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Comparable neutrophil responses for arm and intensity-matched leg exercise

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Short title

Arm vs leg exercise: Neutrophil responses

ABSTRACT

Introduction: Arm exercise is performed at lower absolute intensities than lower body exercise. This may impact on intensity dependent neutrophil responses and it is unknown whether individuals restricted to arm exercise experience the same changes in the neutrophil response as found for lower body exercise. Therefore, we aimed to investigate the importance of exercise modality and relative exercise intensity on the neutrophil response.

Methods: Twelve moderately trained males performed three 45-min constant load exercise trials following determination of peak oxygen uptake for arm exercise ($\dot{V}O_{2\text{peak arms}}$) and cycling ($\dot{V}O_{2\text{peak legs}}$): (1) arm cranking exercise at 60% $\dot{V}O_{2\text{peak arms}}$; (2) moderate cycling at 60% $\dot{V}O_{2\text{peak legs}}$; and (3) easy cycling at 60% $\dot{V}O_{2\text{peak arms}}$.

Results: Neutrophil numbers in the circulation increased for all exercise trials, but were significantly lower for easy cycling when compared with arm exercise ($P=0.009$), mirroring the blunted increase in heart rate and epinephrine during easy cycling. For all trials, exercising heart rate explained some of the variation of the neutrophil number 2h post exercise ($R=0.51-0.69$), epinephrine explaining less of this variation ($R=0.21-0.34$). The number of neutrophils expressing CXCR2 decreased in the recovery from exercise in all trials ($P<0.05$).

Conclusion: Arm and leg exercise elicits the same neutrophil response when performed at the same relative intensity, implying that populations restricted to arm exercise might achieve a similar exercise induced neutrophil response as those performing lower body exercise. A likely explanation for this is the higher sympathetic activation and cardiac output for arm and relative intensity matched leg exercise when compared with easy cycling, which is partly reflected in heart rate. This study further shows that the downregulation of CXCR2 may be implicated in exercise-induced neutrophilia.

Key words: upper body exercise, first line of defence, elastase, CXCR2, PSGL-1, IL-8, neutrophil degranulation

1 INTRODUCTION

Engaging in a moderate amount/intensity of exercise is associated with a lower infection risk when compared with doing no exercise or very high amounts/intensities of exercise (19). This relationship and acute exercise immune responses have been investigated extensively for lower body exercise modalities. However, a limited amount of studies have focussed on populations restricted to upper body exercise (11, 13), which includes the 1.2 million wheelchair users in the UK (18) and the 7.4 million people in the US dependent on wheelchairs or assistive devices to support their mobility (3). Furthermore, direct comparisons of upper vs lower body exercise with respect to immune function are scarce and have so far been limited to cytokine and monocyte responses (10, 14). From a theoretical perspective, this modality comparison is intriguing as arm exercise is usually performed at lower absolute intensities than lower body exercise, which may impact on intensity dependent acute immune responses. Practically, this comparison is relevant for those restricted to the upper body exercise modality, for example for those with a disability or undergoing rehabilitation.

The body of research on lower body exercise modalities such as running or cycling is in support of acute moderate exercise as a means to alter blood borne markers of immunity – this includes cytokine secretion and the leukocyte activation state associated with improved host defence (14, 22, 24). Further, neutrophil numbers increase following exercise with a peak at around 2-4 hours post exercise (19, 28, 31). Neutrophils are part of the first line of defence against invading threats of an infectious nature (25); an increased circulating number following exercise may hence represent a mechanism by which non-specific immunity mechanisms are enhanced (15). This theory is supported by the finding of an increased infection risk in the presence of a reduced number of neutrophils in the

circulation, as found for example as a result of auto-immune diseases or drugs affecting myeloid precursor cells (17).

As with the relationship between exercise and infection risk, exercise intensity appears to be an important mediator of changes in neutrophil numbers and activation by directly affecting modulators such as plasma epinephrine, cortisol, or cytokines (15, 17, 21, 25). This may further impact on the expression of neutrophil surface receptors, such as the interleukin-8 (IL-8) receptor (CXCR2 - involved in neutrophil chemotaxis) or P-selectin glycoprotein ligand-1 (PSGL-1 - promoting neutrophil tethering to endothelial cells) (8) which may also play a crucial role in the appearance of neutrophils in the circulation and their migration and cytotoxic capacity. Given the central role of exercise intensity in mounting neutrophil responses we propose to elucidate whether upper body exercise has a similar capacity to induce these responses as lower body exercise. Hence, the aim of this study was to compare the neutrophil response of arm exercise and cycling, which were matched for (1) relative and (2) absolute intensities.

2 MATERIALS AND METHODS

Blood samples were obtained as part of a study published previously (14). As a consequence, the exercise intervention, participant and exercise characteristics are identical between those studies. The study was approved by the University's Ethics committee.

2.1 Participants and experimental design

Twelve recreationally trained male individuals volunteered to participate (age: 25 ± 4 years, body mass: 76 ± 9 kg, sporting activity 4.0 ± 1.2 h·week⁻¹, $\dot{V}O_{2peak arms}$: 32.1 ± 6.0 mL·kg⁻¹·min⁻¹).

$\text{L} \cdot \text{min}^{-1}$, $\dot{V}\text{O}_{2\text{peak legs}}$: $46.2 \pm 6.8 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). They visited the laboratory on five occasions for two preliminary and three main trials. Initially, body mass and height were determined using scales (model 770, seca, Birmingham, UK) and a Leicester height measure (seca, Birmingham, UK). In the two preliminary trials (visits 1 and 2), peak oxygen consumption ($\dot{V}\text{O}_{2\text{peak}}$) was determined during arm exercise (determining $\dot{V}\text{O}_{2\text{peak arms}}$) using an arm crank ergometer (Angio, Lode, Groningen, Holland) or during cycling exercise (determining $\dot{V}\text{O}_{2\text{peak legs}}$) using a cycle ergometer (Excalibur, Lode Groningen, Holland) in a randomised order. For this, participants performed a graded exercise test to exhaustion, with an initial power output of 35 W (arms) and 70 W (legs), respectively; power output was then increased every three minutes by 15 W (arms) or 30 W (legs) until exhaustion. Arm exercise was performed in a sitting position, the centre of the crank at shoulder level with arms slightly flexed at maximum reach, cycling with legs slightly flexed at maximum reach. Settings were noted and replicated for all main trials.

Main trials were performed in a randomised order after a 24 h food standardisation period without caffeine and with no exercise allowed 24 h before the experiments. To account for diurnal variations exercise tests were performed in the morning (start: 07:45-09:15) for all participants and at the same time of day for each individual participant. The three main trials consisted of 45 min of steady state exercise as follows: (1) arm exercise at $60\% \dot{V}\text{O}_{2\text{peak arms}}$; (2) moderate cycling at $60\% \dot{V}\text{O}_{2\text{peak legs}}$; and (3) easy cycling at $60\% \dot{V}\text{O}_{2\text{peak arms}}$. Exercise intensities and duration were chosen in accordance with exercise prescription guidelines for moderately trained individuals (1). A five minute warm-up was performed at 50% of the start load before each condition. Oxygen uptake was determined in five minute intervals and power output was adjusted if necessary. For all experiments, oxygen uptake was determined using Douglas bags and a gas analyser (Servomex 1440, Servomex Ltd, Crowborough, UK),

and heart rate was continuously monitored using a Polar RS400 (POLAR, Kempele, Finland) monitor. Heart rate data collected in the first 10 minutes of the main trial were excluded from analysis to avoid inclusion of the reduced values at the beginning of exercise. Participants further indicated their rating of perceived exertion (RPE) on a scale ranging from 6 to 20 (2). Water during exercise was given *ad libitum*, with the help of a straw to allow hand free water intake, particularly important for the arm cranking modality. Water intake in the post-exercise period was recorded and replicated for the remaining main trials; food and other drinks than water were not allowed during the main trials. Ten participants were invited to the laboratory for a 4th main trial, which consisted of a 45 min rest period instead of the exercise intervention to carry out flow cytometric analyses at rest.

2.2 Blood collection

Participants were lying in a supine position for venous blood sample collection. Blood was collected into K₃EDTA (for hematology and plasma marker analysis) and heparin (for neutrophil degranulation and flow cytometric analysis) containers from a superficial arm vein by venepuncture. Collection times were before, immediately after, and at 2 h and 4 h after exercise, timings being the same for those participants performing the rest trial. Apart from the collection immediately after exercise, participants rested on a bed for 10 minutes before the blood sample was taken.

2.3 Hematology

Leukocyte subtype numbers, hemoglobin and the hematocrit in whole blood were determined immediately after collection using an automated hematology analyser (Coulter Ac-T 5diff OV; Beckman Coulter, High Wycombe, UK). Blood volume changes were

estimated from hemoglobin values (7), and leukocyte subset numbers corrected for changes in blood volume.

2.4 Plasma markers

Following centrifugation (10 min at 350 g and 4°C) plasma was stored at -20 °C until analysis. Interleukin-8 (R&D systems, Minneapolis, US), epinephrine (IBL International GmBH, Hamburg, Germany) and cortisol (DRG Instruments GmbH, Marburg, Germany) were determined by enzyme-linked immunosorbent assay (ELISA). The within assay coefficient of variation for this analysis performed was $5.6 \pm 4.2\%$ for IL-8, $4.1 \pm 3.9\%$ for epinephrine and $2.7 \pm 2.7\%$ for cortisol. As the focus of this study was on plasma marker concentration affecting leukocytes and other effectors rather than determining the fold change of plasma marker production, plasma concentration was not corrected for plasma volume changes.

2.5 Flow cytometry

The following fluorochromes were used in this study: FITC-conjugated CD182 (also known as CXCR2, the IL-8 receptor), FITC-conjugated IgG1, κ isotype control, PE-conjugated CD16, APC-conjugated IgG2a, κ isotype control (BD, Oxford, UK), and APC-conjugated CD162 (also known as PSGL-1) (eBioscience, Hatfield, UK). Within 2 h of sample collection whole blood (120 μ L) was incubated with the above fluorochromes in duplicate: (1) CD182, CD16, CD162 (2) FITC isotype control, CD16, APC isotype control. Labelling was carried out on ice for 20 min, followed by lysis with FACS lysis buffer (BD, Oxford, UK) and incubation in the dark for another 10 min. Samples were then centrifuged for 6 min at 440 g, the supernatant was removed and the cell pellet re-suspended with 1.5 mL ice-cold

PBS. The centrifugation and supernatant removal steps were repeated, and the cell pellet was re-suspended in 400 μ L ice-cold PBS for immediate analysis with the flow cytometer (FACSCalibur equipped with the CellQuest software package, BD Biosciences, Oxford, UK), collecting 100,000 events per sample. All analyses were performed for neutrophils, defined as the CD16⁺ cells. Histogram plots of CD16⁺ cells incubated with the respective isotype control antibodies alone were used to define the threshold of positive staining for CD182 and CD162 respectively. This threshold was then used to determine the percentage of CD16⁺ cells expressing CD182 and CD162, respectively, and the geometric mean of fluorescence intensity (GMFI) was determined for CD182 and CD162, respectively.

2.6 Neutrophil degranulation

Under sterile conditions, 1 mL of whole blood was incubated with 50 μ L of bacterial stimulant (84015; Sigma-Aldrich, Dorset, UK) or a control medium (50 μ L RPMI 1640, Sigma-Aldrich, Dorset, UK) for 60 min at 37°C; samples were gently inverted before incubation and at 30 min. Following incubation, samples were centrifuged and the plasma stored at -20°C. Plasma elastase concentration was then determined in duplicate by ELISA (2BScientific, Upper Heyford, UK) for the determination of neutrophil degranulation activity, the within assay co-efficient of variation being $2.2 \pm 1.9\%$. The final elastase concentration was corrected for changes in plasma volumes. Net elastase release was calculated by subtracting the stimulated from the unstimulated value, and the elastase release per neutrophil was calculated by dividing this number by the neutrophil number.

2.7 Statistical analyses

The SPSS 23.0 statistical package (SPSS Inc., Chicago IL, USA) was used for all statistical analyses. Means and standard deviations were computed for all variables, and normality was checked with the Shapiro Wilk test. Non-normal data were converted using square root (for CXCR2 GMFI) or logarithmic (for neutrophil number) transformations to achieve normality. A repeated measures two-way (exercise trial, time) analysis of variance (ANOVA) was conducted on normally distributed blood derived variables. Data showing significant interaction effects were further analysed with repeated measures ANOVAs, focussing on time points standing out following visual inspection of plotted data. Huynh-Feldt corrections were applied when sphericity was violated and Sidak adjustments applied for post-hoc comparisons. Non-normal data that were impossible to convert to achieve normality were analysed using repeated, Bonferroni corrected Wilcoxon signed rank tests. Physiological exercise descriptors were analysed using a one-way (exercise trial) repeated measures ANOVA or the non-parametric equivalents for non-normal and RPE data. For main trials, Pearson's correlation coefficients (R) and 2-tailed significances were computed for the bivariate relationships between neutrophil number and the independent variables heart rate, epinephrine and IL-8. Statistical significance was accepted at $P < 0.05$.

3 RESULTS

3.1 Exercise responses

The exercise trials resulted in distinctively different physiological and psychophysiological responses, the lowest power output, heart rate and RPE values found for easy cycling. Arm exercise and easy cycling did not differ with regards to absolute oxygen uptake; arm

exercise and moderate cycling did not differ with regards to relative oxygen uptake (**Table I**).

3.2 Neutrophil and plasma markers

Neutrophil numbers were increased following all exercise trials but also following the rest trial (main effect of time: $P < 0.001$), with a time x trial interaction indicating a blunted response for the rest and easy cycling trial ($P < 0.001$). Arm exercise induced a larger neutrophil number increase in the recovery period when compared with easy cycling ($P = 0.009$), but not when compared with moderate cycling ($P = 0.90$). Neutrophil degranulation, expressed as elastase secretion per neutrophil cell, was significantly reduced at 2 h post exercise (effect of time: $P < 0.02$, effect of condition: $P = 0.53$, condition x time interaction: $P = 0.14$; **Figure 1**).

The plasma epinephrine concentration increased from pre to post exercise for all exercise trials ($P < 0.001$), but the post-exercise epinephrine concentration was higher for arm exercise than for easy cycling ($P = 0.02$). The plasma cortisol concentration was lower in the recovery period for all exercise trials ($P < 0.05$), with no difference between trials. The IL-8 plasma concentration increased from pre to post ($P = 0.01$), but no difference between exercise trials was found for IL-8 ($P = 0.80$; **Figure 2**).

Even though some of the analysed regressions explained up to 52% (R^2) of the random variation between the dependent variable neutrophil number and the independent variables heart rate, epinephrine and IL-8, these relationships were largely found to be insignificant (**Table II**). The dependent variable consistently associated with positive relationships was the neutrophil number 2 h post exercise.

227

228 The CXCR2 and CD162 GMFI showed circadian rhythms, with the lowest values found 2 h
229 and 4 h after the exercise trials but also the rest trial ($P < 0.001$). No differences were found
230 between the three exercise trials and the rest trial ($P > 0.55$). The percentage of neutrophils
231 expressing CXCR2 2 h post exercise was reduced for all exercise trials when compared with
232 the rest trial ($P < 0.05$), but the exercise trials did not differ between each other ($P = 0.17$).
233 The percentage of neutrophils expressing CD162 did not change over time for any trial ($P >$
234 0.05 ; **Figure 3**).

235 **4 DISCUSSION**

236 This is the first study to compare the neutrophil response in intensity matched trials between
237 arm and cycling exercise. It provides further evidence with regards to the exercise modality
238 specific potential of mounting an acute neutrophil response, which is of practical importance
239 for those restricted to upper body exercise. The main finding of this study was that arm
240 exercise and cycling at the same relative exercise intensity induces a comparable acute
241 neutrophil recruitment into the circulation. On the other hand, cycling at the same absolute
242 oxygen uptake as arm exercise results in a blunted response for cycling. Lower responses
243 for easy cycling were further observed for the plasma epinephrine concentration, heart rate,
244 and RPE. Arm exercise also resulted in the same decline of neutrophil degranulation in the
245 recovery period as leg exercise. It therefore appears that the arm cranking modality has the
246 capacity to induce the same acute neutrophil response as the cycling modality.

247

248 A second finding was the presence of circadian rhythms for neutrophil markers: a decline in
249 the GMFI of the neutrophil surface markers CXCR2 and PSGL-1 was observed over the 5 h
250 study period for all exercise and rest conditions.

4.1 *Neutrophil responses*

4.1.1 *Neutrophilia*

The lowest epinephrine concentrations post exercise were found for easy cycling, the trial where the neutrophil response was blunted, corroborating earlier data pointing out the importance of relative exercise intensity in this response (15, 26). It has been suggested levels of epinephrine may impact on the neutrophil response through direct mechanisms, such as epinephrine dependent recruitment of leukocyte subgroups into the circulation (22, 30). Patterns of leukocyte mobilisation similar to the current study have been found when modulating core temperature or exercise intensity, with the modes inducing the most pronounced epinephrine response resulting in the largest increase in neutrophil numbers (27) or cytokine secretion (21, 23). However, neutrophil numbers have been shown not to be affected by epinephrine infusion mimicking the epinephrine increase during exercise (31). Further, it has been shown that epinephrine increases during non-exercise based interventions (hot water immersion) alone are not sufficient to induce neutrophilia as observed during exercise, despite comparable epinephrine levels (12). The results of the present study corroborate these findings: the insignificant correlations between epinephrine concentration post exercise and neutrophil numbers both post ($R \leq 0.13$) and 2 h post exercise ($R \leq 0.28$) imply that epinephrine contributes, if at all, only marginally to exercise induced neutrophilia. A direct causal relationship between the observed larger increases in epinephrine and neutrophil numbers in the arm exercise trial compared to the absolute intensity matched cycling trial is therefore unlikely, and other factors are likely to contribute to this.

Such factors may include neutrophil surface receptors. Given their roles in IL-8 mediated chemotaxis (CXCR2) and tethering to endothelial cells (PSGL-1) it could be hypothesised that these receptors play a role in exercise induced neutrophilia. Indeed, the present results do provide support for a mechanistic role of CXCR2, but not PSGL-1, in this process. Even though exercise is unable to alter the natural decrease of the GMFI over time, a reduction in CXCR2 expression in the exercise trials can be observed 2 h post exercise, which is different from the resting condition. In the context of neutrophilia it could be argued that a reduced expression of CXCR2 allows more neutrophils to remain in the circulation as their IL-8 dependent migration capacity into the extravascular compartment is reduced. This is in line with the observation that intravenous administration of IL-8 inhibits neutrophil accumulation to intradermal sites of inflammation, possibly due to the disruption of concentration gradients (16). The correlations between IL-8 and neutrophil number found in the present study further support this concept: even though not significant for all trials, the positive relationship between IL-8 concentration and neutrophil numbers 2 h post exercise imply that central elevations of IL-8 may help inducing neutrophilia.

Further to this, it has been postulated that the shedding of receptors may serve as an adaptive mechanism for inhibiting excessive inflammatory reactions (21) which may arise given the increased numbers of neutrophils in the circulation. Similarly to CXCR2, other surface markers associated with neutrophil adhesion, such as CD11b (21) or L-selectin (32), have been shown to be downregulated in response to moderate and high intensity exercise. It is important to know, though, that these findings are based on research designs that did not feature a resting control trial - given the findings of the present study a potentially crucial omission given the circadian effects shown for CXCR2 and PSGL-1.

No changes in cortisol were found between trials. Previous evidence suggests that exercise intensities above ~60% $\text{VO}_{2\text{max}}$ can increase cortisol plasma concentrations, whereas intensities below ~50% $\text{VO}_{2\text{max}}$ can result in a decrease, as elimination rates during low intensity exercise are higher than at rest (15). It is therefore possible that the exercise intensities chosen in the current study were not high enough to trigger a cortisol response. Changes in cortisol concentration are hence unlikely to be the main mechanism to govern the neutrophilia observed given the fact that the more strenuous activities resulted in pronounced differences in neutrophilia but no difference in the cortisol response. However, in line with observations in lymphocytes (4, 15) the general decline in cortisol over time coincided with the general increase in neutrophil numbers; importantly, a small degree of neutrophilia was also observed in the rest condition. The reduction in cortisol with its general anti-inflammatory properties may therefore aid the development of neutrophilia over the course of the day, strengthening the first line of defence when the likelihood of infection is increased due to an increased exposure to pathogens.

Further to the discussed plasma and neutrophil markers that are potentially involved in neutrophilia (epinephrine, cortisol, IL-8, or neutrophil receptors), the demargination of neutrophils via the increase in cardiac output (12), increased blood flow through viscera and muscles and a “general” sympathetic activation (15) have been suggested to induce a rise in neutrophil number in the circulation. Cardiac output was not directly assessed in the present study; however, heart rate was significantly higher for arm exercise when compared with the easy cycling trial, and correlations between heart rate and neutrophil number were positive. In fact, heart rate was the only variable with $R > 0.50$ for all trials when related to neutrophil number 2 h post exercise. Whilst we appreciate that heart rate is only an approximation of cardiac output, these data support the notion of cardiac output as an important factor to

induce neutrophilia (12). It is also worth noting that the increase in neutrophil numbers immediately following exercise did not correlate well with any of the presently analysed predictor variables, implying that the effects of adrenaline, IL-8 and heart rate take time (two hours) to manifest in elevated neutrophil numbers. This is in contrast to earlier suggestions that attributed the initial neutrophilia following exercise to factors such as cardiac output and epinephrine, whilst the mechanism underlying delayed neutrophilia following exercise has been suggested to involve glucocorticoids (15).

On a final note, differences in sympathetic activation were also reflected in the RPE responses, lowest for the easy cycling modality. Intensity differences between exercise trials may further explain differences in neutrophilia through their impact on a broader range of markers than catecholamines and glucocorticoids, such as a host of interleukins (14, 23, 29) which ultimately affect neutrophil behaviour (8).

4.1.2 Neutrophil degranulation

In agreement with the present study, similar reductions in bacteria-stimulated neutrophil degranulation, a marker of the reactivity of an individual cell against pathogens, have been reported following exercise (5, 6, 12, 22, 28). This decrease in neutrophil function following aerobic exercise has also been observed when investigating migratory capacity (9, 33). It has been found earlier that exercise and rises in cortisol can induce the release of immature neutrophils from the bone marrow into the circulation (15, 22). As they contain less granular digestive enzymes this may be a mechanism by which exercise reduces average phagocytic activity of the neutrophil population as a whole (22). The present study shows that increases in cortisol are not a requirement to reduce in bacteria-stimulated neutrophil degranulation. In addition, in contrast to previous studies (22) the suppression of neutrophil degranulation

observed in the present study appears to be mode and intensity independent – however, this is likely the result of insufficient statistical power to analyse this detail, as a trend for a reduced depression was seen for easy cycling.

4.2 Future directions

The current study shows rises in neutrophil numbers that are dependent on relative exercise intensity. For future research, it would be of interest to investigate neutrophil subpopulations to assess the origin of the neutrophils released into the circulation following exercise. For this, their maturity could be assessed by morphology (e.g., band vs segmented neutrophils). Another interesting field of research is the investigation of markers involved in neutrophil mobilisation. Whilst we have measured the plasma concentration of the chemokine IL-8, granulocyte colony stimulating factor (G-CSF) has been shown to increase in response to exercise that induces neutrophilia (12) and may explain some of the exercise intensity dependent differences found in the present study. Finally, we have focussed on elastase as a marker for neutrophil degranulation, and it may be of interest to investigate other makers of neutrophil degranulation such as myeloperoxidase (20).

5 CONCLUSION

Arm and leg exercise elicits the same neutrophil response when performed at the same relative intensity, cycling at the same absolute intensity as arm exercise results in a blunted increase in neutrophil numbers, heart rate and epinephrine. Populations restricted to arm exercise therefore have a similar capacity to induce a neutrophil response through exercise, which may play a role in host protection following exercise. Heart rate as an integrated measure of exercise stress / cardiac output was the best predictor of neutrophil numbers 2 h

post for all exercise trials. This study also shows that CXCR2 may be implicated in exercise-induced neutrophilia. Further, the results demonstrate that circadian effects must be considered for neutrophil surface markers enforcing the need for resting control conditions in studies investigating the first line of defence in the circulation.

6 CONFLICT OF INTEREST

The authors declare no conflict of interest.

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8 FIGURE CAPTIONS

FIGURE 1 – Neutrophil number and neutrophil elastase secretion in response to steady state exercise (mean $\pm$ SD). Significant differences ($P < 0.05$): a, different from pre (all trials); b, different from post (all trials); c, different from all time points (all trials); d, difference between arm and leg easy trial.

FIGURE 2 – Plasma epinephrine, cortisol and interleukin-8 concentrations in response to steady state exercise (mean $\pm$ SD). Significant differences ($P < 0.05$): a, different from pre (all trials); b, different from post (all trials); c, difference between arm and leg easy trial.

FIGURE 3 – Neutrophil surface markers in response to steady state exercise (mean $\pm$ SD). GMFI, geometric mean of fluorescence intensity. Significant differences ($P < 0.05$): a, different from pre (all trials); b, different from post (all trials); c, all exercise trials different from rest.

9 TABLE CAPTIONS

Table I – Physiological and psychophysiological exercise descriptors.

Table II – Bivariate correlations of epinephrine, IL-8, heart rate and neutrophil number.

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TABLE I. Physiological and psychophysiological exercise descriptors.

Parameter	Exercise mode		
	Arm exercise	Moderate cycling	Easy cycling
Power output [W]	73 ± 14*	148 ± 30*	101 ± 25*
Heart rate [b·min ⁻¹]	141 ± 9*	150 ± 12*	123 ± 11*
Average RPE	13.3 (12.6, 13.9)	13.2 (12.7, 14.0)	10.6 (10.0, 11.0)*
$\dot{V}O_2$ [L·min ⁻¹]	1.50 ± 0.28	2.16 ± 0.34*	1.50 ± 0.28
% $\dot{V}O_{2peak}$ arms	62.3 ± 1.4	-	62.3 ± 1.1
% $\dot{V}O_{2peak}$ cycling	-	62.3 ± 1.0	43.2 ± 5.4*

RPE, rating of perceived exertion. Data indicate mean ± SD or median (lower quartile, upper

quartile). *Significant difference to both other modalities (P<0.05)

Table II – Bivariate correlations of epinephrine, interleukin-8, heart rate and neutrophil number.

Comparison	Exercise mode		
	Arm exercise	Moderate cycling	Easy cycling
Epinephrine post vs neutrophil number post	R = -0.16 P = 0.63	R = 0.09 P = 0.78	R = 0.13 P = 0.68
Epinephrine post vs neutrophil number 2h post	R = 0.21 P = 0.52	R = 0.24 P = 0.45	R = 0.34 P = 0.28
IL-8 post vs neutrophil number post	R = -0.15 P = 0.65	R = -0.44 P = 0.16	R = 0.08 P = 0.80
IL-8 post vs neutrophil number 2h post	R = 0.72 P = 0.008	R = 0.14 P = 0.67	R = 0.46 P = 0.13
Heart rate vs neutrophil number post	R = 0.19 P = 0.56	R = 0.007 P = 0.98	R = 0.10 P = 0.75
Heart rate vs neutrophil number 2h post	R = 0.51 P = 0.09	R = 0.69 P = 0.01	R = 0.52 P = 0.09

The plasma concentrations of epinephrine and interleukin-8 and the average heart rate during trials were used to compute correlations.

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489 **Figure 1**

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494 **Figure 3**

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