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Abstract: 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 postexercise molecular responses, it is necessary to determine whether cytokine mRNA expression may be diminished after CE. Eight middle-aged men (age, 53.3 Â±1.8 years; body mass index, 29.4 Â± 1.4 kgÂ·m(-2)) randomly completed (balanced for completion or ...

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

Running Title: Cytokine Expression to Exercise Mode.

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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 and 50% AE (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.

Introduction

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

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

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

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

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

Methods

Subjects

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

Baseline Test Procedures

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

Nutritional Procedures

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

Exercise Trial Procedures

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

Machine and ergometer settings documented during baseline testing were respectively standardized for the RE and CE trials (seat height position, seat backrest position, lever arm positioning) and AE

and CE trials (ergometer seat height and handlebar height). During cycling, telemetry-based heart rate (HR) (Vantage NV, Polar, Finland) was recorded every 5min, and pulmonary gas exchange was measured for 5min at 5 and 15min on a metabolic cart (Moxus modular oxygen uptake system; AEI Technologies, Pittsburgh, USA) calibrated for ventilation volume and fractional gas concentration. Rating of perceived exertion (RPE; CR 0-10 scale) was recorded after each set of leg extension exercise, every 5min during cycling exercise, and 10min post-session for all three trials.

Muscle Biopsy Procedures

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

Muscle Glycogen Procedures

Muscle glycogen analysis was carried out according to the acid hydrolysis method (Adamo and Graham 1998). Whilst remaining proximal to a bed of dry-ice, ~5-8mg of freeze-dried muscle was dissected of visible blood, fat or connective tissue. Samples were hydrolysed in weighed tubes with 500µl of 2M HCl and incubated in a heating block for 2h at 99°C. After incubation, tubes were re-weighed and any loss of weight was replaced with water. After weight normalization, 500µl of 2M NaOH was added for pH neutralization and tubes were vortexed for 1min. Samples were measured for glucose concentration (GEM primer 3500; Instrumentation Laboratory, Lexington, MA), of which the data are expressed as a normalized concentration relative to dry weight (Adamo and Graham 1998).

Real-Time Polymerase Chain-Reaction Procedures

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

Statistical Analysis

Data are presented as mean ± standard error of mean (SEM). A within-subject repeated measures design was used for the current study. All data were analysed using two-factor (condition × time) analysis of variance (ANOVA) with repeated measures. Where significant interactions were identified in the ANOVA, Tukey's pairwise comparisons were applied post-hoc to determine differences between means for main effect and interaction. For all analyses, statistical significance was accepted at $P<0.05$. All statistical procedures were conducted using PASW statistics (version 18.0 SPSS Inc, Chicago, IL) and the Relative Expression Software Tool (REST©) (Pfaffl et al. 2002).

Results

Heart Rate, VO_2 Consumption, and RPE

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

Changes of Muscle Glycogen

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

Post-Exercise Cytokine mRNA Expression

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

TNFα mRNA Expression

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

TNF-R1 and TNF-R2 mRNA Expression

TNF-R1 mRNA expression (Figure 3C) increased at 1h post-exercise after AE (1.7 ± 0.1; $P<0.05$ vs. CE) and RE (1.5 ± 0.2) ($P<0.05$); though not after CE ($P>0.05$). TNF-R1 expression at 4h post-

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

IL-1β mRNA Expression

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

IL-1ra mRNA Expression

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

IL-6 and IL-6R mRNA Expression

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

Discussion

Previous investigations have provided valuable context regarding acute cytokine mRNA expression responses to exercise, though typically incorporate young, normal weight, trained populations, and exercise modes that appear inappropriate for untrained, overweight, middle-aged populations (Louis

et al. 2007; Nieman et al. 2004; Nieman et al. 2003; Steensberg et al. 2001; Steensberg et al. 2003; Steensberg et al. 2002; Vella et al. 2011). The data of the current study contributes mode-specific, post-exercise cytokine expression information that may provide scope regarding associated chronic training responses to these modes. Specifically, data from this study suggests that: 1) in comparison to isolated RE or AE completion, duration-matched CE induces a reduced pro-inflammatory (TNFα/IL-1β) expression response during the 1-4h post-exercise period, and as will be discussed, may have implications regarding compensatory molecular mechanisms related to skeletal muscle hypertrophy; 2) RE is capable of up-regulating IL-6 mRNA expression (1-4h) in the absence of muscle glycogen depletion; 3) despite initial up-regulation of IL-6 mRNA expression after CE (1h), expression is ameliorated from 1-4h post-exercise, and may have bio-energetic adaptive implications given the reported role of IL-6 in substrate metabolism; 4) AE up-regulated cytokine receptor mRNA expression the greatest, whereas RE and CE induced a lesser response. Chronic changes in proteins are reportedly the result of cumulative effects of transient changes in mRNA transcription (Yang et al. 2005). As such, reduced receptor expression may prospectively indicate reduced systemic abundance of these receptors, and a diminished capacity to bind or coordinate respective pro-inflammatory member's implicated in chronic low-grade systemic inflammation (i.e. TNFα, IL-1β, etc).

In the present study TNFα and IL-1β mRNA were equivalently up-regulated in expression at 1h post-exercise by all modes. However, expression remained elevated at 4h post-exercise after RE and AE, though returned to non-significant levels after CE. Numerous studies have reported that CE training results in diminished muscle cross-sectional area and strength gains in comparison to RE training (Bell et al. 2000; Kraemer et al. 1995; Nelson et al. 1990). The high-intensity contractions inherent in a bout of RE subject myofibers to injurious forces and the induction of a transient inflammatory response (Tidball 2005; Vella et al. 2011). Respondent mononuclear cells, such as neutrophils and macrophages, can up-regulate TNFα and IL-1β expression and facilitate mechanisms related to cell cycle and apoptosis in compromised myocytes, thus initiating repair and remuneration processes related to hypertrophy (Steensberg et al. 2002; Louis et al. 2007; Vella et al. 2011). Accordingly, it may be that acute cytokine responses assist explaining the modulation of these hypertrophic processes

(Vella et al. 2011). Conversely, a counter view point suggest that as muscle protein synthesis (MPS) is inhibited when ATP availability is compromised (Bylund-Fellenius et al. 1984), the AE component of CE may acutely antagonise MPS responses to CE, and chronically result in an attenuated hypertrophy response in comparison to isolated RE completion (Nader 2006). Regardless, taken together with previous comparisons of duration-matched CE and RE, wherein only RE up-regulated myogenin and differentiation expression (Donges et al. 2012), the reduced post-exercise TNFα/IL-1β expression in the current study may be indicative of diminished gains in muscle mass after CE in comparison to RE.

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

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

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

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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.

Figure Captions

Figure 1. Dual-energy x-ray absorptiometry (DXA); one repetition-maximum (1RM); graded exercise test (GXT); carbohydrate (CHO); resistance exercise (RE): 8 sets x 8 leg extension repetitions at 70% one repetition maximum (1RM), interspersed by 150s rest; Aerobic exercise (AE): 40min of stationary cycling at 55% peak graded exercise test (GXT) workload; concurrent exercise (CE): 50% of RE immediately followed by 50% AE. Muscle specimen collection.

Figure 2. Data are mean ± standard error of mean. (A) Rating of perceived exertion (RPE); RE^1, resistance exercise time-points (S, set); AE^2, aerobic exercise time-points (5min intervals); CE^3, combined exercise, S for RE component and 5min intervals for AE component, respectively. (B) Heart rate (HR). (C) VO_2 measured for 5min at 5 and 15min. *RE different from AE, P<0.05; †CE different from AE, P<0.05; ‡RE different from CE, P<0.05; #AE different from CE, P<0.05.

Figure 3. Data are mean ± standard error of mean fold-change of muscle glycogen concentration (A); mRNA expression of TNFα (B); TNF-R1 (C); TNF-R2 (D); IL-1β (E); IL-1ra (F); IL-6 (G), and IL-6R (H) measured pre-, and 1h and 4h after resistance exercise (RE), aerobic exercise (AE), or concurrent exercise (CE). *Different from Pre, P<0.05; †different to RE 1h, P<0.05; ‡different to RE 1h, P<0.05; #different to RE 1h, P<0.05; ^different to CE 4h, P<0.05.

Figure 4. Data are mean ± standard error of mean 1h and 4h post-exercise fold changes of cytokine mRNA expression as compared to pre-exercise expression (represented as a zero baseline). Fold changes of expression are shown after resistance exercise (RE), aerobic exercise (AE), or concurrent exercise (CE). Graph A includes tumor necrosis factor-α (TNFα), interleukin-6 (IL-6), and IL-1β expression responses. Graph B includes TNF receptor 1 (TNF-R1), TNF-R2, IL-6R, and IL-1 receptor antagonist (IL-1ra) responses.