Cytokine response to eccentric exercise in young and elderly humans

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Departments of 1Infectious Diseases and 2Orthopaedic Medicine and Rehabilitation and 3Copenhagen Muscle Research Center, Rigshospitalet, 2200 Copenhagen N; and 4Department of Rheumatology, Copenhagen Hospital Corporation Amager Hospital, University of Copenhagen, 2300 Copenhagen, Denmark

Toft, Anders Dyhr, Lars Bjørn Jensen, Helle Bruunsgaard, Tobias Ibfelt, Jens Halkjær-Kristensen, Mark Febbraio, and Bente Klarlund Pedersen. Cytokine response to eccentric exercise in young and elderly humans. Am J Physiol Cell Physiol 283: C289–C295, 2002; 10.1152/ajpcell.00583.2001.—To examine the plasma interleukin (IL)-6 response in elderly (E) and young (Y) subjects, 10 E and 10 Y subjects completed 60 min of eccentric lower limb exercise at the same relative oxygen uptake. Plasma IL-6 was measured before, immediately after, and 5 days into recovery from exercise, as were the biochemical markers of muscle damage, creatine kinase (CK), and myoglobin. In both groups, IL-6 increased (P < 0.05) immediately after exercise and peaked 4 h after exercise at 4.35 ± 1.7 vs. 5.05 ± 3.17 pg/ml for E and Y subjects, respectively. However, the increase in IL-6 in both groups was modest relative to the increase in CK peaking at 539 ± 413 vs. 10,301 ± 5,863 U/l for E and Y subjects, respectively. In addition, the increase in IL-6 was less pronounced (P < 0.05) in E subjects compared with Y subjects. These results suggest that IL-6 increases progressively after eccentric exercise, suggesting that this increase is related to muscle damage. However, the modest increase in IL-6, despite large increases in CK, suggests that the IL-6 response to muscle damage does not make an important contribution to the large increase in IL-6 observed during concentric exercise of long duration. Our data also suggest that aging may be associated with impaired repair mechanisms for exercise-induced muscle damage.

interleukin-6; tumor necrosis factor-α; creatine kinase; muscle damage; muscle repair

Several studies have demonstrated that exercise induces a cytokine response that exhibits some similarities to the acute-phase response to sepsis and trauma. Thus exercise has been shown to increase the number of circulating leukocytes and increases in both pro- and anti-inflammatory cytokines. Elevated levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-1 receptor antagonist (IL-1ra), and soluble TNF-receptor type 1 (sTNF-R1) have been observed in response to strenuous exercise (for review, see Refs. 21–23).

During the last few years it has been demonstrated that contracting skeletal muscles produce large amounts of IL-6. Thus IL-6 mRNA is markedly elevated in muscle biopsies obtained after exercise (16, 20, 25, 27). Although one of the earlier studies on eccentric exercise suggested that the exercise-induced increase in IL-6 was associated with muscle damage (4), recent studies did not support this finding (10, 15, 19, 25). It is therefore clear that muscle contractions per se are a stimulus for the production of muscle-derived IL-6. It is, however, also clear that in eccentric exercise the IL-6 kinetics differ greatly from those of concentric exercise (4). Thus, in concentric exercise, the IL-6 levels increase during exercise and decrease immediately after cessation of the exercise (27). In contrast, a modest, prolonged increase in IL-6 is found after eccentric exercise (4), which is likely to be a sign of repair mechanisms after muscle damage. To date, no studies have tracked the response to eccentric exercise several days into recovery. Hence, this was the first aim of this study.

Aging is associated with increased levels of circulating inflammatory components in the blood (3, 6, 7). In a recent study (3) centenarians and 81-year-old subjects were found to have significantly higher plasma concentrations of TNF-α, sTNF-R1, and IL-6 compared with a young control group. In contrast, blood mononuclear cells from elderly subjects have an impaired ability to produce proinflammatory cytokines (5). Thus it is possible that the chronic elevated levels of proinflammatory cytokines induce a negative feedback on the ability of blood mononuclear cells to produce the same cytokines on stimulation. Muscle damage and impaired repair mechanisms after muscle damage may contribute to the impaired muscle function in elderly subjects (9).
In the present study, we hypothesized that elderly subjects have an impaired ability to produce IL-6 and other cytokines in response to muscle damage. Pronounced muscle damage as visualized by increased levels of creatine kinase (CK) can be induced by eccentric exercise (4), an ethically acceptable model for studying muscle damage in elderly vs. young subjects.

**METHODS**

**Protocol**

Ten elderly and ten young subjects, all healthy and not taking any medication, volunteered for this experiment. Median age was 24 yr (20–27 yr) for young subjects and 69 yr (67–75 yr) for elderly subjects. There were no differences between young and elderly subjects regarding height and weight. None of the subjects was specifically trained, but they were recreationally active, performing exercise for 1–3 days a week for 1–2 h. Subjects were screened for systemic diseases via blood sample analysis and physical examination. All elderly subjects underwent an electrocardiogram (ECG) and were screened for arthrosis of lower limb joints by X-ray and physical examination.

For each subject maximal oxygen uptake ($\dot{V}O_2$ max), maximal heart rate, and maximal concentric work load were determined 2 wk before the experiment during an incremental concentric exercise test on the same cycle ergometer as determined 2 wk before the experiment during an incremental age was 24 yr (20–27 yr) for young subjects and 69 yr (67–75 yr) for elderly subjects. There were no differences between young and elderly subjects regarding height and weight.

**Exercise Protocol**

The eccentric exercise consisted of 60 min of opposing the rotation of the pedals down to 60 rpm, performing the following program of six working intervals: 0–6 min at 50%, 6–12 min at 75%, 12–20 min at 100%, 20–25 min at 130%, 25–40 min at 100%, and 40–60 min at 75% of the load eliciting $\dot{V}O_2$ max as previously described (4). The work loads were chosen to give the same relative increases in $\dot{V}O_2$ max and heart rate.

**Experimental Protocol**

The same experimental protocol was used for each trial. The subjects were instructed to abstain from strenuous exercise 7 days before eccentric exercise. After reporting to the laboratory after fasting overnight, subjects rested on the cycle ergometer for 10 min and blood samples (preexercise) were drawn from the antecubital vein. The subjects then performed the eccentric exercise program. ECG and heart rate were continuously monitored throughout the exercise. $\dot{V}O_2$ was recorded after 30 min (during a steady-state period of 100% of the load eliciting concentric $\dot{V}O_2$ max), and work load was noted continuously. Further blood samples were obtained immediately after (0 h), each hour for the following 4 h (+1 h, +2 h, +3 h, +4 h), and 1, 2, and 5 days after exercise. Blood samples were taken at 1200 on the days after exercise. The subjects rested and were provided carbohydrate-rich food and drinks for 4 h after exercise, during which time postexercise samples were taken. Subjects were instructed to refrain from any strenuous and/or organized physical exercise for 5 days after the exercise.

**Cytokines**

For cytokine measurement, one 3-ml blood sample was drawn into a glass tube containing 35 μmol of dipotassium-EDTA and 1,500 kallikrein inactivator units (KIU) of Trypsin (Bayer, Leverkusen, Germany). The tube was kept on ice until centrifugation at 2,500 g for 15 min at 4°C. Plasma was separated from the cells and stored at −80°C until subsequent analysis for TNF-α, IL-6, IL-1ra, and sTNF-R1. According to guidelines from R&D Systems, transforming growth factor (TGF)-β1 was measured in serum treated as follows: one 3-ml blood sample was drawn into a glass tube without anticoagulant. The tube was kept at room temperature (20°C) for 1 h to allow it to clot, and for complete release of TGF-β1 it was stored at 4°C for 24 h, after which it was centrifuged at 2,500 g for 15 min at 4°C. Serum was separated from the cells and stored at −80°C.

All cytokines were analyzed by commercially available enzyme-linked immunosorbent assay (ELISA; R&D Systems Europe, Abingdon, UK). All measurements were performed in duplicate, and high-sensitivity kits were used when avail-
able, which was the case for IL-6 and TNF-α but not for IL-1ra, TGF-β1, and sTNF-R1. According to information provided by R&D Systems, the ELISAs used for measuring IL-6 and TNF-α are insensitive to the addition of the recombinant forms of the soluble receptors (sIL-6R, sTNF-R1, and sTNF-R2, respectively) and these measurements, therefore, correspond to both soluble and receptor-bound cytokine.

Clinical Chemistry Analysis

All clinical chemistry analyses were performed by the Central Laboratory at the University Hospital of Copenhagen. Standard laboratory procedures were employed for the estimation of the levels of CK, myoglobin, leukocyte subsets, hematocrit, lymphocytes, neutrophils, and platelets. Measurements of hematocrit, hemoglobin, leukocytes, neutrophils, and platelets were performed by a cell counter (SE-9000; Toa-Sysmex, Hamburg, Germany). The anticoagulant was EDTA. CK and myoglobin were measured in lithium heparinized plasma with automated enzyme reactions (Hitachi Systems 717; Boehringer Mannheim Diagnostica, Mannheim, Germany).

Changes in hematocrit and hemoglobin were followed to evaluate whether it would be necessary to make corrections for plasma volume shifts caused by dehydration. Because the subjects had access to water during exercise, hemoglobin and hematocrit did not increase (P > 0.5); thus no corrections were necessary.

Statistical Analysis

Data on VO2, pulse rate, work load, and muscle mass were analyzed with a two-sample t-test. The measurements of cytokines, leukocytes, CK, and myoglobin were tested for effects of “time” and “group” in a repeated-measures analysis of variance (RM-ANOVA). The model used was [Measurement] = Constant + Time × Group + Error.

Before we proceeded with the statistical analysis, the residuals in the RM-ANOVA were examined for a normal distribution through investigation of a histogram and a normal plot. If residuals were considered not to be normally distributed, positively skewed data were log transformed and residuals were investigated again. This was the case for IL-6, IL-1ra, TGF-β1, lymphocytes, myoglobin, CK, and work load.

If the effect of time tested significant (P < 0.05), a paired t-test was performed for comparison of the multiple measurements made after exercise with the preexercise value. P values were adjusted with the Bonferroni method.

Correlations were made with Pearson’s simple correlation on log-transformed values. When there were more than two time points, values were adjusted with the Bonferroni method. Statistical calculations were performed with SYSTAT 8.0 for Windows (SPSS, Chicago, IL).

RESULTS

Subject Characteristics

As expected, young subjects had higher lower limb muscle mass (P < 0.01), VO2 max (P < 0.01), and maximal heart rate (P < 0.01). The median concentric work load at VO2 max was 2.2-fold higher (P < 0.01) in young subjects compared with elderly subjects (Table 1).

Eccentric Exercise Program

The prescribed work load was maintained, except for the interval from 20 to 26 min, in which elderly sub-

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<table>
<thead>
<tr>
<th>Interval</th>
<th>Scheduled Workload, %</th>
<th>Young, %</th>
<th>Elderly, %</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–6 min</td>
<td>50</td>
<td>45 ± 19</td>
<td>42 ± 13</td>
<td>0.8</td>
</tr>
<tr>
<td>7–10 min</td>
<td>75</td>
<td>71 ± 17</td>
<td>73 ± 17</td>
<td>0.7</td>
</tr>
<tr>
<td>11–19 min</td>
<td>100</td>
<td>109 ± 16</td>
<td>121 ± 12</td>
<td>0.08</td>
</tr>
<tr>
<td>20–26 min</td>
<td>130</td>
<td>114 ± 32</td>
<td>141 ± 23</td>
<td>0.04</td>
</tr>
<tr>
<td>27–35 min</td>
<td>100</td>
<td>88 ± 25</td>
<td>106 ± 12</td>
<td>0.07</td>
</tr>
<tr>
<td>40–60 min</td>
<td>75</td>
<td>83 ± 24</td>
<td>78 ± 26</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Values are means ± SD.
Table 3. Workload, VO$_2$, and heart rate

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Elderly</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average WL, W</td>
<td>214(141–300)</td>
<td>113(88–164)</td>
<td>0.002</td>
</tr>
<tr>
<td>Average WL relative to concentric WL at VO$<em>2$$</em>{max}$, %</td>
<td>79(54–97)</td>
<td>92(86–111)</td>
<td>0.005</td>
</tr>
<tr>
<td>VO$_2$ at 130% of WL at concentric VO$<em>2$$</em>{max}$, l·kg$^{-1}·$min$^{-1}$</td>
<td>28.9(18.9–32.1)</td>
<td>12.1(7.5–19.7)</td>
<td>10$^{-5}$</td>
</tr>
<tr>
<td>VO$_2$ at 130% of WL at concentric VO$<em>2$$</em>{max}$ relative to VO$<em>2$$</em>{max}$, %</td>
<td>54(34–63)</td>
<td>43(25–62)</td>
<td>0.4</td>
</tr>
<tr>
<td>Average heart rate, beats/min</td>
<td>147(121–165)</td>
<td>108(66–133)</td>
<td>0.001</td>
</tr>
<tr>
<td>Average heart rate relative to heart rate at VO$<em>2$$</em>{max}$, %</td>
<td>75(66–86)</td>
<td>68(50–81)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Values are medians (ranges in parentheses). WL, workload.

young subjects. These increases were 1.4-fold ($P < 0.01$) and 2-fold ($P < 0.01$) for elderly and young subjects, respectively. For the elderly, levels of IL-1ra had returned to preexercise values the day after exercise (Fig. 2C).

TGF-β1. Plasma TGF-β1 levels at rest were elevated ($P < 0.05$) in young compared with elderly subjects, and exercise had a small positive effect ($P < 0.05$) on this cytokine in both groups (Fig. 2D).

Leukocytes

The lymphocyte concentration at rest did not differ between young and elderly subjects and did not increase during exercise. However, the lymphocyte concentration declined by 80% compared with preexercise values ($P < 0.05$) during the following 2 h of recovery but returned to basal levels the day after exercise. The preexercise neutrophil concentration was 1.6-fold higher ($P < 0.05$) in elderly compared with young subjects. The increase in neutrophil concentration was higher ($P < 0.05$) in young compared with elderly subjects during and after exercise, with the increases being 3-fold and 1.6-fold 2 h after exercise, respectively. Neutrophils were still elevated ($P < 0.01$) the day after exercise but had returned to preexercise levels by day 2 (Fig. 3).

Biochemical Markers of Muscle Damage

To determine where CK peaked, we performed pilot experiments in three subjects in whom blood was sampled for 7 days after exercise. These results demonstrated that CK peaked on day 5. Hence, data for CK are presented for 5 days after exercise. CK increased ($P < 0.01$) in both groups, but the increase was more pronounced in young compared with elderly subjects, peaking at 539 ± 413 vs. 10,301 ± 5,863 U/l for elderly and young subjects, respectively (Fig. 4A). Myoglobin levels increased ($P < 0.05$) until day 5 after exercise, with 1.8-fold and 1.3-fold increases in young and elderly subjects, respectively (Fig. 4A). CK correlated positively with average work load on day 2 ($r = 0.6$, $n = 20$, $P = 0.01$) and day 5 (peak CK; $r = 0.7$, $n = 19$, $P = 0.002$) after exercise. Peak myoglobin correlated positively with mean work load ($r = 0.56$, $n = 16$, $P = 0.023$).

Correlations Between IL-6, Muscle Mass, Work Load, CK, and Myoglobin

There was no correlation between the increase in IL-6 and the preexercise values. Furthermore, there was no correlation between peak IL-6 and muscle mass (young: $n = 9$, $r = -0.58$, $P = 0.11$; elderly: $n = 9$, $r = -0.49$, $P = 0.19$; all: $n = 18$, $r = 0.28$, $P = 0.26$) or between peak IL-6 and work load (young: $n = 10$, $r = 0.32$, $P = 0.37$; elderly: $n = 10$, $r = -0.06$, $P = 0.86$; all: $n = 20$, $r = 0.18$, $P = 0.44$).

Peak plasma IL-6 (4 h after exercise) was correlated to CK on day 2 ($n = 10$, $r = 0.7$, $P = 0.025$) within the young group but not within the elderly group ($n = 10$, $r = 0.34$, $P = 0.33$). Peak IL-6 was correlated to myoglobin on day 2 ($n = 10$, $r = 0.69$, $P = 0.028$) within the young group but not within the elderly group ($n = 10$, $r = 0.33$, $P = 0.35$). Peak IL-6 was correlated to myoglobin on day 5 ($n = 10$, $r = 0.72$, $P = 0.28$) within the
The results of this study demonstrate that IL-6 increases progressively after eccentric exercise, consistent with the hypothesis that this increase is related to muscle damage. It is important to note, however, that the increase in IL-6 was modest, despite large increases in CK and myoglobin. Hence, our data provide clear evidence that the IL-6 response to muscle damage does not make an important contribution to large increases in IL-6 observed during exercise of long duration.

Previous studies demonstrated that concentric exercise, such as bicycling, results in a less pronounced increase in IL-6 compared with eccentric exercise, such as running (17, 25). In addition, during intense running, such as a marathon, ~100-fold increases in plasma IL-6 have been consistently observed (19, 20, 26, 29, 30). In one of these previous studies, the authors speculated that the marked increase in IL-6 in the plasma may have been caused by muscle damage because they found postexercise CK levels to be markedly elevated compared with rest (26). However, these authors also speculated that the increase in plasma IL-6 during marathon running may have been caused by endotoxemia secondary to a decreased splanchnic blood flow sufficient to induce ischemia resulting in gut wall bacterial translocation (1, 8). On the basis of the results of the present study, the latter hypothesis is most plausible. In the current study, the eccentric leg kicking model was sufficient to result in plasma CK of ~10,000 U/l with a corresponding peak in IL-6 of ~5 pg/ml in young subjects. In contrast, in the previous marathon running study, in which young subjects were also studied (26), the values for CK and IL-6 were ~3,000 U/l and ~120 pg/ml, respectively. Therefore, this indicates that the large increase in IL-6 that ac-

**DISCUSSION**

The results of this study demonstrate that IL-6 increases progressively after eccentric exercise, consistent with the hypothesis that this increase is related to muscle damage. It is important to note, however, that the increase in IL-6 was modest, despite large increases in CK and myoglobin. Hence, our data provide clear evidence that the IL-6 response to muscle damage does not make an important contribution to large increases in IL-6 observed during exercise of long duration.
companies marathon running is not caused by muscle damage.

Another major finding of the present study was that the increase in IL-6 in response to eccentric exercise was less pronounced in elderly compared with young subjects. Furthermore, the muscle damage as measured by increased plasma levels of CK and myoglobin was significantly less pronounced in elderly subjects. This was despite the fact that the elderly subjects had the same relative increase in V\(\text{O}_2\) and heart rate during the eccentric exercise and a higher relative work load. The latter finding stimulated us to investigate whether the absolute work load, more than the relative work load, determines the exercise-induced increase in IL-6 and muscle damage. A clear association was found between absolute work load on the one hand and peak CK and peak myoglobin on the other. Thus muscle damage is closely related to the intensity of the exercise. However, there was no correlation between work load and plasma IL-6. Furthermore, there was no correlation between muscle mass and plasma IL-6. Thus the differences in IL-6 between young and elderly subjects were not explained by a correlational relationship between IL-6 on the one hand and muscle mass or work load on the other.

According to our two main hypotheses that 1) muscle damage is correlated to IL-6 and 2) aging is associated with impaired repair mechanisms, we investigated whether plasma IL-6 on the one hand was correlated with CK and myoglobin on the other in the elderly and young subjects. Peak plasma IL-6 was correlated with CK on day 2 in young but not elderly subjects. In addition, peak IL-6 was correlated to myoglobin on days 2 and 5 in young but not in elderly subjects. Eccentric exercise induces muscle damage, and the finding that a correlational relationship exists between the prolonged increase in plasma IL-6 and biochemical markers of muscle damage in young subjects indicates that IL-6 may be produced by monocytes infiltrating skeletal muscle. Elderly people have been shown to have impaired cell migration and repair mechanisms for tissue damage (24). On the basis of these results, we speculate that aging is associated with impaired repair mechanisms for exercise-induced muscle damage.

The present study showed that elderly subjects had increased plasma levels at rest for IL-6 and sTNF-R1 and a higher neutrophil concentration and thus confirms previous studies (5, 11, 13). IL-6 stimulates the production of IL-1ra, and the reason why IL-1ra was not significantly elevated in elderly subjects in this study is best explained by a relatively small sample size. Although previous studies demonstrated that elderly people have elevated circulating levels of TNF and TNF receptor (5, 11, 13), the present study only demonstrated elevated levels of TNF receptor but not TNF. This may be because TNF is produced locally, e.g., in atherosclerotic plaques, and that only part of the elevated TNF production is found in the circulation, whereas the locally produced TNF stimulates a systemic production of TNF receptor, which in turn is found in the circulation. We were not able to confirm the hypothesis that high levels of circulating cytokines induce a negative feedback on cytokine production in relation to muscle contractions. The finding that plasma IL-6 was not correlated to muscle damage in the elderly is consistent with the hypothesis that aging is associated with impaired repair mechanisms including cell migration.

In conclusion, our data demonstrate that IL-6 increases progressively after eccentric exercise but that the increase is modest, despite marked increases in CK and myoglobin. Our data, therefore, provide clear evidence that the IL-6 response to muscle damage does not make an important contribution to large increases in IL-6 observed during exercise of long duration. In addition, because IL-6 was related to markers of muscle damage in young but not elderly subjects, our data suggest that aging is associated with impaired repair mechanisms for exercise-induced muscle damage.

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REFERENCES


