Epinephrine infusion increases adipose interleukin-6 gene expression and systemic levels in humans

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Keller, Pernille, Charlotte Keller, Lindsay E. Robinson, and Bente K. Pedersen. Epinephrine infusion increases adipose interleukin-6 gene expression and systemic levels in humans. J Appl Physiol 97: 1309–1312, 2004. First published June 4, 2004; 10.1152/japplphysiol.00284.2004.—Exercise increases IL-6 mRNA in subcutaneous adipose tissue; however, the immediate signal for the IL-6 induction is unknown. We, therefore, explored the possible role of epinephrine in the induction of IL-6 in adipose tissue. Subcutaneous adipose tissue biopsies and blood samples were obtained from eight healthy men (mean age 27 yr, mean height 184 cm, mean weight 83 kg) in response to epinephrine infusion or in response to saline infusion. The rate of epinephrine infusion was such that circulating epinephrine concentrations mimicked that typically seen during exercise. The level of IL-6 mRNA in subcutaneous adipose tissue increased 26-fold (95% confidence interval, 9- to 166-fold) at 3 h of epinephrine infusion compared with controls (P = 0.028). In addition, plasma levels of IL-6 increased in response to epinephrine infusion (P < 0.001). However, epinephrine did not affect the IL-6 receptor mRNA. In conclusion, epinephrine acutely increases IL-6 mRNA levels in subcutaneous adipose tissue as well as circulating IL-6 levels in healthy men.

interleukin-6 receptor; tumor necrosis factor; adipose tissue

PLASMA EPINEPHRINE LEVELS increase markedly in response to physical activity (6), and exercise is known to affect several metabolic genes at both the mRNA and plasma levels (21). However, it is not possible to discriminate whether the exercise-induced metabolic effects are mediated via epinephrine or muscle contraction-induced factors. Recent research suggests that the cytokine IL-6, which is markedly enhanced during muscle contraction-induced factors. Recent research suggests that the cytokine IL-6, which is markedly enhanced during exercise, may play important metabolic roles (29). The exercise-induced increase in systemic levels of IL-6 is thought to originate from contracting skeletal muscles (20, 24). However, adipose tissue may also contribute as IL-6 mRNA expression in subcutaneous fat is increased by exercise (9, 10).

IL-6 secretion increases when human adipocyte cultures are incubated with epinephrine. Moreover, stimulation of β-adrenergic receptors via isoproterenol increases both IL-6 mRNA levels and IL-6 secretion in vitro in human breast adipocytes (18). In rat fibroblasts, epinephrine stimulation increases IL-6 gene expression levels (1, 7) through any of the three epinephrine receptor subtypes, α1A, α1B, and α1D, as shown by microarray analysis (7). In humans, epinephrine infusion induces only a modest increase in plasma IL-6 compared with exercise (23). However, muscle-derived IL-6 is not likely to be induced by epinephrine: in a two-legged human model, IL-6 was released from the working limb, but not from a resting limb, although they were exposed to the same systemic catecholamine levels (24). Thus it is possible that epinephrine stimulates the release of IL-6 from adipose tissue.

Because recombinant human IL-6 infusion in humans increases muscle IL-6 mRNA expression, muscle-derived IL-6 may act in an autocrine manner; this effect is less clear for adipose tissue (10). In mouse 3T3-L1 adipocytes (3) and in other cell culture types (4, 12, 27), an autocrine regulation does exist, and expression of IL-6, the IL-6 receptor (IL-6R), and the signal-transducing gp130 receptor is demonstrated in mature human adipose tissue (18). Thus it may be that this autocrine feedback loop is substantiated by increasing the production of the IL-6R in response to epinephrine infusion. Therefore, the purpose of this study was to test the hypothesis that epinephrine is involved in the regulation of IL-6 gene expression levels in adipose tissue and circulating IL-6 levels in healthy men. In addition, we investigated IL-6R mRNA expression in adipose tissue.

MATERIALS AND METHODS

Subjects. Ten subjects participated in the study [means ± SE: age 27 ± 1 yr, height 184 ± 2 cm, weight 83 ± 3 kg, body mass index (BMI) 24.4 ± 0.7 kg/m²]. The study was approved by the Ethical Committee of the Copenhagen and Frederiksberg Communities, Denmark, and was performed according to the Declaration of Helsinki. Subjects were informed about possible risks and discomfort before giving their informed, written consent to participate. Subjects had no medical history, and physical examination revealed no abnormalities. The subjects did not use any medication and did not have any febrile illness in the fortnight preceding the study. Furthermore, subjects abstained from heavy exercise 2 days in advance of the experiments. Six of the subjects served as their own controls.

On the experimental day, subjects arrived at 0800 after an overnight fast. The femoral vein of one limb was cannulated and used for the infusion. Subjects (n = 8) were infused for 3 h with epinephrine diluted in saline in a total volume of 80 ml. For the first 1.5 h, epinephrine was infused at a rate of 42 ng·kg⁻¹·min⁻¹, and, for the final 1.5 h, the rate was increased to 42 ng·kg⁻¹·min⁻¹ to mimic epinephrine levels seen during intense exercise. Control persons (n = 8) were infused with saline for 3 h. The infusions were performed in a randomized order. Biopsies were obtained from abdominal subcutaneous adipose tissue by using the percutaneous needle biopsy technique with suction preceded by a subcutaneous injection of lidocaine. Biopsies were obtained before infusion, after 1.5 h of infusion, at the cessation of infusion, and 1.5, 3, and 5 h postinfusion. Blood samples were collected before infusion, 0.5 and 1.5 h during the infusion, at the cessation of infusion, and 1.5, 3, and 5 h postinfusion.
Subjects were permitted to consume only water during the experiment.

Adipose tissue samples were analyzed for IL-6 and the IL-6R mRNA levels by real-time PCR with the use of an ABI PRISM 7900 sequence detector (PE Biosystems). IL-6 Taqman probe and primers were as described (10). IL-6R primers and Taqman probe were designed by using the computer program Primer Express 2.0. To ensure the specificity of the primers and probe, a gel was run, in which a single band of the correct size of 126 bp was obtained as follows: IL-6R: forward primer, 5′-AAGACCCCCACTTGGAAACT-3′; reverse primer, 5′-CGTTGATGACACGATGCT-3′; Taqman probe, 5′-ACCATCCATTGTAATGTTTGACGG-3′. The gene expression levels were normalized to the housekeeping gene 18S (obtained from Applied Biosystems). All reactions were run under singleplex conditions. Data were quantitated and normalized using the standard curve method.

Blood samples for measurement of cytokines were drawn into glass tubes containing EDTA. The tubes were spun immediately at 3,500 g for 15 min at 4°C. The plasma was stored at −80°C until analyses were performed. A high-sensitivity ELISA kit from R&D System (Minneapolis, MN) was used to measure IL-6 in plasma. The IL-6 kit does not distinguish between soluble and receptor-bound IL-6 and, therefore, gives a measure of the total IL-6 content in the sample. Free fatty acids (FFA) and glucose levels were determined by COBAS (Fara, Roche) analysis on plasma. Plasma insulin was measured using ELISA kits (no. K6219) from DAKO, UK.

For measurement of plasma epinephrine, blood was drawn into ice-cold glass tubes containing glutathione (1.3 mg/ml blood) and EGTA (1.5 mg/ml blood), pH 6–7, and spun immediately. Plasma was stored at −80°C until analysis by high-performance liquid chromatography (Hewlett-Packard, Waldbronn, Germany) with electrochemical detection.

Statistics. Data on plasma IL-6 and IL-6 mRNA and plasma epinephrine were log transformed, and data on IL-6R mRNA were square root transformed to reach a normal distribution. Data are presented as means ± SE or as geometric means ± SE or as 95% confidence interval with respect to transformed data. Data on FFA plasma levels were normally distributed; however, insulin and glucose plasma data could not be normally distributed, and thus nonparametric statistics were used.

For analysis of data, a two-way repeated-measures (RM)-ANOVA was used to detect changes over time or between groups. Student-Newman-Keuls’ t-test for post hoc analysis was used to detect changes over time from resting values or differences between groups. According to the nonparametric statistics, a RM-ANOVA on ranks was used. P values <0.05 were considered significant. Statistical calculations were performed by using Sigma Stat 3.0 (SPSS, Chicago, IL).

RESULTS

Adipose tissue IL-6 mRNA levels increased ~26-fold (95% confidence interval; 9–166 fold) in response to epinephrine infusion, peaking at the cessation of the 3-h infusion (2-way RM-ANOVA; P = 0.028; Fig. 1). There were no changes in adipose tissue IL-6 mRNA in response to saline infusion.

Plasma IL-6 levels increased to 5 pg/ml at 3 h of epinephrine infusion (2-way RM-ANOVA; P < 0.001) and then decreased after the end of the infusion. IL-6 plasma levels were increased in both groups at 1.5, 3, and 5 h after the end of the infusion period owing to circadian rhythms (see Table 1).

As expected, epinephrine levels increased in the epinephrine infusion trial only (2-way RM-ANOVA; P < 0.001), peaking at ~2.1 nmol/l at the end of the 3-h infusion and returning to baseline by 1.5 h after cessation of the epinephrine infusion (see Table 1).

In contrast to effects on IL-6, epinephrine infusion did not affect adipose tissue mRNA levels of the IL-6R (see Table 2).

In the epinephrine-infused group, insulin plasma levels increased (RM-ANOVA on ranks; P < 0.001) during the infusion period and decreased 3 and 5 h after the cessation of infusion. Insulin levels decreased in the control group (RM-ANOVA on ranks; P = 0.01), although a specific time point could not be detected (see Table 1). The level of FFA increased in response to epinephrine infusion (P < 0.001). Plasma FFA also increased in the control group at 5 h postinfusion (see Table 1). Glucose levels increased in response to epinephrine infusion (P < 0.001) and differed significantly from the control group at the cessation of infusion. After ending the infusion, glucose levels had decreased significantly at 3 and 5 h postinfusion in both groups (P < 0.001) (see Table 1).

DISCUSSION

This study demonstrates that epinephrine infusion increased IL-6 mRNA expression by 26-fold in human subcutaneous adipose tissue. This effect of epinephrine was short term, as the IL-6 mRNA level returned to preinfusion levels at 1.5 h postinfusion. IL-6 mRNA levels in adipose tissue also increase in response to exercise (9); however, this effect is maintained at least 3 h into the exercise recovery phase, suggesting that exercise mediates a longer term effect on IL-6 gene expression. Therefore, the short-term effect of epinephrine on IL-6 mRNA levels in adipose tissue suggests that epinephrine may relay an exercise-induced or stress-induced signal, followed by another exercise-specific substance that may be responsible for maintaining elevated IL-6 gene expression levels in adipose tissue. IL-6 plasma levels were also elevated 3.4-fold following epinephrine infusion, in accordance with previous findings (23); however, the levels are not comparable to a 3-h exercise bout, where circulating IL-6 levels normally increase ~10-fold. In response to exercise, muscle can account for most of the IL-6 secreted into the circulation (24), but IL-6 is also released from adipose tissue during exercise (15). Thus it is possible that the increase in circulating IL-6 observed during the epinephrine infusion is predominantly derived from adipose tissue.

Several studies have demonstrated that adipose tissue secretes IL-6 (5, 11), and plasma levels of IL-6 correlate with BMI, total body fat content (16, 17, 25), and percent body fat (19). Data on catecholamine levels in obesity have been con-
stressing conditions with acute increases in epinephrine, adipose adipocytes increases IL-6 gene expression levels and secretion. Previous in vitro data show that epinephrine treatment of adipose tissue would express the IL-6R. In this study, we detected IL-6R mRNA in adipose tissue; however, macrophages and adipocytes express IL-6 at a similar expression level. Thus it is likely that macrophage contamination explains the high variability in the IL-6 gene expression response to epinephrine. However, as the subjects in this study were lean (BMI = 24.7 kg/m²), macrophages are expected to only constitute a small fraction of the adipose tissue. Moreover, previous in vitro data show that epinephrine treatment of adipocytes increases IL-6 gene expression levels and secretion to the media, which suggests that in stress conditions with acute increases in epinephrine, adipose tissue may be an important contributor to the rise in systemic levels of IL-6.

Recently, our laboratory demonstrated that hyperinsulinemia is also a stimulus for IL-6 production in adipose tissue (13). Because plasma levels of insulin decline during exercise, it is clear that insulin is not involved in the upregulation of IL-6 during exercise. Rather, insulin may be involved in the regulation of IL-6 in patients with obesity and insulin resistance. Insulin levels increased in response to epinephrine infusion, and it is possible that insulin is involved in upregulating adipose tissue IL-6 mRNA levels and plasma levels at rest.

Given that IL-6 is subject to an autocrine regulation (4) and induces adipose tissue lipolysis in vivo (26), as well as when added directly to adipocyte cell cultures (18), we suggested that human adipose tissue would express the IL-6R. In this study, we detected IL-6R mRNA in adipose tissue; however, the expression level of the IL-6R gene was not regulated by epinephrine infusion, suggesting a lack of autocrine regulation and instead points to a targeting at the transcriptional level.

In conclusion, epinephrine infusion induces a rapid and marked increase in IL-6 mRNA in subcutaneous adipose tissue of lean healthy men with a concomitant increase in plasma IL-6. This effect is brief, as IL-6 mRNA and plasma levels return to baseline levels after cessation of the epinephrine infusion.

**ACKNOWLEDGMENTS**

We thank the subjects for their participation. Ruth Rousing, Hanne Villumsen, Carsten Nielsen, and Karin Juel Hansen are acknowledged for excellent technical assistance.

Table 1. **Plasma levels**

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Pre</th>
<th>0.5 h Infusion</th>
<th>1.5 h Infusion</th>
<th>Post</th>
<th>1.5 h Post</th>
<th>3 h Post</th>
<th>5 h Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6, pg/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>1.6 (0.9–3.5)</td>
<td>1.4 (0.8–3.0)</td>
<td>1.5 (0.9–2.9)</td>
<td>2.2 (1.4–3.8)</td>
<td>3.3* (2.3–5.2)</td>
<td>3.6* (2.2–6.4)</td>
<td>3.5* (2.2–6.0)</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>1.3 (0.8–2.4)</td>
<td>1.2 (0.7–2.3)</td>
<td>2.4* (1.6–4.0)</td>
<td>4.4* (2.7–7.9)</td>
<td>2.3* (1.4–4.4)</td>
<td>2.7* (1.8–4.4)</td>
<td>2.4* (1.5–4.2)</td>
</tr>
</tbody>
</table>

Table 2. **Gene expression levels**

<table>
<thead>
<tr>
<th>Gene</th>
<th>mRNA</th>
<th>Pre</th>
<th>1.5 h Infusion</th>
<th>Post</th>
<th>1.5 h Post</th>
<th>3 h Post</th>
<th>5 h Post</th>
</tr>
</thead>
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<tr>
<td>IL-6 receptor</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.0 (1.0–1.0)</td>
<td>1.6 (1.2–2.2)</td>
<td>1.1 (0.8–1.5)</td>
<td>1.2 (0.7–1.9)</td>
<td>1.5 (0.8–2.3)</td>
<td>0.9 (0.7–1.3)</td>
<td></td>
</tr>
<tr>
<td>Epinephrine</td>
<td>1.0 (1.0–1.0)</td>
<td>1.0 (0.6–1.4)</td>
<td>1.7 (0.8–3.0)</td>
<td>1.3 (0.7–2.3)</td>
<td>1.8 (0.9–3.3)</td>
<td>1.4 (0.8–2.1)</td>
<td></td>
</tr>
</tbody>
</table>

**Values are geometric means ± 95% confidence interval (in parentheses), presented as fold change from the preinfusion level. Subcutaneous adipose tissue mRNA levels of the IL-6 receptor in response to epinephrine infusion are shown. There were no significant differences.**
REFERENCES


