Interleukin-6 and associated cytokine responses to an acute bout of high intensity interval exercise: the effect of exercise intensity and volume

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Abstract

Introduction: Acute increases in interleukin (IL)-6 following prolonged exercise are associated with the induction of a transient anti-inflammatory state (e.g. increases in IL-10) that is partly responsible for the health benefits of regular exercise. The purposes of this study were to investigate the IL-6 related inflammatory response to high-intensity interval exercise (HIIE) and to determine the impact of exercise intensity and volume on this response.

Methods: 10 participants (5 males and 5 females) completed 3 exercise bouts of contrasting intensity and volume (LOW, MOD and HIGH). The HIGH protocol was based upon standard HIIE protocols, while the MOD and LOW protocols were designed were designed to enable a comparison of exercise intensity and volume with a fixed duration. Inflammatory cytokine concentrations were measured in plasma (IL-6, IL-10) and also determined at the level of gene expression (IL-6, IL-10, and IL-4R in peripheral blood.

Results: The plasma IL-6 response to exercise (reported as fold changes) was significantly greater in HIGH (2.70 ± 1.51) than LOW (1.40 ± 0.32) ($P=0.04$) and was also positively correlated to the mean exercise $\dot{V}O_2$ ($r=0.54$, $P<0.01$). However, there was no change in anti-inflammatory IL-10 or IL-4R responses, in plasma or at the level of gene expression.

Discussion: HIIE caused a significant increase in IL-6 and was greater than that seen in low intensity exercise of the same duration. The increases in IL-6 were relatively small in magnitude, and appear to have been insufficient to induce the acute systemic anti-inflammatory effects, which are evident following longer duration exercise.

Keywords: Cytokines; anti-inflammatory; HIIT; exercise; high-intensity; interval.

Introduction

Physical inactivity is associated with an increased risk of a number of chronic health conditions such as cardiovascular disease, type 2 diabetes, metabolic syndrome and clinical depression (Booth, Roberts, and Laye 2012). These conditions are associated with chronic low-grade systemic inflammation, which is characterized by 2-4 fold chronic elevations in inflammatory markers such as C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) (Bruunsgaard 2005). Chronic low-grade systemic inflammation appears to be pathologically linked to many of these diseases; it is associated with the development of insulin resistance, atherosclerosis and neurodegeneration (Gleeson et al. 2011; Shoelson, Lee, and Goldfine 2006). It is well known that exercise can protect against the development of many of these chronic diseases (Pedersen and Febbraio 2008), and it has emerged that at least some of the beneficial health effects of regular exercise are due to the induction of a transient anti-inflammatory state post-exercise that assists in the reduction of chronic low-grade inflammation (Pedersen and Saltin 2006; Gleeson et al. 2011). As a result the use of regular exercise as an anti-inflammatory intervention is widely recognized (Beavers, Brinkley, and Nicklas 2010).

There is comprehensive evidence that the anti-inflammatory effect of exercise is induced, in part, by transient elevations in the circulating concentration of IL-6 and the subsequent induction of anti-inflammatory cytokines such as IL-10 by leukocytes, (Reihmane and Dela 2014). IL-6 is released from active skeletal muscle during exercise (Steensberg et al. 2000), and typically, the circulating concentrations of IL-6

and IL-10 peak at the end of exercise (Ostrowski et al. 1999). IL-10 is a potent anti-inflammatory mediator, the primary function of which is to suppress and terminate inflammatory responses (Moore et al. 2001), and in the context of sustained exercise IL-10 appears to be produced by the leukocytes in response to exposure to muscle derived IL-6 (Nieman et al. 2006). It has also been reported that changes in expression of cytokines such as IL-10 can be detected at the mRNA level within purified whole-blood samples (Abbasi et al. 2013) and these responses appear to share the same pattern of regulation as that seen in isolated leukocytes (Nieman et al. 2006). Similarly, it was recently reported that IL-6 can upregulate the expression of the IL-4 receptor (IL-4R) within monocytes, and consequently augment IL-4 mediated anti-inflammatory responses (Mauer et al. 2014). While the literature is not clear whether exercise directly induces increases in circulating IL-4 levels (LaVoy et al. 2013) or not (Nieman et al. 2001), it is possible that exercise-induced increases in IL-6 may increase the gene expression of IL-4R and hence enhance leukocytes' sensitivity to IL-4, thereby presenting another possible anti-inflammatory action of IL-6.

There is extensive literature regarding anti-inflammatory responses to prolonged moderate intensity exercise, and specifically that the magnitude of the IL-6 response is especially sensitive to the duration of exercise (Fischer 2006) in contrast there has been less research investigating anti-inflammatory responses to shorter duration exercise such as high intensity interval training (HIIT). In recent years HIIT has become increasingly popular and research has shown that HIIT induces significant cardiovascular (Wisloff et al. 2007) and metabolic adaptations (Weston et al. 1997). While traditionally the domain of elite athletes, recent studies have shown that HIIT is highly effective and well tolerated in a number of clinical populations such as those with heart failure, and type 2 diabetes (Weston, Wisloff, and Coombes 2014). In

addition there has been considerable interest into how modified HIIT protocols can impact upon beneficial adaptations and their mechanistic underpinnings (Helgerud et al. 2007; Weston et al. 1997); these studies have provided insights into the aspects of training that lead to specific adaptations, thereby aiding the optimization of training programs and interventions.

Importantly, similar work has yet to be conducted in the context of the anti-inflammatory responses to HIIT; in particular, the efficacy of HIIT to reduce markers of low-grade inflammation appears inconsistent (Munk et al. 2011; Tjonna et al. 2013; Boyd et al. 2013). There is evidence that exercise volume may be an important factor that determines anti-inflammatory responses (Balducci et al. 2010). Several studies have reported that increases in IL-10 are related to a relative increase in the volume of exercise performed (Jankord and Jemiolo 2004; Kadoglou et al. 2007). In addition there is evidence that weekly training volume is associated with increased IL-4 and IL-10 responses to antigen challenge (Gleeson et al. 2013; Handzlik et al. 2013). While initial investigations have been conducted into the efficacy of high intensity interval exercise for inducing a post exercise anti-inflammatory state (Zwetsloot et al. 2014; Wadley et al. 2015) these studies have not standardized their exercise protocols for exercise duration. Given the importance of exercise duration in the magnitude of post exercise IL-6 responses (Fischer 2006), this should be considered a limitation of these studies.

Therefore, the aims of this study were firstly to investigate the systemic anti-inflammatory response to an acute bout of high intensity interval cycling, and secondly to investigate the effect of manipulating exercise intensity and volume while controlling for the exercise duration. We aimed to test the hypothesis that high intensity interval exercise would increase the circulating concentrations of IL-6 and

IL-10, and that these increases would be related to exercise volume and intensity. Secondly, based upon previous research (Mauer et al. 2014; Nieman et al. 2006), we aimed to test the hypothesis that increases in plasma IL-6 would be associated with increases in the gene expression of IL-10 and IL-4R in whole blood.

Materials and Methods

Participants

Ten healthy active individuals (5 male, 5 female aged 24 ± 4 years, height 170 ± 9 cm, weight 67 ± 11 kg, $\dot{V}O_{2\ peak}$ 49 ± 5 ml.kg.min-1 gave informed consent to participate in the study. Subjects completed health and physical activity questionnaires to ensure the standardization of exercise and diet for each session. All participants were free of illness and injury for a minimum of one week prior to participation in the study. Ethical approval was obtained from the Cardiff Metropolitan University School of Sport Ethics committee, and all procedures conformed to the declaration of Helsinki.

Preliminary measurements

Upon their first visit to the laboratory participants were tested for maximal oxygen uptake (VO_{2max}) using an incremental exercise test on an electromagnetically braked cycle ergometer (Lode Excalibur, Groningen, Netherlands). Expired gases were measured using an online gas analyzer (OxyconPro, Erich Jaeger GMBH & Co., Hoechberg, Germany), and heart rate was measured continuously via short-range telemetry (RS400, Polar Electro, Finland). Each stage of the incremental exercise lasted 3 minutes and the required power output was increased by 30W at each stage until volitional exhaustion, with participants cycling at a pedal cadence of 80rpm. Males began the test at a required power output of 100W while females began at

50W. VO_{2max} was recorded as the highest 30-s period of oxygen consumption. Oxygen consumption values obtained during the incremental test were used to plot a linear regression of power output versus oxygen consumption. This allowed the calculation of individual power outputs for subsequent testing protocols.

Study Design

Participants completed 3 exercise sessions on a cycle ergometer in a counterbalanced order. These sessions were completed within a period of 2 weeks, with a minimum of 3 days separating each exercise session. The exercise sessions were: (i) 35min cycling at 50% VO_{2max} (LOW), (ii) 5 x 5 minute intervals at 50% VO_{2max} interspersed 5 x 2 minute intervals at 80% VO_{2max} MOD), (iii) 5 x 4 minute intervals at 80% VO_{2max} interspersed with 3 minute intervals at 50% VO_{2max} (HIGH). We chose these three exercise sessions to allow the comparison of the combined effects of exercise intensity and volume (LOW Vs. MOD Vs. HIGH). The HIGH protocol was based upon HIIT protocols that have been extensively reported in the scientific literature (Tjonna et al. 2013), and the MOD and LOW protocols were designed to enable a comparison of exercise intensity and volume with a fixed duration (35 minutes). Expired gases and heart rate (HR) were measured continuously throughout each exercise session. Blood lactate was determined using 20μL capillary blood samples collected from the earlobe at 7-min, 21-min, and 35-min of the exercise, which corresponded with the end of an active recovery bout in the interval exercise sessions. These blood samples were treated immediately and analyzed using a Biosen 5030 (EKF diagnostic, Barlebon, Germany). Additionally, venous blood samples were drawn from the antecubital vein immediately before and after each of the exercise sessions and were used for the analysis of cytokine concentration and gene expression.

XXX Insert Figure 1. XXX

Dietary and physical activity control

Participants were asked to keep a food diary prior to maximal exercise testing and to maintain a similar diet and activity level prior to each of the subsequent exercise sessions. Participants arrived at the laboratory at the same time of day prior to each of their tests and were asked to refrain from eating or drinking (other than water) for the 2 hours prior to testing, and to refrain from alcohol, caffeine and strenuous exercise in the preceding 24 hours.

Enzyme-linked immunosorbent assays

Whole blood samples were collected into K_3EDTA tubes (Greiner Bio-one; Frickenhausen, Germany) and were separated by centrifugation (3,000 x G for 10 min). The resulting plasma was aliquoted and stored at -80°C until analysis. Plasma IL-6 and IL-10 concentrations were analyzed in duplicate using high sensitivity enzyme linked immunosorbent assay technique (ELISA) (Quantikine HS; R&D Systems Ltd., Abingdon, UK). Plasma concentrations of the sIL-6R were measured using a commercially available DuoSet ELISA (R&D Systems Ltd., Abingdon, UK) that was validated for use with plasma samples in a pilot study (data not shown). All additional materials and chemical reagents were purchased from R&D systems (R&D Systems Ltd., Abingdon, UK) and all procedures were carried out as to the manufacturer's instructions. The IL-6 assay has a detection limit of 0.039 pg/ml and an intra-assay coefficient of variation (CV) of 3.8 ± 2.9% across the range 0.15–10 pg/ml. The IL-10 assay has a detection limit of 0.09 pg/ml and an intra assay CV of 1.9 ± 1.7% across a range of 0.78-50 pg/ml. The sIL-6R assay has an intra assay CV

4.8 ± 1.6% across a range of 1.56-100 ng/ml. Protein concentrations were determined in relation to a four-parameter standard curve (GraphPad Prism, San Diego California, USA).

Whole blood mRNA extraction and quantitative real-time PCR analysis

Peripheral whole-blood samples for total RNA extraction were drawn into PAXgene blood RNA tubes (Qiagen, Germany) and frozen at -80°C. Semi-automated RNA extraction was carried out following the guidelines of the PAXgene blood RNA kits using the QIAcube platform (Qiagen, Germany). Whole-blood RNA samples prepared in this way contain RNA extracted from sources such as platelets, reticulocytes, or circulating endothelial cells as well as from leukocytes (Liew et al. 2006). However, it should be noted that similar exercise-associated RNA expression patterns have been reported in whole-blood (Abbasi et al. 2013) to those that have studied leukocyte mRNA gene expression after exercise (Nieman et al. 2006); for this reason, we have utilised a whole-blood sampling approach, and have then made the assumption that leukocytes are the cell-type (or one of the cell-types) within whole-blood samples which are the source of any observed exercise-induced changes in expression with regard to the genes under investigation in our study.

RNA yield was quantified and assessed for purity by reading the absorbance at 260:280nm on the NanoDrop 1000 spectrophotometer (NanoDrop, Wilmington, USA) (all samples had a ratio between 1.9 and 2.3). RNA samples were stored at -80°C before being converted to cDNA using M-MLV reverse transcriptase (Invitrogen, UK) and random hexamer primers (Applied Biosystems, Warrington, UK). Quantitative real-time polymerase chain reaction (RT-PCR) was performed on an Applied Biosystems 7500 Fast real-time PCR system using Taqman fast mastermix gene expression (Applied Biosystems). IL-6, IL-4R, and IL-10 gene expression were

analysed and compared to that of a house keeping gene, β Actin. The following Taqman primer and probe sets for were obtained from Applied Biosystems; IL-6 (ID: Hs00174131_m1), IL-4R (ID: Hs00166237_m1), IL-10 (ID: Hs00174086_m1), β Actin (ID: 4310881E). Following an initial 20s at 95°C, thermocycling consisted of 40 cycles of 3s at 95°C and 30s at 60°C. Gene expression profiles were analysed using ABI software to assign a cycle threshold (C_T), this reflects the cycle number that the cDNA amplification is first detected. This is calculated by the cycle number at which the fluorescent intensity increases beyond a threshold level that is based upon the background fluorescence of the system. Calculation of relative gene expression was performed using the $2^{\Delta\Delta CT}$ method, where the ΔC_T is equal to the difference between values for the gene of interest and the housekeeping gene (Livak and Schmittgen 2001).

Statistical analysis

All data are presented as means ± standard error unless otherwise stated. A within group repeated measures ANOVA was used to analyze the data. There was no significant difference between males and females when measured as absolute cytokine concentrations or the fold change in response to exercise, and therefore all males and females were analyzed together. Statistical significance was set at $P \leq 0.05$. Bonferroni post-hoc tests were performed where appropriate. Cytokine concentrations were non-normally distributed and log-transformed before analysis. Pearson's correlation analyses were used to investigate the relationships between the physiological variables and the fold change in IL-6. SPSS 20.0 was used for all statistical analysis.

Results

The results of the physiological variables for each of the three exercise protocols are summarised in Table 1. The oxygen uptake (mean VO_2 (% max)), heart rate (mean HR (% max), lactate responses were significantly greater for the HIGH trial than the MOD and LOW trials ($P<0.01$), and were significantly greater for the MOD trial than the LOW trial ($P<0.01$). The RER values were significantly higher in the MOD and HIGH trials than the LOW trial. Figure 1 provides an insight into the typical VO_2 response throughout each of the exercise trials.

Throughout the entire study the average concentration of IL-6 across all three conditions increased from 0.57 ± 0.81 pg/ml at rest, to 0.85 ± 0.88 pg/ml ($P<0.01$) immediately following exercise. Compared to pre-exercise, the plasma concentration of IL-6 increased significantly within each of the 3 conditions: 1.4 ± 0.1 fold ($P<0.01$) (LOW), 1.9 ± 0.3 fold ($P<0.01$) (MOD), 2.7 ± 0.6 fold ($P<0.01$) (HIGH). The increase in IL-6 was significantly greater in the HIGH protocol than LOW ($P=0.04$), and showed a trend towards significance when compared to MOD ($P=0.11$) (Fig. 2A). The post-exercise fold change in IL-6 positively correlated with the mean $\dot{V}O_2$ (% max) ($r=0.54$, $P<0.01$), mean HR (% max) ($r=0.39$, $P=0.04$), mean respiratory exchange ratio (RER) ($r=0.61$, $P<0.01$), and the end-test blood lactate concentration ($r=0.56$, $P<0.01$). In contrast, plasma levels of IL-10 and sIL-6R showed no significant change within any of the exercise protocols (Fig. 2B-C).

There was no change in the level of whole-blood gene expression of IL-6, IL-10 or IL-4R following any of the exercise sessions (Figs. 3A-C).

XXX Insert Table 1 XXX

XXX Insert Figure 2. XXX

XXX Insert Figure 3. XXX

Discussion

In this study we report small but significant increases in IL-6 following 3 separate bouts of aerobic exercise lasting 35 minutes. The post-exercise IL-6 response to HIIE (HIGH) was greater than that of steady-state moderate intensity exercise of the same duration (LOW) (Fig. 2A). However, there was no change in the plasma concentrations of IL-10 and sIL-6R (Figs. 2B-C) following any of the three exercise bouts. These results suggest that 35 minutes of HIIE exercise does induce small increases in the circulating concentration of IL-6, but that this is insufficient to induce an increase in the anti-inflammatory cytokine IL-10 which have previously been reported, and attributed to IL-6 in the context of longer duration exercise (Nieman et al. 2001; Suzuki et al. 2003).

In agreement with previous literature (Scott et al. 2011), we found that the post-exercise increase in IL-6 was positively correlated with mean RER ($r=0.61$, $P<0.01$), which is indicative of a greater reliance on carbohydrate as a substrate. This is unsurprising given that one of the primary functions of IL-6 during exercise is to respond to muscle glycogen status and facilitate glucose metabolism (Pedersen and Febbraio 2008). Indeed our results show that RER was significantly greater in the two HIIT bouts (MOD and HIGH) than the steady state exercise bout (LOW), while there was no difference between MOD and HIGH. This suggests that the increase in IL-6 response between the HIIT bouts (MOD and HIGH) and the continuous moderate intensity exercise bout (LOW) could have been due to increased exercise intensity,

and therefore increased reliance on CHO as a substrate. Because post-exercise IL-6 responses were positively correlated with the volume of exercise ($r=0.54$, $P<0.01$) as measured by the mean VO_2 (% max), it is possible that with a larger sample size, a statistically significant difference may have been observed between the MOD and HIGH protocols. Thus, taken together, it appears that a combined increase of both the intensity and volume of exercise is associated with an increased IL-6 response.

It is of note that the IL-6 responses seen in this study (up to 2.7-fold increase) were considerably smaller than those reported following exercise of a longer duration such as a marathon (up to 100-fold increase) (Suzuki et al. 2003). However IL-6 responses are known to be lower following cycling than they are for exercise modes that involve a larger muscle mass, such as running (Fischer 2006). In addition the participants in this study were young (23.7±4.1 yrs.) and relatively fit (VO_{2max} =49.1± 4.5 ml.kg.min-1); thus, given that IL-6 responses to exercise are increased with age and decreased with fitness, it is possible that a larger response would have been seen in older or less fit individuals. Nevertheless, it is probable that the comparatively modest increases in IL-6 seen in our study were primarily due to the relatively short duration of the exercise (Fischer 2006). In our study we saw a 2.7 ± 0.6 fold change in IL-6, while 1hr and 2hrs of cycling at similar mean intensities (70% VO_{2max} and 75% VO_{2max} respectively), albeit steady state, exercise have been reported to induce 5-fold and 40-fold increases in IL-6 respectively (Leggate et al. 2010; Nieman et al. 2006). Given that the IL-6 response to exercise is thought to be exponential with increasing duration (Fischer 2006), our results appear to be in line with the aforementioned studies that used the same mode of exercise and healthy active subjects. Taken together, therefore, while the results of our study show that a combination of both the intensity and volume of the exercise performed do contribute to the IL-6 response, it

appears that 35 minutes of HIIT exercise induces comparatively small increases in IL-6.

Importantly, we saw no increases in the plasma concentration or the gene expression of IL-10, which is in contrast with studies involving more prolonged exercise; for example 26-fold increases in circulating IL-10 protein concentration and 2.7-fold increases in leukocyte gene expression have been reported immediately following 2hrs of cycling (Nieman et al. 2006). Interestingly these considerable anti-inflammatory responses were accompanied by a 40-fold increase in circulating concentration of IL-6. Similarly, while recent evidence has suggested that IL-6 can increase the up regulation of IL-4R in leukocytes, and subsequently augment IL-4 mediated signalling (Mauer et al. 2014), in our study we saw no increase in the gene expression of IL-4R in whole blood following any of the three exercise sessions. Accordingly, it appears that the small increases in IL-6 in our study (2.7 fold) were not sufficient to induce downstream systemic anti-inflammatory responses immediately post exercise, whereas higher concentrations of IL-6 induced by more prolonged exercise appear sufficient to induce up to 26-fold increases in IL-10 immediately following exercise (Nieman et al. 2006). Similarly, although Mauer et al did not focus on exercise (instead using exogenously added 50ng/ml IL-6 as an *in-vitro* stimulus (Mauer et al, 2014)), a similar argument in the case of IL-4R/IL-4 signalling may explain why we did not observe IL-4R upregulation following small (<1pg/ml) exercise-associated increases in IL-6 in the current study.

While the majority of studies have shown that IL-10 peaks immediately post exercise (Ostrowski et al. 1999), these studies have typically been conducted on longer duration exercise. A recently published study has demonstrated that following 20 minutes of aerobic exercise (80% VO_{2max}) IL-6 and IL-10 are increased during the

346 recovery phase, at 30 minutes post-exercise (Wadley et al. 2015). While the increases
347 in IL-6 and IL-10 reported in the study of Wadley et al. (2015) were very small
348 (approximately 1 pg/ml and 0.1 pg/ml respectively) it is possible that, in the current
349 study, elevations in IL-10 may have occurred during recovery from exercise, although
350 given the very small increases detected by Wadley et al. (2015) these increases are
351 likely to have been very small. As such the lack of measurements obtained during
352 recovery from exercise should be considered a weakness of this study, and
353 accordingly it should be noted that the results presented here may not necessarily
354 reflect the responses during recovery from exercise.
355 Several studies have reported the lack of a systemic anti-inflammatory response
356 following moderate exercise of a similar duration (Markovitch, Tyrrell, and
357 Thompson 2008; Nieman et al. 2005); however, there is considerably less evidence
358 for the absence of an acute anti-inflammatory response following high intensity
359 interval exercise. This is an important finding and could provide insight into why
360 some studies have shown no change in resting levels of pro or anti-inflammatory
361 markers following a HIIT programme similar to that employed here (Tjonna et al.
362 2013).
363 It is important to consider that acute increases in IL-6 and the subsequent induction of
364 anti-inflammatory signalling is not the only source of a reduction in chronic
365 inflammation in the context of long-term exercise training (reviewed by Gleeson et
366 al., 2011). Indeed recent evidence has suggested that high intensity interval training in
367 overweight and obese individuals can have an anti-inflammatory effect by reducing
368 the inflammatory profile in adipose tissue, without having any effect on the plasma
369 concentration of inflammatory cytokines (Leggate et al. 2012). Importantly the results
370 of the current study provide further evidence that any beneficial changes in metabolic

health associated with high intensity interval exercise are unlikely to be due to transient systemic anti-inflammatory responses; rather, an extended exercise duration is likely to be necessary for large perturbations in systemic cytokine responses. We propose that there is a minimum duration and intensity of exercise to induce acute beneficial changes in systemic inflammatory responses and based on the results of the current study 35 minutes of high intensity interval exercise may be beneath this threshold for young healthy active individuals.

A limitation to our study was that we did not measure the rate of IL-6 release from the muscle. This is important because, in addition to that produced by myocytes; IL-6 can also be released from the leukocytes in response to tissue damage that could have occurred during exercise (Pedersen and Febbraio 2008). However there was no elevation in the level of IL-6 gene expression in whole blood samples in this study, and given that stationary cycling is unlikely to elicit any significant muscle damage, we would contend that the changes in circulating IL-6 seen in this study were due to release from myocytes, rather than from additional sources such as leukocytes (Steensberg et al. 2000). An additional limitation is that we investigated exercise-associated gene expression in whole-blood samples, rather than in purified leukocytes; however, as stated above, previous studies have reported similar patterns of exercise-associated gene expression in both types of samples (Nieman et al. 2006; Abbasi et al. 2013).

In summary, the results of our study indicate that IL-6 is sensitive to subtle manipulations in intensity and volume of the exercise performed. However, it appears that 35 minutes of high intensity exercise induces comparatively small immediate post-exercise increases in IL-6, which appear to be insufficient to induce the systemic anti-inflammatory effects that are mediated through secondary IL-6 induced

upregulation of anti-inflammatory signaling molecules such as IL-10 (Mauer et al. 2014; Nieman et al. 2006; Nieman et al. 2001; Petersen and Pedersen 2005; Abbasi et al. 2013). Considering the results of this study within the context of the existing literature, it appears that there may be a threshold level of IL-6 required for the induction of the aforementioned beneficial systemic anti-inflammatory responses, and that an extended exercise duration is likely to be an important factor in achieving this. Future research should systematically investigate the required duration to induce beneficial anti-inflammatory signaling responses within different populations, particularly those whose sedentary lifestyles that put them at risk of physical inactivity-related chronic inflammatory conditions such as cardiovascular disease and type-2 diabetes.

The author declares that there are no conflicts of interest.

References

Abbasi, A., E. Fehrenbach, M. Hauth, M. Walter, J. Hudemann, V. Wank, A. M. Niess, and H. Northoff. 2013. 'Changes in spontaneous and LPS-induced ex vivo cytokine production and mRNA expression in male and female athletes following prolonged exhaustive exercise', *Exerc Immunol Rev*, 19: 8-28.

Balducci, S., S. Zanuso, A. Nicolucci, F. Fernando, S. Cavallo, P. Cardelli, S. Fallucca, E. Alessi, C. Letizia, A. Jimenez, F. Fallucca, and G. Pugliese. 2010. 'Anti-inflammatory effect of exercise training in subjects with type 2 diabetes and the metabolic syndrome is dependent on exercise modalities and independent of weight loss', *Nutr Metab Cardiovasc Dis*, 20: 608-17.

Beavers, K. M., T. E. Brinkley, and B. J. Nicklas. 2010. 'Effect of exercise training on chronic inflammation', *Clin Chim Acta*, 411: 785-93.

Booth, F. W., C. K. Roberts, and M. J. Laye. 2012. 'Lack of exercise is a major cause of chronic diseases', *Compr Physiol*, 2: 1143-211.

Boyd, J. C., C. A. Simpson, M. E. Jung, and B. J. Gurd. 2013. 'Reducing the intensity and volume of interval training diminishes cardiovascular adaptation but not mitochondrial biogenesis in overweight/obese men', *PLoS One*, 8: e68091.

Bruunsgaard, H. 2005. 'Physical activity and modulation of systemic low-level inflammation', *J Leukoc Biol*, 78: 819-35.
Fischer, C. P. 2006. 'Interleukin-6 in acute exercise and training: what is the biological relevance?', *Exercise Immunology Review*, 12: 6-33.
Gleeson, M., N. C. Bishop, D. J. Stensel, M. R. Lindley, S. S. Mastana, and M. A. Nimmo. 2011. 'The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease', *Nat Rev Immunol*, 11: 607-15.
Gleeson, M., N. Bishop, M. Oliveira, and P. Tauler. 2013. 'Influence of training load on upper respiratory tract infection incidence and antigen-stimulated cytokine production', *Scand J Med Sci Sports*, 23: 451-7.
Handzlik, M. K., A. J. Shaw, M. Dungey, N. C. Bishop, and M. Gleeson. 2013. 'The influence of exercise training status on antigen-stimulated IL-10 production in whole blood culture and numbers of circulating regulatory T cells', *Eur J Appl Physiol*, 113: 1839-48.
Helgerud, J., K. Hoydal, E. Wang, T. Karlsen, P. Berg, M. Bjerkaas, T. Simonsen, C. Helgesen, N. Hjorth, R. Bach, and J. Hoff. 2007. 'Aerobic high-intensity intervals improve VO2max more than moderate training', *Med Sci Sports Exerc*, 39: 665-71.
Jankord, R., and B. Jemiolo. 2004. 'Influence of physical activity on serum IL-6 and IL-10 levels in healthy older men', *Med Sci Sports Exerc*, 36: 960-4.
Kadoglou, N. P., F. Iliadis, N. Angelopoulou, D. Perrea, G. Ampatzidis, C. D. Liapis, and M. Alevizos. 2007. 'The anti-inflammatory effects of exercise training in patients with type 2 diabetes mellitus', *Eur J Cardiovasc Prev Rehabil*, 14: 837-43.
LaVoy, E. C., J. A. Bosch, T. W. Lowder, and R. J. Simpson. 2013. 'Acute aerobic exercise in humans increases cytokine expression in CD27(-) but not CD27(+) CD8(+) T-cells', *Brain Behav Immun*, 27: 54-62.
Leggate, M., W. G. Carter, M. J. Evans, R. A. Vennard, S. Sribala-Sundaram, and M. A. Nimmo. 2012. 'Determination of inflammatory and prominent proteomic changes in plasma and adipose tissue after high-intensity intermittent training in overweight and obese males', *J Appl Physiol (1985)*, 112: 1353-60.
Leggate, M., M. A. Nowell, S. A. Jones, and M. A. Nimmo. 2010. 'The response of interleukin-6 and soluble interleukin-6 receptor isoforms following intermittent high intensity and continuous moderate intensity cycling', *Cell Stress Chaperones*, 15: 827-33.
Liew, C. C., J. Ma, H. C. Tang, R. Zheng, and A. A. Dempsey. 2006. 'The peripheral blood transcriptome dynamically reflects system wide biology: a potential diagnostic tool', *J Lab Clin Med*, 147: 126-32.
Livak, K. J., and T. D. Schmittgen. 2001. 'Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method', *Methods*, 25: 402-8.
Markovitch, D., R. M. Tyrrell, and D. Thompson. 2008. 'Acute moderate-intensity exercise in middle-aged men has neither an anti- nor proinflammatory effect', *J Appl Physiol (1985)*, 105: 260-5.
Mauer, J., B. Chaurasia, J. Goldau, M. C. Vogt, J. Ruud, K. D. Nguyen, S. Theurich, A. C. Hausen, J. Schmitz, H. S. Bronneke, E. Estevez, T. L. Allen, A. Mesaros, L. Partridge, M. A. Febbraio, A. Chawla, F. T. Wunderlich, and J. C. Bruning. 2014. 'Signaling by IL-6 promotes alternative activation of

macrophages to limit endotoxemia and obesity-associated resistance to insulin', *Nat Immunol*, 15: 423-30.
Moore, K. W., R. de Waal Malefyt, R. L. Coffman, and A. O'Garra. 2001. 'Interleukin-10 and the interleukin-10 receptor', *Annu Rev Immunol*, 19: 683-765.
Munk, P. S., U. M. Breland, P. Aukrust, T. Ueland, J. T. Kvaloy, and A. I. Larsen. 2011. 'High intensity interval training reduces systemic inflammation in post-PCI patients', *Eur J Cardiovasc Prev Rehabil*, 18: 850-7.
Nieman, D. C., D. A. Henson, M. D. Austin, and V. A. Brown. 2005. 'Immune response to a 30-minute walk', *Med Sci Sports Exerc*, 37: 57-62.
Nieman, D. C., D. A. Henson, J. M. Davis, C. L. Dumke, A. C. Utter, E. A. Murphy, S. Pearce, G. Gojanovich, S. R. McAnulty, and L. S. McAnulty. 2006. 'Blood leukocyte mRNA expression for IL-10, IL-1Ra, and IL-8, but not IL-6, increases after exercise', *J Interferon Cytokine Res*, 26: 668-74.
Nieman, D. C., D. A. Henson, L. L. Smith, A. C. Utter, D. M. Vinci, J. M. Davis, D. E. Kaminsky, and M. Shute. 2001. 'Cytokine changes after a marathon race', *J Appl Physiol (1985)*, 91: 109-14.
Ostrowski, K., T. Rohde, S. Asp, P. Schjerling, and B. K. Pedersen. 1999. 'Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans', *J Physiol*, 515 (Pt 1): 287-91.
Pedersen, B. K., and M. A. Febbraio. 2008. 'Muscle as an endocrine organ: focus on muscle-derived interleukin-6', *Physiological Reviews*, 88: 1379-406.
Pedersen, B. K., and B. Saltin. 2006. 'Evidence for prescribing exercise as therapy in chronic disease', *Scand J Med Sci Sports*, 16 Suppl 1: 3-63.
Petersen, A. M., and B. K. Pedersen. 2005. 'The anti-inflammatory effect of exercise', *Journal of Applied Physiology*, 98: 1154-62.
Reihmane, D., and F. Dela. 2014. 'Interleukin-6: possible biological roles during exercise', *Eur J Sport Sci*, 14: 242-50.
Scott, J. P., C. Sale, J. P. Greeves, A. Casey, J. Dutton, and W. D. Fraser. 2011. 'Effect of exercise intensity on the cytokine response to an acute bout of running', *Medicine & Science in Sports & Exercise*, 43: 2297-306.
Shoelson, S. E., J. Lee, and A. B. Goldfine. 2006. 'Inflammation and insulin resistance', *J Clin Invest*, 116: 1793-801.
Steensberg, A., G. van Hall, T. Osada, M. Sacchetti, B. Saltin, and B. Klarlund Pedersen. 2000. 'Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6', *J Physiol*, 529 Pt 1: 237-42.
Suzuki, K., S. Nakaji, M. Yamada, Q. Liu, S. Kurakake, N. Okamura, T. Kumae, T. Umeda, and K. Sugawara. 2003. 'Impact of a competitive marathon race on systemic cytokine and neutrophil responses', *Med Sci Sports Exerc*, 35: 348-55.
Tjonna, A. E., I. M. Leinan, A. T. Bartnes, B. M. Jenssen, M. J. Gibala, R. A. Winett, and U. Wisloff. 2013. 'Low- and high-volume of intensive endurance training significantly improves maximal oxygen uptake after 10-weeks of training in healthy men', *PLoS One*, 8: e65382.
Wadley, A. J., Y. W. Chen, G. Y. Lip, J. P. Fisher, and S. Aldred. 2015. 'Low volume-high intensity interval exercise elicits antioxidant and anti-inflammatory effects in humans', *J Sports Sci*: 1-9.
Weston, A. R., K. H. Myburgh, F. H. Lindsay, S. C. Dennis, T. D. Noakes, and J. A. Hawley. 1997. 'Skeletal muscle buffering capacity and endurance performance

after high-intensity interval training by well-trained cyclists', *Eur J Appl Physiol Occup Physiol*, 75: 7-13.
Weston, K. S., U. Wisloff, and J. S. Coombes. 2014. 'High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis', *Br J Sports Med*, 48: 1227-34.
Wisloff, U., A. Stoylen, J. P. Loennechen, M. Bruvold, O. Rognmo, P. M. Haram, A. E. Tjonna, J. Helgerud, S. A. Slordahl, S. J. Lee, V. Videm, A. Bye, G. L. Smith, S. M. Najjar, O. Ellingsen, and T. Skjaerpe. 2007. 'Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study', *Circulation*, 115: 3086-94.
Zwetsloot, K. A., C. S. John, M. M. Lawrence, R. A. Battista, and R. A. Shanely. 2014. 'High-intensity interval training induces a modest systemic inflammatory response in active, young men', *J Inflamm Res*, 7: 9-17.

Table 1: A summary of the physiological variables describing exercise intensity for each session. Peak interval data represent the average responses recorded during the interval components of the exercise trials, whereas mean data represent the average response across the entire exercise trial. All values are mean ± standard deviation.

Variable	LOW	MOD	HIGH
Mean VO_2 (% max)	50.4 ± 4.6	59.3 ± 3.1*	69.2 ± 2.1*
Peak Interval VO_2 (% max)	N/A	75.2 ± 3.7*	80.4 ± 2.9*
Mean HR (% max)	67.1 ± 6.0	77.3 ± 5.5*	83.1 ± 4.0*
Peak Interval HR (% max)	N/A	85.8 ± 5.3	89.3 ± 3.9
Mean RER	0.88 ± 0.04	0.93 ± 0.04$^{\sharp}$	0.96 ± 0.04$^{\sharp}$
Peak Interval RER	N/A	0.99 ± 0.1	0.99 ± 0.1
End test Blood Lactate (mM)	1.5 ± 0.6	3.0 ± 1.5*	5.8 ± 3.2*

*= Significantly different to the other two exercise sessions ($P<0.01$).

$^{\sharp}$= Significantly different to LOW ($P<0.01$).

Figure Legends

Figure 1. A schematic representation of the three exercise sessions. LOW was 35 min at 50% VO_{2max}, MOD was 5 x 5 minute intervals at 50% VO_{2max} interspersed 5 x 2 minute intervals at 80% VO_{2max} , HIGH was 5 x 4 minute intervals at 80% VO_{2max} interspersed with 3 minute intervals at 50% VO_{2max}.

Figure 2. Typical $\dot{V}O_2$ responses to the three exercise trials.

Table 1. A summary of the physiological variables describing exercise intensity for

each session. All values are mean ± standard deviation. *= Significantly different to the other two exercise sessions (P<0.01). ♯= Significantly different to LOW (P<0.01).

Figure 3. Plasma IL-6 (**A**), IL-10 (**B**) and sIL-6R (**C**) responses to LOW, MOD and HIGH exercise protocols. *=Significantly different to resting (P<0.01). ♯= Significantly greater in HIGH than LOW (P=0.04).