Effect of exercise, training, and glycogen availability on IL-6 receptor expression in human skeletal muscle

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The cytokine interleukin-6 (IL-6) exerts its actions via the IL-6 receptor (IL-6R) in conjunction with the ubiquitously expressed gp130 receptor. IL-6 is tightly regulated in response to exercise, being affected by factors such as exercise intensity and duration, as well as energy availability. Although the IL-6 response to exercise has been extensively studied, little is known about the regulation of the IL-6R response. In the present study, we aimed to investigate the effect of exercise, training, and glycogen availability, factors known to affect IL-6, on the regulation of gene expression of the IL-6R in human skeletal muscle. Human subjects performed either 10 wk of training with an acute exercise bout before and after the training period, or a low-glycogen vs. normal-glycogen acute exercise trial. The IL-6R mRNA response was evaluated in both trials. In response to acute exercise, an increase in IL-6R mRNA levels was observed. Neither training nor intramuscular glycogen levels had an effect on the IL-6R mRNA response to exercise. However, after 10 wk of training, the skeletal muscle expressed a higher mRNA level of IL-6R compared with before training. The present study demonstrated that the IL-6R gene expression levels in skeletal muscle are increased in response to acute exercise, a response that is very well conserved, being affected by neither training status nor intramuscular glycogen levels, as opposed to IL-6. However, after the training period, IL-6R mRNA production was increased in skeletal muscle, suggesting a sensitization of skeletal muscle to IL-6 at rest.

interleukin-6 receptor

cytokines exert their actions via specific receptors, which by themselves can be regulated via local and systemic factors. One of these cytokine and receptor systems is the IL-6/IL-6 receptor (IL-6R) system. IL-6 has various roles in immune function, but it is also produced in response to acute exercise, where it serves a metabolic role. Thus an infusion of IL-6 results in increased lipolysis and oxidation rate (32) and increases hepatic glucose production during exercise (6). IL-6 also increases the insulin-stimulated glucose uptake in myocytes, indicating an autocrine role on the skeletal muscle (2).

The expression of IL-6 is tightly regulated during an acute exercise bout. Its regulation in skeletal muscle depends on exercise intensity and duration, as well as energy availability such as intramuscular glycogen and blood glucose levels (7, 14, 18–22, 31). The skeletal muscle fibers can produce both IL-6 and the IL-6R, and an acute bout of exercise upregulates the expression of both in human skeletal muscle fibers (5, 16), providing further complexity to the regulation of the IL-6 response to exercise. Thus the regulation of the IL-6R locally in skeletal muscle may be as important as the regulation of IL-6 itself, rendering skeletal muscle more or less responsive to the circulating IL-6 levels. Although much research has been devoted to the regulation of IL-6 in response to exercise, little information is currently available on the regulation of the IL-6R in skeletal muscle.

The IL-6R exists both in a soluble and a membrane-bound form. Both forms of the receptor lack an intracellular signaling domain and thus colocalize with the gp130 receptor to convey an intracellular signal. The gp130 receptor is ubiquitously expressed, whereas the IL-6R has restricted expression (30). Association of IL-6 with the soluble IL-6R in plasma can thus render almost all cells responsive to IL-6 via trans signaling (26). The gp130 receptor serves as a signaling conveyor for the entire IL-6 family, including IL-11, oncostatin M, leukemia inhibitory factor and ciliary neurotrophic factor, and cardiotrophin-1 and cardiotrophin-like cytokine (11), differing between cytokines in the binding ratios between the individual cytokines, cytokine receptors, and gp130. Thus IL-6 binds with 2:2:2 ratios between IL-6, the IL-6R, and gp130 (23). The signaling pathway involves activation of the JAK-STAT pathway and subsequently SOCS-1 and SOCS-3 (25). Also the MAPK and the PI3K pathways are activated by binding of IL-6 to its receptor (25). However, whether these are the signaling pathways for IL-6-mediated lipolysis, fat oxidation, and glucose mobilization is currently unknown.

In a recent study, our laboratory found that training attenuates the IL-6 response to an acute bout of exercise in human skeletal muscle (8). This finding is consistent with the fact that low muscle glycogen is a stimulus for muscle-derived IL-6 production and that muscle glycogen is enhanced in skeletal muscle in response to training.

The diminished IL-6 production after training may be compensated for by increased production of the IL-6R, thus rendering the muscle more responsive to the IL-6 produced. The aim of the present study was twofold: first, we aimed to investigate the IL-6R gene expression to both acute exercise and a 10-wk training period. We hypothesized that the skeletal muscle would increase the production of IL-6R mRNA, which may result in increased IL-6R protein expression, suggesting a

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sensitization of the muscle to IL-6. Second, given that the IL-6 gene regulation is highly susceptible to alterations in energy and training status (7, 8, 14), we aimed to investigate the IL-6R mRNA response to glycogen depletion during exercise, as this is a major cost stimulus for IL-6 in response to exercise. We hypothesized that the IL-6R mRNA would be increased during a low-glycogen (LG) trial compared with a control trial during acute exercise, as there is an increased need for substrate uptake and IL-6 signaling when local energy supplies are sparse.

METHODS

All subjects were given both oral and written information about the experimental procedures before giving their written, informed consent. The study was approved by the Copenhagen and Frederiksberg Ethics Committee, Denmark.

Exercise performance tests. One week before the onset of the two studies, a knee extensor exercise performance test was done to determine maximal workload for each subject. After a short warm-up on the knee extensor exercise ergometer, the resistance load was increased every 2 min until a cadence of 60 extensions per minute could no longer be maintained. The highest workload a subject could maintain for 2 min was set as the maximal workload ($W_{\text{max}}$).

Training study. This study has been described previously (8). In short, seven untrained male subjects (26 ± 1 yr, 1.80 ± 0.02 m, 86 ± 9 kg) participated in a 10-wk training program consisting of 1 h of knee extensor exercise with modified Krogh ergometer at 75% of their individual $W_{\text{max}}$. This workload was increased by 5–10% every fortnight, to compensate for increased working capacity. Subjects trained for 1 h five times a week. All training sessions were performed after an overnight fast. Before and after the onset of the training program, an acute exercise bout was performed, consisting of 3 h of two-legged knee extensor exercise at 50% of $W_{\text{max}}$. On both occasions, subjects reported to the lab at 0700 after an overnight fast. A catheter was placed in a femoral artery of one leg, and blood samples were collected preexercise, postexercise, and 2 h into the recovery phase. Skeletal muscle tissue biopsies were obtained from the vastus lateralis muscle by percutaneous needle biopsies with a 2-h recovery period. Skeletal muscle biopsies were obtained from the vastus lateralis muscle by percutaneous needle biopsies with suction at the same time points as blood sampling.

Acute exercise study. This study has been described previously (14). In short, six untrained male subjects (mean age 26 ± 2 yr, mean height 1.87 ± 0.03 m, mean weight 78 ± 3 kg) participated in the study. All subjects performed two trials of two-legged knee extensor exercise in randomized order, separated by at least 2 wk. The experiments had normal intramuscular glycogen levels (control trial), and in the other trial their intramuscular glycogen levels had been lowered by 40% (LG trial), as previously described (14). The glycogen-depletion protocol consisted of 1 h of bicycling followed by 1 h of arm cranking the evening before the experiment. Arm cranking was added to avoid refilling of glycogen stores in skeletal muscle from endogenous hepatic glucose production. The subjects were administered either a low-carbohydrate or high-carbohydrate isocaloric diet, to maintain or reverse the glycogen depletion until the following day. On the experimental days, subjects reported to the lab at 0700 after an overnight fast and performed 3 h of knee extensor exercise at 55–60% of their individual $W_{\text{max}}$. A catheter was placed in a femoral artery of one leg, and blood samples were collected preexercise, postexercise, and after a 2-h recovery period. Skeletal muscle biopsies were obtained from the vastus lateralis muscle by percutaneous needle biopsies with suction at the same time points as blood sampling.

Subjects fasted throughout the three exercise bouts but were allowed to drink water ad libitum.

RNA extraction. Skeletal muscle tissue RNA extraction was performed with Trizol (Invitrogen) according to the manufacturer’s directions. In short, 20–30 mg of skeletal muscle were dissolved in 1 ml of Trizol and homogenized on a Brinkman Polytron (version PT 2100) on setting 26. The aqueous phase was transferred to a fresh tube, and 100 μl of isosamyl/chloroform were added and vigorously shaken. Samples were allowed to sit for 2–3 min and spun at 13,000 rpm for 15 min at 4°C, after which the upper aqueous phase was transferred to a new tube. The aqueous phase was mixed with 0.5 ml of isopropanol, and samples were placed in the freezer for 1 h. Samples were centrifuged at 13,000 rpm for 15 min at 4°C, and the resulting pellet was washed with 0.5 ml of 75% ethanol in diethyl pyrocarbonate-treated water. After centrifugation at 8,000 rpm for 10 min, pellets were redissolved in 15 μl of diethyl pyrocarbonate-treated water and allowed to dissolve on ice, after which samples were ready for reverse transcription.

Reverse transcription. One microgram of total RNA was reverse transcribed using random hexamers from the Applied Biosystems Taqman RT-Kit.

Real-time PCR. IL-6R gene expression was analyzed using semi-quantitative real-time PCR with 18S rRNA as the internal reference gene. The IL-6R primers and probe used had the following sequence: forward primer: 5’-AAGACCCCCACTCTCTGGAAC-3’; reverse primer: 5’-CGTGGATGACAGAGTGTGC-3’; probe: 5’-FAM-ACCATCCATGGTGAATGTCTTTGACCG-TAMRA-3’. Predesigned, primer-limited assay reagents were used for 18s mRNA determination. All PCR reagents were obtained from Applied Biosystems.

A reagent mixture of 35 μl was made up for each sample with 1 × MasterMix, 900-nM IL-6R forward primer, 300-nM reverse primer, 200-nm IL-6R probe, and 50–100 ng of sample and made up to a final volume of 35 μl with water. 18s mix (primers and probe) was run in triplicate in 10–20 ng of sample. Each sample was run in triplicate in a reaction volume of 10 μl for 50 cycles by using standard real-time PCR cycling conditions. Data were analyzed by the standard curve method (9). For the IL-6R, a slope of –3.58 and an $R^2$ value of 1.0 were obtained. The corresponding 18s values were –3.57 and 0.99 for slope and $R^2$, respectively. The $y$-intercept on the standard curves generated represents the cycle threshold value for 100 ng of sample for IL-6R and 10 ng for 18s, which amounted to a value of 26.6 and 11.8, respectively.

Determination of plasma IL-6R. For protein determination, blood was immediately spun at 2,200 g for 15 min at 4°C and stored at −80°C until analysis. To determine the amount of IL-6R protein in samples, high-sensitivity ELISA kits were used (Quantikine, R&D Systems). This kit detects the total amount of IL-6R and has a sensitivity of 6.5 pg/ml.

Statistics. A two-way repeated-measures ANOVA was used to evaluate the effect of time (preexercise, after 3 h of exercise, and after 2 h of recovery) vs. trial (control vs. low glycogen or pre- vs. posttraining) with a Student-Newman-Keuls post hoc t-test. Results were considered significant if $P < 0.05$. mRNA data were log transformed to obtain normal distribution, and the graphs are thus presented as geometric means with 95% confidence intervals (CI). Protein data are presented as regular means with 95% CI.

RESULTS

Effect of training on IL-6R regulation at rest and during acute exercise. A significant difference between resting values of IL-6R mRNA levels at pretraining and posttraining was observed [0.29 arbitrary units (AU), CI: 0.21–0.39 AU pretraining vs. 0.52 AU, CI: 0.36–0.76 AU posttraining, $P = 0.023$] (Fig. 1). IL-6R levels were inducible both before and after the training period, increasing to the same absolute values both after 3 h of exercise (0.77 AU, CI: 0.59–1.02 AU pretraining and 0.64 AU, CI: 0.44–0.95 AU posttraining, $P = 0.014$) and after 2 h of recovery (0.85 AU, CI: 0.75–0.87 AU pretraining and 1.00 AU, CI: 0.88–1.16 AU posttraining, $P <
however, no changes in IL-6R mRNA levels were observed during exercise between trials. No significant changes in IL-6R were observed at the plasma level (Table 1).

Effect of muscle glycogen depletion on IL-6R regulation during acute exercise. A two-way ANOVA demonstrated significant changes over time only ($P < 0.001$), showing no effect of glycogen depletion before exercise (Fig. 2). After 3 h of exercise, IL-6R levels rose in both trials (0.36 AU, CI: 0.26–0.48 AU in the control trial and 0.33–0.63 AU in the LG trial, $P = 0.019$) and again reached the same levels in the two trials after 2 h of recovery (0.66 AU, CI: 0.52–0.86 AU in the control trial and 0.81 AU, CI: 0.54–1.28 AU in the LG trial, $P < 0.001$) (Fig. 2).

No significant changes in IL-6R protein were observed at the plasma level (Table 1).

DISCUSSION

The main finding from the present study was that exercise training increased basal levels of IL-6R mRNA levels in skeletal muscle. A previous study on IL-6R expression in skeletal muscle has demonstrated an increase in IL-6R mRNA upon exercise, which is reflected by an increase in protein levels in the myofibers, as was evidenced by immunohistochemistry (16). Thus the production of IL-6R mRNA in response to exercise seems to result in translation into protein. Together with these data, the present study suggests that skeletal muscle is sensitized to IL-6 after a training period. In addition, we have demonstrated that the acute exercise-induced IL-6R gene expression, in contrast to the IL-6 gene, was influenced by neither intramuscular glycogen levels nor training status of the subjects. IL-6R plasma protein was unaltered in response to acute exercise, indicating that the IL-6R production serves a local role, rather than being shed from myofibers into the circulation. A tendency toward decreased circulating levels of the receptor was observed after the training period. This is in contrast to the increased expression observed in skeletal muscle, further indicating a local role of the IL-6R in skeletal muscle.

The IL-6R mRNA response to muscle contraction seems very consistent and is not affected by factors such as training status or energy availability, in contrast to that of IL-6, which is highly susceptible to such stimuli (7, 8, 14, 15). Both IL-6 and the IL-6R are thus increased by acute exercise (16, 21, 22), but regulation of the IL-6 response with regard to energy availability lies predominantly within the regulation of IL-6 itself, rather than at the receptor gene expression level. This indicates that the induction patterns of IL-6 and its receptor are probably not governed by the same factors. IL-6 is inducible by calcium and low glycogen (12, 14), but knowledge in the area of induction factors of the IL-6R is scarce. In hepatocytes, the IL-6R is increased time and dose dependently at the mRNA level by stimulation with dexamethasone (28), whereas IL-6 production and release is decreased (3, 24, 27), demonstrating differential regulation patterns of IL-6 and its receptor. Upon exercise, cortisol levels increase, a response that could play a role in exercise-induced muscle IL-6R expression. Furthermore, infusion of IL-6 into human subjects results in increased IL-6R expression (16), an effect that is also observed in addition.
cultured hepatocytes (1), indicating that several induction pathways for the IL-6R exist.

In response to prolonged training, there is an adaptive acute exercise response of IL-6, resulting in decreased skeletal muscle IL-6 expression during acute exercise, despite a higher actual workload after the training period (8). The decreased IL-6 expression after training could be due to either an increased capacity to oxidize substrates or an increased sensitization to IL-6. The present study supports the idea that the decreased IL-6 response to exercise in skeletal muscle after prolonged training is not due to increased expression of the IL-6R during exercise. Rather, as regular exercise training results in increased expression of mitochondria proteins and metabolic key enzymes increasing oxidative capacity, such as hexokinase II, PGC-1α, citrate synthase, and 3-hydroxyacyl-CoA dehydrogenase (10), and increased ability to oxidize intramuscular triglycerides (13), the skeletal muscle becomes less dependent on extramuscular energy stores. Thus it seems plausible that the increased ability to oxidize intramuscular triglycerides after a training period renders the muscle less dependent on the hormonal IL-6 response and subsequent energy mobilization from liver and adipose tissue (6, 32).

We have previously demonstrated effects of IL-6 on liver and adipose tissue, yet the direct effects of IL-6 on skeletal muscle are not fully clarified. The myocyte expresses the IL-6R, and during an acute bout of exercise this expression further increases. Given that IL-6 increases metabolite release into the circulation to be taken up by the working muscle, it would seem plausible if IL-6 could convey some effects in skeletal muscle to facilitate the use of these metabolites. A correlation between IL-6 and AMPK has been observed in human contracting skeletal muscle (4), and IL-6 has been demonstrated to increase the AMP kinase activity (17), an energy master switch in skeletal muscle (29), leading to generation of ATP via increased import of free fatty acids into the mitochondria and subsequent oxidation (33). In addition, glucose uptake in L6 myocytes is increased upon stimulation with IL-6 (2). Thus IL-6 can exert metabolic effects on skeletal muscle in exercise situations, as supported by the increase in IL-6R gene expression in skeletal muscle in response to exercise as demonstrated here.

In summary, we have demonstrated that an acute bout of exercise increases IL-6R gene expression in skeletal muscle, a response that is independent of glycogen availability and training status. Furthermore, we have demonstrated that prolonged training elevates basal IL-6R mRNA levels, suggesting a sensitization of skeletal muscle to IL-6 at rest.

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