Arm and intensity-matched leg exercise induce similar inflammatory responses

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Short title

Inflammatory response for arm & leg exercise

ABSTRACT

Introduction: The amount of active muscle mass can influence the acute inflammatory response to exercise, associated with reduced risk for chronic disease. This may affect those restricted to upper body exercise, for example due to injury or disability. The purpose of this study was to compare the inflammatory responses for arm exercise and intensity-matched leg exercise.

Methods: Twelve male individuals performed three 45-min constant load exercise trials following determination of peak oxygen uptake for arm exercise ($\dot{V}O_{2peak\ A}$) and cycling ($\dot{V}O_{2peak\ C}$): (1) arm cranking exercise at 60%$\dot{V}O_{2peak\ A}$; (2) moderate cycling at 60%$\dot{V}O_{2peak\ C}$; and (3) easy cycling at 60%$\dot{V}O_{2peak\ A}$. Cytokine, adrenaline and flow cytometric analysis of monocyte subsets were performed before and up to 4h post exercise.

Results: Plasma IL-6 increased from resting concentrations in all trials, however, post exercise concentrations were higher for arm exercise (1.73±1.04pg·mL^{-1}) and moderate cycling (1.73±0.95pg·mL^{-1}) compared with easy cycling (0.87±0.41pg·mL^{-1},$P<0.04$). Similarly, the plasma IL-1ra concentration in the recovery period was higher for arm exercise (325±139pg·mL^{-1}) and moderate cycling (316±128pg·mL^{-1}) when compared with easy cycling (245±77pg·mL^{-1},$P<0.04$). Arm exercise and moderate cycling induced larger increases in monocyte numbers and larger increases of the classical monocyte subset in the recovery period than easy cycling ($P<0.05$). The post-exercise adrenaline concentration was lowest for easy cycling ($P=0.04$).

Conclusions: Arm exercise and cycling at the same relative exercise intensity induces a comparable acute inflammatory response; however, cycling at the same absolute oxygen uptake as arm exercise results in a blunted cytokine, monocyte and adrenaline response. Relative exercise intensity appears to be more important to the acute inflammatory response than modality, which is of major relevance for populations restricted to upper body exercise.

Key words: cytokines; chemokines; sympathetic activation; inflammation; monocytes; upper body exercise

INTRODUCTION

Cytokines can serve as markers of inflammation, and some have been associated with pro-inflammatory (e.g. interleukin-6 (IL-6), TNF-α), others with anti-inflammatory properties (e.g. IL-10, IL-1ra) (10). Similarly, due to their differential expression of inflammatory markers, monocyte subsets have come into focus to be used as markers of inflammation, CD16-positive monocytes classed as pro-inflammatory due to their limited ability to produce significant amounts of IL-10 (9, 37) but their capacity to produce large amounts of TNF-α (2, 36). In this context, it is important to note that acute and chronic changes in inflammatory markers are not necessarily a result of tissue damage or sepsis; inflammatory markers can be modulated by a range of factors such as stress (e.g., exercise stress), or catecholamines (10).

Exercise is effective in inducing both acute changes in markers of inflammation and monocyte subset numbers. For example, a bout of exercise can acutely increase pro-inflammatory cytokines such as IL-6 (26). Similarly, the pro-inflammatory monocyte subtype is selectively up-regulated immediately following exhaustive exercise (32, 34). Importantly, the first increase in pro-inflammatory markers is followed by longer lasting rises in anti-inflammatory cytokines such as IL-1ra or IL-10 (26). This induction of an anti-inflammatory environment has been suggested to be one of the factors by which exercise may be beneficial in chronically improving an individual's inflammatory status. As a consequence, exercise may represent an effective method in reducing illness risk of conditions associated with inflammatory etiology, such as cardiovascular disease or type 2 diabetes (10).

In addition, exercise can affect leukocyte chemotaxis (20, 35). This may in part be mediated by exercise-induced increases of plasma concentrations of monocyte chemotactic protein 1 (MCP-1), which affects monocyte chemotactic behavior (18, 20). The exercise-induced systemic chemokine increase may disrupt concentration gradients required for chemotaxis (18), rendering monocytes more sensitive to chemo-attractants. For example, monocyte migration activity towards a given amount of MCP-1 can increase following exposure to exercise-induced metabolites such as cortisol (20).

Both the recruitment of CD16-positive monocytes into the circulation (32) and increased concentrations of IL-6 (31) are dependent on adrenergic activation, and catecholamines can independently induce increases in those markers. A major source of IL-6 is contracting muscle, which explains the positive relationship of exercise time and intensity (also associated with adrenergic activation) on circulating plasma IL-6 concentrations (26). However, the effect of involved muscle groups on inflammatory responses has not been studied in great detail. A number of previous upper body exercise interventions failed to increase IL-6 over pre-exercise levels which was suggested to be potentially due to the limited muscle mass investigated (26). However, the exercise stimuli of these interventions were rather low in intensity (3) or involved intense but very brief exercise of the elbow flexors only (13, 19), drastically reducing the muscle mass available in the upper limbs and reducing the time component which is crucial to the IL-6 response (26). Conversely, Helge et al. (12) report a higher IL-6 release from the arms when compared with the legs during whole-body exercise. Also, more recent investigations using upper body exercise bouts of at least 20 min demonstrate an acute cytokine response (16, 21, 22, 33).

To date, the inflammatory effects of upper body exercise with intensity-matched lower body exercise have not yet been compared, and it is hence not possible to transfer any findings derived from lower body exercise into upper body exercise modalities. This is of critical importance for populations that are restricted to these modalities, for example those with a permanent disability or acute injury affecting the lower limbs. Importantly, these more sedentary populations may particularly benefit from potential anti-inflammatory effects of exercise due to their elevated pro-inflammatory resting profile (6).

Therefore, the aim of this study was to compare the inflammatory effects of arm exercise and cycling, which were matched for (1) relative and (2) absolute intensities. (1) For the modalities matched for relative intensities, we hypothesize a similar inflammatory response due to the similar exercise strain. (2) For cycling exercise performed at the same absolute intensity as arm exercise, we hypothesize a blunted inflammatory response due to the lower exercise strain.

METHODS

Participants. Twelve recreationally trained male individuals (age: 25 ± 4 years; body mass: 76 ± 9 kg; $\dot{V}O_{2peak}$ for arm exercise ($\dot{V}O_{2peak\ A}$): 2.41 ± 0.46 L·min^{-1}, 32.1 ± 6.0 mL·kg^{-1}·min^{-1}; $\dot{V}O_{2peak}$ for cycling ($\dot{V}O_{2peak\ C}$): 3.48 ± 0.57 L·min^{-1}, 46.2 ± 6.8 mL·kg^{-1}·min^{-1}) gave written informed consent to participate in this study, which was approved by the University's Ethics committee. Their recreational sports were American football (N=1), cricket (N=1), football (N=3), rugby (N=1), running (N=3), tennis (N=2) and volleyball (N=1) with an average weekly training load of 4.0 ± 1.2 h·week^{-1}.

Experimental design. Participants visited the laboratory on five occasions for two preliminary and three main trials, which were separated by 3 to 10 days. 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), $\dot{V}O_{2peak}$ was determined for arm exercise ($\dot{V}O_{2peak\ A}$) using an arm crank ergometer (Angio, Lode, Groningen, Holland) or for cycling exercise ($\dot{V}O_{2peak\ C}$) using a cycle ergometer (Excalibur, Lode Groningen, Holland) in a randomized order. For this, participants performed a graded exercise test to exhaustion, with an initial power output of 35 W (arm exercise) and 70 W (cycling), respectively; power output was then increased every three minutes by 15 W (arm exercise) or 30 W (cycling) until exhaustion. Arm exercise was performed in a seating position, the center of the crank at shoulder level with arms slightly flexed at maximum reach, cycling with legs slightly flexed at maximum reach. The data of the preliminary tests were used to determine the respective workloads for all main trials, and settings were noted and used for all main trials.

Main trials were performed in a randomized order after a 24 h food standardization period without caffeine and with no exercise allowed 24 h before the experiments. To account for diurnal variations of some of the measured variables (11, 28), 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. Main trials consisted of 45 min of steady state exercise using the following modalities: (1) arm exercise at 60%$\dot{V}O_{2peak\ A}$; (2) cycling at 60%$\dot{V}O_{2peak\ C}$; and (3) cycling at 60%$\dot{V}O_{2peak\ A}$. 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 analyzer (Servomex 1440, Servomex Ltd, Crowborough, UK), and heart rate was continuously monitored using a Polar RS400 (POLAR, Kempele, Finland) monitor. Participants further indicated their rating of perceived exertion (RPE) on a scale

ranging from 6 to 20 (4). Water during exercise was given *ad libitum*, 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 monocyte subpopulation analysis at rest.

Blood collection. Participants were lying in a supine position for venous blood sample collection. Blood was collected into K_3EDTA (for hematology and plasma marker analysis) and heparin (for flow cytometry) containers from a superficial arm vein by venipuncture. Collection times were before, immediately after, and at 2 h and 4 h after exercise. Apart from the collection immediately after exercise, participants rested on a bed for 10 min before the sample was taken.

Hematology. Monocyte numbers, hemoglobin and the hematocrit in whole blood were determined immediately after collection using an automated hematology analyzer (Coulter Ac·T 5diff OV; Beckman Coulter, High Wycombe, UK). Blood volume changes were estimated from hemoglobin values (7) and monocyte numbers corrected for changes in blood volume.

Plasma markers. Following centrifugation (10 min at 3000 rpm and 4ºC) plasma was stored at -20 ºC until analysis. The following analytes were determined in duplicate by enzyme-linked immunosorbent assay (ELISA): IL-6, IL-1ra, MCP-1 (R&D systems, Minneapolis, US), cortisol (DRG Instruments GmbH, Marburg, Germany), and adrenaline (IBL International GmBH, Hamburg, Germany). The within assay co-efficient of variation for the analyses performed were (means ± SD): IL-6 8.0 ± 7.7%, IL-1ra 2.0 ± 2.2%, MCP-1 2.2 ± 1.5%, cortisol 2.7 ± 2.1%, and adrenaline: 4.1 ± 3.9%. As the focus of this study was on plasma marker concentration affecting monocytes and other effectors rather than

175 determining the fold change of plasma marker production, plasma concentration was not
176 corrected for plasma volume changes.
177 **Flow cytometry.** The following fluorochromes were used in this study: PE-conjugated
178 CD16; Alexafluor®647-conjugated CD192 (also known as CCR2, the chemoreceptor
179 binding monocyte chemoattractant proteins); IgG2b,κ AlexaFluor®647-conjugated isotype
180 control (BD, Oxford, UK); and PerCP-conjugated CD14 (abcam, Cambride, UK). Within 2
181 h of sample collection (28), whole blood (120 µL) was incubated with the above
182 fluorochromes in duplicate: (1) CD14, CD16, CD192; (2) CD14, CD16, AlexaFluor®647
183 isotype control. Labelling was carried out on ice for 20 min, followed by lysis with FACS
184 lysis buffer (BD, Oxford, UK) and incubation in the dark for another 10 min. Samples were
185 then centrifuged for 6 min at 3800 rpm, the supernatant was removed and the cell pellet re-
186 suspended with 1.5 mL ice-cold PBS. The centrifugation and supernatant removal steps
187 were repeated, and the cell pellet was re-suspended in 400 µL ice-cold PBS for immediate
188 analysis with the flow cytometer (FACSCalibur equipped with the CellQuest software
189 package, BD Biosciences, Oxford, UK), collecting 100,000 events per sample.

190 Monocyte subsets (classical: CD14++CD16-, intermediate: CD14++CD16+, non-classical:
191 CD14+CD16++ (36)) were determined using the refined gating approach as outlined by
192 Ziegler-Heitbrock and Hofer (38), and their relative fraction was computed. Geometric
193 mean of fluorescence intensity (GMFI) was determined, and receptor expression
194 (percentage of cells) was determined by overlaying and subtracting the receptor distribution
195 from the isotype control distribution.
196 **Statistical analyses.** The SPSS 21.0 statistical package (SPSS Inc., Chicago IL, USA) was
197 used for all statistical analyses. We used the arm crank trial data from Paulson et al. (21) as
198 a foundation for our power calculations. Using GPower 3.1.9.2, we calculated we would
199 need 12 participants to detect a similar change in plasma IL-6 concentration in a repeated

measures design with 3 conditions and 4 measurement time points, with an effect size of 0.74, 90% power, and an α of 5%.

Means and standard deviations were computed for all variables, and normality was checked with the Shapiro Wilk test. Non-normal data were converted using inverse or logarithmic transformations to achieve normality. A repeated measures two-way (exercise modality, time) analysis of variance (ANOVA) was conducted on normally distributed blood derived variables. To compare CCR2 expression and density between monocyte subsets, a repeated measures two-way (exercise modality, subset) ANOVA was conducted on pre data. Huynh-Feldt corrections were applied when sphericity was violated and Sidak adjustments applied for post-hoc comparisons. Data showing significant interaction effects were further analyzed with repeated measures ANOVAs, focusing on time points standing out following visual inspection of plotted data. Non-normal data that were impossible to convert to achieve normality were analyzed using Friedman tests and repeated, Bonferroni corrected Wilcoxon signed rank tests. Physiological exercise descriptors were analysed using a one-way (exercise modality) repeated measures ANOVA or the non-parametric equivalents for non-normal and RPE data. Statistical significance was accepted at $P < 0.05$.

RESULTS

Cytokine and chemokine responses. In all trials, plasma IL-6 increased from resting concentrations ($P < 0.01$), however, an exercise x time interaction ($P = 0.04$) indicated a more pronounced increase immediately post exercise for arm exercise and moderate cycling compared with easy cycling (Figure 1). Similarly, resting plasma IL-1ra concentration rose to higher values for both arm exercise and moderate cycling in the recovery period when compared with easy cycling ($P<0.05$). The MCP-1 plasma concentration increased from pre

to post but was significantly reduced in the recovery period ($P<0.05$), but no modality difference was found ($P = 0.81$). Increases in adrenaline from pre to post exercise were found for all modalities ($P<0.001$), but the post-exercise adrenaline concentration was higher for arm exercise than for easy cycling ($P=0.02$, Table 1). The plasma cortisol concentration was lower in the recovery period for all exercise modalities ($P<0.05$), with no difference between modalities (Table 1).

Blood and plasma volumes were reduced for all exercise modalities and rest at post exercise, with no significant exercise x time interaction effect ($P = 0.16$ and 0.19 for blood and plasma volume, respectively; Table 1).

Monocyte responses. Monocyte numbers were increased in response to all exercise interventions ($P < 0.05$), however, easy cycling resulted in a blunted response (Figure 2). When compared with easy cycling, arm exercise and moderate cycling also induced a larger increase of the classical monocyte subset in the recovery period. All exercise modalities induced a reduction in the intermediate and pro-inflammatory monocyte subset in the recovery period.

Analyzing exercise trials together with the resting trial, no exercise x time interaction effects were found for CCR2 ($P > 0.21$) implying a similar development over time for all modalities. Both CCR2 cell expression and CCR2 GMFI differed between monocyte subsets, and a general decrease of those variables was found in all monocyte subsets in the recovery period (Figure 3, Table 2).

Exercise responses. The exercise intervention resulted in distinctively different physiological and psychophysiological responses, the lowest heart rate and rating of perceived exertion values found for easy cycling (Table 3). 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 their respective relative oxygen uptake.

DISCUSSION

The main finding of this study was that arm exercise and cycling at the same relative exercise intensity induces a comparable acute systemic inflammatory response; however, cycling at the same absolute oxygen uptake as arm exercise results in a blunted response. This is evidenced for IL-6 and IL-1ra plasma concentration, the monocyte counts and the increase of the percentage of classical monocytes. Lower responses for easy cycling were also observed for plasma adrenaline concentration, heart rate, and the rating of perceived exertion. The largest change for most anti-inflammatory markers was found at 2 h post exercise, with many returning towards baseline levels by 4 h post exercise. These results are in line with the proposed hypotheses and support the usefulness of upper body exercise as a means to induce an acute inflammatory response.

This is the first study to compare the cytokine (IL-6, IL-1ra) and chemokine (MCP-1) response in intensity-matched trials between arm and cycling exercise. Another novelty is the investigation of the monocyte subset response in this exercise modality comparison. Finally, as a range of anti-inflammatory markers are induced with a time lag following exercise (26), observing responses up to 4 h post exercise is an advantage over a number of exercise studies limiting their analysis to up to an hour into the recovery period (22, 23, 25, 32, 34).

Cytokine and chemokine responses. Consistent with previous research, the acute IL-6 response was followed by increases in IL-1ra for all modalities, as IL-6 can independently up-regulate anti-inflammatory cytokines such as IL-10 or IL-1ra (30). The arm exercise modality investigated in the present study further allowed the analysis of inflammatory

responses induced by a smaller muscle group. Upper body exercise interventions that showed cytokine responses in able-bodied and disabled populations demonstrate a link to sympathetic activation (16, 22, 33). For example, both the adrenaline and the IL-6 response are blunted in individuals with sympathetic dysfunction (16, 22). This reinforces the role of adrenaline as an important factor to increase plasma IL-6 concentration, as it can independently induce an IL-6 response (31). The present results corroborate these data: The lowest adrenaline response was found for easy cycling, the modality with a blunted inflammatory response. Low circulating levels of adrenaline may affect the inflammatory response through direct mechanisms, such as their action on adrenergic receptors on leukocytes, governing cytokine secretion (17) or by adrenaline dependent recruitment of leukocyte subgroups into the circulation (24, 29). Both adrenaline (15) and IL-6 (26) are involved in glucose metabolism; adrenaline may therefore also indirectly influence the inflammatory response through potential interaction effects. Further to differences in the adrenaline response, differences in sympathetic activation between exercise modalities were also reflected in the heart rate and RPE responses, which were lowest for the easy cycling modality.

The strain on individual muscle fibers was likely to be smallest during the easy cycling modality. Muscle is a producer of IL-6, and calcium-dependent pathways of cytokine secretion are essential for a normal physiological response (14). Muscle contractions are accompanied by increases in intracellular calcium levels; the easy cycling modality with the least intense contractions is therefore expected to result in lower amounts of IL-6 secreted by muscle. As the upper and lower body exercise modalities were matched for relative and absolute $\dot{V}O_2$, it therefore seems that relative, rather than absolute exercise intensity influences the inflammatory response to a greater extent. Corroborating this, Helge et al.

(12) showed that full body exercise simultaneously using the arms and legs at the same relative intensity resulted in a similar absolute IL-6 release in the upper body compared with the lower body, despite the muscle mass in the upper body in their investigation being ~3 times smaller than the muscle mass of the lower body. However, it must be pointed out that the structure and function of the exercising skeletal muscle is likely to differ between modalities. For example, differences in the fibre type distribution may exist between arm and leg muscles, which may explain the lower citrate synthase activity, indicative of aerobic capacity, that has been found previously in arms when compared with legs in a similar population to the present study (12). This again may be associated with the higher rates of glycogenolysis during arm exercise at the same relative intensity as leg exercise (1). Higher rates of glycogenolysis deplete glycogen stores more quickly which in turn is associated with enhanced IL-6 secretion (26). This may hence also represent a mechanism by which upper body exercise induces an inflammatory response. Furthermore, the recreational training status of the participants of the present study meant that arm cranking related training was not part of their routine, whereas lower extremity activities were more consistent with their sports. This difference in training status of the arms and legs may result in higher physiological strain during arm exercise when compared with cycling at the same relative intensity, which may also contribute to the significant difference in adrenaline found between arm exercise and easy cycling, but not between moderate and easy cycling. It must hence be acknowledged that arm and leg muscles are potentially functionally different in the studied participant group. We therefore conclude that the extent of the inflammatory response was independent of exercise *modality* (arm cranking vs cycling) when performed at the same relative intensity. However, it would be misleading to state that the inflammatory response is independent of muscle mass *per se*, as the most dramatic increases

in IL-6 to date have been observed when exercising with large muscle groups, such as during running (26).

The exercise-induced increase in the plasma concentrations of the chemokine MCP-1 are in line with previous research (34). In the present study, this increase was independent of modality, but in contrast to IL-6, also independent of intensity. The chemotactic capacity of MCP-1 is mediated by its interaction with the CCR2 receptor found on monocytes; MCP-1 further induces the production of IL-6 by monocytes (18). Together with adrenaline and cortisol related mechanisms, the increase of MCP-1 post exercise may therefore initiate increases in monocyte numbers into the circulation and be partly responsible for the pro-inflammatory environment immediately post exercise. In the recovery phase, the down-regulation of MCP-1 below resting levels may help to suppress the inflammatory response and represent another factor that helps creating the anti-inflammatory environment associated with the health benefits of exercise (10).

Monocyte responses. In line with the present results, Shantsila et al. (28) report a selective up-regulation of the classical monocyte subset following short (~12-15 min) exhaustive running exercise, with a down-regulation of non-classical monocytes in the recovery period. Other investigators failed to measure responses in the recovery period, but found exercise intensity to be positively related to the acute changes in monocyte subsets (34), even though these responses differed to those reported in the present study: Very intense and short exercise can up-regulate the non-classical monocyte subset immediately after exercise, as shown during 1-min exhaustive cycling (32) or 12-15-min cycling to exhaustion (34). The discrepancy in the monocyte response post exercise may stem from the major increases (279%) in adrenaline (32), compared with the ~50% increase in the present study for arm

exercise and moderate cycling. Indeed, blocking β-adrenergic receptors significantly reduces the exercise-induced mobilization of non-classical monocytes into the circulation (32). Similar patterns of leukocyte mobilization have been found when modulating core temperature or exercise intensity, with the modes inducing the most pronounced adrenaline response resulting in the largest increase in monocyte numbers (27) or cytokine secretion (23, 25). The non-selective up-regulation of leukocyte numbers in the recovery period is likely due to the exercise-induced increase in cardiac output which is related to leukocyte demargination (8) – even though not measured in the present study, arm exercise is associated with lower maximum cardiac output than leg exercise at both submaximal and maximal intensities (5), which may explain the differences found in absolute circulating monocyte numbers.

Circadian rhythms were found for CCR2, with higher values in the morning and a subsequent reduction of both CCR2 expression and GMFI on monocyte subsets. CCR2 expression was not influenced by exercise, and the development over time followed the resting condition for all exercise modalities. This stresses the need for a resting control condition for any future study investigating these markers over time. It is also an indication that monocytes are unlikely to exhibit an altered chemotactic behavior governed by CCR2 due to the exercise stimulus, as an increase in those surface markers would suggest a more pronounced response to the chemo-attractant MCP-1. In line with the present results, exercise of a similar intensity and duration to the present study did not alter CCR2 expression on monocytes (20). However, incubation of blood with cortisol for 24 h resulted in increased CCR2 expression and migration activity (20). A more potent stimulus than ~ 45 min of exercise alone therefore seems to be required. The cortisol plasma concentration in the present study was not increased as a result of the exercise intervention and decreased

progressively throughout the intervention, following its reported circadian rhythm (11). The inability of the present exercise interventions to disturb this circadian rhythm may be a further reason that CCR2 expression was unaffected; longer and/or more strenuous exercise interventions may be required to achieve this goal.

CONCLUSION

Arm cranking and cycling exercise induce a similar inflammatory and anti-inflammatory response when performed at the same relative exercise intensity. Populations restricted to upper body exercise modalities due to injury or disability may hence experience the same positive anti-inflammatory effects of exercise as found for lower body exercise. This is of major relevance as these populations are at a higher risk for diseases of inflammatory etiology. Reduction of the relative exercise intensity results in a blunted inflammatory and adrenaline response, consistent with the previously reported role of sympathetic activation in inflammation. The most pronounced anti-inflammatory responses occur 2 hours post exercise, which should be considered in future protocol design.

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Figure captions

FIGURE 1 – Cytokine and chemokine response. Data indicate means and SD.

Effects of time: Significant difference to [a]pre and [b]post ($P<0.05$).

Effect of trial: [A]significant difference between the easy cycling and the other two modalities ($P<0.05$).

FIGURE 2 – Monocyte count and monocyte subset proportions. Data indicate means and SD.

Effect of time: Significant difference to [a]pre ($P<0.05$).

Effects of trial: Significant difference to [A]rest, [B]easy cycling ($P<0.05$).

FIGURE 3 – Monocyte CCR2 expression. Data indicate means and SD.

Effects of time: Significant difference to [a]pre and [b]post ($P<0.05$).

Table captions

TABLE 1. Hormones and changes in blood and plasma volume.

TABLE 2. The chemokine receptor CCR2.

TABLE 3. Physiological and psychophysiological exercise descriptors.

TABLE 1. Hormones and changes in blood and plasma volume.

Parameter	**Modality**	**Time**			
		Pre	Post	2h post	4h post
Adrenaline (pg/mL)	Arm	31 ± 10	46 ± 10[a,A]	N/A	N/A
	Moderate cycling	30 ± 9	43 ± 16[a]	N/A	N/A
	Easy cycling	29 ± 7	35 ± 11[a]	N/A	N/A
Cortisol (ng/mL)	Arm	198±106	152±78	118±64[a]	112±50[a,b]
	Moderate cycling	198±85	192±135	123±70[a]	106±34[a,b]
	Easy cycling	197±107	154±88	111±51[a]	104±43[a,b]
Plasma volume change compared with pre (%)	Arm	N/A	92±4	99±4[b]	101±4[b]
	Moderate cycling	N/A	93±3	98±6[b]	101±6[b]
	Easy cycling	N/A	96±5	99±4[b]	100±5[b]
	Rest	N/A	95±2	100±4[b]	99±5[b]
Blood volume change compared with pre (%)	Arm	N/A	96±2	99±3[b]	101±4[b]
	Moderate cycling	N/A	96±2	99±3[b]	101±4[b]
	Easy cycling	N/A	98±2	100±2[b]	100±2[b]
	Rest	N/A	97±1	100±2[b]	99±3[b]

Data indicate means±SD. Significant difference to [a]pre, [b]post, and [A]easy cycling ($P<0.05$)

TABLE 2. The chemokine receptor CCR2 (geometric mean of fluorescence intensity).

Monocyte subset	**Modality**	**Time**				**Significant effects of time (P<0.05)**
		Pre	Post	2h post	4h post	
Classical*	Arm exercise	66±9	69±30	67±37	59±25	2h, 4h < pre, post
	Moderate cycling	64±12	55±7	50±5	52±8	
	Easy cycling	60±14	60±7	51±8	50±8	
	Rest	47±17	45±18	47±11	43±12	
Inter-mediate*	Arm exercise	53±8	56±25	61±33	47±23	2h, 4h < pre
	Moderate cycling	52±11	44±6	40±8	42±7	
	Easy cycling	50±12	51±8	45±8	42±6	
	Rest	38±16	38±16	35±8	35±9	
Non-classical*	Arm exercise	12±2	11±5	16±8	11±5	post, 4h < pre, 2h
	Moderate cycling	14±5	10±3	12±3	10±2	
	Easy cycling	13±4	11±2	12±3	10±2	
	Rest	8±3	8±3	9±2	7±3	

Data indicate means±SD. *Significant difference between the monocyte subpopulations (group effect; P<0.001)

TABLE 3. Physiological and psychophysiological exercise descriptors.

Parameter	**Exercise mode**		
	Arm exercise	Moderate cycling	Easy cycling
Power output [W]	73 ± 14*	148 ± 30*	101 ± 25*
VO_2 [L·min^{-1}]	1.50 ± 0.28	2.16 ± 0.34*	1.50 ± 0.28
$\%\dot{V}O_{2peak\ A}$	62.3 ± 1.4	-	62.3 ± 1.1
$\%\dot{V}O_{2peak\ C}$	-	62.3 ± 1.0	43.2 ± 5.4*
Heart rate [b·min^{-1}]	141 ± 9*	150 ± 12*	123 ± 11*
RPE (whole duration)	13.3 (12.6, 13.9)	13.2 (12.7, 14.0)	10.6 (10.0, 11.0)*
RPE (30-45min)	14.5 (13.0, 15.8)	14.0 (13.0, 16.8)	11.5 (10.8, 12.0)*

A, arm; C, cycling; RPE, rating of perceived exertion. Data indicate means±SD or median (lower quartile, upper quartile). *Significant difference to both other modalities ($P<0.05$)

Figure 1

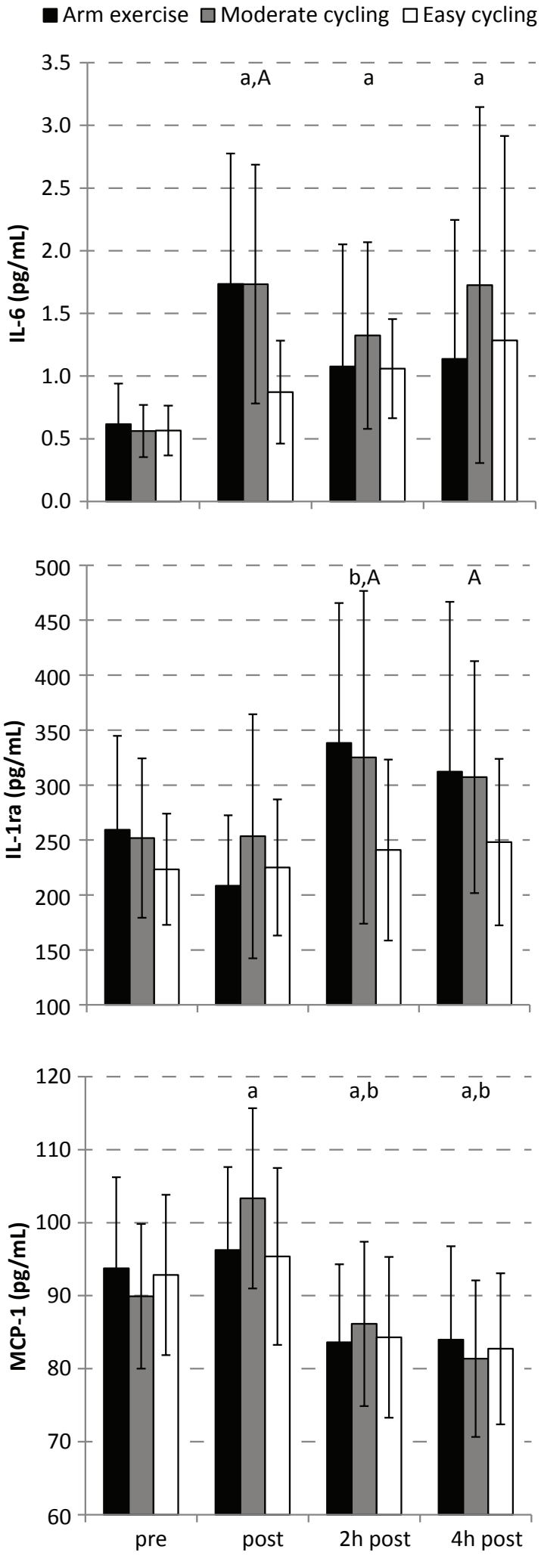

Arm exercise
Moderate cycling
Easy cycling
IL-6 (pg/mL)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
a,A
a
a
IL-1ra (pg/mL)
500
450
400
350
300
250
200
150
100
b,A
A
MCP-1 (pg/mL)
120
110
100
90
80
70
60
a
a,b
a,b
pre
post
2h post
4h post

Figure 2

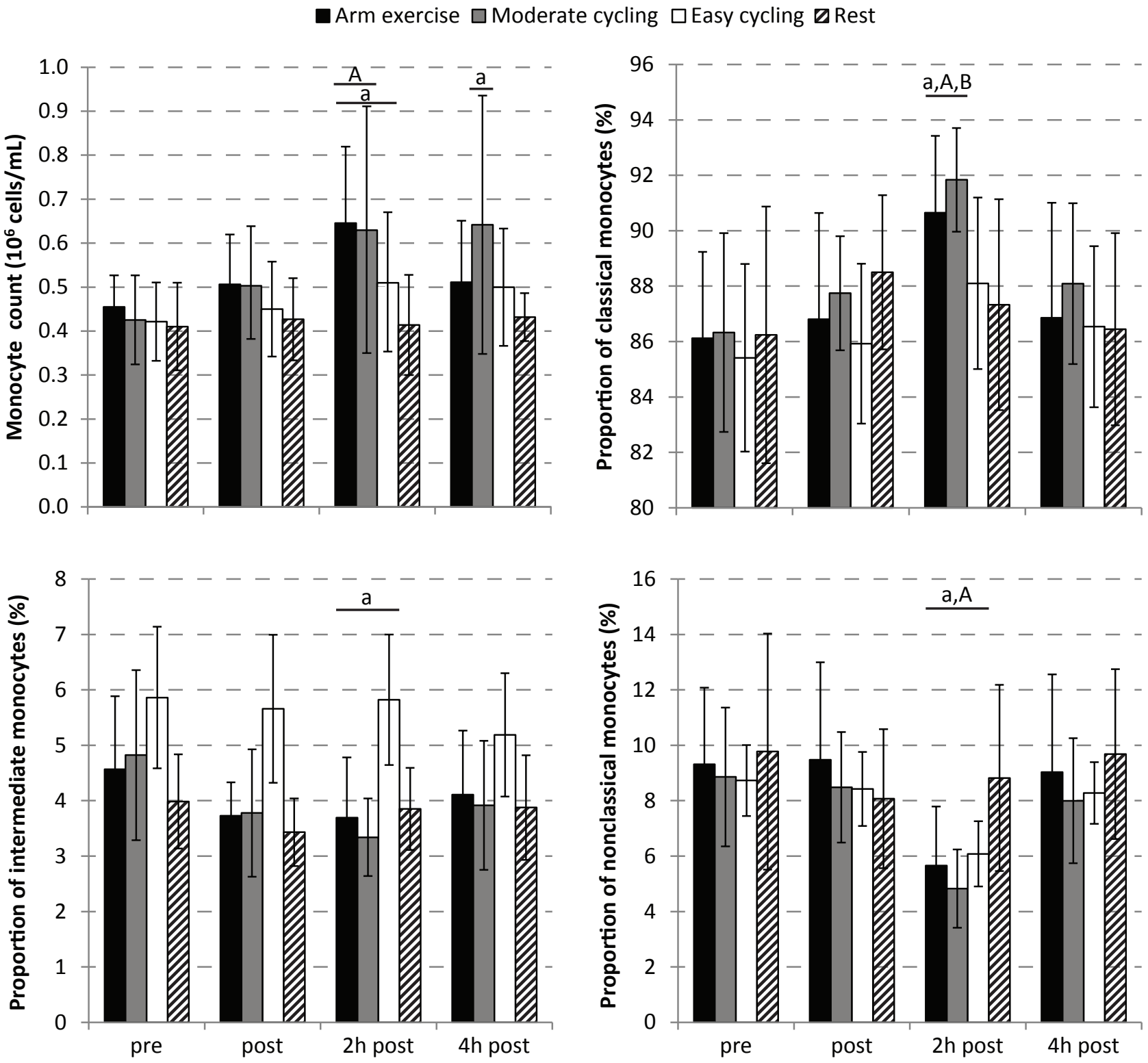
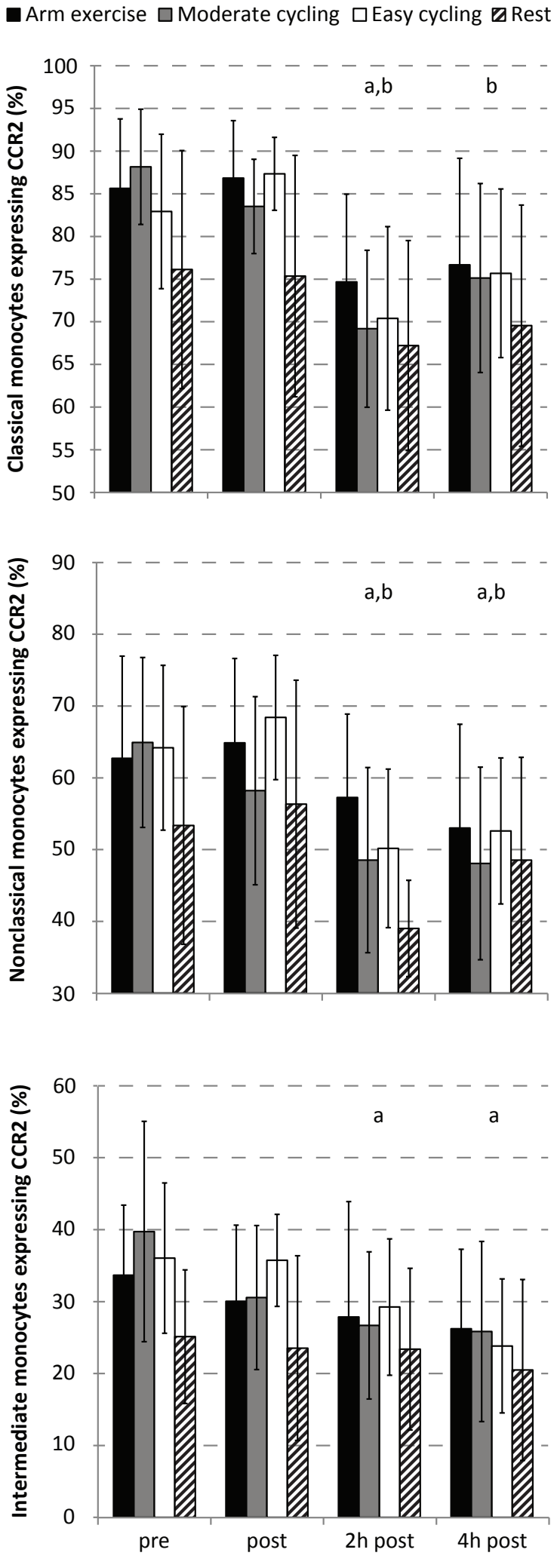

Arm exercise
Moderate cycling
Easy cycling
Rest
Monocyte count (10^6 cells/mL)
A
a
a
Proportion of classical monocytes (%)
a,A,B
Proportion of intermediate monocytes (%)
a
Proportion of nonclassical monocytes (%)
a,A
pre
post
2h post
4h post

Figure 3


Arm exercise
Moderate cycling
Easy cycling
Rest
Classical monocytes expressing CCR2 (%)
100
95
90
85
80
75
70
65
60
55
50
a,b
b
Nonclassical monocytes expressing CCR2 (%)
90
80
70
60
50
40
30
a,b
a,b
Intermediate monocytes expressing CCR2 (%)
60
50
40
30
20
10
0
a
a
pre
post
2h post
4h post