Recombinant human interleukin-6 infusion during low-intensity exercise does not enhance whole body lipolysis or fat oxidation in humans

N. Hiscock, C. P. Fischer, M. Sacchetti, G. van Hall, M. A. Febbraio, and B. K. Pedersen. Recombinant human interleukin-6 infusion during low-intensity exercise does not enhance whole body lipolysis or fat oxidation in humans. Am J Physiol Endocrinol Metab 289: E2–E7, 2005. First published March 1, 2005; doi:10.1152/ajpendo.00274.2004.—The present study examined the role of the cytokine IL-6 in the regulation of fatty acid metabolism during exercise in humans. Six well-trained males completed three trials of 120 min of cycle ergometry at 70% peak O2 consumption (V̇O2 peak; MOD) and 40% V̇O2 peak with (LOW + IL-6) and without (LOW) infusion of recombinant human (rh)IL-6. The dose of rhIL-6 during LOW + IL-6 elicited IL-6 concentration similar to those during MOD but without altering the circulating hormonal milieu seen in MOD. Palmitate rate of appearance (Rₐ), rate of disappearance (Rₜ), and oxidation were measured by means of a constant infusion of [U-13C]palmitate (0.015 μmol/kg) and oxidation were not affected by rhIL-6 infusion during LOW + IL-6. The increase in plasma IL-6 seen during exercise is to increase lipolysis and subsequent fat oxidation.

INTERLEUKIN-6 (IL-6) is a multifunctional immune-modulating cytokine produced mainly by monocytes/macrophages, fibroblasts and vascular endothelial cells (1). However, growing evidence suggests that IL-6 can also influence metabolic processes, including the regulation of lipid metabolism. IL-6-deficient mice develop mature-onset obesity within 9 mo of age, which is reversed by 18 days of intraperitoneal IL-6 treatment (24), whereas rats fed a high-fat diet display a decreased body fat content following 2 wk of daily IL-6 injections, not shown in saline-treated, control rats (25). There is also evidence in humans that IL-6 is involved in the regulation of lipolysis in vivo. We (23) and others (20, 10) have shown that recombinant human (rh)IL-6 infusion increases whole body lipolysis and/or fat oxidation in resting humans. Although this effect may be mediated via the effect of IL-6 on lipolytic hormones (8), in preliminary studies we have shown that IL-6 directly increases lipolysis in 3T3-L1 adipocytes and oxidation in L6 myotubes (27).

It has been known for some time that IL-6 is released from adipose tissue under noninflammatory conditions, contributing ~30% of the circulating IL-6 levels at rest (11). Work from our laboratories has focused on the IL-6 response within skeletal muscle and its influence on metabolic processes (4). Recently, we (5, 15) have shown that IL-6 is produced in skeletal myocytes during contraction. Moreover, IL-6 is released into the circulation during contraction in marked quantities (19). However, the precise biological roles of the skeletal muscle-derived increase in circulating IL-6 are presently not fully elucidated.

It is well known that, at the onset of moderate-intensity exercise, there is an increase in the rate of appearance (Rₐ) and disappearance (Rₜ) of fatty acids (FA) that is progressively enhanced as exercise progresses (9). Although this effect is largely mediated by a decline in insulin levels and increases in catecholamine concentrations, it is possible that IL-6 acts in an “endocrine-like” fashion to increase circulating lipid. In support of this hypothesis, we (6) have recently shown that pharmacological inhibition of lipolysis during exercise markedly increases circulating IL-6 and IL-6 mRNA in skeletal muscle and adipose tissue. The purpose of this study was, therefore, to test the hypothesis that one functional role of the increase in plasma IL-6 seen during exercise is to increase lipolysis and subsequent fat oxidation.

METHODS

Subjects. Six physically active males age (mean ± SE) 24 ± 1 yr, body mass 75.8 ± 2.2 kg, and height 182 ± 2 cm were recruited to participate in this study from the student population of the University of Copenhagen, Denmark. Written consent was obtained from all subjects after a verbal and written description of all procedures and risks associated with the investigation. Each subject underwent a full physical examination and full blood screening before participation. This study was approved by the local ethical committee of the Copenhagen and Frederiksberg Communities.

Determination of peak O2 consumption. Peak O2 consumption (V̇O2 peak) was determined during bicycle exercise on an electrically braked, cadence-independent cycle ergometer (Monark 839E; Monark, Varberg, Sweden). Subjects cycled at progressively higher workloads (increasing 5 W every 3 min for 12 min and then increasing 25 W every minute) until volitional exhaustion. Expired oxygen (O2) and carbon dioxide (CO2) concentrations were recorded every minute (S-3A/1 O2 Analyzer; Amatek, Applied Electrochemistry, Pittsburgh, PA, and L8–2 Medical Gas Analyzer; Fullerton, CA) using gas analyzers calibrated against known gas mixtures before each test. Inspired volume was recorded each minute by use of a turbine ventilometer (R-1, Applied Electrochemistry) calibrated against a known volume before each test.

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IL-6 and Lipolysis During Exercise in Humans

Experimental protocol. Subjects completed three experimental trials. In the first trial, subjects were asked to complete 120 min of cycle ergometry at 70% $V_{O2_{peak}}$ (MOD). During exercise, subjects were infused with human albumin (30 ml/h). Previous studies in our laboratory have shown that an identical exercise protocol results in a significant increase in plasma IL-6 concentration. After the completion of this first trial, plasma IL-6 concentration was measured. Subjects were asked to return to the laboratory on two subsequent occasions to complete two randomly assigned, single-blind experimental trials. On both occasions, subjects were asked to perform 120 min of cycle ergometry at 40% $V_{O2_{peak}}$. In one trial, subjects were infused with rhIL-6 in albumin (30 ml/h) at a concentration calculated to elicit a plasma IL-6 concentration similar to that measured in the MOD experimental trial (LOW + IL-6). In the other trial, subjects were infused with human albumin alone at an identical dose (30 ml/h; LOW).

For all trials, subjects reported to the exercise laboratory at 0800, in a fasted and rested state, on the day of each experimental trial. After a change of clothing to hospital attire, subjects were asked to lie supine while indwelling catheters were placed into the right forearm vein and an antecubital vein in both arms by use of aseptic techniques. Regular flushing of these catheters, when not in use for infusions, was performed using 0.9% sodium chloride to maintain patency. After a background enrichment blood sample, a constant infusion of potassium-[U-13C]palmitate (0.015 μmol·min⁻¹·kg⁻¹, prime NaHCO₃, 1 μmol/kg) was started into the right antecubital vein. The isotope was purchased from Cambridge Isotope Laboratories, Andover, MA. Subjects remained supine for 120 min before the onset of exercise and/or rhIL-6 infusion. The palmitate infusion rate was doubled during the exercise period in all trials.

Blood samples were then collected every half-hour from the beginning of exercise (Pre0) throughout the exercise period (Ex30, Ex60, Ex90), immediately after exercise (Ex120) and after 30 min (Rec30) and 60 min (Rec60) of recovery. Blood samples were collected from the left antecubital vein after a heating cuff was placed over the cannulated site for 5 min before blood sampling to enable collection of an arterial-venous-mixed blood sample. To ensure that this occurred, blood gases were measured using an automated spectrophotometric analyzer (ABL; Radiometer, Copenhagen, Denmark) throughout the exercise period, and $S_{O2}$ was greater than 90% on all occasions.

rhIL-6 infusion. rhIL-6 (Sandoz Pharma, Basel, Switzerland) was reconstituted to a concentration of 0.75 mg/ml. Dilutions were made in human albumin to concentrations suitable for the further dilution and preparation of rhIL-6 infuses and to avoid repeated freeze-thawing. Dilutions were sterile-filtered during preparation, and the entire process was performed using aseptic techniques. On the morning of each LOW + IL-6 trial, the rhIL-6 infusate was prepared by taking an aliquot prepared from the initial dilutions and further diluted in human albumin to a concentration calculated to elicit an elevation in plasma IL-6 concentration equal to that measured during the MOD experimental trial, on the basis of preliminary dose-response studies in our laboratory. Infusion rate was set at 30 ml/h. During the MOD and LOW trials, human albumin was infused during the 120-min exercise period at the same infusion rate of 30 ml/h. During the LOW + IL-6 trial, a sample of the IL-6-albumin infusate was collected at Pre0, Ex60, and Ex120 for the measurement of IL-6 concentration to ensure that the rhIL-6 was not degraded or lost via adherence to the infusion lines during the infusion period.

Expired gases were measured at each blood collection time point during each trial using the equipment outlined above. In addition, expired air was collected into an expired-gas bag and transferred into SST tubes (Vacutainer; Becton Dickinson, North Ryde, Australia) for later analysis of ¹³C enrichment in CO₂. $V_{O2}$ was closely monitored throughout the exercise period of each trial to ensure that subjects were exercising at the correct exercise intensity to elicit the desired workload.

Tracer analysis. Plasma [U-13C]palmitate was analyzed in our laboratory as described previously (22). Palmitate concentration was measured by gas chromatography (Autosystem XL, Perkin Elmer), and plasma [¹³C]palmitate enrichment was measured by gas chromatography-combustion isotope ratio mass spectrometry (GC-C-IRMS; Hewlett Packard 5890 Finnigan GC combustion III, Finnigan Delta+es, Finnigan MAT, Bremen, Germany). The $R_a$ and $R_d$ of palmitate were calculated using the method of Steele (18), adapted to stable isotope analysis by Wolfe (26), using the following formula:

$$R_a = \frac{F - pV[(C_{a0} - C_{a1})/2(E_{a2} - E_{a1})/(t_2 - t_1)]}{(E_{a2} + E_{a1})/2}$$

$$R_d = R_a - pV[(C_{d0} - C_{d1})/(t_2 - t_1)]$$

where $F$ = the isotopic infusion rate, $E_{a1}$ and $E_{a2}$ are the plasma palmitate isotopic enrichments, $C_{a1}$ and $C_{a2}$ are the plasma palmitate concentrations, $pV$ is the distribution volume (0.04 liter/kg body mass for palmitate), and $t_1$ and $t_2$ are plasma concentrations at time 1 and time 2, respectively.

Plasma IL-6. Plasma IL-6 concentration was measured in EDTA-treated, arterialized venous plasma by using high-sensitivity enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s instructions (R&D Systems, Abingdon, UK).

Hormones. Plasma epinephrine, norepinephrine, insulin, cortisol, and glucagon were measured by RIA according to the manufacturer’s instructions and as previously reported (23).

Plasma FA and glycerol. Plasma FA and glycerol were measured enzymatically by an automated analyzer (Cobas Fara, Roche, Switzerland).

Statistical analysis. Statistical analysis was performed using SigmaStat for Windows (version 2.03). All data were normally distributed and were therefore expressed as means ± SE. A two-way (treatment and time) ANOVA with repeated measures was used to measure the effect of rhIL-6 infusion and exercise. The Student-Newman-Keuls post hoc analysis for pairwise multiple comparison procedure was performed to identify the source of any significant differences. At all times, $P < 0.05$ was used to indicate statistical significance.

RESULTS

All subjects completed the experimental trials, and no adverse effects of the rhIL-6 infusion were reported by any of the subjects. Exercising heart rate was higher throughout exercise in the MOD trial compared with the LOW + IL-6 and LOW trials ($P < 0.05$). There was no difference, however, between the LOW + IL-6 and LOW trials. Similarly, mean exercising workload was higher in the MOD trial compared with the LOW + IL-6 and LOW trials and was identical between the latter two trials.

Plasma IL-6 concentration. Plasma IL-6 concentration was not elevated during the LOW trial. During the MOD trial, plasma IL-6 concentration increased during the 2nd h of exercise and remained higher than Pre0 levels 1 h after the cessation of exercise. Infusion of rhIL-6 was able to successfully mimic the plasma IL-6 levels reported during the MOD trial (Fig. 1). During the 1st h of IL-6 infusion, plasma IL-6 concentration was higher during the LOW + IL-6 trial compared with the MOD trial; however, these levels were similar in the second hour of infusion/exercise and after 60 min of recovery. Importantly, mean plasma IL-6 concentration and peak plasma IL-6 concentration were almost identical in the MOD and LOW + IL-6 trials.
Mean $\dot{V}O_2$, $\dot{V}CO_2$, and respiratory exchange ratio (RER) during the exercise period were higher in the MOD trial compared with the LOW and LOW trials ($P < 0.05$; Table 1). There was no difference in palmitate $R_a$ between groups at any time ($P = 0.156$; Fig. 2, middle). Palmitate oxidation increased in all groups during exercise and recovery and was significantly higher in the MOD trial compared with the LOW + IL-6 and LOW trials during the 2nd h of exercise ($P < 0.05$). After 1 h of recovery, oxidation in all three trials had returned to basal levels (Fig. 2, bottom). Importantly, at no time was palmitate $R_a$, $R_d$, or oxidation different between the LOW + IL-6 and LOW trials.

**Fig. 2.** Palmitate rate of appearance ($R_a$), rate of disappearance ($R_d$), and oxidation before (Pre0) and after 30 (Ex30), 60 (Ex60), 90 (Ex90), and 120 min (Ex120) and during recovery from cycle ergometry at 70% [peak $O_2$ consumption ($V_{O_2\text{peak}}$); MOD, black diamonds] and 40% $V_{O_2\text{peak}}$ with (LOW + IL-6, open squares) and without (LOW, grey circles) recombinant human (rh)IL-6 infusion. Data are expressed as means ± SE. #Difference from LOW + IL-6 and MOD; *difference from MOD.

Table 1. Mean $\dot{V}O_2$, $\dot{V}CO_2$, RER, heart rate, and workload during 120 min of cycle ergometry in MOD, LOW, and LOW + IL-6 groups.

<table>
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<th>MOD</th>
<th>LOW</th>
<th>LOW + IL-6</th>
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<tr>
<td>$\dot{V}O_2$, ml/min</td>
<td>2.718±0.40</td>
<td>1.587±0.53*</td>
<td>1.652±0.33*</td>
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<tr>
<td>$\dot{V}CO_2$, ml/min</td>
<td>2.517±0.49</td>
<td>1.274±0.45*</td>
<td>1.383±0.30*</td>
</tr>
<tr>
<td>RER</td>
<td>1.274±0.45*</td>
<td>1.274±0.45*</td>
<td>1.383±0.30*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>140±1.9</td>
<td>113±2.9*</td>
<td>113±2.3*</td>
</tr>
<tr>
<td>Workload, W</td>
<td>191±4.7</td>
<td>110±3.2*</td>
<td>110±3.9*</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. $V_{O_2\text{peak}}$, $V_{CO_2\text{peak}}$, respiratory exchange ratio; MOD, moderate-intensity (70% $V_{O_2\text{peak}}$) exercise; LOW, low-intensity (40% $V_{O_2\text{peak}}$) exercise without IL-6 infusion; LOW + IL-6, LOW with IL-6 infusion. *Difference from IL-6 and LOW.

Elevated during the recovery period compared with Pre0. $R_a$ was significantly higher in the MOD trial compared with the LOW + IL-6 and LOW trials at the cessation of exercise (Ex120) and after 1 h of recovery (Rec60) ($P < 0.05$; Fig. 2, top). There was no difference in palmitate $R_d$ between groups at any time ($P = 0.156$; Fig. 2, middle). Palmitate oxidation increased in all groups during exercise and recovery and was significantly higher in the MOD trial compared with the LOW + IL-6 and LOW trials during the 2nd h of exercise ($P < 0.05$). After 1 h of recovery, oxidation in all three trials had returned to basal levels (Fig. 2, bottom). Importantly, at no time was palmitate $R_a$, $R_d$, or oxidation different between the LOW + IL-6 and LOW trials.

**Fig. 1.** Plasma IL-6 concentration before (Pre0) and after 60 (Ex60), 90 (Ex90), and 120 min (Ex120) of exercise and 60 min of recovery from cycle ergometry at 70% [peak $O_2$ consumption ($V_{O_2\text{peak}}$); MOD, black diamonds] and 40% $V_{O_2\text{peak}}$ with (LOW + IL-6, open squares) and without (LOW, grey circles) recombinant human (rh)IL-6 infusion. Data are expressed as means ± SE. #Difference from LOW + IL-6 and MOD; *difference from MOD.
**DISCUSSION**

The major finding of this investigation was that a low dose of rhIL-6, administered during low-intensity exercise to mimic circulating levels of IL-6 reported during moderate-intensity exercise, did not alter FA metabolism compared with levels shown during low intensity exercise alone. Furthermore, rhIL-6 infusion during low-intensity exercise at the dose given in this study did not alter the hormonal milieu compared with low intensity exercise alone.

To our knowledge, this is the first study to date that has examined the direct effect of rhIL-6 infusion on FA metabolism during exercise. Our study design allowed us to manipulate circulating the IL-6 concentration during low-intensity exercise without altering the hormonal milieu. Our data clearly show that low-dose rhIL-6, when infused during exercise of low intensity, cannot mimic the effect of moderate exercise on lipid metabolism, indicating that exercise-induced lipolysis (at low intensity) is likely to be mediated via other factors. However, we (23) previously demonstrated that, at resting conditions, rhIL-6 infusion increased fat oxidation and lipolysis in healthy humans, an effect that did not appear to be mediated via known lipolytic hormones.

It is well known that lipolysis is controlled by many factors, including changes in the hormonal milieu, and it has been suggested that any effect of IL-6 on fat metabolism occurs via alteration in hormone levels (8). Epinephrine is a powerful lipolytic inducer (2) and has been shown to be elevated with rhIL-6 infusion (23, 20). In the present study, epinephrine increased in the MOD trial but not in the LOW + IL-6 and LOW trials. Similarly, the hormone insulin, which decreases lipolysis, was decreased in the MOD trial to a further degree than in the LOW + IL-6 and LOW trials. In our previous study in healthy males at rest (23) and recent work from our laboratory in both elderly and type 2 diabetes patients (27), rhIL-6 infusion induced an increase in circulating cortisol level. Glucocorticoids have been shown to both increase lipolysis in vivo (3) and decrease lipolysis in vivo and in cultured adipocytes (7, 14) and are considered less powerful regulators of lipolysis. In the current study, cortisol was increased only in the MOD trial.

In response to our previous work, Jensen (8) proposed that the increase in whole body lipolysis reported during rhIL-6 infusion might be due to an increase in growth hormone concentration. Growth hormone has been shown to be increased in some (21), but not all (16), rhIL-6 infusion studies and is known to be a strong regulator of lipolysis (12). During acute exercise, circulating growth hormone level is elevated and may therefore be directly involved in exercise-induced increases in lipolysis. In the current study, growth hormone was elevated to a greater degree in the MOD trial than in the LOW + IL-6 and LOW trials, and rhIL-6 infusion did not alter the growth hormone response. Thus growth hormone may be involved in mediating lipolysis during exercise. However, data from our group (15a) demonstrate that lipolysis takes place during IL-6 infusion in patients with type 2 diabetes without an increase in plasma growth hormone.

It appears unlikely from our data that skeletal muscle-derived IL-6 released during moderate exercise plays a major role in FA metabolism. Our palmitate data are con-
consistent with others’ (22) showing that lipolysis is rapidly increased during acute exercise to meet the increased demands of lipids for fuel. Plasma IL-6 concentration typically increases at a much slower rate, and even prolonged exhaustive exercise, such as a marathon (13, 17), usually elicits far lower circulating plasma IL-6 concentrations than those reported in previous rhIL-6 infusion studies. The dose of rhIL-6 administered during low-intensity exercise (LOW + IL-6) in this study was aimed to mimic levels measured during moderate-intensity exercise in this study (MOD). The average circulating concentration of IL-6 in the MOD trial was ~9 pg/ml, far lower than levels reported during sepsis and more prolonged, exhaustive exercise such as a marathon. The dose of rhIL-6 infused in the study by van Hall et al. (23) elicited a plasma IL-6 concentration at least 15-fold the values measured in the current investigation. It is possible that the low “physiological” increase in circulating IL-6 elicited by our rhIL-6 infusion dose was simply not sufficient to promote changes in FA metabolism reported in previous studies that have used a higher rhIL-6 dose. This then brings into question whether IL-6 produced during low- to moderate-intensity acute exercise can directly affect FA kinetics, and it is perhaps the “pharmacological” doses used in resting rhIL-6 studies and also increases in circulating IL-6 concentration during high-intensity exercise that may be more beneficial. It is also possible that the shorter rhIL-6 infusion time in the current study (120 min) was not adequate to elicit changes in lipolysis. In previous rhIL-6 infusion studies, the length of infusion was greater (180 – 240 min), and some of the changes occurred only after 120 min of infusion (23).

One further factor worth considering in the current study is that, despite similar circulating plasma IL-6 levels in the MOD and LOW + IL-6 trials, there may have been differences in local tissue levels (endogenous production) of IL-6. A greater production of IL-6 within the contracting muscle may have further elevated local lipolytic effects that may alter whole body FA kinetics reported in this study.

In conclusion, our data indicate that IL-6 is not a major contributor to fatty acid utilization during 120 min of moder-
ate-intensity exercise. During this type of exercise, hormones such as epinephrine and growth hormone are likely to mediate the exercise effect on lipid metabolism.

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GRANTS

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