Interleukin-6 release from human skeletal muscle during exercise: relation to AMPK activity

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IL-6 has been proposed to play a signaling role between contracting muscle and hepatic glucose production as well as adipose tissue lipolysis during exercise (2, 12). A role for IL-6 in regulation of glucose homeostasis during exercise has also been suggested (2, 3).

The molecular signaling leading to IL-6 release from contracting muscle is not established. However, the fact that IL-6 release seems to depend on exercise intensity (6) and is stimulated by low muscle glycogen levels (14) may suggest that IL-6 release is related to AMPK activity. AMPK is a ubiquitously expressed sensor of cellular energy charge. The central concept is that the AMPK system protects cells by acting as a “low fuel warning” system. On activation, AMPK switches off ATP-consuming anabolic processes and turns on ATP-producing catabolic processes, via phosphorylation of several downstream metabolic enzymes and via effects on gene expression (4, 5). Two catalytic subunits of AMPK are known: The α1 isoform is widely distributed in different body tissues, and the α2 isoform is primarily expressed in skeletal muscle, heart, and liver (4). It has also been shown that α2-AMPK activity both at rest and during exercise is higher in muscles with low than in muscles with high glycogen content (19, 20). Thus the pattern of activation of AMPK during exercise fits with the pattern of IL-6 release from muscle.

In the present study, we tested the hypothesis that IL-6 release from muscle during exercise may be related to muscle AMPK activity. For this reason subjects were studied under conditions in which muscle glycogen content was either low or high in this way creating conditions in which both AMPK activity and IL-6 release would be expected to differ between the trials. It was hypothesized that covariation between AMPK activity and IL-6 release might be obtained. If so, this would support that AMPK activity and IL-6 release might be related.

METHODS

Subjects. Eight young (28 ± 1 yr), healthy, endurance-trained men gave their written, informed consent to partici-
The glycogen-depleting cycle exercise protocol combining leg and arm exercise was performed 2 days before each experiment. The subjects were instructed to eat a mixed diet and to avoid exercise 2 wk. The subjects went through two experimental trials separated by 2 wk. The subjects were allowed to drink unlimited water and eat the specified diet until 11 PM.

Main experiment. The next morning the subjects ingested a light breakfast (75 E% carbohydrate, 8 E% fat, and 17 E% protein; total energy intake: 713 KJ) and arrived at the laboratory 2 h later using a minimum of physical activity. After 45 min of rest, lidocaine, and resting pulmonary oxygen consumption was measured by use of the constant-infusion thermodilution method as previously described (1). Then a needle biopsy was taken through two incisions spaced 5 cm from the inguinal ligament in one femoral artery and one vein under local analgesia with lidocaine (1%). When 4 h had elapsed since breakfast, blood samples were drawn simultaneously from the arterial and venous catheters, and leg blood flow was measured by using the bolus-infusion thermodilution method as previously described (1). Then a needle biopsy was taken from the vastus lateralis under local analgesia with lidocaine, and resting pulmonary oxygen consumption was measured by using an on-line gas and airflow analyzer (Medgraphics). The subjects then performed exercise on a cycle ergometer test (V\textsubscript{O2 peak} = 65 ± 1 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}). The results presented in this study are part of a larger study dealing with the effects of varying muscle glycogen levels on muscle glucose uptake and AMPK activity, which has partly been published previously (20).

Preexperimental treatment. The subjects randomly underwent two experimental trials separated by 2 wk. The subjects were instructed to eat a mixed diet and to avoid exercise training 2 days before each experiment. At noon the day before an experiment, the subjects underwent a glycogen-depleting cycle exercise protocol combining leg and arm exercise at varying power outputs. The glycogen-depleting exercise lasted for 5 h on average and was well tolerated by all the subjects. The subjects then left the laboratory and were instructed to eat a specified, controlled, isoenergetic diet for dinner and during the evening, consisting of 80 energy percent (E%) carbohydrate, 7 E% fat, and 13 E% protein (total energy intake: 17.4 MJ) in one of the trials and in the other trial 2 E% carbohydrate, 86 E% fat, and 12 E% protein (total energy intake: 16.3 MJ). The subjects were allowed to drink unlimited water and eat the specified diet until 11 PM.

RESULTS

Muscle biopsies obtained before the experimental trial demonstrated that the depletion and diet protocol were successful in creating vastly different glycogen concentrations in the low-glycogen (LG; 163 ± 12 mmol/kg dry wt (dw)) and high-glycogen (HG; 909 ± 75 mmol/kg dw) conditions (Table 1). During the 60 min of cycle exercise, muscle glycogen content decreased progressively in the HG trial only, but at all time points it was higher than in the LG trial (Table 1).

At rest, the respiratory exchange ratio was lower in the LG compared with the HG condition (0.81 ± 0.003 vs. 0.84 ± 0.007, respectively; P < 0.05). During exercise, the average respiratory exchange ratio value was significantly lower in the LG condition (0.73 ± 0.01) compared with the HG condition (0.93 ± 0.01) (P < 0.001). Pulmonary oxygen uptake during exercise was on average found to be significantly higher in the LG (47 ± 0.3 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}) compared with the HG trial (45 ± 0.3 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}) (P < 0.001) even though the average work load was exactly the same in the two trials (256 ± 9 W).

Muscle biopsies were quickly frozen in liquid nitrogen while still situated in the biopsy needles. After termination of exercise, 30 s elapsed before the “exercise” biopsy was frozen. The frozen biopsies were freeze-dried and dissected free of visible blood, fat, and connective tissue before any analysis was performed.

Glycogen content was determined as glycosyl units after acid hydrolysis (10). Muscle AMPK activities were measured in muscle lysates prepared as described previously (21). α-Isos-form-specific AMPK activity was measured in immunoprecipitates from 200 μg of muscle lysate protein using anti-α\textsubscript{1}- or anti-α\textsubscript{2}-antibodies kindly provided by D. G. Hardie. A p81-filter paper assay using SAMS-peptide (HMRSAMS-GLHVKRR) (200 μmol/l) as substrate was used to measure AMPK activity as previously described (21). Calculated before and after assay variation was accounted for by expressing the data relative to these samples. Net IL-6 release across the limb was calculated by multiplying arteriovenous differences with plasma flow. Data are expressed as means ± SE. Statistical evaluation was performed by two-way ANOVA with repeated measures. When ANOVA revealed significant differences, a post hoc test was used to correct for multiple comparisons (Student-Newman-Keuls test). Pearson’s correlation analysis was performed to establish the presence of correlations. Differences between groups were considered statistically significant when P < 0.05.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Rest</th>
<th>Ex 10 min</th>
<th>Ex 60 min</th>
<th>Δ Rest-Ex 60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>HG</td>
<td>909 ± 75*</td>
<td>679 ± 68*†</td>
<td>391 ± 24*†</td>
<td>518 ± 76*</td>
</tr>
<tr>
<td>LG</td>
<td>163 ± 12</td>
<td>149 ± 10</td>
<td>119 ± 9</td>
<td>43 ± 9</td>
</tr>
</tbody>
</table>

Values are means ± SE of 8 observations (in mmol/kg dry wt (dw)). Ex, exercise; HG, high glycogen. *P < 0.05 compared with low-glycogen (LG) trial. †P < 0.05 compared with rest. These data have been published previously (20).
Table 2. Arterial plasma catecholamine concentrations before and after 10 and 60 min exercise in the HG and LG trials

<table>
<thead>
<tr>
<th>Condition</th>
<th>Rest</th>
<th>Ex 10 min</th>
<th>Ex 60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HG</td>
<td>0.7 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.1*</td>
</tr>
<tr>
<td>LG</td>
<td>0.8 ± 0.2</td>
<td>2.2 ± 0.2†</td>
<td>4.8 ± 0.9†</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HG</td>
<td>1.1 ± 0.1</td>
<td>8.2 ± 0.9†</td>
<td>12.3 ± 0.8†</td>
</tr>
<tr>
<td>LG</td>
<td>0.9 ± 0.2</td>
<td>10.8 ± 1.2†</td>
<td>15.2 ± 1.6†</td>
</tr>
</tbody>
</table>

Values are means ± SE (in nM); n = 8. *P < 0.05 compared with LG trial. †P < 0.05 compared with LG trial.

DISCUSSION

The main finding in the present study is that in endurance-trained subjects IL-6 release across the leg after 1 h of exercise at 70% of \( V_{\text{O2 peak}} \) in the glycogen-depleted state correlates with \( \alpha_2 \)-AMPK activity in the exercising muscle. Furthermore, despite differences in leg IL-6 release, arterial IL-6 concentrations rose similarly in the two trials. Finally, a novel observation is that IL-6 is released from the leg already after 10 min of exercise when preexercise muscle glycogen stores are low. Thus, in this respect the present findings extend and confirm previous findings indicating that IL-6 release from an exercising limb depends on the preexercise glycogen store (14). In that study, IL-6 release was measured after 1 h of exercise in the glycogen-depleted leg, whereas 2 h elapsed before release was detectable in the leg with normal preexercise glycogen levels.

Table 3. \( \alpha_1 \) and \( \alpha_2 \)-AMPK activity in vastus lateralis muscle at rest and after 10 and 60 min exercise in the HG and LG conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>Rest</th>
<th>Ex 10</th>
<th>Ex 60</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha_2 )-AMPK</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HG</td>
<td>1.1 ± 0.2*</td>
<td>1.4 ± 0.3*</td>
<td>1.6 ± 0.3*</td>
</tr>
<tr>
<td>LG</td>
<td>1.6 ± 0.2</td>
<td>2.4 ± 0.4†</td>
<td>2.6 ± 0.3†</td>
</tr>
<tr>
<td>( \alpha_1 )-AMPK</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HG</td>
<td>3.0 ± 0.3*</td>
<td>3.0 ± 0.3*</td>
<td>3.5 ± 0.2*</td>
</tr>
<tr>
<td>LG</td>
<td>4.7 ± 0.5</td>
<td>4.4 ± 0.4</td>
<td>3.8 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE (in pmol·mg\(^{-1}\)·min\(^{-1}\) of 8 observations. *P < 0.05 compared with LG trial. †P < 0.05 compared with rest. These data have been published previously (20).
Low glycogen levels have been shown to induce IL-6 gene transcription in skeletal muscle during exercise (8). The fact that IL-6 was released already after 10 min of exercise in the LG condition indicates that release must have been due to IL-6 stored in the muscle rather than to acute exercise-induced increase in IL-6 gene transcription. Supposedly gene transcription was activated during the glycogen-depletion trial the day before and was probably maintained during the low-carbohydrate diet consumed after the glycogen-depletion trial, whereas the glycogen supercompensation resulting from the carbohydrate-rich diet presumably inhibited IL-6 gene transcription. Nevertheless, because IL-6 release was identical at rest in the two trials, the rapid rise in IL-6 release in the LG condition indicates the existence of specific mechanisms for IL-6 release activated by muscle contractions when glycogen stores are low. So far, the underlying signaling mechanisms behind exercise-induced IL-6 release have not been clarified. Nevertheless, in the present experiment, α2-AMPK activity and IL-6 release only increased above resting values in the LG trial and, furthermore, a fairly strong positive correlation was found when the individual values for IL-6 release in LG at 60 min of exercise were correlated against the individual values of α2-AMPK activity at the same time (r = 0.87, P = 0.006). A similar strong correlation was obtained when average IL-6 release over the entire 60 min was correlated to α2-AMPK activity at 60 min (r = 0.86, P = 0.006). Whereas correlation is not necessarily indicating a causative relationship between α2-AMPK activity and IL-6 release, the present findings may implicate such a relationship.

It has previously been estimated that the increase in plasma IL-6 concentration during 5 h of knee extensor exercise could be fully accounted for by release from the working muscle (17), and it was hypothesized that leg release might signal to increase hepatic glucose output during exercise (2, 3). In the present study the increase in arterial IL-6 during 60 min of exercise was identical in the two conditions despite the fact that the leg only released IL-6 in the LG condition. This indicates that other sources of IL-6 than exercising muscle contribute to the increase in plasma IL-6 during 60 min of exercise at 70% of VO₂peak. These may include the adipose tissue (7), the peritendinous tissue (9), and the brain (11). The possibility also exists that clearance of IL-6 was lower in the HG trial than in the LG trial. The similar increase in plasma IL-6 in the two trials indicates that the liver is exposed to similar IL-6 concentrations during the two trials despite different leg release of IL-6. Therefore, leg release of IL-6 cannot play an important signaling role to liver glucose output at least during exercise of up to 60 min. This is in agreement with recent findings from one of our laboratories that infusion of recombinant IL-6 into healthy resting volunteers does not increase hepatic glucose production (15). However, IL-6 may have other signaling properties because infusion of IL-6 has been shown to increase lipolysis and fat oxidation (18).

It was recently found that IL-6 release during knee extensor exercise correlated with the arterial plasma epinephrine concentration (6). Also, infusion of epinephrine at rest, at a rate resulting in arterial plasma epinephrine concentrations observed during strenuous whole body exercise, has been shown to increase the arterial IL-6 concentration. However, the increase was markedly smaller than the increase seen during strenuous exercise (16). In addition, in a study of one-legged exercise, IL-6 release was found in the exercising but not in the resting leg (17), pointing toward local factors as being more important than adrenaline in release of IL-6 during exercise. In the present study, IL-6 release during the LG trial did not correlate significantly with plasma epinephrine concentration, supporting the importance of local mechanisms in IL-6 release during exercise.
In conclusion, the present study has shown that, when preexercise muscle glycogen concentration is low, IL-6 release across the leg is measurable already after 10 min of dynamic exercise of moderate intensity and that after 60 min of exercise release of IL-6 correlated significantly with muscle activity of α2-AMPK. In contrast, when preexercise muscle glycogen level is high, IL-6 release and α2-AMPK activity do not increase for 1 h at this exercise intensity. Despite the difference in leg release of IL-6, arterial IL-6 concentrations increased similarly in the two trials, suggesting that either IL-6 is released mainly from other organs than muscle during exercise of this duration or that clearance of IL-6 is affected differently in the two trials. The present data are compatible with a role for AMPK in IL-6 release during exercise or a role for IL-6 in activating AMPK. Alternatively, both AMPK and IL-6 are independent sensors of a low muscle glycogen concentration during exercise.

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DISCLOSURES

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REFERENCES