CX₃CL1—a macrophage chemoattractant induced by a single bout of exercise in human skeletal muscle

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A transient increase in inflammatory cells is seen in the muscle tissue after acute muscle damage. These cells aid in the repair process by removing cellular debris and providing a regenerative milieu. In particular, the importance of monocytes (MOs) and macrophages (MΦs) in skeletal muscle repair and remodeling has been demonstrated in several studies (2, 5, 6, 19, 24, 27, 29). Indeed, both MO depletion and selective inhibition of MΦ infiltration lead to severely impaired regeneration following muscle injury in mice (2, 29), which is characterized by fibrosis and delayed satellite cell proliferation (2). Current data provide evidence that the MOs recruited to damaged muscle are proinflammatory, and act to phagocytose cellular debris and induce myoblast proliferation (2). These MOs then differentiate into MΦs and switch to an anti-inflammatory phenotype, which promotes myoblast differentiation and angiogenesis (5, 6, 19, 24, 27).

The mechanisms behind skeletal muscle recruitment of MOs and/or of MΦs are just beginning to be discovered. Animal models of muscle injury have established the importance of chemokines expressed in the muscle tissue for the recruitment of MOs/MΦs and muscle regeneration (32, 35). Moreover, a previous study investigating the interaction between human myogenic precursor cells and MOs/MΦs reported that human myoblasts in culture produced the chemokines CX₃CL1, CCL22, and CCL2, which selectively attracted MOs (10). Further, these factors were demonstrated to account for a large part of MO chemotaxis and to be expressed by activated human satellite cells in vivo, suggesting that they may be responsible for attracting MOs to human skeletal muscle.

Importantly, since skeletal muscle remodeling occurs also after nondamaging type of exercise, a plausible hypothesis is that such a stimulus also induces the production of inflammatory cell chemoattractants. In support of this hypothesis, it was shown recently that a prolonged, low-intensity endurance exercise bout in well-trained subjects induced skeletal muscle infiltration of inflammatory cells (22). Furthermore, the number of MΦs increased in mouse skeletal muscle after concentric contractions that did not cause any signs of muscle injury (22, 23). Thus, in the current study, the overall aims were to investigate the expression, localization, and plausible biological effects of chemoattractants with suggested importance for skeletal muscle remodeling i.e., CX₃CL1, CCL2, and CCL22. We hypothesized that a single endurance exercise bout in humans would increase the expression of these chemoattractants, presumably localizing to the satellite cells and muscle fibers. Moreover, on the basis of the knowledge that MOs exhibiting a proinflammatory profile arrive first within regenerating muscle, we hypothesized that these chemoattractants could induce a proinflammatory phenotype in monocytic cells.

METHODS

Ethical Approval

The regional ethical review board in Stockholm, Sweden, approved the study. The subjects gave written informed consent before participating in the respective experimental setup. The study conformed to the standards set by the Declaration of Helsinki.

Exercise and Muscle Biopsies

Seventeen male and female moderately active subjects were included in the study and divided into an exercise group (n = 12) and a control group (n = 5). All subjects reported that they exercised 1–3
times per week. The subjects did not use any medications and were nonsmokers. Prior to the intervention, their maximal oxygen uptake (\(\text{VO}_2\) max) was determined using an incremental cycle ergometer test until exhaustion, with respiratory gases continuously analyzed (SensorMedics Vmax 229, IntraMedic AB, Bälsta, Sweden). For inclusion to the study, the upper limit for \(\text{VO}_2\) max was set to 60 ml kg\(^{-1}\) min\(^{-1}\) for men, and 50 ml kg\(^{-1}\) min\(^{-1}\) for women. The mean (SD) age, height, weight, and \(\text{VO}_2\) max for the cycling subjects were 25.9 (3.5) yr, 178.8 (7.2) cm, 77.9 (10.6) kg, and 48.1 (6.6) ml kg\(^{-1}\) min\(^{-1}\). For the control subjects, the mean (SD) age, height, weight and \(\text{VO}_2\) max were 24.2 (1.9) yr, 176.6 (11.1) cm, 68.8 (6.9) kg, and 48.3 (5.8) ml kg\(^{-1}\) min\(^{-1}\). No significant differences were found between the groups regarding age, height, weight, or \(\text{VO}_2\) max. The group as a whole \((n = 17)\) had an average (SD) BMI of 23.6 (2.5) kg/m\(^2\). The average of the males was 24.3 (2.1) and that of the women was 22.4 (2.9) kg/m\(^2\). All subjects were Caucasian.

**Experimental procedure.** The subjects were instructed to abstain from alcohol and exercise for two days before the test. When arriving at the laboratory, the subjects were randomized to either exercise (8 males and 4 females) or control (3 males and 2 females) groups. The exercise group performed a 1-h cycling bout consisting of 20 min at a work load corresponding to 50% of \(\text{VO}_2\) max and 40 min at a work load corresponding to 65% of \(\text{VO}_2\) max, while the control group rested. To measure the perceived effort of the exercising subjects, the Borg scale (rating exertion on a scale of 6–20) was used every 10 min. The following protocol was used to change load: At 30 min, if the effort rated <13, the workload was increased by 10 Watts (W). At 40 min, if effort rated <15, the workload was increased by 10 W, if effort were rated 17, workload was decreased by 10 W, and if it were \(\geq\)18, workload decreased by 20 W. At 50 min, if effort rated <16, workload increased by 10 W, and if effort rated \(\geq\)18, workload decreased by 10 W. Muscle biopsies were obtained using the percutaneous needle biopsy technique from the vastus lateralis muscle of both legs. Before the 1-h cycling/resting, one biopsy was obtained from each leg. Biopsies also were obtained at 30 min, 2, 6, 10, and 24 h after the cycling/resting; the biopsies at 30 min and 6 h were taken from one leg, while biopsies at 2 h and 24 h were obtained from the other. The biopsies were immediately frozen in isopentane, cooled in liquid nitrogen, and stored at \(-80^\circ\)C. Both groups remained in the laboratory until after the 6-h biopsy, and then returned the morning after for the 24-h biopsy. All subjects were given standardized meals the night before, during the day of, and in the morning after the intervention (Table 1).

**Exercise and Microdialysis**

Three healthy male subjects with a mean (range) age, height, and weight of 23 (19–26) yr, 182 (175–190) cm, and 77 (71–85) kg, participated in this part of the study. They did not use any medications and were nonsmokers. Subjects performed dynamic constant load one-legged knee extension exercise (60 rpm) in the sitting position using a modified cycle ergometer using the dominant leg (1). One week prior to the experiment, subjects performed a one-legged exercise test with incremental workload: after 1 min at 60 rpm with 25 W, workload was increased with 5 W every minute until exhaustion. Subjects were instructed to abstain from exhaustive exercise during the week prior to the experiment. On the day of the experiment, initial workload was chosen to be 60% of maximal workload (range 25–30 W). After 20 and 40 min, workload was adjusted on the basis of effort assessed with the Borg scale. At 20 min, if effort rated <11, workload was increased by 10 W, if it rated \(\geq\)16, workload decreased by 10 W. At 40 min, effort rated <13, workload increased by 5 W, and if effort rated \(\geq\)16, workload decreased by 5 W. Two of the subjects worked at 30 W during the whole 1-h bout, while one subject worked at 30 W the first 40 min and then at 25 W for the last 20 min. All subjects were Caucasian.

**Experimental procedure.** Microdialysis was obtained from the vastus lateralis muscle of both legs after one-legged exercise using CMA 71 high cut-off brain microdialysis catheters with a cut-off of 100 kDa (CMA Microdialysis AB, Solna, Sweden). After local anesthesia, the catheters were inserted with a guided cannula and perfused with Ringer’s acetate at a rate of 2 \(\mu\)l/min. During the 4-h sampling, the subjects rested in a supine position. The dialysate was collected in vials that were switched every 30 min, and then the collected sample was transferred to \(-80^\circ\)C. During the first 30 min after insertion, the catheters were infused at a rate of 5 \(\mu\)l/min. The samples collected during the first 60 min postinsertion were not used for the stimulation assay.

**Cell Culture Experiments**

**Stimulation of human umbilical vein endothelial cells with microdialysate.** Human umbilical vein endothelial cells (HUVECs), (C-003-5C; Life Technologies, Carlsbad, CA) were cultured in 24-well plates in Medium 200 (M200) supplemented with low-serum growth supplement (LSGS), and 1% antibiotic-antimycotic (AbAm) (Life Technologies). When confluent, the cells were stimulated for 2 h with microdialysate diluted 1:2 in Ringer’s acetate. Two wells were

<table>
<thead>
<tr>
<th>Table 1. Prescribed diet</th>
<th>Meal</th>
<th>Food Eaten</th>
<th>When Eaten</th>
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<tbody>
<tr>
<td>Evening meal (× 2 days)</td>
<td>Yogurt drink 350 ml–245 kcal, 1.75 g fat, 45 g carbohydrates, 10.5 g protein, 0.3 g salt</td>
<td>The subjects were given the food the day before the experiment and were instructed to have the evening meal at 8 PM after their dinner.</td>
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<tr>
<td>Breakfast (× 2 days)</td>
<td>1 banana</td>
<td>The subjects were instructed to eat the breakfast at home at least 1 h before arriving to the laboratory.</td>
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<tr>
<td>Breakfast</td>
<td>200 g fruit yogurt–160 kcal, 4 g fat, 26 g carbohydrates, 6.6 g protein, 0.2 g salt</td>
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<td></td>
<td>Kellogg’s corn flakes one serving–108 kcal, 2.2 g fat, 22 g carbohydrates, 0.8 g protein, 0.1 g salt.</td>
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<td>Orange juice 200 g–90 kcal, &lt;0.5 g fat, 18 g carbohydrates, 2 g protein, &lt;0.1 g salt.</td>
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<td>½ cheese sandwich 38 g–127 kcal, 5.5 g fat, 14 g carbohydrates, 5 g protein, 0.7 g salt</td>
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<td>Lunch (× 1 day)</td>
<td>Pasta salad with chicken 380 g–kcal not stated, 39% of contents is pasta with pesto, 18% chicken filet, 16% seasonal salad, 8% cherry tomato, 8% beans in rape seed oil, 5% white cabbage</td>
<td>The subjects had lunch after the biopsy obtained 2 h after cycling/resting.</td>
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<tr>
<td>Fruit (× 1 day)</td>
<td>1 apple</td>
<td>The subjects had the fruits after the biopsy obtained 30 min after cycling/resting.</td>
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<td></td>
<td>1 banana</td>
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All subjects, exercise and control, were provided the same diet during the two days of the experiment. Water was given ad libitum.

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used per condition (resting leg, exercised leg), which were pooled in TRIzol (Life Technologies) at cell harvest prior to RNA extraction.

Stimulation of THP-1 cells with CX3CL1. THP-1 monocytic cells (a human acute monocytic leukemia cell line) (Sigma-Aldrich, St. Louis, MO) were cultured in RPMI 1640 medium GlutaMAX (Life Technologies) supplemented with 1% AbAm, 10% FBS, 1 mM sodium pyruvate, and 0.05 mM 2-mercaptoethanol. For the stimulation assay, cells were suspended at 1 × 10⁶ cells/ml in serum-free RPMI medium, and 2 ml were plated in each well in a six-well plate, with 0, 10, or 100 ng/ml recombinant human CX3CL1 (R&D Systems, Minneapolis, MN). The rationale behind using 10 ng/ml and 100 ng/ml is based on a previous article, in which similar dosing was used to evaluate the proangiogenic effect of CX3CL1 in HUVECs (33).

After 5 h, the cell suspensions were centrifuged at 6,000 g, and the cell pellets were resuspended in 1 ml of TRIzol and kept at −80°C until RNA extraction. The experiment was performed four times in duplicate.

Stimulation of human primary myoblasts and myotubes with CX3CL1. Four healthy, nonsmoking subjects (20–31 yr old, 2 females, 2 males) were recruited specifically to obtain muscle biopsies for isolation of primary human myoblasts. All subjects were Caucasian. A single biopsy from musculus vastus lateralis was obtained from each subject using the percutaneous needle biopsy technique. In brief, the biopsy was digested in 5 ml of 0.25% trypsin 1 mM EDTA at 37°C and 5% CO₂ with gentle agitation for 20 min, and the supernatant was diluted in proliferation medium (DMEM/F-12) supplemented with 1% AbAm and 20% FBS (Life Technologies). Digestion of the slurry was repeated twice. After preplating the cells in petri dishes for 20 min, the cells were cultured in T75 flasks (Sarstedt, Göteborg, Sweden), and growth medium was changed every 3rd or 4th day until cells reached 60% confluence. The myoblasts then were trypsinized and seeded in six-well plates at a density of 80,000 cells per well, in proliferation medium. All cultures had a desmin positivity >90% analyzed using cytocentrifuged cells. After 2 days, the medium was changed to low-serum medium (DMEM/F-12 containing 2% FBS, 1% AbAm), and the cells were stimulated with 0, 10, or 100 ng/ml of CX3CL1 for 5 h, with two wells per condition. For myoblast experiments, myoblasts in six-well plates were allowed to reach 90% confluence, and then the medium was changed to a low-serum medium. After 3 days, the myoblasts were stimulated with CX3CL1 using the same protocol as for the myoblasts. After stimulation, the cells from each condition were pooled in 1 ml of TRIzol, and RNA extraction was performed.

Myoblast proliferation assay. Primary human myoblasts were cultured in 96-well plates in DMEM/F-12 containing 20% FBS and 1% AbAm. After 2 days, the cells received fresh DMEM medium supplemented with 0, 10, or 100 ng/ml of CX3CL1, and BrdU was added to all wells. After 20 h, analysis of BrdU incorporation was performed according to the manufacturer’s instructions (F. Hoffmann-La Roche, Basel, Switzerland). The experiment was performed four times in triplicate.

Analysis
Gene expression analysis. Total RNA from skeletal muscle, HUVECs, THP-1 cells, primary human myoblasts, and myotubes were prepared by the acid phenol method and quantified by measuring absorbance at 260 nm using NanoDrop 2000 (Thermo Scientific, Basel, Switzerland). The frozen muscle biopsies were cut into 5-μm sections and placed on Superfrost/Plus microscope slides (Thermo Scientific). The sections were fixed for 10 min in 4% phosphate-buffered formaldehyde and then washed 3 min in PBS, 3 min in 0.3% Triton-X/PBS, and 3 min in PBS. The tissue sections were blocked in 4% FBS/PBS for 30 min, and then incubated with the primary antibody overnight at 4°C in a humid chamber. The antibodies used were goat anti-human CX3CL1 at 1:250 (Santa Cruz Biotechnology, Dallas, TX), mouse anti-human CD31 at 1:300, (Dako Sweden AB, Stockholm, Sweden), mouse anti-human CD56 (Clone MY31; BD Biosciences, Franklin Lakes, NJ) at 1:20, mouse anti-human caveolin-3 (A-3) at 1:500 (Santa Cruz Biotechnology), goat anti-human IL-8 at 1:20 (Abcam, Cambridge, UK) and rabbit anti-human collagen-4 at 1:1,000 (Rockland, Limerick, PA). After washing in PBS, sections were incubated with the donkey anti-mouse Alexa Fluor 568 antibody or the donkey anti-rabbit Alexa Fluor 488 antibody at 1:800, and the donkey anti-goat Alexa Fluor 488 at 1:800 (Life Technologies) for 50 min in room temperature, washed again, and mounted in ProLong Gold antifade reagent (Life Technologies) containing the DNA stain DAPI. Both primary and secondary antibodies were diluted in 1% FBS/PBS. Fluorescent signals (Fig. 2, A–D) were detected with a Zeiss LSM710 laser scanning microscope in combination with the ZEN2011 software (Zeiss, Jena, Germany) using the laser lines 405 (DAPI), 488 (Alexa Fluor 488) and 561 (Alexa Fluor 568) for fluorescence excitation. Image rendering (Fig. 2, B–D) was done with Bitplane Imaris software using the volume tool. A Leica DMLA microscope equipped with a Leica DFC 450 digital camera (Leica Microsystems AB, Sweden) was used to obtain conventional fluorescent images (Figs. 2, E–G, 3, D–G) using an oil immersion objective with magnification ×63 1.25.

Statistics
The effect of exercise on gene and protein levels in human skeletal muscle was analyzed using two-way repeated-measures ANOVA with the factors condition (exercise and control) × time (pre, post 30, post 2 h, post 6 h, and post 24 h). The Holm-Sidak all pairwise comparison was used as a post hoc test to locate the points of interaction. The effect of CX3CL1 stimulation on gene expression in myoblasts, myotubes, and THP-1 cells was analyzed using one-way repeated-measures ANOVA, with the Holm-Sidak all pairwise comparison used as a post hoc test. Gene expression in HUVECs was analyzed using the two-tailed t-test. These statistical analyses were performed using SigmaPlot 13.0 (Systat Software, San Jose, CA). The mRNA data from muscle biopsies were not normally distributed, as assessed by the Shapiro-Wilk’s test. To address this, the Mauchly test for sphericity together with the Greenhouse-Geisser correction was utilized to correct the degrees of freedom for the F-distribution in the two-way repeated-measures ANOVA. This analysis was performed using SPSS v.22 (SPSS, Chicago, IL). Differences were considered statistically significant at P < 0.05.
RESULTS

Increase in CX3CL1 mRNA and Protein in Human Skeletal Muscle with Exercise

The mRNA level of CX3CL1 increased in the skeletal muscle tissue at 30 min postexercise compared with preexercise levels. No change in CX3CL1 mRNA levels was seen in the control group (Fig. 1A, interaction between exercise and control P = 0.002). For the CCL2 and CCL22 mRNA levels, no significant interaction was found between the groups (Fig. 1, C and D, P = 0.48 and P = 0.6, respectively). No significant difference was seen between males vs. females in the mRNA levels of CX3CL1, MDC, or MCP-1. The expression levels of CCL22 were low with a mean CT value of 33.2 in the preexercise biopsies in cycling and 32.8 in control subjects at cDNA dilution 1:10 corresponding to 18 ng RNA per well. Since no changes were seen between exercise and rest in the gene expression of CCL2 and CCL22, the remainder of the study focused on CX3CL1. The protein level of CX3CL1 in the muscle homogenate was increased at 2 h postexercise and returned to resting levels at 24 h postexercise. The mean (SD) value expressed as picograms of CX3CL1 per microgram of protein was 0.07 (0.04), 0.12 (0.06), 0.21 (0.11), 0.13 (0.04), and 0.09 (0.08) in the exercise subjects. No change in CX3CL1 protein levels was seen in the control group (Fig. 1B, interaction between exercise and control, P = 0.04).

CX3CL1 Is Expressed on Endothelium in Human Skeletal Muscle

To visualize the localization of CX3CL1, immunofluorescent staining for CX3CL1 was performed on muscle sections (Fig. 2A). Confocal imaging showed CX3CL1 colocalized with the endothelial cell marker CD31 and, in addition, was detectable within endothelial cell nuclei (Fig. 2, B–D). Faint CX3CL1 staining also was associated with muscle fibers, next to the endothelium, and the endomysium, which could represent shed CX3CL1. The staining level of CX3CL1 was slightly increased 2 h postexercise compared with preexercise levels (Fig. 2, E and F). Satellite cells showed no or very weak CX3CL1 staining (Fig. 2G). To investigate whether factors in the skeletal muscle tissue fluid could induce the expression of CX3CL1 in endothelial cells, HUVECs were stimulated with microdialysate obtained from exercised muscle. The level of CX3CL1 mRNA increased 1.9-fold in HUVECs stimulated with exercise microdialysate compared with microdialysate from the resting leg, suggesting that at least part of the induction in CX3CL1 may be due to factors increasing in the muscle interstitial fluid with exercise (Fig. 2H; P = 0.02).

CX3CL1 Induces Factors Involved in Skeletal Muscle Remodeling in Human Monocytic and Myogenic Cells

THP-1 monocytic cells were stimulated with CX3CL1 to assess whether CX3CL1 could modulate their inflammatory profile. The expression of proinflammatory TNF-α (P = 0.005) and IL-6 (P = 0.02) and anti-inflammatory IL-10 (P = 0.01) were increased significantly in cells treated with 100 ng/ml CX3CL1, and there was a trend toward an increased level of VEGF-A (P = 0.08). For MMP9 and TGF-β, the ANOVA detected a significant treatment effect, which, however, could not be located with the post hoc test (Fig. 3A). iNOS was not expressed in any condition.

To further investigate plausible biological effects of CX3CL1, the expression of factors involved in muscle remodeling was measured in primary human myoblasts and myotubes following CX3CL1 stimulation. In myoblasts, the expression of IL-6 was increased in cells treated with 100 ng/ml of CX3CL1 (P = 0.02), and there was a trend toward increased CCL2 (P = 0.07) and IL-8 (P = 0.1). 100 ng/ml CX3CL1 decreased
the level of MyoD (P = 0.04), but it did not alter myogenin (P = 0.47) or VEGF-A (P = 0.69). Myostatin (P = 0.14) and MMP9 (P = 0.11) exhibited a tendency to increase, although MMP9 was virtually not expressed, with an average CT value of 35.4 at cDNA dilution 1:5 corresponding to 36 ng RNA per well (Fig. 3B). Myotubes increased their expression of IL-8 (P = 0.008), IL-6 (P < 0.001), MMP9 (P = 0.03), and CCL2 (P = 0.003) at 100 ng/ml, while no change was seen for VEGF-A (P = 0.78), myostatin (P = 0.55), MyoD (P = 0.75), or myogenin (P = 0.15) (Fig. 3C). Because IL-8 was highly induced in myotubes with CX3CL1 treatment, immunofluorescent staining of IL-8 was performed in the skeletal muscle tissue before and after exercise. The staining for IL-8 in skeletal muscle sections was increased at 2 h postexercise compared with preexercise levels (Fig. 3D–G). Because IL-8 was highly induced in myotubes with CX3CL1 treatment, immunofluorescent staining of IL-8 was performed in the skeletal muscle tissue before and after exercise. The staining for IL-8 in skeletal muscle sections was increased at 2 h postexercise compared with preexercise levels (Fig. 3D–G). Because IL-8 was highly induced in myotubes with CX3CL1 treatment, immunofluorescent staining of IL-8 was performed in the skeletal muscle tissue before and after exercise. The staining for IL-8 in skeletal muscle sections was increased at 2 h postexercise compared with preexercise levels (Fig. 3D–G).

**DISCUSSION**

The key observations of the current study were 1) CX3CL1 is increased in human skeletal muscle at the mRNA and protein level after one bout of endurance exercise, 2) CX3CL1 expression is localized mainly to the skeletal muscle endothelium and increases in HUVECs stimulated with tissue fluid from exercised muscle, and 3) when cultured in the presence of CX3CL1, THP-1 monocytes and human primary myoblasts and myotubes increased their expression of proinflammatory, proangiogenic, and chemotactic factors.

Following acute muscle injury, muscle regeneration is largely dependent on the recruitment of MOs and MΦs to create a regenerative milieu. Although this is well established in the setting of muscle injury, less is known regarding recruitment of MOs/MΦs to muscle in response to physiological stimuli associated with muscle remodeling processes, such as exercise. The importance of several chemokines for the recruitment of MOs and MΦs to muscle has been demonstrated in both rodents (32, 35) and in human cell models (10). In the latter, CX3CL1, CCL2, and CCL2 were described to be important MO/MΦ chemoattractants produced by human muscle cells (10). In the present study, an
increase in CX3CL1 at both the mRNA and protein level was observed in the exercised human skeletal muscle, demonstrating that the production of MO/MΦ-recruiting chemokines is increased following a single bout of exercise in humans. In contrast with CX3CL1, no significant interaction was found between the exercise and control groups for changes in CCL2 or CCL22. Because of this, it was not possible to distinguish biopsy vs. exercise effects, and changes in CCL2 or CCL22 in the exercised muscle could not be evaluated.

Although the cellular source underlying the production of chemokines in muscle tissue is unknown, cultured human satellite cells can produce chemoattractants (including CX3CL1, CCL22, and CCL2) that selectively attract MOs and amplify the chemotactic activity of MΦs (10). This finding indicates a secretory role for the satellite cell and suggests that satellite cells may be one source of muscle tissue-derived chemokines that contributes to the recruitment of MOs/MΦs. On the basis of these findings, we hypothesized CX3CL1 to be localized to the skeletal muscle fibers and/or satellite cells of human muscle tissue (10). However, in contrast to our hypothesis, our immunostaining showed a clear localization of CX3CL1 to the endothelium, with only a weak staining visible on the muscle fiber membranes. Thus, the induced expression of CX3CL1 in the exercised skeletal muscle presumably reflects an increase in the endothelium. Endothelial expression of CX3CL1 is known to be induced by the proinflammatory factors TNF-α, IFN-γ, and IL-1 (4, 18), factors demonstrated to be released from human myogenic cells (3, 7, 28). In fact, the increased expression of CX3CL1 in HUVECs stimulated with...
We investigated the role of CX3CL1 in human primary myoblasts and myotubes stimulated with 0 (Ctrl), 10 or 100 ng/ml CX3CL1 [mean (SD), n = 4].

CX3CL1 stimulation of THP-1 monocytes resulted in a strong increase in the factors TNF-α, IL-6, and MMP9, all markers of a proinflammatory profile, together with an increase in the anti-inflammatory factor IL-10. Interestingly, both IL-6 and TNF-α also are known to stimulate proliferation of satellite cells (21, 30); thus, this could be one indirect mechanism by which CX3CL1 acts as a mitogen for muscle cells. Altogether, in addition to serving as a chemoattractant for MOs, as well as expression of proinflammatory, proangiogenic, and chemotactic factors in myogenic cells.

The present findings suggest a CX3CL1-dependent bidirectional signaling between endothelium and cells in the muscle tissue with exercise. This is in line with previous data demonstrating a cross talk between endothelial cells, MOs, and myogenic cells in skeletal muscle remodeling (10, 12). Our results corroborate earlier studies demonstrating that noninjurious exercise promotes inflammatory processes in the skeletal muscle and suggest that these are part of the physiological adaptation to increased physical activity. However, it is unclear whether a larger induction of inflammatory processes translates to a greater adaptation to exercise, or whether it could be deleterious to the remodeling process. This question is of importance in understanding the skeletal muscle adaptation in young vs. elderly, the latter of which may, thereby, serve to further increase recruitment of MOs/MΦs. The current finding is supported by a recent study by Catoire et al. (8), in which exercise-induced myokines with chemotactic properties were identified in a more unbiased strategy by use of microarray analysis (8). Even though their analysis employed a low fold-change cut-off value, their results support exercise-induced expression of genes encoding chemoattractants. In fact, both CX3CL1 and CCL2 were major factors in their identified networks, and both factors increased at mRNA levels in skeletal muscle tissue, which was followed by increased plasma protein levels. The latter supports secretion of these factors from the skeletal muscle tissue. In our study, we could not detect any difference in CCL2 expression between exercise and controls when all time points were included in the analysis. However, if only the time points preexercise and 30 min postexercise were compared, similar to the study by Catoire et al. (8), an increase of CCL2 with exercise relative to controls also was observed in the present study. Thus, the present study indirectly supports an exercise-induced expression of CCL2, but to accurately establish changes in the expression of this factor, future studies should consider a design that does not use repetitive biopsy sampling. It is concluded that 1) CX3CL1 increases in human skeletal muscle with exercise and is mainly localized to the endothelium; 2) exercise-induced factors in the skeletal muscle participate in this regulation; and 3) CX3CL1 increases the expression of proinflammatory and chemotactic factors in MOs, as well as expression of proinflammatory, proangiogenic, and chemotactic factors in myogenic cells.

Perspectives and Significance

The present findings suggest a CX3CL1-dependent bidirectional signaling between endothelium and cells in the muscle tissue with exercise. This is in line with previous data demonstrating a cross talk between endothelial cells, MOs, and myogenic cells in skeletal muscle remodeling (10, 12). Our results corroborate earlier studies demonstrating that noninjurious exercise promotes inflammatory processes in the skeletal muscle and suggest that these are part of the physiological adaptation to increased physical activity. However, it is unclear whether a larger induction of inflammatory processes translates to a greater adaptation to exercise, or whether it could be deleterious to the remodeling process. This question is of importance in understanding the skeletal muscle adaptation in young vs. elderly, the latter of which may have a chronic low-grade inflammation, as well as in the context of nonsteroidal anti-inflammatory drugs that are known to be used to a high degree by both recreational and professional athletes.
GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

REFERENCES
6. T.G. approved final version of manuscript.