Applied Physiology, Nutrition, and Metabolism

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Journal:	<i>Applied Physiology, Nutrition, and Metabolism</i>
Manuscript ID	apnm-2018-0693.R2
Manuscript Type:	Article
Date Submitted by the Author:	21-Dec-2018
Complete List of Authors:	Hoekstra, Sven; Loughborough University, School of Sport, Exercise and Health Sciences Westerman, Matthew; Loughborough University, School of Sport, Exercise and Health Sciences Beke, Flavio; Loughborough University, School of Sport, Exercise and Health Sciences Bishop, Nicolette; Loughborough University, Leicht, Christof; Loughborough University, SSEHS
Keyword:	heat shock protein 72, flow cytometry, interleukin-6, upper-body exercise, monocytes, Chronic low-grade inflammation
Is the invited manuscript for consideration in a Special Issue? :	Not applicable (regular submission)

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Modality-specific training adaptations – do they lead to a dampened acute inflammatory response to exercise?

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Abstract

While adaptations to a short-term training program can dampen the acute inflammatory response to exercise, less is known about the influence of chronic modality-specific adaptations to training. This study compares the acute inflammatory response to upper- and lower-body interval exercise in individuals chronically trained in these respective modalities. Ninety minutes of interval exercise matched for relative power output on an arm-crank (ARM) and cycle ergometer (LEG) was performed by 8 trained paddlers and 8 trained cyclists. Blood samples were taken pre- and post-exercise. Interleukin-6 (IL-6) concentrations were analysed in plasma, while the expression of intracellular Hsp72 was assessed in monocytes (iHsp72). Interleukin-6 was increased following both modalities (fold change ARM: 7.23 ± 3.56 , $p < 0.001$; LEG: 9.03 ± 4.82 , $p < 0.001$), in both groups (cyclists $p < 0.001$; paddlers $p < 0.001$), but the increase was smaller in ARM compared with LEG (Time x Modality $p < 0.001$). ARM induced a smaller iHsp72 response compared with LEG (fold change ARM: 1.07 ± 0.14 , $p = 0.102$; LEG: 1.18 ± 0.14 , $p < 0.001$, Time x Modality $p = 0.039$). Following ARM, iHsp72 expression was increased in the cyclists only (fold change cyclists: 1.12 ± 0.11 , $p = 0.018$; paddlers: 1.03 ± 0.17 , $p = 0.647$), while iHsp72 expression following LEG was increased in both groups (fold change cyclists: 1.14 ± 0.15 , $p = 0.027$; paddlers: 1.22 ± 0.13 , $p < 0.001$). Taken together, the acute inflammatory response to lower-body interval exercise was larger compared with work-matched upper-body interval exercise. Moreover, adaptations to upper-body exercise training dampened the iHsp72 response to this modality. Therefore, exercise may be less effective in reducing chronic low-grade inflammation in individuals relying on their upper body, such as wheelchair users.

Keywords: heat shock protein 72; flow cytometry; interleukin-6; upper-body exercise; monocytes; chronic low-grade inflammation

Introduction

The acute inflammatory response to exercise is suggested to be partly responsible for the protective effects of exercise against a range of chronic diseases, such as type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) (Pedersen 2006). It is characterised by an immediate increase in pleiotropic markers such as interleukin (IL)-6 and extracellular heat shock protein (eHsp72), followed by a longer lasting increase in anti-inflammatory cytokines such as IL-1ra and intracellular heat shock protein (iHsp72). Simultaneously, in contrast to the inflammatory response observed following infection, the concentrations of pro-inflammatory markers such as tumour necrosis factor- α and IL-1 β are suppressed (Gleeson et al. 2011). Together, the anti-inflammatory milieu created following each exercise bout is suggested to contribute to the reduction of, among other characteristics of chronic low-grade inflammation, resting concentrations of pro-inflammatory proteins, Toll-like receptor expression on immune cells, and the proportion of cluster of differentiation (CD)16 positive monocyte subsets in the circulation; potentially contributing to a reduced risk for T2DM and CVD following chronic exercise training (Gleeson et al. 2011). The amplitude of the acute inflammatory response following exercise is intensity and duration-dependent. In addition, the muscle mass involved in the activity seems to be of influence, suggested by the larger increases in IL-6 following running compared to other sports involving a smaller muscle mass (Fischer 2006).

Forms of exercise that are characterised by a small active muscle mass are activities using only the upper body, such as arm-cranking, handcycling and wheelchair sports; all activities suited for individuals with an impaired ability to engage in lower-body exercise. Due to the smaller muscle mass as well as the differences in structural and functional characteristics of the arm musculature, the acute inflammatory response to upper-body

exercise may therefore be different when compared with lower-body exercise (Fischer 2006). This may make exercise less effective in reducing chronic low-grade inflammation in wheelchair users, a population at a heightened risk for this condition (Manns et al. 2005).

Despite the smaller muscle mass involved in upper-body exercise, Helge et al. (2011) showed in a whole-body exercise model that the arms release more IL-6 into the circulation compared with the legs and in a direct comparison between intensity-matched upper-and lower-body endurance exercise at a moderate intensity no differences in the inflammatory response between both modalities were found (Leicht et al. 2016). However, the training status of the exercising muscles may impact on this response. Indeed, eight weeks of endurance training dampens the acute iHsp72 response to an acute bout of exercise until exhaustion in the soleus muscle of mice (Smolka et al. 2000), while a 10-week training intervention attenuates the acute IL-6 mRNA response following three hours of knee extension endurance exercise in humans (Fischer et al. 2004). Therefore, as previous studies on the acute inflammatory response to upper-body exercise have often included participants unaccustomed to this form of exercise (Leicht et al. 2016; Paulson et al. 2015), the potential of upper-body exercise to induce this beneficial response may be smaller in individuals adapted to this modality.

Since it is now widely acknowledged that skeletal muscle plays a major role in the inflammatory response to exercise (Fischer 2006; Gleeson et al. 2011), local adaptations resulting from exercise training are likely to play a role in the potentially altered acute inflammatory response to exercise following chronic training. Adaptations in the exercising muscle that may affect this response include an increased glycogen content, capacity to oxidise fat (Coffey and Hawley 2006) and elevated iHsp72 expression (Morton et al. 2009). However, while previous studies suggest an impact of physical fitness on the acute inflammatory response to exercise (Fischer et al. 2004; Gokhale et al. 2007; Smolka et al.

2000), no studies have investigated the influence of exercise modality-specific adaptations to chronic exercise training. As even in sedentary wheelchair users the upper-body musculature is relatively well-trained compared with able-bodied individuals from the general population (Jacobs et al. 2013), such insight could provide more understanding on the potential of upper-body exercise to induce the beneficial acute inflammatory response in people adapted to this form of exercise.

Therefore, to investigate the impact of local training status on the acute inflammatory response to upper- as well as lower-body exercise, this study assesses the acute elevation of circulating inflammatory markers following cycling and arm-cranking in athletes specifically trained for either modality.

Methods

Eight chronically upper body trained males (i.e. paddlers) and 8 chronically lower body trained males (i.e. cyclists) volunteered to participate in this study. Participants had at least 2 years of experience in their specific sport and reported to train 4 times per week or more. Exclusion criteria were: engagement in more than once a week cross-training (e.g. cycling or running for the paddlers or upper-body strength and conditioning for the cyclists), smoking and the use of anti-inflammatory drugs. After being informed about the procedures of the study, all participants gave written informed consent at the start of the first visit. The study was approved by the local ethics committee of Loughborough University, in accordance with the Declaration of Helsinki.

Study design

Participants visited the laboratory on four occasions. In the first two preliminary visits, peak exercise capacity was assessed for the upper- as well as lower-body, using a graded

incremental exercise test (GXT) on an arm-crank and cycle ergometer, respectively. Thereafter, two main trials consisting of a 90 min interval protocol were conducted on the arm-crank (ARM) and cycle ergometer (LEG). The order in which participants performed the GXTs and ARM and LEG was counter-balanced. In the 24 h prior to each visit, participants refrained from strenuous exercise, caffeine and alcohol and standardised their diet using a food diary. Participants consumed a carbohydrate-rich meal before each visit. The main trials all started between 8:00-9:30 am, at the same time for each participant to account for a circadian rhythm in any of the outcome measures.

Preliminary visits

On arrival at the laboratory, percentage body fat using 4 site skinfold measurements (Durnin and Womersley 1973), body mass and height were measured. In addition, leg and arm volume were estimated using the methods described by Katch and Katch (1974) and Brorson et al. (2012), respectively. Briefly, the circumference of the arm and leg was taken at 6 sites, as well as the length between them. This was used to calculate the volume of the resulting truncated cones.

Peak exercise capacity for the upper- and lower body was assessed using a GXT on an arm-crank (Angio, Lode, Groningen, The Netherlands) and cycle ergometer (Excalibur, Lode, Groningen, The Netherlands), respectively. A 5 min warm-up was performed at 5 W (arm-crank ergometer) or 40 W (cycle ergometer) prior to the GXT. Following a 2 min rest, the test started at 5 W (arm-crank ergometer) or 40 W (cycle ergometer), which was increased by 15 W (arm-crank ergometer) or 35 W (cycle ergometer) every 3 min. Cadence was kept in the range between 70-90 rpm for both modalities. Heart rate (HR) was measured continuously throughout the tests (Polar RS400, Kempele, Finland), whilst oxygen uptake (VO_2) was determined in the last minute of each incremental stage using Douglas bags and a

gas analyser (Servomex 1440, Crowborough, UK). Local, central and overall ratings of perceived exertion (differentiated RPE) were reported and capillary blood was taken from the earlobe in the last 30 s of each incremental stage for the assessment of blood lactate concentration (BLa), using a Biosen C-line (Biosen, Barleben, Germany). The tests were terminated at volitional exhaustion or when the requested cadence could no longer be maintained.

Immediately after completion of the tests, RPE was reported and a capillary blood sample was taken. The HR averaged over the last 30 s of the test was defined as HR_{peak} , while the highest VO_2 value during the test was considered VO_{2peak} . The peak power output (PO_{peak}) was determined as the PO of the last stage fully completed plus the fraction of the stage in which the test was terminated. The GXTs were regarded valid when the respiratory exchange ratio ≥ 1.10 or $HR_{peak} \geq 95\%$ of the predicted HR_{peak} for the specific modality in combination with a final RPE ≥ 19 . One participant was excluded from participation in the main trials because of two non-valid GXTs.

Main trials

All participants performed 90 min interval exercise on both ARM and LEG. After 10 min of seated rest, a blood sample was taken from an antecubital vein. The exercise session consisted of 10 blocks of 9 min starting with a 3 min stage at 40% PO_{peak} , followed by a 4.75 min stage at 60% PO_{peak} , a 1 min stage at 90% PO_{peak} and 15 s passive rest. Temperature and humidity during the trials was $21.8 \pm 1.1^\circ\text{C}$ and $50 \pm 4\%$ for ARM and $21.9 \pm 0.9^\circ\text{C}$ and $47 \pm 5\%$ for LEG, respectively ($p > 0.431$).

During the trial HR and skin temperature at four sites (T_{skin} ; Ramanathan 1964) using skin thermistors (I-buttons, Homechip Ltd, Milton Keynes, UK) were measured continuously,

while expired air was collected from min 2 until min 3 of the 40%PO_{peak} and 60%PO_{peak} stage of block 1, 5 and 9. Capillary blood was taken from the earlobe at the end of those same blocks. Perceptual responses were reported and core temperature (T_{core}), using a telemetry pill (CorTemp, Palmetto, Florida), was measured at the end of the 40%PO_{peak}, 60%PO_{peak} and 90%PO_{peak} stages of block 1, 3, 5, 7 and 9. The perceptual responses assessed were affect using the Feeling Scale (FS) (Hardy and Rejeski 1989), thermal sensation (Epstein and Moran 2006) and differentiated RPE (Paulson et al. 2013). Participants were allowed to drink water *ad libitum*, which was provided using a bladder to avoid differences in the convenience of drinking between ARM and LEG. Nude body mass was measured before and directly following the exercise bout to calculate sweat loss, corrected for the volume of water consumed.

Immediately following completion of the exercise bout, a second blood sample was taken. Thirty minutes following the cessation of exercise, participants filled-out the Physical Activity Enjoyment Scale (PACES) (Kendzierski and DeCarlo 1991) and reported a session RPE (sRPE)(Foster et al. 2001) of the completed exercise bout. Two hours after completion of the exercise, the last blood sample was taken following 10 minutes of seated rest. Participants were allowed to drink water *ad libitum* but refrained from food in the time between completion of the exercise and the 2 h post blood sample.

Blood analyses

Blood was drawn from an antecubital vein into a K₃EDTA tube. The blood was spun down immediately for 5 min at 1500 g, and plasma was stored at -80°C until analyses by enzyme linked immunosorbent assay (eHsp72: Amp'd HSP70 high-sensitivity, Enzo life sciences, Farmingdale, US; IL-6: High-sensitivity, RnD systems, Abington, UK). Plasma IL-6 concentrations were only assessed in the pre and immediately post-exercise samples as previous literature indicates that plasma IL-6 concentrations peak immediately after and

return to baseline 2 h following exercise similar to the protocol employed in the present study (Fischer 2006). The intra-plate coefficients of variations were 7.1% and 6.4% for eHsp72 and IL-6, respectively. A whole blood count was obtained using a Yumizen H500 cell counter (Horiba Medical, Montpellier, France) for the determination of leukocyte subsets and haemoglobin, while haematocrit was determined using a microcentrifuge. The latter two were used to correct the post and post+2h plasma IL-6 and eHsp72 concentrations for changes in plasma volume (Dill & Costill 1974).

Flow cytometry was used to assess changes in iHsp72 in total CD14⁺ monocytes and the distribution in monocyte subsets. Sixty μ l of whole blood was incubated together with 5 μ l PerCP-conjugated cluster of differentiation (CD)14 and 2.5 μ l PE-conjugated CD16 antibodies in the dark at room temperature for 15 min. Thereafter, samples were lysed by FACS lysing solution (BD biosciences, San Diego, US), washed with phosphate buffered saline (PBS) and fixed using Leucoperm (BD biosciences). Following permeabilisation (Leucoperm, BD biosciences) samples were incubated with 4 μ l FITC-conjugated Hsp70 antibody or isotype control for 30 min. Finally, samples were washed and resuspended in PBS prior to running through the Flow Calibur (BD biosciences). The staining procedure was started immediately after blood sample collection and were run through the flow cytometer directly upon completion.

All antibodies except CD16 (BD biosciences) were purchased from Miltenyi Biotec (Teterow, Germany). Cell Quest software (BD biosciences) was used for the analysis. Monocytes were selected based on their CD14 expression, the percentage of monocyte subsets (CD14⁺⁺CD16⁻ classical monocytes, CD14⁺CD16⁺ intermediate monocytes and CD14⁺CD16⁺⁺ non-classical monocytes) was determined using the trapezoid method (Zawada et al. 2015). The iHsp72 expression in monocytes was determined using the

geometric mean fluorescence intensity (GMFI) following subtraction of the isotype control GMFI.

Statistical analysis

Participant characteristics and outcome measures are given in means and standard deviations. When the assumption of normality was violated, identified by the Shapiro Wilk test, data were log transformed before analysis. A Greenhouse-Geisser correction was applied when the assumption of sphericity was violated, which was tested with Mauchley's sphericity test. For both modalities, a repeated measures ANOVA was used to test for differences between time points and groups, while the same method was used to test for a Time x Modality effect with all participants grouped together. Post-hoc Bonferroni corrected tests were used for further inspection when statistical significance was detected. Differences between the modalities and the groups in personal characteristics and averaged outcome measures of the main trials were assessed using independent and paired sample T-tests, respectively. Correlations between physiological and inflammatory markers were assessed using Pearson's r tests. As the latter was performed as an explorative analysis and a type II error was therefore not deemed problematic, no Bonferroni adjustments were made for significance testing of the correlational analyses (Perneger 1998). R was calculated as the post-exercise fold change in the eHsp72/iHsp72 ratio compared with the ratio at baseline (Krause et al. 2015). The effect of exercise on R was tested using Bonferroni-corrected one-sample T-tests with 1 (i.e. the eHsp72/iHsp72 ratio at baseline) as reference value. The neutrophil-to-lymphocyte ratio was calculated as the ratio between both leukocyte subtypes at each time point (Forget et al. 2017). Statistical significance was set at $p < 0.05$. The 23rd version of the statistical software package SPSS (SPSS inc, Chicago, IL) was used for all analyses.

Results

Participants

The paddlers (age: 20.3 ± 1.9 yrs; height: 179 ± 5 cm; body mass: 72.4 ± 7.5 kg; body fat percentage: $8.9 \pm 1.7\%$) and cyclists (age: 27.5 ± 10.1 yrs; height: 179 ± 7 cm; body mass: 68.8 ± 5.8 kg; body fat percentage: $8.5 \pm 1.6\%$) differed in $\dot{V}O_{2\text{peak}}$ on the arm-crank and cycle ergometer, with higher values for both groups on their respective familiar exercise modality (arm-crank ergometer; paddlers: 3.38 ± 0.57 L/min, cyclists: 2.56 ± 0.29 L/min, $p = 0.019$; cycle ergometer; paddlers: 3.57 ± 0.47 L/min, cyclists: 4.13 ± 0.41 L/min, $p = 0.007$). The volume of the arms was larger in the paddlers compared with the cyclists (paddlers: 3.1 ± 0.8 L, cyclists: 2.3 ± 0.4 L, $p = 0.023$), while there was no difference in leg volume between the 2 groups (paddlers: 10.6 ± 1.5 L, cyclists: 9.8 ± 1.7 L, $p = 0.315$). Absolute PO and $\dot{V}O_2$ was higher during LEG compared with ARM ($p < 0.001$). The paddlers and cyclists exercised at a higher absolute PO in their familiar trial compared with the group for whom the exercise modality was unfamiliar ($p < 0.005$)(Table 1).

There were no differences in final BL_a between ARM and LEG for either of the groups ($p > 0.539$). Fig. 1 shows the T_{core} during the trials for both groups. For both groups LEG induced a larger increase in T_{core} compared with ARM (Time x Modality; $p < 0.001$), but there was no difference between both groups during either modality (Time x Group; $p > 0.152$). LEG induced a larger sweat loss compared with ARM ($p = 0.003$). There was a Modality x Group interaction for sweat loss, with the cyclists sweating more compared with the paddlers during LEG ($p = 0.006$)(Table 1).

Both in the paddlers and cyclists iHsp72 expression was increased following LEG (Time; $p < 0.027$). However, iHsp72 expression following ARM was increased in the cyclists only (Time; paddlers $p = 0.647$, cyclists $p = 0.018$). However, this did not result in a Time x Group

interaction in either of the modalities (ARM $p = 0.396$, LEG $p = 0.175$). When all participants were grouped together, only LEG induced an increase in iHsp72 expression immediately post-exercise (Time; ARM $p = 0.100$, LEG $p < 0.001$), resulting in a Modality x Time interaction ($p = 0.039$)(Fig. 2). At 2 h post-exercise, iHsp72 expression had returned to baseline for both modalities, in both groups (pre versus 2 h post-exercise $p > 0.208$).

Interleukin-6 concentrations were elevated in both groups, following both modalities (Time; $p < 0.001$ in both groups for ARM and LEG). There were no differences in the acute IL-6 response between the groups for either modality (Time x Group; ARM $p = 0.113$; LEG $p = 0.480$)(Fig. 2). Plasma IL-6 concentrations increased to a larger extent following LEG compared with ARM when all participants were grouped together (Time x Modality; $p < 0.001$).

Neither ARM or LEG induced an increase in eHsp72 concentrations immediately post-exercise (Table 2, Time; $p = 0.339$), while there was a trend for a decrease in eHsp72 concentrations following both ARM (Time; $p = 0.060$) and LEG (Time; $p = 0.069$) at 2 h post-exercise. This effect was similar between the paddlers and cyclists (Time x Group; $p > 0.558$). After LEG, R was different from 1 in the paddlers only ($p = 0.004$), while R differed from 1 in neither of both groups after ARM ($p > 0.086$). There was no difference in R between both groups at any time point in either modality ($p > 0.114$). The distribution of the monocyte subsets was altered 2 h post-exercise, with an increase of the percentage of classical monocytes (Time; $p < 0.001$), and a decrease in the percentage of intermediate and non-classical subsets (Time; intermediate monocytes $p = 0.007$; non-classical monocytes $p < 0.001$). There were no differences between the groups in either of the modalities (Time x Group; $p > 0.286$), nor was there a difference in the change in monocyte subset distribution between ARM and LEG when all participants were grouped together (Time x Modality; $p > 0.130$)(Table 2).

The whole blood count before and after the trials is shown in Table 3. For both ARM and LEG there were no differences in the leukocyte responses between the paddlers and cyclists (Time x Group; $p > 0.115$). LEG induced a larger increase in leukocyte (Time x Modality; $p < 0.001$), monocyte (Time x Modality; $p = 0.009$), neutrophil (Time x Modality; $p < 0.001$), lymphocyte numbers (Time x Modality; $p < 0.001$) and the neutrophil-to-lymphocyte ratio ($p < 0.001$) compared with ARM when all participants were grouped together.

Perceptual responses

The perceptual responses during the trials for both groups are shown in Fig. 3 and Table 1. Local, central as well as overall RPE increased during ARM (Time; $p < 0.001$) and LEG (Time; $p < 0.001$) in both groups. During LEG and ARM there were no differences in any of the RPE measures between both groups (Time x Group; $p > 0.245$). When all participants were grouped together, there were no differences in any of the RPE measures between ARM and LEG (Time x Modality; $p > 0.330$). There was also no difference between the groups in the sRPE reported following ARM ($p = 0.914$) and LEG ($p = 0.277$), nor was there a difference between both modalities when all participants were grouped together ($p = 0.189$).

During ARM and LEG, basic affect decreased throughout both exercise trials (Time; $p = 0.005$), resulting in lower scores on the FS in block 9 compared with block 1 during 40%, 60% and 90% PO_{peak} ($p < 0.036$). This progression was similar between the paddlers and cyclists in both modalities (Time x Group; $p > 0.253$). Ratings of enjoyment after both ARM or LEG did not differ between the paddlers and cyclists (Table 1; $p > 0.294$).

Correlations

There was a significant correlation between T_{core} attained at the end of the exercise bout and the IL-6 response for both modalities (T_{core} final ARM - Δ IL-6 ARM: $r = 0.54$, $p = 0.04$; T_{core} final LEG - Δ IL-6 LEG: $r = 0.58$, $p = 0.03$). This association with final T_{core} was not present for the acute iHsp72 response following exercise (T_{core} final ARM - Δ iHsp72 ARM: $r = 0.23$, $p = 0.41$; T_{core} final LEG - Δ iHsp72 LEG: $r = 0.39$, $p = 0.13$). There was no significant correlation between the acute iHsp72 and IL-6 response (Δ pre – immediately post-exercise) following ARM nor LEG (Δ iHsp72 - Δ IL-6 ARM: $r = 0.05$, $p = 0.85$; Δ iHsp72 - Δ IL-6 LEG: $r = -0.04$, $p = 0.88$). Furthermore, there was no significant correlation between PO_{peak} and the iHsp72 or IL-6 response for either of the two modalities (PO_{peak} ARM - Δ iHsp72 ARM: $r = -0.37$, $p = 0.15$; PO_{peak} LEG - Δ iHsp72 LEG: $r = -0.26$, $p = 0.33$; PO_{peak} ARM - Δ IL-6 ARM: $r = -0.38$, $p = 0.14$; PO_{peak} LEG - Δ IL-6 LEG: $r = 0.03$, $p = 0.93$).

Discussion

This study showed that chronic upper-body training attenuates the acute iHsp72 response to a bout of upper-body interval exercise. However, chronic training does not influence the acute IL-6 or eHsp72 response, nor does it influence the change in monocyte subset distribution following exercise. While several studies have reported a dampened acute inflammatory response to exercise in physically fit or chronically trained individuals (Fischer et al. 2004; Gokhale et al. 2007), the findings of the present study indicate that modality-specific adaptations also impact on these acute responses to exercise. This has implications for the interpretation of research findings gathered from cycling studies and upper-body exercise studies using able-bodied, active individuals unaccustomed to upper-body exercise as well as for the creation of exercise programs for individuals accustomed to upper-body exercise (e.g. wheelchair users).

The absence of an acute iHsp72 response to upper-body exercise in individuals adapted to this form of exercise is in accordance with exercise intervention studies in mice. For instance, Smolka et al. (2000) reported an attenuated acute increase in iHsp72 expression in soleus muscle of mice who were trained for eight weeks compared with sedentary control mice following treadmill running. The attenuated iHsp72 response was accompanied by an increase in anti-oxidative enzymes and an attenuated increase in reactive oxygen species following the exercise bout, suggesting that the dampened oxidative stress response following exercise in the trained mice reduces the need for an iHsp72 response. Although oxidative stress was not measured in the present study, this may have mediated the finding in the current study as also in humans short- (Fisher et al. 2011) and long-term (Falone et al. 2010) exercise training can attenuate the acute oxidative stress response following exercise. Additionally, chronic training elevates skeletal muscle glycogen content at rest and reduces glycogenolysis during exercise for a given relative intensity (Hickner et al. 1997). As glycogen depletion can impact on the acute iHsp72 response to exercise (Febbraio et al. 2002), this may be an additional explanation for the dampened iHsp72 response following ARM in the paddlers. The absence of a dampened iHsp72 response in the cyclists following LEG may be explained by the fact that humans rely on their legs for locomotion and the difference in adaptations in the legs between the paddlers and cyclists may not have been as pronounced as in the upper body.

As acute IL-6 and iHsp72 elevations are partly induced by similar stressors (e.g. hyperthermia, oxidative stress, acidosis; (Welc et al. 2012)), it may be somewhat surprising that there was no difference in the IL-6 response between both groups for either modality. Indeed, exercise intervention studies have reported an attenuation of the acute IL-6 response following the training period (Croft et al. 2009; Yfanti et al. 2012), while chronically trained athletes also show a dampened acute IL-6 response compared with sedentary individuals

(Gokhale et al. 2007). It is possible, however, that the measurement of systemic IL-6 (i.e. plasma concentration) is not sensitive enough to detect any differences in the acute response to exercise resulting from local adaptations in the exercising muscle of the cyclists and paddlers of the present study. For instance, while Fischer et al. (2004) found a markedly attenuated acute IL-6 mRNA response in skeletal muscle following the 10-week intervention period, no difference was observed in plasma IL-6 concentrations. Interestingly, for both ARM and LEG there was a significant correlation between final T_{core} and the acute IL-6 response, suggesting that the elevation of plasma IL-6 concentrations following exercise are indeed influenced by more than contraction related processes only. As hyperthermia alone can induce elevations in plasma IL-6 concentrations (Hoekstra et al. 2018), the elevated circulating IL-6 concentration following the exercise bouts may therefore have also originated from other sites than the exercising muscle groups.

Regardless of specific training status, LEG induced a larger acute iHsp72 and IL-6 response when compared with relative intensity-matched ARM. This contrasts earlier studies that have found a similar acute IL-6 response after 45 min of cycling and arm-cranking at 60% VO_{2peak} (Leicht et al. 2016) and a similar contribution of the arms and legs to circulating levels of IL-6 during whole-body exercise (Helge et al. 2011). The difference in exercise duration and intensity between these studies and the present investigation may explain the discrepancy in the findings. For instance, exercise in the study of Leicht et al. (2016) comprised of 45 min of moderate intensity cycling or arm-cranking, which is not likely to induce large elevations in T_{core} in either modality. The relatively intense and prolonged interval protocol employed in the present study may have exacerbated the differences in T_{core} and related physiological stressors (e.g. dehydration) between upper- and lower-body exercise, resulting in a larger acute inflammatory response following LEG compared with ARM. Therefore, it may be conceivable that when exercise is more prolonged and performed

at a high intensity, upper-body exercise may be less effective than lower-body exercise in inducing an acute inflammatory response.

While knowledge on the acute inflammatory response to exercise may aid in the creation of more effective exercise strategies to promote cardiometabolic health, such interventions can only be successful when sufficiently adhered to in the long-term. The perceptual responses to exercise are suggested to be important for exercise adherence (Ekkekakis et al. 2011). Although physical fitness is positively associated with favourable perceptual responses during an acute bout of exercise (Frazão et al. 2016), the present study shows that familiarity to a specific exercise modality does not influence the acute affective response to or enjoyment of exercise. Therefore, a history of training or physical fitness per se may be more important than familiarity to a specific exercise modality for the perceptual responses during exercise. Consequently, having to change between modalities, for instance due to injury, does not necessarily lead to more negative perceptual responses during unaccustomed exercise. This may facilitate adherence to rehabilitation programs of injured athletes or in people that are forced to change exercise modality due to sudden disability.

In the present study both groups exercised at the same relative intensity during both exercise modalities (i.e. similar $\%PO_{peak}$ and $\%VO_{2peak}$ between paddlers and cyclists during both ARM and LEG). Therefore, the attenuated iHsp72 response following ARM in the paddlers is not the result of a reduced relative workload compared with the cyclists. With regards to the ARM versus LEG comparison, however, it should be noted that because of its intermittent character the exercise in the present study was prescribed relative to PO_{peak} . This was chosen as regulating VO_2 during intermittent exercise is problematic due to the time needed to reach a steady-state VO_2 during exercise. Therefore, VO_2 was not controlled and the relative VO_2 was in fact smaller during ARM compared with LEG. Thus, in contrast to the comparison between the cyclists and paddlers, the relative *internal* workload

(i.e. %VO_{2peak}, as opposed to %PO_{peak} as a measure of *external* workload) differed between both modalities, which can be regarded as a limitation. This did, however, not result in a difference in BL_a or RPE between both modalities. Additionally, while iHsp72 expression following ARM was increased in the group unaccustomed to upper-body exercise only, no significant Time x Group interaction was observed for this modality, potentially due to an inadequate statistical power resulting from the relatively small sample size. Therefore, although this study provides support for the influence of local training status on the acute inflammatory response to upper-body exercise, future studies including a larger number of participants may be needed to confirm this finding. Finally, future studies may assess a broader range of inflammatory markers, focussing in particular on anti-inflammatory cytokines such as IL-1ra and IL-10.

In summary, the acute inflammatory response to upper-body exercise is smaller when compared with lower-body exercise matched for relative power output. Moreover, chronic upper-body exercise training can further attenuate the acute iHsp72 response to upper-body exercise. Since the acute inflammatory response to exercise is suggested to induce a range of health-promoting adaptations, including improvements in insulin sensitivity and vascular health, the attenuated acute iHsp72 response to upper-body exercise in people adapted to this modality may dampen the beneficial effects of exercise in these individuals. Therefore, longer or more intense upper-body exercise may be needed in individuals accustomed to this modality to gain these benefits associated with exercise. This might warrant additional strategies to maintain or improve cardiometabolic health in wheelchair users, a population that is at an increased risk for chronic low-grade inflammation and related chronic diseases.

Acknowledgements

The authors thank Courtney Thorpe-Mott, Ellie Newman, Juan Soto-Restrepo, Michael Higbee, Ollie McKeag and Sotiris Lafis for their excellent assistance during the process of data collection. The authors acknowledge the support of the National Institute for Health Research (NIHR) Leicester Biomedical Research Centre. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health. Most of all, the authors are grateful for the effort and time the participants have dedicated to this research project.

Conflict of interest

The authors have no conflict of interest to report.

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Table 1. Physiological and perceptual responses to the main trials. Data presented as mean $\pm$ SD.

Parameter	ARM		LEG	
	Paddlers	Cyclists	Paddlers	Cyclists
VO ₂ at 40%PO _{peak} (L/min)	1.29 $\pm$ 0.26	1.15 $\pm$ 0.20	1.78 $\pm$ 0.25	2.08 $\pm$ 0.19* [^]
VO ₂ at 60%PO _{peak} (L/min)	1.68 $\pm$ 0.32	1.45 $\pm$ 0.19	2.27 $\pm$ 0.34	2.74 $\pm$ 0.22* [^]
%VO _{2peak} at 40%PO _{peak}	39.7 $\pm$ 5.8	43.7 $\pm$ 7.7	49.8 $\pm$ 3.1	50.6 $\pm$ 3.7*
%VO _{2peak} at 60%PO _{peak}	51.7 $\pm$ 6.6	55.0 $\pm$ 7.6	63.2 $\pm$ 4.2	66.8 $\pm$ 5.1*
PO at 40%PO _{peak} (W)	59 $\pm$ 11	46 $\pm$ 2 [^]	99 $\pm$ 13	135 $\pm$ 15* [^]
PO at 60%PO _{peak} (W)	89 $\pm$ 16	70 $\pm$ 3 [^]	149 $\pm$ 19	203 $\pm$ 22* [^]
PO at 90%PO _{peak} (W)	133 $\pm$ 25	104 $\pm$ 4 [^]	223 $\pm$ 28	304 $\pm$ 33* [^]
Mean HR (b/min)	142 $\pm$ 9	126 $\pm$ 11 [^]	157 $\pm$ 9	158 $\pm$ 12*
Final BL _a (mmol/L)	3.1 $\pm$ 1.2	3.5 $\pm$ 0.7	3.3 $\pm$ 1.0	3.5 $\pm$ 0.7
Mean T _{skin} (°C)	32.8 $\pm$ 0.8	32.8 $\pm$ 0.8	33.3 $\pm$ 0.9	32.9 $\pm$ 0.9
Sweat loss (L)	1.2 $\pm$ 0.4	1.1 $\pm$ 0.4	1.4 $\pm$ 0.4	2.0 $\pm$ 0.4* [^]
PACES	93.1 $\pm$ 10.6	83.1 $\pm$ 23.7	90.3 $\pm$ 11.4	86.1 $\pm$ 21.9
Final RPE O (6-20)	16.1 $\pm$ 2.5	15.3 $\pm$ 2.4	17.3 $\pm$ 1.8	16.4 $\pm$ 2.0
sRPE (6-20)	14.9 $\pm$ 1.5	14.8 $\pm$ 2.2	16.3 $\pm$ 1.5	15.4 $\pm$ 1.6

Abbreviations: PO: power output; VO₂: oxygen uptake; HR: heart rate; BL_a: blood lactate; T_{skin}: mean skin temperature; PACES: physical activity enjoyment scale; RPE O: overall rating of perceived exertion; sRPE: session rating of perceived exertion. Significantly different from (*) other modality or ([^]) group

Table 2. Changes in the distribution of monocyte subsets, eHsp72 and *R* following ARM and LEG in the paddlers and cyclists. Data presented as mean $\pm$ SD.

Parameter	Time point	ARM		LEG	
		Paddlers	Cyclists	Paddlers	Cyclists
CD14 ⁺⁺ CD16 ⁻ mon (%)	Pre	91.2 $\pm$ 2.8	91.5 $\pm$ 2.2	92.0 $\pm$ 4.6	91.9 $\pm$ 2.6
	Post	91.3 $\pm$ 1.8	92.5 $\pm$ 3.3	91.3 $\pm$ 4.9	91.4 $\pm$ 2.5
	Post 2h	93.2 $\pm$ 2.5*	94.6 $\pm$ 1.2*	94.1 $\pm$ 4.9*	95.5 $\pm$ 1.3*
CD14 ⁺⁺ CD16 ⁺ mon (%)	Pre	1.88 $\pm$ 0.95	1.78 $\pm$ 0.66	2.43 $\pm$ 2.09	2.22 $\pm$ 1.16
	Post	1.79 $\pm$ 0.78	1.76 $\pm$ 0.93	2.65 $\pm$ 1.75	1.46 $\pm$ 0.62
	Post 2h	1.60 $\pm$ 0.57	1.48 $\pm$ 0.70	1.84 $\pm$ 2.22*	1.13 $\pm$ 0.36*
CD14 ⁺ CD16 ⁺⁺ mon (%)	Pre	4.72 $\pm$ 2.19	4.55 $\pm$ 1.75	4.20 $\pm$ 2.65	4.63 $\pm$ 1.77
	Post	4.82 $\pm$ 1.50	4.27 $\pm$ 2.48	4.78 $\pm$ 3.33	4.27 $\pm$ 1.76
	Post 2h	3.33 $\pm$ 1.64*	2.24 $\pm$ 0.65*	2.20 $\pm$ 2.35*	1.80 $\pm$ 0.79*
eHsp72 (fold change)	Pre	N/A	N/A	N/A	N/A
	Post	1.22 $\pm$ 0.24	1.86 $\pm$ 1.24	0.92 $\pm$ 0.18	1.19 $\pm$ 0.89
	Post 2h	0.81 $\pm$ 0.97	0.41 $\pm$ 0.38	0.37 $\pm$ 0.35	0.72 $\pm$ 1.14
eHsp72 (ng/ml)	Pre	1.91 $\pm$ 2.29	1.52 $\pm$ 1.51	2.04 $\pm$ 2.63	1.90 $\pm$ 2.11
	Post	2.64 $\pm$ 2.27	2.40 $\pm$ 1.71	1.96 $\pm$ 2.14	1.94 $\pm$ 2.10
	Post 2h	1.03 $\pm$ 1.19	0.96 $\pm$ 1.01	1.19 $\pm$ 2.18	1.63 $\pm$ 1.85
<i>R</i>	Pre	N/A	N/A	N/A	N/A
	Post	1.16 $\pm$ 0.31	1.70 $\pm$ 1.15	1.07 $\pm$ 0.85	1.10 $\pm$ 0.71
	Post 2h	0.93 $\pm$ 0.87	0.43 $\pm$ 0.38	0.37 $\pm$ 0.31*	0.79 $\pm$ 0.80

Abbreviations: mon: monocytes; eHsp72: extracellular heat shock protein 72; *R*: fold change in eHsp72/iHsp72 ratio from pre

* Significantly different from Pre

Table 3. Haematological markers in response to ARM and LEG for the paddlers and cyclists. Data presented as mean $\pm$ SD.

Parameter	ARM						LEG					
	Paddlers			Cyclists			Paddlers			Cyclists		
	Pre	Post	P+2h	Pre	Post	P+2h	Pre	Post	P+2h	Pre	Post	P+2h
WBC ($10^9/L$)	4.83 $\pm$ 0.47	7.71 $\pm$ 4.45*	7.90 $\pm$ 0.61*	4.80 $\pm$ 1.66	7.04 $\pm$ 2.60*	8.68 $\pm$ 2.16*	5.31 $\pm$ 0.81	9.73 $\pm$ 2.36*	10.70 $\pm$ 2.21*	5.07 $\pm$ 1.48	9.89 $\pm$ 2.95*	10.78 $\pm$ 2.13*
Lymphocytes ($10^9/L$)	1.60 $\pm$ 0.32	2.49 $\pm$ 0.50*	1.69 $\pm$ 0.26	1.67 $\pm$ 0.45	2.66 $\pm$ 1.19*	1.88 $\pm$ 0.46	1.71 $\pm$ 0.56	3.15 $\pm$ 1.18*	1.45 $\pm$ 0.27	1.65 $\pm$ 0.40	3.86 $\pm$ 1.27*	1.50 $\pm$ 0.34
Monocytes ($10^9/L$)	0.47 $\pm$ 0.09	0.74 $\pm$ 0.22*	0.62 $\pm$ 0.14*	0.48 $\pm$ 0.16	0.65 $\pm$ 0.25*	0.61 $\pm$ 0.16*	0.58 $\pm$ 0.19	0.97 $\pm$ 0.34*	0.80 $\pm$ 0.15*	0.46 $\pm$ 0.10	0.86 $\pm$ 0.26*	0.75 $\pm$ 0.20*
Neutrophils ($10^9/L$)	2.46 $\pm$ 0.48	4.16 $\pm$ 1.28*	5.64 $\pm$ 1.04*	2.51 $\pm$ 1.00	3.49 $\pm$ 1.28*	5.90 $\pm$ 1.69*	2.79 $\pm$ 0.57	5.20 $\pm$ 1.70*	8.19 $\pm$ 2.26*	2.64 $\pm$ 1.14	5.17 $\pm$ 1.64*	8.51 $\pm$ 1.41*
Neutrophil/lymphocyte	1.61 $\pm$ 0.51	1.74 $\pm$ 0.64	3.45 $\pm$ 1.05*	1.48 $\pm$ 0.41	1.39 $\pm$ 0.45	3.22 $\pm$ 0.93*	1.84 $\pm$ 0.90	1.77 $\pm$ 0.66	5.79 $\pm$ 1.84*	1.67 $\pm$ 0.80	1.45 $\pm$ 0.60	5.92 $\pm$ 1.49*
Hct (%)	45.8 $\pm$ 1.2	48.3 $\pm$ 1.3*	45.7 $\pm$ 1.1	45.1 $\pm$ 1.8	48.7 $\pm$ 2.2*	45.5 $\pm$ 1.6	45.4 $\pm$ 1.7	48.3 $\pm$ 2.2*	45.1 $\pm$ 1.8	45.2 $\pm$ 1.3	48.3 $\pm$ 2.1*	45.1 $\pm$ 1.4

Abbreviations: WBC: white blood cells; Hct: haematocrit

* Significantly different from Pre

Fig. 1 Core temperature during the trials for the paddlers and cyclists. Data are presented as mean $\pm$ SD. Significantly different from (^) ARM or (*) Pre

Fig. 2 The acute iHsp72 and IL-6 responses following ARM and LEG for the paddlers and cyclists. Lines represent individual participants, while bars represent group means. * Significantly different from pre

Fig. 3 Perceptual responses to ARM and LEG for both groups. RPE L = local ratings of perceived exertion, RPE C = central ratings of perceived exertion, FS: feeling scale, TS = thermal sensation. Data represent responses during exercise blocks 1, 3, 5, 7 and 9. * Significantly different from first 9-min exercise block. ^ Significantly different from 40% PO_{peak} .

Draft