 211 ± 38 (LV-HIIE). Total energy expenditure for the LV-HIIE (190 ± 30) bout was
238 significantly lower ($p < .0001$) than both MOD (264 ± 39) and HIGH (266 ± 39).

239

240 Oxidative Stress

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

251

252 *[insert Figure 2 here]*

253

254 Cytokines

255 **The effect of exercise on IL-6 and IL-10 concentrations can be seen in Figure 3(a-b). IL-**
256 **6 concentrations increased at the end of exercise in HIGH and LV-HIIE (p<0.05), with**
257 **elevations post+30, relative to baseline in all bouts (p<0.016). The concentration of IL-6 post+30**
258 **was significantly higher in HIGH compared to MOD (group x time interaction effect; p=0.037).**
259 **IL-10 concentrations were unchanged at the end of exercise, however increases were observed**
260 **post+30, relative to baseline (p=0.05) and the end of exercise (p=0.05) in LV-HIIE and HIGH**
261 **respectively (group x time interaction effect; p=0.015). IL-10 concentrations post+30 were**
262 **higher in HIGH than MOD (group x time interaction effect; p=0.05). No statistical differences**
263 **in IL-6 and IL-10 responses were observed between LV-HIIE and HIGH. Further analysis of**
264 **the individual exercise bouts using one-way ANOVAs revealed that IL-10 concentration**
265 **decreased post+30 relative to baseline in MOD (p=0.01).**

266

267 *[insert Figure 3 here]*

268

269 Other physiological measures

270 *Total Peripheral Blood Lymphocytes and plasma adrenaline*

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

279

280 *[insert Table 1 here]*

281

282 *Heart Rate and Blood Pressure*

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

288

289 *[insert Figure 4 here]*

290

291 **Discussion**

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

327 **comparable to HIGH, indicating that these exercise bouts were more effective than MOD in**
328 **stimulating an anti-inflammatory response to exercise** (Figure 3). Aside from the classical role of
329 IL-6 to signal and facilitate the inflammatory response, there is evidence to suggest that its release
330 during exercise can inhibit pro-inflammatory cytokine production (Nimmo, Leggate, Viana, & King,
331 2013) and up-regulate the transcription of anti-inflammatory cytokines such as IL-10 (Fischer, 2006).
332 The present results are in support of these studies, with IL-10 elevation seen thirty minutes following
333 the increase in IL-6 in both LV-HIIE and HIGH. Previous evidence has highlighted that exercise
334 intensity may be the key factor governing IL-6 release when considering shorter bouts of steady state
335 exercise under an hour (Fischer, 2006; Ostrowski et al., 2000). Despite the higher peak intensity of
336 LV-HIIE, the intermittent nature of the stimulus may have aided the clearance of IL-6 by the liver and
337 kidneys (Febbraio et al., 2003) during the active rest intervals. Nevertheless, these data indicate that
338 LV-HIIE and short duration high intensity, steady state exercise can elicit comparable **IL-6 and IL-**
339 **10** responses in untrained participants.

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

347

348 **Conclusions**

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

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501 **Conflict of Interest:** None of the authors declare a conflict of interest and have no financial
502 interest in the study.

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519 Tables

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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 (×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

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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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