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Transcriptional activation of the IL-6 gene in human contracting skeletal muscle: influence of muscle glycogen content

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ABSTRACT

In humans, the plasma interleukin 6 (IL-6) concentration increases dramatically during low-intensity exercise. Measurements across the working limb indicate that skeletal muscle is the source of IL-6 production. To determine whether energy availability influences the regulation of IL-6 expression during prolonged exercise, six male subjects completed two trials consisting of 180 min of two-legged dynamic knee extensor with either normal or low (~60% of control) pre-exercise muscle glycogen levels. Increases in plasma IL-6 during exercise were significantly higher ($P<0.05$) in the low-glycogen (16-fold) trial verses the control (10-fold) trial. Transcriptional activation of the IL-6 gene in skeletal muscle was also higher in the low-glycogen trial; it increased by about 40-fold after 90 min of exercise and about 60-fold after 180 min of exercise. Muscle IL-6 mRNA followed a similar but delayed pattern, increasing by more than 100-fold in the low-glycogen trial and by about 30-fold in the control trial. These data demonstrate that exercise activates transcription of the IL-6 gene in working skeletal muscle, a response that is dramatically enhanced when glycogen levels are low. These findings also support the hypothesis that IL-6 may be produced by contracting myofibers when glycogen levels become critically low as a means of signaling the liver to increase glucose production.

Key words: interleukin 6 • transcription • exercise • physical activity

Interleukins are polypeptide proteins secreted primarily by macrophages and lymphocytes that coordinate the actions of other cells within the immune system in response to injury or infection. Several studies have demonstrated that circulating levels of interleukin-6 (IL-6) also increase dramatically in response to strenuous physical activity (1-7). On the basis of direct correlation with other forms of tissue injury or trauma, IL-6 production in response to exercise

has generally been regarded as a consequence of damage to muscle tissue induced by high-intensity and/or eccentric contractile activity (4, 8, 9). Similar to its role in the immune system, the exercise-induced release of IL-6 is assumed to regulate components of the acute phase response, including acute phase protein synthesis by the liver and glucocorticoid release via stimulation of the hypothalamus-pituitary-adrenal axis (2, 10).

More recent studies, however, have indicated that the production of IL-6 during exercise may not be related to exercise-induced muscle damage. Croisier et al. (7) reported that a single bout of eccentric exercise in untrained subjects elicited a marked increase in plasma IL-6 (~8-fold) that was associated with severe levels of delayed-onset muscle soreness and a greater than 100-fold increase in serum myoglobin, both indicators of muscle damage. However, after subjects completed 3 wk of exercise training, the same bout of eccentric exercise elicited an identical marked increase in IL-6 despite no significant change in perceived soreness or serum myoglobin, which suggests that the production of IL-6 in response to exercise is not a function of damage to contracting myofibers. Steensberg et al. (11) found that circulating IL-6 levels also increase dramatically during low-intensity (40% of a maximum 2-min power output), one-legged knee extensor exercise, a form of exercise that evokes little to no increase in creatine kinase levels. Moreover, on the basis of femoral arteriovenous IL-6 concentration and blood flow measurements, it was found that the net release of IL-6 from the exercising leg could account for the entire increase in circulating plasma IL-6 concentration (11), which suggests that active muscle tissue rather than circulating monocytes (12) is the source of IL-6 production during exercise. Although a number of different cell types present in muscle tissue are capable of cytokine production, IL-6 has been found to be expressed in primary human myoblasts subjected to inflammatory stimuli (13) and in skeletal muscle isolated from rat hindlimb after electrical stimulation (14), providing indirect evidence that contracting myofibers may in fact be the primary source of IL-6 production during exercise.

Although the physiological significance of IL-6 production during exercise is not known, several lines of evidence suggest that IL-6 may be functioning in a hormone-like fashion to help regulate glucose homeostasis. In the study by Steensberg et al. (11), net release of IL-6 from the working muscle was low through 2 h of exercise, after which it rose exponentially to a value nearly 1000-fold above resting levels after 5 h of exercise. Exercise of this duration and intensity results in a progressive decline in muscle glycogen concentration and a gradual increase in the reliance of contracting myofibers on blood glucose and free fatty acids for oxidative metabolism (15). It is interesting that ingesting carbohydrate during exercise significantly attenuates the increase in plasma IL-6 (16). IL-6 has recently been shown to stimulate liver glycogenolysis and gluconeogenesis in primary hepatocytes (17, 18), to activate transcription of the glucose-6-phosphatase gene in mice (19), and to stimulate hepatic glucose output in humans (20, 21). These results raise the intriguing possibility that muscle-derived IL-6 may be released when glycogen levels become critically low as a means of signaling the liver to enhance glucose production (11, 22).

The purpose of the present study was to determine whether exercise activates transcription of the IL-6 gene in skeletal muscle and to determine the potential influence of muscle glycogen content on the IL-6 response to prolonged exercise in humans. Specifically, we tested the hypothesis

that activation of the IL-6 gene and rise in plasma IL-6 concentration are enhanced and/or accelerated during exercise when pre-exercise muscle glycogen levels are reduced.

MATERIALS AND METHODS

Subjects and experimental design

Six healthy, physically active but untrained male subjects (mean age 26 years [range 22-33 years], mean weight 78.1 kg [range 70-93 kg], mean height 1.87 m [range 1.75-1.93 m]) were recruited to participate in the study. The subjects were given both oral and written information about the experimental procedures before they gave their written informed consent. The study was approved by the Copenhagen and Frederiksberg Ethics Committee, in Denmark, and the Human Investigations Committee of Yale University, in New Haven, CT.

The subjects performed two trials of two-legged knee extensor exercise on a modified Krogh cycle ergometer (23) in a randomized order and separated by at least 2 wk. The trials were identical in design with the exception that leg muscle glycogen content was either normal (control trial) or reduced by about 40% (low-glycogen trial) prior to exercise. One week before the first trial, a two-legged knee extensor exercise test was performed to determine the maximal work load for each subject. The resistance load was increased every 2 min until a cadence of 60 extensions/min could no longer be maintained. The highest work load that the subjects could maintain for 2 min was set as the maximum work load.

On the afternoon before each trial, the subjects completed 60 min of cycling exercise at a work load equivalent to about 70% of their maximal work load. To further lower whole body glycogen stores (liver and upper body muscles), the subjects completed an additional 60 min of two-arm cycling exercise. After this glycogen depletion exercise protocol, subjects consumed either a low-carbohydrate (low-glycogen trial) or a high-carbohydrate (normal-glycogen trial) meal in isocaloric amounts. The subjects reported to the laboratory the following day after an overnight fast. Catheters were placed in the femoral vein of one leg and a muscle sample (pre-exercise) was obtained from the middle portion of the vastus lateralis muscle by use of the percutaneous needle biopsy technique with suction. The subjects performed the two-legged knee extensor exercise at about 60% of their maximum 2-min work load for 180 min. Blood samples were drawn into precooled tubes containing EDTA at 0, 30, 60, 90, 120, and 180 min of exercise. The tubes were mixed and immediately centrifuged at 2200*g* for 15 min at 4°C. The plasma was stored at –80°C until analysis. Additional muscle biopsy samples were taken after 30, 90, and 180 min of exercise. Biopsy samples were immediately placed on an ice-cold glass plate, cleaned of connective tissue and blood, weighed, and separated for nuclei isolation (120-130 mg) or quick frozen in liquid N_2 (20-40 mg) for RNA and glycogen determination.

Isolation of nuclei and transcription analysis

Nuclei were isolated from muscle biopsy samples as previously described (24). Fresh muscle tissue was placed in 35 ml of ice-cold buffer A (15 mM HEPES, 60 mM KCl, 3 mg/ml bovine serum albumin [BSA], 300 mM sucrose, 5 mM each of EDTA and EGTA, 1 mM dithiothreitol [DTT], 0.5 mM spermidine, 0.15 mM spermine, 2 μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride), thoroughly minced, rotated for 5 min at 4°C, and gently

homogenized for 20 s (setting 15, Kinematica Polytron PT2100, Kinematica AG, Lucerne, Switzerland). Samples were allowed to settle on ice for 5 min and then were centrifuged at 700*g* for 10 min at 4°C. The crude nuclear pellets were gently resuspended in 10 ml of buffer B (15 mM HEPES, 60 mM KCl, 3 mg/ml BSA, 300 mM sucrose, 0.1 mM each of EDTA and EGTA, 0.5 % Triton X-100, 1 mM DTT, 0.5 mM spermidine, 0.15 mM spermine, 2 μg/ml leupeptin, and 2 μg/ml aprotinin) and filtered through prewetted cheesecloth. Nuclei were repelleted (700*g*, 10 min, 4°C), gently resuspended in 10 ml of buffer C (15 mM HEPES, 60 mM KCl, 5 mM magnesium acetate [$MgAc^-$], 3 mg/ml BSA, 300 mM sucrose, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 0.5 mM spermidine, 0.15 mM spermine, 2 μg/ml leupeptin, and 2 μg/ml aprotinin) and repelleted (700*g*, 10 min, 4°C). Final nuclear pellets were resuspended in 200 μl of storage buffer (75 mM HEPES, 60 mM KCl, 15 mM NaCl, 5 mM $MgAc^-$, 0.1 mM EDTA, 0.1 mM EGTA, 40 % glycerol, 1 mM DTT, 0.5 mM spermidine, 0.15 mM spermine, 2 μg/ml leupeptin, and 2 μg/ml aprotinin), quick frozen in liquid N_2, and stored at –80°C.

Relative transcription of genes was determined by a reverse transcriptase-polymerase chain reaction (RT-PCR)-based nuclear run-on technique as previously described (24, 25). Briefly, incomplete transcripts were allowed to proceed to completion in the presence of nonradioactive nucleotides CTP, GTP, UTP, and ATP. After thorough digestion of both genomic DNA and protein, nascent RNA transcripts were isolated by extraction (TRIzol, Invitrogen, Carlsbad, CA) and precipitation and were resuspended overnight (4°C) in 22 μl of 10 mM Tris (pH 8.0)/0.1mM EDTA. Nascent RNA transcripts (18 μl) were converted to a double strand by using the Superscript II RNase H^- Reverse Transcriptase (Invitrogen) in a reaction volume of 30 μl according to the manufacturer's instructions and then were diluted to 50 μl. Relative β-actin transcription was determined for samples within a given set (subject) by amplifying 2.5 μl (in duplicate) from each sample with human-specific primers (forward primer 5'-CCCAAGGCCAACCGCGAGAAGAT-3'; reverse primer 5'-GTCCCGGCCAGCCAGGTCCAG-3'), established reaction conditions [1.5 mM $MgCl_2$, (24)], and the following cycle profile: 94°C for 2 min (94°C for 30 s, 61°C for 50 s, 72°C for 50 s) × 10 followed by (94°C for 30 s, 61°C for 50 s, 72°C for 50 s + 20 s extension/cycle) × 12-15 cycles. PCR products were separated by gel (2.5% agarose) electrophoresis, stained with ethidium bromide, visualized by ultraviolet exposure with a CCD integrating camera (Gel Doc, Bio-Rad Laboratories, Hercules, CA), and quantified under nonsaturating conditions using analysis software (Molecular Analyst, Bio-Rad). To account for differences in overall transcriptional activity among samples (primarily reflecting variations in original nuclei content), final dilution volumes (average 150 μl) were adjusted on the basis of relative β-actin transcript content. Relative transcription of the IL-6 gene was determined with primers (forward primer 5'-AAAGAGGCA CTGGCAGAAAACAAC-3'; reverse primer 5'-TTAAAGCTGCGCAGAATGAGATGA-3') designed from the human IL-6 gene sequence (GeneBank No. M54894), yielding a product of 353 bp. PCR reaction conditions for IL-6 included a 10-μl sample (4× normal), 1.0 mM primers (2× normal), 2.0 mM $MgCl_2$, and a "touchdown" PCR cycle profile of 94°C for 2 min (94°C for 30 s, 59°C [annealing temperature + 2°C] for 15 min, 72°C for 50 s) × 10 followed by (94°C for 15 s, 57°C for 5 min, 72°C for 50 s) × 10 and (94°C for 15 s, 57°C for 50 s, 72°C for 50 s + 20 s extension/cycle) × 15. IL-6 PCR products were quantified as described above and expressed as relative to β-actin.

RNA analysis

Total RNA was isolated from 12-35 mg of muscle tissue by a modified guanidinium thiocyanate phenol-chloroform extraction method adapted from Chomczynski and Sacchi (26) as previously described (24). Final RNA pellets were dissolved in 0.1 mM EDTA (2 μl/mg original wet weight). Reverse transcription reactions were carried out on 22 μl of sample using the Superscript II RNase H^- Reverse Transcriptase (Invitrogen) in a reaction volume of 40 μl. All samples were diluted with 160 μl of nuclease-free water. The β-actin mRNA content was determined (in duplicate) by PCR amplification of 2.5 μl of RT product (14 cycles, reaction profile described above). Final dilution volumes of all samples within a given set (subject) were normalized to β-actin mRNA and set to an average of 400 μl. The IL-6 mRNA content was determined with 2.5 μl of diluted sample by PCR using the following cycle profile: 94°C for 2 min, (94°C for 30 s, 57°C for 50 s, 72°C for 50 s) × 10 followed by (94°C for 30 s, 57°C for 50 s, 72°C for 50 s + 20 s extension/cycle) × 17 cycles.

Determination of plasma IL-6

To determine IL-6 content in samples, ELISA kits from R & D Systems (Minneapolis, MN) were used. This kit does not distinguish between soluble and receptor-bound IL-6 and therefore gives a measure of the total IL-6 content in the sample.

Determination of glycogen

Frozen muscle samples (10-20 mg) were freeze-dried, dissected free of connective tissue, weighed, and hydrolyzed in 1 M HCl. Glycogen concentrations were determined by a standard enzymatic technique with fluorometric detection (27)

Statistical analysis

All data are expressed as means ± SE. Two-way ANOVA for repeated measures was used to evaluate the effect of exercise (time) and trial (control vs. low glycogen). Specific differences across time within a given trial and between trials at a given time were located by a Student-Newman-Keuls post-hoc test. Differences were considered significant at $P < 0.05$.

RESULTS

Glycogen concentration

Pre-exercise muscle glycogen concentration averaged 398 ± 56 μmol/g dry weight in the control trial and 240 ± 42 in the low-glycogen trial, a difference ($P<0.05$) of about 40% (Fig. 1). Despite a slightly higher rate of glycogen utilization in the control trial during exercise, this difference in glycogen concentration between trials persisted through the first 90 min of exercise. However by the end of exercise (180 min), glycogen concentrations were reduced to similar levels, averaging 153 ± 55 and 101 ± 48 μmol/g dry weight in the control and low-glycogen trials, respectively.

IL-6 concentration

The plasma concentration of IL-6 was similar in the two trials before exercise, averaging 0.6 ± 0.2 ng/L in the control trial and 0.7 ± 0.1 ng/L in the low-glycogen trial. Exercise induced a progressive rise in plasma IL-6 concentration in both trials; however, the increase occurred earlier and was much greater during the low-glycogen trial (Fig. 2). After 120 min of exercise, plasma IL-6 concentrations were more than two times higher in the low-glycogen trial (8.3 ± 1.9 ng/L) than in the control trial (3.8 ± 1.1 ng/L) and remained significantly higher throughout the end of exercise (at 180 min: 10.1 ± 1.3 vs. 6.3 ± 0.7 ng/L, respectively).

IL-6 transcription and mRNA content

To determine whether the sharp increase in plasma IL-6 concentration during exercise is mediated by activation of the IL-6 gene in muscle, an RT-PCR-based nuclear run-on procedure was performed with nuclei isolated from skeletal muscle biopsy samples. Consistent with the low pre-exercise plasma IL-6 concentrations, transcription of the IL-6 gene was nearly undetectable before exercise and was not different in the control and low-glycogen trials (Fig. 3). In the control trial, exercise induced an increase in transcription of the IL-6 gene, although the responses were variable among subjects. In contrast, IL-6 transcription was elevated in all subjects in the low-glycogen trial. After 90 and 180 min of exercise, the average IL-6 transcription was significantly higher in the low-glycogen trial as compared with the control trial. In agreement with the transcription data, IL-6 mRNA content was significantly higher after 180 min of exercise in the low-glycogen trial as compared with the control trial (Fig. 4).

DISCUSSION

The results from the present study demonstrate that prolonged exercise activates transcription of the IL-6 gene in skeletal muscle of humans, a response that is dramatically enhanced when muscle glycogen concentrations are low. Based on measurements of net IL-6 release across both the working and the nonworking limbs during one-legged knee extensor exercise, Steensberg et al. (11) recently established that contracting skeletal muscle tissue is the source of IL-6 production during prolonged exercise. The findings in the present study provide further evidence that induction of IL-6 expression in contracting myofibers may be largely responsible for the dramatic rise in circulating IL-6 concentration during prolonged exercise and that muscle glycogen concentration may be a critical determinant regulating the IL-6 response to exercise.

The acute phase response to exercise is characterized by elevations of a number of circulating proinflammatory and anti-inflammatory cytokines as well as several naturally occurring cytokine inhibitors (2). This integrated cytokine response has generally been considered to be induced by damage to specific contracting myofibers (4, 8, 9), similar to the acute phase response associated with other types of tissue injury or trauma. Indeed, in a direct comparison of short-duration (30 min), high-intensity concentric versus eccentric exercise, Bruunsgaard et al. (9) found that plasma IL-6 levels increased fivefold only in response to eccentric exercise. Elevations in circulating IL-6 were significantly correlated with increases in plasma creatine kinase in the days after exercise, which supported the notion that cytokine production may be related to the degree

of muscle damage occurring during the exercise (9). However, in more recent studies, significant increases in plasma IL-6 have been detected after as little as 6 min of high-intensity rowing (28) and 30 min of treadmill running, even in well-trained athletes (5). Even more striking is that plasma IL-6 concentrations continue to rise dramatically (10- to 25-fold) as exercise duration extends from 3 to 5 h (11), reaching levels comparable to those seen with severe infections (29). Exercise of this type (concentric, low intensity, and long duration) elicits only slight elevations in plasma creatine kinase during the subsequent 24-48 h of recovery, which suggests that production of IL-6 is not due to exercise-induced damage to skeletal muscle (7).

The source of IL-6 production during exercise is of obvious interest and importance. Previous work has established that IL-6 mRNA is markedly increased in human skeletal muscle after strenuous exercise (4, 11) and in rat hindlimb muscle after electrically induced contractile activity (14). Direct evidence that contracting skeletal muscle is the major source of IL-6 production comes from the recent study by Steensberg et al. (11) in which femoral arterial and venous IL-6 concentrations and leg blood flow were determined in subjects performing one-legged low-intensity knee extensor exercise for 5 h. Net IL-6 release was detected only across the exercising leg, increasing by about 1000-fold over the course of the 5 h and accounting for the entire corresponding 19-fold increase in arterial plasma IL-6 concentration. We used a similar exercise model in the present study, and our findings clearly demonstrate that the induction of IL-6 in exercising human skeletal muscle is mediated by transcriptional activation of the IL-6 gene and, collectively with previous data (11), suggest that contracting skeletal muscle tissue is the sole source of IL-6 production during exercise. It is conceivable that any of the many different cell types within skeletal muscle (i.e., myofibers, satellite, neuronal, endothelial, smooth muscle, and fibroblast) could be the source of IL-6. Although neutrophils and lymphocytes are mobilized into the circulation during exercise, no change in IL-6 production is detected in mononuclear cells isolated from blood after exercise (4, 12). Although definitive identification of the cell type(s) responsible for IL-6 production during exercise will require immunolocalization studies, primary cultures of human myoblasts have been shown to actively express and secrete IL-6 in response to inflammatory stimuli (13), providing at least indirect evidence that contracting myofibers may be a primary source of IL-6 production during exercise.

A second major finding from the present study was that lowering muscle glycogen content prior to exercise enhanced the induction of IL-6 transcription and mRNA in exercising skeletal muscle. When exercise is performed at a very low intensity, the net release of IL-6 across the working muscle is fairly minimal during the initial 2-3 h of exercise but increases dramatically as exercise duration extends from 3 to 5 h (11). The fact that this latter period of exercise is also characterized by low muscle glycogen content and a decreased reliance on glycogenolysis led to the hypothesis addressed in the present study that activation of the IL-6 gene during exercise may be sensitive to muscle glycogen content. To test this hypothesis, subjects completed identical exercise and glycogen depletion protocols on the day preceding each trial, either with or without carbohydrate replacement. The resulting 40% difference in glycogen content between trials before exercise (Fig. 1) was associated with an overall 40% greater increase in plasma IL-6 concentration (Fig. 2). One possibility is that differences between trials in blood-borne substrates and/or hormones may have accounted for differences in the induction of IL-6. However, this possibility was recently excluded in a related study in which subjects performed

two-legged exercise with only one leg depleted of glycogen prior to exercise. Both legs performed the same amount of work and were exposed to the same arterial substrate and hormonal milieu, yet IL-6 mRNA and protein increased to a greater extent in the glycogen-depleted leg (A. Steensberg, M. Febbraio, T. Osada, P. Schjerling, G. van Hall, B. Saltin, and B. Pedersen, unpublished data). Thus, these findings suggest that glycogen content rather than circulatory factors per se may be a critical factor in triggering the activation of IL-6 expression in muscle and the consequent increase in plasma IL-6 concentration during exercise.

The mechanism by which glycogen content may influence gene expression remains open to speculation but may be a function of the extensive signaling and regulatory mechanisms exerted over glycogen metabolism. A number of enzymes with key roles in regulating glycogen synthesis and breakdown are bound to regulatory proteins that target the enzymes to glycogen particles in response to different intracellular signals or cellular conditions (30). For example, protein phosphatase 1 ($PP\text{-}1_G$) catalyzes the dephosphorylation and activation of glycogen synthase, the rate-limiting enzyme controlling glycogen synthesis. The $PP\text{-}1_G$ holoenzyme exists in vivo as a complex with a catalytic subunit ($PP\text{-}1_C$) bound to one of three targeting subunits specific for glycogen particles (G_M, PTG , U5). However, $PP\text{-}1_C$ also associates with a number of other regulatory subunits that target the enzyme to various other subcellular locations, including the sarcoplasmic reticulum, the myofibrils, and the nucleus. Thus, it is reasonable to speculate that proteins such as PP-1 that are normally bound to the glycogen scaffold may be liberated as glycogenolysis proceeds during exercise and therefore free to interact with other targeting proteins, some of which may lead to the transcriptional regulation of specific genes.

The physiological importance of IL-6 has perhaps been most well characterized with respect to liver regeneration. Following hepatic injury or partial hepatectomy (removal of two-thirds of the liver), gluconeogenic and acute phase response genes are rapidly activated to maintain whole body metabolic homeostasis and promote tissue repair (31). IL-6 is considered an essential component of this process, in that normal liver regeneration and repair after partial hepatectomy are severely compromised in IL-6 knockout mice (−/−) but can be completely restored by injection of recombinant IL-6 (31, 32). In cultured hepatocytes, IL-6 stimulates glycogenolysis by simultaneously increasing glycogen phosphorylase activity and inhibiting glycogen synthase activity (18). IL-6 also mediates the transcriptional activation of the glucose-6-phosphatase gene through a signaling system involving hepatocyte nuclear factor 1 (HNF-1)/STAT3/AP-1 interaction with the HNF-1 DNA binding site (19). Consistent with these data from cultured hepatocytes, infusion of recombinant human IL-6 in humans has been shown to increase hepatic glucose output (20, 21). Thus, there is strong evidence indicating that IL-6 stimulates glucose production in liver. The results from the present study clearly demonstrate that the exercise-induced activation of IL-6 expression in muscle and consequent rise in plasma IL-6 concentration are inversely related to the glycogen content within the active muscle. Taken together, these findings support the hypothesis proposed by Steensberg et al. (11) that production of IL-6 by contracting skeletal muscle may serve, in a counterregulatory hormone-like manner, to enhance hepatic glucose production in an effort to support the increased reliance of skeletal muscle on blood glucose during prolonged exercise.

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Fig. 1

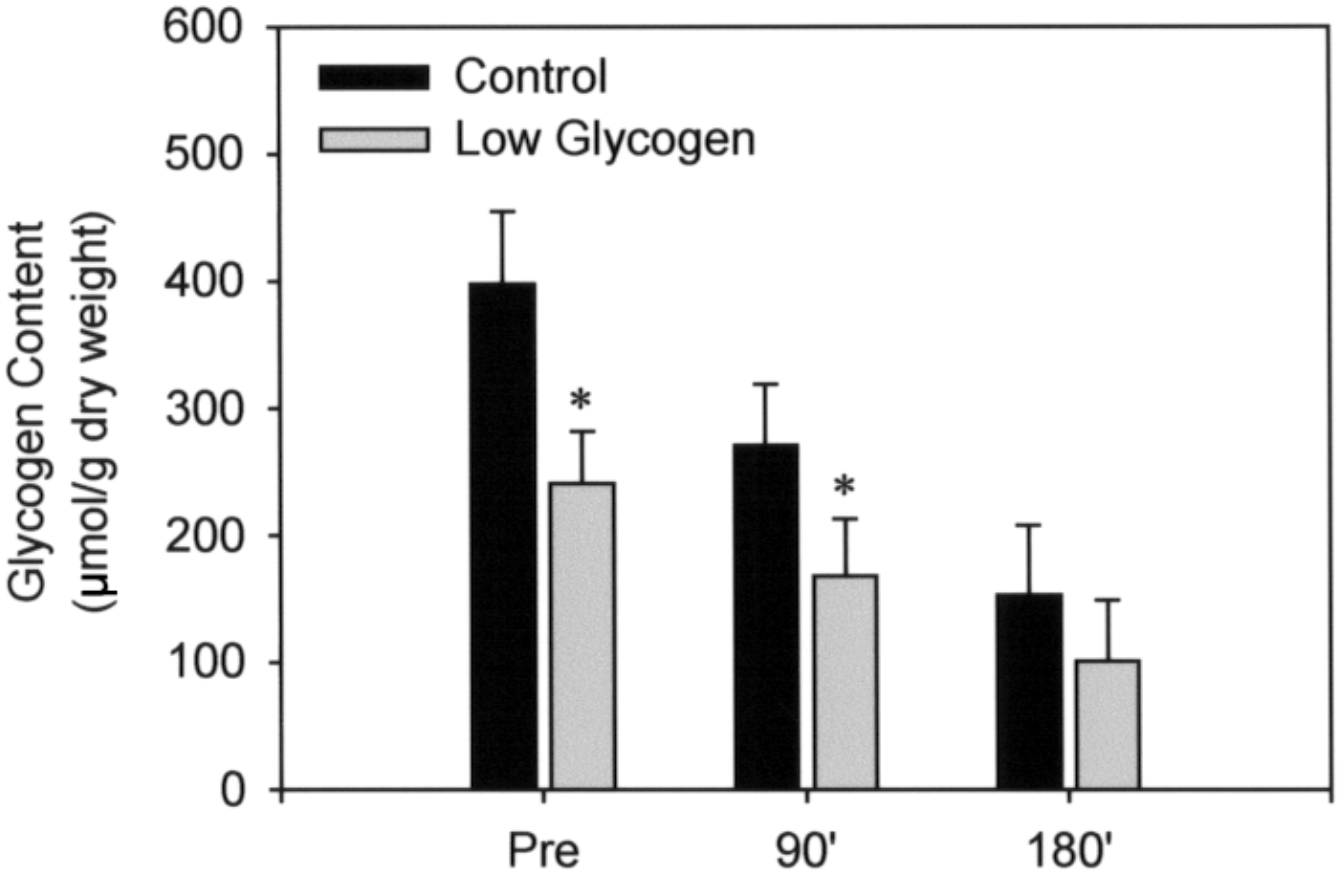

Figure 1. Muscle glycogen content before (Pre) and after 90 and 180 min of a two-legged knee extensor exercise performed with normal (control, dark bars) or low (gray bars) pre-exercise levels of muscle glycogen. Data are presented as means ± SE. *Significantly (P<0.05) different from control.

Fig. 2

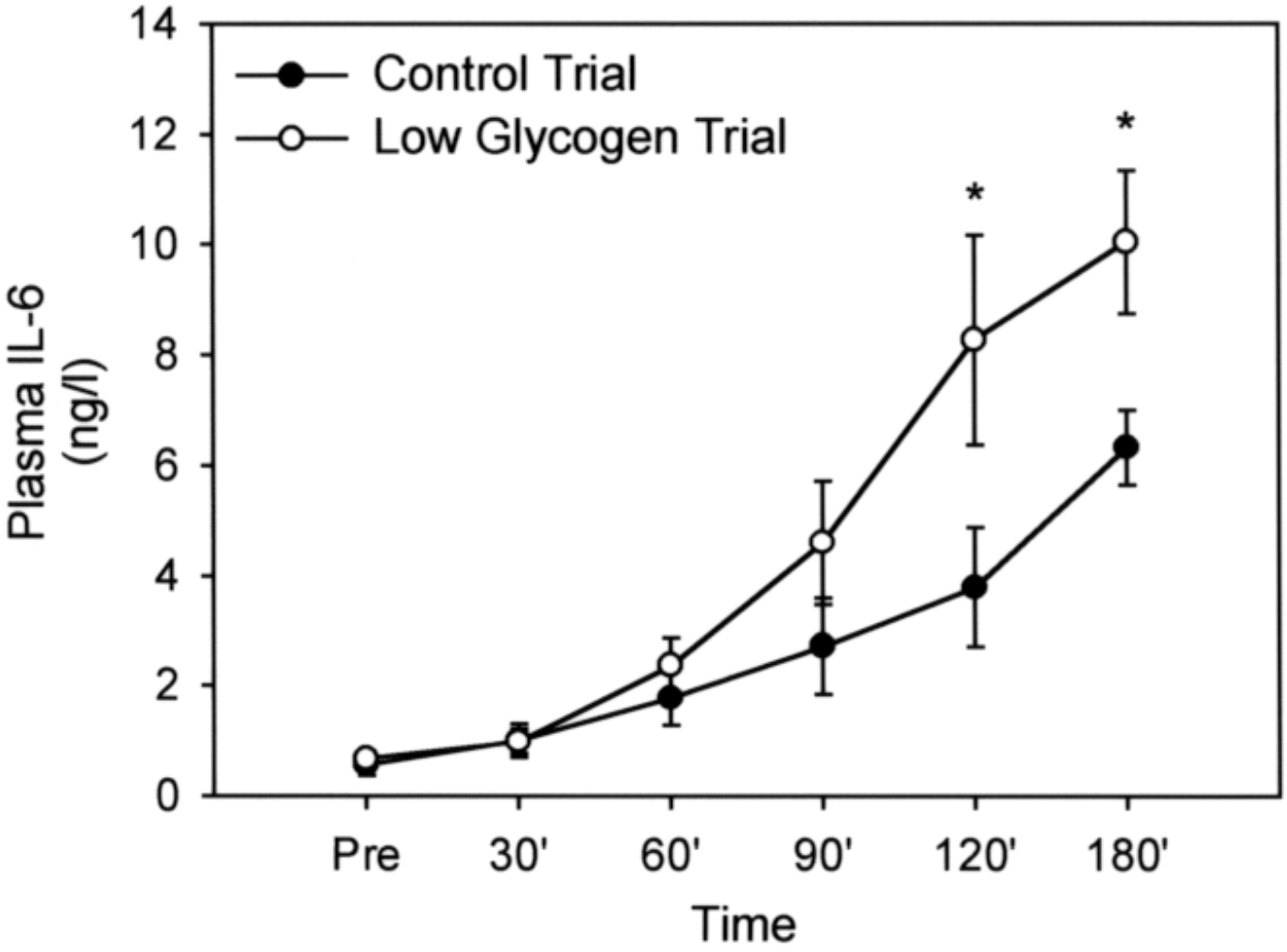

Figure 2. Plasma IL-6 concentration before (Pre) and during 180 min of a two-legged knee extensor exercise performed with normal (control, filled circles) or low (open circles) pre-exercise levels of muscle glycogen. Data are presented as means ± SE. *Significantly (P<0.05) different from control.

Fig. 3

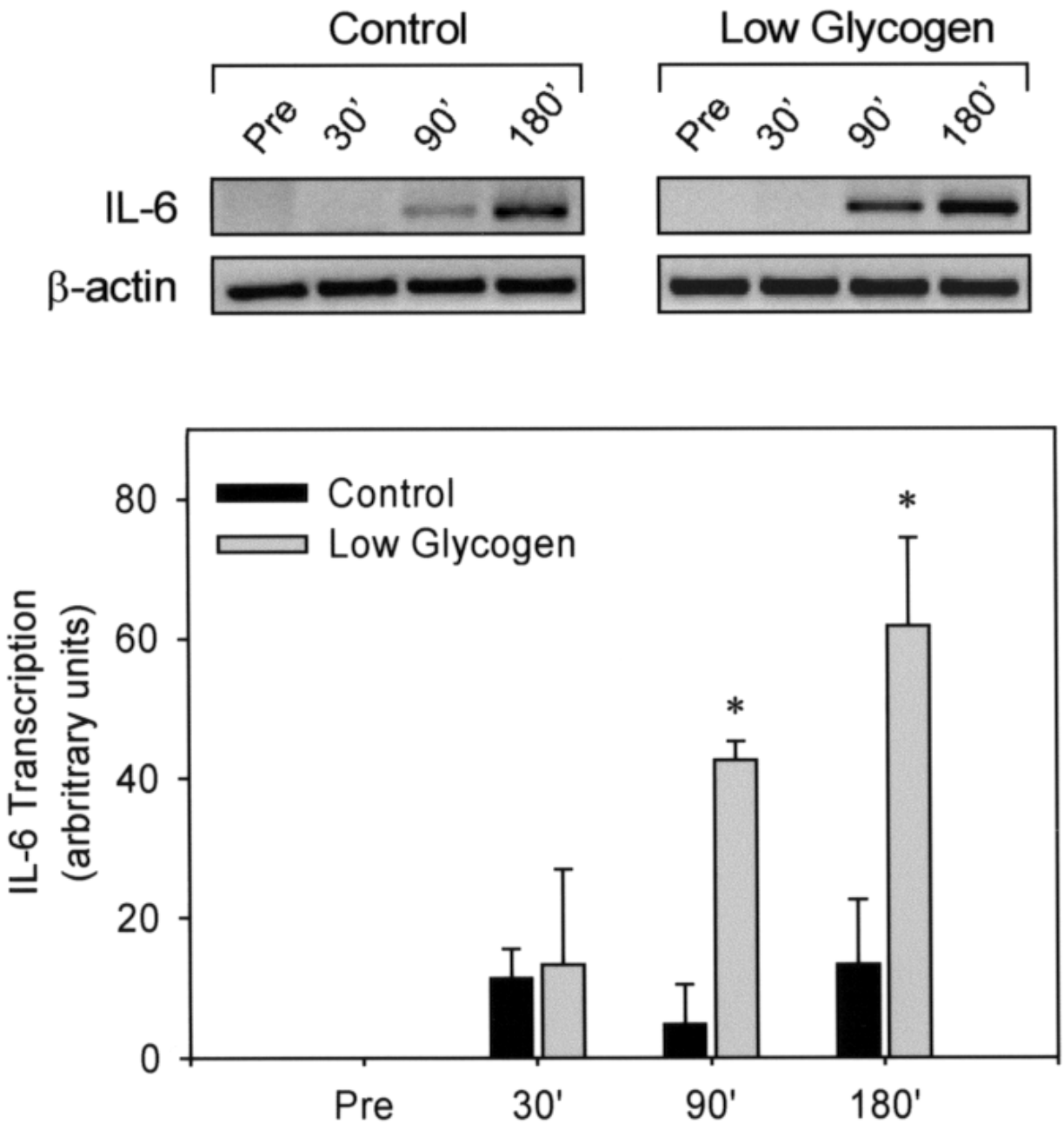

Figure 3. Muscle IL-6 transcription before (Pre) and after 30, 90, and 180 min of a two-legged knee extensor exercise with normal or low pre-exercise levels of muscle glycogen. Nuclei were isolated from biopsy samples of the vastus lateralis muscle at the indicated time points and were subjected to an RT-PCR-based nuclear run-on analysis. The top panels show representative negative images of PCR products (stained with ethidium bromide) for the IL-6 and β-actin genes, and the graph at the bottom shows the data (mean ± SE) for IL-6 transcription (relative to β-actin) during exercise with normal (control, dark bars) or low (gray bars) pre-exercise levels of muscle glycogen. *Significantly (P<0.05) different from control.

Fig. 4

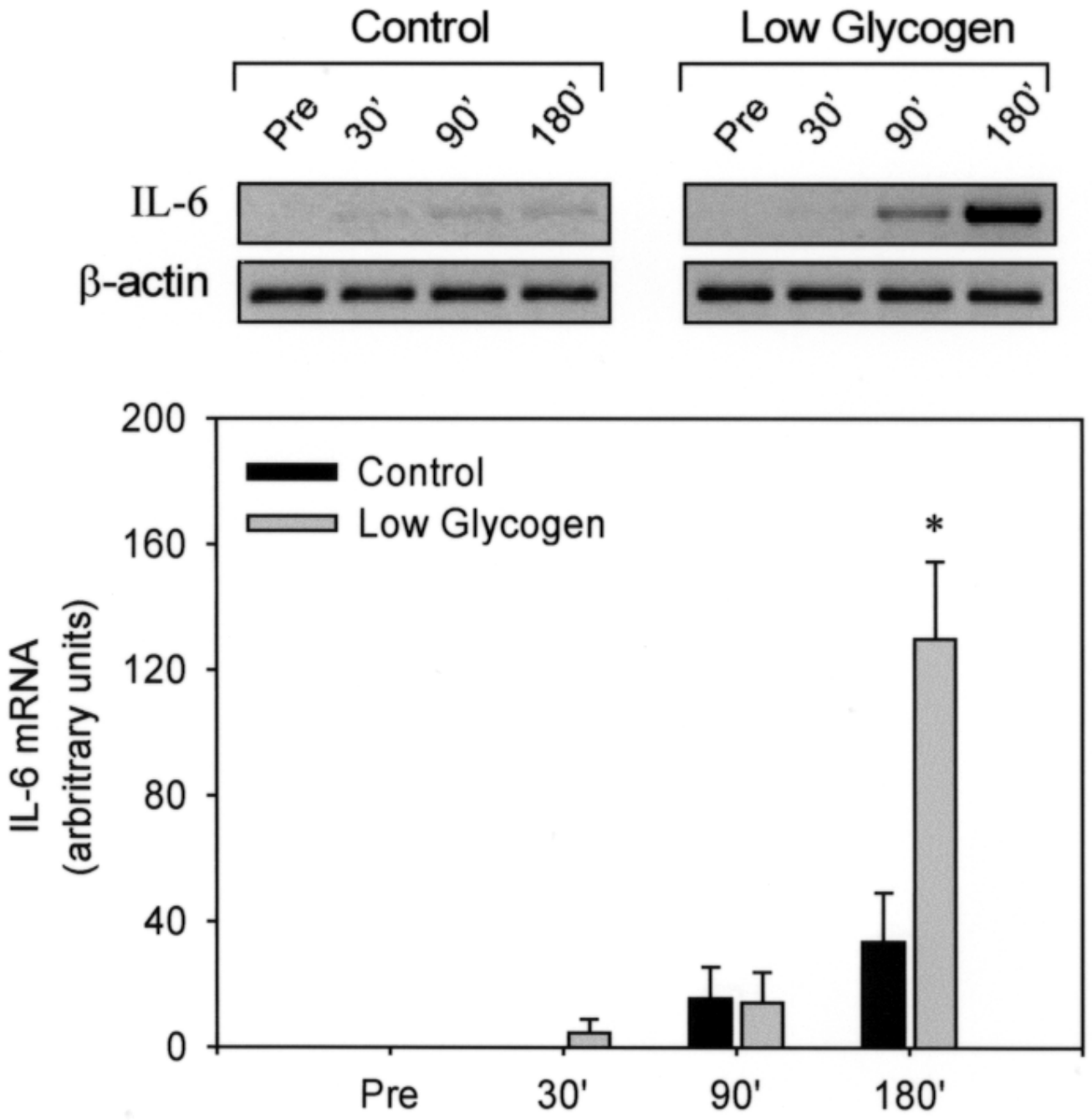

Figure 4. IL-6 mRNA content before (Pre) and after 30, 90, and 180 min of a two-legged knee extensor exercise with normal or low pre-exercise levels of muscle glycogen. Total RNA was isolated from biopsy samples of the vastus lateralis muscle at the indicated time points and subjected to RT-PCR. The top panels show representative negative images of PCR products (stained with ethidium bromide) for the IL-6 and β-actin genes, and the graph at the bottom shows the data (mean ± SE) for IL-6 mRNA (relative to β-actin) during exercise with normal (control, dark bars) or low (gray bars) pre-exercise levels of muscle glycogen. *Significantly (P<0.05) different from control.