Substantial elevation of interleukin-6 concentration in peritendinous tissue, in contrast to muscle, following prolonged exercise in humans

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Plasma interleukin-6 (IL-6) concentration has been shown to increase with exercise and various cell types and tissues have been suggested to be responsible for this increase. At present no studies have measured the interstitial concentration of IL-6 in skeletal muscle and connective tissue. The present study represents the first attempt to simultaneously measure IL-6 in plasma, skeletal muscle and peritendinous connective tissue in response to prolonged exercise. Six healthy well-trained volunteers completed a 36 km run (flat, 12 km h⁻¹). IL-6 was measured before, 2 h post-exercise and 24 h, 48 h, 72 h and 96 h post-exercise in both the medial gastrocnemius muscle (not measured at rest due to risk of disabling the subsequent exercise, and 24 h and 72 h post-exercise) and the peritendinous tissue around the Achilles tendon using microdialysis catheters with a high molecular mass cut-off value (3000 kDa). The plasma concentration of IL-6 was measured simultaneously, and in addition every hour during the exercise, by enzyme-linked immunosorbent assay (ELISA). The plasma concentration of IL-6 was found to increase throughout the exercise, reaching peak values immediately after completion of the run (50-fold increase). Using the microdialysis technique, the interstitial concentration of IL-6 was found to increase dramatically from 0 ± 0 pg ml⁻¹ to 3618 ± 1239 pg ml⁻¹ in the peritendinous tissue in the hours following the exercise. The pattern of changes was similar in plasma and peritendinous tissue, although approximately 100-fold higher in the latter. For comparison the interstitial muscle concentration was found to be 465 ± 176 pg ml⁻¹ when measured 2 h post-exercise and 223 ± 113 pg ml⁻¹ and 198 ± 96 pg ml⁻¹ 48 h and 96 h post-exercise, respectively. The present study demonstrates that the connective tissue around the human Achilles tendon produces significant amounts of IL-6 in response to prolonged physical activity, which might contribute to the exercise-induced increase in IL-6 found in plasma.

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Several studies have demonstrated that strenuous physical exercise in humans is accompanied by an increase in circulating levels of inflammatory cytokines, and that the cytokine that demonstrates by far the largest exercise induced increase is interleukin-6 (IL-6) (Drenth et al. 1995; Nehlsen-Cannarella et al. 1997; Castell et al. 1997; Ostrowski et al. 1998a, 2000; Jeukendrup et al. 2000; Steensberg et al. 2001; Pedersen et al. 2001a, b; Starkie et al. 2001). This finding has recently led several authors to suggest that IL-6 works in a hormone-like fashion during exercise (Pedersen et al. 2001a, b) and that it may play a key role in regulation of glucose homeostasis and adipose tissue lipolysis (Mohamed-Ali et al. 1997; Fried et al. 1998). The primary sources of IL-6 have been suggested to be stimulated monocytes, and endothelial cells (Ostrowski et al. 2000), whereas circulating monocytes have not been found to be the source of elevations in plasma IL-6 after prolonged running (Starkie et al. 2001b). In addition to this, IL-6 is known to be released from fibroblasts (Van Snick, 1990), and has been suggested to be involved in collagen metabolism in bone tissue (Greenfield et al. 1999). With the use of the arterio-venous concentration difference technique, a release of IL-6 from an exercising lower extremity has been demonstrated during prolonged muscular contraction (Steensberg et al. 2000), and the release has been suggested to account for the exercise-induced increase in plasma IL-6 content (Steensberg et al. 2000; Pedersen et al. 2001b). From these findings, and supported by the demonstration of an exercise-induced increase in mRNA for IL-6 from skeletal muscle biopsy homogenates (Ostrowski et al. 1998b) and by in situ hybridization of injured skeletal muscles (Kami & Senba, 1998), skeletal muscle has been suggested to be the primary site of IL-6 formation and release during exercise. However, from the available data it cannot be excluded that other tissues in exercising extremities, such as
to determine interstitial tissue concentrations of the glycosylated protein IL-6 (~27 kDa) simultaneously in both skeletal muscle, in the peritendinous region, and in circulating plasma. We studied tissue and plasma IL-6 concentrations during rest and in response (over 4 days) to prolonged running exercise. The hypothesis was that physical activity will increase tissue concentration in both muscle and peritendon, and that these responses contribute to changes in circulating IL-6 responses to muscular contractions.

METHODS

Six healthy well-trained volunteers (males, aged 30 ± 3 years) participated after obtaining informed consent in the present study, which was approved by the Ethical Committee of Copenhagen ((KF) 01–215/99) and confirmed to the Declaration of Helsinki.

Microdialysis

Four microdialysis catheters with a high molecular mass cut-off value (3000 kDa; membrane length 30 mm; catheter outer diameter 0.05 mm) were positioned with two catheters in the peritendinous area around the Achilles tendon, as previously described (Langberg et al. 1999), and with two catheters in the m. gastrocnemius medialis. In the muscle the catheters were positioned parallel to the muscle fibres by the use of ultrasound guidance to minimize the risk of traumatizing the tissue. Ringer acetate solution (Fresenius Kabl AB, Uppsala, Sweden) was used as perfusate (perfusion rate 5 μl min⁻¹), and 3 nM [³H]human type IV collagen (130 kDa; specific activity 7.0 TBq mg⁻¹; NEN, Boston, USA) was added to the perfusate to mimic the in vivo recovery of IL-6 (molecular mass of 22–27 kDa) using the internal reference method (Scheller & Kolb, 1991; Langberg et al. 1999) as no radioactive labelled IL-6 was commercially available.

Experimental protocol

All experiments were started at 09.00 h. The subjects were told not to perform any kind of exercise, either 24 h prior to the experiment or during the five following days (between measurements), except for ordinary daily working activities (students or sedentary office jobs). The experiment consisted of a resting period of 240 min, an exercising period of 180 min and a recovery period of 240 min following the acute exercise, as well as an additional resting period of 240 min every day for the 4 days following the exercise. The exercise intervention consisted of 36 km of outdoor running. Arterial blood samples were drawn every 30 min during both rest and recovery periods, and every hour during running (during a 1 min stop), as well as 24 h, 48 h, 72 h and 96 h post-exercise. Microdialysis was performed before, after and 24 h, 48 h, 72 h and 96 h post-exercise in the peritendinous tissue around the Achilles tendon and in the medial gastrocnemius muscle 2 h and 48 h, and 96 h post-exercise.

intramuscular- and tendon/ligament-related connective tissue, as well as endothelial tissue in the vasculature, are also important sources of IL-6 release during exercise. It is at present unknown to what extent the interstitial tissue concentration of IL-6 in fibroblast-rich areas like the peritendinous region and/or in skeletal muscle changes during exercise, and what its relative change is compared with alterations in circulating blood. In the present study, it has been possible, with the use of microdialysis catheters with a high molecular mass cut-off value (3000 kDa),

**Table 1. Experimental protocol**

<table>
<thead>
<tr>
<th>Time pre/post exercise (h)</th>
<th>−2</th>
<th>36 km run</th>
<th>2</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
</tr>
</thead>
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<td>Blood sample</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<td>X</td>
</tr>
<tr>
<td>Microdialysis in peritendon</td>
<td>X</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Microdialysis in muscle</td>
<td>X</td>
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<td>X</td>
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<td>X</td>
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</table>

The experiment consisted of a resting period of 240 min, an exercising period of 180 min and a recovery period of 240 min following the acute exercise, as well as an additional resting period of 240 min every day for the 4 days following the exercise. The exercise intervention consisted of 36 km of outdoor running. Arterial blood samples were drawn every 30 min during both rest and recovery periods, and every hour during running (during a 1 min stop), as well as 24 h, 48 h, 72 h and 96 h post-exercise. Microdialysis was performed before, after and 24 h, 48 h, 72 h and 96 h post-exercise in the peritendinous tissue around the Achilles tendon and in the medial gastrocnemius muscle 2 h and 48 h, and 96 h post-exercise.

![IL-6 in serum](image)

**Figure 1. Plasma IL-6 data from six healthy well-trained males measured before, every hour during the 3 h run, immediately after, and during the 4 days following the exercise**

Plasma IL-6 concentration is shown to increase throughout the exercise bout, peaking at the end of the bout. The concentration diminished during the hour following the run and was found to return to pre-exercising level 48 h post-exercise. Data are given as means ± S.E.M.; *P < 0.05 vs. before.
regular flushing with isotonic saline containing heparin (10 U ml⁻¹). Arterial blood samples were drawn every 30 min during both rest and recovery periods, and every hour during running (during a 1 min stop). Haematocrit was determined by the microhaematocrit method. Arterial samples for plasma IL-6 concentration analysis were drawn into glass tubes containing EDTA. The tubes were centrifuged immediately at 2000 g for 10 min at 4°C and the plasma was stored at −80°C until analyses were performed. Of the microdialysis samples only 5–10 μl of dialysate was needed for the IL-6 determination. Enzyme-linked immunosorbent assay (ELISA) high sensible kits (R & D Systems, Minneapolis, MN, USA; coefficient of variation (CV) = 2.6%), which detect both soluble and receptor-bound IL-6, were used to quantify plasma IL-6 as well as interstitial IL-6, using chemiluminescence.

**Statistics**
Statistical analysis with the Friedman test of repeated measures and subsequent Wilcoxon’s signed rank tests were used. Data are expressed as means ± S.E.M. P < 0.05 was considered significant.

**RESULTS**

Exercise resulted in a graded increase in plasma IL-6 concentrations, already significant after 1 h of running (Fig. 1). At the end of the 3 h exercise bout, plasma IL-6 was ~50-fold higher compared with pre-exercise levels (Fig. 1). In the recovery phase plasma IL-6 decreased gradually, being similar to basal levels when measured 48 h after completion of running (Fig. 1).

Using microdialysis, the relative recovery (labelled type IV collagen) was found to be 42 ± 5 % (n = 36) in the peritendinous area and 58 ± 4 % (n = 21) in the muscle. No significant differences were found between muscle and peritendon determinations, or between sampling time points.

Pre-exercise levels of IL-6 in peritendinous tissue were undetectable, and no microdialysis sampling was performed prior to exercise in muscle (due to the risk of tissue damage influencing the subsequent running bout). In response to 3 h exercise, interstitial IL-6 concentrations in peritendinous tissue rose markedly up to 3618 ± 1239 pg ml⁻¹ in dialysate sampled from 120 min to 150 min after stopping of exercise. This value is ~90–100-fold higher than in plasma (Figs 1, 2 and 3). Peritendinous IL-6 levels remained significantly elevated above pre-exercise values for at least 48 h (24 h post-exercise, 1427 ± 482 pg ml⁻¹; 48 h post-exercise, 72 ± 26 pg ml⁻¹), whereas values obtained at 72 h and 96 h post-exercise were not significantly different from pre-exercise levels. Muscle interstitial (dialysate) IL-6 concentration after exercise did numerically decrease by ~50 % from 2 h post-exercise to 48 h post-exercise (465 ± 176 pg ml⁻¹ to 223 ± 113 pg ml⁻¹), but the change was not significant (Fig. 2). Whereas tissue dialysate IL-6 concentrations were significantly ~7–8-fold higher in the peritendon region vs. skeletal muscle immediately after exercise, at later time points the concentrations in muscle were found to be higher compared with the peritendon region (Figs 2 and 3). Interestingly, at 96 h post-exercise Tissue concentrations of IL-6 in response to exercise
muscle concentrations of IL-6 remained higher than in plasma (Figs 1 and 2).

**DISCUSSION**

The present study indicates that the peritendinous tissue produces substantial amounts of IL-6 during exercise in humans. This has been demonstrated using simultaneous determination of plasma and interstitial IL-6 concentrations in both peritendinous tissue and skeletal muscle using the microdialysis technique. The changes found in plasma IL-6 concentration are in agreement with previous studies on IL-6 release during prolonged exercise (Nehlsen-Cannarella et al. 1997; Ostrowski et al. 1998a, b, 1999, 2000; Croisier et al. 1999; Jeukendrup et al. 2000; Steensberg et al. 2001; Starkie et al. 2001a, b). From these studies it has been concluded that exercising muscle is the major site for IL-6 production (Steensberg et al. 2000; Pedersen et al. 2001b). The fact that the interstitial concentration of IL-6 in skeletal muscle throughout the present study was found to be higher than in the circulating blood could support this notion. However, in the period following exercise the IL-6 concentration in the peritendinous area around the Achilles tendon reached concentrations approximately 10-fold higher than the concentration measured in the skeletal muscle, making it unlikely that the skeletal muscle alone is responsible for the rise in plasma IL-6. It is, furthermore, an interesting observation that the tissue concentrations obtained 2 h after exercise are, to our knowledge, the highest concentrations of IL-6 ever reported in healthy humans. In accordance with the view that the IL-6 produced by tendons could contribute to the changes in plasma concentration, we found a similar time pattern of response in both connective tissue and plasma.

The higher concentration of IL-6 in the peritendinous tissue could be a result of a low perfusion of the peritendinous tissue during exercise, which could limit the wash-out of the produced IL-6, thus favouring a gradually increase in interstitial IL-6 concentration during prolonged exercise. However, in a study by the present authors, the blood flow to the calf muscle was found to be 2.6 ml min⁻¹ (100 g tissue)⁻¹ during rest (Boushel et al. 2000). The corresponding value around the Achilles tendon was, in the same study, found to be 2.1 ml min⁻¹ (100 g tissue)⁻¹. During exercise the flow around the Achilles tendon increased to approximately one-third of that of the skeletal muscles (Boushel et al. 2000), indicating that the flow around the Achilles tendon is not negligible, but on the other hand not as high as in the muscle. Based on these data the differences in interstitial IL-6 concentration determined in the present study cannot alone be explained by differences in blood flow and thus wash-out of substances. In contrast to this, it has been concluded, based on studies using the arterial–venous concentration difference method, that the release of IL-6 from skeletal muscle alone could explain the rise in plasma IL-6 during exercise (Steensberg et al. 2000). However, from these studies it is not clear which cell type releases IL-6 and although IL-6 mRNA has been shown to increase in homogenates of skeletal muscle biopsies (including some intramuscular connective tissue; Ostrowski et al. 1998b), it is likely that the reported IL-6 release somewhat overestimates the true production within the muscle fibre itself. In accordance with this idea cell types known to be localized in between muscle fibres have been demonstrated to secrete IL-6 (Mohamed-Ali et al. 1997; Eckelberg et al. 1997; Fried et al. 1998; Knight, 2001) and thus it cannot be excluded that some of the IL-6 released from skeletal muscle originates from connective tissue, adipose tissue or vasculature within the muscle.

The finding of increased levels of IL-6 in peritendinous tissue points towards an increased release from fibroblasts (Skutek et al. 2001). However, it cannot be excluded that adipose tissue situated in the region also contributes. It has been shown that adipose tissue is able to release IL-6, using both the arterial–venous in vivo differences technique (Mohamed-Ali et al. 1997) and in vitro measurement in human adipose biopsies (Fried et al. 1998). Furthermore, IL-6 release from adipose tissue has been shown to be inducible by changes in nutritional status and hormonal levels (Fried et al. 1998). However, it has been shown by isolating the adipose cells from adipose tissue that these only produce 10% of the total IL-6 release from intact adipose tissue, supporting the view that the tissue adjacent to the adipose cells, and which is removed by collageneases during preparation, contributes largely to the IL-6 release (Fried et al. 1998). In spite of this, the IL-6 release in relation to exercise in adipose tissue responses has never been measured and it is therefore not known how responsive this tissue is to muscular contraction.

We used radiolabelled type IV collagen as a marker for relative recovery of IL-6, and it was demonstrated that relative recovery was high (42–58%) and similar in both the skeletal muscle and the peritendinous tissue dialysed. In spite of a marked difference in molecular mass, type IV collagen was used, as no radioactive-labelled IL-6 was commercially available. This could lead to an underestimation of the relative recovery and thus an overestimation of the interstitial concentration of IL-6. The fact that no significant differences in relative recovery were found between the tissues dialysed underlines that a potential minor overestimation of the interstitial concentrations would not influence the conclusions drawn.

In the present study we used the microdialysis technique to determine interstitial concentrations of IL-6, and it cannot be excluded that the measured interstitial concentrations of IL-6 could be influenced by a trauma induced by the
insertion of the microdialysis catheters. In rats it has been demonstrated that the concentration of the mRNA of IL-6 increases after a blunt trauma (Kami & Senba, 1998). On the other hand studies in humans have failed to show an association between peak IL-6 concentration and prolonged muscle damage, measured as creatine kinase (Ostrowski et al. 1998a, 1999; Croisier et al. 1999). In the present study the subjects rested for at least 90 min after positioning of the microdialysis catheters and before starting the experiment to minimize the possible effects of the insertion, as determined from the concentration of inflammatory mediators (Langberg et al. 1999). Furthermore, the data from the present study indicate that, at least in the peritendinous area, the influence of the insertion on IL-6 production is negligible as the concentration before exercise was close to zero, both before exercise and at day 3 and day 4, in spite of repeated insertions being performed.

In conclusion the present study represents the first attempt to measure IL-6 in plasma, muscle and the peritendinous area simultaneously. The study shows a dramatic increase in peritendinous tissue IL-6 concentration that peaks post-exercise. This indicates that the peritendinous tissue is an IL-6-producing region during prolonged exercise, and suggests that connective tissue may contribute to the rise in IL-6 concentration observed in plasma in response to exercise.

REFERENCES


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