Increase in interstitial interleukin-6 of human skeletal muscle with repetitive low-force exercise

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IN RECENT YEARS, THE INTERLEUKIN (IL)-6 response to intense or prolonged exercise has been characterized as well as its possible biological role. Increases in plasma IL-6 were found in response to intense exercise (13, 20), and it was suggested that exercise-induced increase in plasma IL-6 is due to muscle damage from intense eccentric contractions (3). However, increases were also found during nondamaging intense exercise (2, 27). It has been demonstrated that IL-6 is produced by muscle tissue in response to exercise and that local production in the exercising muscles can account for the observed increase in plasma IL-6 (17, 27). A recent study (8) applying the microdialysis technique to connective tissue demonstrated that this tissue may also contribute to the plasma elevations in IL-6 during intense exercise. There seems to be a time delay for the exercise increase in IL-6 in the blood during exercise under normal conditions. For example, during combined concentric and eccentric contractions, plasma IL-6 was significantly increased after 30 min (16), and in a concentric, one-legged exercise model, IL-6 was significantly increased after 3 h (27). However, it is unknown whether this time delay is due to a reduced initial release rate or to a delayed production. Recently, a rapid IL-6 release was demonstrated after 10 min of exercise when the subjects were exercising in a glycogen-depleted state (11). IL-6 has several possible biological effects. Recent data suggest that IL-6 should be classified as an anti-inflammatory cytokine due to its inhibitory effect on low-grade inflammation via suppressing tumor necrosis factor (TNF)-α. TNF-α is thought to play a central role in diseases associated with low-grade inflammation such as cardiovascular disease and Type 2 diabetes (12). In a human in vivo model, exercise-induced increases in IL-6 as well as infusion of recombinant human IL-6 were shown to blunt endotoxin-induced increases in TNF-α (25), and thus it has been suggested that IL-6 mediates the beneficial effects of exercise on diseases associated with low-grade inflammation (19, 25). IL-6 is also speculated to work in hormone-like fashion, exerting metabolic control by increasing energy supply during exercise (22, 26). It has been shown that IL-6 inhibits glycogen synthase activity and facilitates glycogen phosphorylase activity (6) and that IL-6 gene transcription rates increase rapidly during exercise when the muscle glycogen content is low (7). Furthermore, IL-6 induces lipolysis in humans (10), and in support of the importance of this metabolic effect, it has been shown that IL-6-deficient mice develop mature-onset obesity (32). Thus IL-6 has been substantiated as an important metabolic cytokine released from muscle tissue during intense exercise.

During low-force exercise, systemic changes in markers of metabolism are rarely detected. However, our group recently described increased lactate and pyruvate levels locally in upper extremity muscle tissue measured by microdialysis, without simultaneous systemic changes, in response to a repetitive, low-force exercise task (23). Therefore, we speculated that IL-6 may be released concomitantly due to its metabolic effects. The microdialysis technique offers a unique opportunity for in vivo measurement of the time pattern for cytokine release locally in muscular tissue during exercise. On this background, the aim of the present study was to evaluate the local and systemic IL-6 response to a repetitive, low-force exercise task in humans. Our hypothesis is that the IL-6 response to 20 min of repetitive low-force exercise...

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concentration, as during intense exercise, is increased locally in muscle tissue in response to repetitive low-force exercise.

Because the microdialysis technique is used to study the local muscle IL-6 response to exercise, a small trauma is introduced during insertion of the microdialysis catheters. Therefore, a control study characterizing the cytokine response to the insertion trauma alone was also conducted.

MATERIALS AND METHODS

Participants. Six healthy men with an average age of 31 yr (range of 26–40 yr), height of 180 cm (range of 176–184 cm), and weight of 81.5 kg (range of 75–89 kg) participated in the control study. Six healthy men with an average age of 30 yr (range of 28–33 yr), height of 180 cm (range of 173–188 cm), and weight of 81.5 kg (range of 68–89 kg) participated in the exercise study. Three participants took part in both studies. All participants were without known muscle disorders, and there was no significant difference in participant characteristics between studies. Data from the exercise study have been reported previously (23). The participants were instructed not to perform any shoulder- or neck-straining exercise for 48 h before the study, except for ordinary daily working activities. The participants were all students or had sedentary office jobs and did not take any medication. None of the participants experienced discomfort or tenderness in the upper body region during the week before the study. All participants gave their informed, written consent, and the study conformed to The Declaration of Helsinki and was approved by the Ethical Committee of Copenhagen (KF 01-152/01).

Experimental protocol. In both studies, the participants reported to the laboratory at 0900 after an overnight fast. During the studies, the participants were only allowed to drink water.

In the control study, the participants had a microdialysis catheter inserted into the upper trapezius muscle (see below) after which they rested while sitting upright for 120 min. The control study was conducted to investigate the local muscle IL-6 responses to the minimal trauma caused by inserting a microdialysis catheter. Microdialysis was sampled over 10-min intervals during rest, and IL-6 was measured at the following time points: 5 min (representing the collection interval 0–10 min), 25 min (20–30 min), 65 min (60–70 min), and 105 min (100–110 min).

In the exercise study, a microdialysis catheter was inserted into the upper trapezius muscle on the dominant side and a venous catheter into the antecubital vein of the nondominant arm, after which the participants rested while sitting upright for 60 min. The resting period was followed by a 20-min repetitive, low-force exercise period performed with the dominant arm and hand. Short wooden sticks (12 cm, 23 g) were moved back and forth between standardized positions 30 cm apart on a giant pegboard at a frequency of 1 Hz, indicated by an electronic metronome (Zen-on music, Tokyo, Japan). The participants performed the exercise in a seated position with the pegboard placed 30 cm in front of them, measured from the elbow with the upper arm hanging vertically and the elbow in a 90° flexion. The exercise load was characterized by electromyography measurement and was found to be 8–9% maximal electromyography, which has been reported elsewhere (23). After exercise, the participants rested for 30 min (recovery). IL-6 was measured at the following time points: during rest at 5 min (representing the collection interval 0–10 min), 25 min (20–30 min), and 55 min (50–60 min); during exercise at 65 min (60–70 min) and 75 min (70–80 min); and during recovery at 85 min (80–90 min) and 105 min (100–110 min).

Microdialysis and calculations. Microdialysis was performed following the principles described by Lönnroth and coworkers (9). A custom-made microdialysis catheter (membrane length: 30 mm; molecular cutoff: 3,000 kDa) was inserted with ultrasound guidance into the upper trapezius muscle at a standard anatomic point on the dominant side. The catheter was placed in the trapezius muscle parallel to the muscle fibers, verified by ultrasonography. The insertion point was located on the center of the descending part of the trapezius muscle, midway between the processus spinosus of the seventh cervical vertebra and the lateral end of acromion. The skin and the subcutaneous tissues where the catheter entered and exited the trapezius muscle were anesthetized with a local injection (0.2–0.5 ml) of Xylocaine (20 mg/ml) without epinephrine, and care was taken not to anesthetize the underlying muscle. The distance between the entrance and exit sites of the catheters in the skin was ~7 cm with at least 5 cm of the catheter in the trapezius muscle, ensuring that the entire 30-mm membrane was within the muscle. The catheters were constructed and were sterilized by ethylene oxide before use as previously described (8). The microdialysis catheter was perfused via a high-precision syringe pump (CMA 100; Carnegie Medicine, Solna, Sweden) at a rate of 5 μl/min with a Ringer acetate solution (Phar- macia & Upjohn, Copenhagen, Denmark) containing 3 mM glucose and 0.5 mM lactate. Then, 0.25 μg/ml 3H labeled-human type IV collagen (130 kDa; specific activity of 5.9 MBq/mg; NEN, Boston, MA) was added to the perfusate to mimic the in vivo relative recovery (RR) of IL-6 as previously described (8), because no radioactive-labeled IL-6 was commercially available. The distal exteriorized tip of the microdialysis catheter was placed in a 200-μl micropip refill for dialysis collection. Dialysate collection was timed to adjust for the transition time of the dialysate in the nonpermeable outlet part of the catheter. The sampling periods were 10 min. To ensure that the actual flow within the catheter was 5 μl/min, each microvial was weighed before and immediately after each collection on a high-precision electronic weight (Sartorius BP 211 D, Bie & Berntsen, Copenhagen, Denmark). Deviation from the intended dialysate volume by more than ±15% would result in discarding the sample. No samples were discarded in the present studies. Dialysate samples were collected, and the samples were immediately frozen and stored at –80°C until the analyses were performed.

To determine the RR of each sample, 3 μl of dialysate was pipetted into a counting vial, 3 ml of scintillation fluid (High-flash Point LSC Cocktail UCH maGold Packard Bioscience, Gronningen, The Netherlands) was added, and the samples were counted in a beta counter. RR was calculated for each microdialysis catheter as RR = (cpmD − cpmP)/cpmP, where cpmD and cpmP were counts/min in the perfusate and the dialysate, respectively. It was assumed that the RR from the interstitial fluid to the perfusate of an unlabeled metabolite equals relative loss from the perfusate to the interstitial fluid of a labeled metabolite. The interstitial concentrations were calculated using the internal reference calibration method (24) equaling (C4 – C0)/RR + C4, where C4 was dialysate concentration and C0 was perfuse concentration.

Blood samples. In the exercise study, a polyethylene catheter was inserted into the antecubital vein of the nondominant control arm, and blood was sampled in glass tubes containing EDTA before insertion of the microdialysis catheters as well as before, during, and after the repetitive low-force exercise period. The blood samples were immediately iced and centrifuged at 3,000 rpm for 15 min at 4°C, and plasma was stored at –80°C until analysis.

Chemical analysis. IL-6 was measured by a high-sensitivity Quanti- tine assay (R&D systems, Minneapolis, MN). To meet the minimum sample volume requirements of the IL-6 assay, dialysate samples were diluted 100-fold with calibrator diluent, whereas plasma samples were measured undiluted. The lowest calibration standard was used as the detection limit. Hence, the detection limit for cytokine in the dialysate was 15.6 pg/ml for IL-6 when adjusting for the dilution factor. If the measured concentration was less than the level of detection, one-half of that value (8 pg/ml) was used as the result. Accuracy of the assay was checked by spiking buffer and a low sample in duplicate with IL-6 international standard (NIBSC 89/548). The measured recovery of the spike did not differ substantially between the two sample types. The average RR (mean ± SD) was 93 ± 9% (n = 4).

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Statistics. Data are presented as means ± SE. The IL-6 data were log-transformed to serve the criteria of a normal distribution of the residuals in the statistical model. One-way ANOVA for repeated measures approached by general linear modeling was used to test for time effect during the two studies. If the ANOVA test revealed significant changes, post hoc analyses by Tukey’s multiple comparison test were used to compare specific pairs of means. To examine whether IL-6 concentrations and participant characteristics differed between the two studies, the independent sample $t$-test was used (SPSS standard version 11.0). A probability of $<0.05$ (2 tailed) was accepted as criteria for significance.

RESULTS

The interstitial muscle IL-6 concentration was below the detection level ($<15.6$ pg/ml) in 11 of 12 participants (control study + exercise study) in the first microdialysis sample (5 min). In the control study, interstitial muscle IL-6 increased in response to the trauma caused by inserting a microdialysis catheter into the trapezius muscle. The initial value (mean ± SE) was 8 ± 0 pg/ml, which increased after 65 min to 359 ± 171 pg/ml ($P < 0.001$; Fig 1), but the subsequent increase to 484 ± 202 pg/ml after 110 min was not significant compared with the 65-min value ($P = 0.694$). In the exercise study, interstitial muscle IL-6 also increased over time and reached a value of 289 ± 128 pg/ml at 55 min of rest (Fig 1). In response to the repetitive low-force exercise, IL-6 further increased, reaching a mean of 1,246 ± 461 pg/ml during the last 10 min of the 20-min exercise period. Of note is that IL-6 levels continued to increase after the cessation of exercise and reached the highest level of 2,132 ± 477 pg/ml during the last 10 min of the 30-min recovery (Fig 1). The increase in IL-6 during and after exercise (75, 85, 105 min) was highly significant compared with the preexercise level at 55 min ($P < 0.0001$; Fig 1) and also significantly higher than the IL-6 concentrations reached in the control study at corresponding times relative to insertion of the microdialysis catheter (all $P < 0.05$). In contrast, although the average plasma IL-6 numerically increased by 45% over time in the exercise study, this was not statistically significant (Fig 1).

The average RR of the microdialysis catheters (labeled type IV collagen) was found to be 61 ± 3% (control study) and 62 ± 2% (exercise study) without significant difference between either study or periods within each study.

DISCUSSION

The novel findings from the present study are 1) the fast and marked increase in interstitial IL-6 locally in the trapezius muscle during repetitive low-force exercise that is not reflected systemically and 2) the high absolute IL-6 concentrations achieved in the muscle interstitium.

It has previously been shown that IL-6 is produced by skeletal muscle during intense exercise and that overflow from muscle to plasma can account for the exercise-induced increase in plasma IL-6 (17). However, it has recently been speculated that release from connective tissue may also contribute to the increase in plasma IL-6 during intense exercise (8). The increasing number of studies on the IL-6 response to exercise has focused on intense or prolonged exercise. The only exception to our knowledge is a study investigating the local and systemic production of cytokines in response to a 10-min, moderate-intensity, unilateral wrist-flexion exercise (14). The method used was venous effluent blood from the exercising arm (referred to as local) and venous blood from the contralateral arm (systemic). The study showed no change in IL-6 during exercise but a small increase of ~2 pg/ml during the postexercise period in venous blood from both arms. The authors suggested that the increase in IL-6 was due to a systemic response rather than a local response (14). The increase in systemic IL-6 during the moderate-intensity exercise protocol is comparable to the numerical systemic increase found in the present study (4 pg/ml), although our study did not reach statistical significance. It is noteworthy that, in the above-mentioned study (14), actual muscle IL-6 levels were not determined, and one limitation of the used method is that venous effluent blood only reflects local IL-6 levels if the IL-6 is released from the local tissue to the blood.

Biological effects of IL-6. The biological role of IL-6 is not fully elucidated, but it is speculated that IL-6 released from muscle to blood during exercise is acting in a hormonelike fashion exerting metabolic control (22). Supporting this idea, it has been shown that IL-6 has the capability to increase hepatic
glucose release and to induce lipolysis in adipose tissue, thereby increasing the total substrate availability (6, 21, 28, 29). In the present study, circulating IL-6 was not statistically changed, which could suggest that the exercise performed did not necessitate a systemic recruitment of substrates for metabolism. However, since effluent venous blood was not measured, it is not possible to determine whether IL-6 was not released from muscle to blood and/or whether hepatic clearance (4) was sufficiently high to maintain systemic plasma IL-6 at a fairly constant level.

To what extent IL-6 exerts local effects on the exercising muscle itself cannot be proved in this study. However, it has been shown that IL-6 stimulates myoblast proliferation and enhances myoblast differentiation (15), and it has been suggested that IL-6 is a hypertrophying factor released during resistance exercise (31). In contrast to this idea is the notion that IL-6 primarily is released during endurance-type exercise (18) and that transgenic mice overexpressing IL-6 are in a muscle atrophic state due to increased protein catabolism (30). Interestingly, it has also been suggested that IL-6 may potentially function as a local metabolic regulator during exercise, having glucose-releasing and lipolytic properties (22), thus increasing substrate availability during exercise. It is an intriguing idea that IL-6 may exert local metabolic effects during exercise, and we have found some support for this in the previously reported data from the low-force exercise protocol (23). The metabolic activity locally in the trapezius muscle was shown to be relatively high during exercise despite the low force demands. We showed that the interstitial muscle lactate concentration increase by 50% during and after exercise, followed by an increase in pyruvate, indicating an exercise-induced rise in anaerobic metabolism (23). Furthermore, the local changes in metabolism were devoid of a systemic response, perhaps due to the small absolute muscle mass activated. Although it is speculative, the local increase in IL-6 found in the present study may contribute to an acceleration of local muscle glycogenolysis by releasing glucose to meet the increased metabolic demands during exercise. The effect of metabolic demand and metabolic status during exercise on local muscle IL-6 levels has been analyzed in a study by Pedersen and colleagues (7) in which an increase in IL-6 mRNA was found after 30 min of exercise together with a more pronounced increase in the gene transcription rate when the muscle glycogen content was reduced. Recently, it has also been shown that IL-6 is only released from exercising muscle when the muscle is in a glycogen-depleted state (11).

Regular exercise has been shown to be protective against insulin resistance, Type 2 diabetes, and cardiovascular disease (1), and it has been speculated that the anti-inflammatory effect of IL-6 by suppressing low-grade inflammation via TNF-α may be one mechanism by which physical exercise has a positive effect on these diseases (25). The substantial increase in muscle IL-6 demonstrated in the present study could indicate that low- to moderate-intensity physical exercise, utilizing many muscle groups, may have an effect on low-grade inflammation and insulin sensitivity and thereby yield positive health effects.

**Control vs. exercise.** The muscle concentrations of IL-6 during and after exercise were high compared with plasma levels and up to 15- to 20-fold higher than peak plasma levels previously reported during intense exercise (8, 13, 17), and this is the first study to demonstrate an increase in interstitial IL-6 of an upper extremity muscle group in response to low-force exercise.

Because we use the microdialysis technique to study local events, a local trauma is unavoidable when the microdialysis catheter is inserted into the muscle, and thus one of the aims of this investigation was to determine to what extent the insertion trauma affects local IL-6 concentrations. The data demonstrate that the insertion trauma caused a local increase in IL-6 in the muscle, which was significant 65 min after the insertion and remained at approximately the same level 40 min later. In rats, it has previously been shown that the concentration of IL-6 mRNA increases after a blunt trauma (5). In early exercise studies, it was hypothesized that the increase in IL-6 in response to intense exercise was due to muscle damage (3), and the trauma data in the present control and exercise studies support the idea that muscle damage per se results in an IL-6 increase. Because we have used the microdialysis technique to determine the intramuscular IL-6 response to exercise, it cannot be excluded that friction between catheter and the surrounding tissue could create a small additional trauma during exercise; however, the friction is most likely limited because the trapezius muscle is primarily functioning as a stabilizer of the scapula during the hand and arm movement, as well as a stabilizer of the neck, and is thus exerting minimal length changes. This is also demonstrated by the lack of fluctuations and oscillations in trapezius electromyography in the present low-force exercise protocol (23). Furthermore, the increase in the interstitial muscle IL-6 concentration in response to the insertion trauma only accounted for 20–30% of the increase seen at comparable time points during and after exercise, and therefore the major IL-6 contribution was most likely due to the exercise per se.

In conclusion, a substantial increase in interstitial muscle IL-6 was demonstrated in response to repetitive, low-force exercise of the upper extremity that was not reflected systematically. An increase in muscle IL-6 was also demonstrated in response to the insertion trauma, but it only accounted for 20–30% of the exercise-induced increase. Thus muscle IL-6 seems to be highly responsive to exercise, even at low force levels, which may be attributable to local changes in metabolic demands during exercise.

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**GRANTS**

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