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Heat stress, cytokines, and the immune response to exercise

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Abstract

To examine the effect of exercise and heat stress on cytokine production, seven males (77 ± 2 kg; $VO_{2peak} = 4.7 \pm 0.4$ L min^{-1}) completed two (15 °C; CON or 35 °C; HEAT) 90 min cycling trials at 70% VO_{2peak}. Blood samples were collected throughout and analysed for spontaneous, and LPS-stimulated intracellular monocyte cytokine production, plasma cytokine levels, and circulating stress hormone concentration. Plasma epinephrine, norepinephrine, and cortisol concentration were elevated ($P < .05$) as a result of exercise in CON. HEAT increased ($P < .05$) epinephrine and norepinephrine levels, however, cortisol concentration was not different between the two trials. Exercise had no effect on the concentration of circulating monocytes spontaneously producing IL-6, TNF-α or IL-1α, however, there was a decrease in the amount of TNF-α per cell post-compared with pre-exercise. HEAT had no effect on spontaneous intracellular cytokine production. Circulating levels of both IL-6 and TNF-α were elevated in HEAT, but not in CON. Upon stimulation with LPS, the concentration of monocytes positive for IL-6, TNF-α, and IL-1α production was elevated ($P < .01$) post- and 2 h post-compared with pre-exercise. Stimulated cells, however, produced less ($P < .05$) TNF-α post-exercise and less ($P < .05$) TNF-α and IL-6 2 h post-exercise. HEAT resulted in an increase ($P < .05$) in the concentration of stimulated cells positive for TNF-α and IL-1α, however, did not affect the amount of cytokine produced by stimulated monocytes. These results demonstrate that exercise decreases the amount of cytokine produced by LPS-stimulated monocytes, possibly due to elevated levels of circulating stress hormones. Heat stress did not, however, augment the suppression in the amount of cytokine produced by circulating monocytes upon stimulation, despite elevated catecholamines.

Keywords: Flow cytometry; Monocytes; Interleukin-6; Tumour necrosis factor-α; Interleukin-1α

1. Introduction

The effect of catecholamines and glucocorticoids on cytokine production in the subject of controversy. Incubation of whole blood with epinephrine decreases lipopolysaccharide (LPS)-induced TNF-α, IL-1, and IL-6 production in vitro (Bergmann et al., 1999). However, norepinephrine treatment has been shown to either decrease cytokine production when incubated concomitantly with LPS (van der Poll et al., 1994) or increase TNF-α via stimulation of α2 adrenoreceptors (Bergmann and Sautner, 2002). Likewise while cortisol treatment (DERijk et al., 1997) have also been demonstrated to decrease LPS-stimulated cytokine production, this is not always the case (for review see Yeager et al., 2004). The controversy in the literature with respect to the effect of hormones on monocyte intracellular cytokine production in vitro also seems to pervade in vivo. Previous work from our laboratory has demonstrated that there is a decrease in the amount of IL-1α, IL-6, and TNF-α produced by LPS-stimulated monocytes post-compared with pre-exercise when stress hormones are elevated (Starkie et al., 2000a,b). Rhind et al. (2001), however, have reported an increase in LPS-stimulated monocyte intracellular expression of IL-1α, IL-6, and

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TNF-α after exercise, and associated these changes with sympathoadrenal hormones (Rhind et al., 2001).

The level of circulating epinephrine, norepinephrine, and cortisol all increase as a result of strenuous exercise, and these hormonal changes are exacerbated by heat stress (Hargreaves et al., 1996a). Hence, exercise combined with heat stress provides one way to study the additive effects of exercise and stress hormones on cytokine production. The effects of exercise and heat stress on cytokine production is unclear since exercise and heat stress may (Brenner et al., 1999) or may not (Montain et al., 2000; Niess et al., 1999) increase circulating IL-6. No previous studies have examined the effect of exercise and heat stress on cytokine production within leukocytes although Cross et al. (1996) showed that both exercise per se and the rise in body core temperature contributed to leukocytosis indicating that such a combination may effect leukocyte intracellular cytokine production.

Hence, the aim of the present study was to investigate the effect of submaximal, prolonged cycling, and heat stress, which increases circulating stress hormone concentration, on spontaneous and stimulated monocyte cytokine production and the plasma cytokine response. We hypothesised that exercise would decrease monocyte cytokine production due to increased epinephrine, norepinephrine, and cortisol concentrations, and heat stress would augment these changes, due to further elevations in hormones and temperature. In contrast, we hypothesised that heat stress would exacerbate the plasma cytokine response due to elevated metabolic stress.

2. Methods

2.1. Subjects

Seven endurance trained men (26 ± 2 years; 77 ± 2 kg; $VO_{2peak} = 4.7 \pm 0.4\,L\,min^{-1}$, mean ± SD) volunteered to participate in this study. The study was conducted in the winter months to minimize any natural heat acclimatization. Each subject was informed of the experimental protocol, made aware of possible risks and signed a letter of informed consent prior to participation. Subjects had been free of infection for 6 weeks prior to the study were exempt from symptoms of respiratory illness and were not on any medication. Experiments were approved by the Deakin University Human Research Ethics Committee.

2.2. Experimental procedures

On arrival at the laboratory, subjects were weighed, and a single use, flexible thermistor (Monatherm Mallinckrodt Medical, St. Louis, MO, USA) was inserted 15 cm beyond the anal sphincter to allow continual monitoring of core temperature. A venous catheter (Terumo, Tokyo, Japan) was inserted into a vein in the antecubital space for continuous blood sampling. Subjects then completed a 90 min cycling trial at 70% VO_{2peak} at either 15 °C (CON) or 35 °C (HEAT) in a randomised counterbalanced fashion. Both trials were conducted at 30% humidity. Venous blood samples, heart rate (HR), rating of perceived exertion (RPE), and rectal temperature (T_{rec}) were collected pre-exercise, at 15 min intervals during exercise, immediately post-exercise and 2 h post-exercise. Temperature of the vastus lateralis muscle (T_{mus}) was obtained pre- and post-exercise in three subjects, using a needle thermistor (YSI 525, Yellow Springs Instruments, Yellow Springs OH, USA) inserted 4 cm into the muscle belly. Trials were conducted at least one week apart and commenced early morning (6:30–7 a.m.) to avoid circadian variations in hormones.

Pre-, post-, and 2 h post-exercise blood samples were analysed for alterations in leukocyte counts and spontaneous and LPS-stimulated monocyte cytokine production. Samples collected pre-, 45 min, post-, and 2 h post-exercise were analysed for plasma glucose, lactate, cortisol, catecholamines, IL-6, and TNF-α.

Subjects consumed $3.5\,ml\,kg^{-1}$ (270 ± 7 ml, mean ± SE) of water every 15 min during exercise and a total of $16.2\,ml\,kg^{-1}$ (1248 ± 30 ml, mean ± SE) during the 2 h recovery period following exercise. Trials were randomised.

2.3. Leukocyte counts

Three millilitres of blood were placed in ethylenediaminetetraacetic acid (EDTA) tubes and analysed for differential white cell counts as routinely performed by the haematology laboratory at the Alfred Hospital (Melbourne, VIC, Australia). This included determination of total white blood cell (WBC) numbers and neutrophil, monocyte, and lymphocyte numbers to detect changes in circulating white blood cell populations with exercise.

2.4. Intracellular and plasma cytokines

Two millilitres of blood were placed in sodium heparin tubes and kept at room temperature until the end of the experiment for measurement of intracellular cytokine production. The tubes were gently inverted and rolled periodically. Whole blood was incubated for 4 h with (stimulated) or without (spontaneous) $1\,\mu g\,ml^{-1}$ lipopolysaccharide (LPS) at 37 °C in a humidified incubator. Brefeldin-A ($10\,\mu g\,ml^{-1}$) was added at the commencement of the incubation to all samples to inhibit intracellular transport of proteins, thus retaining cytokines produced within the cell. Hundred microlitre aliquots of stimulated and unstimulated blood was then incubated for 30 min with CD33 (PECy5) conjugated monoclonal antibody (Immunotech, Marseille, France) for staining of monocytes. In preliminary experiments in non-LPS-stimulated blood, we showed that >95% of