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RESEARCH ARTICLE

Arachidonic acid supplementation transiently augments the acute inflammatory response to resistance exercise in trained men

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Markworth JF, D'Souza RF, Aasen KM, Mitchell SM, Durainayagam BR, Sinclair AJ, Peake JM, Egner IM, Raastad T, Cameron-Smith D, Mitchell CJ. Arachidonic acid supplementation transiently augments the acute inflammatory response to resistance exercise in trained men. *J Appl Physiol* 125: 271–286, 2018. First published April 26, 2018; doi:10.1152/japplphysiol.00169.2018.—Strenuous exercise can result in skeletal muscle damage, leading to the systemic mobilization, activation, and intramuscular accumulation of blood leukocytes. Eicosanoid metabolites of arachidonic acid (ARA) are potent inflammatory mediators, but whether changes in dietary ARA intake influence exercise-induced inflammation is not known. This study investigated the effect of 4 wk of dietary supplementation with 1.5 g/day ARA ($n = 9$, 24 ± 1.5 yr) or corn-soy oil placebo ($n = 10$, 26 ± 1.3 yr) on systemic and intramuscular inflammatory responses to an acute bout of resistance exercise (8 sets each of leg press and extension at 80% one-repetition maximum) in previously trained men. Whole EDTA blood, serum, peripheral blood mononuclear cells (PMBCs), and skeletal muscle biopsies were collected before exercise, immediately postexercise, and at 2, 4, and 48 h of recovery. ARA supplementation resulted in higher exercise-stimulated serum creatine kinase activity [incremental area under the curve (iAUC) $P = 0.046$] and blood leukocyte counts (iAUC for total white cells, $P < 0.001$; neutrophils: $P = 0.007$; monocytes: $P = 0.015$). The exercise-induced fold change in peripheral blood mononuclear cell mRNA expression of interleukin-1β (*IL1B*), CD11b (*ITGAM*), and neutrophil elastase (*ELANE*), as well as muscle mRNA expression of the chemokines interleukin-8 (*CXCL8*) and monocyte chemoattractant protein 1 (*CCL2*) was also greater in the ARA group than placebo. Despite this, ARA supplementation did not influence the histological presence of leukocytes within muscle, perceived muscle soreness, or the extent and duration of muscle force loss. These data show that ARA supplementation transiently increased the inflammatory response to acute resistance exercise but did not impair recovery.

NEW & NOTEWORTHY Daily arachidonic acid supplementation for 4 wk in trained men augmented the acute systemic and intramuscular inflammatory response to a subsequent bout of resistance exercise. Greater exercise-induced inflammatory responses in men receiving arachidonic acid supplementation were not accompanied by increased symptoms of exercise-induced muscle damage. Although increased dietary arachidonic acid intake does not appear to influence basal inflammation in humans, the acute inflammatory response to exercise stress is transiently increased following arachidonic acid supplementation.

inflammation; omega-6; PUFA; skeletal muscle; supplement

INTRODUCTION

Intense or unaccustomed exercise involving eccentric muscular contractions can result in skeletal muscle damage, characterized by an efflux of intramuscular proteins, temporary loss of muscle force-generating capacity, and delayed onset muscle soreness (55). Proinflammatory mediators, including cytokines/chemokines (53) and eicosanoids (42), transiently increase in blood and muscle following resistance exercise. In response to these chemical signals, white blood cells (leukocytes) are mobilized into the systemic circulation (20) and recruited to the exercised musculature (55). Neutrophils accumulate within muscle in the early hours of recovery (56, 60, 84). This is followed by the migration of blood monocytes, which (depending on the extent of tissue damage inflicted) may infiltrate within muscle and differentiate locally into tissue macrophages (52). Inflammation has classically been considered to contribute to exercise-induced muscle pain, swelling, and loss of function (72). However, it is now generally accepted that the acute inflammatory response also plays an important supportive role in muscle growth and regeneration (15, 54, 78).

Arachidonic acid (ARA) (20:4n-6) is a long-chain omega-6 (n-6) polyunsaturated fatty acid (PUFA) and a key component of mammalian cells (45). ARA is typically consumed at ~0.15 g/day in standard Western diets (27, 41). Dietary ARA is rapidly incorporated into plasma and tissue membrane phospholipids, where it remains esterified at rest (43, 86). In response to injurious or inflammatory stimuli, ARA is released from membrane phospholipids through the action of phospholipase A_2 from both leukocytes (3, 38) and muscle cells (32,

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83). Free intracellular ARA can then be converted to prostaglandins (e.g., PGE_2) through the cyclooxygenase (COX)-1 and -2 pathway and to leukotrienes (e.g., LTB_4) through the 5-lipoxygenase (5-LOX) pathway (31). PGE_2 and LTB_4 both have a number of potentially proinflammatory actions, including increasing vascular permeability (19, 88) and vasodilation (87), neutrophil chemotaxis (1, 35), sensation of pain (37), and cytokine release by leukocytes (23, 36, 66) and muscle cells (34, 74). Furthermore, ARA itself can also act directly as a second messenger, independent of eicosanoid biosynthesis, to stimulate leukocyte adhesion (8), degranulation (8, 73), oxidative burst (10, 46, 65), and cytokine release (5).

On the basis of the key role of ARA in the inflammatory response, it has been suggested that an excessive dietary ARA intake may promote inflammation (71). Contrary to this hypothesis, a number of randomized controlled human trials have found that substantial increases in dietary ARA intake appear to have little (or no) effect on basal levels of systemic inflammation in otherwise healthy adults (13, 28). We recently reported that 4 wk of dietary supplementation with 1.5 g/day ARA modulated plasma and muscle lipid composition but had no greater effect than corn-soy oil placebo on systemic and intramuscular inflammation at rest in young men participating in resistance exercise training (43). In rodent studies, the abundance of ARA in leukocyte phospholipids has been shown to be directly related to the capacity of those cells to release free ARA and produce PGE_2 and LTB_4 in response to an inflammatory challenge (57, 86). Therefore, although dietary ARA supplementation has little effect on basal inflammation in otherwise healthy individuals, increases in tissue ARA content that occur following a period increased dietary intake may transiently modulate immune cell responses to an acute injurious or inflammatory stimulus such as exercise-induced muscle damage.

Therefore, the aim of the current study was to investigate the effect of 4 wk of dietary supplementation with 1.5 g/day ARA or corn-oil placebo on the acute inflammatory response to a subsequent bout of resistance exercise. It was hypothesized that previously trained men who received dietary ARA supplementation daily for 4 wk prior would transiently exhibit a greater inflammatory response to a bout of resistance exercise and that greater acute inflammation would be associated with increased symptoms of exercise-induced muscle damage.

METHODS

Participants

Twenty-one healthy young active men (18−35 yr of age) were recruited from the Auckland, New Zealand region. All participants had been undertaking a regular resistance exercise training program for ≥1 yr, which incorporated at least one leg-based training session per week. Participants were free of any existing injuries, cardiovascular, musculoskeletal, or metabolic disease and were not taking any medications or performance enhancing drugs. The experimental protocol was explained to the participants, and informed written consent to participate was obtained. Ethics approval was provided by the Northern Health and Disability Ethics Committee (New Zealand) (14/NTA/147). The clinical trial was registered with the Australian New Zealand Clinical Trial Registry (ANZCTR) (ACTRN1261-5000710527).

Experimental Design

Participants were randomized using sequences generated using the website https://www.random.org to receive dietary supplementation with 1.5 g/day ARA ($n = 11$) or placebo ($n = 10$) for 4 wk. Both the participants and investigators were blinded to the identity of the supplement capsules for the duration of the study and until completion of sample analysis. The dose of ARA tested was based on a recent study that reported apparent chronic ergogenic effects of 1.5 g/day ARA supplementation in men participating in a resistance training program (18). Additionally, this amount of ARA is typical of that provided in a daily recommended dose of dietary supplements currently marketed to athletes and body builders (e.g., 18, 64). Two participants randomized to the ARA group were lost to follow-up (43). Therefore, results are presented here for $n = 10$ in the placebo group and $n = 9$ in the ARA group. The fatty acid composition of the ARA and placebo capsules, changes in resting blood and muscle fatty acid profile, body composition, clinical parameters, as well as systemic and intramuscular markers of basal inflammation in these same participants in a resting state before and after the 4-wk supplementation period have been previously reported (43). Briefly, ARA supplementation increased the ARA content of both plasma and muscle tissue lipids to a greater extent than placebo, and this was associated with a reduction in the relative abundance of the n-3 PUFA eicosapentaenoic acid in plasma and α-linolenic acid in muscle (43). At the beginning of the supplementation period, strength testing was performed to determine the participants' one-repetition maximum (1RM). The maximal weight that participants could lift for three to six repetitions (3–6 RM) on the leg press and leg extension exercises was determined, and the participants' 1RM was estimated using the Brzycki equation (62). At the completion of the 4-wk supplementation period, the participants returned to the laboratory to perform an acute resistance exercise trial. The participants were instructed to schedule their habitual training regimen so as to abstain from any leg-based resistance training for at least 72 h before the experimental trial day and to avoid any vigorous physical activity or consumption of alcohol in the preceding 24 h, as well as throughout subsequent 48 h postexercise recovery monitoring period. On the evening before each laboratory visit, the participants fasted from 10 PM onwards.

Dietary Supplements

ARASCO capsules (ARASCO, 1 g VegCap, 396 mg ARA), containing a mixture of high-oleic sunflower oil and an ARA-enriched oil extracted from the unicellular fungus *Mortierella alpina* and placebo capsules (Pbo, Corn-Soy, 1 g VegCap) containing a soy-corn oil blend, were both provided by DSM Nutritional Products (Heerlen, The Netherlands). The complete fatty acid composition of the placebo and ARA capsules as determined by GC-MS analysis has been reported previously (43). The ARA group received an estimated dose of 1.5 g/day ARA through oral ingestion of four 1-g ARASCO capsules each day. The placebo group received 4 g/day of corn-soy oil blend administered as four 1-g placebo capsules daily. One dose of two 1-g capsules was consumed in the morning, and a second dose of two 1-g capsules was consumed in the evening each day for 28 days, during which time the participants were instructed to maintain their habitual diet and resistance exercise training regimen.

Acute Resistance Exercise Trial

Following completion of the 4-wk dietary supplementation period, the participants arrived at the laboratory ~7 AM in a rested and fasted stated. An intravenous cannula (20-gauge) was inserted into an antecubital vein, and a slow saline drip was used to keep the cannula patent. During a 2-h preexercise period, body composition and resting measures of isometric muscle force and muscle soreness were obtained (see below). Participants then rested in a supine position for ~30 min before collection of a preexercise muscle biopsy and blood

sample. The participants then performed a single bout of bilateral resistance exercise consisting of eight sets of horizontal leg press, followed by eight sets of seated knee extensions. The first 2 sets of leg press were performed for 10 repetitions, with the load increasing from 50−70% of 1RM as a warm-up. The remaining 6 sets of horizontal leg press, and 8 sets of seated knee extensions were performed at 80% of predetermined 1RM for 8−10 repetitions. Exercises were performed with 2 min of rest between each set and between exercises. If participants were able to complete ≥10 repetitions on the final set of each exercise, they were verbally encouraged to continue until muscular failure. The exercise protocol took ~45 min to complete. Following completion of the exercise protocol, the participants rested in a supine position throughout an acute 4-h recovery period. The participants returned to the laboratory at 24 and 48 h postexercise in a fasted stated for follow up testing. The participants continued to consume the allocated dietary supplements throughout the monitored 48-h postexercise recovery period but consumed their allocated morning doses following completion of morning laboratory tests so as to avoid any potential acute effects of ARA ingestion on experimental outcomes.

Blood Collection and Handling

Venous blood samples were collected before exercise, immediately postexercise (within 30 s of muscular failure), and again at 2, 4, 24, and 48 h of recovery. Blood samples were collected into BD Vacutainer K_2 EDTA tubes (BD 367525) for whole blood complete cell counts and isolation of peripheral blood mononuclear cells (PBMCs) or BD Vacutainer Plastic Serum Tubes (Silica; BD 368975) for serum separation. Serum tubes were allowed to clot at room temperature for 15 min before centrifugation at 1,500 *g* for 15 min at 4°C. Aliquots (~1 ml) of serum were immediately prepared, flash frozen in liquid nitrogen and stored at −80°C until further analysis.

Serum and Whole Blood Analyses

Blood serum samples were assayed for creatine kinase (CK) activity and myoglobin concentration as systemic markers of muscle damage using a Roche C311 autoanalyzer (Roche, Mannheim, Germany) by enzymatic colorimetric assay. Complete blood cell counts were performed on whole EDTA blood (1 ml) by LabPLUS (Auckland, New Zealand) to determine circulating leukocyte number using a Sysmex XN-10 hematology analyzer (Auckland, New Zealand). For isolation of PBMCs, whole EDTA blood (2 ml) was layered over 2 ml of Histopaque solution (Sigma-Aldrich, St. Louis, MO) and centrifuged for 30 min at 400 *g* at room temperature. PBMCs were aspirated from the upper layer interface, washed twice with phosphate buffered saline, and pelleted by centrifugation at 250 *g* for 10 min at room temperature. PBMC cell pellets were immediately homogenized with 600 μl RLT plus RNA lysis buffer (Qiagen, Hilden, Germany), and then stored at −80°C until subsequent RNA extraction.

Skeletal Muscle Biopsy Sampling

Skeletal muscle biopsies (~100 mg) were obtained at rest before exercise, within 10 min postexercise, and again at 2, 4, and 48 h of recovery. All biopsy samples were taken from the m. vastus lateralis while subjects were under local anesthesia (1% Xylocaine) by percutaneous needle biopsy technique modified for manual suction using a 5-mm Bergstöm biopsy needle. Biopsy samples for molecular analysis were rapidly frozen in liquid nitrogen and stored at −80°C until further analyses. A portion of the preexercise and 48 h postexercise biopsies was prepared for immunohistological staining by mounting the muscle tissue in optimal cutting temperature compound and then freezing the tissue rapidly in isopentane cooled on liquid nitrogen. The preexercise, immediately postexercise, and 2- and 4-h recovery biopsies were obtained from the nondominant limb through separate incisions 2−3 cm apart moving proximally to distally with sequential biopsies. The 48-h postexercise biopsy was obtained from the contralateral (dominant) limb.

RNA Extraction from PBMCs and Muscle Tissue

Total RNA was extracted from PBMC cell pellets and ~20 mg of frozen muscle tissue using the AllPrep DNA/RNA/miRNA Universal Kit (Qiagen, Hilden, Germany), as previously described (16). RNA concentration was measured using a NanoPhotometer N60 (Implen, Westlake Village, CA). RNA (0.5 μg for PBMCs and 1 μg for muscle) was reverse transcribed to cDNA using a High Capacity RNA-to-cDNA Kit (Life Technologies, Carlsbad, CA).

Real-Time polymerase Chain Reaction Analysis

RT-PCR was performed on a LightCycler 480 (Roche Applied Science, Penzberg, Germany) using SYBR Green I DNA-binding dye. Samples were analyzed in duplicate 10 μL reaction volumes. Results are expressed as each participant's fold change in mRNA expression from their respective resting preexercise sample following normalization to an endogenous control using the $2^{-\Delta\Delta Ct}$ method. The geometric mean of human charged multivesicular body protein 2A (*CHMP2A*), hypoxanthine guanine phosphoribosyl transferase (*HPRT*), and valosin containing protein (*VCP*) mRNA expression was used as the endogenous control. Primers (Table 1) were obtained from Invitrogen (Life Technologies, Carlsbad, CA).

Table 1. *RT-PCR primer sequences*

Target	Primer Sequence
ITGAM	
Forward	TCAGGTGGTGAAAGGCAAGG
Reverse	ATCTGTCCTTCTCTTAGCCGA
ELANE	
Forward	CGTGGCGAATGTAAACGTCC
Reverse	TTTTCGAAGATGCGCTGCAC
CD68	
Forward	GCTACTGGCAGCCCAGG
Reverse	CGTGAAGGATGGCAGCAAAG
CD163	
Forward	GCGGCTTGCAGTTTCCTCAA
Reverse	CTGAAATCAGCTGACTCATGGGA
MRC1	
Forward	CGATCCGACCCTTCCTTGAC
Reverse	TGTCTCCGCTTCATGCCAT
IL1B	
Forward	TTCGAGGCACAAGGCACAA
Reverse	TGGCTGCTTCAGACACTTGAG
IL6	
Forward	TCAATGAGGAGACTTGCCTGG
Reverse	GGGTCAGGGGTGGTTATTGC
TNF	
Forward	AGCCCATGTTGTAGCAAACC
Reverse	TGAGGTACAGGCCCTCTGAT
CXCL2	
Forward	GAAAGCTTGTCTCAACCCCG
Reverse	TGGTCAGTTGGATTTGCCATTTT
CXCL8	
Forward	ACCGGAAGGAACCATCTCAC
Reverse	GGCAAAACTGCACCTTCACAC
CCL2	
Forward	GCAATCAATGCCCCAGTCAC
Reverse	CTTGAAGATCACAGCTTCTTTGGG
HPRT	
Forward	CCTGGCGTCGTGATTAGTGAT
Reverse	TCGAGCAAGACGTTCAGTCC
CHMP2A	
Forward	CGCTATGTGCGCAAGTTTGT
Reverse	GGGGCAACTTCAGCTGTCTG
VCP	
Forward	AAACTCATGGCGAGGTGGAG
Reverse	TGTCAAAGCGACCAAATCGC

Recovery of Muscle Force

Unilateral maximal isometric knee extension and flexion torque was tested at a knee angle of 90° using a Biodex System 4 Quickset isokinetic dynamometer (Shirley, NY). Before the exercise bout on the morning of the experimental trial day, participants performed three 5-s maximal voluntary knee extension and knee flexion contractions (MVC) with 30-s of rest between repetitions. Further single repetition 5-s knee extension and knee flexion MVCs were performed immediately postexercise (≤5 min) and then again at 4, 24, and 48 h of recovery. All muscle force measurements were performed on the dominant limb to avoid any potential influence of the preexercise and early postexercise biopsies on muscle force-generating capacity. The final MVC at 48 h of recovery was also performed on the dominant limb but before the final 48 h postexercise biopsy (which was also collected from the dominant limb) to avoid any potential effect of the biopsy procedure on muscle force-generating capacity.

Muscle Soreness

Participants were asked to rate the extent of perceived muscle soreness by marking a vertical line on a 0−100 mm visual analog scale (VAS), with 0 representing no muscle soreness and 100 mm representing extreme muscle soreness. Muscle soreness was assessed while seated (at rest), slowly rising from a chair (concentric loading), and slowly lowering into a chair (eccentric loading). VAS assessments were completed before exercise, immediately postexercise (≤2 min), and again at 4, 24, and 48 h of recovery. For all measures of muscle soreness, participants were told to focus on sensation in their nonbiopsied (dominant limb) to avoid any potential confounding influence of the muscle biopsy procedure on perception of exercise-induced muscle soreness. Muscle soreness at 48 h of recovery was also assessed on the dominant limb prior but before the final 48 h MVC testing and muscle biopsy (which was also collected from the dominant limb) so as to avoid any confounding influence of these tests on perceived muscle soreness.

Immunohistochemistry

A portion of the preexercise and 48 h postexercise muscle biopsies was used for immunohistological analysis of intramuscular leukocytes (neutrophils and macrophages). Optimal cutting temperature embedded muscle tissue was cryosectioned at 8 μm, air-dried at room temperature, and stored at −80°C. Before immunostaining, frozen sections were air dried at room temperature and subsequently fixed with 1% PFA for 7 min. Sections were permeabilized with 0.2% Triton-X in PBS for 20 min and blocked with 1% bovine serum albumin/20% goat serum/1% dry milk/0.2% Triton-X in PBS for 1 h at room temperature. Sections were then incubated with primary antibodies at 4°C overnight followed by secondary antibodies for 1 h at room temperature. The following primary antibodies were used: rabbit anti-laminin (1:100; Z0097; Dako) to stain the basal lamina surrounding each myofiber, mouse anti-CD66b (1:100; No. CLB-B13.9; Sanquin Reagents) to stain for neutrophils, and mouse anti-CD68 (1:100; No. EBM-11; DakoCytomation) to stain for macrophages. Secondary antibodies used were goat anti-mouse (1:200; Alexa Fluor 488; Invitrogen) and goat anti-rabbit (1:200; Alexa Fluor 594; Invitrogen). Nuclei were counterstained with DAPI (ProLong Gold Antifade Reagent with DAPI, P36935; Invitrogen). Sections were imaged using an Axi-ocam camera (Zeiss, Oberkochen, Germany) mounted on an Axioskop-2 light microscope (Zeiss). Immune cells were identified by DAPI staining surrounded by anti-CD68 or anti-CD66b staining and are presented as the number of positive cells per myofiber.

Statistical Analysis

All data were checked for normality with Shapiro-Wilk test. Where necessary, data were log transformed to obtain a normal distribution before statistical analysis. Differences were assessed with a two-way ANOVA with time as a within-participant factor and group as a between-participant factor. Following a significant main effect of time or time × group interaction effect, changes over time from preexercise were assessed with Holm-Sidak post hoc tests. Following a significant main effect of group or time × group interaction effect, differences between the placebo and ARA group were tested at each individual time point with Holm-Sidak post hoc tests. Differences in cumulative responses over time between groups were determined by calculating the incremental area under the curve (iAUC), which was compared between groups with two-tailed independent samples *t*-tests. Statistical analysis was performed using SigmaPlot 12.3 (Systat Software, Chicago, IL). All data are reported as means ± SE, and statistical significance was determined at $P \leq 0.05$.

RESULTS

Muscle Damage and force

Blood markers of muscle damage are presented in Fig. 1. Time × group interaction effects were observed for serum CK activity ($P = 0.008$) (Fig. 1*A*) and serum myoglobin concentration ($P = 0.016$) (Fig. 1*B*). Serum CK activity increased at 4 h postexercise in the ARA group ($P = 0.029$) but was unchanged in the placebo group ($P = 0.539$) (Fig. 1*A*). Serum CK activity was increased in both groups at 24 h ($P < 0.001$) but remained elevated at 48 h of recovery only in the ARA group (ARA: $P < 0.001$; placebo: $P = 0.324$) (Fig. 1*A*). The iAUC for the overall serum CK response was greater in the ARA group compared with the placebo group ($P = 0.046$). Serum myoglobin concentration increased in both groups at 2 h (placebo: $P = 0.003$; ARA: $P < 0.001$) and 4 h (placebo: $P = 0.028$; ARA: $P < 0.001$) but was higher in the ARA group compared with placebo at both 2 h ($P = 0.008$) and 4 h ($P = 0.020$) (Fig. 1*B*). Serum myoglobin concentration no longer differed from preexercise at 24 h in either the placebo group ($P = 0.060$) or the ARA group ($P = 0.141$). There was no difference between groups in the iAUC for the overall serum myoglobin response ($P = 0.117$).

Muscle force produced during maximal isometric knee extension is displayed in Fig. 1*C*. There was a main effect of time ($P < 0.001$) but no effect of group ($P = 0.480$) or time × group interaction effect ($P = 0.750$). Muscle force decreased immediately postexercise in both the placebo group ($P < 0.001$) and the ARA group ($P < 0.001$). Force remained reduced at 4 h in the placebo group ($P = 0.008$) but no longer differed from preexercise levels in the ARA group ($P = 0.346$). Muscle force had recovered to preexercise levels at 24 and 48 h of recovery in both the placebo group (24 h: $P = 0.838$; 48 h: $P = 0.722$) and the ARA group (24 h: $P = 0.760$; 48 h: $P = 760$). The overall iAUC for loss knee extension MVC did not differ between groups ($P = 0.499$).

Muscle Soreness

Muscle soreness as determined by VAS is displayed in Fig. 2. Main effects of time were found for muscle soreness at rest (time: $P < 0.001$; group: $P = 0.154$; time × group: $P = 0.187$) (Fig. 2*A*), during concentric (CON) loading (time: $P < 0.001$; group: $P = 0.221$; time × group: $P = 0.085$) (Fig. 2*B*), and during eccentric (ECC) loading (time: $P < 0.001$; group: $P = 0.143$; time × group: $P = 0.303$) (Fig. 2*C*). Resting soreness

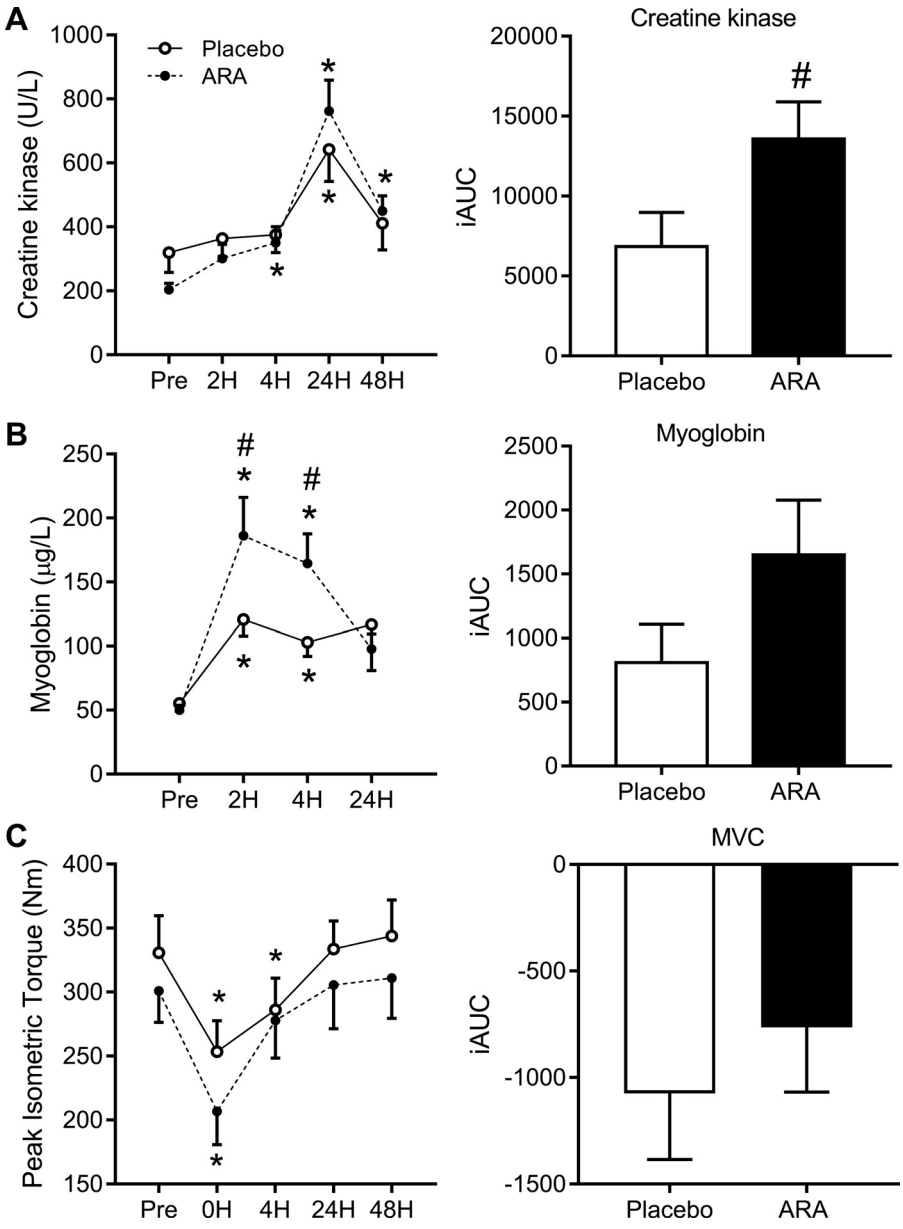

Fig. 1. Blood markers of muscle damage. Serum creatine kinase activity (U/l; *A*), serum myoglobin concentration (μg/l; *B*), and peak torque generated during isometric knee extension (*C*) following an acute bout of resistance exercise in men receiving dietary supplementation with arachidonic acid (ARA) or placebo. iAUC, incremental area under the curve. Values are means ± SE. *$P < 0.05$, different vs. preexercise within group. #$P < 0.05$, different between groups.

increased immediately postexercise in the placebo group ($P < 0.001$) and persisted until 48 h of recovery ($P = 0.014$) (Fig. 2*A*). Resting soreness also increased immediately postexercise in the ARA group ($P = 0.001$) but was no longer elevated at 4 h ($P = 0.404$), 24 h ($P = 0.237$), or 48 h ($P = 0.371$) of recovery (Fig. 2*A*). Soreness under load increased immediately postexercise in the placebo group (CON: $P < 0.001$; ECC: $P < 0.001$) and persisted at 48 h (CON $P = 0.021$ ECC $P = 0.013$) (Fig. 2, *B* and *C*). Soreness under load also increased immediately postexercise in the ARA group also (CON: $P < 0.001$; ECC: $P = 0.015$), and persisted at 24 h (CON: $P = 0.018$; ECC: $P = 0.042$) but was no longer elevated at 48 h (CON: $P = 0.278$; ECC: $P = 0.180$) (Fig. 2, *B* and *C*). There were statistical trends toward lower muscle soreness iAUC in the ARA group than the placebo group when resting ($P = 0.092$) and during concentric loading ($P = 0.096$) but not during eccentric loading ($P = 0.173$).

Blood Leukocyte Counts

Whole blood immune cell counts for total white cells, neutrophils, and monocytes are displayed in Fig. 3. Time × group interaction effects were observed for total circulating white blood cells ($P = 0.022$) (Fig. 3*A*) and blood monocytes ($P = 0.037$) (Fig. 3*C*). Additionally, a main effect of time ($P < 0.001$) but no effect of group ($P = 0.292$) or time × group interaction ($P = 0.126$) was observed for blood neutrophils (Fig. 3*B*). Total white cells increased at 2 h ($P < 0.001$) and 4 h ($P < 0.001$) postexercise in both groups but remained elevated at 24 h only in the ARA group (ARA: $P < 0.001$; placebo: $P = 0.719$) (Fig. 3*A*). Neutrophils also increased from preexercise in both groups at 2 h ($P < 0.001$) and 4 h ($P < 0.001$) but remained elevated at 24 h only in the ARA group (ARA: $P = 0.002$; placebo: $P = 0.873$) (Fig. 2*B*). Monocytes increased in the ARA group at 2 h ($P < 0.001$), 4 h ($P = 0.022$), and 24 h

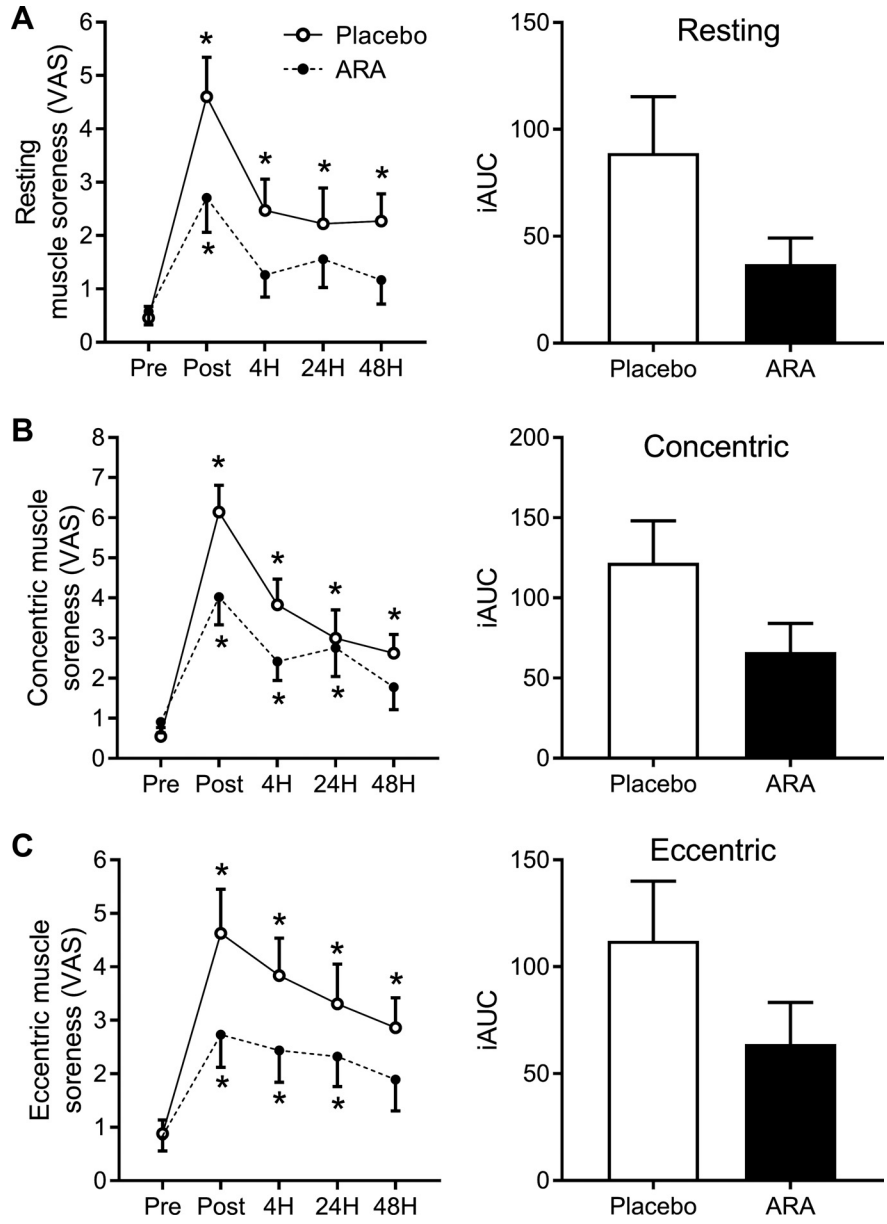

Fig. 2. Perceived muscle soreness. Muscle soreness determined by visual analog scale (VAS) while seated (resting) (*A*), rising from a chair (concentric loading) (*B*), and descending into a chair (eccentric loading) (*C*) following an acute bout of resistance exercise in men receiving dietary supplementation with arachidonic acid (ARA) or placebo. iAUC, incremental area under the curve. Values are means ± SE. *$P < 0.05$, different vs. preexercise within group.

($P < 0.001$) but were not changed in the placebo group at 2 h ($P = 0.771$), 4 h ($P = 0.954$), 24 h ($P = 0.914$), and 48 h ($P = 0.934$) (Fig. 2*C*). Following 48 h of recovery, total white blood cell (placebo: $P = 0.719$; ARA: $P = 0.147$) and neutrophil (placebo: $P = 0.869$; ARA: $P = 0.269$) counts no longer differed from preexercise in either group. There was, however, a statistical trend for blood monocytes to remain elevated at 48 h in the ARA group ($P = 0.063$) but not the placebo group ($P = 0.934$). The cumulative change in blood leukocyte number throughout 0−48 h of recovery (as determined by iAUC) was greater in the ARA group than the placebo group for total white blood cells ($P < 0.001$), blood neutrophils ($P < 0.001$), and blood monocytes ($P = 0.030$).

Whole blood immune cell counts for lymphocytes, eosinophils, and basophils are displayed in Fig. 4. Blood lymphocytes and eosinophils showed main effects of time (both $P < 0.001$) but no effect of group ($P = 0.244$ and $P = 0.947$, respectively) or time × group interaction effects ($P = 0.498$ and $P = 0.738$, respectively). Lymphocyte counts decreased at 2 h postexercise in both the placebo group ($P < 0.001$) and the ARA group ($P = 0.001$) but had returned to resting levels by 4 h of recovery in both groups (placebo: $P = 0.086$; ARA: $P = 0.981$) (Fig. 4*A*). Eosinophil counts decreased in both groups at 2 h (both $P = 0.001$) and 4 h postexercise (both $P < 0.001$) but returned to resting levels at 24 h (placebo: $P = 0.255$; ARA: $P = 0.954$) and 48 h (placebo: $P = 0.886$; ARA: $P = 0.840$) of recovery (Fig. 4*B*). Basophil counts showed no effect of time ($P = 0.456$), group ($P = 0.726$), or group × time interaction ($P = 0.834$) (Fig. 4*C*). There were no differences between groups in the iAUC for blood lymphocytes ($P = 0.228$), eosinophils ($P = 0.741$), or basophils ($P = 0.713$).

Inflammatory Gene Expression

Immune cell markers. The expression of the general leukocyte marker CD11b (*ITGAM*), the granulocyte marker neutro-

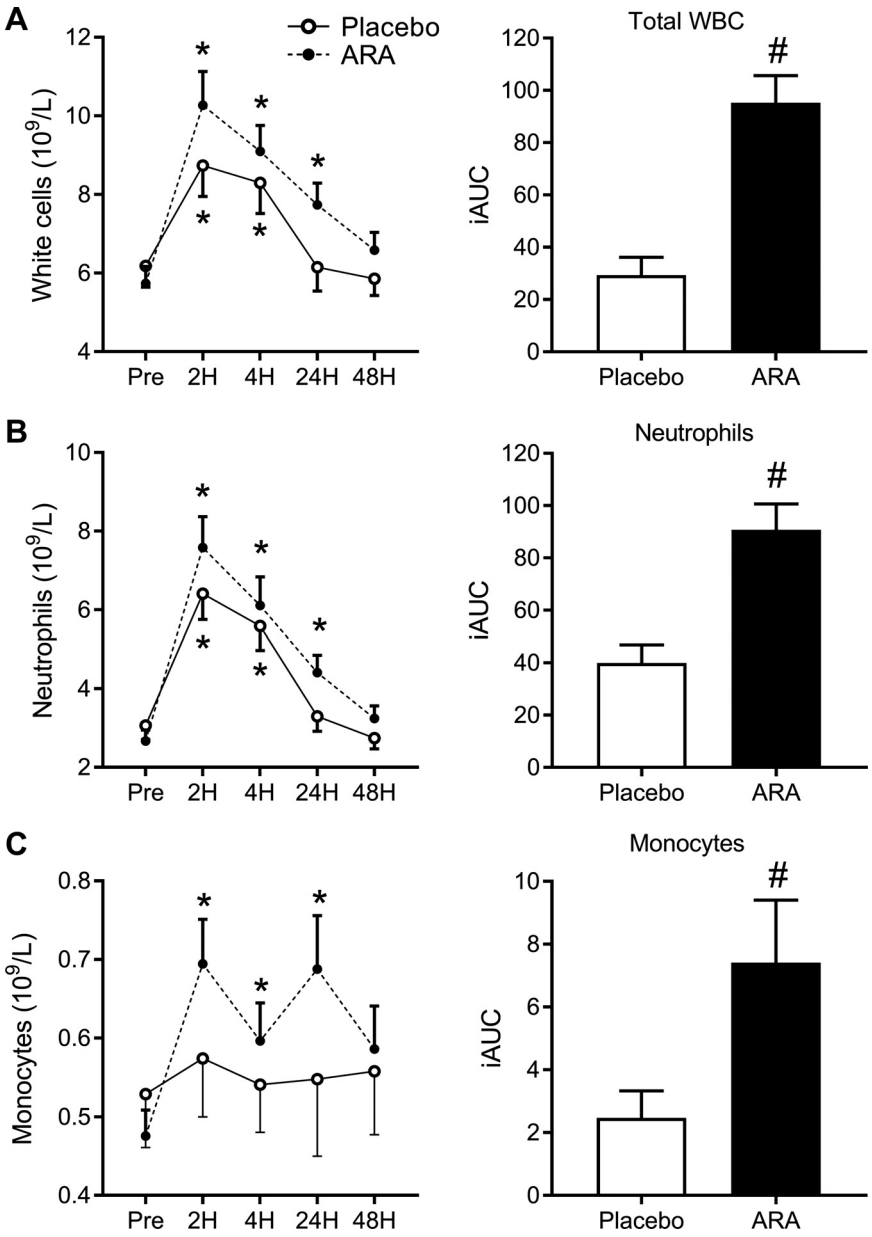

Fig. 3. Resistance exercise-induced neutrophilia and monocytosis. Total white blood cell counts (*A*), blood neutrophil counts (*B*), and blood monocyte counts (*C*) during recovery from an acute bout of resistance exercise in in men receiving dietary supplementation with arachidonic acid (ARA) or placebo. iAUC, incremental area under the curve. Values are means ± SE. $*P < 0.05$, different vs. preexercise within group. $\#P < 0.05$, different between groups.

phil elastase (*ELANE*), the pan monocyte marker CD68 (*CD68*), and the M2 monocyte/macrophage markers CD163 (*CD163*) and CD206 (*MRC*) in circulating PBMCs and muscle is shown in Fig. 5, *A* and *B*, respectively. There were main effects of time for PBMC mRNA expression of *ELANE* ($P = 0.025$), *CD68* ($P = 0.006$), *CD163* ($P = 0.009$) (Fig. 5*A*), and *MRC1* ($P = 0.006$) (data not shown). Additionally, main effects of group were found for *ELANE* ($P = 0.013$) and *ITGAM* ($P = 0.032$) (Fig. 5*A*). *ITGAM* did not change significantly over time in either group but was overall higher in the ARA group than the placebo group throughout recovery. *ELANE* increased in the ARA group but not the placebo group at 2 h postexercise (placebo: $P = 0.820$; ARA: $P = 0.013$) and was more highly expressed in the ARA group than the placebo group at 2 h ($P = 0.019$), 4 h ($P = 0.004$), and 48 h ($P = 0.035$). *CD68* increased at 2 h in the placebo group ($P = 0.028$), with a similar trend observed for the ARA group also ($P = 0.080$). *CD163* increased in the ARA group but not the placebo group at 2 h (placebo: $P = 0.153$; ARA: $P = 0.032$) and 24 h (placebo: $P = 0.865$; ARA: $= 0.040$). There was also a statistical trend for increased *MRC1* at 24 h postexercise in the ARA group ($P = 0.074$) but not the placebo group ($P = 0.635$) (data not shown).

Main effects of time but no effect of group or time × group interactions, were observed for muscle expression of *CD68* ($P < 0.001$) and *MRC1* ($P = 0.008$) (Fig. 5*B*). Muscle *CD68* increased in both the placebo group ($P = 0.010$) and the ARA group ($P < 0.001$) at 4 h postexercise and remained elevated at 48 h of recovery in the ARA group ($P = 0.005$) but not the placebo group ($P = 0.317$). *MRC1* was also increased at 48 h in the ARA group ($P = 0.042$) but was unchanged in the placebo group ($P = 0.378$). There was no effect of resistance exercise or ARA supplementation on muscle expression of *ITGAM* (time: $P = 0.558$; group: $P = 0.503$; time × group:

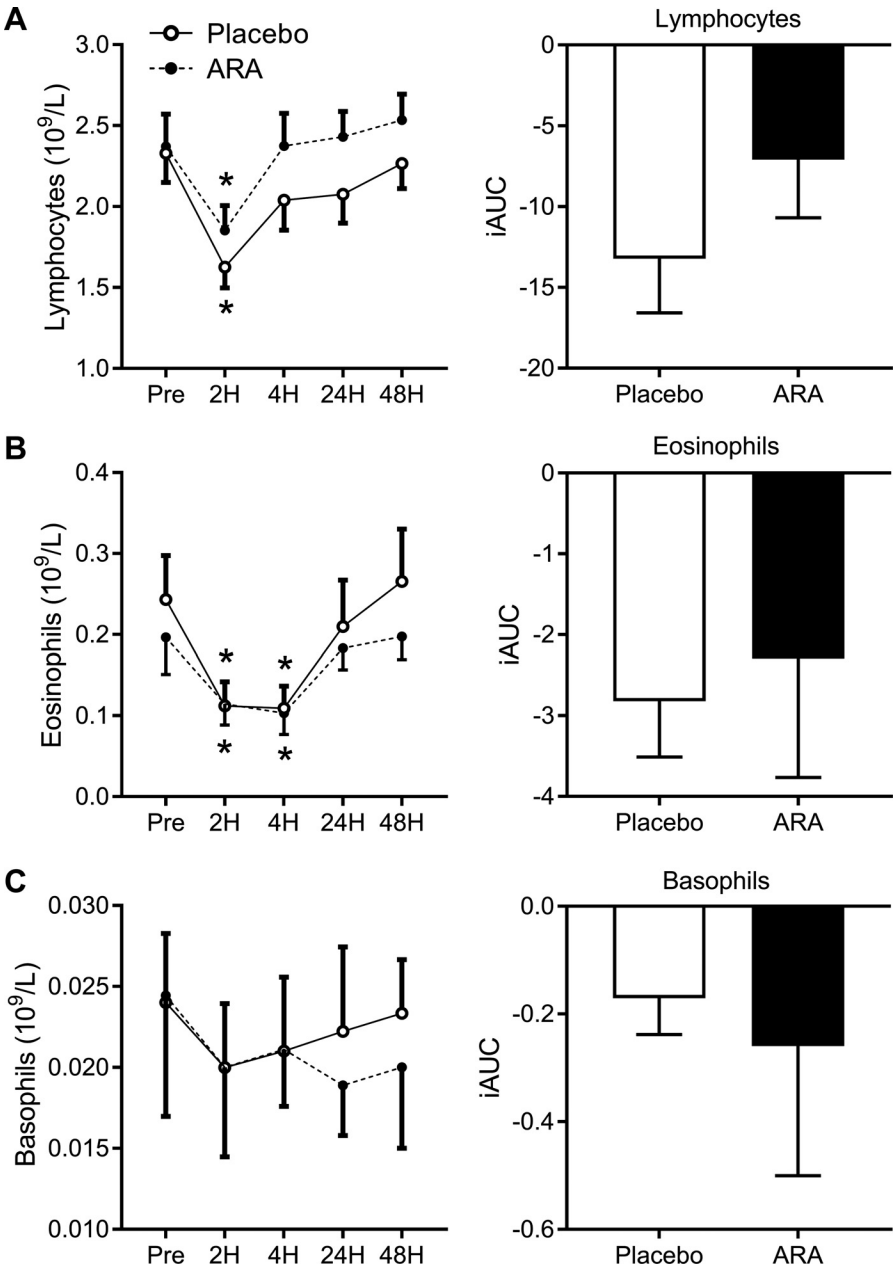

Fig. 4. Resistance exercise-induced lymphopenia and eosinopenia. Blood lymphocyte counts (*A*), blood eosinophil counts (*B*), and blood basophil counts (*C*) during recovery from an acute bout of resistance exercise in in men receiving dietary supplementation with arachidonic acid (ARA) (1.5 g/day) or placebo. iAUC, incremental area under the curve. Values are means ± SE. *$P < 0.05$, different vs. preexercise within group.

$P = 0.467$), *ELANE* (time: $P = 0.261$; group: $P = 696$; time × group: $P = 0.313$) (Fig. 5*B*), or *CD163* (time: $P = 0.162$; group: $P = 0.649$; time × group: $P = 0.943$) (data not shown).

Inflammatory cytokines. mRNA expression of the inflammatory cytokines interleukin-1β (*IL1B*), tumor necrosis factor-α (*TNF*), and interleukin-6 (*IL6*) in circulating PBMCs and muscle tissue is shown in Fig. 6, *A* and *B*, respectively. There was a main effect of group ($P = 0.002$) but no effect of time (0.477) or time × group interaction ($P = 0.384$) for PBMC expression of *IL1B* (Fig. 6*A*). PBMC *IL1B* was greater in the ARA group than the placebo group at 2 h ($P = 0.028$), 4 h ($P = 0.045$), 24 h ($P = 0.024$), and 48 h ($P = 0.040$) (Fig. 6*A*). There were no differences over time or between groups for PBMC mRNA expression of *IL6* (time: $P = 0.292$; group: $P = 0.673$; group × time: $P = 0.513$) or *TNF* (time: $P = 0.280$; group: $P = 0.752$; group × time: $P = 0.478$) (Fig. 6*A*).

There were main effects of time for muscle *IL1B* ($P < 0.001$), *IL6* ($P < 0.001$), and *TNF* ($P < 0.001$) (Fig. 6*B*). Additionally, statistical trends toward a main effect of group were found for *IL-6* ($P = 0.076$) and *TNF* ($P = 0.084$) (Fig. 6*B*). Muscle *IL1B* increased at 2 h in the both the placebo group ($P < 0.001$) and the ARA group ($P < 0.001$) but remained elevated at 4 h only in the ARA group (placebo: $P = 0.324$; ARA: $P = 0.002$). *TNF* increased immediately postexercise in both the placebo group ($P = 0.002$) and the ARA group ($P < 0.001$) but tended to be higher in the ARA group than placebo immediately postexercise ($P = 0.064$) and at 4 h of recovery ($P = 0.060$). *IL6* increased immediately postexercise in the ARA group ($P < 0.001$) with a statistical trend

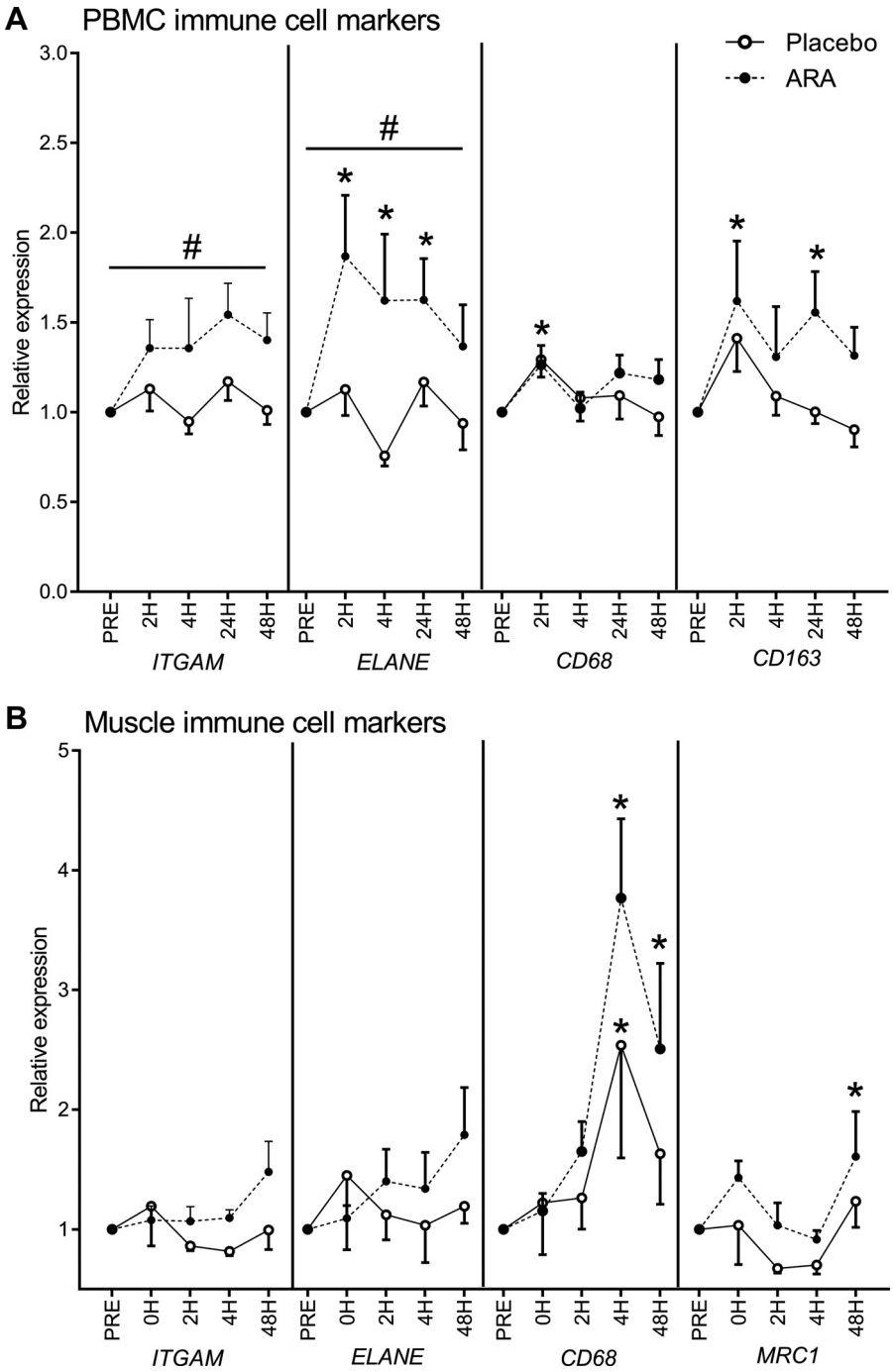

Fig. 5. Immune cell marker mRNA expression in circulating peripheral blood mononuclear cells (PBMCs) and skeletal muscle biopsies. Relative PBMC (*A*) and skeletal muscle (*B*) mRNA expression (fold-change from preexercise) for integrin-α M/CD11b (*ITGAM*), neutrophil elastase (*ELANE*), CD68 (*CD68*), and CD163 (*CD163*) in men receiving dietary supplementation with arachidonic acid (ARA) or placebo. Values are means ± SE. $*P < 0.05$, different vs. preexercise within group. $\#P < 0.05$, different between groups.

toward an increase in the placebo group ($P = 0.086$). Muscle *IL6* remained increased in both groups at 2 h (placebo: $P = 0.019$; ARA: $P < 0.001$) and 4 h (placebo: $P = 0.019$; ARA: $P < 0.001$) but tended to be higher in the ARA group than the placebo immediately postexercise ($P = 0.084$) and at 2 h of recovery ($P = 0.069$). At 48 h postexercise, muscle *IL1B* (placebo: $P = 0.931$; ARA: $P = 0.583$), *TNF* (placebo: $P = 0.922$; ARA: $P = 0.615$), and *IL6* (placebo: $P = 0.463$; ARA: $P = 0.638$) no longer differed from preexercise in either group.

Chemokines. mRNA expression of leukocyte chemotactic factors including C-X-C motif chemokine ligand 2 (*CXCL2*), C-X-C motif chemokine ligand 8 [*CXCL8*, also known as interleukin-8 (IL-8)], and C-C-motif chemokine ligand 2 [*CCL2*, also known as monocyte chemoattractant protein 1 (MCP-1)] by PBMCs and muscle is shown in Fig. 7, *A* and *B*, respectively. PBMC *CXCL2* showed a main effect of time ($P = 0.040$) but no effect of group ($P = 0.409$) or group × time interaction ($P = 0.721$). Despite this, PBMC *CXCL2* did not achieve a statistically significant change from preexercise at any time-point in either group. PBMC *CXCL8* showed a main effect of time ($P = 0.050$) and a statistical trend toward a group × time interaction effect ($P = 0.090$) (Fig. 7*A*). *CXCL8* increased in the ARA group but not the placebo group at 2 h (ARA: $P = 0.026$; placebo: $P = 0.852$) and 24 h postexercise

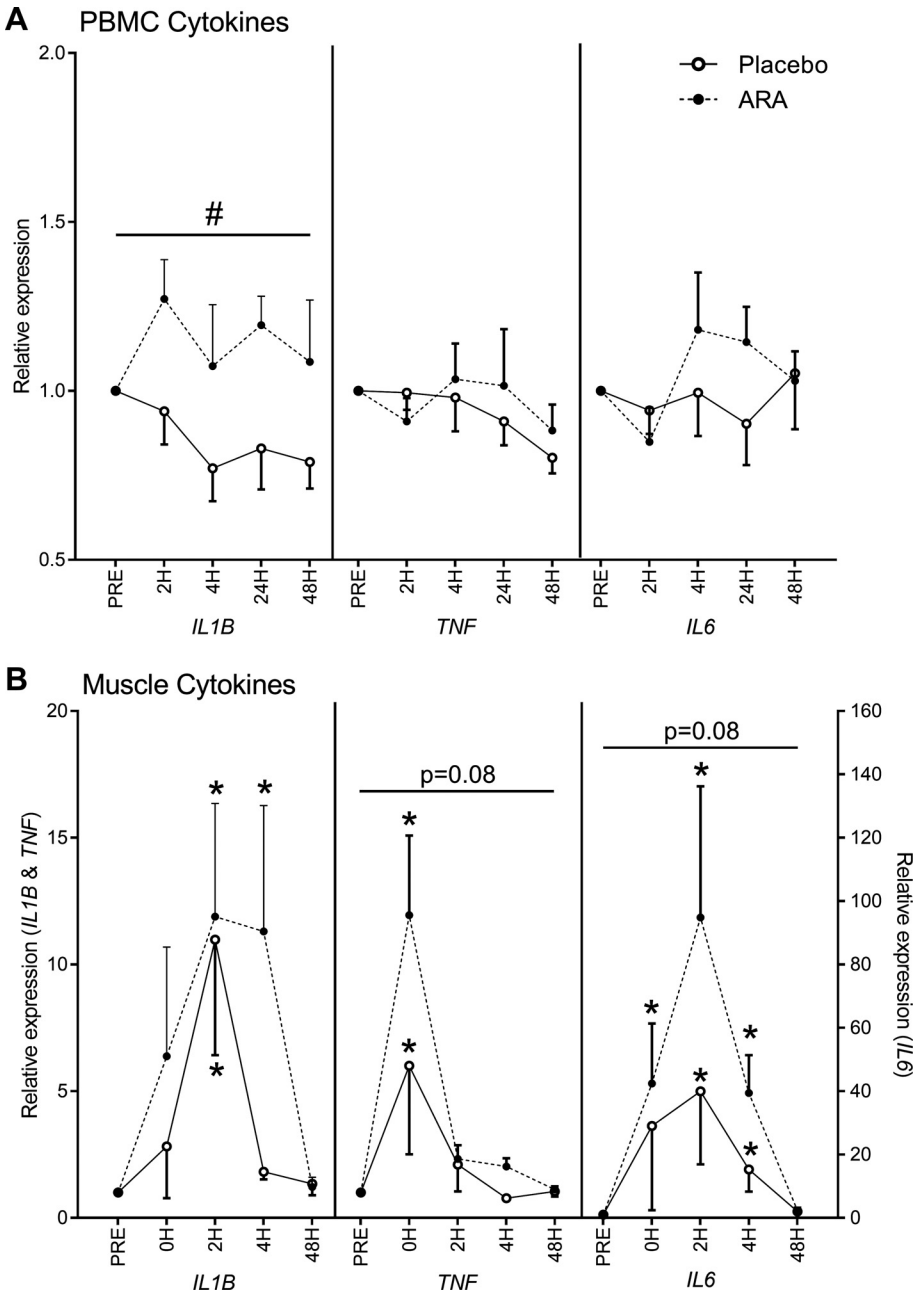

Fig. 6. Inflammatory cytokine mRNA expression in circulating peripheral blood mononuclear cells (PBMCs) and skeletal muscle biopsies. Relative PBMC (*A*) and skeletal muscle (*B*) mRNA expression (fold-change from preexercise) for interleukin-1β (*IL1B*), tumor necrosis factor alpha (*TNF*) and interleukin 6 (*IL6*) during recovery from an acute bout of resistance exercise in men receiving dietary supplementation with arachidonic acid (ARA) or placebo. Values are means ± SE. $*P < 0.05$, different vs. preexercise within group. $\#P < 0.05$, different between groups.

(ARA: $P = 0.023$, placebo $P = 0.986$). *CXCL8* was also greater in the ARA group compared with the placebo at 24 h ($P = 0.019$). There were no differences over time or between groups for PBMC mRNA expression of *CCL2* (time $P = 0.658$, group $P = 0.354$, group × time $P = 0.567$) (Fig. 7*A*).

Main effects of time were found for muscle mRNA expression of *CXCL2* ($P < 0.001$), *CXCL8* ($P < 0.001$), and *CCL2* ($P < 0.001$) (Fig. 7*B*). Additionally, there were main effects of group for muscle *CXCL8* ($P = 0.05$) and *CCL2* ($P = 0.004$) (Fig. 7*B*). *CXCL2* increased immediately postexercise in both groups ($P < 0.001$) and remained elevated ARA group (but not the placebo group) at 2 h (ARA: $P = 0.004$; placebo: $P = 0.130$) and 4 h (ARA: $P = 0.004$; placebo: $P = 0.274$). *CXCL8* increased in both the placebo group ($P = 0.010$) and the ARA group ($P < 0.001$) immediately postexercise (placebo: $P = 0.01$; ARA: $P < 0.001$) and remained elevated in both groups at 2 h ($P < 0.001$) and 4 h of recovery ($P < 0.001$). *CXCL8* was overall higher in the ARA group compared with the placebo group throughout recovery, and statistical trends to this effect were found at 4 h ($P = 0.088$) and 48 h ($P = 0.074$) specifically. *CCL2* mRNA increased in both the placebo group ($P = 0.033$) and the ARA group ($P < 0.001$) immediately postexercise and remained elevated in both groups at 2 h ($P < 0.001$) and 4 h of recovery ($P < 0.001$). *CCL2* expression was also higher in the ARA group compared with the placebo group at 2 h ($P = 0.016$) and 4 h ($P < 0.001$). By 48 h of recovery, mRNA expression no longer differed from preexercise levels for *CXCL2* (placebo: $P = 0.845$; ARA: $P = 0.098$), *CXCL8* (placebo: $P = 0.327$; ARA: $P = 0.257$), or *CCL2* (placebo: $P = 0.672$; ARA: $P = 0.366$).

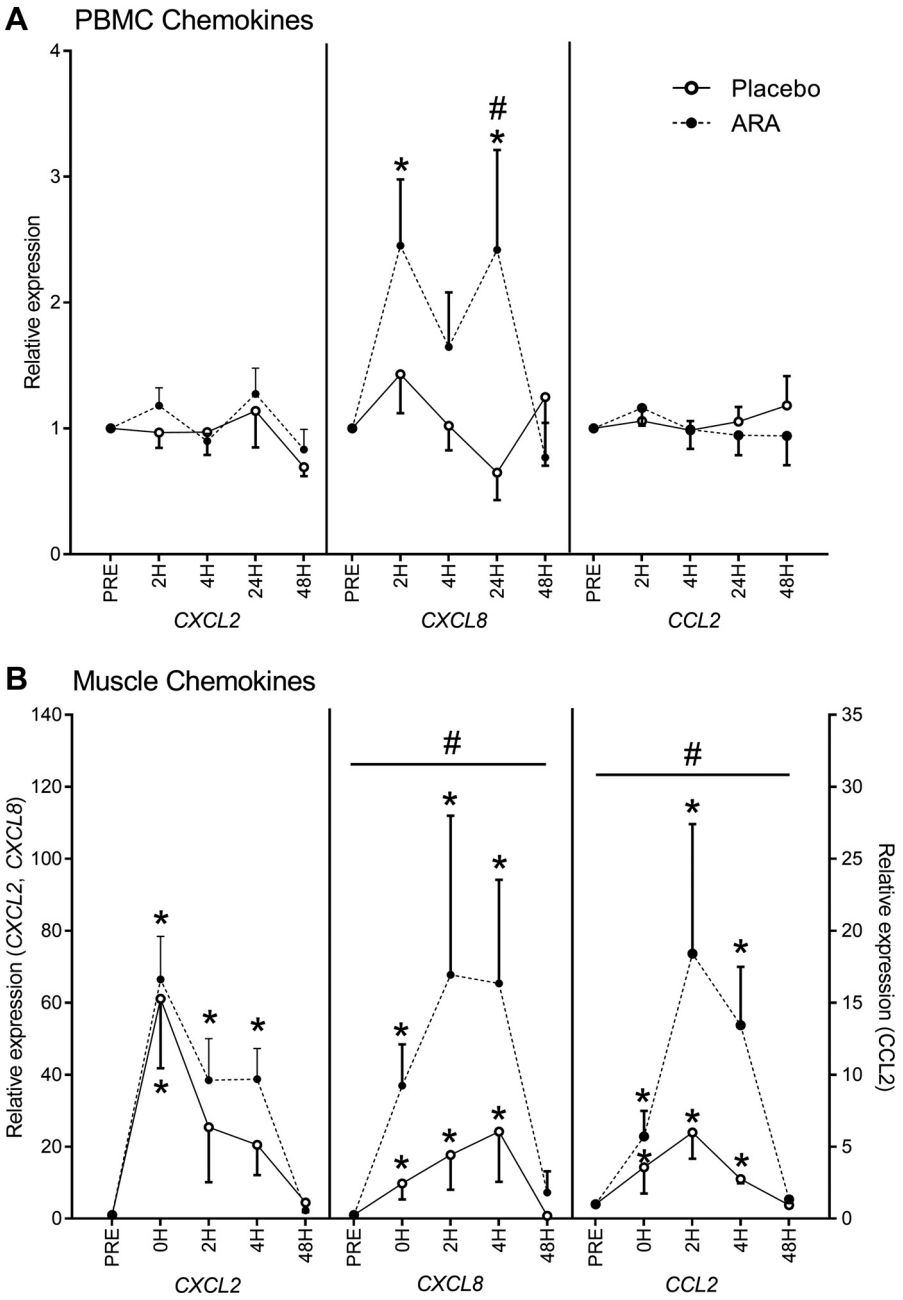

Fig. 7. Chemokine mRNA expression in circulating peripheral blood mononuclear cells (PBMCs) and skeletal muscle biopsies. Relative PBMC (*A*) and skeletal muscle (*B*) mRNA expression (fold-change from preexercise) for C-X-C motif chemokine ligand 2 (*CXCL2*), C-CX-C motif chemokine ligand 8 [CXCL8, also known as interleukin-8 (IL-8)], and C-C motif chemokine ligand 2 [*CCL2*, also known as monocyte chemoattractant protein (MCP)-1] during recovery from an acute bout of resistance exercise in men receiving dietary supplementation with arachidonic acid (ARA) or placebo. Values are means ± SE. $*P < 0.05$, different vs. preexercise within group. $\#P < 0.05$, different between groups.

Muscle immune cell staining

The number of cells within muscle positive for the neutrophil marker CD66b and the macrophage marker CD68 as determined by immunohistochemical staining of tissue sections is presented in Fig. 8. On average 209 ± 29 fibers were analyzed preexercise and 265 ± 23 fibers were analyzed at 48 h postexercise. A group × time interaction effect was found for the number of $CD66b^+$ cells per 100 myofibers ($P = 0.012$). The number of $CD66b^+$ cells in muscle decreased from preexercise at 48 h of recovery in the ARA group ($P = 0.009$) but was unchanged in the placebo group ($P = 0.802$) (Fig. 8*A*). Despite this, there was no significant difference between groups for muscle $CD66b^+$ cells number before exercise ($P = 0.529$) or at 48 h of recovery ($P = 0.939$). There was no effect of time ($P = 0.622$), group ($P = 0.363$), or time × group interaction ($P = 0.331$) for muscle CD68 counts (Fig. 8*B*).

DISCUSSION

This study investigated the effect of 4 wk of dietary ARA supplementation on acute inflammatory responses to a subsequent bout of resistance exercise in previously trained men. Compared with the corn-soy oil placebo, ARA supplementation resulted in greater exercise-induced serum markers of muscle damage; systemic leukocytosis; PBMC expression of *IL1B, CXCL8*, *ITGAM*, and *ELANE* mRNA; as well as muscle expression of *CXCL8* and *CCL2* mRNA. Muscle expression of monocyte/macrophage marker mRNA (*CD68* and *MRC1)* also increased above resting levels at 48 h of recovery in the ARA

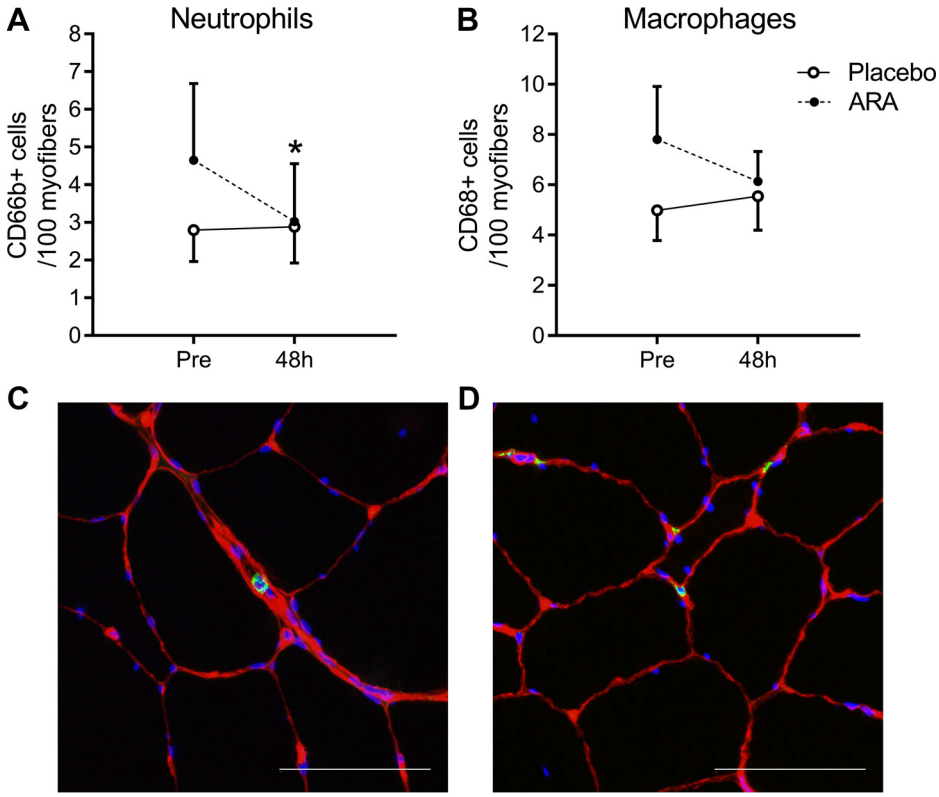

Fig. 8. Immunofluorescent staining of neutrophils (CD66b$^+$ cells) and macrophages (CD68$^+$ cells) in muscle tissue at 48 h of recovery from resistance exercise. Numbers of neutrophils (CD66b$^+$ cells) (*A*) and macrophages (CD68$^+$ cells) (*B*) per 100 myofibers before (pre) and 48 h following an acute bout of resistance exercise in men receiving dietary supplementation with arachidonic acid (ARA) or placebo. Values are means ± SE. *$P < 0.05$, different vs. preexercise within group. *C*: representative staining of neutrophils in human muscle tissue cross sections showing CD66b (green), laminin (red), and DAPI (blue). *D*: representative staining of macrophages in human muscle tissue cross sections showing CD68 (green), laminin (red), DAPI (blue). Scale bar = 100 μm.

group. Despite these changes, ARA supplementation did not alter the histological presence of leukocytes within muscle tissue, the severity of perceived muscle soreness, or the extent of loss of muscle force generating capacity.

Marked increases in blood neutrophils and total leukocytes were observed in both groups in the current study, but overall, resistance exercise-induced neutrophilia was of a higher relative magnitude and more prolonged in participants receiving ARA supplementation. Blood monocyte counts also increased throughout recovery but only in the ARA group. These data are consistent with prior studies showing that cells of the innate immune system, including blood neutrophils and monocytes, often exhibit a transient and delayed elevation in circulation in the early hours of recovery from intense resistance exercise (20, 76). However, to our knowledge no prior studies examining the systemic leukocyte response to acute resistance exercise have found blood neutrophils or monocytes to remain elevated beyond ~3−6 h into recovery (9, 22, 29, 40, 49, 63). Therefore, the prolonged duration of exercise-induced leukocytosis in trained men receiving dietary ARA supplementation is considerable. Differences in the exercise bout or participant training status between studies are unlikely to explain this effect, considering that the placebo group in the current study exhibited a typical response (9, 29, 63). In contrast to the effect of ARA on neutrophilia and monocytosis, blood lymphocytes and eosinophils decreased below preexercise levels similarly in both groups. Such a transient and delayed lymphopenia is commonly observed following intense resistance exercise (14, 61, 63). Eosinopenia has also been reported in some (51) but not all prior studies (33, 70). Collectively, these data show that ARA supplementation specifically enhanced resistance exercise-induced mobilization of major innate immune cell populations involved in the acute inflammatory response (neutrophils and monocytes).

The mechanisms responsible for the systemic leukocyte response to resistance exercise are largely unknown but may involve the release of innate immune cell populations from bone marrow stores during in response to chemotactic humoral factors (63), similar to that which occurs in response to injury or infection (4, 39). The capacity to induce systemic neutrophilia is a property common to a number of inflammatory cytokines/chemokines including IL-1β (81), IL-6 (80), CXCL8 (IL-8) (26, 77), TNFα (25, 81), CXCL1 (KC) (44, 85), and CXCL2 (MIP-2) (12, 85). In contrast, mobilization of monocytes from bone marrow is specifically controlled by C-C motif chemokine receptor type 2 (CCR2) ligands such as CCL2 (MCP-1) (39, 69). In the current study, ARA supplementation resulted in greater PBMC *IL1B* and *CXCL8* mRNA expression, whereas it did not influence PBMC expression of *IL6*, *TNF*, *CXCL2*, or *CCL2*. These data are consistent with prior studies showing that ARA supplementation can prime human monocytes to transiently produce greater IL-1β upon exposure to an acute inflammatory stimuli in vitro (5, 17, 67). Additionally, ARA has been established to be key second messenger in the induction of leukocyte *CXCL8* (IL-8) transcription (47). In turn, IL-1β itself promotes mobilization of neutrophils from bone marrow indirectly by stimulating release of ARA from membrane phospholipids (81). ARA metabolites including PGE_2 (79), $PGF_{2\alpha}$ (79, 82), LTB_4 (26), and platelet-activating factor (26) themselves can also directly stimulate neutrophilia when injected in rodents. Therefore, the mechanism by which ARA supplementation enhanced exercise-induced neutrophilia is likely to be mediated by reciprocal effects of ARA on inflammatory cytokines and lipid mediators. The mechanism

responsible for enhanced monocytosis following ARA supplementation is less clear given the lack of effect of ARA on PBMC *CCL2* expression. However, it is possible that CCL2 produced by exercised muscle tissue may signal bone marrow monocyte release, as has been demonstrated following muscle injury in mice (39).

In addition to effects on leukocyte mobilization, we observed relatively greater integrin-α M/CD11b (*ITGAM*) and neutrophil elastase (*ELANE*) mRNA expression in circulating PBMCs during exercise recovery in the ARA group. CD11b is a β_2-integrin expressed on both neutrophils and monocytes, which is upregulated upon leukocyte activation to permit adhesion to the endothelium and subsequent extravasation from the blood stream to local sites of inflammation (89). Similarly, ELANE is a serine protease released from activated leukocytes during degranulation to destroy invading pathogens and necrotic tissue (21). These data are consistent with prior studies showing that ARA supplementation stimulates expression of CD11b and release of elastase by neutrophils in vitro (6, 7, 24), resulting in increased neutrophil adhesion, degranulation, and leukocyte-mediated cellular damage (6–8). Although no between group differences were evident, PBMC expression of *CD163* mRNA also increased above preexercise levels at 24 h of recovery in the ARA group in the current study. CD163 is a scavenger receptor expressed exclusively on the monocytes/macrophages, which is inducible in response to stimulation by anti-inflammatory cytokines (e.g., IL-10) (11). This result may indicate that monocytes that were mobilized postexercise in the ARA group switched from a classically activated proinflammatory (e.g., "M1") to an alternatively activated anti-inflammatory (e.g., "M2") phenotype during the latter stages of recovery (78).

Serum markers of skeletal muscle damage including creatine kinase activity and myoglobin concentration were greater during recovery from resistance exercise in the ARA group. These data are consistent with rodent studies showing that certain leukocyte populations (e.g., neutrophils) may contribute to exercise-induced muscle damage (30, 58), and that ARA supplementation primes human neutrophils in vitro for increased respiratory burst (10, 46, 65) and degranulation (8, 73), resulting in increased neutrophil-mediated damage of cultured endothelial cells (6, 8). Collectively, these data suggest that dietary ARA supplementation may potentially exacerbate neutrophil-mediated damage of host tissues during acute inflammatory reactions. Despite this, we actually observed statistical trends toward an overall reduction in exercise-induced muscle soreness in the ARA group. Additionally, while no significant differences between groups were found, the ARA group had recovered muscle force by 4 h of recovery, at which time a significant force deficit remained in the placebo group. Therefore, there was no evidence to support our initial hypothesis that dietary ARA supplementation would increase the extent of perceived muscle soreness or delay the recovery of muscle force during exercise recovery. ARA is the precursor not only to proinflammatory lipid mediators (e.g., the prostaglandins and leukotrienes) but also anti-inflammatory/proresolving mediators with opposing effects (e.g., the lipoxins; reviewed in Ref. 42). Notably, the lipoxins possess potent pain inhibitory properties (75). It is therefore possible that ARA supplementation may unexpectedly have had a modest net suppressive effect on delayed onset muscle soreness. These data do not support the hypothesis that inflammation contributes to symptoms of exercise-induced muscle damage as has been classically suggested by some researchers (72).

Consistent with the effect of ARA on the systemic inflammatory response to resistance exercise, there was an overall greater (*CXCL8* and *CCL2*) or a more prolonged (*IL1B* and *CXCL2*) local muscle cytokine/chemokine mRNA response in men receiving ARA supplementation., Similar statistical trends were also found for muscle expression of *TNF* and *IL6*. Contracting muscle cells have been shown to release a variety of inflammatory cytokines and chemokines, suggesting that muscle tissue may be a major source of leukocyte chemotactic factors during exercise recovery (53). Indeed, changes in muscle expression of inflammatory cytokines and chemokines were much greater in magnitude than in circulating PBMCs in the current study. In addition to playing an essential role in mobilization of monocytes from bone marrow, CCL2 (MCP-1) has been shown to be required for the local recruitment of blood monocytes to injured skeletal muscle tissue in mice (39). In the current study, muscle mRNA expression of the monocyte/macrophage marker *CD68* was increased in both groups at 4 h postexercise. Following 48 h of recovery, however, muscle *CD68* mRNA remained elevated above resting levels only in the ARA group, together with increased expression of the anti-inflammatory M2 macrophage marker CD206 (*MRC1*). These data lend some support to the concept that blood monocytes that are recruited to injured muscle initially exhibit a proinflammatory ("M1") phenotype before transitioning to become anti-inflammatory ("M2") macrophages, which play an active role in tissue regeneration (2, 78). However, despite these changes at the gene level, we did not observe any evidence of infiltration of neutrophils ($CD66b^+$ cells) or macrophages ($CD68^+$ cells) within muscle tissue by immunohistological analysis in either group. Resident human muscle macrophages predominantly express CD68 together with the M2 markers CD163 and CD206 (50, 59, 68). Therefore, a lack of a change in CD68 cell number, combined with increased muscle expression of *CD68* and *MRC1* mRNA expression, may potentially suggest a change in the activation or polarization of the resident macrophages, even in the absence of infiltration of monocytes from the blood stream, as had been previously suggested (59).

Histological evidence of leukocyte infiltration of muscle has been most commonly observed following single joint, maximal and unaccustomed eccentric contractions which can result in severe muscle damage characterized by a ≥50% reduction in force that persists for days to weeks (52). In contrast, traditional resistance exercise stimuli with equal concentric and eccentric loads of 70−90% 1RM similar to that used in the current study typically inflicts only mild to moderate muscle damage, which often has not resulted in obvious evidence of leukocyte infiltration (52). According to the criteria proposed by Paulsen et al. (52), participants in the current study experienced only mild muscle damage, characterized by a ~30% loss of muscle force, which was completely recovered within 48 h irrespective of group. Therefore, it is perhaps not surprising that there was no macrophage infiltration found within muscle tissue after exercise in the current study. We have, however, previously reported a transient increase in $CD66b^+$ and $CD68^+$ cell number within muscle tissue sections using the same antibody staining protocol at 2 and 24 h following a

similar bout of resistance exercise in men of comparable training status (56). It is therefore possible that macrophages infiltrated muscle before the 48 - postexercise biopsy sampling time point used for immunohistochemical analysis in the current study.

Acute inflammation is a normal physiological response to resistance exercise (55) and may play an important role in muscular adaptation (15, 54, 78). However, excessive or persistent inflammation can have deleterious effects on muscle mass and function, as is commonly observed in states of chronic inflammatory disease (31). Importantly, the effects of ARA supplementation on exercise-induced leukocytosis and inflammatory gene expression in the current study were transient and had fully resolved within 48 h. There were also little or no apparent long-term effects of 4 wk of ARA supplementation on basal systemic and intramuscular inflammation and a range of clinical parameter nor negative effects on skeletal muscle mass and function in these participants (reported in Ref. 43). Longer periods (e.g., 8 wk) of ARA supplementation in young men participating in resistance training have actually been reported to enhance gains in muscle mass/force (18) and/or and power output (18, 64). We also observed that 4 wk of prior ARA supplementation potentiated some, but not other, acute molecular mechanisms related to muscle hypertrophy during recovery from a bout of resistance exercise in these same participants (reported in Ref. 48). Therefore, evidence that transiently increased inflammatory responses to acute resistance exercise in healthy young men receiving ARA supplementation may have deleterious effects when repeated over time as part of a regular training program is currently lacking. Despite this, the effect of dietary ARA intake in on acute immune responses to physiological stress and long-term impact on muscle health in individuals with preexisting inflammatory conditions remains unknown.

In conclusion, dietary supplementation with 1.5 g/day ARA for 4 wk resulted in greater acute inflammatory responses to an acute bout of resistance exercise in previously trained young healthy men. The effects of ARA on exercise-induced inflammation were transient, however, and had fully resolved within 48 h. There was also no evidence that the increased inflammatory response to exercise stress in men receiving dietary ARA supplementation impeded recovery of muscle force or increased symptoms of muscle soreness. These data show for the first time that dietary ARA intake can transiently modulate immune cell responses in human participants in vivo but that exposure to an acute inflammatory stimulus, such as exercise-induced muscle damage, is required to reveal this effect.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.F.M., A.J.S., D.C.-S., and C.J.M. conceived and designed research; J.F.M., R.F.D., K.M.A., S.M.M., B.R.D., I.M.E., and C.J.M. performed experiments; J.F.M. and I.M.E. analyzed data; J.F.M., R.F.D., A.J.S., J.M.P., T.R., and C.J.M. interpreted results of experiments; J.F.M. and I.M.E. prepared figures; J.F.M. and C.J.M. drafted manuscript; J.F.M., R.F.D., J.M.P., I.M.E., T.R., D.C.-S., and C.J.M. edited and revised manuscript; J.F.M., R.F.D., K.M.A., S.M.M., B.R.D., A.J.S., J.M.P., I.M.E., T.R., D.C.-S., and C.J.M. approved final version of manuscript.

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