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Myokine Expression in Muscle and Myotubes in Response to Exercise Stimulation

Jeffrey D. Covington[1,2], **Charmaine S. Tam**[3], **Sudip Bajpeyi**[4], **Jose E. Galgani**[5], **Robert C. Noland**[1], **Steven R. Smith**[4], **Leanne M. Redman**[1], and **Eric Ravussin**[1]

[1]Pennington Biomedical Research Center, Baton Rouge, La

[2]School Of Medicine, Louisiana State University Health Sciences Center, New Orleans, La

[3]The Charles Perkins Centre And The School Of Biological Sciences, University Of Sydney, Nsw, Australia

[4]Department Of Kinesiology, University Of Texas In El Paso, El Paso, Tx

[5]School Of Medicine, Pontificia Universidad Catolica De Chile, Santiago, Chile

Abstract

Purpose—Myokines have been shown to affect muscle physiology and exert systemic effects. We endeavored to investigate a panel of myokine mRNA expression after a single exercise bout (studies 1 and 2) to measure myokine mRNA in primary human myotubes in an *in vitro* exercise model (study 2).

Methods— *Vastus lateralis* muscle biopsies were obtained from 20 healthy males (age, 24.0 ± 4.5 yr; BMI, 23.6 ± 1.8 kgIm^{-2}) before and after a single exercise bout (650 kcal at 50% $\dot{V}O_{2max}$). Primary myotubes from active and sedentary male donors were treated with a pharmacological cocktail (palmitate, forskolin, and ionomycin (PFI)) to mimic exercise-stimulated contractions *in vitro*.

Results—Interleukin 6 and 8 (IL-6 and IL-8), leukocyte-inducing factor, and connective tissue growth factor (CTGF) mRNA levels increased approximately 10-fold after a single exercise bout (all $P < 0.001$), whereas myostatin levels decreased ($P < 0.05$). Key correlations between myokine expression and parameters of muscle and whole-body physiology were found: myostatin versus skeletal muscle citrate synthase activity ($r = -0.69$, $P < 0.001$), $\dot{V}O_{2max}$ ($r = -0.64$, $P = 0.002$) and the percentage of Type I fibers ($r = -0.55$, $P = 0.01$); IL-6 versus the RER ($r = 0.45$, $P = 0.04$), homeostatic model assessment of insulin resistance ($r = 0.44$, $P = 0.05$), and serum lactate ($r = 0.50$, $P = 0.02$). Myokine expressions in myotubes from sedentary donors for CTGF and myostatin decreased, whereas IL-6 and IL-8 increased after PFI treatment. In myotubes from active donors, myokine expression increased for IL-6, CTGF, and myostatin but decreased for IL-8 after PFI treatment.

Address for correspondence: Eric Ravussin, Ph.D., Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA 70808; eric.ravussin@pbrc.edu.

The authors have nothing to disclose. The results of the present study do not constitute endorsement by the American College of Sports Medicine.

Conclusion—These data offer insight into the differences in regulation of myokine expression and their possible physiologic relationships.

Keywords

MYOSTATIN; CYTOKINE; INTERLEUKIN; MYOBLAST; ENDURANCE

Skeletal muscle is now recognized as an endocrine organ with proteins that are both expressed by and secreted from skeletal muscle called myokines. Studies using computational analysis have identified hundreds of potentially secreted proteins from human skeletal muscle (5,11) and in myotubes (17). It has been suggested that the contractile activity of muscle affects its secretory function linking physical activity to myokine secretory response. However, much of the physiologic function and how these myokines are secreted from the contractile activity of skeletal muscle remain unknown.

The majority of studies have focused on select myokines, and few studies have examined associations between myokine gene expression and anthropometric, metabolic, or skeletal muscle functional parameters. Myostatin, a member of the transforming growth factor β superfamily, was the first myokine discovered (30) and found to inhibit muscle growth (37, 38). Interleukin 6 (IL-6), a cytokine with known proinflammatory functions (8), is secreted after muscle contraction from skeletal muscle (40). Extensively reviewed by Pedersen and Febbraio (31), muscle IL-6 has been speculated to contribute to the antiinflammatory effects of exercise, regulate glycogen content in skeletal muscle, and contribute to insulin sensitivity through AMP kinase activation. IL-15 and leukocyte-inducing factor (LIF) have been speculated to regulate muscle cell growth and satellite cell proliferation (7,18), with IL-15 playing a potential role in adipose tissue lipolysis (33). IL-8 and connective tissue growth factor (CTGF) have been hypothesized to regulate angiogenesis within the skeletal muscle (3,6,16,22). These aforementioned myokines have not been examined together in one study, and few studies have examined associations between myokine gene expressions and anthropometric, metabolic, or skeletal muscle functional parameters. In this study, we examined myokine gene expressions (myostatin, IL-6, IL-15, LIF, and CTGF) in human skeletal muscle before and after a single bout of exercise. Additionally, we performed *in vitro* experiments using primary skeletal muscle cultures taken from healthy, lean, and sedentary as well as physically active male volunteers. Myokine expressions were measured before and after treatment with an exercise pharmacological cocktail (palmitate, forskolin, and ionomycin (PFI)) known to stimulate contraction of myotubes (36).

METHODS

Study 1: endurance exercise bout in human participants

Twenty healthy, normoglycemic sedentary male participants (16 Caucasians, 3 African Americans, and 1 nonspecified race), who were not engaged in sports at a competitive level, were recruited to participate in this trial. The institutional review board of Pennington Biomedical Research Center approved all aspects of this study in accordance to the Declaration of Helsinki, and all participants provided written informed consent. Detailed aspects of this exercise trial have been reported (14). Participant characteristics are provided

in Table 1. Body composition was assessed by dual x-ray absorptiometry (QDR 4500A; Hologic, Waltham, MA), and $\dot{V}O_{2max}$ was measured on a stationary bicycle ergometer (Lode Excalibur, Groningen, the Netherlands) using an incremental workload protocol with simultaneous gas exchange measurements using a metabolic cart (TrueOne 2400; ParvoMedics, Sandy, UT).

$\dot{V}O_{2max}$ and dual x-ray absorptiometry measurements were assessed at a period of no greater than 2 d before the exercise intervention to prevent any confounding acute effects of exercise on baseline measurements. The night before the exercise bout, participants were admitted to the institutional in-patient unit. After an overnight fast, resting metabolic rate was measured using a DeltaTrac metabolic cart, and a percutaneous skeletal muscle biopsy of the *vastus lateralis* muscle was performed. Gas exchange while exercising was assessed from expired air collected by a mouthpiece using the same TrueOne 2400 ParvoMedics metabolic cart. Total energy expenditure and substrate oxidation were calculated as previously described (13). Participants then exercised on a stationary bike at 50% of their $\dot{V}O_{2max}$ until they had expended 650 kcal. Indirect calorimetry measures were performed after the estimated 8%, 20%, 40%, 60%, and 80% and right before exercise completion to gauge when 650 kcal of energy had been expended. Blood was drawn at regular intervals coupled to indirect calorimetry measures before and after the exercise bout with serum glucose, insulin, and lactate by an enzymatic assay on a Beckman Coulter DXC 600 (Beckman Coulter, Brea, CA). All blood parameters were measured in a certified clinical chemistry laboratory, and the manufacturer's protocols were followed for all the serum measurements (Table 2). Immediately after the exercise bout, a second percutaneous skeletal muscle biopsy was obtained proximal to the first biopsy.

Skeletal muscle biopsy procedure

After local anesthesia with 2% lidocaine/0.5% bupivacaine (1/1 ratio), *vastus lateralis* samples were collected using the Bergstrom technique with suction. Two separate incisions were made to collect tissues at baseline and postexercise. The second biopsy was obtained immediately after the completion of exercise (<3 min). Muscle samples were visually assessed and cleaned of intramuscular adipose tissue. Muscle biopsies were snap frozen in liquid nitrogen for subsequent mRNA and protein analyses, or blotted dry and then mounted in a mixture of optimal cutting temperature compound (Thermo Scientific, Waltham, MA) and tragacanth powder (Acros, Geel, Belgium) for immunohistochemical measures of glycogen, intramyocellular lipid (IMCL), and fiber typing. Another sample was collected for measurements of *ex vivo* palmitate oxidation.

Immunohistochemical measures

Measures of fiber typing and IMCL were performed as previously described using immunofluorescence techniques (14). Images were taken using a multiphoton confocal microscope (Leica TCS SP5 AOBS; Leica Microsystems, Wetzlar, Germany) and Type I fibers were counted. IMCL was determined using the Sigma Scan Pro 5 software (SPSS, Chicago, IL) by delineating BODIPY staining within the myofibers. Glycogen content was measured using periodic acid–Schiff staining and analyzed using the Sigma Scan Pro 5 software (2). For all histology measures, three cross-sectional slices were obtained within

the tissue. Not less than 50 fibers were assessed from each cross-sectional slice for IMCL content, fiber type, and glycogen.

Ex vivo palmitate oxidation and pyruvate oxidation measures in skeletal muscle

A palmitate oxidation assay was performed as previously described (14). Data were adjusted to total protein content obtained from muscle homogenate as determined through the bicinchoninic acid assay (Pierce BCA, Thermo Scientific).

Maximal citrate synthase activity in skeletal muscle

About 80 mg of skeletal muscle was diluted 20-fold in the extraction buffer (0.1 M KH_2PO_4/Na_2PHO_4 and 2 mM EDTA (pH 7.2)) and then homogenized (Glas Col, Terre Haute, IN). Activity was measured at 37°C in a 0.1M Tris–HCl (pH 8.3) assay buffer containing 0.12 mM 5,59-dithio-bis-2-nitrobenzoic acid and 0.6 mM oxaloacetate. After an initial 2-min absorbance reading at 412 nm, the reaction was initiated by adding 3 mM acetyl-CoA, and the change in absorbance was measured every 10 s for 7 min. Values were adjusted for total protein.

Study 2: establishment of primary human skeletal muscle cultures

Primary muscle cultures were established from muscle biopsies obtained from the *vastus lateralis* in five lean, healthy, and sedentary Caucasian male donors (age, 23.0 ± 1.9 yr; BMI, 24.2 ± 0.6 $kg \cdot m^{-2}$; and percent fat, 8.4% ± 6.4%) from the EAT trial (Clinicaltrials.gov number: NCT01672632) (20) and four active donors (age, 23.0 ± 1.0 yr; BMI, 25.1 ± 2.5 $kg \cdot m^{-2}$; percent fat, 13.2 ± 2.0; and $\dot{V}O_{2max}$, 50.1 ± 3.7 $mL \cdot min^{-1} \cdot kg^{-1}$ fat-free mass (FFM)) from the ACTIV trial (Clinicaltrials.gov number: NCT00401791) (1). Establishment of the human primary muscle culture was performed as previously described (12).

In vitro PFI treatment in primary human myotubes

Myotubes were treated with 30 μM palmitate, 4 μM forskolin, and 0.5 μM ionomycin (PFI)—all purchased from Sigma (St. Louis, MO). We previously showed that PFI treatment in myotubes increased palmitate oxidation, increased mitochondrial oxidative phosphorylation complex expression, and improved insulin-stimulated glucose uptake (36). Briefly, myotubes were maintained in differentiation media for 4 d and then treated with PFI for three additional days ($1\ h \cdot d^{-1}$). Differentiation media were similarly changed each day, without PFI, for control cells. Total protein and mRNA were collected immediately before PFI (0 min) and at 15 min, 30 min, 1 h, 8 h, and 24 h after PFI treatment. Total mRNA was collected using QIAzol (Qiagen, Germantown, MD).

Gene expression measures in skeletal muscle tissue and primary human myotubes

The total mRNA from both *in vivo* and *in vitro* experiments was extracted using the miRNEasy Mini Kit (Qiagen); cDNA was made using the High Capacity cDNA Kit (Applied Biosystems, Foster City, CA). Detection of gene expression was performed using TaqMan Gene Expression Assays-on-Demand (Applied Biosystems). The list of assay catalogue numbers are as follows: RPLPO (Hs99999902_m1), IL-6 (Hs00985639_m1), IL-8 (Hs00174103), IL-15 (Hs01003716_m1), Myostatin (Hs00976237_m1), CTGF

(Hs01026927_m1), and LIF (Hs01055668_m1). Real-time PCR was carried out using the 7900HT Fast Real-Time PCR System (Applied Biosystems), and expression levels were determined against a standard curve. Skeletal muscle and myotube gene expression were adjusted to the expression of RPLPO.

Statistical analysis

Data were analyzed using the PRISM GraphPad Software, version 6.0 (GraphPad Software, La Jolla, CA). Data were checked for normality using the Shapiro–Wilk normality test. Normally distributed data were analyzed using paired *t*-tests, whereas not normally distributed data were analyzed using the Wilcoxon signed-rank paired test. A two-way ANOVA with Tukey post hoc test was used to test for differences in myokine expression in myotubes from lean and sedentary donors versus athlete donors. Correlational analyses were performed using Pearson's correlations. A P value ≤0.05 was considered statistically significant.

RESULTS

Changes in myokine expression after a single exercise bout

Myostatin gene expression decreased on average 20% after exercise ($P < 0.05$, Fig. 1A). IL-6, IL-8, LIF, and CTGF gene expressions all increased robustly after exercise (all markers, $P < 0.001$, Fig. 1B–E). There were no significant changes in IL-15 (Fig. 1F).

Correlations between myokine expression and metabolic parameters

Next, we examined correlations between myokine expression and factors related to whole body and skeletal muscle metabolism, physical fitness, and body composition at baseline and after exercise. At baseline, myostatin gene expression was inversely related to citrate synthase activity in the *vastus lateralis* ($r = -0.69$, $P < 0.001$) and $\dot{V}O_{2max}$ ($r = -0.64$, $P = 0.002$, Table 3). Additionally, myostatin was positively related to the percent of Type II, fast-twitch fibers and negatively associated to the percentage of Type I, slow-twitch fibers in vastus lateralis ($r = 0.55$ and $r = -0.55$, respectively, both $P = 0.01$, Table 3). Baseline IL-6 expression was positively related to the markers of glucose metabolism including the homeostatic model assessment of insulin resistance (HOMA-IR), homeostatic measure of insulin resistance ($r = 0.44$, $P = 0.05$), baseline RER ($r = 0.45$, $P = 0.04$), serum lactate levels ($r = 0.50$, $P = 0.02$, Table 3), and change in IL-6 inversely associated with the change in glycogen content with exercise ($r = -0.57$, $P = 0.01$, Table 3).

IL-15 mRNA expression at rest was inversely associated with $\dot{V}O_{2max}$ ($r = -0.47$, $P = 0.03$) as well as positively associated with fat mass ($r = 0.43$, $P = 0.05$), and the change in IL-15 expression with exercise was inversely associated with the change in total IMCL in the *vastus lateralis* ($r = -0.61$, $P = 0.007$, Table 3). CTGF was associated with markers of glucose metabolism with resting levels being associated with serum glucose ($r = 0.52$, $P = 0.02$) and with changes in *ex vivo* pyruvate oxidation measured in the vastus lateralis ($r = -0.65$, $P = 0.005$, Table 3). The change in CTGF with exercise was positively associated with percent body fat ($r = 0.64$, $P = 0.003$, Table 3).

Primary myotube expression of myokines from athlete and sedentary donor with PFI stimulation

Given that IL-6, IL-8, and CTGF mRNA levels increased and myostatin decreased after a single bout of exercise, we next examined whether contraction in primary skeletal muscle myotubes from active and sedentary lean donors would stimulate similar adaptations in these myokines. Both resting and contraction-stimulated (PFI-treated) mRNA myokine expressions were examined over 24 h after PFI treatment. We found significant time, group, and interaction effects in IL-6, IL-8, CTGF, and myostatin mRNA levels (all $P < 0.001$, Fig. 2). Compared with unstimulated controls, IL-6, IL-8, and CTGF all increased with PFI stimulation and returned to almost basal expression levels 24 h after PFI treatment. However, myostatin levels significantly rose with PFI in myotubes from active donors and were significantly above myostatin expression in sedentary donors (Fig. 2B). This higher gene expression of myostatin in myotubes from active donors was maintained even 24 h after PFI treatment, whereas PFI treatment seemed to suppress its expression in myotubes from sedentary donors.

DISCUSSION

Skeletal muscle secreted myokines, whose functional role still remains elusive, provide evidence that skeletal muscle operates as an endocrine organ. Here, we examined the gene expression of seven known myokines in 20 healthy lean males before and after an endurance exercise bout, and identified associations between myokine expression and factors related to whole body physical fitness, skeletal muscle metabolism, and glucose homeostasis. Furthermore, we examined the gene expression of four of those myokines in an *in vitro* pharmacologic exercise model of contraction induced in primary human myotubes taken from healthy, lean, and sedentary males as well as active males. Similar to previous studies (28,40), our study found 10-fold increases in IL-6 and a significant decrease in myostatin expression. Additionally, IL-8, CTGF, and LIF expressions increased substantially after exercise. Furthermore, we found significant associations between myokine mRNA levels and factors of whole body metabolism and skeletal muscle metabolism, such as RER, $\dot{V}O_{2max}$, HOMA-IR, substrate oxidation, and citrate synthase activity. Together, these data provide insight into the clinical and anthropometric characteristics related to myokine expression as well as alterations in expression based on the exercise stimulus and physical fitness.

Of the two most extensively studied myokines, IL-6 and myostatin expressions have been shown to respond with exercise (reviewed by Pedersen and Febbraio [31,32]). We found a significant decrease in myostatin and a marked increase in IL-6 expression. The novelty of our study is that we report IL-6 and myostatin expression in human primary myotubes cultured from both physically active and lean sedentary donors. Our data show fundamental differences in post-PFI exercise mimetic cocktail treatment in myotubes from different types of donors, particularly with regard to myostatin. IL-6 gene expression seems to follow a similar pattern in both sedentary and active donors with increasing expression with PFI, peaking between 15 and 30 min after PFI, and subsiding to basal expression levels 1 to 24 h after PFI treatment. However, myostatin gene expression is differentially regulated with PFI

treatment, with lean sedentary myotubes having decreasing expression as would be hypothesized, but myotubes from active individuals having markedly increased expression of myostatin that is maintained 24 h post-PFI treatment.

This unexpected finding in myotubes from active donors provides speculative insight into the function of myostatin. Myostatin is involved in regulating the inhibition of skeletal muscle growth (38). Patients with type 2 diabetes (39) and elderly individuals with muscle wasting (41) possess elevated levels of myostatin. However, physical activity can reduce expression of myostatin allowing for muscle growth and bulk (27,28). One potential mechanism that has been proposed is that myostatin operates by inhibiting the differentiation and fusion of skeletal muscle satellite cell progenitors for the formation of new myofibers rather than acting directly on mature, preexisting muscle fibers themselves (15,23,29). It may be that endurance-trained individuals might release excess myostatin to maintain a substantial pool of already matured “trained” muscle fibers and perhaps to maintain relative fiber type composition in a mixed muscle such as the *vastus lateralis*. In fact, we found an association between higher myostatin levels, a lower percentage of Type I fibers and a higher percentage of Type II fibers in our clinical study (Table 3). Furthermore, on the whole body level, we see an inverse relationship between baseline myostatin gene expression and $\dot{V}O_{2max}$ (Table 3). Of fundamental interest is how basal myostatin gene expression is inversely associated with citrate synthase activity, which serves as a surrogate marker for mitochondrial content. Because Type I, slow-twitch fibers have higher levels of mitochondria and are associated with higher $\dot{V}O_{2max}$, myostatin could have an effect in determining fiber type composition. Further investigations in the effects of myostatin on fiber type composition are required.

IL-6 is secreted in large amounts from muscle as a fuel sensor of skeletal muscle glycogen content and glucose oxidation (21) and secreted as an endocrine factor from the muscle as glycogen is depleted to enhance gluconeogenesis and lipolysis in the liver and adipose tissue, respectively (reviewed by Pedersen and Febbraio, ref. 31). In fact, IL-6 levels were blunted in participants who underwent endurance exercise with glucose supplementation during exercise (4). Our data seem to partly support the association between IL-6 function and glucose utilization. IL-6 is positively associated with resting RER and with serum lactate levels, suggesting that higher levels of IL-6 are associated with higher levels of carbohydrate oxidation. Likewise, the change in IL-6 with exercise is inversely associated with the change in glycogen in the muscle, implying that IL-6 expression is increased as glycogen levels fall with exercise. Seemingly contrary, but connected with this concept that IL-6 is a fuel sensor (glucose utilization and glucose need) in skeletal muscle, is how IL-6 expression is positively associated with HOMA-IR, a measure of insulin resistance (Table 3). It has also been postulated that IL-6 is involved in the regulation of AMP kinase (10), a fuel-sensitive kinase that is partly responsible for exercise-induced, insulin-independent glucose uptake into skeletal muscle (19,34). Because the proinflammatory functions of IL-6 have been shown to blunt insulin sensitivity (9,35), the myokine function of IL-6 might be to inhibit systemic insulin uptake of glucose from other tissues in favor of exercise-induced, insulin-independent uptake of glucose into the skeletal muscle where it would be most needed during endurance exercise. Additional experiments would be needed to test these hypotheses.

We found increased CTGF with exercise in muscle tissue (Fig. 1E) and after *in vitro* exercise mimetic stimulation in myotubes with the PFI cocktail (Fig. 2D). Since CTGF is involved in recruiting and differentiating fibroblasts (24) and angiogenesis (6,16), higher levels of CTGF might be involved in the production of fibroblast proliferation to buffer muscle interstitial fluid from higher serum glucose levels and angiogenesis with exercise.

Although we observed large increases in IL-8 and LIF expression (Fig. 1C and D) with exercise and differential IL-8 expression patterns post-PFI treatment in myotubes from sedentary and active donors (Fig. 2C), we did not find any correlations or significant associations of LIF or IL-8 expression with any clinical or molecular parameters. LIF has been shown to contribute to myoblast proliferation and myotube differentiation (7). Studies into the myokine functions of IL-8 have suggested that it might be involved in angiogenesis (3,22). However, due in part to limited biopsy materials, further examination of these effects fell outside of the scope of our investigation. Further studies would be needed to understand the role IL-8 and LIF might play in response to exercise-mediated expression.

One of the major limitations of our study is that our observations are based solely on the mRNA expression of myokines rather than on the serum content of secreted myokines. Malm et al. (26) reported that multiple biopsies could result in increased inflammatory serum markers as well as increased IL-6 protein content detected by immunohistochemistry. However, Lundby et al. (25) showed that alterations of mRNA expression in skeletal muscle are not altered by the effect of multiple biopsies. We opted therefore to base our analysis on mRNA levels from skeletal muscle issue so that we could address whether alterations to myokine mRNA expression were the results of the exercise bout and not due to potential effects of the biopsy procedure. Furthermore, by utilizing mRNA expression from the muscle tissue, we can rule out the possibility that the inflammatory markers are coming from some other distant source—a possibility that we could not rule out if we observe serum concentrations of the myokines.

In conclusion, we investigated the gene expression changes in 20 healthy lean males in response to an acute exercise bout and discovered large increases in IL-6, IL-8, LIF, and CTGF gene expressions with a decrease in myostatin gene expression. Additionally, we found some associations between myokine expression with certain markers of physical fitness, lipid and glycogen muscle metabolism, mitochondrial function, and body composition. We also examined the expression of myokines in myotubes from active and sedentary donors both basally and after pharmacologically induced myotube contraction using the PFI treatment representing an *in vitro* exercise mimetic. We found differential expression of myokines from active and sedentary myotubes, particularly with myostatin expression. These data offer insights into the potential functions of exercise-induced myokine expression.

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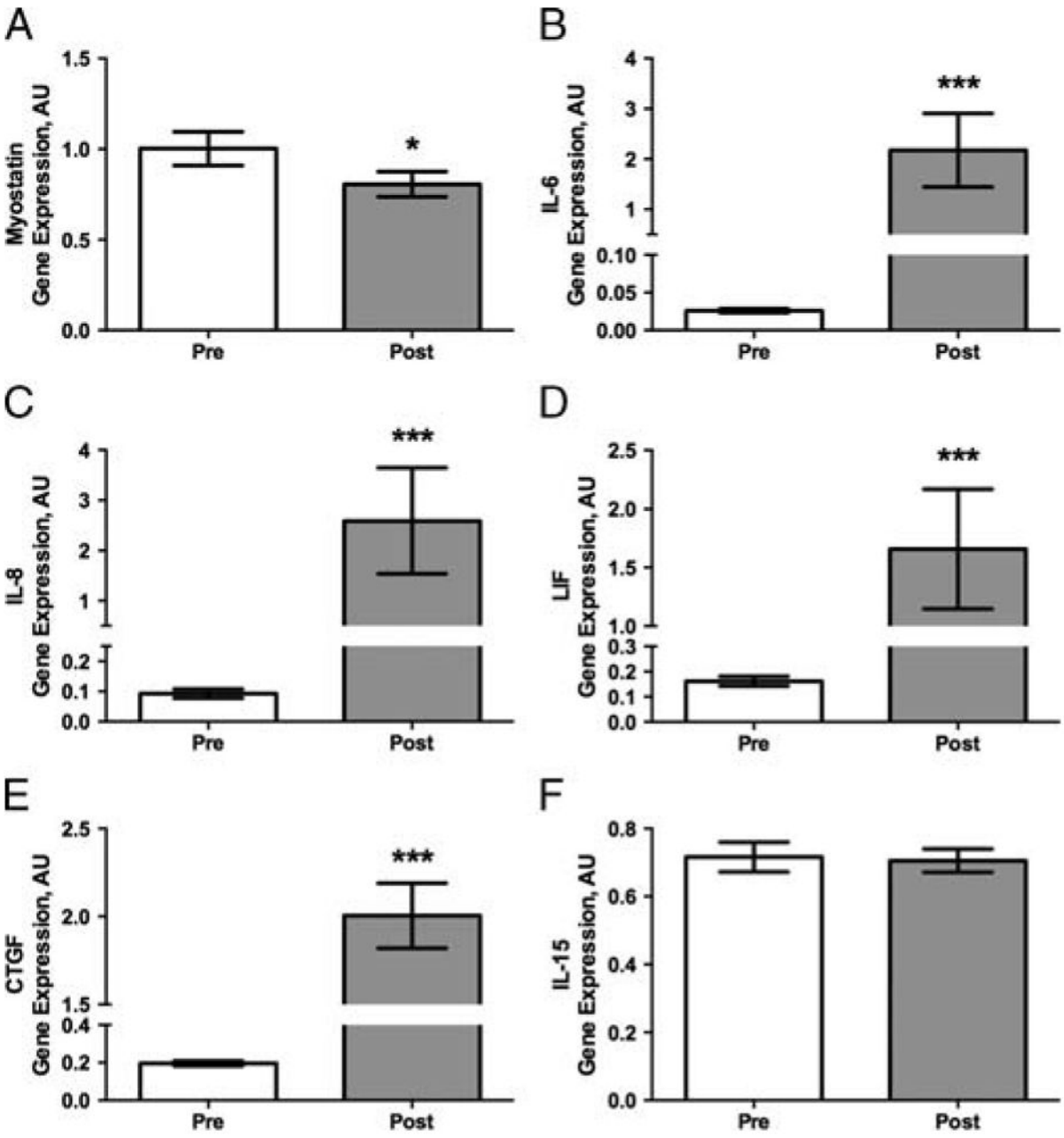

FIGURE 1.

Myokine gene expression in the *vastus lateralis* before and after the endurance exercise bout. A, Myostatin gene expression decreased on average 20% with endurance exercise. B, IL-6 increased eightfold with endurance exercise. C, IL-8 increased 25-fold with endurance exercise. D, LIF mRNA increased approximately 8.5-fold from preexercise levels. E, CTGF increased 10-fold over preexercise expression levels. F, IL-15 did not significantly change with a single endurance exercise bout. Data represent mean ± SEM. $*P < 0.05$ and $***P < 0.001$.

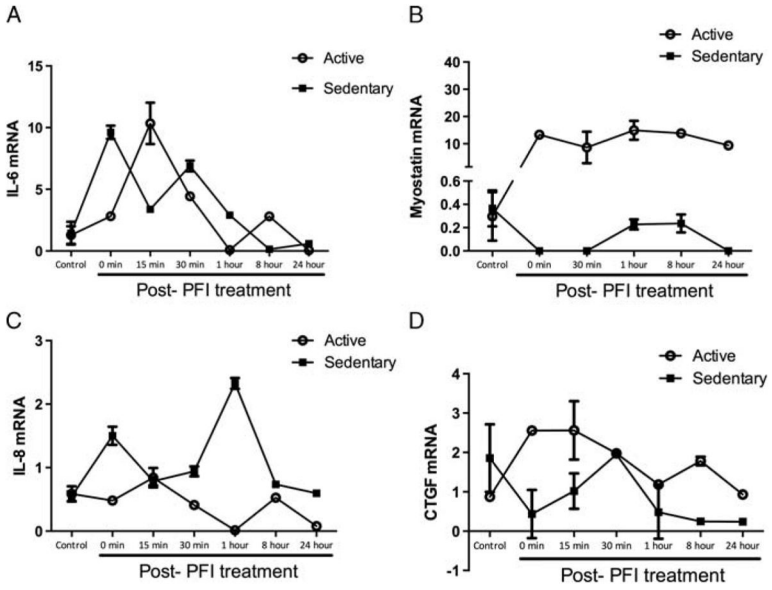

FIGURE 2.

Myokine gene expression in human primary myotubes from active and sedentary donors. Myotubes were treated with the pharmacologic cocktail of 30 KM palmitate, 4 KM forskolin, and 0.5 KM ionomycin (PFI) for 1 h a day for 3 d to stimulate muscle contractions. mRNA was collected across a time course after the last day of PFI treatment. A, IL-6 increased significantly in both active and sedentary donors up until 1 h after PFI treatment, with the active donors increasing above basal condition at the 8-h time point and returning to control conditions by the 24-h time point. B, Myostatin gene expression increased significantly in the active donors with PFI treatment and was maintained up to 24 h post-PFI treatment, whereas sedentary donor myotubes decreased myostatin gene expression immediately after and 30 min after PFI treatment, and again 24 h post-PFI treatment. C, Sedentary myotubes increased expression of IL-8 immediately after and 1 h after PFI treatment. Athletes decreased IL-8 expression significantly at 1 and 24 h post-PFI treatment. D, CTGF expression was significantly elevated in active donors immediately after and 15 min after PFI treatment, whereas sedentary myotubes had a decrease in expression from control levels 8 and 24 h after PFI treatment. Data represent mean T SEM of experiments performed in triplicate.

TABLE 1

Anthropometric and serum characteristics of male participants in endurance exercise study.

	Mean ± SD
Age (yr)	24.0 ± 4.5
Weight (kg)	76.7 ± 6.5
Height (cm)	180.3 T 5.4
BMI ($kg \cdot m^{-2}$)	23.6 ± 1.8
Percent body fat	16.6 ± 3.2
Fat mass (kg)	12.8 ± 3.1
Fat free mass (kg)	63.9 ± 4.7
$\dot{V}O_{2max}$ ($mL \cdot min^{-1} \cdot kg^{-1}$ FFM)	47.2 ± 5.7
Fasting glucose ($mg \cdot dL^{-1}$)	88.0 ± 4.6
Fasting insulin ($mU \cdot dL^{-1}$)	3.6 ± 1.6
HOMA-IR	0.78 ± 0.37
Fasting lactate ($mmol \cdot L^{-1}$)	1.01 ± 0.31
Percentage of Type I fibers	35.9 ± 11.7
Percentage of Type II fibers	64.2 ± 11.7

TABLE 2

Clinical and skeletal muscle parameters before and after the endurance exercise bout.

	Baseline (Mean ± SD)	Postexercise (Mean ± SD)	P
RER	0.95 ± 0.04	0.89 ± 0.03	< 0.001
Palmitate oxidation, *ex vivo* (nmol·h^{-1}·mg^{-1} protein)	615.9 ± 375.9	887.3 ± 404.3	0.01
Pyruvate oxidation, ex vivo (nmol·h^{-1}·mg^{-1} protein)	1153.0 ± 767.8	1840.0 ± 990.0	0.02
IMCL content (AU)	27.7 ± 27.5	21.3 ± 19.4	0.21
Glycogen content (AU)	8.40 ± 0.79	7.32 ± 0.68	0.001
Serum lactate (mmol·L^{-1})	1.01 ± 0.31	2.61 ± 0.79	<0.001

TABLE 3

Correlational matrix between myokine mRNA expression and metabolic/ anthropometric characteristics.

				Pearson *r*	*P*
Myostatin	BL	Citrate synthase activity (nmol·min^{-1}·mg^{-1} protein)	BL	−0.69	<0.001
	BL	$\dot{V}O_{2max}$ (mL·min^{-1}·kg^{-1} FFM)	BL	−0.64	0.002
	BL	% Type I fibers	BL	−0.55	0.01
	BL	% Type II fibers	BL	0.55	0.01
IL-6	BL	HOMA-IR	BL	0.44	0.05
	BL	RER	BL	0.45	0.04
	BL	Serum lactate (mmol·L^{-1})	BL	0.50	0.02
	Δ	Glycogen content (AU)	Δ	−0.57	0.01
IL-15	BL	Fat mass (kg)	BL	0.43	0.05
	BL	$\dot{V}O_{2max}$ (mL·min^{-1}·kg^{-1} FFM)	BL	−0.47	0.03
	Δ	Total IMCL (AU)	Δ	−0.61	0.007
CTGF	BL	Serum glucose (mg·dL^{-1})	BL	0.52	0.02
	BL	Pyruvate oxidation, *ex vivo* (nmol·h^{-1}·mg^{-1} protein)	Δ	−0.65	0.005
	Δ	Percent fat	BL	0.64	0.003

Data are represented as either the baseline (BL) mRNA expression or the change (Δ) in mRNA expression in participants who participated in the single endurance exercise bout. These data are correlated with either the baseline (BL) or the change (Δ) in the measure of metabolic/anthropometric characteristics of these participants.