

# Cerebrospinal fluid IL-6, HSP72, and TNF- $\alpha$ in exercising humans

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## Abstract

During exercise the concentration of interleukin (IL)-6 and of heat shock protein (HSP) 72 increases in plasma, especially in fasting subjects. Both IL-6 and HSP72 are involved in a variety of metabolic and immunological processes, including some within the central nervous system and, accordingly, they are present not only in plasma but also in the cerebrospinal fluid (CSF). To evaluate whether the two pools equilibrate we determined the levels of IL-6 and HSP72 in CSF, at a time when their plasma levels were increased due to exercise. Measurements of TNF- $\alpha$  served as a control, as its plasma level remains stable during exercise. Two groups of healthy, fit males performed 2 h of strenuous exercise with either carbohydrate ingestion ( $n=8$ ) or placebo ( $n=8$ ). The concentration of IL-6, HSP72, and TNF- $\alpha$  was measured in arterial blood and in the CSF obtained by a lumbar puncture immediately after exercise. A third group of subjects served as resting controls ( $n=8$ ). At rest, CSF levels of IL-6 and HSP72 were 2- and 3-fold higher than the plasma levels, respectively ( $P<.05$ ). During exercise, with and without carbohydrate ingestion, plasma IL-6 increased 8- and 18-fold, respectively, and HSP72 increased 5-fold ( $P<.05$ ). However, the concentrations of IL-6 and HSP72 in CSF did not change with exercise and were therefore below their corresponding plasma levels. The concentration of TNF- $\alpha$  in CSF was below that in plasma and both remained stable during exercise. The findings that resting CSF levels of IL-6 and HSP72 are higher than in plasma and that they remain stable despite exercise-induced, profound increases in their systemic levels, suggest that the CSF pool is segregated from that in blood.

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## 1. Introduction

During exercise the plasma IL-6 concentration increases several fold (Nieman et al., 1998). The majority of the plasma IL-6 is released from contracting skeletal muscles in a hormone-like fashion (Steensberg et al., 2000) mediating hepatic glucose production (Febbraio et al., 2004) and lipolysis (van Hall et al., 2003). Circulating IL-6 also stimulates the activity of the hypothalamic–pituitary–adrenal axis (Tsigos et al., 1997) and it is implicated in the induction of fever (Weber et al., 1993) and fatigue (Robson-Ansley

et al., 2004) indicating that IL-6 has an effect on the central nervous system (CNS).

We considered whether the cerebral response to changes in the plasma IL-6 concentration depends on the ability of this cytokine to cross the blood–brain barrier (BBB) (Banks et al., 1994, 1995). Alternatively, neurons in the median eminence and other circumventricular organs, lacking a BBB, might be exposed to blood borne IL-6 (McCann et al., 1994) without altering the content within the cerebrospinal fluid (CSF). Within the CSF IL-6 arises from either uptake from the blood, as mediated by facilitated transport, or local production. In the latter case, exercise may increase the CSF IL-6 content independently of plasma IL-6, whereas, in the first case, CSF IL-6 follows changes in plasma IL-6. Within the brain both astrocytes and neurons produce IL-6 (Benveniste, 1998; Spangelo et al., 1990b) and

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under basal conditions, the CSF IL-6 concentration surpasses the plasma IL-6 concentration, especially in lean subjects (Stenlof et al., 2003).

Exercise offers a model to study whether equilibrium is established between the increasing level of IL-6 in plasma and the CSF. During exercise, the increase in plasma IL-6 is attenuated by ingestion of carbohydrate (Nieman et al., 1998). If exercise elevates CSF IL-6 independently of plasma IL-6 (e.g., the CSF levels increase to the same extent during exercise with and without carbohydrate ingestion) this argues for a local CNS production, rather than transport across the BBB.

The HSP are ubiquitously expressed intracellular proteins that protect proteins and DNA from stress-induced damage (Asea et al., 2000). The HSP also serve extracellular functions (Asea et al., 2000), and are released from several tissues during exercise (Fehrenbach et al., 2005). They are crucial in the generation of some aspects of the adaptive immune response. For example, the inducible HSP72 increases monocyte IL-6 production (Asea et al., 2000), which raises the possibility that HSP72 could play a role in brain-derived IL-6 production. In exercise, the liver is partly responsible for the increase in circulating HSP72 (Febbraio et al., 2002) together with the leukocytes, the heart and the kidney (Fehrenbach et al., 2005), and a release from the brain is suggested (Lancaster et al., 2004). Whether, during exercise, HSP72 increases within the CSF or it crosses the BBB is unknown. In addition, increased levels of HSP72 within the CSF during exercise may benefit the brain, making it more resistant to stress-induced cell damage (Campisi et al., 2003).

Inasmuch as exercise-induced increases in circulating IL-6 may affect the brain, we investigated the effect of exercise on CSF IL-6 concentrations. The plasma IL-6 response was attenuated in one group of exercising subjects by ingestion of carbohydrate, another group served as control (placebo). We hypothesised during exercise CSF IL-6 would increase to a concentration as that in plasma. Also, the CSF TNF- $\alpha$  and HSP72 levels were measured.

## 2. Methods

Twenty-four healthy physically active males were randomised into three groups. Two groups performed exercise with either placebo (Ex-PLA;  $n = 8$ ) or carbohydrate ingestion (Ex-CHO;  $n = 8$ ), whilst the last group served as resting controls (REST;  $n = 8$ ). The study was approved by the Ethical Committee of the Copenhagen and Frederiksberg Communities (KF 01-034/02), and performed according to The Declaration of Helsinki. The subjects were informed about potential risks and discomfort and written informed consent was obtained.

### 2.1. Protocol

Each subject underwent a maximal exercise test on a cycle ergometer (Monach, Stockholm, Sweden). Heart rate (Polar, Finland) and maximal whole-body oxygen consumption ( $\text{VO}_{2, \text{max}}$ ; Jaeger, Germany) were measured and rating of perceived exertion (RPE; Borg Scale) was expressed during the exercise. After 5 min of rest the subjects cycled for 4 min at 100 W, where after the intensity was increased by 35 W every second minute until exhaustion; a workload corresponding to 60% of individual  $\text{VO}_{2, \text{max}}$  was calculated.

On the experimental day the subjects reported to the laboratory at 9:00 a.m. after an overnight fast. They changed into appropriate hospital attire and the brachial artery of the nondominant arm was cannulated for blood sampling. After 15 min in the supine position blood was sampled and then subject started cycling. Each individual cycled for 2 h at a workload corresponding to 60% of  $\text{VO}_{2, \text{max}}$ . Each subject consumed 250 ml of either artificially sweetened placebo or 6% carbohydrate drink before and every 15 min during the exercise, at which time points, also heart rate and RPE were obtained. CSF was obtained immediately after exercise (within 10 min) with the use of a 25 gauge pencil-point cannula (Braun, Melsungen, Germany) inserted between the third and fourth lumbar vertebrae until penetration of dura. Local anaesthesia with 2% lidocaine was used prior to the procedure. There were no complications, except for one subject who transiently developed headache 2 days after the experiment.

The subjects serving as resting controls fasted for the same period and abstained from physical activity on the experimental day and were cannulated for sampling of arterial blood and CSF (between 11 and 12 a.m.). To avoid multiple lumbar punctures CSF at rest and post-exercise was obtained in different groups of subjects.

### 2.2. IL-6, TNF- $\alpha$ , HSP 72, and glucose

Glucose, IL-6, and TNF- $\alpha$  were analysed in arterial plasma (obtained in EDTA glass tubes spun at 2200g for 15 min at 4 °C) and CSF samples, by using an automatic analyser (Cobas Fara, Roche, Switzerland) and commercially available high sensitivity enzyme-linked immunosorbent-assay (ELISA; R&D Systems Europe, Oxon, UK), respectively. All measurements were performed in duplicate.

To obtain serum samples for HSP72, arterial blood samples were spun at 2200g for 15 min at 4 °C in tubes containing a clot-inducing plug (Vacutainer Systems Europe, Meylan, Cedex France). A High sensitivity ELISA was used to determine the HSP72 content in serum and in the CSF (EKS-700 Stressgen, Victoria, BC, Canada).

### 2.3. Statistics

Data are presented as mean  $\pm$  SEM unless otherwise indicated. Changes in plasma IL-6, TNF- $\alpha$ , glucose, and serum HSP72 during exercise were evaluated by a two-way RM-ANOVA. Differences between plasma and CSF samples obtained at the end of exercise and differences compared to REST were evaluated by a one-way ANOVA. If statistically significant, a Newman-Keuls post hoc test was used to identify specific differences. Within each group (Ex-CHO, Ex-PLA, and REST) differences between the last plasma and the CSF samples were evaluated using a Student's  $t$  test.  $P < .05$  was set as the significance level.

## 3. Results

The age, weight, height, and BMI of subjects were similar between groups (Table 1) and also the workloads during the exercise trials were similar (Ex-PLA  $190 \pm 7$  W, Ex-CHO

Table 1  
Age, weight, height, and body mass index (BMI) for the resting control group (REST) and the two groups exercising with (Ex-CHO) and without (Ex-PLA) carbohydrate ingestion

	REST	Ex-CHO	Ex-PLA
Age (years)	23 (20–25)	24 (21–27)	24 (21–29)
Weight (kg)	79 (70–88)	79 (67–90)	75 (63–80)
Height (m)	182 (178–189)	181 (172–195)	180 (172–186)
BMI ( $\text{kg}/\text{m}^2$ )	23 (22–28)	23 (22–26)	23 (21–25)

There were no significant differences in these parameters when comparing the three groups. Values are medians and ranges for 8 subjects.

186 ± 10 W). Exercise increased heart rate to 162 ± 3 (Ex-PLA) and 158 ± 3 (Ex-CHO) beats min<sup>-1</sup>, respectively. The subjects had more difficulty to complete the Ex-PLA trial than the Ex-CHO. During the last hour of exercise in Ex-PLA RPE was higher (median 18, range 15–20) than during Ex-CHO (median 16, 14–18) ( $P < .05$ ).

During exercise plasma glucose was maintained by ingestion of the carbohydrate drink, whereas it decreased by ~25% in the placebo trial, resulting in lower plasma glucose levels at the end of Ex-PLA compared to REST and Ex-CHO ( $P < .05$  for time  $X$  treatment; Table 2). CFS glucose level was lower than the corresponding plasma level in all groups. Following exercise with placebo ingestion CSF glucose levels were similar to those obtained at REST. In contrast, glucose ingestion during exercise elevated CSF glucose levels compared to REST ( $P < .05$ ) in spite of no significant differences in plasma glucose levels.

Plasma IL-6 increased more during Ex-PLA (18-fold) than during Ex-CHO (8-fold) ( $P < .05$  for time  $X$  treatment; Fig. 1); the resting plasma IL-6 concentration was ~0.7 pg ml<sup>-1</sup> in all groups. CSF IL-6 concentrations averaged 1.4 ± 0.2, 1.2 ± 0.2, and 1.2 ± 0.3 pg ml<sup>-1</sup> following Ex-PLA, Ex-CHO, and REST, respectively (Fig. 2). At rest, the CSF IL-6 concentration was ~2-fold higher than in plasma ( $P < .05$ ).

Exercise did not change plasma TNF- $\alpha$  levels (Table 2). The CSF TNF- $\alpha$  content was below the detection limit, and thus below plasma TNF- $\alpha$  levels. Serum HSP72 increased ~5-fold during exercise ( $P < .05$  for time; Table 2) with no significant difference between Ex-PLA and Ex-CHO. The

Table 2

Glucose, TNF- $\alpha$ , and heat shock protein (HSP) 72 plasma and cerebrospinal fluid (CSF) concentrations during 120 min exercise, with (Ex-CHO) and without (Ex-PLA) carbohydrate ingestion and resting controls (REST)

	Time (min)			CSF (120)
	0	60	120	
<b>Glucose (mmol L<sup>-1</sup>)</b>				
REST	—	—	4.79 ± 0.11	2.72 ± 0.08 <sup>b</sup>
Ex-CHO	5.31 ± 0.08	5.35 ± 0.08	5.14 ± 0.24	3.12 ± 0.08 <sup>b,c</sup>
Ex-PLA	5.19 ± 0.10	4.40 ± 0.16 <sup>a,d</sup>	3.87 ± 0.19 <sup>a,c,d</sup>	2.97 ± 0.06 <sup>b</sup>
<b>TNF-<math>\alpha</math> (pg ml<sup>-1</sup>)</b>				
REST	—	—	4.0 ± 1.0	0.0 ± 0.0
Ex-CHO	4.4 ± 0.7	4.8 ± 0.9	5.0 ± 0.8	0.1 ± 0.0
Ex-PLA	3.4 ± 0.4	3.8 ± 0.4	4.1 ± 0.5	0.0 ± 0.0
<b>HSP 72 (ng ml<sup>-1</sup>)</b>				
REST	—	—	0.08 ± 0.08	0.29 ± 0.05 <sup>b</sup>
Ex-CHO	0.00 ± 0.00	0.36 ± 0.18 <sup>a</sup>	0.64 ± 0.32 <sup>a,c</sup>	0.21 ± 0.05 <sup>b</sup>
Ex-PLA	0.04 ± 0.03	0.35 ± 0.18 <sup>a</sup>	0.69 ± 0.34 <sup>a,c</sup>	0.28 ± 0.07 <sup>b</sup>

Exercise did not affect plasma or CSF TNF- $\alpha$  levels. Resting CSF HSP72 levels were 3-fold higher compared with resting plasma HSP72 levels. Exercise increased plasma HSP72 content to levels above CSF HSP72, whereas CSF HSP72 levels did not change during exercise.

<sup>a</sup> Different from before exercise.

<sup>b</sup> Different from plasma levels ( $P < .05$ ).

<sup>c</sup> Different from REST ( $P < .05$ ).

<sup>d</sup> Different from Ex-CHO ( $P < .05$ ). Values are means ± SEM for 8 subjects.

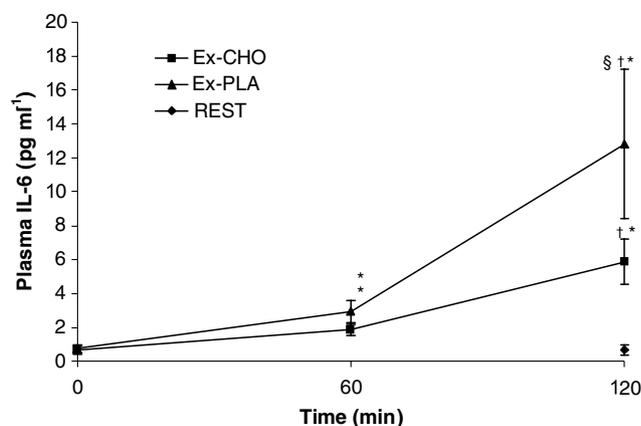


Fig. 1. Plasma interleukin (IL)-6 during 120 min of exercise with (Ex-CHO) and without (Ex-PLA) carbohydrate ingestion and resting controls (REST). Plasma IL-6 increased 8- and 18-fold during, respectively, Ex-CHO and Ex-PLA. \*Different from before exercise ( $P < .05$ ). †Different from Ex-CHO ( $P < .05$ ). ‡Different from REST ( $P < .05$ ). §Different from Ex-CHO ( $P < .05$ ). Values are means ± SEM for 8 subjects.

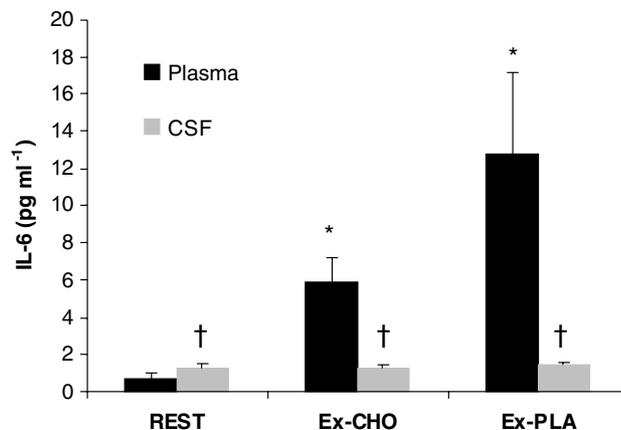


Fig. 2. Plasma and cerebrospinal fluid (CSF) interleukin (IL)-6 levels following exercise with (Ex-CHO) and without (Ex-PLA) carbohydrate ingestion and in resting controls (REST). Plasma IL-6 was 8- and 18-fold higher following Ex-CHO and Ex-PLA, respectively, compared to REST. CSF IL-6 content did not differ between groups, but the resting CSF level was ~2-fold higher than the corresponding plasma levels. \*Different from REST ( $P < .05$ ). †Different from plasma levels ( $P < .05$ ). Values are means ± SEM for 8 subjects.

resting CSF HSP72 was ~3-fold higher than the corresponding plasma levels ( $P < .05$ ) with no significant effect of exercise.

#### 4. Discussion

This study demonstrates that an acute, physiological elevation in circulating IL-6 and HSP72 does not affect their concentration in the CSF. The CSF IL-6 concentration did not equilibrate with plasma IL-6, and even an 18-fold increase in circulating IL-6 was not associated with elevated CSF IL-6 in exercising subjects. Accordingly, the release of IL-6 from the brain during exercise (Nybo et al., 2002) is not through efflux along with CSF, but more likely

a local release from the pituitary gland or endothelial cells. We demonstrate that the resting CSF HSP72 concentration exceeds that in plasma by a factor of 3. However, the CSF levels of HSP72 and TNF- $\alpha$  are not affected by 2 h of exhausting exercise.

The BBB contains bidirectional IL-6 transport molecules (Banks et al., 1994), but only 0.2% of radio-labeled IL-6 enter the CSF when injected intravenously in mice, and the CSF IL-6 content remained stable despite an 18-fold increase in its plasma level. It can therefore be questioned, whether systemic IL-6 affects the mammalian brain, at least in the levels obtained in this study. Whereas obesity is associated with an elevated level of IL-6 in plasma, that in CSF is attenuated (Stenlof et al., 2003). Such a difference could result from a defect transport system for IL-6 in the BBB. The present data suggests, however, that the low CSF IL-6 in obese subjects is caused by a decreased local production within the brain. In support, the concentration of IL-6 in CSF exceeds that in plasma (Stenlof et al., 2003) as verified in this study.

Even though IL-6 in plasma seems not to alter the CSF IL-6 content, it may affect brain functions by alternative routes. Neurons within the median eminence and other circumventricular organs in the hypothalamus are devoid of a BBB, thus representing a way for hormones to exert an effect irrespectively of the concentration in CSF (McCann et al., 1994). Alternatively, IL-6 may act via second messengers or afferent nerves. However, in IL-6 knock-out mice, infusion of IL-6 affects energy expenditure only when administered centrally (Wallenius et al., 2002). In humans, plasma IL-6 levels above 40 pg ml<sup>-1</sup> robustly increases, e.g., adreno-corticotrop hormone, growth hormone and prolactin (Robson-Ansley et al., 2004), whereas acute elevations of IL-6 to 15 pg ml<sup>-1</sup> do not activate the hypothalamic–pituitary–adrenal axis (Tsigos et al., 1997). Furthermore, in humans, only high levels of circulating IL-6 increase energy expenditure (Stouthard et al., 1995). Plasma IL-6 concentrations exceed 40 pg ml<sup>-1</sup> only during infections or prolonged strenuous exercise, such as marathon running (Febbraio and Pedersen, 2002). IL-6 plasma levels around 40 pg ml<sup>-1</sup> impairs running performance, without affecting heart rate, body temperature, blood glucose or lactate, indicating a central effect on fatigue (Robson-Ansley et al., 2004). Thus, in physiological doses above 30–40 pg ml<sup>-1</sup> plasma IL-6 exerts effects on the pituitary gland and some cerebral processes. When circulating in lower concentrations plasma IL-6 seems to exert only little effect on the brain, indicating segregated roles for IL-6 within the CSF and in the circulation in most physiological conditions.

During exercise the brain releases small amounts of IL-6 (Nybo et al., 2002) but its level in CSF remains stable, as demonstrated in the present study supporting a local release of IL-6, most likely from either the luminal side of the endothelial cells (Joseph et al., 1993) or from the pituitary gland. The pituitary gland is a likely site of IL-6 production as, *in vitro*, it produces substantial amounts of IL-6

(Spangelo et al., 1990a,b). Furthermore, the pituitary gland has its own blood supply and drainage (Porter et al., 1983), whereby IL-6 can be released without altering the IL-6 CSF content. Astrocytes and neurons constitutively produce IL-6 (Spangelo et al., 1990b; Benveniste, 1998). Both HSP72 (Asea et al., 2000) and TNF- $\alpha$  (Jablons et al., 1989) are involved in the cytokine cascade yielding IL-6 during infections, and their CSF concentrations were measured to investigate whether these substances could play a role in CNS IL-6 production during exercise. Neither TNF- $\alpha$  nor HSP72 increased in CSF, thus, exhaustive exercise does not provoke an immune cascade within the brain. Resting HSP72 concentration in CSF was ~3-fold higher than in plasma, perhaps suggesting enhanced neuronal stress tolerance (Guzhova et al., 2001).

It would be of interest to examine CSF HSP72 levels in obese and in sedentary subjects. In sedentary mice the CNS HSP72 response to stress is less than that in physically trained mice (Campisi et al., 2003). In the present study, whilst CSF HSP72 did not change, plasma levels increased throughout the exercise trials, thus, seemingly, this protein is not transported across the BBB within the time frame of this study. Also, this implies that the suggested release of HSP72 from the human brain following exercise (Lancaster et al., 2004) is not through efflux with CSF. In contrast, endothelium cells of the cerebral vasculature, or possibly the pituitary gland, may produce HSP72. Notably, the release of HSP72 was found in only 3 out of 6 subjects and did not reach statistical significance (Lancaster et al., 2004), which may argue for unknown subject-specificity. Indeed, it is not known whether CSF IL-6 could have been elevated in these 3 subjects, whereas in the present study no subjects ( $n=16$ ) had elevated CSF IL-6 content following exercise. In fact, CSF HSP72 levels were very similar amongst subjects.

The CSF TNF- $\alpha$  content was below the detection limit, whereas TNF- $\alpha$  was detectable in plasma. Therefore, as opposed to the CSF concentration of IL-6 and HSP72, that of TNF- $\alpha$  is lower than in plasma. This supports that TNF- $\alpha$  is involved only in inflammatory events, whereas IL-6 and HSP72 seem to regulate metabolic pathways within the brain.

In conclusion, the CSF levels of IL-6 and HSP72 are higher than in plasma and they remain stable despite profound increases in the plasma levels during exercise, suggesting that IL-6 and HSP72 do not equilibrate between CSF and blood. Thus, a low IL-6 CSF content in obese subjects is likely due to a diminished central production, rather than to reduced transport across the blood–brain barrier.

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