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Elevated SOCS3 and altered IL-6 signaling is associated with age-related human muscle stem cell dysfunction

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McKay BR, Ogborn DI, Baker JM, Toth KG, Tarnopolsky MA, Parise G. Elevated SOCS3 and altered IL-6 signaling is associated with age-related human muscle stem cell dysfunction. *Am J Physiol Cell Physiol* 304: C717–C728, 2013. First published February 7, 2013; doi:10.1152/ajpcell.00305.2012.—Aging is associated with increased circulating interleukin-6 (IL-6) and a reduced myogenic capacity, marked by reduced muscle stem cell [satellite cell (SC)] activity. Although IL-6 is important for normal SC function, it is unclear whether elevated IL-6 associated with aging alters SC function. We hypothesized that mild chronically elevated IL-6 would be associated with a blunted SC response through altered IL-6 signaling and elevated suppressor of cytokine signaling-3 (SOCS3) in the elderly. Nine healthy older adult men (OA; 69.6 ± 3.9 yr) and 9 young male controls (YC; 21. 3 ± 3.1 yr) completed 4 sets of 10 repetitions of unilateral leg press and knee extension (75% of 1-RM). Muscle biopsies and blood were obtained before and 3, 24, and 48 h after exercise. Basal SC number was 33% lower in OA vs. YC, and the response was blunted in OA. IL-6$^+$/Pax7$^+$ cells demonstrated a divergent response in OA, with YC increasing to 69% at 3 h and peaking at 24 h (72%), while IL-6$^+$/Pax7$^+$ cells were not increased until 48 h in OA (61%). Type II fiber-associated phosphorylated signal transducer and activator of transcription (pSTAT3)$^+$/Pax7$^+$ cells demonstrated a similar delay in OA, not increasing until 48 h (vs. 3 h in YC). SOCS3 protein was 86% higher in OA. These data demonstrate an age-related impairment in normal SC function that appears to be influenced by SOCS3 protein and delayed induction of IL-6 and pSTAT3 in the SCs of OA. Collectively, these data suggest dysregulated IL-6 signaling as a consequence of aging contributes to the blunted muscle stem cell response.

aging; STAT3; SOCS1; muscle damage; Pax7

THE PROGRESSIVE LOSS OF SKELETAL muscle mass and strength (termed sarcopenia) due to the normal aging process is of significant clinical relevance. The normal aging process has a profound effect on the structure and function of skeletal muscle. Aged muscle undergoes progressive atrophy, with a decrease in cross-sectional area caused by a decrease in muscle fiber number and fiber size (24, 29). Muscle function also appears to be reduced in aged muscle, displaying a decrease in power and maximum force production (6). In addition, aged muscle is more susceptible to injury compared with younger muscle and displays a decreased capacity for regeneration (7, 24).

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The myogenic response to a robust stimulus such as unaccustomed heavy resistance exercise involves a rapid coordinated response of the tissue-resident stem cells, the muscle satellite cells (SCs). In adult muscle, SCs comprise only a small fraction of the total myonuclear complement (~2–4%) yet possess an incredible proliferative capacity, capable of repairing extensive muscle injury (27). The activation, proliferation, and subsequent differentiation of these cells are controlled by a unique and complex set of transcriptional networks, the myogenic regulatory factors (MRFs). The MRFs are basic helix-loop-helix (bHLH) transcription factors that form heterodimeric DNA binding complexes for genes that encode cell-cycle machinery or encode terminal differentiation factors (11, 17, 39, 45). There are four main MRFs that control normal myogenesis: Myf5, MyoD, MRF4, and myogenin. The paired box transcription factor Pax7 is a key protein that coordinates the transcription of the MRFs and progression of SCs through the developmental pathway known as the myogenic program (8, 16, 39, 43, 44). SCs have been implicated as a significant factor contributing to the inability of aged muscle to repair/remodel. An age-related impairment of the myogenic program (either impaired activation and/or progression of SCs through the myogenic program) is hypothesized to be a major factor of SC dysfunction, contributing to the onset or progression of sarcopenia (54).

The myogenic program is influenced by a host of growth factors and cytokines (14, 27, 33). Recently, IL-6 has been investigated as a positive regulator of SC proliferation in both animals and humans through its interactions with the Janus activated kinase/signal transducer and activator of transcription (JAK/STAT) signaling cascade (35, 53, 56). Transient increases in local IL-6 concentrations following acute exercise-induced myotrauma are associated with SC proliferation and increased transcription of cell-cycle regulatory genes as well as increased pSTAT3 and cMyc localized to the SCs during the proliferative phase of the myogenic response (35, 56). Furthermore, the genetic loss of IL-6 in a murine model was shown to abolish SC proliferation and inhibit muscle hypertrophy (53). Increased IL-6 signaling induces increases in the negative-feedback regulatory proteins: suppressors of cytokine signaling (SOCS), which bind phosphorylated JAKs, blocking phosphorylation of STAT proteins, turning off the pathway (49). Elevations in SOCS proteins are essential for the transition of SCs from proliferation to differentiation by inhibiting JAK/STAT-induced SC proliferation (18).

IL-6 is a pleiotropic cytokine with divergent effects on many tissues depending on local and temporal changes in concentration (22, 28, 46). Importantly chronically elevated systemic IL-6 is associated with proinflammatory and muscle wasting conditions such as aging and cancer cachexia (50, 51). Data from animal infusion studies demonstrate that elevated IL-6 induces a catabolic environment, promotes muscle atrophy, and impairs normal muscle growth (3, 25). Human studies have demonstrated strong associations between elevated serum IL-6 and lower muscle strength and muscle mass associated with the aging process (47, 60). Furthermore, it appears that systemic IL-6 is elevated in healthy older (70.6 ± 7.9 yr) individuals (57), suggesting IL-6 is increased as a consequence of normal human aging. Although the exact mechanisms detailing how chronically elevated IL-6 promotes muscle catabolism remain unclear, the role of SOCS proteins has been implicated in reducing the efficacy of anabolic signaling pathways such as IGF-1 in skeletal muscle (1). However, whether the modest chronic elevation in systemic IL-6 concentrations observed in normal human aging impairs normal muscle stem cell function, in vivo, remains to be elucidated. We hypothesize that elevated SOCS proteins (SOCS1 and SOCS3), as a result of altered IL-6/STAT3 signaling and chronic systemic IL-6 elevation in older men, would be associated with a reduced SC response to acute muscle damage compared with young controls.

MATERIALS AND METHODS

Subjects. Nine healthy young male control subjects (YC) aged 21.3 ± 3.1 yr, 91.65 ± 21.96 kg and nine healthy older adult men (OA) aged 69.6 ± 3.9 yr, 87.64 ± 11.54 kg were recruited from the McMaster University community. No statistical differences were observed between the subject groups for height, weight, fat mass, or lean body mass (not shown). Subjects underwent a routine screening, completed a health questionnaire and were not involved in a lower-body resistance exercise training program for at least 6 mo before participating in the study. Exclusion criteria included evidence of coronary heart disease, respiratory disease, uncontrolled hypertension, renal disease, diabetes, major orthopedic disability, and smoking. All subjects were informed of the procedures and potential risks associated with the study and gave their written informed consent to participate. This study was approved by the Hamilton Health Sciences Research Ethics Board and conformed to all declarations on the use of human subjects as research participants. Subjects were told to refrain from consuming alcohol, caffeine, and nonsteroidal anti-inflammatory medications and exercising throughout the time course of the study.

Baseline measures and familiarization. One week before the acute exercise protocol, subjects visited the laboratory for a baseline blood collection, body composition assessment via dual-energy X-ray absorptiometry (DEXA; GE Medical), and familiarization of baseline strength measurement procedures [maximal voluntary contractions; (MVC)] using a Biodex Dynamometer (Biodex-System 3; Biodex Medical Systems) and the exercise protocol. All subjects were instructed to consume the same breakfast before each visit to minimize any potential the effect of nutrition on the outcome variables.

Acute exercise protocol. Subjects arrived to the clinic at the McMaster University Medical Centre at 0600 and underwent a series of three MVC tests separated with 3 min rest. After completion of the baseline strength, subjects conducted a unilateral incremental 1 repetition maximum (1-RM) test for leg press and knee extension. Following 1-RM assessment, subjects completed 4 sets of 10 repetitions of unilateral leg press and unilateral knee extension at 75% of their 1-RM separated by 2 min of rest between sets. This protocol has been used previously to elicit a detectable level of muscle damage and has been illustrated to induce an SC response in both elderly and young men (37). The goal of the acute protocol was to elicit a modest degree of muscle damage as was completed in a unilateral manner for safety considerations for the older adults. All exercise was conducted under the supervision of two trained investigators. Tissue and blood was collected following the exercise at 3, 24, and 48 h with the unexercised leg acting as the nonexercise control biopsy for baseline measurements (PRE).

Muscle biopsies. A total of four percutaneous needle biopsies were obtained from the mid-portion of the vastus lateralis under local anesthetic (1% lidocaine) using manual suction (55). One muscle biopsy was obtained from the nonworking leg for baseline analysis (PRE). Other biopsies were obtained from the working leg 3, 24, and 48 h postintervention. Incisions and biopsies on the exercise leg were spaced by ~3 cm, and the order of distal, mid, and proximal incision was randomized to minimize any effect of the previous incision on the biopsy. From each biopsy, ~25 mg of the sample were mounted in optimum cutting temperature compound and frozen in isopentane cooled in liquid nitrogen for histological analysis. The remaining portion was split into two pieces for mRNA and protein analysis.

RNA isolation. RNA was isolated from homogenized muscle samples using the TRIzol/RNeasy method (35). Briefly, ~25 mg of each muscle sample were homogenized in a total of 1.0 ml of TRIzol Reagent (Invitrogen) using an electric homogenizer (Pro200 BioGen). After the addition of chloroform, RNA was isolated as the liquid phase supernatant. Seven-hundred microliters of the liquid phase were transferred into a Qiagen RNeasy mini spin column and RNA was purified by using the RNeasy mini kit (cat. no. 74106), according to the manufacturer's instructions (Qiagen Sciences). RNA was quantified using a spectrophotometer (NanoDrop 1000; Thermo Scientific), and RNA integrity was assessed using a bioanalyzer (Agilent 2100 Bioanalyzer; Agilent Technologies). Average RNA Integrity Number (RIN) values were 9.2 ± 0.6.

Reverse transcription. Individual samples were reverse transcribed in 20-μl reactions using a commercially available kit (Applied Biosystems High Capacity cDNA Reverse Transcription Kit; Applied Biosystems) according to the manufacturer's instructions. The cDNA synthesis reaction was carried out on an Eppendorf Mastercycle ep gradient thermal cycler (Eppendorf).

Quantitative PCR. Individual 25-μl reactions were prepared in 0.2-ml Eppendorf twin.tec PCR plates (Eppendorf) and run in duplicate for each time point. Primers are listed in (Table 1) and were

Table 1. *Primer sequences*

Gene Name	Forward Sequence	Reverse Sequence	NCBI Gene ID
IL-6	5′-GAAAGCAGCAAAGAGGCACT-3′	5′-AGCTCTGGCTTGTTCCTCAC-3′	3569
SOCS3	5′-GACCAGCGCCACTTCTTCA-3′	5′-CTGGATGCGCAGGTTCTTG-3′	9021
SOCS1	5′-TTGGAGGGAGCGGATGGGTGTAG-3′	5′-AGAGGTAGGAGGTGCGAGTTCAGGTC-3	8651
cMyc	5′-CGTCTCCACACATCAGCACAA-3′	5′-TCTTGGCAGCAGGATAGTCCTT-3′	4609
MyoD	5′-GGTCCCTCGCGCCCAAAAGAT-3′	5′-CAGTTCTCCCGCCTCTCCTAC-3′	4654
Myogenin	5′-CAGTGCACTGGAGTTCAGCG-3′	5′-TTCATCTGGGAAGGCCACAGA-3′	4656
β2M	5′-ATGAGTATGCCTGCCGTGTGA-3′	5′-GGCATCTTCAAACCTCCATG-3′	567

IL-6, interleukin-6; SOCS1 and 3, suppressors of cytokine signaling-1 and -3; β2M, β2-microglobulin; NCBI, National Center for Biotechnology Information.

resuspended in 1× TE buffer (10 mM Tris·HCl, 0.11 mM EDTA) and stored at −20°C before use. In each reaction tube, 1.0 μl of cDNA (25 ng/μl) and 7.5 μl of ddH_2O were added to 16.5 μl of a master mix containing 12.5 μl of RT^2 Real-Time SYBR Green qPCR master mix (SuperArray Bioscience) along with 2 μl of the specific forward and reverse primers. Quantitative RT-PCR reactions were carried out using a Eppendorf Mastercycler ep realplex^2 real-time PCR system (Eppendorf). Relative mRNA expression was calculated using the ΔCt method ($2^{-\Delta Ct}$; Ref. 48), and fold change from baseline was calculated using the delta-delta ΔΔCt method ($2^{-\Delta\Delta Ct}$; Ref. 32). Gene expression was normalized to the housekeeping gene β_2-microglobulin (32, 48). mRNA values were expressed as either total mRNA expression (for between groups comparisons) and/or fold change from PRE for within group comparisons (means ± SE). β_2-Microglobulin was not different from PRE at any of the postintervention time points or between age groups at any time point.

Immunofluorescence. Seven-micrometer muscle cross sections were stained with antibodies against Pax7 (neat; cell supernatant from cells obtained from the Developmental Studies Hybridoma Bank); IL-6 (500 ng/ml, MAB 2061; R&D Systems); p-STAT3 (1:100; Cell Signaling Technologies); A4.951 [myosin heavy chain slow (MYH1) isoform; neat; cell supernatant from cells obtained from the Developmental Studies Hybridoma Bank]; and laminin [1:1,000, L8271 (Sigma-Aldrich) and ab11575 (Abcam)].Secondary antibodies used were as follows: Pax7 (AlexaFluor 594, 1:500; Invitrogen, Molecular Probes); IL-6, immunoglobulin biotinylated secondary antibody [1:200 (Dako); followed by a streptavidin-FITC fluorochrome, 1:100 (Biosource)]; A4.951(Dylight 488, 1:500, Thermo Scientific); laminin (AlexaFluor 647, 1:500, or AlexaFluor 488, 1:200; Invitrogen, Molecular Probes); and p-STAT3 (AlexaFluor 647, 1:500; Invitrogen, Molecular Probes). Immunofluorescence methods were adapted from previously published methods from our laboratory (35, 36, 42). Briefly, for coimmunofluorescent staining, sections were fixed with 2% paraformaldehyde (Sigma-Aldrich) for 10 min followed by several washes in PBS. Sections were then covered for 60 min in a blocking solution containing 2% BSA (Santa Cruz Biotechnology), 5% FBS (GIBCO), 0.2% Triton-X 100 (Sigma-Aldrich), 0.1% sodium azide (Sigma-Aldrich), and 5% goat serum (Sigma-Aldrich). Following blocking, sections were incubated in the primary antibody cocktail (i.e., MYH1 and laminin diluted in 1% BSA) at 4°C overnight. After several washes, sections were then incubated in the appropriate secondary antibodies. Sections were then refixed in 2% paraformaldehyde to prevent migration of the secondary antibodies and reblocked in 10% goat serum in 0.01% Triton-X 100. The sections were then incubated in the second primary antibody cocktail, followed by incubation in the appropriate secondary antibodies. Sections were then washed with PBS followed by 4′,6-diamidino-2-phenylindole (DAPI, 1:20,000; Sigma-Aldrich) for nuclear staining. Staining was verified using the appropriate positive and negative controls to ensure specificity of staining. Multiple secondary only controls were used for stains where multiple antigens were probed on the same slide. Stained slides were viewed with the Nikon Eclipse Ti Microscope (Nikon Instruments), and images were captured and analyzed using the Nikon NIS Elements AR 3.2 software (Nikon Instruments). For all analyses, the investigator was blinded to both the group and the time points; 250 ± 15 muscle fibers were analyzed per section for each time point for each subject for all analyses.

Immunofluorescent data analysis. For muscle cross-sectional area, images were taken with the ×40 objective and images were stitched using Nikon NIS Elements AR 3.2 software (Nikon Instruments). The stitched image comprised all of the myofibers in the muscle cross section (>200 fibers per subject per time point). CSA was determined using Nikon NIS Elements AR 3.2 software by an appropriately blinded and trained investigator from images obtained from tissue stained with Pax7, laminin, and MYH1. SC number was assessed using a Pax7, laminin, myosin heavy chain-I, and DAPI coimmunofluorescent stain as published previously (37). Briefly, images were obtained with the × 40 objective as for the CSA analysis. Each $Pax7^+$ cell was verified as being beneath the basal lamina and was also quantified based on the associated MYH1 staining (presence of MYH1 in the myofiber was type I positive, absence was type II positive). Total myofiber number and total myonuclear number were also quantified at this time (total myonuclei were DAPI-positive cells under the basal lamina, and interstitial nuclei were not included).

Protein analysis. Protein concentration was determined using the bichinconic acid method as per the manufacturer's recommendations (Pierce, Rockford, IL) with a spectrophotometer (Benchmark Plus; Bio-Rad, Hercules, CA). Ten micrograms of total protein were run per lane on a 12.5% gel. After transfer, both membranes were blocked for 1 h; 5% skim milk powder in TBS-Tween for SOCS1 and 5% BSA in TBS-Tween for SOCS3. Membranes were then incubated for 1 h at room temperature with 1 ug/ml ab3691 for SOCS1 (Abcam) or overnight at 4°C with 1 ug/ml ab16030 for SOCS3 (Abcam). After being washed, both membranes were probed with 1:10,000 ab6721 (Abcam) for 1 h at room temperature. Membranes were then treated with SuperSignal West (34075; Thermo Scientific), visualized using a FluorChem SP, and quantified using the AlphaEase software FC Software, Version 5.0.2 (both from ProteinSimple, formerly Alpha Innotech). Ponceau S (p7170; Sigma-Aldrich) staining was used as a loading control.

Blood measures. A resting blood sample was obtained from the antecubital vein immediately before the intervention and at 3, 24, and 48 h post. Approximately 20 ml of blood were collected, and serum and plasma samples were obtained for each time point. Samples were separated into 50-μl aliquots and stored at −80°C for analysis at a later date. Serum samples were thawed on ice and analyzed for muscle creatine kinase using a commercially available kit according to manufacturers' instructions (Pointe Scientific). Serum IL-6 was analyzed using a commercially available high sensitivity Quantikine Enzyme-Linked ImmunoSorbent Assay Kit according to the manufacturer's instructions (R&D Systems).

Statistical analysis. Statistical analysis was performed using SigmaStat 3.1.0 analysis software (Systat Software). A two-way repeated-measures ANOVA with one factor for time and one for age was conducted for all measures except PRE subject characteristics where a *t*-test was used for differences between age groups. Significant interactions and main effects were analyzed using the Tukey's honestly significant difference post hoc test. All results are presented as means ± SE. Statistical significance was accepted at $P < 0.05$.

RESULTS

Baseline measurements. Before commencing the experimental protocol, subjects were assessed for baseline strength characteristics. The elderly men (OA) generated 41% less force compared with the young controls (YC) during the isometric maximal voluntary contraction tests (OA: 228.3 ± 45.1 N·m vs. YC: 324.6 ± 85.4 N·m; $P = 0.0038$). As illustrated in Fig. 1, baseline assessment of cross-sectional area of type I and type II muscle fibers illustrated a 27% lesser type II fiber mean cross-sectional area (OA: 6,641.2 ± 1,788.8 μm^2 vs. YC: 8,407.9 ± 2,007.1 μm^2; $P = 0.034$). There was no difference in the mean cross-sectional area of the type I fibers between age groups (Fig. 1). To elucidate any age-related difference in the response to acute muscle damage, subjects completed a bout of unilateral leg exercise, with muscle biopsies collected throughout the postexercise time course.

Muscle response to acute resistance exercise is blunted with age. To verify a deficit in the myogenic response of OA, $Pax7^+$ cells were analyzed in muscle cross sections. SC number was 33.5% lower at baseline in OA [OA: 9.2 ± 1.2 $Pax7^+$ cells per 100 myofibers (cells/100MF) vs. YC: 13.8 ± 1.6 $Pax7^+$

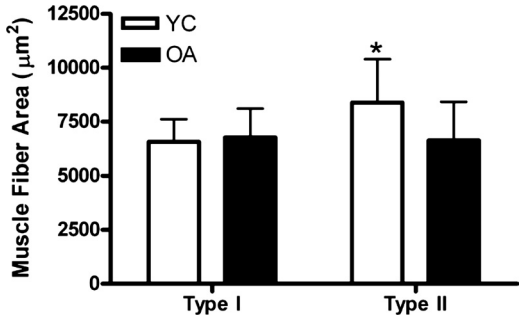

Fig. 1. Baseline muscle cross-sectional area (CSA) by fiber-type. Type I muscle fiber area and type II muscle fiber area in young controls (YC; open bar) and older adults (OA; closed bar). *$P < 0.05$ vs. OA; data are presented as means ± SD.

cells/100MF; $P = 0.02$; Fig. 2*A*]. SC number increased 20% in YC at 24 h ($P = 0.04$) and was 33% higher than baseline (PRE) 48 h after exercise ($P < 0.05$; Fig. 2*A*). In contrast, SCs of OA did not increase until 48 h postexercise ($P = 0.04$) and remained ~35% lower compared with YC at 3, 24, and 48 h (OA: 11.9 ± 0.8 $Pax7^+$ cells/100MF 48 h; YC: 18.4 ± 1.5 $Pax7^+$ cells/100MF 48 h; $P < 0.05$; Fig. 2*A*). This response was further characterized by fiber type-specific immunofluorescent microscopy. The type I fiber-specific response was similar in both groups, tending to increase by 24–48 h in YC ($P = 0.08$) and by 48 h in OA ($P = 0.08$; Fig. 2*C*). Baseline type I-associated SCs were not different between groups. Type II-associated SCs were 42.5% lower in OA ($P = 0.044$ vs. YC) and did not significantly increase over time despite a 40% increase by 48 h ($P = 0.1$; Fig. 2*D*). YC demonstrated a 62% increase in type II-associated SCs by 48 h ($P = 0.031$; Fig. 2*D*) and were significantly higher vs. OA at 24 h ($P = 0.012$) and 48 h ($P = 0.002$). Although type II-associated SCs were not significantly elevated at 24 h, there was a trend towards an increase in YC (41% increase, $P = 0.079$). The number of myonuclei per myofiber was not different over time or between groups (data not shown).

MyoD and myogenin, two key MRFs instrumental in myogenic differentiation, were examined at the mRNA level in whole muscle. There were no significant changes in MyoD between age groups or across time; however, significant variability in the response may have masked any acute changes (data not shown). Whole muscle myogenin was increased in both OA and YC at 24 h and 48 h ($P < 0.05$; Fig. 2*B*). Although both groups demonstrated a significant increase in myogenin expression, the absolute expression of myogenin in OA was 65% lower at 48 h compared with YC (strong trend, $P = 0.06$; Fig. 2*B*). These data, taken together, illustrate a blunted SC response in aged muscle, which appears to be restricted to the type II fibers. To determine possible causative factors for this impairment, we examined the response of both systemic and local levels of IL-6 and the downstream targets of the IL-6/STAT3 axis in relation to the SC response.

IL-6 response is differentially regulated with age. It is well established that aging is associated with an increase in chronic inflammatory states that in many cases leads to an increase in circulating IL-6 concentration in the blood. In the present

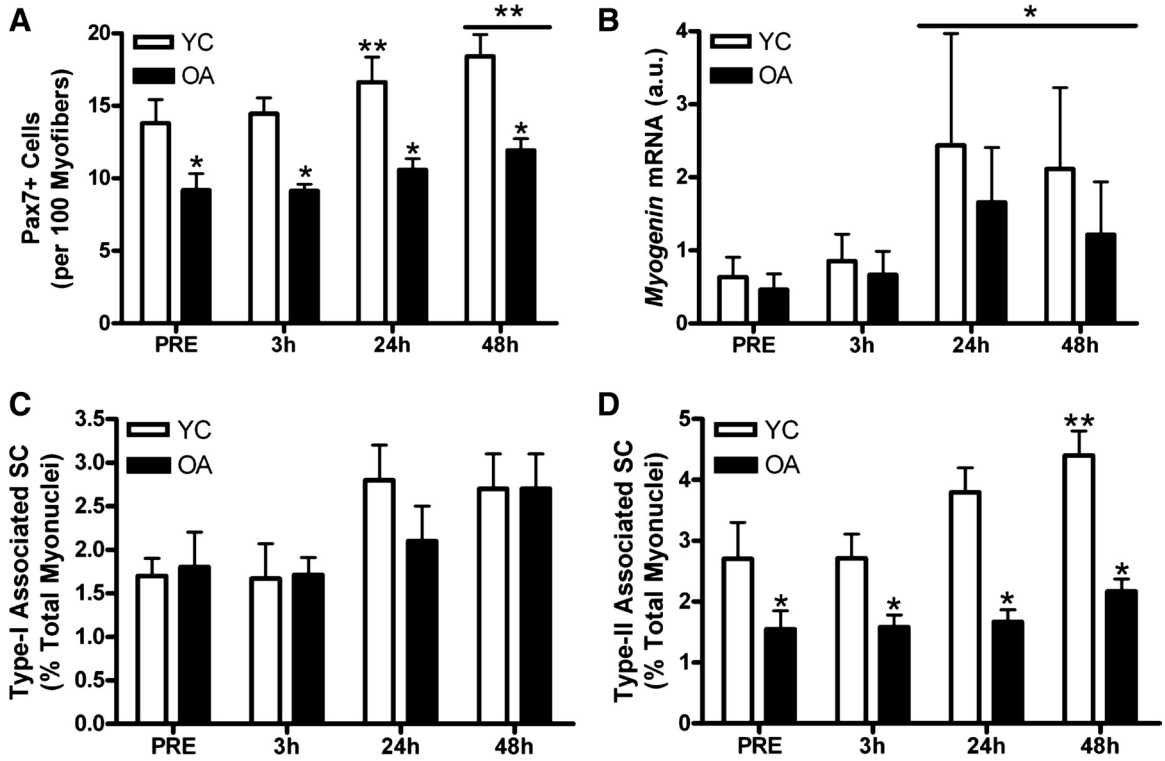

Fig. 2. Muscle satellite cell (SC) response to acute resistance exercise is blunted with age. *A*: number of $Pax7^+$ cells (SCs) per 100 myofibers. *Effect of age, $P < 0.05$ vs. YC. **Effect of time, $P < 0.05$ vs. baseline measurements (PRE; bar denotes both YC and OA different from PRE at 48 h). *B*: whole muscle mRNA expression of myogenin in YC (open bars) and OA (closed bars); a.u., arbitrary units. *Effect of time, $P < 0.05$ vs. PRE (bar denotes both groups are different from PRE). Type I-associated SCs (*C*) and type II-associated SCs (*D*) expressed as a percentage of total myonuclei in YC (open bars) and OA (closed bars). *Effect of age, $P < 0.05$ vs. YC. **Effect of time, $P < 0.05$ in YC only. Data are presented as baseline (PRE), 3, 24, and 48 h postexercise; means ± SE.

study, there was a trend for resting serum IL-6 concentrations to be elevated in OA (51%) compared with YC (2.04 ± 0.44 pg/ml OA vs. 1.35 ± 0.46 pg/ml YC; $P = 0.07$; Fig. 3*A*). Following the same relative muscle loading stimulus, serum IL-6 increased 57% ($P = 0.027$) at 3 h and 125% ($P < 0.01$) at 48 h postexercise in OA, while serum IL-6 was not elevated in YC until 48 h after exercise, increasing 99% from basal levels ($P < 0.05$; Fig. 3*A*). Despite a similar increase in serum creatine kinase (a surrogate marker of muscle damage) between OA and YC (~200% increase; data not shown), serum IL-6 was 107% greater at 24 h and 70% greater at 48 h in the OA compared with YC ($P < 0.05$; Fig. 3*A*). Whole muscle *IL-6* mRNA displayed a similar albeit exaggerated response. Muscle *IL-6* mRNA was 264% higher in the basal condition in OA compared with YC ($P < 0.001$; Fig. 3*B*). *IL-6* mRNA increased in a similar temporal manner in both groups; however, the magnitude of the response in the OA was much greater (Fig. 3*C*). OA demonstrated a progressive increase in *IL-6* mRNA, reaching a 21-fold increase at 48 h ($P < 0.001$; Fig. 3*C*). *IL-6* mRNA increased in YC at 24 and 48 h, reaching a 5.9-fold increase from PRE at 48 h ($P < 0.05$; Fig. 3*C*). The robust response observed in the OA translated into a 12-fold greater level of *IL-6* mRNA at 48 h vs. YC ($P < 0.001$; Fig. 3*C*). Figure 3*D* illustrates the temporal *IL-6* mRNA response in the YC only as the relationship is not easily visible in Fig. 3*C*. As demonstrated previously (35), the temporal response of serum IL-6 and muscle *IL-6* mRNA was very similar. To investigate if the altered IL-6 response observed systemically and at the level of the gene was also affecting the SC pool following heavy resistance exercise, we examined the SC-specific IL-6 response.

Using immunofluorescent microscopy (Fig. 4*A*), we determined that there were no differences in the proportion of $Pax7^+$ cells that were positive for IL-6 protein at baseline. However, there was a slight trend for a greater proportion of IL-6^+ SCs in OA (27% greater in OA, $P = 0.09$; Fig. 4*B*). In YC, the number of $Pax7^+$/IL-6^+ cells increased from 24.3 ± 6.2 to 69.7 ± 12.9% by 3 h ($P < 0.001$; Fig. 4*B*), peaking at 24 h (72.4 ± 7.6%, $P < 0.001$). By 48 h, the proportion of SCs positive of IL-6 had dropped down to 41.8 ± 6.8%, which was not significantly different from basal levels (Fig. 4*B*). The response was drastically different in OA, with $Pax7^+$/IL-6^+ cells remaining significantly lower than YC at 3 h (62% lower, $P < 0.001$) and 24 h (46% lower, $P = 0.004$). By 48 h the number of $Pax7^+$/IL-6^+ cells had increased from 30.9 ± 7.5% at baseline to 61.9 ± 8.8% ($P = 0.02$) in OA. There was a trend for the number of $Pax7^+$/IL-6^+ cells in OA to be elevated with respect to YC at 48 h ($P = 0.068$). Figure 4, *C* (YC) and *D*, (OA) illustrates the proportion of $Pax7^+$ cells that were co-positive for IL-6 and the total $Pax7^+$ cell (SC) response across time. This figure illustrates the normal temporal nature of IL-6 in this cell population in response to the acute exercise in YC (38, 56) and blunted or delayed response in the OA. Taken together these data suggest that the IL-6 response in muscle

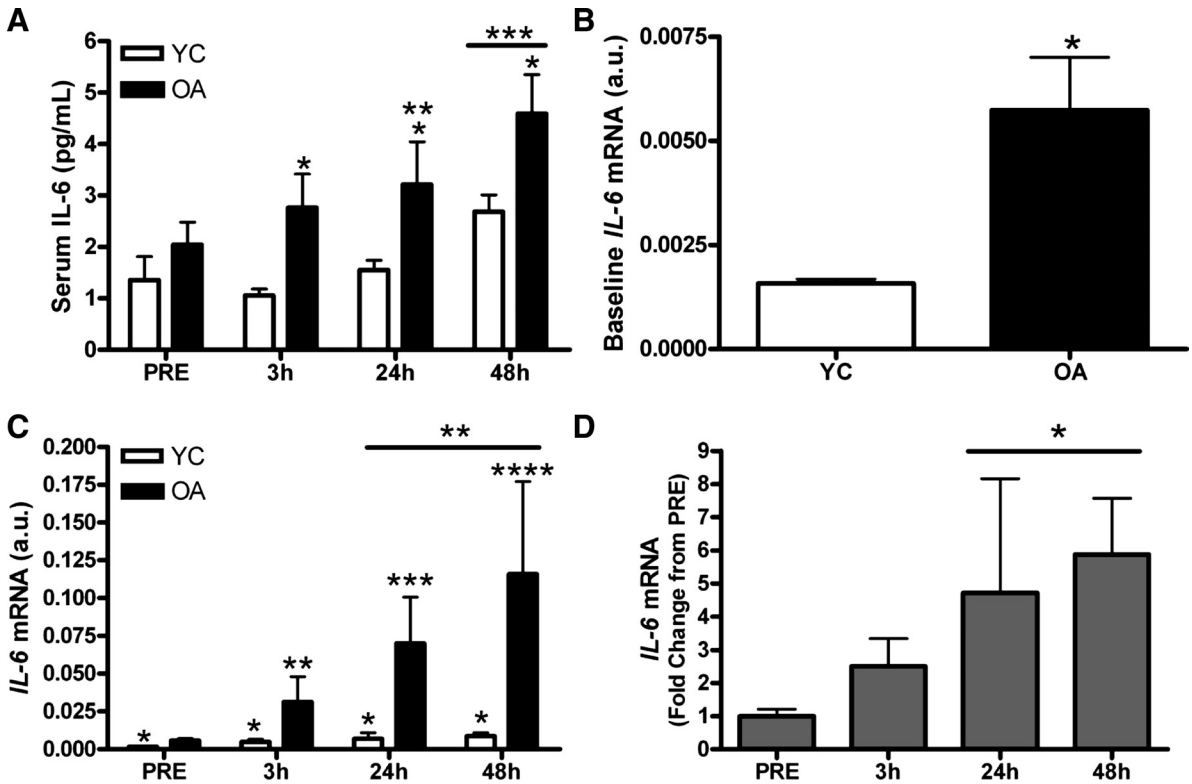

Fig. 3. IL-6 is elevated in the elderly. *A*: serum IL-6 concentration in YC (open bars) and OA (closed bars). *Effect of age, $P < 0.05$ vs. YC. **Effect of time, $P < 0.05$ vs. PRE in YC. ***Effect of time, $P < 0.05$ vs. PRE and 3 h (bar denotes effect of time in both YC and OA). *B*: baseline whole muscle mRNA expression of IL-6 in YC (open bars) and OA (closed bars). *Effect of age, $P < 0.05$ vs. YC. *C*: whole muscle mRNA expression of IL-6 through the study time course in YC (open bars) and OA (closed bars). *Effect of age, $P < 0.05$ vs. YC. **Effect of time, $P < 0.05$ vs. PRE (bar denotes effect in both groups). ***Effect of time, $P < 0.05$ vs. PRE and 3 h in OA only. ****Effect of time, $P < 0.05$ vs. all other time points in OA only. *D*: whole muscle mRNA expression of IL-6 in YC only to illustrate the effect of time (fold change from PRE). *$P < 0.05$ vs. PRE. All data are presented as baseline (PRE), 3, 24, and 48 h postexercise; means ± SE.

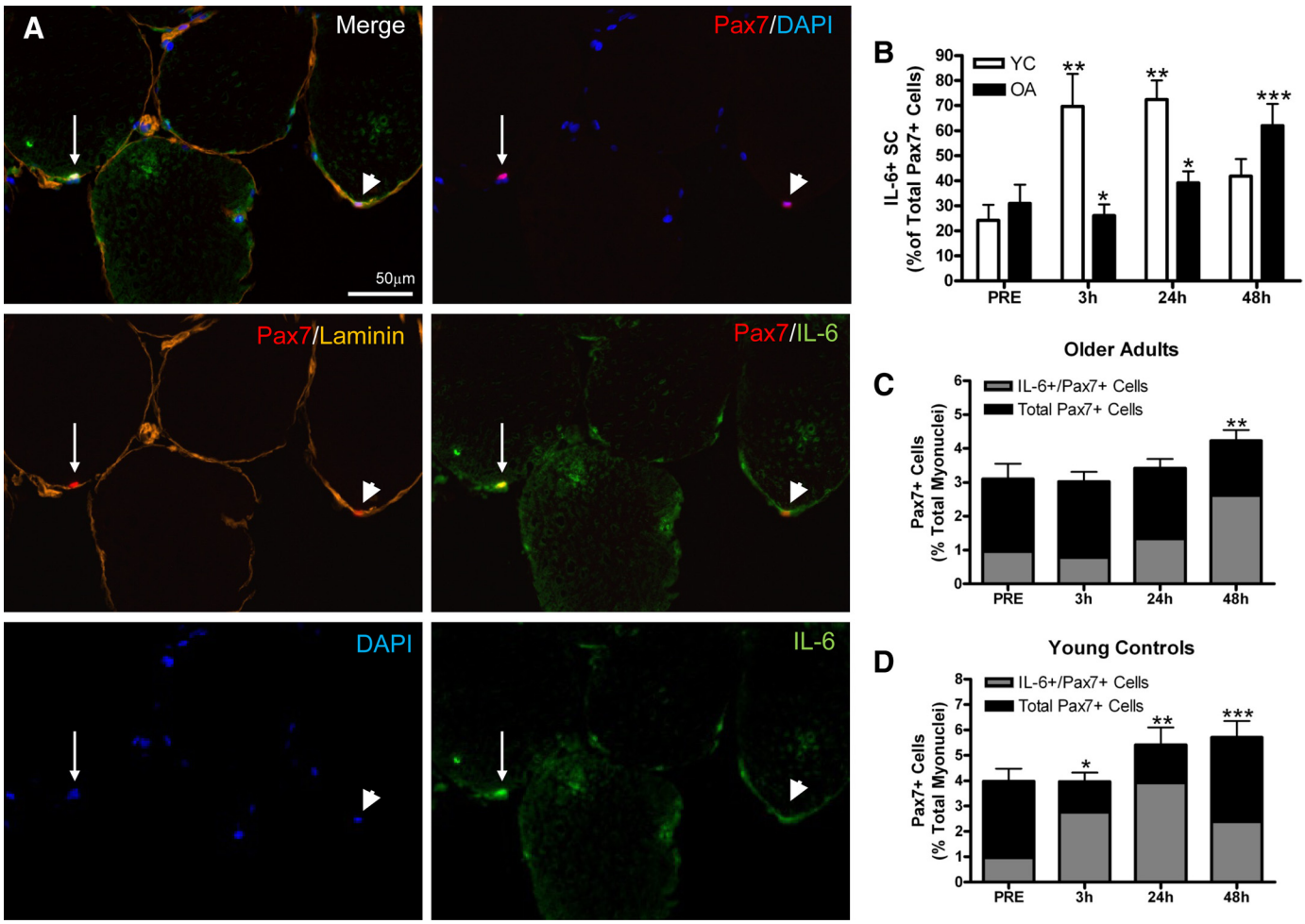

Fig. 4. SC-specific IL-6 is differentially regulated with age. *A*: immunofluorescent image of a muscle cross section stained for Pax7 (red), laminin (orange), IL-6 (green), and nuclei (DAPI, blue). Various panels illustrate an IL-6$^+$/Pax7$^+$ cell (long arrow) and an IL-6$^-$/Pax7$^+$ cell (arrowhead). *B*: quantification of the proportion of SCs staining positive for IL-6 (as a percentage of total SC number) in YC (open bars) and OA (closed bars). *Effect of age, $P < 0.05$ vs. YC. **Effect of time for YC, $P < 0.05$ vs. PRE and 48 h. ***Effect of time for OA, $P < 0.05$ vs. PRE, 3, and 24 h. *C*: proportion of total SCs (black) positive of IL-6 (grey) in OA across time. **Effect of time, $P < 0.05$ vs. PRE for both IL-6 and Pax7. *D*: proportion of total SCs (black) positive of IL-6 (grey) in YC across time. *Effect of time for IL-6 at 3 h, $P < 0.05$ vs. PRE. **Effect of time, $P < 0.05$ vs. PRE for both IL-6 and Pax7. ***Effect of time, $P < 0.05$ vs. PRE for Pax7 only (bar denotes effect in both groups). All data are presented as baseline (PRE), 3, 24 and 48 h postexercise; means ± SE.

and in the SCs themselves is differentially altered with age, associated with a blunted or delayed induction of IL-6 in the SCs following acute resistance exercise. To determine if the differential IL-6 response was impacting downstream JAK/STAT signaling, we investigated the SC-localized phosphorylated STAT3 (pSTAT3) response in a fiber type-specific manner.

Fiber type-specific pSTAT3 response is altered with age. Using immunofluorescence microscopy (Fig. 5*A*), we preformed fiber type-specific analysis of pSTAT3 in SCs. The pSTAT3 response in the type I-associated SCs was also not significantly different with age (Fig. 5*B*). The proportion of pSTAT3$^+$ type I SCs (type I fiber-associated pSTAT3$^+$/Pax7$^+$ cells) increased from 27.7 ± 4.4% (YC) and 26.2 ± 3.9% (OA) at baseline to 67.9 ± 2.9% (YC) and 56.5 ± 5.5% (OA) at 24 h ($P < 0.026$; Fig. 5*B*). Both groups demonstrated a reduction in pSTAT3$^+$ type I SCs at 48 h (YC: 46.9 ± 6.2% and OA: 47.5 ± 5.9%, OA). In the type II fibers, there was no effect of age at baseline (YC: 21.9 ± 3.3% vs. OA: 23.3 ± 5.6% pSTAT3$^+$ type II SCs; Fig. 5*C*). At 3 h, pSTAT3$^+$ type II SCs increased in YC only (YC: 57.2 ± 4.5% pSTAT3$^+$ type II SCs; $P < 0.05$). The number of pSTAT3$^+$ type II SCs in OA was 32% lower compared with YC at 3 h [38.8 ± 3.7% pSTAT3$^+$ type II SCs; $P = 0.027$ (age); Fig. 5*C*]. At 24 h, the number of pSTAT3$^+$ type II SCs were elevated in both groups; however, OA remained 17% lower compared with YC (trend, $P = 0.09$). There was no change in the number of pSTAT3$^+$ type II SCs in OA from 24 h to 48 h; however, this number dropped significantly in the YC (67.7 ± 5.4% at 24 h to 40.7 ± 3.6% at 48 h; $P < 0.05$). These data illustrate a delay in the appearance of pSTAT3 in the type II SCs of OA compared with YC and a lower peak percentage of pSTAT3$^+$ type II SCs (YC: 67.7 ± 5.4% vs. OA: 56.2 ± 3.4% at 24 h). To investigate whether this delayed response impacted the downstream targets of STAT3 signaling, we examined three key genes involved in cell proliferation and signaling.

Downstream gene expression of the IL-6/STAT3 pathway is altered with age. The specific target genes of the JAK/STAT pathway *cMyc, SOCS1*, and *SOCS3* were chosen specifically because their direct actions influence cell cycle progression (*cMyc*) and negative feedback to halt IL-6/JAK/STAT3 signal-

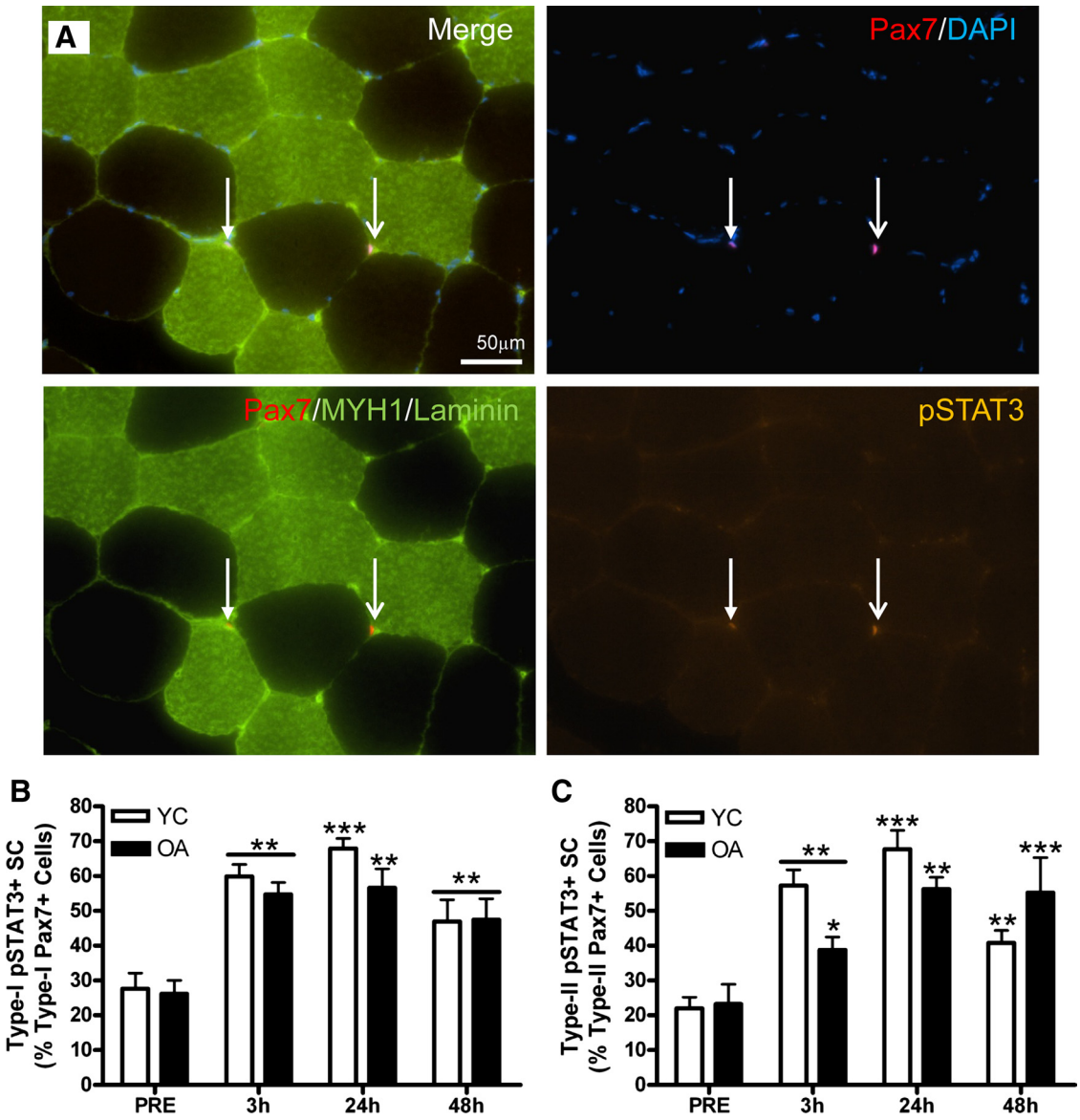

Fig. 5. Fiber-type specific phosphorylated signal transducer and activator of transcription (pSTAT3) response is altered with age. *A*: immunofluorescent image of a muscle cross section stained for Pax7 (red), laminin (green), myosin heavy chain type-I (MYH1, green), phosphorylated STAT3 (pSTAT3, orange), and nuclei (DAPI, blue). Various panels illustrate a type I-associated pSTAT3$^+$/Pax7$^+$ cell (closed arrow) and a type II-associated pSTAT3$^+$/Pax7$^+$ cell (open arrow). Type I SC specific pSTAT3 (*B*) and Type II SC specific pSTAT3 (*C*) response in YC (open bars) and OA (closed bars). *Effect of age, $P < 0.05$ vs. YC. **Effect of time, $P < 0.05$ vs. PRE. ***Effect of time, $P < 0.05$ vs. PRE and 3 h in YC only (bar denotes effect in both groups). All data are presented as baseline (PRE), 3, 24, and 48 h postexercise; means ± SE.

ing (*SOCS*; Ref. 49). *cMyc* mRNA was not different at baseline between OA and YC and was significantly increased ~22-fold 3 h postexercise in both groups ($P < 0.0001$; Fig. 6*A*). However, the absolute response of the OA was 40% lower at 3 h compared with YC ($P < 0.05$; Fig. 6*A*). *cMyc* mRNA was decreased from 3 h at both 24 h and 48 h but remained elevated from baseline with no differences between groups ($P < 0.05$). *SOCS1* mRNA was not different at baseline between OA and YC and increased at 3 h to the same relative level (160% increase in both groups, $P < 0.01$; Fig. 6*B*). There was no difference between the mRNA expression at 3 and 24 h; however, at 48 h the expression of *SOCS1* was 78% higher in OA ($P = 0.007$; Fig. 6*B*). *SOCS3* mRNA was not different at baseline between groups (Fig. 6*C*). *SOCS3* mRNA in both OA and YC increased fourfold 3 h postexercise ($P < 0.05$; Fig. 6*D*). The expression of *SOCS3* increased in both groups at 24 and 48 h ($P < 0.05$); however, *SOCS3* expression in OA was 149% higher at 24 h ($P < 0.001$) and remained 104% higher at 48 h vs. YC ($P < 0.05$; Fig. 6*C*). These data suggest that specific target genes of IL-6/STAT3 signaling are differentially regulated with age, leading to increased *SOCS* expression and a less robust *cMyc* response to the same relative exercise stimulus. To determine whether the differences in *SOCS* gene expression translated into observable changes at the protein level, Western blot analysis was conducted on whole muscle homogenates.

SOCS protein is higher in aging muscle. SOCS1 protein was 38% higher in OA at baseline ($P = 0.034$, Fig. 7*A*). Although

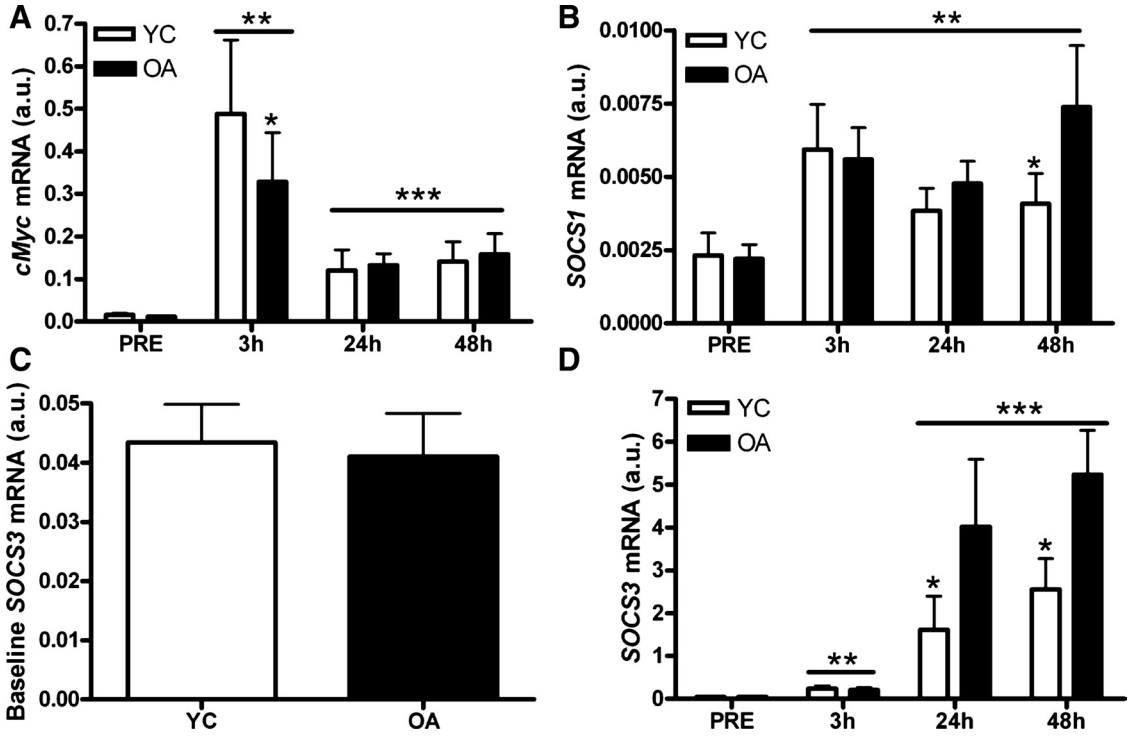

Fig. 6. pSTAT3 gene targets are differentially expressed with age. Whole muscle mRNA expression of cMyc (*A*), suppressor of cytokine signaling-1 (SOCS1; *B*), baseline SOCS3 (*C*), and SOCS3 (*D*) in YC (open bars) and OA (closed bars). *Effect of age, $P < 0.05$ vs. YC. **Effect of time, $P < 0.05$ vs. PRE. ***Effect of time, $P < 0.05$ vs. PRE and 3 h (bar denotes effect in both groups). All data are presented as baseline (PRE), 3, 24, and 48 h postexercise; means ± SE.

there was no effect for time, SOCS1 protein tended to decrease at 3 h postexercise in OA (33% lower vs. PRE; $P = 0.08$; Fig. 7*A*). 24 h postexercise SOCS1 was 64% lower vs. YC ($P = 0.002$). At 48 h postexercise, SOCS1 protein concentration was similar between OA and YC but not different from any other time point (Fig. 7*A*). Representative blots for SOCS1 and SOCS3 are shown in Fig. 7*B*. SOCS3 protein was 86% higher at baseline in OA vs. YC (0.93 ± 0.09 vs. 0.50 ± 0.04 arbitrary units, respectively; $P < 0.001$; Fig. 7*C*). SOCS3 protein concentration was not different from baseline in either group at 3 and 24 h and remained ~85% higher in OA vs. YC ($P < 0.001$; Fig. 7*D*). 48 h after exercise SOCS3 protein concentration decreased 17% from 3 h in OA ($P = 0.008$) and remained 74% higher compared with YC ($P = 0.006$; Fig. 7*D*). Together these data illustrate a differential regulation in protein expression with age. There appears to be higher SOCS1 protein in OA at rest and until 24 h postexercise, and SOCS3 appears to be chronically elevated in muscle from OA. Together these data suggest that the proteins responsible for inhibiting STAT3 signaling are differentially regulated with age.

DISCUSSION

There are multiple theories and mechanisms to explain human aging, and it appears that aging is a complex multifaceted process involving accumulated damage from oxidative stress as well as genetic and autoimmune processes that effect various organ systems in different ways (12). The maintenance of muscle mass with age is imperative for health, autonomy, and injury prevention. Understanding the cellular and molecular processes that underpin normal skeletal muscle maintenance is critical for elucidating strategies to attenuate or prevent the seemingly inevitable decline in muscle mass in the later stages of life. In the present study we identify the IL-6/STAT3/SOCS3 axis as a key pathway that is differentially regulated with age and appears to be associated with a dysfunctional SC response to a physiological stimulus.

It is clear from several previous studies that there is a preferential loss of type II fibers and reduction in type II fiber CSA with age (31, 59). Furthermore, recent data using fiber type-specific immunofluorescent microscopy demonstrate that in addition to a preferential loss of type II fiber CSA there is a type II specific loss of SCs per myofiber in older muscle (37, 59). These data suggest that the loss of type II fiber SC content may be important in the etiology of type II fiber atrophy in older adults. Furthermore, chronic exercise training may reverse some of the age-related alterations in gene expression and restore the type II SC content (40, 58); however, the mechanisms remain to be elucidated.

In the present study older men had 42% less force production, associated with a 27% reduction in type II fiber CSA, 42.5% fewer type II-associated SCs, and a 33.5% reduction in total SC content compared with young controls. These data are in agreement with previous studies comparing older men (~70 yr) with younger men (20–25 yr), which together demonstrate a 44–47% reduction in type II-associated SC content in older men (37, 59). The dampened whole muscle myogenin mRNA response adds further evidence to support a signaling deficit in elderly muscle, contributing to the blunted or delayed SC response. Although myogenin was measured in whole muscle homogenates, the trend for less myogenin mRNA in OA at 48 h supports our data illustrating a blunted or delayed induction of proliferation and thus differentiation. Currently, a paucity of data exists to explain the fiber type-specific deficit in the

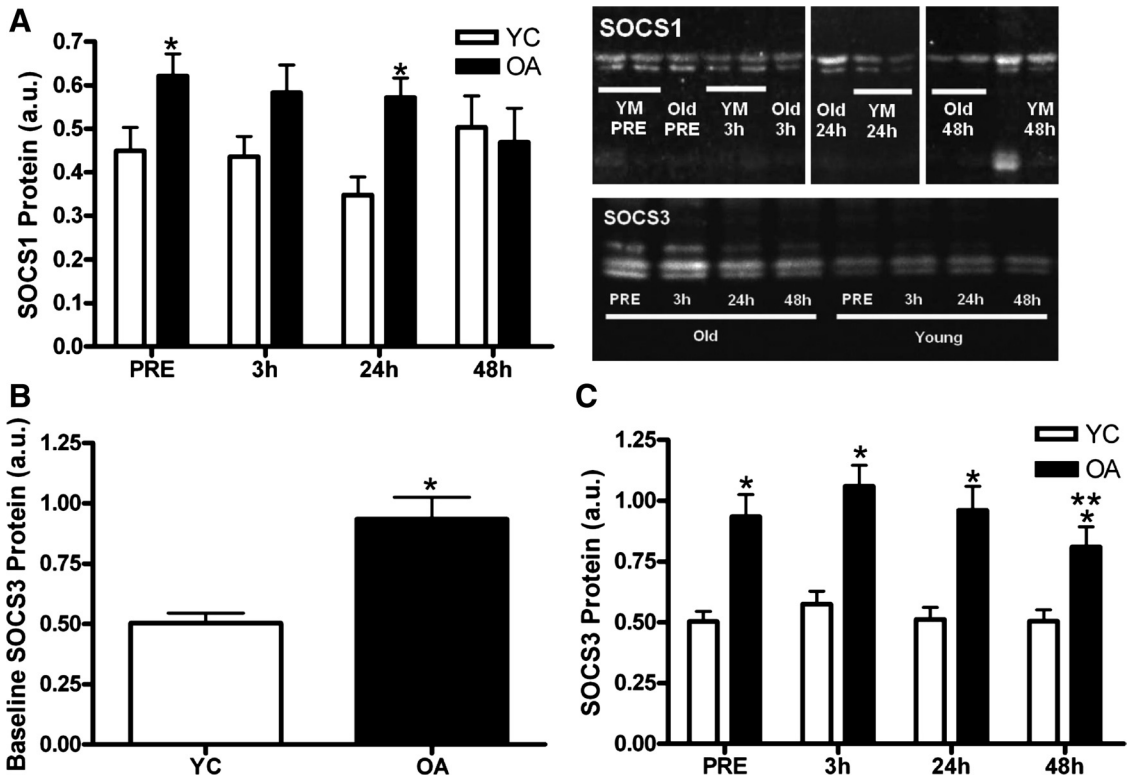

Fig. 7. SOCS1 and SOCS3 protein content is elevated in elderly muscle. SOCS1 protein (*A*), baseline SOCS3 protein (*B*), and SOCS3 protein content (*C*) in YC (open bars) and OA (closed bars) with the associated representative blots. *Effect of age, $P < 0.05$ vs. YC. **Effect of time, $P < 0.05$ vs. 3 h. All data are presented as baseline (PRE), 3, 24, and 48 h postexercise; means ± SE.

SC pool. Previous experiments on SCs isolated from human muscle demonstrate that SCs from type I or type II fibers are not genetically programmed to differentiate in a fiber type-specific fashion and SCs express similar myosin heavy chain isoforms in vitro (4). Thus there do not appear to be distinct myosin heavy chain-specific populations of human SCs (4). However, distinct populations may exist based on adhesion characteristics or other molecular markers yet to be fully classified. Little is known about the regulation of fiber-specific SCs in vivo and whether these cells are differentially regulated. Differences in the microenvironment and interactions with the myofiber may be important factors influencing fiber type-specific SC responses. Myostatin appears to be a key factor in regulating human SC function (34, 37). We have recently demonstrated that elevated myostatin and elevated SC-specific myostatin levels in type II SCs may be one of the key factors impairing the myogenic capacity of muscle in older humans (37). Although elevated myostatin appears to be, in part, responsible for the age-related SC dysfunction, aging is also associated with higher muscle SOCS3 protein and circulating inflammatory cytokines such as IL-6 and IL-1β (30, 57). Currently, it is unclear if these are mutually exclusive or are related processes. It has been shown previously that IL-6 is a key regulatory factor for SC proliferation in young men and animals (35, 53, 56). However, the appearance of elevated IL-6 was transient, with *SOCS3* mRNA being induced rapidly (24 h) following muscle damage, likely to inhibit the STAT3 cascade and limit proliferation to allow for differentiation to begin (18, 35, 56). In healthy older humans, IL-6 has been shown to be chronically elevated (57), which may impair the normal IL-6 response and the sensitivity of the SCs to IL-6.

Although the basal serum IL-6 concentration in older men was 51% higher ($P = 0.07$), this failed to reach statistical significance compared with young controls. This may be partially explained by our exclusion criteria that selected for older men that had no identified comorbidities and were active. An older cohort or a cohort that included more frail individuals would likely have demonstrated a larger basal difference. In addition to a trend for higher basal systemic IL-6, muscle *IL-6* mRNA was 264% higher in older muscle compared with the young controls. Although some of these outcomes were strong trends, collectively these data demonstrate that aging muscle appears to be in a proinflammatory state. IL-6 has been shown to be produced by the myofiber following a range of exercise interventions and in response to muscle injury (46). The release in IL-6 from the fiber may help initiate the SC response following exercise and/or myotrauma aiding it the adaptive response; however, this theory has not been fully tested and requires further work to elucidate the communication events between the myofiber and the SC in response to induced myofiber damage. In agreement with the observed elevation of muscle and systemic IL-6, muscle SOCS3 protein was 86% higher in older muscle. These alterations have been noted in previous studies and appear to be associated with normal aging (30, 57), which are further elevated in disease states such as cancer cachexia (5). In animals, 14 days of IL-6 infusion increased the transcription of *atrogin*, *SOCS3*, and *TNF-α*, which promoted a proinflammatory state and blunted muscle growth in young

animals and induced atrophy in adults (3, 25). However, the effect of elevated IL-6 on SC function was not assessed in those studies.

Unfortunately, due to technical limitations we were unable to determine the fiber type-specific IL-6/SC response. However, the mixed muscle response clearly illustrates a blunted induction of IL-6 within the SC pool in OA. In young controls, IL-6 in the SC compartment increased (transiently) as early as 3 h postexercise and peaked at 24 h and began to decrease by 48 h. This is consistent with previous work in young subjects following exercise-induced muscle injury (35, 56). This transient rapid induction of IL-6, presumably to enhance SC proliferation (35, 56), is under tight control and rapidly downregulated to allow for SC differentiation to occur (56). Interestingly, the IL-6 response in elderly SCs was significantly blunted, not increasing until 48 h postexercise despite completing the same relative work and demonstrating the same relative creatine kinase response. A delay in the induction of IL-6 in the SCs was in contrast to the whole muscle and systemic response, which was more rapid (significant increases at 3 h in both mRNA and serum) and more robust compared with the young controls. This may be partially explained by the elevated basal SOCS3 in the muscle, which may have desensitized the SCs to IL-6 (20, 62). The muscle stem cell niche is sensitive to age-related alterations in signaling molecules (15, 23) and thus chronically elevated muscle IL-6 (as demonstrated at the mRNA level) may have negatively impacted the microenvironment of the SCs, impairing normal IL-6 signaling. This hypothesis is supported by the blunted induction of pSTAT3 in the type II-associated SCs in older muscle at 3 h postexercise. Conversely, the young controls demonstrated a robust increase in $pSTAT3^+$ SCs early in the postexercise time course and an earlier peak in the response (24 h vs. 48 h in OA). The impaired type II pSTAT3 response in the early part of the time course likely contributed to the 40% lower *cMyc* mRNA response in older men. Therefore, the impaired induction of pSTAT3 in older adults SCs and thus reduced *cMyc* expression appear to be a significant factor impairing type II SC proliferation following exercise. Interestingly, myoblasts isolated from obese humans with type 2 diabetes and chronically elevated systemic IL-6 displayed an altered response to IL-6 administration, demonstrating a suppressed induction of pSTAT3 compared with controls (52). Therefore, it appears the chronic systemic elevation of IL-6 may fundamentally alter the normal response of SCs, regardless of age. The relative contribution of SCs to the systemic IL-6 concentration is unknown but likely negligible due to a relatively small volume of SCs compared with all other IL-6 producing tissues. However, it appears that the circulating concentration of IL-6 is of major importance to the regulation of downstream IL-6 signaling in the SC. Further research is warranted to determine the precise mechanisms by which chronically elevated IL-6 impacts muscle stem cell function.

The rapid increase in IL-6 in older men observed in the present study may have been enhanced by an elevation in IL-1β, which has been previously demonstrated to be elevated in healthy men aged 70 yr (57). IL-1β has been shown to induce the transcription of IL-6 via activator protein-1 (AP-1) induction and phosphorylation of transforming growth factor-β-activated kinase-1 (TAK1; Refs. 9, 63). IL-6 is also induced by AP-1 in muscle in response to muscle contractions (61); thus it is possible that the proinflammatory state of older muscle and repeated muscle contractions from the acute exercise may act synergistically, increasing AP-1 induction to a greater degree leading to a more robust IL-6 response to exercise. In addition, tissue resident macrophages may also contribute to the production of IL-6 in response to muscle damage (2), further contributing to the increase in IL-6 following our protocol. Clearly, further research is warranted in this area to elucidate the role of inflammation and SC function.

The main negative regulator of IL-6/STAT3 signaling is SOCS3, which is induced by pSTAT3 as a negative feedback mechanism (49). In the present study, the basal concentration of SOCS3 protein in elderly muscle was 86% higher compared with young controls and remained significantly higher over time. SOCS3 protein levels did not change significantly over the 48 h postexercise time from baseline. *SOCS3* mRNA was not different between groups initially but at 24 and 48 h *SOCS3* mRNA was 149 and 104% higher in older men. This increase mirrors that of systemic IL-6 and muscle *IL-6* mRNA observed in older men following exercise. Considering the inhibitory functions of SOCS3, the high SOCS3 protein expression and enhanced *SOCS3* gene response observed in older muscle in the present study may be a contributing factor to the etiology of sarcopenia by inhibition of muscle anabolic signaling (1, 30) and a blunted muscle myogenic response to muscle loading. Although SOCS3 mRNA was elevated 24–48 h postexercise in YC, which is similar to previous reports (35), SOCS3 protein was not changed. This may have been due to the timing of tissue sampling, where the peak in SOCS3 mRNA (48 h in both YC and OA) occurred within the 48-h time course whereas the protein may have peaked after 48 h. A longer time course may be required in the future to fully investigate the SOCS3 response in the muscle fiber. Importantly, however, the SOCS3 protein response in OA remained significantly higher than YC, which appears to be an important contributing factor to the delay in the initial SC response in the OA. Previous aging studies investigating SOCS3 have demonstrated increased expression of negative regulators of muscle mass such as myostatin and atrogin-1 (30); however, further work is needed to confirm whether elevated SOCS3 is in fact a key contributing factor in the onset and/or progression of sarcopenia in humans. Basal SOCS1 protein concentration was also significantly higher in elderly muscle. SOCS1 protein remained elevated in older subjects until 48 h where the protein levels were similar between the age groups. Interestingly *SOCS1* mRNA was not different between ages until 48 h postexercise where it was 78% higher in older muscle, despite a similar response profile of the mRNA postexercise. IL-6 has been shown to upregulate SOCS1 in $CD4^+$ T cells (19) to inhibit STAT3 signaling by inhibiting JAK2 (21). SOCS1 also appears to be required for phosphorylation of the cell-cycle inhibitor p53 (10). Therefore, the increased SOCS1 protein content of elderly skeletal muscle may have acted to inhibit cell-cycle progression through interactions with both STAT3 and p53 signaling pathways, blunting the early SC response. SOCS1 and SOCS3 proteins may also interfere with IGF-1 receptor signaling (13). The inhibition or dysregulation of IGF-1 signaling in older adults following resistance exercise has been shown previously and may also contribute to the impaired myogenic response (26, 41). Elevated SOCS3 in cultured myoblasts as a result of treatment with IL-6 has been shown to block IGF-1 signaling through the ERK1/2 and Akt pathways via inhibitory interactions with IRS-1, suggesting

IL-6 and specifically SOCS3 may inhibit classic growth factor signaling in skeletal muscle (1). Thus chronically elevated SOCS3 likely as a result of increased local and systemic IL-6 may impair proper SC function by blunting the induction of pSTAT3 and *cMyc* and also interfering with IGF-1 signaling through both SOCS1 and SOCS3. Further research into SC-specific IGF-1 signaling and the role of SOCS proteins in older adults is necessary to fully understand the relationship among chronically elevated IL-6, impaired myogenic capacity, and sarcopenia. Unfortunately, the analysis of IL-6 and pSTAT3 is limited to the presence or absence of pSTAT3 or IL-6 in the SCs due to the lack of appropriate techniques for detection of protein concentration in SCs in cross section. Colocalization analysis fails to address the specific level of pSTAT3 in each SC and the potential differences between fiber types. The use of flow cytometry may provide insight into the SC-specific concentrations of key signaling molecules in the future. It should be noted that compared with previous research in humans using 300 maximal eccentric contractions to induce a SC response (35, 56), the present study used a much lower intensity of exercise as the stimulus; thus many of the analyses were less robust when comparing to our previous data, which may explain why there were many trends that did not reach statistical significance. However, the associations and relationships among IL-6, muscle damage, and the SC response are conserved, albeit at a lesser magnitude in the present study. Future research at a higher intensity may provide key insight into the factors effecting recovery from myotrauma and/or exercise in aging humans.

Understanding the mechanisms responsible for the progressive loss of muscle mass in advanced age and loss of myogenic capacity of aged muscle is necessary for determining effective clinical treatments for the maintenance of muscle mass and is fundamentally necessary for our basic understanding of human aging. In the present study we demonstrate the association between an elevation in SOCS protein (muscle milieu) and the impaired SC-specific IL-6/pSTAT3 signaling (compartmentalized effect of IL-6), leading to a blunted myogenic response in older adults compared with young controls. Importantly, the divergent responses of many key signaling factors as well as important differences in the baseline levels of these factors, including SOCS3, appear to be important factors in the dysfunctional muscle stem cell response in aged muscle. Therefore, it appears that low-level systemic inflammation associated with normal human aging is at least, in part, responsible for the reduced SC response and may be implicated in the progression of sarcopenia by reducing the regenerative/adaptive potential of skeletal muscle. Further research is needed to understand the relationships between the IL-6/STAT3/SOCS3, IGF-1, and myostatin signaling pathways, which appear to be dysregulated in aged muscle.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: B.R.M., D.I.O., K.G.T., M.A.T., and G.P. conception and design of research; B.R.M., D.I.O., J.M.B., and K.G.T. performed experiments; B.R.M., D.I.O., J.M.B., and K.G.T. analyzed data; B.R.M., J.M.B., M.A.T., and G.P. interpreted results of experiments; B.R.M. and J.M.B. prepared figures; B.R.M. drafted manuscript; B.R.M., D.I.O., M.A.T., and G.P. edited and revised manuscript; B.R.M., D.I.O., J.M.B., K.G.T., M.A.T., and G.P. approved final version of manuscript.

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