Hepatosplanchnic clearance of interleukin-6 in humans during exercise

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The cytokine interleukin (IL)-6 increases markedly in the circulation during exercise, but whether the liver is a source of this increase is unknown. The aim of this study was to measure IL-6 flux across the hepatosplanchnic tissues in humans. To elevate systemic concentrations of IL-6, six healthy male subjects performed 120 min of semirecumbent cycling, and blood samples were simultaneously obtained from a brachial artery and the hepatic vein before and during exercise for the analysis of IL-6. Hepatosplanchnic blood flow (HBF) was measured using the indocyanine green infusion technique. Net hepatosplanchnic IL-6 balance was calculated from these measures. HBF was 1.3 ± 0.1 l/min at rest and was not reduced throughout exercise, averaging 1.1 ± 0.2 l/min. Arterial plasma IL-6 markedly increased (P < 0.05) from 1.8 ± 0.6 ng/l at rest to 14.3 ± 3.2 ng/l after 120 min of exercise. The hepatosplanchnic viscera did not contribute to this increase, since there was a net hepatosplanchnic IL-6 uptake (0.8 ± 0.3 vs. 5.5 ± 1.9 ng/min, rest vs. 120 min; P < 0.05). These data demonstrate that the hepatosplanchnic viscera remove IL-6 from the circulation in humans. This removal may constitute a mechanism limiting the negative chronic metabolic action of chronically elevated circulating IL-6.

Despite the fact that myocytes are likely to contribute largely to the exercise-induced increase in circulating IL-6, it is also possible that other cells and/or organs may contribute to this increase. One such organ is the liver. IL-6 is produced in isolated perfused rodent livers subjected to corticosterone (17), epinephrine (18), partial hepactectomy (12), thermal injury (30), and endotoxin (21). In addition, the protein "proteolysis-inducing factor" increases the production of IL-6 in cultured human hepatocytes (46). Therefore, during physiological or pathophysiological stress, the liver may contribute to elevations in circulating IL-6 via increased production and subsequent net release into the blood. However, after the cessation of muscle contractions (35) or IL-6 infusion (36, 43), the reduction in circulating IL-6 is marked, suggesting that IL-6 is rapidly cleared. Because the liver is a major organ responsible for clearing blood-borne substances, it is also possible that IL-6 is cleared by hepatocytes when the systemic concentration is increased. Although transient increases in IL-6 may aid in the maintenance of metabolic homeostasis (6, 29), chronic elevations in IL-6 characteristic of diseases such as acquired immunodeficiency syndrome (42) and type 2 diabetes mellitus (44) may be detrimental to metabolism and immune function via dysregulation of endocrine receptor activity (4). Thus hepatosplanchnic clearance of IL-6

THE CYTOKINE INTERLEUKIN (IL)-6 increases markedly in the circulation when metabolic homeostasis is altered by physiological stressors (28) to act as an "endocrine-like" factor in mediating substrate metabolism (6, 27, 43). Although it has been thought that the immune cells are primarily responsible for the increased systemic IL-6 with stress, the cellular origin of this increase has not been fully elucidated. Recent work has used exercise as a model for increasing the systemic concentration of IL-6 in humans (6). During exercise, neither the immune cells (34) nor the adipose tissue (19) contributes to the increase in plasma IL-6, and contribution from the brain (24) and the peritendons (16) is small. Rather, muscle contraction rapidly increases intramuscular IL-6 gene expression (7, 15, 23, 33, 35, 37) and the nuclear transcriptional activity of IL-6 (15). Importantly, IL-6 protein is released from skeletal muscle during prolonged exercise (7, 8, 32, 33, 35), and cultured human primary muscle cells are capable of increasing IL-6 mRNA when incubated with the calcium ionophore ionomycin (14). Therefore, it is likely that myocytes produce IL-6 in response to physical stress, and production of IL-6 by such tissue can account for most of the exercise-induced increase in plasma levels of this cytokine.

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may constitute an important mechanism for limiting the negative systemic actions of chronic elevations in this cytokine. In addition, it is well known that patients with hepatic cirrhosis have compromised immune and/or metabolic function as well as elevated levels of IL-6 (3), and it has been hypothesized that lack of hepatic clearance of IL-6 may be the cause of the elevation in IL-6 in these patients (1).

To our knowledge, IL-6 has not been measured across the intact human hepatosplanchnic viscera, but whether this tissue bed releases or clears IL-6 is important, not only for understanding the IL-6 response to exercise but also in relation to the study of metabolic, hepatic, and immune diseases. The aim of this study was to measure IL-6 across the human hepatosplanchnic viscera by use of exercise as a model for increasing systemic IL-6 concentration. We hypothesized that the hepatosplanchnic viscera are clearance organs for IL-6 when the systemic concentration is elevated during exercise.

METHODS

Subjects. Six healthy, active men [20.8 ± 1.8 (SD) yr; 181.4 ± 5.6 cm; 80.8 ± 13.8 kg; maximal oxygen uptake (VO2max) 3.96 ± 0.29 l/min] participated in the study. The study was approved by the Ethics Committee of the Copenhagen and Frederiksborg Communities, Denmark, and performed according to the Declaration of Helsinki. Subjects were informed about the possible risks and discomfort involved before their written consent was obtained.

Experimental procedures. Volunteers underwent a preliminary medical screening and were exempted from the study if they presented contraindications. After the medical screening, each subject underwent a VO2max test on a semirecumbent cycle ergometer. From this test, a workload was calculated that would elicit ~65% of each individual's VO2max. Forty-eight hours before the experimental trial, subjects reported to the laboratory and completed 45 min of upright cycling exercise at a workload corresponding to 65% of maximal heart rate. Thereafter, the subjects were provided with a food package, which they consumed for the following days (~16 MJ per day, 70% carbohydrate, 15% protein, 15% fat). During this period, subjects were asked to adhere to the diet and to refrain from strenuous exercise and the intake of alcohol, tobacco, and caffeine.

On the day of the experiment, the subjects reported to the laboratory at 0730 after a 12- to 14-h overnight fast. They voided, changed into appropriate exercise attire, and rested supine for 10 min. After this time, a liver venous 7-Fr catheter (Cournand) was inserted (22). During the initial experimental trials, the liver venous catheter was introduced via the right median cubital vein and was guided with the subject supine. The position of the catheter was confirmed with fluoroscopy in the body position used during cycling. To ensure that ventilation (Ve) did not displace the catheter, the position was also confirmed after maximal voluntary Ve. Despite these efforts, the catheter dislodged during exercise on three occasions, and we were forced to repeat these experiments. To reduce the likelihood of this occurring subsequently to these initial experiments, we introduced the catheter via the right femoral vein. This procedure ensured that the catheters remained in the liver vein. After this procedure, a 20-gauge catheter (1.0 mm ID) was placed in the left brachial artery. The catheters were kept patent by continuous infusion of isotonic saline (3 ml/h) and were connected to a pressure monitoring kit (Baxter Healthcare, Maurepas, France) positioned at the level of the heart.

When the catheters were positioned, a constant infusion of indocyanine green (ICG; 0.18 ± 0.02 μmol/L; Cardio-Green; Becton Dickinson, Cockeysville, MD) was administered into a vein by a peristaltic roller pump (type 104; Ole Dich, Hillerodon, Denmark) (22) and was maintained for 30 min to secure a steady-state plasma concentration of ICG. After 30 min, blood samples were collected simultaneously from the brachial artery and hepatic vein every 5 min for the subsequent 30 min. These samples were analyzed for ICG concentration. In addition, samples collected at 10-min intervals during this period were analyzed for hemoglobin and hematocrit (Hct) (see Hepatosplanchnic blood flow).

After basal samples were collected for 30 min, subjects commenced a 5-min warm-up consisting of semirecumbent cycling at 50% VO2max. On completion of the warm-up, the subjects cycled for a further 115 min at ~65% VO2max. During exercise, blood samples were collected simultaneously from the brachial arterial and hepatic vein every 10 min for the measurement of ICG concentration and hemoglobin and Hct. In addition, immediately before exercise and at 30-min intervals during exercise, blood samples were also collected for the measurement of plasma IL-6. Immediately before sampling, oxygen uptake (VO2), respiratory exchange ratio (RER), heart rate (HR), and mean arterial pressure (MAP) were recorded.

Hepatosplanchnic blood flow. For description of hepatic blood flow and blood variables obtained from the hepatic vein, we used the term “hepatosplanchnic” to indicate that blood from the hepatic vein also represents portal blood, whereas ICG is eliminated exclusively by the liver. The estimated mean hepatosplanchnic blood flow (HBF) at rest and during exercise was calculated

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\text{HBF} = \frac{(\text{IR} \times \text{Ve}) \times (\text{dC} \div \text{dt})}{(\text{Ca} \times \text{Cv}) \times (1/(1-\text{Hct}))}
\]

where IR is the infusion rate of ICG; Ca and Cv are the concentrations of ICG in the brachial arterial and in the hepatic vein, respectively; dC/dt is the Ca accumulation rate; VdICG is the volume of distribution of ICG (plasma volume); dC/dt × VdICG represents a correction for minor deviations from steady-state conditions (25). VdICG was estimated as 0.05 × body weight (kg), and dC/dt was expressed as the linear regression for the five samples (25, 26).

Blood analysis. The ICG dye concentration was determined by high-performance liquid chromatography with a detection limit of 0.01 μmol/L (25). Paired samples of arterial and hepatosplanchnic venous blood were collected in heparinized syringes (Q550; Radiometer, Copenhagen, Denmark). Blood samples were kept on ice until analysis for hemoglobin and Hct by use of an ABL apparatus (model 615; Radiometer). IL-6 was analyzed by commercially available enzyme-linked immunosorbent assay (ELISA; R&D Systems Europe, Oxon, UK) (32, 35). All measurements were performed in duplicate, and high-sensitivity kits (detection limit ≥0.1 ng/l) were used. According to information provided by R&D Systems, the kit used for measuring IL-6 is insensitive to the addition of the recombinant form of the soluble receptor sIL-6R, and the measurements, therefore, correspond to both soluble and receptor-bound IL-6. The inter- and intra-assay coefficients of variation for this analysis are both <3% (32).

Physiological measures. Expired pulmonary VO2 and carbon dioxide production were measured on-line using a Medgraphics CPX/D metabolic cart (St. Paul, MN). HR was measured with the brachial artery catheter connected to a sterile disposable pressure transducer (Baxter, Uden, The Netherlands).
The Netherlands) interfaced with a pressure monitor (Danico Electronic-Dialogue 2000, Denmark) and acquired using a beat-to-beat customized software data acquisition system interfaced with a personnel computer.

Calculations and statistics. Net hepatosplanchnic IL-6 balance is expressed as the hepatosplanchnic venous-arterial IL-6 difference times the HBF. Comparative data are expressed as means ± SE. A one-way analysis of variance (ANOVA) with repeated measures on the time factor was used to compute the statistics (Statistica, Tulsa, OK), with significance accepted with a *P* value of < 0.05. If analyses revealed a significant interaction, a Newman-Keuls post hoc test was used to locate specific differences.

RESULTS

Subjects exercised at a $\dot{V}O_2$ of $2.47 \pm 0.15$ l/min, which was equivalent to $62 \pm 2\%$ of $V_{O_2 max}$. As expected, RER and HR were both higher (*P* < 0.05) during exercise compared with rest, averaging $0.87 \pm 0.03$ and $153 \pm 1$ beats/min, respectively (Table 1).

HBF averaged $1.3 \pm 0.1$ l/min at rest and was maintained throughout exercise, averaging $1.1 \pm 0.2$ l/min (Fig. 1). Arterial IL-6 averaged $1.8 \pm 0.6$ ng/l at rest and increased progressively throughout exercise to $14.3 \pm 3.2$ ng/l at 120 min (*P* < 0.05; Fig. 2). The hepatosplanchnic venous-arterial difference was slightly negative at rest ($-0.6 \pm 0.2$ ng/l), and this was gradually augmented such that the value at 120 min, $-5.9 \pm 1.2$ ng/l was different (*P* < 0.05) compared with rest (Fig. 2). This resulted in a net increase (*P* < 0.05) in hepatosplanchnic IL-6 uptake that averaged $5.5 \pm 1.9$ ng/min at 120 min (Fig. 2).

DISCUSSION

The results from this study demonstrate that, rather than contributing to the elevation in circulating IL-6, the hepatosplanchnic tissues are clearance organs dur-

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Table 1. $\dot{V}O_2$, RER, HR, and MAP before (0 min) and during 120 min of semirecumbent cycling at $62 \pm 2\%$ of $V_{O_2 max}$

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<tr>
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<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
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<tbody>
<tr>
<td>$\dot{V}O_2$, l/min</td>
<td>$0.26 \pm 0.04$</td>
<td>$2.51 \pm 0.11^*$</td>
<td>$2.49 \pm 0.10^*$</td>
<td>$2.57 \pm 0.10^*$</td>
<td>$2.54 \pm 0.09^*$</td>
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<tr>
<td>RER</td>
<td>$0.80 \pm 0.04$</td>
<td>$0.90 \pm 0.03^*$</td>
<td>$0.89 \pm 0.03^*$</td>
<td>$0.87 \pm 0.03^*$</td>
<td>$0.83 \pm 0.04^*$</td>
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<tr>
<td>HR, beats/min</td>
<td>$64 \pm 1$</td>
<td>$147 \pm 1^*$</td>
<td>$151 \pm 1^*$</td>
<td>$156 \pm 1^*$</td>
<td>$158 \pm 1^*$</td>
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<tr>
<td>MAP, mmHg</td>
<td>$96 \pm 1$</td>
<td>$99 \pm 1$</td>
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Values are means ± SE (*n* = 6). $\dot{V}O_2$, oxygen uptake; RER, respiratory exchange ratio; HR, heart rate; MAP, mean arterial pressure; $V_{O_2 max}$, maximal $\dot{V}O_2$. *Significant difference (*P* < 0.05) from 0 min.

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Fig. 1. Hepatosplanchnic blood flow before (0 min) and during 120 min of semirecumbent cycling at $62 \pm 2\%$ of maximal oxygen uptake. Values are means ± SE (*n* = 6).

Fig. 2. Arterial interleukin-6 (IL-6) concentration (top), hepatosplanchnic vein-arterial (hv-a) IL-6 concentration (middle), and net hepatosplanchnic IL-6 uptake (bottom) before (0 min) and during 120 min of semirecumbent cycling at $62 \pm 2\%$ of maximal oxygen uptake. *Significant difference (*P* < 0.05) from 0 min. Data are means ± SE (*n* = 6).
ing muscle contraction. Hence, we have shown, for the first time, that when physiological stress increases IL-6 production with subsequent release from tissue beds, the hepatosplanchnic viscera are responsible for attenuating the systemic concentration of this cytokine.

No studies have measured IL-6 flux across the intact human liver. However, two previous studies have measured IL-6 uptake by the liver in intact rats (2, 31). In these studies, radiolabeled IL-6 was injected intravenously into rats, and the IL-6 progressively disappeared from the plasma, with most of the recovered IL-6 found in the liver. The results from the present study extend these findings to demonstrate that systemic, endogenously produced IL-6 is removed by the intact human hepatosplanchnic tissues. It is important to note that, during the study by Castell et al. (2), the radiolabeled IL-6 was localized exclusively on the surface of the parenchymal cells, suggesting the existence of an IL-6 receptor on hepatocytes. Hence, the possibility exists that, rather than simply clearing IL-6 from the systemic circulation, liver cells may take up IL-6 to play an important biological role within this organ. Indeed, it is known that IL-6 has a significant role in the maintenance of liver homeostasis, and because of this, IL-6 has been suggested as a possible therapeutic agent in the treatment of fulminant hepatic failure (10).

During exercise, the contracting muscle is primarily responsible for the systemic increase in plasma IL-6 (6), although a small contribution is made by the peri-tendon (16) and brain (24). In the current study, we did not report the release of IL-6 from these tissue beds, but it is clear that, although IL-6 was extracted by the hepatosplanchnic viscera, there was a mismatch between clearance and production, because exercise resulted in an elevated systemic IL-6 concentration (Fig. 2). When IL-6 is elevated by exercise (35) or recombinant human (rh)IL-6 infusion (36, 43), the decline in systemic IL-6 upon removal of the stimulus is rapid, with values returning to baseline within hours. Although we did not measure hepatosplanchnic removal of IL-6 during recovery, the data from the present study together with previous work (35, 36, 43) suggest that hepatosplanchnic removal of IL-6 during recovery may continue, even though IL-6 production and release have ceased. It is tempting to speculate why there is a mismatch between IL-6 release and clearance during exercise. It is possible that the capacity for hepatosplanchnic IL-6 uptake cannot match the rate of contracting limb IL-6 release, because of the large differences in blood flow to these regions during exercise. We have previously measured leg blood flow during similar exercise to be at least threefold higher (7, 8) than the hepatosplanchnic blood flow reported in the present study (Fig. 1). It is, however, also possible that IL-6 kinetics may be tightly regulated, in that small elevations in circulating IL-6 during exercise may serve functions in the maintenance of metabolic homeostasis (29). In fact, there are many studies that demonstrate a bioactive role for this cytokine. It has recently been demonstrated that an IL-6-deficient mouse developed mature-onset obesity and insulin resistance, a situation that was partially reversed with phasic IL-6 treatment (45). In addition, we (43) and others (20) have recently shown that acute rhIL-6 infusion results in an increase in lipolysis and fatty acid oxidation. IL-6 also appears to affect glucose metabolism. Tsigos et al. (41) demonstrated that rhIL-6 administration to healthy volunteers increased circulating plasma glucose in a dose-response manner. In addition, Stouthard et al. (40) studied patients with metastatic renal cell cancer receiving rhIL-6 infusion and observed an increase in glucose appearance and whole body glucose disposal when the isotopic tracer dilution method was used. In addition, Stouthard et al. (39) demonstrated that IL-6 enhanced both basal and insulin-stimulated glucose uptake in cultured 3T3-L1 adipocytes, and Hardin et al. (11) observed increased glucose transport in jejunal tissue incubated with IL-6 compared with controls. Therefore, a phasic increase in IL-6 may have an important biological role, as previously suggested (6, 27). However, chronic IL-6 hypersecretion may exert pathogenesis in age-related diseases such as obesity, atherosclerosis, and type 2 diabetes (4). Specifically, chronically elevated IL-6 can result in upregulation of glucocorticoid receptors, leading to abnormal hormonal function (5). Thus our observation of hepatosplanchnic clearance of IL-6 may constitute an important mechanism for limiting the negative systemic actions of chronic elevations in this cytokine.

Many physiological and pharmacological stressors, such as corticosterone (17), epinephrine (18), partial hepectectomy (12), thermal stress (30), and endotoxin (21), have been demonstrated to increase IL-6 production in rodent liver cells. Although we measured net IL-6 flux across the intact human hepatosplanchnic tissues, we cannot determine whether IL-6 production within the liver increased, because we did not sample liver tissue. Whether acute exercise increases liver IL-6 production is unknown; however, given the fact that circulating cortisol and epinephrine are markedly elevated with acute exercise (9), this scenario is possible.

In conclusion, we have demonstrated, for the first time, that rather than releasing IL-6 during exercise, the hepatosplanchnic tissues clear this protein in these circumstances. However, the hepatosplanchnic uptake does not match the release of IL-6, giving rise to an elevation in systemic IL-6 concentration.

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DISCLOSURES

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