Effects of Aging and Lifelong Aerobic Exercise on Basal and Exercise-Induced Inflammation

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ABSTRACT

Age-associated chronic basal inflammation compromises muscle mass and adaptability, but exercise training may exert an anti-inflammatory effect. This investigation assessed basal and exercise-induced inflammation in three cohorts of men: young exercisers (YE, *n*=10, 25±1y, VO_2max:53±3mL/kg/min, quadriceps area:78±3cm^2), old healthy non-exercisers (OH, *n*=10, 75±1y, VO_2max:22±1mL/kg/min, quadriceps area:56±3cm^2), and lifelong exercisers with a 53±1y aerobic training history (LLE, *n*=21, 74±1y, VO_2max:34±1mL/kg/min, quadriceps area:67±2cm^2). Resting serum IL-6, TNF-α, CRP, and IGF-1 were measured. Vastus lateralis muscle biopsies were obtained at rest (basal) and 4h after an acute exercise challenge (3x10reps, 70%1RM) to assess gene expression of cytokines (IL-6, TNF-α, IL-1β, IL-10, IL-4, IL-1Ra, TGF-β), chemokines (IL-8, MCP-1), cyclooxygenase enzymes (COX-1, COX-2), prostaglandin E_2 synthases (mPGES-1, cPGES) and receptors (EP3-4), and macrophage markers (CD16b, CD163), as well as basal macrophage abundance ($CD68^+$ cells). Aging led to higher ($P \leq 0.05$) circulating IL-6 and skeletal muscle COX-1, mPGES-1, and CD163 expression. However, LLE had significantly lower serum IL-6 ($P \leq 0.05$ vs. OH) and a predominantly anti-inflammatory muscle profile [higher IL-10 ($P \leq 0.05$ vs. YE), TNF-α, TGF-β, and EP4 ($P \leq 0.05$ vs. OH)]. In OH only, acute exercise increased expression of pro-inflammatory factors TNF-α, TGF-β, and IL-8 ($P \leq 0.05$). LLE had postexercise gene expression similar to YE, except lower IL-10 ($P \leq 0.10$), mPGES-1, and EP3 ($P \leq 0.05$). Thus, while aging led to a pro-inflammatory profile within blood and muscle, lifelong exercise partially prevented this and generally preserved the acute inflammatory response to exercise seen in young exercising men. Lifelong exercise may positively impact muscle health throughout aging by promoting anti-inflammation in skeletal muscle.

KEYWORDS

Inflammation, inflammaging, acute exercise, lifelong exercise, skeletal muscle

NEW AND NOTEWORTHY

This study assessed a unique population of lifelong aerobic exercising men and demonstrated that their activity status exerts an anti-inflammatory effect in skeletal muscle and circulation. Further, we provide evidence that the inflammatory response to acute exercise is dysregulated by aging but preserved with lifelong exercise, which might improve skeletal muscle resilience to unaccustomed loading and adaptability into late life.

INTRODUCTION

Chronic low-grade inflammation throughout aging ("inflammaging") threatens functional capacity, independence, and quality of life in older individuals (20, 21). Inflammaging has also been associated with sarcopenia, muscle atrophy, and accompanying functional deficits in older adults (66, 67, 85), likely through its negative impact on muscle protein balance (33, 70, 84). Further supporting this connection, chronic anti-inflammatory drug consumption in older adults appears beneficial for muscle mass and performance (6, 32, 39).

Skeletal muscle plays an important role in inflammatory signaling: factors such as cytokines, prostaglandins, and chemokines can be both released (37, 52, 55) and taken up (51, 84) by muscle at rest and following exercise. Aging has been shown to alter these factors in skeletal muscle (35, 56), which may contribute to poor communication with inflammatory cells (e.g., macrophages), dysregulated protein balance, and impaired resolution of inflammation following a stimulus, such as muscle loading. In aging individuals, exaggerated or sustained inflammation following exercise may contribute to suboptimal muscle adaptations (17, 38, 68). Our laboratory previously found that older adults taking anti-inflammatory drugs throughout resistance training demonstrated superior muscle growth over placebo (78, 80), suggesting that chronic low-grade muscle inflammation may dysregulate the exercise response and interfere with adaptation. Thus, in order to reverse age-related muscle mass losses, basal inflammation may need to be controlled (1) or exercise training prescriptions may need to be designed to overcome the basal inflammatory burden (3, 65). Resistance exercise in particular is a potent anti-sarcopenic stimulus that targets both slow (oxidative, type I)

and fast (glycolytic, type II) muscle fibers (31, 80), the latter of which are a notable casualty of the aging process (50).

However, while short-term training studies have demonstrated reductions in basal systemic inflammation in older adults (4), this finding is not universal within muscle (17, 81). Thus, rather than attempt to reverse age-related declines in muscle health, the optimal exercise strategy might seek to prevent them. Recent research has examined the impact of lifelong exercise (adherence to a structured exercise program throughout the adult lifespan into and beyond retirement age) (13, 24). Previous studies have demonstrated superior cardiovascular health and skeletal muscle mass in lifelong aerobic exercisers in comparison to age-matched non-exercising control subjects (12, 41, 76). Though limited, evidence from 50–65y-old populations suggests that these benefits are accompanied by a more favorable circulating inflammatory profile (42, 43) and an improved molecular environment within muscle (64). Thus, the aims of this investigation were to examine skeletal muscle to assess the basal inflammatory profile and inflammatory response to an acute resistance exercise challenge in lifelong exercisers, young exercisers, and old healthy non-exercisers. These objectives were met with a comprehensive analysis of 23 targets related to inflammation in muscle, macrophages, and circulation.

METHODS

Subjects

Old lifelong exercisers (LLE, *n*=21), old healthy non-exercisers (OH, *n*=10), and young exercisers (YE, *n*=10) were included in this investigation (Table 1). Subjects were recruited from the greater Muncie, Indiana area by newspaper advertisements, mailed flyers, and personal interaction. More extensive subject characteristics and more details regarding the recruitment and screening process, along with cardiovascular and skeletal muscle profiles are presented by our research team elsewhere (13, 24). Enrolled individuals were free from acute or chronic illness (cardiac, pulmonary, liver, or kidney abnormalities, cancer, uncontrolled hypertension, insulin- or non-insulin dependent diabetes or other known metabolic disorders), free from orthopedic limitations (including any artificial joints), and they did not smoke or participate in other forms of tobacco use. The study was approved by the Institutional Review Board of Ball State University. All study procedures, risks, and benefits were explained to the subjects before giving written consent to participate.

Exercise history of the subjects was carefully evaluated using a comprehensive questionnaire and confirmed through personal interviews (Table 2). The LLE cohort consisted primarily of cyclists and runners that reported ~50 years of structured exercise. LLE trained ~5 d and ~7 h per week. Exercise history of LLE subjects was extensively reviewed for frequency, duration, intensity, and athletic achievements. As such, two clear LLE sub-groups emerged: one group that participated in lower intensity training for physical fitness (Fitness, LLE-F; *n*=7) and another group that trained more vigorously and often participated in competitive events (Performance, LLE-P; *n*=14).

Serum Inflammatory Markers

A resting, fasted blood draw was taken for measurement of circulating inflammatory markers. Samples were analyzed (LabCorp, Muncie, IN) for serum C-reactive protein (latex immunoturbidimetry), IL-6 and TNF-α (enzyme-linked immunosorbent assay), and IGF-1 (immunochemiluminometric assay).

Acute Exercise Trial and Skeletal Muscle Biopsies

Subjects completed a resistance exercise challenge of the knee extensors, consisting of 3 sets of 10 repetitions at 70% 1RM, with 2 min rest between each set. When completed chronically, this acute exercise stimulus elicits significant increases in muscle size and strength in young and old individuals (61, 68, 75, 77, 88). Muscle biopsies (5) of the vastus lateralis were obtained before and 4 h after the resistance exercise challenge. This postexercise timepoint reflects an optimal time for interrogating expression of numerous intramuscular regulators of muscle adaptation, including cytokine activity (37, 58-60, 62, 89). All biopsies were obtained in the fasted state (≥10 h), after at least 30 min of supine rest. Subjects remained in the lab and rested quietly during the 4 h postexercise period. Subjects also refrained from structured exercise and aspirin consumption for 72 h, alcohol consumption for 24 h, and caffeine the morning of the trial.

Following each muscle biopsy, excess blood, visible fat, and connective tissue were removed, and a portion of the muscle (~20 mg) to be used for mRNA analysis was immediately frozen and then stored in liquid nitrogen. Prior to analysis, the muscle was transferred to 0.5 mL of RNAlater-ICE (Ambion, Austin, TX) at stored at -20°C until

152 analysis. A portion of the muscle to be used for immunohistochemistry was oriented
153 longitudinally in a mounting medium (tragacanth gum, Sigma, St. Louis, MO) atop a
154 cork, frozen in isopentane cooled in liquid nitrogen, and subsequently stored in liquid
155 nitrogen until analysis.
156
157 *Gene Expression Measurements*
158 Inflammatory factors listed in Table 3 were assessed in vastus lateralis skeletal
159 muscle homogenates using real-time quantitative polymerase chain reaction (qPCR).
160 Muscle mRNA analyses were completed on all 41 subjects for basal expression (i.e.,
161 preexercise) and on 39 subjects for expression 4 h postexercise (i.e., 2 individuals did
162 not undergo the postexercise biopsy: 1 from LLE and 1 from OH).
163 *Total RNA Extraction and Quality Check.* Total RNA was extracted in TRI
164 Reagent RT (Molecular Research Center, Cincinnati, OH). The quality and integrity
165 (RIN = 8.34 ± 0.05) of extracted RNA (94.24 ± 3.97 ng/μL) were evaluated using a RNA
166 6000 Nano LabChip kit on an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa
167 Clara, CA) as previously described (29, 78).
168 *Real-Time Polymerase Chain Reaction.* Oligo (dT) primed first-strand cDNA was
169 synthesized (96–144 ng of total RNA, depending on magnitude of target gene
170 expression) using SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, CA). For
171 each target, quantification of mRNA levels was performed in duplicate in a 72-well
172 Rotor-Gene Q Centrifugal Real-Time Cycler (Qiagen, Germantown, MD). Ribosomal
173 protein lateral stalk subunit P0 (RPLP0) was selected as a housekeeping/reference
174 gene, as previously done in human muscle (48). RPLP0 was similar among the three

175 groups at baseline (C_T: 19.02 ± 0.03) and stable after exercise (C_T: 19.01 ± 0.03). All
176 primers used in this study were mRNA specific (on different exons and/or crossing over
177 an intron) and designed for qPCR [Vector NTI Advance 9 software (Invitrogen) and
178 Primer Design Tool (Entrez) NCBI/Primer-BLAST program] using SYBR Green
179 chemistry (29). Primers details are presented in Table 3. A melting curve analysis was
180 generated for all PCR runs to validate that only one product was present. For each run,
181 a serial dilution curve was made using cDNA from a known amount (500–2000 ng) of
182 human skeletal muscle RNA (Ambion, Austin, TX) or from human muscle samples
183 collected in our laboratory. The amplification calculated by the Rotor-Gene software was
184 specific and highly efficient (efficiency = 1.02 ± 0.01; R^2 = 0.98 ± 0.00; slope = 3.29 ±
185 0.03). Basal gene expression among YE, LLE, and OH was compared using the $2^{-\Delta C_T}$
186 (arbitrary units) method. Gene expression before and after the resistance exercise
187 challenge was compared using the $2^{-\Delta\Delta C_T}$ (fold change) relative quantification method,
188 as previously described (36, 37, 70, 81). Based on the principle of the calculation, the
189 preexercise value and the associated variability should be very close to one for each
190 group. In the current study, this was true of all genes analyzed, and preexercise $2^{-\Delta\Delta C_T}$
191 values were not statistically different among the three groups or between subgroups
192 (P>0.05). Therefore, to simplify interpretation, preexercise expression for each gene is
193 graphically represented as a dotted line at 1.0-fold.
194
195 *Immunohistochemistry*
196 For histochemical detection of skeletal muscle macrophages, transverse sections
197 (7 μm) for histochemical analysis were cut on a microtome-cryostat (HM 525, Microm,

198 Walldorf, Germany) at -20 ºC. Prior to staining, sections were air-dried in a humidified
199 chamber for 30 min, then fixed in cold (-20 ºC) acetone for 10 min and rehydrated in
200 PBS for 5 min. Endogenous peroxidase activity was quenched with 0.3% peroxide.
201 Sections were incubated in anti-CD68 primary antibody (1:100 dilution in PBS, M0718,
202 Dako, Carpinteria, CA) at 4 ºC overnight (15 h) in a humidified chamber. Sections were
203 treated using HistoStain Kit (Invitrogen, Frederick, MD) and visualized using aminoethyl
204 carbazole (AEC) single solution substrate (Invitrogen), then counterstained with
205 hematoxylin (Gill No. 3, Sigma) for 30 seconds. All analyses included a CD68 negative
206 control (no primary antibody during incubation) and an internal positive control [skeletal
207 muscle biopsy obtained following a damaging exercise protocol similar to those shown
208 to elicit macrophage infiltration (87)]. Positive and negative control slides were included
209 in all analyses. A sample image from a lifelong exercise subject is shown in Figure 1.
210 Macrophage abundance is represented as $CD68^+$ cells/100 fibers [the number of $CD68^+$
211 cells relative to the number of muscle fibers assessed (109 ± 4 fibers per subject),
212 multiplied by a factor of 100] and $CD68^+$ cell density [number of $CD68^+$ cells in an
213 analyzed area of muscle (645,271 ± 6,721 μm^2 per subject)]. All measurements were
214 completed by two independent investigators and averaged to represent each sample.
215
216 *Statistical Analyses*
217 Data were analyzed with a one-way analysis of variance (ANOVA) to compare
218 subject characteristics, training histories, serum inflammatory factor levels, macrophage
219 parameters, and basal gene expression ($2^{-\Delta C_T}$ method) among the three main groups
220 (YE, LLE, and OH) and between LLE subgroups (LLE-F and LLE-P). A two-way ANOVA

(group x time) was completed to evaluate gene expression ($2^{-\Delta\Delta C_T}$ method) in response to exercise among the three main groups and LLE subgroups. Follow-up one-way ANOVAs were used to compare basal gene expression and postexercise expression levels between YE and both old groups combined (LLE and OH). Post-hoc comparisons were made with Tukey's test. Significance was accepted at $P \leq 0.05$. Data are presented as mean ± SE.

RESULTS

Basal Circulating Inflammatory Factors

Serum concentrations of inflammatory factors are shown in Table 4. IL-6 was lower ($P \leq 0.05$) in young exercisers (YE) and lifelong exercisers (LLE) than in old healthy (OH) men. Serum TNF-α and CRP were not different among groups ($P > 0.05$). Both LLE and OH had 43% lower ($P \leq 0.05$) IGF-1 than YE. However, the performance subgroup (LLE-P) had higher ($P \leq 0.05$) IGF-1 (+23%) than the fitness subgroup (LLE-F). IL-6, TNF-α, and CRP were not different ($P > 0.05$) between LLE-F and LLE-P.

Basal Muscle Macrophage Abundance

Skeletal muscle macrophage parameters also appear in Table 4. $CD68^+$ cells per 100 fibers and $CD68^+$ cell density were similar ($P > 0.05$) across all three groups and between the LLE subgroups.

Basal Skeletal Muscle Inflammation

Basal muscle cytokine expression among YE, LLE, and OH is presented in Figure 2A (pro-inflammatory) and 3A (anti-inflammatory). TNF-α expression tended ($P \leq 0.10$) to be lower in OH than in YE (-55%) and was significantly lower ($P \leq 0.05$) than in LLE (-62%). LLE also had higher expression ($P \leq 0.05$) of anti-inflammatory IL-10 (+43% vs. YE) and TGF-β (+66% vs. OH). As shown in the LLE subgroup summary (Table 5), higher ($P \leq 0.05$) gene expression in LLE-F than in LLE-P may have contributed to these

findings in TNF-α (+59%) and TGF-β (+48%). No differences were found in expression of IL-6, IL-1β, IL-4, or IL-1Ra.

Within the PGE_2/COX pathway, both COX-1v2 (Figure 4A, +37%) and mPGES-1 (Figure 5A, +69%) were higher ($P \leq 0.05$) in the older cohorts (LLE and OH combined) than in YE. The downstream anti-inflammatory receptor EP4 was differentially expressed across the groups (Figure 5A), with higher ($P \leq 0.05$) expression in LLE (+21%) and lower ($P \leq 0.05$) expression in OH (-51%) compared to YE. COX-1v1, COX-2, cPGES, and EP3 were not different across the groups ($P > 0.05$). The LLE-P subgroup showed a trend ($P \leq 0.10$) for higher expression of COX-2 (+69% vs. LLE-F), although no differences ($P > 0.05$) were found for COX-1, cPGES, mPGES-1, EP3, or EP4 (Table 5).

Basal expression of chemokines IL-8 and MCP-1 was similar ($P > 0.05$) among the three groups (Figure 6A) and between the LLE subgroups (Table 5). LLE (+98%) and OH (+125%) each had significantly higher ($P \leq 0.05$) expression of CD163 than YE (Figure 6A), and there was no difference between LLE subgroups (Table 5). No differences ($P > 0.05$) were found in expression of CD16b (Figure 6A, Table 5).

Effects of Acute Resistance Exercise

After resistance exercise, expression of TNF-α was significantly elevated ($P \leq 0.05$) only in the OH group (2.1-fold; Figure 2B). There was a trend for a larger increase in IL-6 expression in the older groups ($P \leq 0.10$), likely explained by a 1.4-fold increase in LLE. IL-1β was unaffected by exercise across the groups ($P > 0.05$). For the anti-inflammatory genes (Figure 3B), IL-10 tended to be higher ($P \leq 0.10$) after exercise in YE (2.0-fold) compared to LLE. Exercise led to a 1.7-fold increase ($P \leq 0.05$) in TGF-β

272 in OH, and LLE was significantly lower than OH postexercise (1.2-fold). IL-1Ra was
273 unchanged (P>0.05) after exercise. IL-4 was also unchanged (P>0.05) across the three
274 groups, although the LLE subgroups (Table 6) showed a 2.5-fold change after exercise
275 (P≤0.05, main effect). No differences (P>0.05) were found between the LLE subgroups
276 for expression of any cytokines postexercise (Table 6).

277 COX-1v1 and v2 were unchanged with exercise in YE, LLE, OH (Figure 4B), and
278 both LLE subgroups (Table 6). There was a trend (P≤0.10, main effect) for the LLE
279 subgroups to decrease expression of COX-1v2 following exercise (Table 6).
280 Conversely, exercise increased (P≤0.05, main effect) expression of COX-2 in the three
281 groups and the LLE subgroups (~1.9-fold).

282 Downstream PGE_2/COX pathway components were differentially responsive to
283 exercise across the groups (Figure 5B). cPGES tended to be higher (P≤0.10) after
284 exercise in the aging cohorts combined (1.1-fold) than YE. LLE had lower (P≤0.05)
285 expression of mPGES-1 (0.8-fold) compared to YE (1.5-fold) following exercise, which
286 was supported by an overall decrease (P≤0.05, main effect) in the LLE subgroups
287 (Table 6). Exercise led to a 3.4-fold increase (P≤0.05) in EP3 in YE, and LLE had lower
288 (P≤0.05) EP3 expression than YE postexercise (1.5-fold). EP4 increased after exercise
289 for all three groups (P≤0.05, main effect). cPGES, EP3, and EP4 were not different
290 (P>0.05) between the subgroups (Table 6).

291 Postexercise skeletal muscle IL-8 expression (Figure 6B) tended to be higher
292 (P≤0.10) in OH (2.2-fold) than YE (0.9-fold) or LLE (1.0-fold). With the LLE subgroups,
293 IL-8 tended (P≤0.10, main effect) to decrease overall, primarily driven by a 0.5-fold
294 change in the LLE-F subgroup (Table 6). MCP-1 increased overall after exercise

295 ($P \leq 0.05$, main effect), but YE showed a significantly ($P \leq 0.05$) greater response (1.8-fold
296 increase) than the older groups (1.3-fold increase). Muscle macrophage surface
297 markers CD16b and CD163 (Figure 6B, Table 6) both approached ($P \leq 0.10$, main effect)
298 or attained ($P \leq 0.05$, main effect) significantly higher expression after exercise across all
299 groups.

DISCUSSION

This study examined the influences of aging and lifelong exercise on inflammation in circulation and skeletal muscle at baseline and after acute resistance exercise. This investigation arose given the negative impact of chronic basal inflammation on muscle size and function in older individuals (66, 67, 85), along with the established anti-inflammatory benefits of exercise training (4, 42, 43). Findings from this study show that aging led to a pro-inflammatory profile within the blood and muscle. Lifelong exercise partially protected against this effect and favored a generally anti-inflammatory profile within muscle. A resistance exercise bout was chosen to provide a potent anti-sarcopenic stimulus and present an unaccustomed exercise challenge to all groups. Our laboratory has historically been interested in resistance training as a tool to combat sarcopenia in aging adults. The present study builds on this tenet, along with previous work from us (78, 80, 81) and others (18, 38) showing that skeletal muscle inflammation may preclude optimal adaptations. We provide evidence that highly aerobically trained older adults display a preserved response to exercise, which may indicate they are better prepared to adapt to resistance training. Further research is needed to understand whether this advantage persists into the ninth decade of life, where exercise adaptations appear to be blunted (23, 61, 68).

Basal Inflammatory Profile

The impressive and unique training history of the lifelong exercisers (LLE) resulted in an anti-inflammatory muscle environment, which complements findings of higher anti-inflammatory factors in circulation of older exercise-trained individuals (28,

43). Relative to OH, higher basal IL-10 (+43%) and TGF-β (+66%) in LLE likely contribute to suppression of the transcription and signaling activity of pro-inflammatory factors. For example, IL-10 and TGF-β have regulatory relationships with the pro-inflammatory cytokine TNF-α (7, 15), which may explain the tendency of these cytokines to track together in the present study. Both YE and LLE had higher basal expression of TNF-α than OH, suggesting that exercise training increases its expression. Other studies have also demonstrated higher TNF-α in trained compared to untrained individuals, including circulation of older men (41) and skeletal muscle of young men (51). Likewise, short-term (12 wk) resistance training in older adults leads to elevated expression of muscle TNF-α, along with a number of other cytokines (81). The typically proteolytic effects of TNF-α are likely moderated in trained individuals as a result of the overall anti-inflammatory profile.

Despite lower expression of muscle TNF-α, OH had circulating TNF-α levels similar to the other groups. IL-6 was similarly expressed in muscle across the three groups but highest in the circulation of OH. Other studies have also shown an apparent disconnect between muscle and circulating inflammation (44, 72). Thus, assuming that muscle cytokine mRNA is translated and released into circulation similarly across the groups, a source other than muscle [e.g., a differential circulating immune cell population (19) or pro-inflammatory effects of greater adipose tissue mass (47) in OH] likely contributed to the observed patterns in circulating IL-6 and TNF-α. Nevertheless, the deleterious effects of inflammation on skeletal muscle were apparent in a negative association between circulating IL-6 and quadriceps muscle cross-sectional area across the groups (Figure 7). Large-scale studies (66, 67, 85) have often reported similar

346 relationships, positing that sarcopenia may be a long-term consequence of the negative
347 impact of IL-6 on muscle protein metabolism (9, 25, 84). Lifelong exercise apparently
348 exerts a positive influence on this trend, preventing the age-related increase in
349 circulating IL-6 and thereby partially attenuating the decrease in whole muscle mass
350 (13). Overall reductions in muscle mass in both older groups might also be related to
351 the age-related decrease in circulating IGF-1. This factor is known to promote
352 anabolism and reduce cytokine production in skeletal muscle (34). Despite a minimally
353 protective effect of higher lifelong training intensity in LLE-P compared to LLE-F, lifelong
354 aerobic exercise was not able to rescue the decline of IGF-1.

355 Aging may also be accompanied by an increased capacity for production of
356 PGE_2 in skeletal muscle (35). Produced in the COX pathway, this lipid-based
357 inflammatory mediator can promote protein breakdown (63) and activate pro-
358 inflammatory signaling within muscle (70). Fittingly, long-term use of COX-inhibiting
359 drugs may aid in combatting sarcopenia (32) and skeletal muscle dysfunction during
360 inflammatory conditions (6, 39) in older adults. In the present study, there was an
361 overall effect of aging (LLE and OH combined) in increasing the expression of several
362 components of the PGE_2/COX pathway. Most apparently, aging increased expression of
363 COX-1 and the PGE_2-specific synthase mPGES-1. EP3, a pro-inflammatory receptor for
364 PGE_2 (22, 46), followed a similar though non-significant pattern (+74% in OH vs. YE).
365 Comparable to previous findings at the protein level in sarcopenic muscle (35), the OH
366 group had lower expression of EP4, a purportedly anti-inflammatory PGE_2 receptor.
367 Existing data in human tissues other than skeletal muscle have demonstrated that PGE_2
368 signaling through EP4 may enhance IL-10 activity (14), reduce chemokine production

(74), and inhibit maturation of IL-1β (69). Therefore, age-related increases in pro-inflammatory flux through the PGE_2/COX pathway could have consequences for regulation of muscle mass (32), protein turnover, and exercise adaptations (78-80).

Despite an apparent age-related increase in PGE_2/COX pathway expression, LLE had higher expression of EP4. The effect of training on increasing expression of this receptor is supported in the literature (81). Given its anti-inflammatory roles, higher EP4 expression in LLE muscle could negatively regulate pro-inflammatory activity at rest and during periods of heightened PGE_2 availability (e.g., exercise). High basal expression of EP4, along with several anti-inflammatory cytokines generally supports that lifelong exercise fosters an anti-inflammatory profile, potentially as a positive adaptation to long-term aerobic training.

Subdivision of the LLE group provided insight into the effects of lifetime training intensity on basal inflammation. Within LLE, higher intensity training in LLE-P reduced basal expression of TNF-α and TGF-β, further supporting that these likely modulate one another's activity. Higher training intensity also led to upregulation of COX-2. While not typically measurable at the protein level in healthy human muscle (35, 87), COX-2 can be induced during challenging inflammatory conditions (57). Thus, higher expression of COX-2 may indicate an adaptation to intense muscular exercise for many decades in the LLE-P group. Likewise, short-term training has been shown to lead to increased basal muscle COX-2 expression in older adults (78). Despite this potential for heightened COX pathway activity in LLE-P, any increased PGE_2 production would likely result in a downstream anti-inflammatory response due to high EP4 expression in both LLE subgroups.

To provide insight into the capacity of muscle for intercellular signaling with inflammatory cells, basal macrophage abundance and gene expression of muscle chemokines and macrophage surface markers were assessed. Heightened basal expression of chemokines or elevated macrophage abundance in skeletal muscle could indicate the presence of unresolved inflammation (73). Interestingly, a general non-significant pattern for reduction in muscle chemokine expression was observed in OH (IL-8: -53%, MCP-1: -24% vs. YE), with LLE partially mitigating these effects. Both older groups had higher basal expression of anti-inflammatory (M2) macrophage surface marker CD163. However, because no differences in intramuscular macrophage abundance were detected among the groups, higher CD163 receptor density for a given number of resident macrophages might indicate heightened capacity for CD163-mediated signaling. Given its established roles in anti-inflammatory signal transduction and cytokine production (10, 53), CD163 may partially contribute to the overall anti-inflammatory profile of the LLE muscle. However, this relationship was not seen in OH, which may suggest the presence of regulatory defects between muscle and inflammatory cells (56). Such dysfunctions could contribute to impaired ability to resolve exercise-induced inflammation and create a resting environment that favors muscle atrophy in OH.

Response to Acute Resistance Exercise

Recent evidence suggests that failure to resolve inflammation after exercise may partially limit adaptability to short-term training in older adults (38, 78, 79). We sought insight into this phenomenon by examining the response to an exercise bout previously

shown to lead to adaptations when completed chronically (75, 78). The present investigation found an exaggerated response for TNF-α and TGF-β in OH muscle. TNF-α may engage proteolytic pathways within the muscle (e.g., NF-κB, MAPK) (30), and the pleiotropic nature of TGF-β may contribute to chemotaxis of inflammatory cells or aid in tissue repair following mechanical stress (26, 86). Thus, it appears that the bout of muscle loading presented a challenge to the OH muscle, whereas both YE and LLE had adapted through exercise training to better tolerate the exercise stress.

Furthermore, increased expression of cPGES in OH, coupled with higher basal expression of COX pathway components, could enable greater PGE_2 production following exercise. Previous work has demonstrated that PGE_2 leads to transcription of inflammatory and proteolytic factors within muscle (70). Thus, in combination with elevated postexercise expression of muscle cytokines, elevated PGE_2 production capacity within OH muscle could lead to heightened activity of and cross-talk between inflammatory signaling pathways. Failure to resolve this might contribute to a sustained pro-inflammatory environment after exercise. This may partially explain why COX-inhibiting drugs have successfully modulated inflammation and enhanced muscle growth in older adults undergoing resistance training (79, 80).

Production of skeletal muscle chemokines IL-8 and MCP-1 is often increased after exercise to recruit inflammatory cells to the site of insult (16). Given the role of IL-8 in signaling with inflammatory cells (2), the exercise-induced increase in IL-8 seen in only OH further suggests that the untrained aging muscle was less prepared than the trained groups to tolerate the exercise stress. This may be problematic, since other investigations have demonstrated that aging may impair the responsiveness of

inflammatory cells after exercise (11, 56). The present study did not demonstrate a difference in postexercise macrophage surface marker gene expression across groups. However, both older groups had lower expression of MCP-1 compared to YE after exercise. Thus, while aging may preserve the ability of resident macrophages to respond to exercise, there may be an age-related disconnect or delay in the ability to recruit more macrophages to aid in resolution of exercise-induced inflammation within muscle tissue. In OH, impairments in intercellular communication might lead to a sustained cytokine environment and contribute to longer duration impairments in protein balance following exercise.

Only LLE showed a modest elevation (1.4-fold) in IL-6 after exercise. The fact that no change in IL-6 was seen in the other groups is not an uncommon finding (38, 83). However, our laboratory has previously demonstrated a dramatic increase in muscle IL-6 expression (791-fold) 4 h following an exercise bout identical to the present study (37). These differences may be due to the familiarity of the stimulus to the resistance-trained subjects in the previous study (37). In support of this, muscle samples from this previous time course investigation were re-analyzed using the current qPCR conditions and confirmed the different findings between these studies. IL-6 is often highly upregulated following endurance exercise (≥1 h) (49, 71) because of its important roles in intra- and intercellular inflammatory signaling and glucose metabolism (27). Thus, the apparently higher sensitivity of LLE muscle to transcribe IL-6 after exercise may be a product of their decades of aerobic training and/or the pleiotropic nature of IL-6. A better understanding of the capacity of highly trained muscle to mount an inflammatory response might be gained by examining time points outside of the

current 4h window or by imposing a different exercise stimulus (e.g., longer duration or more familiar activity). This could also provide insight into the effect of lifelong training intensity, as the current investigation did not demonstrate any effect of training intensity on IL-6 or any other genes measured following acute exercise.

Some responses in LLE suggest that a heightened threshold may have been established as an adaptation to repeated exposure to the stress of exercise. For example, IL-10, EP3, and mPGES-1 were lower after exercise in LLE than YE, indicating a blunted response. The potent effects of IL-10 in reducing pro-inflammatory cytokine transcription and signaling (8, 45) are implicated in resolution of acute inflammation. While no data exist on the typical response to exercise for EP3 and mPGES-1, these pro-inflammatory PGE_2/COX pathway components may be involved in PGE_2 signaling after exercise to aid in muscle protein turnover necessary for remodeling (54, 82). Further research is necessary to clarify whether differences in postexercise gene expression contribute to differences in responsiveness to a resistance exercise regimen between young and lifelong exercisers.

Summary

This study supports the recent evidence that exercise training is anti-inflammatory. While aging contributes to the elevation of pro-inflammatory factors in blood and muscle, lifelong aerobic exercise training partially reduces these effects and promotes an overall anti-inflammatory profile. Lifelong training intensity appears to have a minimal effect on this pattern. Future studies may expand on these findings in a muscle fiber type-specific manner, given that slow and fast muscle fibers may have

484 differential inflammatory profiles in healthy young muscle (35, 55, 80). Aging also results
485 in an altered inflammatory response to acute exercise, which may have implications for
486 the ability to increase muscle mass and handle a loading stress. However, this effect is
487 largely rescued by lifelong exercise, with no additional influence of lifelong training
488 intensity. Thus, further investigation into whether lifelong aerobic exercise improves
489 skeletal muscle adaptability to resistance training (i.e., size and strength gains) would
490 provide considerable insight. Additionally, further work is needed to establish whether
491 LLE-induced patterns in the inflammatory profile of older skeletal muscle at baseline
492 and following exercise mirror differences between young exercisers and young
493 untrained individuals. Combined with previous studies on basal inflammation in
494 individuals with a shorter training history (40, 41), this would provide further insight into
495 whether an individual's age, training status, and/or duration of training interact to
496 provide the anti-inflammatory benefits observed here. The results of the present study
497 help to understand the long-term benefits of exercise for avoidance of a chronic
498 inflammatory state that may contribute to poor health and functional decline in aging
499 adults.

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DISCLOSURES

None.

AUTHOR CONTRIBUTIONS

Conception and design of research: KML, RKP, BJ, UR, SWT, TAT

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REFERENCES

1. **Alturki M, Beyer I, Mets T, Bautmans I**. Impact of drugs with anti-inflammatory effects on skeletal muscle and inflammation: A systematic literature review. *Exp Gerontol*. 114: 33-49, 2018.
2. **Baggiolini M, Clark-Lewis I**. Interleukin-8, a chemotactic and inflammatory cytokine. *FEBS Lett*. 307: 97-101, 1992.
3. **Bamman MM, Ferrando AA, Evans RP, Stec MJ, Kelly NA, Gruenwald JM, Corrick KL, Trump JR, Singh JA**. Muscle inflammation susceptibility: a prognostic index of recovery potential after hip arthroplasty? *Am J Physiol Endocrinol Metab*. 308: E670-679, 2015.
4. **Beavers KM, Brinkley TE, Nicklas BJ**. Effect of exercise training on chronic inflammation. *Clin Chim Acta*. 411: 785-793, 2010.
5. **Bergström J**. Muscle electrolytes in man. *Scand J Clin Lab Invest*. 14: 1-110, 1962.
6. **Beyer I, Bautmans I, Njemini R, Demanet C, Bergmann P, Mets T**. Effects on muscle performance of NSAID treatment with piroxicam versus placebo in geriatric patients with acute infection-induced inflammation. A double blind randomized controlled trial. *BMC Musculoskelet Disord*. 12: 292, 2011.
7. **Bitzer M, von Gersdorff G, Liang D, Dominguez-Rosales A, Beg AA, Rojkind M, Bottinger EP**. A mechanism of suppression of TGF-beta/SMAD signaling by NF-kappa B/RelA. *Genes Dev*. 14: 187-197, 2000.
8. **Bogdan C, Vodovotz Y, Nathan C**. Macrophage deactivation by interleukin 10. *J Exp Med*. 174: 1549-1555, 1991.
9. **Braun DA, Fribourg M, Sealfon SC**. Cytokine Response Is Determined by Duration of Receptor and Signal Transducers and Activators of Transcription 3 (STAT3) Activation. *J Biol Chem*. 288: 2986-2993, 2013.
10. **Buechler C, Ritter M, Orso E, Langmann T, Klucken J, Schmitz G**. Regulation of scavenger receptor CD163 expression in human monocytes and macrophages by pro- and antiinflammatory stimuli. *J Leukoc Biol*. 67: 97-103, 2000.
11. **Cannon JG, Orencole SF, Fielding RA, Meydani M, Meydani SN, Fiatarone MA, Blumberg JB, Evans WJ**. Acute phase response in exercise: interaction of age and vitamin E on neutrophils and muscle enzyme release. *Am J Physiol*. 259: R1214-1219, 1990.
12. **Carrick-Ranson G, Hastings JL, Bhella PS, Fujimoto N, Shibata S, Palmer MD, Boyd K, Livingston S, Dijk E, Levine BD**. The effect of lifelong exercise dose on cardiovascular function during exercise. *J Appl Physiol*. 116: 736-745, 2014.
13. **Chambers TL, Burnett TR, Raue U, Lee G, Finch WH, Graham B, Trappe TA, Trappe S**. Skeletal muscle size, function, and adiposity with lifelong aerobic exercise. *J Appl Physiol.* In review.
14. **Cheon H, Rho YH, Choi SJ, Lee YH, Song GG, Sohn J, Won NH, Ji JD**. Prostaglandin E2 augments IL-10 signaling and function. *J Immunol*. 177: 1092-1100, 2006.

15. **Clarke CJ, Hales A, Hunt A, Foxwell BM**. IL-10-mediated suppression of TNF-alpha production is independent of its ability to inhibit NF kappa B activity. *Eur J Immunol*. 28: 1719-1726, 1998.
16. **Della Gatta PA, Cameron-Smith D, Peake JM**. Acute resistance exercise increases the expression of chemotactic factors within skeletal muscle. *Eur J Appl Physiol*. 114: 2157-2167, 2014.
17. **Della Gatta PA, Garnham AP, Peake JM, Cameron-Smith D**. Effect of exercise training on skeletal muscle cytokine expression in the elderly. *Brain Behav Immun*. 39: 80-86, 2014.
18. **Dennis RA, Zhu H, Kortebein PM, Bush HM, Harvey JF, Sullivan DH, Peterson CA**. Muscle expression of genes associated with inflammation, growth, and remodeling is strongly correlated in older adults with resistance training outcomes. *Physiol Genomics*. 38: 169-175, 2009.
19. **Duggal NA, Pollock RD, Lazarus NR, Harridge S, Lord JM**. Major features of immunesenescence, including reduced thymic output, are ameliorated by high levels of physical activity in adulthood. *Aging Cell*. 17: 2018.
20. **Ferrucci L, Corsi A, Lauretani F, Bandinelli S, Bartali B, Taub D, Guralnik JM, Longo D**. The origins of age-related proinflammatory state. *Blood*. 105: 2294-2299, 2005.
21. **Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, Ottaviani E, De Benedictis G**. Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci*. 908: 244-254, 2000.
22. **Goulet JL, Pace AJ, Key ML, Byrum RS, Nguyen M, Tilley SL, Morham SG, Langenbach R, Stock JL, McNeish JD, Smithies O, Coffman TM, Koller BH**. E-prostanoid-3 receptors mediate the proinflammatory actions of prostaglandin E2 in acute cutaneous inflammation. *J Immunol*. 173: 1321-1326, 2004.
23. **Greig CA, Gray C, Rankin D, Young A, Mann V, Noble B, Atherton PJ**. Blunting of adaptive responses to resistance exercise training in women over 75y. *Exp Gerontol*. 46: 884-890, 2011.
24. **Gries KJ, Raue U, Perkins RK, Lavin KM, Overstreet BS, D'Acquisto LJ, Graham B, Finch WH, Kaminski LJ, Trappe TA, Trappe S**. Cardiovascular and skeletal muscle health with lifelong exercise. *J Appl Physiol (1985).* 125: 1636-1645, 2018.
25. **Haddad F, Zaldivar F, Cooper DM, Adams GR**. IL-6-induced skeletal muscle atrophy. *J Appl Physiol (1985)*. 98: 911-917, 2005.
26. **Heinemeier K, Langberg H, Kjaer M**. Exercise-induced changes in circulating levels of transforming growth factor-beta-1 in humans: methodological considerations. *Eur J Appl Physiol*. 90: 171-177, 2003.
27. **Helge JW, Stallknecht B, Pedersen BK, Galbo H, Kiens B, Richter EA**. The effect of graded exercise on IL-6 release and glucose uptake in human skeletal muscle. *J Physiol*. 546: 299-305, 2004.
28. **Jankord R, Jemiolo B**. Influence of physical activity on serum IL-6 and IL-10 levels in healthy older men. *Med Sci Sports Exerc*. 36: 960-964, 2004.
29. **Jemiolo B, Trappe S**. Single muscle fiber gene expression in human skeletal muscle: validation of internal control with exercise. *Biochem Biophys Res Commun*. 320: 1043-1050, 2004.

30. **Kandarian SC, Jackman RW**. Intracellular signaling during skeletal muscle atrophy. *Muscle Nerve*. 33: 155-165, 2006.
31. **Kosek DJ, Kim JS, Petrella JK, Cross JM, Bamman MM**. Efficacy of 3 days/wk resistance training on myofiber hypertrophy and myogenic mechanisms in young vs. older adults. *J Appl Physiol*. 101: 531-544, 2006.
32. **Landi F, Marzetti E, Liperoti R, Pahor M, Russo A, Martone AM, Colloca G, Capoluongo E, Bernabei R**. Nonsteroidal anti-inflammatory drug (NSAID) use and sarcopenia in older people: results from the ilSIRENTE study. *J Am Med Dir Assoc*. 14: 626.e629-e613, 2013.
33. **Lang CH, Frost RA, Vary TC**. Regulation of muscle protein synthesis during sepsis and inflammation. *Am J Physiol Endocrinol Metab*. 293: E453-E459, 2007.
34. **Lee WJ**. IGF-I exerts an anti-inflammatory effect on skeletal muscle cells through down-regulation of TLR4 signaling. *Immune Netw*. 11: 223-226, 2011.
35. **Liu SZ, Jemiolo B, Lavin KM, Lester BE, Trappe SW, Trappe TA**. Prostaglandin E2/cyclooxygenase pathway in human skeletal muscle: influence of muscle fiber type and age. *J Appl Physiol*. 120: 546-551, 2016.
36. **Livak KJ, Schmittgen TD**. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods*. 25: 402-408, 2001.
37. **Louis E, Raue U, Yang Y, Jemiolo B, Trappe S**. Time course of proteolytic, cytokine, and myostatin gene expression after acute exercise in human skeletal muscle. *J Appl Physiol*. 103: 1744-1751, 2007.
38. **Merritt EK, Stec MJ, Thalacker-Mercer A, Windham ST, Cross JM, Shelley DP, Craig Tuggle S, Kosek DJ, Kim JS, Bamman MM**. Heightened muscle inflammation susceptibility may impair regenerative capacity in aging humans. *J Appl Physiol*. 115: 937-948, 2013.
39. **Mets T, Bautmans I, Njemini R, Lambert M, Demanet C**. The influence of celecoxib on muscle fatigue resistance and mobility in elderly patients with inflammation. *Am J Geriatr Pharmacother*. 2: 230-238, 2004.
40. **Mikkelsen UR, Agergaard J, Couppe C, Grosset JF, Karlsen A, Magnusson SP, Schjerling P, Kjaer M, Mackey AL**. Skeletal muscle morphology and regulatory signalling in endurance-trained and sedentary individuals: The influence of ageing. *Exp Gerontol*. 93: 54-67, 2017.
41. **Mikkelsen UR, Couppé C, Karlsen A, Grosset JF, Schjerling P, Mackey AL, Klausen HH, Magnusson SP, Kjaer M**. Life-long endurance exercise in humans: Circulating levels of inflammatory markers and leg muscle size. *Mech Ageing Dev*. 134: 531-540, 2013.
42. **Minuzzi LG, Chupel MU, Rama L, Rosado F, Munoz VR, Gaspar RC, Kuga GK, Furtado GE, Pauli JR, Teixeira AM**. Lifelong exercise practice and immunosenescence: Master athletes cytokine response to acute exercise. *Cytokine*. 115: 1-7, 2019.
43. **Minuzzi LG, Rama L, Bishop NC, Rosado F, Martinho A, Paiva A, Teixeira AM**. Lifelong training improves anti-inflammatory environment and maintains the number of regulatory T cells in masters athletes. *Eur J Appl Physiol*. 117: 1131-1140, 2017.

44. **Moldoveanu AI, Shephard RJ, Shek PN**. Exercise elevates plasma levels but not gene expression of IL-1beta, IL-6, and TNF-alpha in blood mononuclear cells. *J Appl Physiol*. 89: 1499-1504, 2000.
45. **Moore KW, O'Garra A, Malefyt R, Vieira P, Mosmann TR**. Interleukin 10. *Annu Rev Immunol*. 11: 165-190, 1993.
46. **Morimoto K, Shirata N, Taketomi Y, Tsuchiya S, Segi-Nishida E, Inazumi T, Kabashima K, Tanaka S, Murakami M, Narumiya S, Sugimoto Y**. Prostaglandin E2-EP3 signaling induces inflammatory swelling by mast cell activation. *J Immunol*. 192: 1130-1137, 2014.
47. **Mraz M, Haluzik M**. The role of adipose tissue immune cells in obesity and low-grade inflammation. *J Endocrinol*. 222: R113-127, 2014.
48. **Murach K, Raue U, Wilkerson B, Minchev K, Jemiolo B, Bagley J, Luden N, Trappe S**. Single muscle fiber gene expression with run taper. *PLoS One*. 9: e108547, 2014.
49. **Nieman DC, Davis JM, Henson DA, Gross SJ, Dumke CL, Utter AC, Vinci DM, Carson JA, Brown A, McAnulty SR, McAnulty LS, Triplett NT**. Muscle cytokine mRNA changes after 2.5 h of cycling: influence of carbohydrate. *Med Sci Sports Exerc*. 37: 1283-1290, 2005.
50. **Nilwik R, Snijders T, Leenders M, Groen BB, van Kranenburg J, Verdijk LB, van Loon LJ**. The decline in skeletal muscle mass with aging is mainly attributed to a reduction in type II muscle fiber size. *Exp Gerontol*. 48: 492-498, 2013.
51. **Olesen J, Biensø RS, Meinertz S, van Hauen L, Rasmussen SM, Gliemann L, Plomgaard P, Pilegaard H**. Impact of training status on LPS-induced acute inflammation in humans. *J Appl Physiol*. 118: 818-829, 2015.
52. **Pedersen BK, Steensberg A, Schjerling P**. Muscle-derived interleukin-6: possible biological effects. *J Physiol*. 536: 329-337, 2001.
53. **Philippidis P, Mason JC, Evans BJ, Nadra I, Taylor KM, Haskard DO, Landis RC**. Hemoglobin scavenger receptor CD163 mediates interleukin-10 release and heme oxygenase-1 synthesis: antiinflammatory monocyte-macrophage responses in vitro, in resolving skin blisters in vivo, and after cardiopulmonary bypass surgery. *Circ Res*. 94: 119-126, 2004.
54. **Phillips SM, Tipton KD, Aarsland A, Wolf SE, Wolfe RR**. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *Am J Physiol*. 273 (Pt 1): E99-E107, 1997.
55. **Plomgaard P, Penkowa M, Pedersen BK**. Fiber type specific expression of TNF-alpha, IL-6 and IL-18 in human skeletal muscles. *Exerc Immunol Rev*. 11: 53-63, 2005.
56. **Przybyla B, Gurley C, Harvey J, Bearden E, Kortebein P, Evans W, Sullivan DH, Peterson CA, Dennis RA**. Aging alters macrophage properties in human skeletal muscle both at rest and in response to acute resistance exercise. *Exp Gerontol*. 41: 320-327, 2006.
57. **Rabuel C, Renaud E, Brealey D, Ratajczak P, Damy T, Alves A, Habib A, Singer M, Payen D, Mebazaa A**. Human septic myopathy: induction of cyclooxygenase, heme oxygenase and activation of the ubiquitin proteolytic pathway. *Anesthesiology*. 101: 583-590, 2004.

58. **Raue U, Jemiolo B, Yang Y, Trappe S**. TWEAK-Fn14 pathway activation after exercise in human skeletal muscle: insights from two exercise modes and a time course investigation. *J Appl Physiol (1985)*. 118: 569-578, 2015.
59. **Raue U, Slivka D, Jemiolo B, Hollon C, Trappe S**. Myogenic gene expression at rest and after a bout of resistance exercise in young (18-30 yr) and old (80-89 yr) women. *J Appl Physiol (1985)*. 101: 53-59, 2006.
60. **Raue U, Slivka D, Jemiolo B, Hollon C, Trappe S**. Proteolytic gene expression differs at rest and after resistance exercise between young and old women. *J Gerontol A Biol Sci Med Sci*. 62: 1407-1412, 2007.
61. **Raue U, Slivka D, Minchev K, Trappe S**. Improvements in whole muscle and myocellular function are limited with high-intensity resistance training in octogenarian women. *J Appl Physiol (1985)*. 106: 1611-1617, 2009.
62. **Raue U, Trappe TA, Estrem ST, Qian HR, Helvering LM, Smith RC, Trappe S**. Transcriptome signature of resistance exercise adaptations: mixed muscle and fiber type specific profiles in young and old adults. *J Appl Physiol (1985)*. 112: 1625-1636, 2012.
63. **Rodemann HP, Goldberg AL**. Arachidonic acid, prostaglandin E and F2a influence rates of protein turnover in skeletal and cardiac muscle. *J Biol Chem*. 257: 1632-1638, 1982.
64. **Sailani MR, Halling JF, Moller HD, Lee H, Plomgaard P, Pilegaard H, Snyder MP, Regenberg B**. Lifelong physical activity is associated with promoter hypomethylation of genes involved in metabolism, myogenesis, contractile properties and oxidative stress resistance in aged human skeletal muscle. *Sci Rep*. 9: 3272, 2019.
65. **Sardeli AV, Tomeleri CM, Cyrino ES, Fernhall B, Cavaglieri CR, Chacon-Mikahil MPT**. Effect of resistance training on inflammatory markers of older adults: A meta-analysis. *Exp Gerontol*. 111: 188-196, 2018.
66. **Schaap LA, Pluijm SM, Deeg DJ, Harris TB, Kritchevsky SB, Newman AB, Colbert LH, Pahor M, Rubin SM, Tylavsky FA, Visser M**. Higher inflammatory marker levels in older persons: associations with 5-year change in muscle mass and muscle strength. *J Gerontol A Biol Sci Med Sci*. 64A: 1183-1189, 2009.
67. **Schaap LA, Pluijm SM, Deeg DJ, Visser M**. Inflammatory markers and loss of muscle mass (sarcopenia) and strength. *Am J Med*. 119: 526.e529-e517, 2006.
68. **Slivka D, Raue U, Hollon C, Minchev K, Trappe S**. Single muscle fiber adaptations to resistance training in old (>80 yr) men: evidence for limited skeletal muscle plasticity. *Am J Physiol Regul Integr Comp Physiol*. 295: R273-R280, 2008.
69. **Sokolowska M, Chen LY, Liu Y, Martinez-Anton A, Qi HY, Logun C, Alsaaty S, Park YH, Kastner DL, Chae JJ, Shelhamer JH**. Prostaglandin E2 inhibits NLRP3 inflammasome activation through EP4 receptor and intracellular cyclic AMP in human macrophages. *J Immunol*. 194: 5472-5487, 2015.
70. **Standley RA, Liu SZ, Jemiolo B, Trappe SW, Trappe TA**. Prostaglandin E2 induces transcription of skeletal muscle mass regulators interleukin-6 and muscle RING finger-1 in humans. *Prostaglandins Leukot Essent Fatty Acids*. 88: 361-364, 2013.

71. **Steensberg A, Keller C, Starkie RL, Osada T, Febbraio MA, Pedersen BK**. IL-6 and TNF-alpha expression in, and release from, contracting human skeletal muscle. *Am J Physiol Endocrinol Metab*. 283: E1272-1278, 2002.
72. **Steensberg A, van Hall G, Osada T, Sacchetti M, Saltin B, Klarlund Pedersen B**. Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *J Physiol*. 529 Pt 1: 237-242, 2000.
73. **Sugimoto MA, Sousa LP, Pinho V, Perretti M, Teixeira MM**. Resolution of inflammation: what controls its onset? *Front Immunol*. 7: 771-718, 2016.
74. **Takayama K, Garcia-Cardena G, Sukhova GK, Comander J, Gimbrone MA, Jr., Libby P**. Prostaglandin E2 suppresses chemokine production in human macrophages through the EP4 receptor. *J Biol Chem*. 277: 44147-44154, 2002.
75. **Trappe S, Godard M, Gallagher P, Carroll C, Rowden G, Porter D**. Resistance training improves single muscle fiber contractile function in older women. *Am J Physiol Cell Physiol*. 281: C398-406, 2001.
76. **Trappe S, Hayes E, Galpin A, Kaminsky L, Jemiolo B, Fink W, Trappe T, Jansson A, Gustafsson T, Tesch P**. New records in aerobic power among octogenarian lifelong endurance athletes. *J Appl Physiol*. 114: 3-10, 2013.
77. **Trappe S, Williamson D, Godard M, Porter D, Rowden G, Costill D**. Effect of resistance training on single muscle fiber contractile function in older men. *J Appl Physiol (1985)*. 89: 143-152, 2000.
78. **Trappe TA, Carroll CC, Dickinson JM, LeMoine JK, Haus JM, Sullivan BE, Lee JD, Jemiolo B, Weinheimer EM, Hollon CJ**. Influence of acetaminophen and ibuprofen on skeletal muscle adaptations to resistance exercise in older adults. *Am J Physiol Regul Integr Comp Physiol*. 300: R655-R662, 2011.
79. **Trappe TA, Liu SZ**. Effects of prostaglandins and COX-inhibiting drugs on skeletal muscle adaptations to exercise. *J Appl Physiol*. 115: 909-919, 2013.
80. **Trappe TA, Ratchford SM, Brower BE, Liu SZ, Lavin KM, Carroll CC, Jemiolo B, Trappe SW**. COX inhibitor influence on skeletal muscle fiber size and metabolic adaptations to resistance exercise in older adults. *J Gerontol A Biol Sci Med Sci*. 71: 1289-1294, 2016.
81. **Trappe TA, Standley RA, Jemiolo B, Carroll CC, Trappe SW**. Prostaglandin and myokine involvement in the cyclooxygenase-inhibiting drug enhancement of skeletal muscle adaptations to resistance exercise in older adults. *Am J Physiol Regul Integr Comp Physiol*. 304: R198-R205, 2013.
82. **Trappe TA, White F, Lambert CP, Cesar D, Hellerstein M, Evans WJ**. Effect of ibuprofen and acetaminophen on postexercise muscle protein synthesis. *Am J Physiol Endocrinol Metab*. 282: E551-E556, 2002.
83. **Tsintzas K, Stephens FB, Snijders T, Wall BT, Cooper S, Mallinson J, Verdijk LB, van Loon LJC**. Intramyocellular lipid content and lipogenic gene expression responses following a single bout of resistance type exercise differ between young and older men. *Exp Gerontol*. 93: 36-45, 2017.
84. **van Hall G, Steensberg A, Fischer C, Keller C, Møller K, Moseley P, Pedersen BK**. Interleukin-6 markedly decreases skeletal muscle protein turnover and increases nonmuscle amino acid utilization in healthy individuals. *J Clin Endocrinol Metab*. 93: 2851-2858, 2008.

85. **Visser M, Pahor M, Taaffe DR, Goodpaster BH, Simonsick EM, Newman AB, Nevitt M, Harris TB**. Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: the Health ABC Study. *J Gerontol A Biol Sci Med Sci*. 57: M326-332, 2002.
86. **Wahl SM, Hunt DA, Wakefield LM, McCartney-Francis N, Wahl LM, Roberts AB, Sporn MB**. Transforming growth factor type beta induces monocyte chemotaxis and growth factor production. *Proc Natl Acad Sci*. 84: 5788-5792, 1987.
87. **Weinheimer EM, Jemiolo B, Carroll CC, Harber MP, Haus JM, Burd NA, LeMoine JK, Trappe SW, Trappe TA**. Resistance exercise and cyclooxygenase (COX) expression in human skeletal muscle: implications for COX-inhibiting drugs and protein synthesis. *Am J Physiol Regul Integr Comp Physiol*. 292: R2241-2248, 2007.
88. **Williamson DL, Gallagher PM, Carroll CC, Raue U, Trappe SW**. Reduction in hybrid single muscle fiber proportions with resistance training in humans. *J Appl Physiol (1985)*. 91: 1955-1961, 2001.
89. **Yang Y, Creer A, Jemiolo B, Trappe S**. Time course of myogenic and metabolic gene expression in response to acute exercise in human skeletal muscle. *J Appl Physiol (1985)*. 98: 1745-1752, 2005.

FIGURE CAPTIONS

Figure 1. Representative cross-section from vastus lateralis skeletal muscle of a lifelong exerciser. Immunohistochemically stained $CD68^+$ cells appear red, and hematoxylin-stained nuclei appear purple.

Figure 2. (A) Basal expression and (B) exercise-induced fold-change in expression of pro-inflammatory cytokines in vastus lateralis skeletal muscle homogenate of young exercisers (YE), lifelong exercisers (LLE), and old healthy (OH). The dashed line at 1.0-fold represents the preexercise fold-change for each group, derived from the $2^{-\Delta\Delta C_T}$ calculation (see methods). AU: arbitrary units; TNF-α: tumor necrosis factor α. ‡$P \leq 0.05$ vs. OH, †$P \leq 0.10$ vs. YE, **$P \leq 0.05$ vs. preexercise.

Figure 3. (A) Basal expression and (B) exercise-induced fold-change in expression of anti-inflammatory cytokines in vastus lateralis skeletal muscle homogenate of (A) young exercisers (YE), lifelong exercisers (LLE), and old healthy (OH). The dashed line at 1.0-fold represents the preexercise fold-change for each group, derived from the $2^{-\Delta\Delta C_T}$ calculation (see methods). AU: arbitrary units; IL-1Ra: interleukin 1 receptor antagonist; TGF-β: transforming growth factor β. *$P \leq 0.05$ vs. YE, ‡$P \leq 0.05$ vs. OH, †$P \leq 0.10$ vs. YE, **$P \leq 0.05$ vs. preexercise.

Figure 4. (A) Basal expression and (B) exercise-induced fold-change in expression of cyclooxygenase (COX) enzymes in vastus lateralis skeletal muscle homogenate of young exercisers (YE), lifelong exercisers (LLE), and old healthy (OH). The dashed line at 1.0-fold represents the preexercise fold-change for each group, derived from the $2^{-\Delta\Delta C_T}$ calculation (see methods). AU: arbitrary units; v1: variant 1; v2: variant 2. *$P \leq 0.05$ vs. YE, **$P \leq 0.05$ vs. preexercise.

Figure 5. (A) Basal expression and (B) exercise-induced fold-change in expression of PGE_2/COX pathway components in in vastus lateralis skeletal muscle homogenate of young exercisers (YE), lifelong exercisers (LLE), and old healthy (OH). The dashed line at 1.0-fold represents the preexercise fold-change for each group, derived from the $2^{-\Delta\Delta C_T}$ calculation (see methods). AU: arbitrary units; cPGES: cytosolic prostaglandin E_2 synthase; m: microsomal prostaglandin E_2 synthase; EP: E-prostanoid receptor. *$P \leq 0.05$ vs. YE, ‡$P \leq 0.05$ vs. OH, †$P \leq 0.10$ vs. YE, **$P \leq 0.05$ vs. preexercise.

Figure 6. (A) Basal expression and (B) exercise-induced fold-change in expression of chemokines and macrophage surface markers in vastus lateralis skeletal muscle homogenate of young exercisers (YE), lifelong exercisers (LLE), and old healthy (OH). The dashed line at 1.0-fold represents the preexercise fold-change for each group, derived from the $2^{-\Delta\Delta C_T}$ calculation (see methods). AU: arbitrary units; IL: interleukin; MCP-1: monocyte chemoattractant protein 1; CD: cluster of differentiation. *$P \leq 0.05$ vs. YE, †$P \leq 0.10$ vs. YE and LLE, **$P \leq 0.05$ vs. preexercise, §$P \leq 0.10$ vs. preexercise.

855 **Figure 7.** Association between serum interleukin (IL-6) and quadriceps muscle cross-
856 sectional area (CSA) in young exercisers (YE), lifelong exercisers (LLE), and old
857 healthy (OH) men. This finding is in agreement with the work of others suggesting a
858 negative impact of circulating inflammation on skeletal muscle (41, 66, 67, 85).

Table 1. Subject Characteristics

		Lifelong Exercisers			
	YE	**Combined**	**LLE-P**	**LLE-F**	**OH**
N	10	21	14	7	10
Age (y)	25 ± 1*	74 ± 1	74 ± 1	75 ± 2	75 ± 1
Height (cm)	181 ± 2	180 ± 2	179 ± 2	182 ± 3	177 ± 2
Weight (kg)	75 ± 3	79 ± 2	77 ± 2	83 ± 5	88 ± 3*
BMI (kg/m^2)	23 ± 1	24 ± 1	24 ± 1	25 ± 1	28 ± 1*
Body fat (%)	18 ± 2*	24 ± 1†	22 ± 1‡	27 ± 1	32 ± 1
VO_2max (mL/kg/min)	53 ± 3*	34 ± 1†	38 ± 1‡	27 ± 2	22 ± 1
Quadriceps size (cm^2)	78 ± 3*	67 ± 2†	68 ± 2	65 ± 3	56 ± 3
Quadriceps strength (N)	596 ± 29*	478 ± 16†	481 ± 19	474 ± 29	387 ± 25
Quadriceps power (W)	699 ± 30*	370 ± 19	365 ± 13	377 ± 50	318 ± 42
Handgrip strength (kg)	51 ± 3	46 ± 2	48 ± 3	43 ± 2	44 ± 1
Steps per day	9404 ± 635	9560 ± 619	9369 ± 725	10006 ± 1265	5813 ± 488*

Values are mean ± SE. YE: Young Exercisers, LLE: Lifelong Exercisers, LLE-P: Lifelong Exercisers-Performance, LLE-F: Lifelong Exercisers-Fitness, OH: Old Healthy. *$P \leq 0.05$ vs. main groups, †$P \leq 0.05$ vs. OH, ‡$P \leq 0.05$ LLE-P vs. LLE-F. Additional cardiovascular and skeletal muscle data, as well as details of the body fat (DXA), VO_2max, muscle size (MRI) and function, and steps per day measurements are presented by us elsewhere (13, 24).

Table 2. Exercise Training Histories

		Lifelong Exercisers			
	YE	**Combined**	**LLE-P**	**LLE-F**	**OH**
Total Training Years	5 ± 1*	53 ± 1	53 ± 1	53 ± 3	-
Competitive Focus[1]	Yes	-	Yes	No	-
Lifetime Average					
Frequency (d/wk)	-	4.5 ± 0.2	4.4 ± 0.2	4.6 ± 0.3	-
Duration (h/wk)	-	7.3 ± 0.5	7.6 ± 0.7	6.6 ± 0.9	-
Intensity[2]	-	2.0 ± 0.1	2.1 ± 0.1‡	1.8 ± 0.1	-
Current Decade					
Frequency (d/wk)	5.1 ± 0.2	4.7 ± 0.3	4.5 ± 0.3	4.9 ± 0.7	-
Duration (h/wk)	7.0 ± 0.7	8.1 ± 1.1	8.5 ± 1.4	7.4 ± 1.9	-
Intensity[2]	2.8 ± 0.1*	2.0 ± 0.1	2.2 ± 0.1‡	1.5 ± 0.2	-

Values are mean ± SE. [1]Competitive focus indicates exercise training for the purpose of competition was currently or once a primary goal for the majority of the group. Lifetime average reflects current decade exercise habits for YE. [2]Levels of self-reported training intensity were: 1 (Light), 2 (Moderate), and 3 (Hard). In the case that a subject reported more than one training intensity, values were weighted and averaged (e.g., 80% of training at a 2 and 20% of training at a 3 resulted in an overall intensity of 2.2). *$P \leq 0.05$ vs. LLE Combined, ‡$P \leq 0.05$ LLE-P vs. LLE-F. More detailed exercise training histories are presented by us elsewhere (24).

Table 3. Nomenclature, gene information, and mRNA primer characteristics

Common Name	Gene Name	Accession #	Sequence (5'→3')	Amplicon Size, bp	mRNA Region, bp	Annealing Temp, ºC
Pro-Inflammatory Cytokines						
IL-1β	IL1B	NM_000576.2	GGATATGGAGCAACAAGTGGTG CGCAGGACAGGTACAGATTCT	113	661–773	61
TNF-α	TNF	NM_000594.3	CCCAGGCAGTCAGATCATCTTCTCGAA CTGGTTATCTCTCAGCTCCACGCCATT	149	390–538	60
Anti-Inflammatory Cytokines						
IL-10	IL10	NM_000572.2	GGCGCTGTCATCGATTTCTTCC GGCTTTGTAGATGCCTTTCTCTTG	101	430–530	60
IL-4	IL4	NM_000589.3[a]	TCTTCCTGCTAGCATGTGCC TGTTACGGTCAACTCGGTGC	128	100–227	60
IL-1Ra	IL1RN	NM_173842.2[b]	AGCTGGAGGCAGTTAACATCA ACTCAAAACTGGTGGTGGGG	102	375–476	60
Pleiotropic Cytokines						
IL-6	IL6	NM_000600.4	CTATGAACTCCTTCTCCACAAGCGCCTT GGGGCGGCTACATCTTTGGAATCTT	127	61–187	60
TGF-β	TGFB1	NM_000660.6	ACCAACTATTGCTTCAGCTCCA GAAGTTGGCATGGTAGCCCT	120	1683–1802	60
PGE_2/COX Pathway Components						
COX-1 v1[1]	PTGS1	NM_000962	CCCAGGAGTACAGCTACGAGCAGTTCTT CCAGCAATCTGGCGAGAGAAGGCAT	101	1327–1427	60
COX-1 v2[1*]	PTGS1	NM_080591	GTCCAGTTCCAATACCGCAACCGCAT CCACCGATCTTGAAGGAGTCAGGCAT	92	1237–1328	60
COX-2[2]	PTGS2	NM_000963.3	TTGCTGGCAGGGTTGCTGGTGGTA CATCTGCCTGCTCTGGTCAATGGAA	86	1381–1466	60
cPGES[3]	PTGES3	NM_006601.6	AGGCCCGCCCACCAGTTCGC AGTCCCTTCGATCGTACCACTTTGCAG	82	254–335	60
mPGES-1[4]	PTGES	NM_004878.4	CGGAAGAAGGCCTTTGCCAACC GGGTAGATGGTCTCCATGTCGTTCC	125	171–295	60
EP3[5]	PTGER3	NM_198715.2[c]	CTGGTCTCCGCTCCTGATAA TTCAGTGAAGCCAGGCGAAC	132	1113–1244	60
EP4[6]	PTGER4	NM_000958.2	GCTCGTGGTGCGAGTATTCGTCAACC TCCAGGGGTCTAGGATGGGGTTCA	122	1453–1574	60
Chemokines and Macrophage Surface Markers						
IL-8[7]	CXCL8	NM_000584.3	GCTCTGTGTGAAGGTGCAGTTTTGCCAA GGCGCAGTGTGGTCCACTCTCAAT	135	153–287	60
MCP-1[8]	CCL2	NM_002982.3	GCAATCAATGCCCCAGTCAC CTTGAAGATCACAGCTTCTTTGGG	123	152–274	60
CD16b[9]	FCGR3B	NM_001271037.1	CCAGGCCTCGAGCTACTTCA TGCCAAACCGATATGGACTTCT	121	441 –561	60
CD163	CD163	NM_004244.5[d]	CCCAGTGAGTTCAGCCTTTA TCAGCAGCAGTCTTAGGAATC	140	3600–3739	60

Other aliases: [1]Prostaglandin-endoperoxidase synthase 1; [2]Prostaglandin-endoperoxidase synthase 2; [3]Prostaglandin E synthase 3; [4]Prostaglandin E synthase; [5]Prostaglandin E receptor 3; [6]Prostaglandin E receptor 4; [7]C-X-C motif chemokine ligand 8; [8]C-C motif chemokine ligand 2; [9]Fc fragment of IgG receptor IIIb. *One primer was designed for each variant of COX-1 based on our previous research (W,07). Top sequence reflects the Forward primer and bottom sequence reflects the Reverse primer. [a]Primers detect variant 1 isoform 1 (NM_000589.3). [b]Primers detect all variants: variant 1 isoform 1 (NM_173842.2), variant 2 isoform 2 (NM_173841.2), variant 3 isoform 3 (NM_000577.4), variant 4 isoform 4 (NM_173843.2), and variant 5 isoform 4 (NM_001318914.1). [c]Primer detects variant 4 isoform 4 (NM_198714.1), variant 5 isoform 5 (NM_198715.2), variant 6 isoform 6 (NM_198716.1), variant 7 isoform 7 (NM_198717.1), variant 8 isoform 8 (NM_198718.1), variant 9 isoform 4 (NM_198719.1) and variant 11 isoform 4 (NM_001126044.1). [d]Primer detects both variant 1 isoform a (NM_004244.5) and variant 2 isoform b (NM_203416.3). See text for definitions of abbreviations.

Table 4. Basal serum concentrations of inflammatory factors and intramuscular macrophage abundance

		Lifelong Exercisers			
	YE	Combined	LLE-P	LLE-F	OH
Serum Inflammatory Factors					
IL-6 (pg/mL)	0.9 ± 0.1	2.0 ± 0.2†	2.1 ± 0.2	1.8 ± 0.4	3.9 ± 1.2*
TNF-α (pg/mL)	1.7 ± 0.2	1.7 ± 0.1	1.6 ± 0.1	1.9 ± 0.3	1.3 ± 0.2
CRP (mg/L)	0.6 ± 0.2	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.3	0.8 ± 0.1
IGF-1 (ng/mL)	204 ± 14	116 ± 5*	124 ± 6‡	101 ± 5	117 ± 12*
Intramuscular Macrophage Abundance					
$CD68^+$ cells/100 fibers	7.8 ± 1.4	8.0 ± 0.7	8.3 ± 0.8	7.3 ± 1.4	6.8 ± 1.1
$CD68^+$ cells/mm^2	13.0 ± 2.4	12.8 ± 1.2	13.4 ± 1.5	11.2 ± 2.0	11.7 ± 2.0

Values are mean ± SE. *$P \leq 0.05$ vs. YE; †$P \leq 0.05$ vs. OH; ‡$P \leq 0.05$ LLE-P vs. LLE-F.

Table 5. Basal gene expression in LLE subgroups

	LLE-P	LLE-F
Pro-Inflammatory Cytokines		
IL-6	0.16 ± 0.03	0.17 ± 0.04
TNF-α*	0.21 ± 0.03	0.33 ± 0.06
IL-1β	0.88 ± 0.14	0.66 ± 0.12
Anti-Inflammatory Cytokines		
IL-10	0.24 ± 0.04	0.19 ± 0.04
IL-4	0.87 ± 0.17	0.59 ± 0.32
IL-1Ra	0.83 ± 0.16	1.46 ± 0.60
TGF-β*	60.3 ± 3.7	89.3 ± 16.2
PGE_2/COX Pathway Components		
COX-1v1	2.71 ± 0.22	2.90 ± 0.27
COX-1v2	4.89 ± 0.43	5.94 ± 0.60
COX-2†	0.22 ± 0.03	0.13 ± 0.03
cPGES	214 ± 17	225 ± 22
mPGES-1	3.97 ± 0.62	4.75 ± 0.76
EP3	3.36 ± 0.90	4.02 ± 0.83
EP4	9.54 ± 0.58	9.03 ± 0.51
Chemokines and Macrophage Markers		
IL-8	0.22 ± 0.04	0.23 ± 0.05
MCP-1	51.3 ± 5.2	53.5 ± 14.7
CD16b	2.56 ± 0.29	3.02 ± 1.28
CD163	54.1 ± 7.0	41.3 ± 5.9

Values are in arbitrary units and presented as group mean ± SE.
*$P \leq 0.05$ between groups; †$P \leq 0.10$ between groups.

Table 6. Change in gene expression in LLE subgroups

		LLE-P	LLE-F
Pro-Inflammatory Cytokines			
IL-6	Pre	1.24 ± 0.21	1.25 ± 0.32
	Post	1.55 ± 0.28	1.23 ± 0.23
TNF-α	Pre	1.08 ± 0.12	1.12 ± 0.19
	Post	1.25 ± 0.14	0.94 ± 0.11
IL-1β	Pre	1.22 ± 0.24	1.10 ± 0.21
	Post	0.88 ± 0.13	1.23 ± 0.26
Anti-Inflammatory Cytokines			
IL-10	Pre	1.20 ± 0.21	1.10 ± 0.21
	Post	0.95 ± 0.13	1.23 ± 0.25
IL-4	Pre	1.31 ± 0.28	1.84 ± 1.01
	Post*	2.55 ± 0.62	2.45 ± 0.88
IL-1Ra	Pre	1.23 ± 0.25	1.37 ± 0.57
	Post	1.22 ± 0.12	0.76 ± 0.15
TGF-β	Pre	1.03 ± 0.07	1.09 ± 0.20
	Post	1.35 ± 0.16	1.00 ± 0.12
PGE_2/COX Pathway Components			
COX-1v1	Pre	1.05 ± 0.90	1.03 ± 0.90
	Post	0.94 ± 0.09	0.82 ± 0.11
COX-1v2	Pre	1.05 ± 0.11	1.03 ± 0.10
	Post†	0.91 ± 0.10	0.78 ± 0.11
COX-2	Pre	1.20 ± 0.20	1.21 ± 0.26
	Post*	1.58 ± 0.22	2.15 ± 0.28
cPGES	Pre	1.04 ± 0.08	1.03 ± 0.10
	Post	1.17 ± 0.06	1.08 ± 0.10
mPGES-1	Pre	1.21 ± 0.23	1.09 ± 0.18
	Post*	0.70 ± 0.11	0.80 ± 0.12
EP3	Pre	1.52 ± 0.51	1.63 ± 0.34
	Post	1.75 ± 0.22	1.62 ± 0.16
EP4	Pre	1.02 ± 0.06	1.01 ± 0.06
	Post	1.11 ± 0.08	1.10 ± 0.11
Chemokines and Macrophage Markers			
IL-8	Pre	1.28 ± 0.28	1.20 ± 0.27
	Post†	1.18 ± 0.18	0.53 ± 0.11
MCP-1	Pre	1.07 ± 0.12	1.17 ± 0.32
	Post	1.30 ± 0.13	1.32 ± 0.20
CD16b	Pre	1.09 ± 0.13	1.48 ± 0.63
	Post†	1.68 ± 0.23	1.64 ± 0.48
CD163	Pre	1.08 ± 0.14	1.05 ± 0.15
	Post*	1.28 ± 0.21	1.57 ± 0.26

Values represent fold-change from preexercise and are presented as mean ± SE. *$P \leq 0.05$ vs. preexercise; †$P \leq 0.10$ vs. preexercise

Figure 1

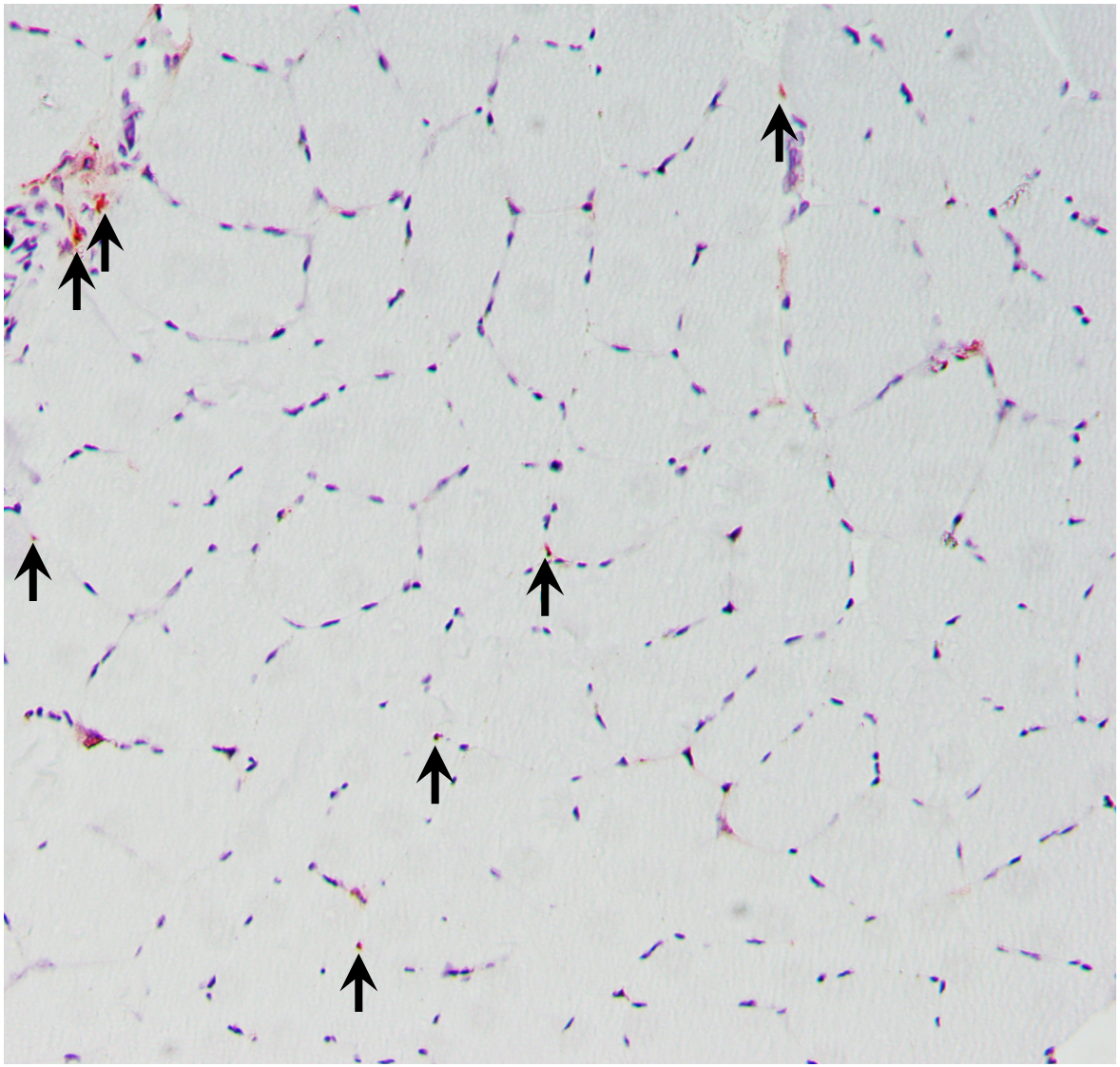

Figure 2

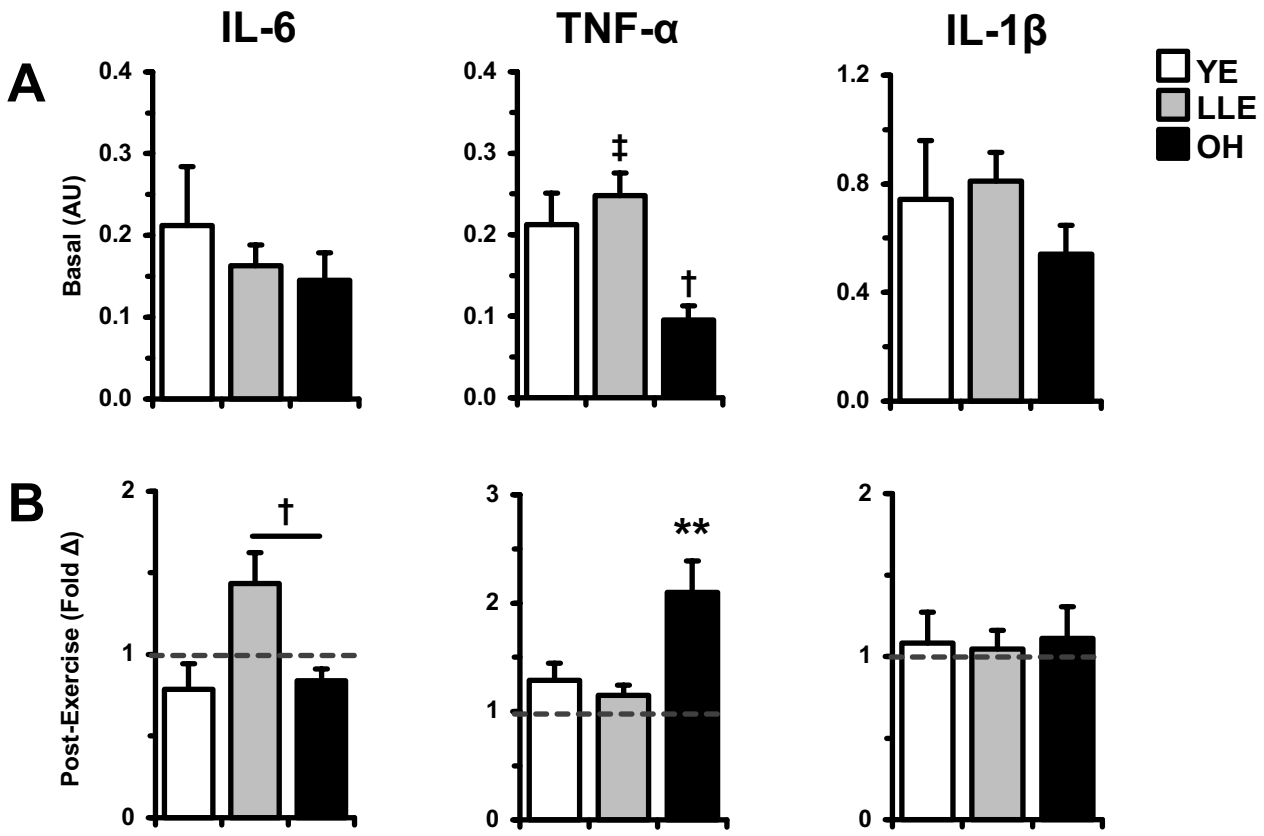

Figure 3

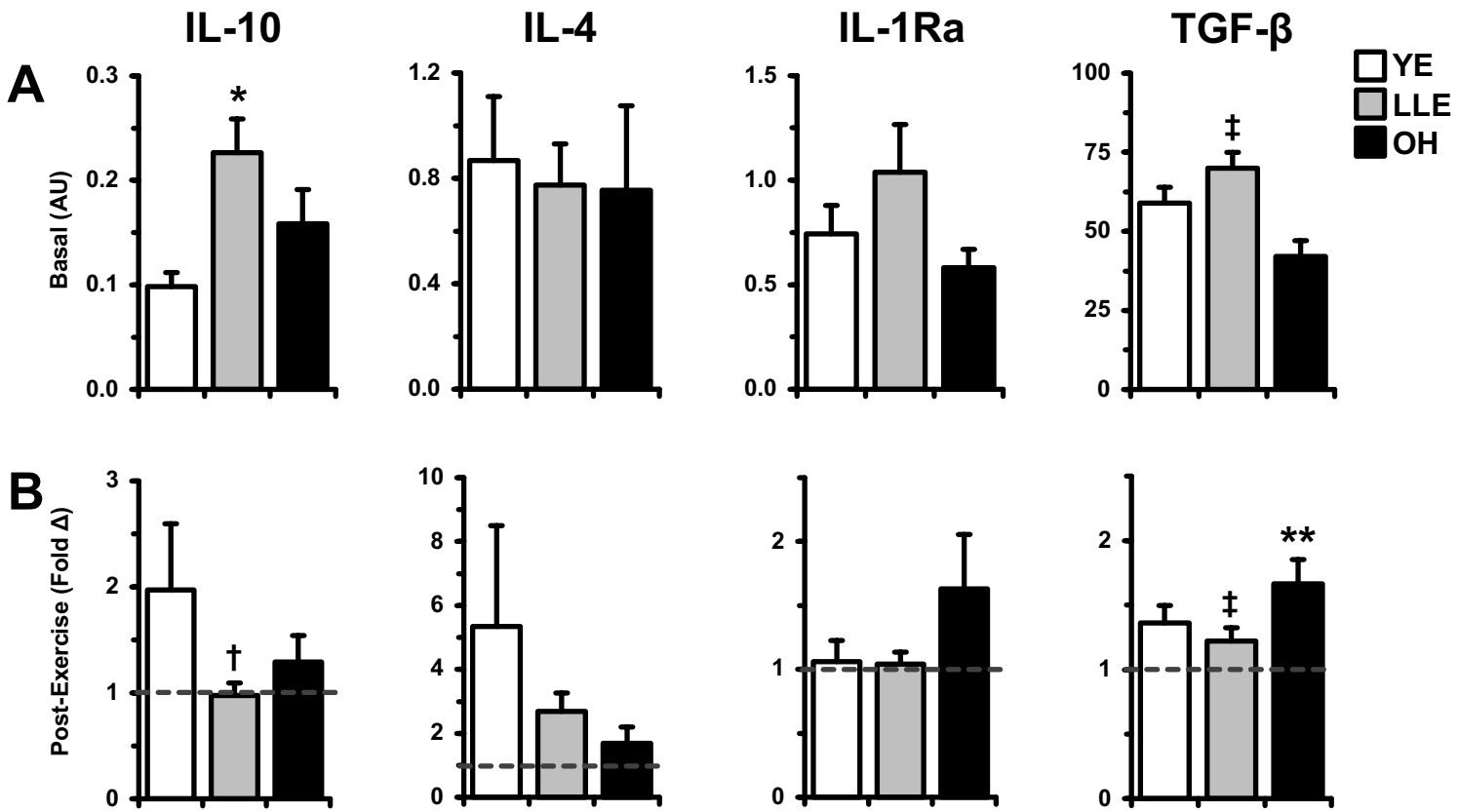

Figure 4

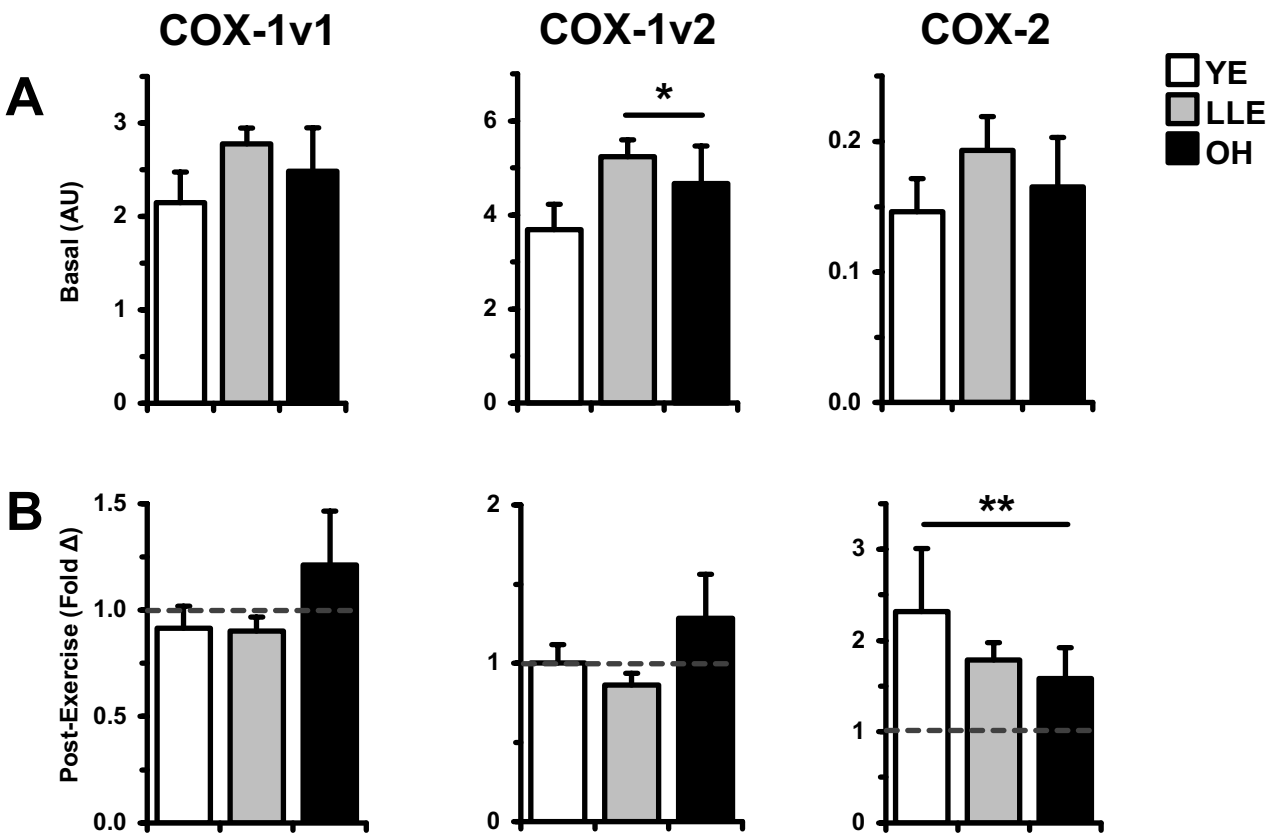

Figure 5

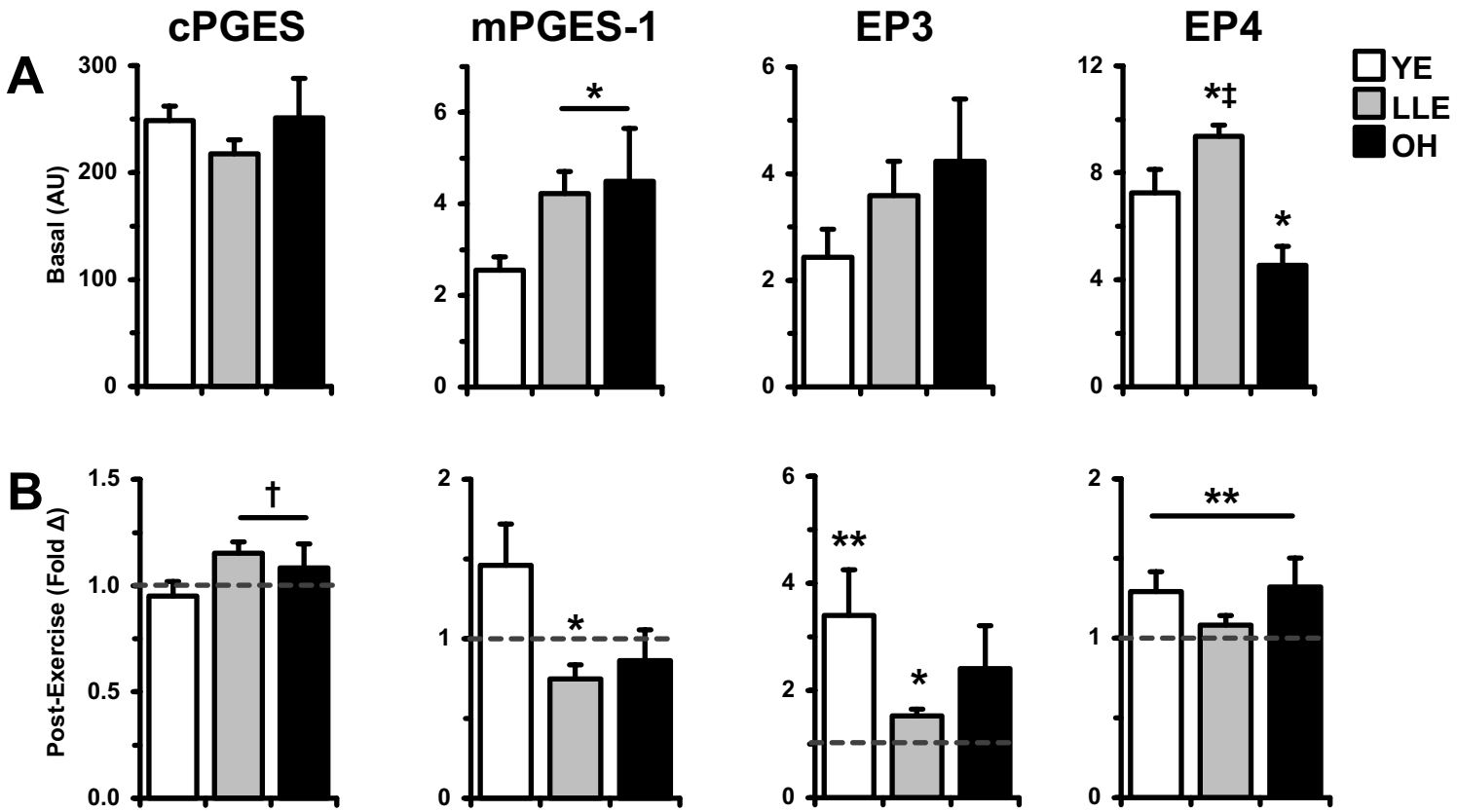

Figure 6

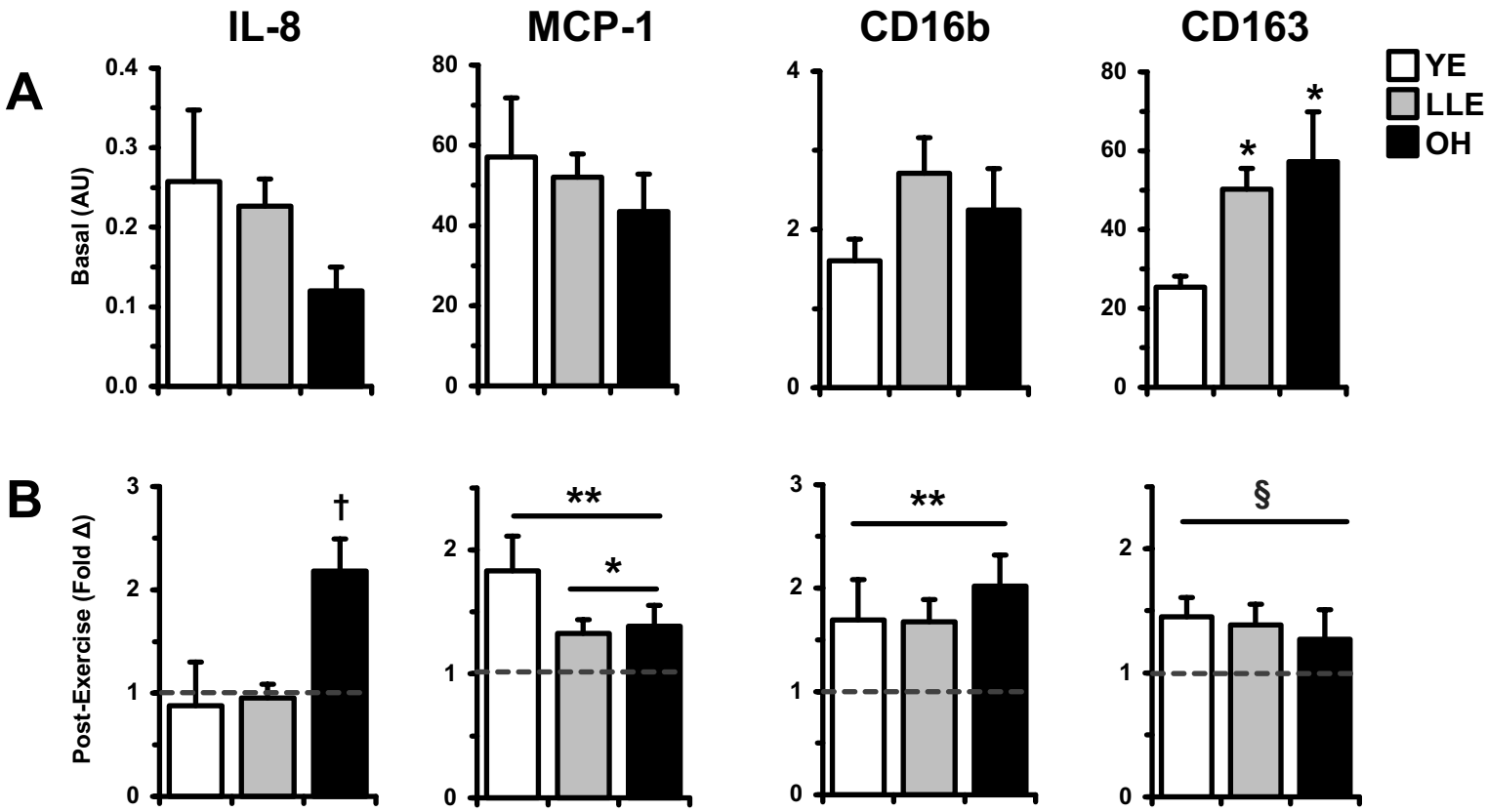

Figure 7