Title: Cytokine mRNA Expression Responses to Resistance, Aerobic and Concurrent Exercise in Sedentary Middle-Aged Men.

Running Title: Cytokine Expression to Exercise Mode.

Authors: Cheyne E. Donges[1], Rob Duffield[1,2], Greg C. Smith[3], Michael J. Short[1], and Johann A. Edge[4‡].

Affiliation: [1]School of Human Movement Studies, Charles Sturt University, Australia.

[2]Sport and Exercise Discipline Group, Faculty of Health, University of Technology, Sydney, Australia.

[3]Department of Molecular Medicine and Pathology, The University of Auckland, New Zealand.

[4]Department of Sport and Exercise Science, The University of Auckland, New Zealand.

[‡]Deceased

Correspondence: Cheyne Donges,

School of Human Movement Studies, Charles Sturt University, Panorama Avenue, Bathurst, Australia, 2795.

Phone: +61 2 6338 4048

Fax: +61 2 6338 4065

Email: cdonges@csu.edu.au

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Abstract

Purpose: Concurrent resistance and aerobic exercise (CE) is recommended to ageing populations; though is postulated to induce diminished acute molecular responses. Given that contraction-induced cytokine mRNA expression reportedly mediates remunerative post-exercise molecular responses, it is necessary to determine whether cytokine mRNA expression may be diminished after CE.

Methods: Eight middle-aged men (53.3±1.8y; 29.4±1.4kg·m^2) randomly completed (balanced for completion order) 8×8 leg extensions at 70% maximal-strength (RE), 40min cycling at 55% of peak aerobic-workload (AE), or (workload-matched) 50% RE (4×8 leg extensions) and 50% AE (20min cycling) (CE). Muscle (*v.* lateralis) was obtained pre-exercise, and 1h and 4h post-exercise, and analyzed for changes of glycogen concentration, tumor necrosis factor (TNF)α, TNF receptor-1 and 2 (TNF-R1/TNF-R2), interleukin (IL)-6, IL-6R, IL-1β, and IL-1 receptor-antagonist (IL-1ra).

Results: All exercise modes up-regulated cytokine mRNA expression at 1h post-exercise comparably (TNFα, TNF-R1, TNF-R2, IL-1β, IL-6) ($P<0.05$). Expression remained elevated at 4h after RE and AE ($P<0.05$), though returned to pre-exercise levels after CE ($P>0.05$). Moreover, AE and RE up-regulated IL-1β and IL-1ra expression, whereas CE up-regulated IL-1β expression only ($P<0.05$). Only AE reduced muscle glycogen concentration ($P<0.05$), whilst up-regulating receptor expression the greatest; though, IL-6R expression remained unchanged after all modes ($P>0.05$).

Conclusions: In middle-aged men, all modes induced commensurate cytokine mRNA expression at 1h post-exercise; however, only CE resulted in ameliorated expression at 4h post-exercise. Whether the RE or AE components of CE are independently or cumulatively sufficient to up-regulate cytokine responses, or whether they collectively inhibit cytokine mRNA expression, remains to be determined**.**

Keywords: inflammation; TNF; interleukin; concurrent exercise; aerobic exercise; resistance exercise.

27 **Introduction**

28 Exercise-induced skeletal muscle contraction is capable of up-regulating mRNA expression of many
29 inflammatory cytokines in the post-exercise period (Nieman et al. 2003; Louis et al. 2007; Buford et
30 al. 2009b; Nieman et al. 2004; Buford et al. 2009a; Vella et al. 2011). Importantly, it is during this
31 period that mechanical and biochemical re-modelling and adaptive processes occur, many of which
32 are reportedly initiated and modulated via cytokine interactions (Pedersen 2009; Kramer and
33 Goodyear 2007; Gleeson et al. 2011; Tidball 2005). Inherent to these acute adaptive processes are
34 mode-specific effects of the contractile stimulus; including myocyte injury and glycogen depletion,
35 which are induced by resistance exercise (RE) and aerobic exercise (AE), respectively (Steensberg et
36 al. 2001; Steensberg et al. 2002; Vella et al. 2011). Given that RE and AE occupy opposing ends of
37 the strength-endurance continuum (Hawley 2009; Nader 2006), it has been postulated that serial
38 completion of these diverse contractile stimuli, i.e. concurrent exercise (CE), promotes acute post-
39 exercise molecular signalling convergence and diminished adaptive responses (Hawley 2009; Nader
40 2006). Thus, CE training is theorized to eventuate reduced mode-specific adaptations in comparison
41 to RE (muscle mass and force production) or AE (oxidative and endurance capacity) alone (Hawley
42 2009; Nader 2006). Despite these assumptions, it was recently shown in an acute study of untrained,
43 middle-aged men that CE performed as 50% RE and 50% AE, respectively increased myofibrillar and
44 mitochondrial muscle protein synthesis equivalently to RE or AE alone (Donges et al. 2012).

45 An understanding of how cytokine mRNA expression responses are affected by the exercise mode is
46 pertinent; especially in sedentary middle-aged populations at risk of chronic diseases related to
47 reductions of muscle mass and oxidative capacity (Griewe et al. 2001; Rooyackers et al. 1996; Evans
48 2010; Petersen and Pedersen 2005). Evidence supports that disease progression related to age- and
49 dysfunctional cytokine-related diseases such as sarcopenia (Griewe et al. 2001), type II diabetes
50 (T2D) (Pradhan et al. 2001), and cardiovascular disease (CVD) (Ridker et al. 2000) may be inhibited
51 and/or attenuated via cytokine interactions. Problematically though, the predominance of literature
52 pertaining to the acute cytokine mRNA response to exercise are derived from studies incorporating
53 young, normal weight, active populations, or methodology that are physically (i.e. downhill running,

54 leg kicking) or temporally (2-5h) inappropriate (Louis et al. 2007; Nieman et al. 2004; Nieman et al.
55 2003; Steensberg et al. 2001; Steensberg et al. 2003; Steensberg et al. 2002; Vella et al. 2011). Whilst
56 these studies contribute valuable insight regarding cytokine expression after exercise; evidence of the
57 effect of more age-appropriate exercise methodology for RE, AE, or CE in initially sedentary middle-
58 aged populations are necessary (Haskell et al. 2007; Ross et al. 2012; Donnelly et al. 2009).

59 Currently, it remains unclear how appropriate mode-specific (Ross et al. 2012; Haskell et al. 2007)
60 exercise-induced responses affect cytokine expression in sedentary middle-aged humans. Tumor
61 necrosis factor (TNF)-α and interleukin (IL)-1β are mediators of apoptosis and immunity (Dinarello
62 1996) that respond to myocyte injury and mononuclear cell activation, as classically induced by RE
63 (Louis et al. 2007; Nieman et al. 2004), though their response to CE remains unexamined.
64 Furthermore, whether receptors associated to TNFα (TNF-R1/TNF-R2) and IL-1β (IL-1 receptor
65 antagonist [IL-1ra]) are expressed in accordance with TNFα and IL-1β remains unclear. While debate
66 continues as to whether IL-6 retains pro- (adipose-derived) or anti-inflammatory (contraction-derived)
67 localized and wider systemic actions; evidence shows that IL-6 is exponentially expressed according
68 to glycogen depletion (Keller et al. 2001), as typically induced by AE (Steensberg et al. 2001; Nieman
69 et al. 2003). However, as type II muscle fibres are the predominant source of IL-6 mRNA inducement
70 (Hiscock et al. 2004), evidence indicates that RE may activate IL-6 in an intensity-based, as well as
71 glycogen-based manner (Mendham et al. 2011). To date, these comparisons have not been determined
72 between AE and RE, nor CE. Lastly, many of the substrate-based effects of IL-6 are exerted through
73 the IL-6 receptor (IL-6R) (Keller et al. 2005). As IL-6R appears in accordance with IL-6 (Gray et al.
74 2008; Keller et al. 2005), it may respond more to AE than RE; yet evidence for this response is
75 lacking, and further the effect of CE on IL-6 and IL-6R expression has also not been examined.

76 Thus given the aforementioned lack of data related to mode-induced cytokine expression, the purpose
77 of the present study of sedentary middle-aged men was to examine the acute effects of combining RE
78 and AE on post-exercise cytokine mRNA expression. Despite previous suggestions of molecular
79 convergence with CE, based upon recent findings of equivalent molecular responses between modes,
80 we hypothesized that CE would induce cytokine mRNA expression equivalently to full RE or AE.

81 **Methods**

82 ***Subjects***

83 Eight sedentary middle-aged men (age range: 45-60y) men (data presented in Table 1) were recruited
84 for the study. Subjects were not involved in regular or incidental physical activity (>30min on >1d ·
85 wk^{-1}) in the preceding 12 months. A physician overviewed subject's medical history and baseline data
86 for diabetes, cardiovascular disease, renal or hepatic disorders, arthritis, pulmonary disease, abnormal
87 leukocyte sub-population count, periodontal disease, or any other condition associated with a systemic
88 inflammatory response. Subjects confirmed with these conditions or those that were tobacco smokers
89 or recently taking potentially confounding medications were not involved in the study. All subjects
90 provided written informed consent prior to becoming involved in the study, which was approved by
91 The University of Auckland Human Subjects Ethics Committee and conformed to standards for the
92 use of human subjects in research as outlined in the Declaration of Helsinki.

93 ***Baseline Test Procedures***

94 A schematic diagram of all study procedures is presented in Figure 1. Following pre-screening and
95 recruitment, subjects underwent anthropometric measures (height, mass, waist and hip girth) and
96 supine whole-body dual-energy x-ray absorptiometry (model DPX+ with software version 3.6y; GE-
97 Lunar, Madison, WI, USA) for estimation of absolute fat and fat-free mass. During this visit, subjects
98 completed familiarization procedures in the Exercise Science Laboratories, including explanation,
99 demonstration and practice of all exercise testing and exercise trial procedures. One week later,
100 subjects returned and completed (in order; separated by 30min) one repetition-maximum (1RM)
101 testing of the quadriceps muscle group on a leg extension machine (Fitness Works, Auckland, New
102 Zealand) and an incremental graded exercise test (GXT) on an electronically-braked cycle ergometer
103 (Velotron, RacerMate Inc., Seattle, Washington, USA). The GXT commenced at 2.0W · kg^{-1} body
104 mass for 150s, increased by 50W for 150s for the 2nd stage, and increased by 25W every 150s for
105 subsequent stages until volitional exhaustion to determine peak oxygen consumption ($VO_{2\ peak}$) and
106 power output associated with $VO_{2\ peak}$. Pulmonary gas exchange was determined by measuring O_2 and
107 CO_2 concentrations and ventilation to calculate VO_2 using a calibrated metabolic gas analysis system
108 (Moxus modular oxygen uptake system; AEI Technologies, Pittsburgh, USA).

109 ***Nutritional Procedures***

110 After baseline testing (Figure 1), subjects completed three exercise trials (separated by ≥7 d recovery),
111 that were randomized for order of completion to ameliorate potential for order effects in study data.
112 For the 24h prior to the first exercise trial, macronutrient composition of all ingested food and
113 beverages was documented in a diary provided and overviewed by the research team. To ensure
114 homogeneity regarding each pre-trial dietary preparation, for the night prior to testing of all three
115 trials subjects were provided with and consumed the same meal (beef lasagne 400g; 407cal. [1700
116 kJ]; 56.4g carbohydrate; 10.0g fat; 19.6g protein). Given that intra-muscular and intra-hepatic
117 glycogen stores are critical regulators of ensuing exercise-induced cytokine mRNA responses
118 (Steensberg et al. 2001), dietary intake was further supplemented with additional carbohydrate at a
119 rate of $3g \cdot kg^{-1}$ of body mass (270 ± 27g) to assist pre-trial saturation. Nutritional composition of the
120 additional carbohydrate source was: 1466g total mass; 1319cal. (5498 kJ); 270.9g carbohydrate; 37.9g
121 fat; 24.0g protein. For the two ensuing exercise trials, subjects replicated documented macronutrient
122 dietary intake from the 24h prior to the first trial in addition to the supplemental carbohydrate intake.

123 ***Exercise Trial Procedures***

124 After a 10h overnight fast from the provided meal, subjects arrived at the Laboratory for the first of
125 three exercise trials, including: **1)** a RE trial consisting of 8 sets of 8 repetitions of machine-based leg
126 extension exercise at 70% of 1RM, with sets separated by 150s rest. The RE trial had a total duration
127 of ~24 min (8×25s sets + 7×150s rest periods), and total exercise duration of 3min 20s; **2)** an AE trial
128 consisting of 40min of stationary ergometer cycling at 55% of the peak aerobic workload identified in
129 the GXT; **3)** a CE trial which comprised 50% of the RE and AE trials. Accordingly, 4 sets of 8
130 repetitions of leg extension exercise at 70% of 1RM (with 150s rest) were initially completed, and
131 promptly after the fourth set, 20min stationary ergometer cycling at 55% of peak aerobic workload
132 was undertaken. The CE trial had a total duration of ~30 min (4×25s sets + 3×150s rest periods = ~9
133 min + 1 min change-over from RE to AE + 20 min cycling), and total exercise duration of 21.5 min.
134 Of the 8 subjects, 3 completed RE, 3 completed AE, and 2 completed CE as their first trial. The
135 ensuing two trials were again randomized and balanced as evenly as possible (e.g. 3,3, and 2).

136 Machine and ergometer settings documented during baseline testing were respectively standardized
137 for the RE and CE trials (seat height position, seat backrest position, lever arm positioning) and AE
138 and CE trials (ergometer seat height and handlebar height). During cycling, telemetry-based heart rate
139 (HR) (Vantage NV, Polar, Finland) was recorded every 5min, and pulmonary gas exchange was
140 measured for 5min at 5 and 15min on a metabolic cart (Moxus modular oxygen uptake system; AEI
141 Technologies, Pittsburgh, USA) calibrated for ventilation volume and fractional gas concentration.
142 Rating of perceived exertion (RPE; CR 0-10 scale) was recorded after each set of leg extension
143 exercise, every 5min during cycling exercise, and 10min post-session for all three trials.

144 ***Muscle Biopsy Procedures***

145 As described previously (Donges et al. 2012), as a means of alleviating unnecessary soreness to
146 subjects, a pre-exercise muscle biopsy was collected for trial 1 only (Figure 1). Thus for the remaining
147 two trials, muscle was collected at 1h and 4h post-exercise only. Given evidence that fine-needle
148 muscle biopsy procedures may influence inflammatory responses independent of performed exercise
149 (Friedmann-Bette et al. 2012); we chose to collect muscle from *m.* vastus lateralis in an alternating
150 manner (trial 1 and 3 on the same leg; trial 2 the opposing leg) in order to allow 2 weeks recovery
151 between sampling of a potentially confounding site (with respect to chronic inflammatory processes).
152 After administration of local anaesthetic (2% Lignocaine) at a site ~ 15cm superior to the patella, a
153 5mm Bergstrom needle modified with suction was inserted into the incision site for collection of a
154 specimen which upon excision was promptly blotted on filter paper, removed of visible fat or
155 connective tissue, frozen in liquid nitrogen, and stored at -80°C for ensuing real-time PCR analyses.

156 ***Muscle Glycogen Procedures***

157 Muscle glycogen analysis was carried out according to the acid hydrolysis method (Adamo and
158 Graham 1998). Whilst remaining proximal to a bed of dry-ice, ~5-8mg of freeze-dried muscle was
159 dissected of visible blood, fat or connective tissue. Samples were hydrolysed in weighed tubes with
160 500μl of 2M HCl and incubated in a heating block for 2h at 99°C. After incubation, tubes were re-
161 weighed and any loss of weight was replaced with water. After weight normalization, 500μl of 2M
162 NaOH was added for pH neutralization and tubes were vortexed for 1min. Samples were measured for

163 glucose concentration (GEM primer 3500; Instrumentation Laboratory, Lexington, MA), of which the
164 data are expressed as a normalized concentration relative to dry weight (Adamo and Graham 1998).

165 ***Real-Time Polymerase Chain-Reaction Procedures***

166 RT-PCR procedures utilized in this study have been reported in full previously (Donges et al. 2012);
167 though an abbreviated description is provided here. Muscle was homogenized and RNA isolated with
168 TRIzol®Plus reagent (Invitrogen, Carlsbad, CA, USA) and chloroform, respectively. Isolated RNA
169 was mixed with glycogen in diethylpyrocarbonate treated water (DEPC-tx H_20) and 1-Propanol to
170 precipitate the RNA, which was tested for concentration and purity (NanoDrop® 1000 UV-Vis
171 spectrophotometry, NanoDrop Technologies, New Zealand) and size and density (Agilent 2100
172 Expert Bioanalyser, Agilent technologies, Palo Alto, California, USA). Mean RNA integrity number
173 (RIN) of RNA included in the study was 8.8±0.4; range of RIN: 7.4-9.2. RNA were then treated with
174 DNase1 (Invitrogen, Carlsbad, CA, USA), reverse-transcribed using a TaqMan® SuperScript™ VILO
175 cDNA synthesis kit (Invitrogen, Carlsbad, CA, USA). TaqMan® Universal PCR Master Mix™ and
176 TaqMan® Gene Expression assays (Applied Biosystems, Foster City, CA, USA) were used to analyze
177 mRNA of TNFα (Hs01113624_g1); TNF-R1 (Hs01042313_m1); TNF-R2 (Hs00961749_m1); IL-1β
178 (Hs01555410_m1); IL-1ra (Hs00893626_m1); IL-6 (Hs00985639_m1); IL-6R (Hs01075666_m1) and
179 GAPDH as a control. For each subject, all samples were simultaneously analyzed in triplicate on the
180 same plate. PCR was performed using a7900HT Fast Real-Time PCR System and SDS 2.3 software
181 (Applied Biosystems, Foster City, CA, USA). Measurements of relative distribution of the target gene
182 were performed, a cycle threshold (C_T) value obtained by subtracting GAPDH C_T values from target
183 C_T values, and expression of the target was evaluated by the $^{\Delta\Delta}C_T$ algorithm (Pfaffl et al. 2002).

184 ***Statistical Analysis***

185 Data are presented as mean ± standard error of mean (SEM). A within-subject repeated measures
186 design was used for the current study. All data were analysed using two-factor (condition × time)
187 analysis of variance (ANOVA) with repeated measures. Where significant interactions were identified
188 in the ANOVA, Tukey's pairwise comparisons were applied post-hoc to determine differences
189 between means for main effect and interaction. For all analyses, statistical significance was accepted

190 at $P<0.05$. All statistical procedures were conducted using PASW statistics (version 18.0 SPSS Inc,
191 Chicago, IL) and the Relative Expression Software Tool (REST©) (Pfaffl et al. 2002).

192 **Results**

193 ***Heart Rate, VO_2 Consumption, and RPE***

194 HR (5, 10, 15, 20min) and VO_2 (5, 15min) (Figure 2) were not different between AE and CE at any
195 time-point ($P>0.05$). As the resistance lifted for each set of RE and CE were identical, there was no
196 difference in the applied load between trials ($P>0.05$). Differences were observed between trials for
197 RPE (Figure 2), with subjects rating RE more strenuous than AE trial at all time-points ($P<0.05$).
198 Within the CE condition, subjects rated the AE component as less strenuous compared to the RE
199 component ($P<0.05$) and the latter half of the AE condition (Figure 2; 25-35min time-points; $P<0.05$).

200 ***Changes of Muscle Glycogen***

201 Changes of muscle glycogen concentration are presented in Figure 3A. Pre-exercise glycogen
202 concentration (286 ± 40 $mmol·L^{-1}$) was not reduced after RE (1h = 257 ± 48 $mmol·L^{-1}$; 4h = 244 ± 45
203 $mmol·L^{-1}$) or CE (1h = 256 ± 38 $mmol·L^{-1}$; 4h = 234 ± 46 $mmol·L^{-1}$) ($P>0.05$); though was
204 significantly reduced at 1h after AE (1h = 186 ± 34.0 $mmol·L^{-1}$) ($P<0.05$). The 4h post-exercise
205 concentration (4h = 191 ± 30.6 $mmol·L^{-1}$) after AE was not significantly different to pre-exercise
206 concentration ($P>0.05$).

207 ***Post-Exercise Cytokine mRNA Expression***

208 Cytokine mRNA expression are presented in Figure 3 for mode-based fold-change comparisons, and
209 Figure 4 for cytokine and cytokine receptor time-course responses.

210 *TNFα mRNA Expression*

211 All exercise modes up-regulated TNFα mRNA expression (Figure 3B) at 1h post-exercise (RE = 2.7 ±
212 0.5; AE = 1.8 ± 0.3; CE = 2.5 ± 0.3 ($P<0.05$); however, expression only remained elevated at 4h post-
213 exercise after RE (3.0 ± 0.7) and AE (2.4 ± 0.4) ($P<0.05$; Figure 3B and Figure 4A). Accordingly, at
214 4h post-exercise, expression of TNFα was significantly greater for RE than CE ($P<0.05$).

215 *TNF-R1 and TNF-R2 mRNA Expression*

216 TNF-R1 mRNA expression (Figure 3C) increased at 1h post-exercise after AE (1.7 ± 0.1; P<0.05 vs.
217 CE) and RE (1.5 ± 0.2) (P<0.05); though not after CE (P>0.05). TNF-R1 expression at 4h post-
218 exercise was not increased above pre-exercise levels by any mode (P>0.05; Figure 3C and Figure 4B).
219 For TNF-R2 (Figure 3D), all modes increased mRNA expression at 1h post-exercise (RE = 2.3 ± 0.4;
220 AE = 3.1 ± 0.5; CE = 2.6 ± 0.4) (P<0.05); though at 4h post-exercise, expression remained elevated
221 after AE only (1.9 ± 0.4; P<0.05) (Figure 3D and Figure 4B).

222 *IL-1β mRNA Expression*

223 All modes up-regulated IL-1β mRNA expression (Figure 3E) at 1h post-exercise (RE = 2.0 ± 0.4; AE
224 = 3.1 ± 0.6; CE = 2.9 ± 0.8) (P<0.05); with expression maintained to 4h after RE (4.4 ± 1.1) and AE
225 (4.1 ± 1.1) (P<0.05), though not CE (P>0.05) (Figure 3E and Figure 4B). Expression of IL-1β at 4h
226 post-exercise after AE was greater than CE (P<0.05), with RE showing a trend for the same (p=0.07).

227 *IL-1ra mRNA Expression*

228 AE up-regulated IL-1ra mRNA expression (Figure 3F) at 1h post-exercise (4.9 ± 0.9; P<0.05); though
229 there was no change in expression after RE or CE (P>0.05). At 4h post-exercise, IL-1ra expression
230 remained increased in response to AE (4.4 ± 1.5; P<0.05), and for RE, increased to significant levels
231 compared to pre-exercise (3.3 ± 1.1; P<0.05; Figure 3F and Figure 4B). The expression of IL-1ra at
232 4h post-exercise after AE was significantly greater than that after CE (P<0.05).

233 *IL-6 and IL-6R mRNA Expression*

234 All exercise modes up-regulated IL-6 expression (Figure 3G) at 1h post-exercise (CE = 4.0 ± 0.7; RE
235 = 3.0 ± 0.6; AE = 3.4 ± 0.5) (P<0.05); with expression maintained to 4h post-exercise after RE (3.4 ±
236 0.6) and AE (2.6 ± 0.7), though not CE (2.0 ± 0.4) (P>0.05) (Figure 3G and Figure 4). The mRNA
237 expression of IL-6 at 4h post-exercise after RE was significantly greater than after CE (P<0.05). The
238 mRNA expression of IL-6R was not altered in response to the exercise modes (P>0.05) (Figure 3H).

239 **Discussion**

240 Previous investigations have provided valuable context regarding acute cytokine mRNA expression
241 responses to exercise, though typically incorporate young, normal weight, trained populations, and
242 exercise modes that appear inappropriate for untrained, overweight, middle-aged populations (Louis
243 et al. 2007; Nieman et al. 2004; Nieman et al. 2003; Steensberg et al. 2001; Steensberg et al. 2003;
244 Steensberg et al. 2002; Vella et al. 2011). The data of the current study contributes mode-specific,
245 post-exercise cytokine expression information that may provide scope regarding associated chronic
246 training responses to these modes. Specifically, data from this study suggests that: 1) in comparison to
247 isolated RE or AE completion, duration-matched CE induces a reduced pro-inflammatory (TNFα/IL-
248 1β) expression response during the 1-4h post-exercise period, and as will be discussed, may have
249 implications regarding compensatory molecular mechanisms related to skeletal muscle hypertrophy;
250 2) RE is capable of up-regulating IL-6 mRNA expression (1-4h) in the absence of muscle glycogen
251 depletion; 3) despite initial up-regulation of IL-6 mRNA expression after CE (1h), expression is
252 ameliorated from 1-4h post-exercise, and may have bio-energetic adaptive implications given the
253 reported role of IL-6 in substrate metabolism; 4) AE up-regulated cytokine receptor mRNA
254 expression the greatest, whereas RE and CE induced a lesser response. Chronic changes in proteins
255 are reportedly the result of cumulative effects of transient changes in mRNA transcription (Yang et al.
256 2005). As such, reduced receptor expression may prospectively indicate reduced systemic abundance
257 of these receptors, and a diminished capacity to bind or coordinate respective pro-inflammatory
258 member's implicated in chronic low-grade systemic inflammation (i.e. TNFα, IL-1β, etc).

259 In the present study TNFα and IL-1β mRNA were equivalently up-regulated in expression at 1h post-
260 exercise by all modes. However, expression remained elevated at 4h post-exercise after RE and AE,
261 though returned to non-significant levels after CE. Numerous studies have reported that CE training
262 results in diminished muscle cross-sectional area and strength gains in comparison to RE training
263 (Bell et al. 2000; Kraemer et al. 1995; Nelson et al. 1990). The high-intensity contractions inherent in
264 a bout of RE subject myofibers to injurious forces and the induction of a transient inflammatory
265 response (Tidball 2005; Vella et al. 2011). Respondent mononuclear cells, such as neutrophils and

266 macrophages, can up-regulate TNFα and IL-1β expression and facilitate mechanisms related to cell
267 cycle and apoptosis in compromised myocytes, thus initiating repair and remuneration processes
268 related to hypertrophy (Steensberg et al. 2002; Louis et al. 2007; Vella et al. 2011). Accordingly, it
269 may be that acute cytokine responses assist explaining the modulation of these hypertrophic processes
270 (Vella et al. 2011). Conversely, a counter view point suggest that as muscle protein synthesis (MPS) is
271 inhibited when ATP availability is compromised (Bylund-Fellenius et al. 1984), the AE component of
272 CE may acutely antagonise MPS responses to CE, and chronically result in an attenuated hypertrophy
273 response in comparison to isolated RE completion (Nader 2006). Regardless, taken together with
274 previous comparisons of duration-matched CE and RE, wherein only RE up-regulated myogenin and
275 differentiation expression (Donges et al. 2012), the reduced post-exercise TNFα/IL-1β expression in
276 the current study may be indicative of diminished gains in muscle mass after CE in comparison to RE.

277 The present study highlights exercise-induced up-regulation of IL-6 mRNA expression, wherein all
278 modes induced comparable expression at 1h, yet CE resulted in ameliorated expression by 4h post-
279 exercise. Debate continues as to whether IL-6 retains pro- or anti-inflammatory mechanisms of action
280 (Petersen and Pedersen 2005; Krook 2008). However, when induced via muscle contraction, IL-6 is
281 reported to facilitate insulin action and glucose uptake, in addition to lipid oxidation and turnover
282 (Petersen and Pedersen 2005; Pedersen 2009; Steensberg et al. 2002; Kramer and Goodyear 2007).
283 Accordingly, IL-6 mRNA are expressed exponentially based on muscle glycogen depletion, as is
284 classically induced by AE (Steensberg et al. 2001; Nieman et al. 2003; Keller et al. 2001). In contrast,
285 a previous finding that type II fibres are the predominant source of IL-6 mRNA inducement (Hiscock
286 et al. 2004) implies that RE may up-regulate IL-6 expression in an intensity-based manner. These
287 suppositions may be supported by a recent study of untrained middle-aged men, which showed an
288 equivalent plasma IL-6 response between duration-matched RE and AE (Mendham et al. 2011). In the
289 current study, the finding that AE, but not RE, reduced muscle glycogen, concomitant with similar
290 expression of IL-6 between modes, provides evidence that RE can up-regulate IL-6 mRNA expression
291 in the absence of glycogen depletion. In addition, our data shows for the first time that CE results in
292 acute diminishment of IL-6 mRNA expression in comparison to RE or AE; which is novel given that

293 AE and RE were equivalent in IL-6 expression when undertaken in isolation. Given that IL-6 may
294 operate as an energy sensor and signal to numerous cellular targets involved in substrate metabolism
295 (MacDonald et al. 2003), the finding that CE results in ameliorated post-exercise expression may have
296 implications related to post-exercise metabolism and chronic oxidative adaptations (Krook 2008).

297 Accumulating evidence implicates TNFα, IL-1β and IL-6 (adipose tissue macrophage-derived) in the
298 aetiological progression of insulin resistance and T2D (Pradhan et al. 2001), as well as atherosclerosis
299 and CVD (Ridker et al. 2000). The pro-inflammatory actions of IL-6, TNFα, and IL-1β are under
300 inhibitory and coordinative control via their respective cytokine receptors (i.e. IL-6R, TNF-R1/R2,
301 IL-1ra) (Dinarello 1996; Febbraio et al. 2010). Evidence suggests that chronic systemic inflammation
302 and associated disease conditions (T2D, CVD) may be exacerbated when these receptor proteins are
303 insufficient in systemic presence (Dinarello 1996; Febbraio et al. 2010). Chronic adaptive responses
304 that govern such maintenance at the cellular level appear to be the result of cumulative effects of
305 transient changes in mRNA transcription (Yang et al. 2005). Thus, acute exercise-induced receptor
306 mRNA expression may explain chronic reductions in pro-inflammatory cytokines after training;
307 however *in-vivo* evidence for these proposed effects in humans remains unclear (Smith et al. 1999).

308 In the current study, IL-6R mRNA expression did not change in response to exercise, which is in
309 opposition to that observed by others (Keller et al. 2005). In contrast, TNF-R1, TNF-R2, and IL-1ra
310 expression were up-regulated post-exercise. Given that IL-6 is capable of activating the expression of
311 the aforesaid receptors (Steensberg et al. 2003; Petersen and Pedersen 2005), it is surprising that RE
312 induced a lesser receptor response when compared to AE. This is particularly the case given that RE
313 induced IL-6 expression comparable to AE at 1h, and further up-regulated expression at 4h more so
314 than AE. In addition, CE had minimal effect on the respective receptors, up-regulating only TNF-R2;
315 yet as was the case for other up-regulated cytokines, CE resulted in ameliorated expression at 4h post-
316 exercise. Collectively, these mode-based data for cytokine receptor expression suggests that it may be
317 the depletion of muscle glycogen which is influential (Keller et al. 2005). In support, RE and CE did
318 not reduce glycogen concentration, and as mentioned, may be seen to have had little effect on receptor

expression. Future research is needed to examine and provide further verification as to whether this indeed was the case. Nevertheless, the data indicate that AE shows the greatest capacity to induce cytokine receptor expression and may provide further support for AE regarding reported systemic reductions of pro-inflammatory cytokines (Stewart et al. 2007; Smith et al. 1999; Conraads et al. 2002).

In conclusion, in untrained middle-aged men AE demonstrated the greatest capacity to up-regulate cytokine mRNA expression, and was the only mode to reduce muscle glycogen. Though there was no effect of any exercise mode on IL-6R mRNA expression, AE up-regulated receptor-based cytokine expression (TNF-R1, TNF-R2, IL-1ra) to a greater extent than RE and CE. RE induced comparable IL-6 mRNA expression as AE, though in contrast, this occurred in the absence of glycogen reduction. We have shown for the first time that duration-matched concurrent AE and RE results in ameliorated acute cytokine mRNA expression from 1-4h post-exercise, and warrants further research as to whether these acute findings may have chronic implications regarding exercise-induced adaptive processes. As such, in comparison to AE or RE, future research should determine whether it is a lesser dose (i.e. 50%) of each respective contractile stimulus, or the addition of these divergent stimuli that promotes reduced cytokine mRNA expression. Further enquiry should also appraise the relationship between intra-muscular glycogen reduction and cytokine receptor (TNF-R1/R2, IL-1ra) expression.

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Conflicts of Interest

There Authors wish to declare that there are no conflicts of interest associated with this manuscript.

References

1. Adamo K. & Graham T. 1998. Comparison of traditional measurements with macroglycogen and proglycogen analysis of muscle glycogen. J Appl. Physiol. 84(3):908-913.
2. Bell G., Syrotuik D., Martin T., Burnham R., & Quinney H. 2000. Effect of concurrent strength and endurance training on skeletal muscle properties and hormone concentrations in humans. Eur. J. Appl. Physiol. 81(5):418-427.
3. Buford T.W., Cooke M.B. & Willoughby D.S. 2009a. Resistance exercise-induced changes of inflammatory gene expression within human skeletal muscle. Eur J Appl. Physiol. 107(4):463-471.
4. Buford T.W., Cooke M.B., Shelmadine B.D., Hudson G.M., Redd L.R. & Willoughby D.S. 2009b Effects of eccentric treadmill exercise on inflammatory gene expression in human skeletal muscle. Appl. Physiol. Nutr. Metab. 34(4):745-753.
5. Bylund-Fellenius A.C., Ojamaa K.M., Flaim K.E., Li J.B., Wassner S.J., Jefferson L.S. 1984. Protein synthesis versus energy state in contracting muscles of perfused rat hindlimb. Am. J. Physiol-Endocrinol. Metab. 246(4):E297-E305.
6. Conraads V., Beckers P., Bosmans J., De Clerck L.S., Stevens W., Vrints C., Brutsaert D.L. 2002. Combined endurance/resistance training reduces plasma TNF-α receptor levels in patients with chronic heart failure and coronary artery disease. Eur. Heart. J. 23(23):1854-1860.
7. Dinarello, C. 1996. Biologic basis for interleukin-1 in disease. Blood. 87(6):2095-2147.
8. Donges C.E., Burd N.A., Duffield R., Smith G.C., West D.W.D., Short M.J., Mackenzie R.W., Plank L.D., Shepherd P.R. & Phillips S.M. 2012. Concurrent resistance and aerobic exercise stimulates both myofibrillar and mitochondrial protein synthesis in sedentary middle-aged men. J. Appl. Physiol. 112(12):1992-2001.
9. Donnelly J.E., Blair S.N., Jakacic J.M., Manore M.M., Rankin J.W. & Smith B.K. 2009. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. Med.Sci. Sport. Exerc. 41(2):459-471.
10. Evans W.J. 2010. Skeletal muscle loss: cachexia, sarcopenia, and inactivity. Am. J. Clin. Nutr. 91(4):1123-1127S.
11. Febbraio M., Rose-John S. & Pedersen B. 2010. Is interleukin-6 receptor blockade the Holy Grail for inflammatory diseases? Clin. Pharmacol. & Therapeut. 87 (4):396-398.
12. Friedmann-Bette B., Schwartz F.R., Eckhardt H., Billeter R., Bonaterra G., Kinscherf R. 2012. Similar changes of gene expression in human skeletal muscle after resistance exercise and multiple fine needle biopsies. J. Appl. Physiol.112(2):289-295.
13. Gleeson M., Bishop N.C., Stensel D.J., Lindley M.R., Mastana S.S., Nimmo M.A. 2011. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. Nature. Rev. Immunol. 11 (9):607-615.
14. Gray S., Robinson M. & Nimmo M. 2008. Response of plasma IL-6 and its soluble receptors during submaximal exercise to fatigue in sedentary middle-aged men. Cell Stress Chaper. 13 (2):247-251.
15. Griewe J.S., Cheng B., Rubin D.C., Yarasheski K.E. & Semenkovich C.F. 2001. Resistance exercise decreases skeletal muscle tumor necrosis factor α in frail elderly humans. FASEB J. 15 (2):475-482.
16. Haskell W.L., Lee I., Pate R.R., Powell K.E., Blair S.N., Franklin B.A., Macera C.A., Heath G.W., Thompson P.D. & Bauman A. 2007. Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. Med. Sci. Sport. Exerc. 39 (8):1423-1425.
17. Hawley, J.A. 2009. Molecular responses to strength and endurance training: Are they incompatible? Appl. Physiol. Nutr. Metabol. 34 (3):355-361.

18. Hiscock N., Chan M.H.S., Bisucci T., Darby I.A. & Febbraio M.A. 2004. Skeletal myocytes are a source of interleukin-6 mRNA expression and protein release during contraction: evidence of fiber type specificity. FASEB J. 18 (9):992-994.
19. Keller C., Steensberg A., Hansen A.K., Fischer C.P., Plomgaard P. & Pedersen B.K. 2005a. Effect of exercise, training, and glycogen availability on IL-6 receptor expression in human skeletal muscle. J Appl. Physiol. 99 (6):2075-2079.
20. Keller C., Steensberg A., Pilegaard H., Osada T., Saltin B., Pedersen B.K. & Neufer P.D. 2001. Transcriptional activation of the IL-6 gene in human contracting skeletal muscle: influence of muscle glycogen content. FASEB J. 15(14):2748-2750.
21. Keller P., Penkowa M., Keller C., Steensberg A., Fischer C.P., Giralt M., Hidalgo J. & Pedersen B.K. 2005b. Interleukin-6 receptor expression in contracting human skeletal muscle: regulating role of IL-6. FASEB J. 19(9):1181-1183.
22. Krook A. 2008. IL-6 and metabolism-new evidence and new questions. Diabetologia 51(7):1097-1099.
23. Louis E., Raue U., Yang Y., Jemiolo B. & Trappe S. 2007. Time course of proteolytic, cytokine, and myostatin gene expression after acute exercise in human skeletal muscle. J Appl. Physiol. 103(5):1744-1751.
24. MacDonald C., Wojtaszewski J.F., Pedersen B.K., Kiens B., & Richter E.A. 2003. Interleukin-6 release from human skeletal muscle during exercise: relation to AMPK activity. J. Appl. Physiol. 95(6):2273-2277.
25. Mendham A.E., Donges C.E., Liberts E.A. & Duffield R. 2011. Effects of mode and intensity on the acute exercise-induced IL-6 and CRP responses in a sedentary, overweight population. Eur. J. Appl. Physiol. 111(6):1035-1045.
26. Nader G.A. 2006. Concurrent strength and endurance training: from molecules to man. Med. Sci. Sport. Exerc. 38(11):1965-1971.
27. Nieman D.C., Davis J., Brown V.A., Henson D.A., Dumke C.L., Utter A.C., Vinci D.M., Downs M.F., Smith J.C., & Carson J. 2004. Influence of carbohydrate ingestion on immune changes after 2 h of intensive resistance training. J. Appl. Physiol. 96(4):1292-1298.
28. Nieman D.C., Davis J.M., Henson D.A., Walberg-Rankin J., Shute M., Dumke C.L., Utter A.C., Vinci D.M., Carson J.A. & Brown A. 2003. Carbohydrate ingestion influences skeletal muscle cytokine mRNA and plasma cytokine levels after a 3-h run. J. Appl. Physiol. 94(5):1917-1925.
29. Pedersen B.K. 2009. Edward F. Adolph distinguished lecture: muscle as an endocrine organ: IL-6 and other myokines. J. Appl. Physiol. 107(4):1006-1014.
30. Petersen, A.M.W. & Pedersen B.K. 2005. The anti-inflammatory effect of exercise. J. Appl. Physiol. 98(4):1154-1162.
31. Pfaffl M.W., Horgan G.W. & Dempfle L. 2002. Relative expression software tool (REST©) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic. Acid. Res. 30(9):e36-e36.
32. Pradhan A.D., Manson J.A.E., Rifai N., Buring J.E., & Ridker P.M. 2001. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA. 286(3):327-334.
33. Ridker P.M., Hennekens C.H., Buring J.E., & Rifai N. 2000. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. New. Eng. J. Med. 342 (12):836-843.
34. Rooyackers O.E., Adey D.B., Ades P.A. & Nair K.S. 1996. Effect of age on in vivo rates of mitochondrial protein synthesis in human skeletal muscle. Pro. Nat. Acad. Sci. 93 (26):15364-15369.
35. Ross R., Hudson R., Day A.G. & Lam M. 2012. Dose–response effects of exercise on abdominal obesity and risk factors for cardiovascular disease in adults: Study rationale, design and methods. Cont. Clin. Trials. 34(1): 155-160

36. Smith J.K., Dykes R., Douglas J.E., Krishnaswamy G., & Berk S. 1999. Long-term exercise and atherogenic activity of blood mononuclear cells in persons at risk of developing ischemic heart disease. JAMA. 281(18):1722-1727.
37. Steensberg A., Febbraio M.A., Osada T., Schjerling P., Van Hall G., Saltin B., & Pedersen B.K. 2001. Interleukin-6 production in contracting human skeletal muscle is influenced by pre-exercise muscle glycogen content. J. Physiol. 537(2):633-639.
38. Steensberg A., Fischer C.P., Keller C., Møller K. & Pedersen B.K. 2003. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. Am. J. Physiol.-Endocrinol. Metab.285(2):E433-E437.
39. Steensberg A., Keller C., Starkie R.L., Osada T., Febbraio M.A. & Pedersen B.K. 2002. IL-6 and TNF-α expression in, and release from, contracting human skeletal muscle. Am. J. Physiol.-Endocrinol. Metab. 283(6):E1272-E1278.
40. Stewart L.K., Flynn M.G., Campbell W.W., Craig B.A., Robinson J.P., Timmerman K.L., McFarlin B.K., Coen P.M. & Talbert E. 2007. The influence of exercise training on inflammatory cytokines and C-reactive protein. Med. Sci. Sport. Exerc. 39(10):1714-1719.
41. Tidball J.G. 2005. Inflammatory processes in muscle injury and repair. Am.J. Physiol.-Regul. Int. Comp. Physiol. 288(2):R345-353.
42. Vella LD, Caldow MK, Larsen AE, Tassoni DM, Della-Gatta PA, Gran P, Russell AP, Cameron-Smith D (2011) Resistance exercise increases nuclear factor-kappa B activity in human skeletal muscle. Am.J. Physiol.-Regul. Int. Comp. Physiol. 302(6): R667-673.
43. Yang Y., Creer A., Jemiolo B., & Trappe S. 2005. Time course of myogenic and metabolic gene expression in response to acute exercise in human skeletal muscle. J. Appl. Physiol. 98(5):1745-1752.

Table 1 – Subject baseline data.

Measure	**Value**
Age (*y*)	53.3 ± 1.8
Height (*cm*)	176.5 ± 2.0
Mass (*kg*)	90.2 ± 3.1
Body fat (*kg*)	27.0 ± 2.3
Body fat (*%*)	30.5 ± 1.7
Waist girth (*cm*)	100.0 ± 2.8
Waist : hip ratio	0.96 ± 0.02
Systolic BP (*mmHg*)	125 ± 3
Diastolic BP (*mmHg*)	82 ± 2
VO_{2peak} ($ml \cdot kg^{-1} \cdot min^{-1}$)	39.1 ± 2.9
W_{peak} (*W*)	235 ± 20
Leg extension 1RM (*kg*)	76 ± 5

Data are mean ± standard error of the mean (*n*=8). BP, blood pressure; W_{peak}, peak workload identified during graded exercise testing; 1RM, one-repetition maximum.