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Relationship of inflammatory response and mood to high-intensity interval exercise.

Short Running Title: Mood response to high intensity exercise

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Abstract

The current study compared the acute inflammatory response and the relationship to mood following two intensities of high-intensity interval exercise (HIIE). Eight physically active males (25±6 years; $\dot{V}O_2$max 49.02±5.53 ml·kg^{-1}·min^{-1}) undertook two 20 min HIIE trials (10 x 1 min intervals at 80% (HIIE80) and 90% (HIIE90) $\dot{V}O_2$max interspersed with 1 min active recovery). Plasma interleukin-6 (IL-6), leukocyte counts, and Brunel Mood Scale (BRUMS) mood ratings were collected before (pre), immediately after (post), 30 min (post30) and 60 min (post60) post-exercise with an additional measure of mood 24h post-exercise (post24h). Feelings of tension were significantly reduced post30 (P=0.003), post60 (P=0.001) and post24h (P=0.01) following HIIE80. Correlations between IL-6 and mood identified a significant negative relationship between IL-6 and fatigue 30 min after HIIE80 (r=-0.78, P=0.02). Inflammatory response did not significantly differ between exercise intensities, however, only HIIE90 was sufficient to elicit a significant transient increase in IL-6 (2.64 fold) which may provide an effective strategy to target inflammatory dysregulation. Future studies are required to establish the long-term implications of the anti-inflammatory properties of HIIE on mood.

Keywords: inflammation, interleukin-6, anti-inflammatory, tension, fatigue

Introduction

Exercise is a readily available and potent means of improving mood across both healthy and clinical populations[1] as well as in treatment-resistant depressive patients[2]. One neurobiological pathway which putatively links exercise to improvements in mood is inflammation[3]. Pro-inflammatory cytokines (PICs) have been suggested to directly contribute to the symptomatology of mood disorders including alterations in mood, sleep, energy, cognition and motivation through disrupting monoamine metabolism, hypothalamic-pituitary-adrenal (HPA) axis activation and neuroplasticity[4]. Accordingly, numerous studies have reported elevated levels of PICs such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), interleukin-1beta (IL-1β) and C-reactive protein (CRP) centrally and peripherally in patients with major depressive disorder (MDD)[5], generalised anxiety[6] and bipolar disorder[7]. Pre-clinical studies have also demonstrated a causal role for inflammation with the administration of lipopolysaccharide (LPS), a prototypical pathogen known to activate the innate immune system, resulting in increases in PICs and depressive-like behaviours in healthy individuals[8].

Exercise with its potential to alter the number and function of immune cells has a profound effect on the production of cytokines[9]. During skeletal muscle contractions, IL-6 is released in an exponential fashion[10]. In turn, the transient increase in circulating IL-6 upregulates the transcription of anti-inflammatory cytokines interleukin-10 (IL-10) and interleukin-1 receptor antagonist (IL-1ra) and inhibits the production of the pro-inflammatory cytokine TNF-α[11]. Therefore, despite IL-6 being classified as a pro- and anti- inflammatory pleiotropic cytokine[12], in the context of exercise it has been suggested that IL-6 exerts anti-inflammatory effects[13]. Indeed, this mechanism may explain why baseline levels of pro-inflammatory cytokines are lower in those individuals who are most physically active[14]. Importantly, since these cytokines have been shown to readily cross the blood-brain barrier, the exercise-induced reduction in peripheral inflammation may be extended to the central nervous system[15]. Thus, IL-6 may provide a possible biomarker for the mood enhancing response to exercise

and be used as a therapeutic target for the prevention and alleviation of mood disorders by ameliorating neuroinflammation.

High-intensity interval exercise (HIIE), characterised by repeated bouts of short duration high-intensity exercise interspersed with periods of rest or low-intensity exercise for recovery[16], has been identified as a time-efficient strategy to improve inflammatory outcomes[17, 18]. Notably, despite its reduced exercise volume, HIIE has been demonstrated to elicit a greater transient increase in IL-6 and anti-inflammatory IL-10 in comparison to moderate-intensity (60% $\dot{V}O_2$max) steady-state exercise[19, 20]. However, the efficacy of HIIE as an anti-inflammatory strategy appears inconsistent [21, 22, 23]. These equivocal outcomes may be attributed to the diversity of HIIE protocols applied, with particular discrepancies in exercise intensity[24]. As a result, deciphering the peak workload intensity required to elicit a transient increase in IL-6 and promote an anti-inflammatory effect is required before HIIE can be implemented as a possible strategy for the prevention and rehabilitation of mood disorders.

In particular, determining whether lower intensities of HIIE (80% $\dot{V}O_2$max) elicit a comparable inflammatory response to higher intensities (90% $\dot{V}O_2$max) would be beneficial, as this may provide a more suitable intervention strategy to improve mood within a wider population as opposed to only well-trained individuals. Therefore, the aims of the present study were firstly to compare the effect of two different intensities (80% and 90% $\dot{V}O_2$max) of HIIE on acute inflammatory response, and secondly to investigate if the inflammatory response was associated with changes in mood.

Materials and Methods

Participants

Eight healthy, physically active (defined as self-reported 4 ± 2 days.week^{-1}) male volunteers (mean ± SD; age 25 ± 6 years, body mass 79.96 ± 13.90 kg, stature 177.63 ± 8.72 cm and $\dot{V}O_2$max 49.02 ± 5.53 ml·kg^{-1}·min^{-1}) gave written informed consent to participate in all components of the study. Ethical approval was obtained from Kingston University Faculty Ethics Committee, and all procedures conformed to the Declaration of Helsinki (2013).

Experimental design

The present study applied a repeated measures cross-over design where participants were required to attend the laboratory on three separate occasions. Following a preliminary aerobic capacity test, the two experimental trials were (i) 10 x 1 min intervals at 80% $\dot{V}O_2$max interspersed with 1 min recovery intervals at 40% $\dot{V}O_2$max (HIIE80); and (ii) 10 x 1 min intervals at 90% $\dot{V}O_2$max interspersed with 1 min recovery intervals at 40% $\dot{V}O_2$max (HIIE90).

Preliminary assessments

Anthropometric measures of stature (cm) and mass (kg) were recorded on arrival to the laboratory and the Beck Depression Inventory (BDI)[25] was administered to screen for depressive symptoms. Aerobic capacity for maximal oxygen consumption ($\mathbf{\dot{V}}O_2$max) was determined by a continuous incremental exercise test on an electromagnetically braked cycle ergometer (Lode Excalibur, Groningen, Netherlands) as described ([23]). From this, individual power outputs (80% and 90% $\mathbf{\dot{V}}O_2$max) were calculated for subsequent HIIE testing. The average power outputs for the two

exercise trials were 189 ± 40 W [range 140 - 263 W] (HIIE80) and 216 ± 48 W [range 161 - 308 W] (HIIE90).

Experimental procedures

Before both exercise trials, participants completed the 24-item Brunel Mood Scale (BRUMS[26]) to determine their baseline mood profile (pre). A total mood disturbance (TMD) score was calculated by subtracting vigour from the sum of the five negative measures of mood and adding a constant of 100 to eliminate negative values[27]. In addition, baseline venous blood samples were collected as detailed below. Participants then undertook a 5 min warm up at 40% $\dot{V}O_2$max followed by one of two HIIE trials: (i) 10 x 1 min intervals at 80% $\dot{V}O_2$max interspersed with 1 min recovery intervals at 40% $\dot{V}O_2$max (HIIE80); or (ii) 10 x 1 min intervals at 90% $\dot{V}O_2$max interspersed with 1 min recovery intervals at 40% $\dot{V}O_2$max (HIIE90)[21]. Expired gases were measured continuously throughout each 20 min HIIE trial by breath-by-breath analysis using the Oxycon metabolic system (OxyconPro, Jaeger, Germany) to determine energy expenditure (EE). Immediately after the exercise trial a second blood sample and mood rating were collected (post) in addition to an 18-item physical activity enjoyment scale (PACES[28]) and repeated again at 30 min (post30) and 60 min post-exercise (post60). One further mood rating was completed 24 h after completion of the exercise (post24h).

Blood collection and analysis

Whole blood samples were collected via venepuncture in K_3EDTA vacutainer tubes (Bender Dickinson, Plymouth, Devon) before exercise (pre), immediately after (post), 30 min (post30) and 60 min (post60) after completion of the HIIE. The samples were centrifuged and plasma stored at -80°C for subsequent analysis. Systemic concentrations of IL-6 ($pg \cdot ml^{-1}$) were assessed using high-sensitivity ELISA commercial kits (R&D Systems, assay sensitivity: 0.11 $pg \cdot ml^{-1}$) and measured at 490nm with a background correction of 690nm using an Infinite® M200 Magellan pro microplate reader (Tecan, USA) with Magellan 7.2 software. The inter- and intra-assay coefficient of variation

(CV) was 7.8% and 7.4%, respectively. In addition, whole blood cell count analysis, specifically total white blood cell (WBC), lymphocyte, monocyte and neutrophil counts were assessed using a coulter analyser (Coulter Analyser, Beckman-Coulter, High Wycombe, UK). All of the variables measured were adjusted according to the formula proposed by Dill & Costill[29] to account for exercise-induced plasma volume changes using haemoglobin ($g \cdot dl^{-1}$) and haematocrit (%) levels.

Statistical analyses

Data are presented as mean ± standard deviation (SD) and normality was verified using the Shapiro-Wilk test. Two-way repeated measures ANOVA [intensity of HIIE (80% $\dot{V}O_2max$, 90% $\dot{V}O_2max$) x time point (pre, post, post30, post60)] were used to analyse the differences in inflammatory response and mood. Following a significant *F*-ratio, step down Bonferroni pairwise comparisons were performed. In addition, paired sample t-tests were used to analyse the differences in total workload, energy expenditure and enjoyment ratings between the two HIIE trials. Pearson's correlation analyses were used to investigate the relationship between the change in IL-6 concentration ($pg \cdot ml^{-1}$) and mood. All statistical analyses were performed using SPSS 24.0 software (SPSS Inc., Chicago, IL, USA).

Results

Total workload and energy expenditure

The average workload was significantly greater for the HIIE90 trial (216 ± 48 W) compared to the HIIE80 trial (189 ± 40 W) (P<0.001, 95% Confidence Interval (CI)=20.98, 34.02). This workload ranged from 140 to 263 W for the HIIE80 trial compared to 161 to 308 W for the HIIE90 trial. Similarly, the total energy expenditure was significantly greater by 25 kcal in HIIE90 (328.6 ± 65.8 kcal) compared to HIIE80 (352.7 ± 71.5 kcal) (P=0.002, 95% CI=-35.51, -12.57). This translated

to an energy expenditure of 16.4 ± 3.3 and 17.6 ± 3.6 kcal.min^{-1} for HIIE80 and HIIE90, respectively (P=0.002, 95% CI=-1.78, -0.63).

Inflammatory response

The effect of the two exercise intensities on IL-6 concentrations are displayed in Figure 1. IL-6 levels increased by 164.15% from pre (0.32 ± 0.12 pg·ml^{-1}) to post30 (0.84 ± 0.33 pg·ml^{-1}) in the HIIE90 trial (P=0.03, 95% CI=-0.10, -0.04). Despite IL-6 levels also increasing in response to HIIE80 by 35.10% post30 and 80.93% post60 relative to pre, this increase was not significant (F (3, 21)=1.38, P=0.28, η^2=0.16, observed power=0.31). No statistical differences in IL-6 responses were observed between HIIE80 and HIIE90 (F (1, 7)=3.28, P=1.1, η^2=0.32, observed power=0.35) and no significant changes were seen in absolute IL-6 delta (Δ) from pre to post30 between HIIE80 (2.12 pg.ml^{-1}) and HIIE90 2.54 pg.ml^{-1}; P=0.64, 95% CI=-2.46, -1.6).

Table 1 presents the change in leukocyte counts in response to the two exercise intensities. There was significant lymphocytosis at the end of both exercise trials (F (1, 8)=27.33, P=0.001, η^2=0.80, observed power=1.00), with lymphocyte counts increasing by 48.17% post HIIE80 and 55.32% post HIIE90, relative to pre. Significant increases in WBC's post (P=0.01, 95% CI=-4.21, -0.87) and neutrophils post (P=0.01, 95% CI=-1.5, -0.29) and post60 (P=0.02, 95% CI=-3.00, -0.24) were observed following HIIE90 only. Conversely, no significant differences in monocyte counts were present following either exercise trial (P=0.09). There were no statistical differences across leukocyte responses between HIIE80 and HIIE90 (main effect by trial; P>0.05).

Mood response

Participants at the initiation of the study had a BDI score of 7.75 ± 5.33 which is indicative of minimal depression. TMD did not significantly differ between trials (F (1, 7)=0.52, P=0.50, η^2=0.07, observed power=0.10) or time points (F (2, 14)=3.04, P=0.08, η^2=0.30, observed power=0.50) (Figure 2). However, further analysis of the six subscales individually revealed that relative to pre-exercise, feelings of tension were significantly reduced post 30 (P=0.003, 95% CI=1.10, 4.65), post 60

(*P*=0.001, 95% CI=1.68, 5.07) and post 24 h (*P*=0.01, 95% CI=0.78, 3.73) following HIIE80 (Table 2). Levels of enjoyment, as measured by PACES score, did not significantly differ between HIIE80 (87.63 ± 22.70) and HIIE90 (80.63 ± 12.33) (F (1, 7)=0.63, P=0.45, η^2=0.08, observed power=0.15). Total TMD delta (Δ) from pre to post24 for HIIE80 was -1.98 and for HIIE90 was 0.10 (*P*=0.35, 95% CI=-6.97, 2.82).

Relationship between changes in IL-6 and mood

As shown in Figure 3, there was no significant relationship between IL-6 levels and TMD in the HIIE80 trial at pre (*r*=-0.39, *P*=0.33), post (*r*=-0.05, *P*=0.91), post30 (*r*=-0.68, *P*=0.07) or post60 (*r*=-0.51, *P*=0.20). Similarly, there was no significant relationship between IL-6 levels and TMD in the HIIE90 trial at pre (*r*=-0.11, *P*=0.80), post (*r*=-0.17, *P*=0.70), post30 (*r*=0.07, *P*=0.87) or post60 (*r*=0.01, *P*=0.98) (Figure 3). Further analysis of the IL-6 – mood relationship for each of the six subscales revealed a large and significant negative relationship between IL-6 and fatigue at 30 min after HIIE80 (*r*=-0.78, *P*=0.02, Table 3).

Discussion

To date, this is the first study to compare the acute inflammatory response to two different intensities of HIIE (80% and 90% $\dot{V}O_2$max) while controlling for exercise duration. The main finding was that IL-6 and leukocyte response did not significantly differ between the two exercise intensities (*P*>0.05). However, only the HIIE90 trial was sufficient to significantly increase IL-6 concentration with a 164.15% (2.64 fold) increase observed 30 min post exercise relative to baseline (*P*<0.05, 95% CI=-0.10, -0.04). This inflammatory response following HIIE90 was further supported by a significant increase in WBCs, lymphocytes and neutrophils post exercise (*P*<0.01) (Table 1). Given that a transient increase in IL-6 and subsequent activation of such immune cells has been previously demonstrated to upregulate the transcription of anti-inflammatory cytokines IL-10[219, 21] and IL-1ra[30], it

can be suggested that HIIE performed at 90% $\dot{V}O_2$max may provide an effective strategy to ameliorate low-grade systemic inflammation.

It should be noted that the 0.52 pg·ml^{-1} (2.64 fold) increase in IL-6 was almost half of that detected by Wadley e*t al.*[21] (approx. 1.00 pg·ml^{-1}) despite utilising the same protocol. This is of importance since analogous increases in IL-6 (2.7 fold) have been demonstrated to be insufficient to promote beneficial anti-inflammatory effects with no increases in plasma concentration or gene expression of IL-10 observed[23]. However, this limited IL-10 response may be attributed to the omission of measurements during the short-term recovery from exercise. While previous research has suggested that IL-6 and IL-10 levels peak immediately post exercise[31], these studies have typically been conducted on prolonged exercise i.e. marathons. Concurring with the present study, when a similar protocol of short duration (20 min) of HIIE was applied, IL-6 levels peaked 30 min post exercise[21]. This delayed inflammatory response was further illustrated in the HIIE80 trial whereby IL-6 concentration continued to increase from 0.41 pg·ml^{-1} at post30 to 0.55 pg·ml^{-1} at post60 (Figure 1). Therefore, it can be speculated that an IL-10 response would have been observed by Cullen *et al.*[23] had measurements been obtained 30 min and 60 min post exercise. As such, a caveat of the present study was that IL-10 was not measured. Future research is warranted to investigate the magnitude of IL-6 response required to trigger a cascade of anti-inflammatory cytokines following HIIE.

Despite the significant inflammatory response present following HIIE90, this was not associated with changes in TMD (Figure 3). This appears to contradict the hypothesis that IL-6 may provide an underlying mechanism for the mood enhancing to response to exercise[32]. However, given that IL-6 and mood were measured simultaneously, the lack of relationship between the two may be indicative of a time delay for the cytokines to cross the blood-brain barrier and exert their effects on the central nervous system[16]. In support of this, longitudinal studies have demonstrated that chronic reductions in pro-inflammatory cytokines IL-1β and IL-6 were associated with parallel reductions in

depressive symptoms following a 12-week exercise intervention[32, 33]. While these studies provide support for the involvement of cytokines in the mood enhancing effect of exercise, both were conducted on a clinical population. This is of relevance given that such populations have been documented to have higher baseline levels of pro-inflammatory cytokines which have been positively associated with improvements in mood following exercise[32]. This may explain why IL-6 mediated improvements in TMD were not observed in the present study as all participants were asymptomatic of mood disorders (BDI score 7.75 ± 5.33). Taken together, it can be postulated that the anti-inflammatory properties of HIIE90 may have implications in the long-term mood enhancing effect of regular exercise, particularly within a clinical population in which baseline levels of IL-6 are elevated. This is therefore an avenue of interest for future investigation. In addition, the findings provide preliminary evidence for the use of a short 20 min bout of HIIE performed at 80% $\dot{V}O_2$max as a potent means of reducing tension 30 min up to 24h post-exercise in a healthy population. However, the short duration of exercise (20min) and measure of mood over short time points (pre to post30 and post60) was non-significant (P>0.05), and TMD ∆-change post-24 showed no significant difference (P>0.05). Therefore further investigation of the long-term effects of regular HIIE is required to determine the impact on TMD.

Perspectives

Given that HIIE promotes greater enjoyment in comparison to moderate-intensity continuous exercise [34]and that enjoyment has been widely associated with exercise adherence ([35]), the findings herein support the use of HIIE (80% $\dot{V}O_2$max) in exercise prescription to reduce tension, particularly in individuals with depressive symptoms in which non-compliance rates are salient[36]. In addition, the large and significant negative relationship between IL-6 and fatigue 30 mins after HIIE80 indicates that IL-6 may provide a biomarker and therapeutic target to alleviate symptoms of fatigue, which are commonly present in mood disorders. These findings accord with previous research highlighting IL-6

as a possible neurobiological mechanism for the beneficial effects of exercise on fatigue in the context of patients undergoing cancer treatment ([37])..

Despite these acute effects of HIIE80 on tension and fatigue, only HIIE performed at 90% $\dot{V}O_2$max was sufficient to elicit a significant transient increase IL-6 which may be utilised as an effective strategy to target inflammatory dysregulation. Future studies employing longitudinal designs are required to establish the long-term implications of the anti-inflammatory properties of HIIE90 on mood particularly in those with symptomatic mood disorders.

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Table 1: Leukocyte counts ($10^3 \cdot \mu l^{-1}$) in response to the two high-intensity interval exercise trials.

HIIE80	**Pre**	**Post**	**Post30**	**Post60**
WBC	6.81 ± 1.67	8.38 ± 2.77	6.16 ± 1.39	7.01 ± 1.89
Lymphocyte	2.18 ± 0.52	3.23 ± 1.61*	1.70 ± 0.31	1.42 ± 0.25*
Monocyte	0.67 ± 0.18	0.82 ± 0.22	0.61 ± 0.15	0.62 ± 0.20
Neutrophil	4.44 ± 1.98	4.72 ± 2.45	3.98 ± 1.31	5.06 ± 1.68
HIIE90	**Pre**	**Post**	**Post30**	**Post60**
WBC	6.27 ± 1.48	8.81 ± 2.50*	6.12 ± 1.69	7.21 ± 1.84
Lymphocyte	2.35 ± 0.54	3.65 ± 1.08*	1.74 ± 0.52*	1.54 ± 0.41*
Monocyte	0.67 ± 0.24	0.90 ± 0.36	0.61 ± 0.21	0.64 ± 0.21
Neutrophil	3.05 ± 1.09	3.93 ± 1.47*	3.58 ± 1.25	4.65 ± 1.52*

Note: WBC= white blood cell, * denotes significant differences relative to pre-exercise ($P < 0.05$).

Table 2: Mood state changes across the six subscales in response to the two high-intensity interval trials.

HIIE80	**Pre**	**Post**	**Post30**	**Post60**	**Post24h**
Anger	3.13 ± 3.80	3.13 ± 4.32	1.38 ± 1.69	1.50 ± 1.77	2.13 ± 3.36
Confusion	5.00 ± 3.42	4.74 ± 5.01	3.13 ± 3.27	1.38 ± 1.41	1.63 ± 2.00
Depression	3.25 ± 3.24	2.00 ± 2.56	2.00 ± 1.51	1.75 ± 1.58	0.88 ± 1.46
Fatigue	4.00 ± 2.20	9.25 ± 2.92	6.75 ± 3.06	7.00 ± 4.00	3.00 ± 2.73**
Tension	3.63 ± 1.41	3.00 ± 2.39	0.75 ± 0.71*	0.25 ± 0.46*	1.38 ± 0.74*
Vigour	11.00 ± 2.83	9.29 ± 3.82	11.29 ± 2.43	10.86 ± 3.72	11.14 ± 3.02
HIIE90	**Pre**	**Post**	**Post30**	**Post60**	**Post24h**
Anger	2.50 ± 3.46	2.75 ± 3.54	1.38 ± 3.50	1.38 ± 2.50	1.38 ± 1.50
Confusion	2.75 ± 3.45	2.38 ± 3.20	1.63 ± 1.69	1.00 ± 1.41	2.75 ± 2.87
Depression	2.63 ± 2.88	2.50 ± 2.51	1.38 ± 1.60	1.00 ± 1.20	1.00 ± 1.20
Fatigue	4.00 ± 3.12	8.38 ± 4.00	6.50 ± 3.82	5.38 ± 3.25	4.63 ± 4.50
Tension	2.37 ± 2.07	1.88 ± 3.09	1.00 ± 1.41	0.75 ± 1.04	1.88 ± 1.73
Vigour	8.14 ± 2.34	10.86 ± 2.97	10.86 ± 2.61	11.29 ± 3.45	9.71 ± 3.40

Note: * denotes significant differences relative to pre-exercise ($P < 0.01$). ** denotes significant differences relative to post-exercise ($P < 0.01$).

Table 3: Relationship between change in plasma IL-6 concentration and mood across the six subscales.

HIIE80	**Pre**		**Post**		**Post30**		**Post60**	
	r=	*P*=	*r*=	*P*=	*r*=	*P*=	*r*=	*P*=
Anger – IL6	0.02	0.963	-0.19	0.662	-0.42	0.295	0.29	0.478
Confusion – IL6	-0.43	0.294	-0.11	0.802	-0.48	0.226	-0.24	0.574
Depression – IL6	-0.18	0.677	-0.11	0.804	-0.62	0.101	-0.39	0.343
Fatigue – IL6	-0.19	0.658	0.16	0.552	-0.73*	0.024*	-0.70	0.056
Tension – IL6	0.16	0.704	-0.23	0.577	-0.09	0.831	0.33	0.423
Vigour – IL6	0.48	0.232	-0.21	0.623	0.18	0.665	0.56	0.150
HIIE90	**Pre**		**Post**		**Post30**		**Post60**	
	r=	*P*=	*r*=	*P*=	*r*=	*P*=	*r*=	*P*=
Anger – IL6	-0.19	0.651	-0.30	0.471	-0.31	0.454	-0.54	0.172
Confusion – IL6	0.06	0.894	-0.39	0.342	-0.26	0.537	-0.20	0.631
Depression – IL6	-0.21	0.611	-0.05	0.903	0.53	0.177	0.24	0.568
Fatigue – IL6	0.43	0.286	-0.33	0.427	0.28	0.495	0.52	0.190
Tension – IL6	-0.19	0.651	-0.30	0.471	-0.31	0.454	-0.54	0.172
Vigour – IL6	-0.13	0.232	-0.16	0.750	0.23	0.665	0.10	0.150

Note: All values are Pearson's correlation (*r*). * denotes significant relationship ($P < 0.05$).

Figure Legends

Figure 1: Plasma IL-6 concentration changes in response to the two high-intensity interval exercise trials. * denotes significant differences relative to pre-exercise ($P < 0.05$).

Figure 2: Total mood disturbance (TMD) changes in response to the two high-intensity interval exercise trials. A constant of 100 was added to all scores to eliminate negative values.

Figure 3: Relationship between plasma IL-6 concentration and total mood disturbance (a) before, (b) immediately after, (c) 30min and (d) 60 min after the two high-intensity interval exercise trials.