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Sweating by Exercise Controls Body Temperature through Increase of Interleukin-1beta

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This study was designed to investigate the expression and production of interleukin-1beta (IL-1 β) in human peripheral blood of trained runners and untrained controls after temporary moderate intensity exercise. Male long-distance trained runners (TR) and untrained sedentary control subjects (SED) ran for 1 h at 70% of heart rate reserve (HRR). IL-1 β gene and protein expressions were significantly higher in TR than those with SED at all 3 intervals examined independently. Significant increases in total sweat volume and oral temperature were observed after exercise in both groups, however, there were some differences between the groups. We conclude, therefore, that sweating due to exercise is associated with increase of IL-1 β and it is correlated with decrease of oral temperature.

Key Words: Sweating, Exercise, Body temperature, Interleukin-1beta, Trained runner

INTRODUCTION

Cytokines are glycosylated polypeptides that are secreted by, and influence the action of, most cells of the body (Turnbull & Rivier, 1999). The proinflammatory cytokines, including interleukin (IL)-1 β, IL-6, and tumor necrosis factor- α (TNF- α), modulate immune cell function and migration, initiating and amplifying the acute-phase and stress responses, and pyrogenesis (Baumann & Gauldie, 1994; Dinarello, 1994; Cohen & Cohen, 1996). The local production of these molecules coordinates the function of innate and adaptive immune cells, including the interactions with vascular endothelial cells, differential expression of cell surface effector molecules, growth, and differentiation (Martin et al, 1997). Prolonged endurance exercise elevates plasma concentrations of IL-1 β, IL-6, and TNF- α, but it does not change the accumulation of the corresponding cytokine messenger RNAs in peripheral blood mononuclear cells (PBMCs) (Moldoveanu et al, 2000).

Dinarello (2004) showed that many cell types, including monocytes and macrophages secrete active IL-1 β. IL-1 β enters the circulation and triggers IL-1 receptors on the hypothalamic vascular network resulting in synthesis of cyclooxygenase-2 (COX-2), which increases brain levels of prostaglandin E_2 (PGE_2), thus activating the thermoregulatory center for fever (heat) production.

We previously demonstrated that leukocytes and neutrophils were significantly increased immediately after exercise and 30 min after that (Shin et al, 2004). In this study, blood samples were obtained from the TR and SED subjects after exercise. The aims of this study were to determine the decrease of body temperature due to sweating and probable difference between TR and SED. We also evaluated whether the protein and RNA levels of IL-1 β were higher in TR than those of SED.

METHODS

Subjects

Ten well-trained male athletes (TR, 21.3±1.1 yrs; 174.3 ±7.1 cm high; 62.6±3.9 kg body weight) were recruited from Korea National Sport University. Athletes lived in a dormitory and trained regularly. They usually trained 3 hours a day, 6 days a week throughout the year. Ten healthy but sedentary age-matched male college students were selected as controls (SED, 22.4±1.5 yrs; 174.9±5.3 cm high; 72.7±11.0 kg body weight). SED had not engaged regular physical exercise for at least 3 years before the study. All subjects did not have any physiological stress activity during the last 3 days before the collection of basal blood samples. All Subjects read and signed an informed consent about the experimental purpose and procedure. We paid a great attention to the subjects in accordance with the Helsinki declaration of 1975.

Experimental procedure

All experiments were performed in a thermostatically controlled experimental room maintained at 24±0.5°C and 60± 3.0% relative humidity. Upon arrival at the experimental

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ABBREVIATIONS: IL, interleukin; TR, trained runners; SED, sedentary control subjects; HRR, heart rate reserve; TNF- α, tumor necrosis factor- α; PBMCs, peripheral blood mononuclear cells; COX-2, cyclooxygenase-2; PGE_2, prostaglandin E_2; LPS, lipopolysaccharide; HSTF, heat-shock transcription factor; HSE, heat shock element.

room, subjects wore light clothing and rested quietly for 60 min before the experiment commenced. On experimental day, subjects ate breakfast and arrived at the laboratory at 2~5 p.m. Subjects carried out 1 hour running on a treadmill (Quinton, USA) at 70% intensity of heart rate reserve (HRR). Each target heart rate was calculated from Karvonen formula (Karvonen et al, 1957) and maintained during the run. Heart rates were monitored by wireless all throughout the experiment. Body weight and oral temperature were measured immediately before and after exercise, and 30 min after exercise. When was measured of subjects the body weight, they wore only running pants.

Blood

Peripheral blood samples (20 ml) were drawn from antecubital vein by using a EDTA treated disposable syringe immediately before and after exercise, and 30 min after exercise. Total and differential counts of white blood cells were determined using an automated hematology analyzer.

Isolation of RNA and polymerase chain reaction (PCR)

Total RNA was extracted from blood using Trizol reagent (Invitrogen Corp., Carlsbad, CA) and was dissolved in diethylpyrocarbonate (DEPC, Sigma) treated water. RNA concentration was quantified by measuring optical density at 260 nm. RNA samples (1 μg) were reversibly transcribed to cDNA using Ready-To-Go™ T-Primed First-Strand Kit (Pharmacia Biotech, Uppsala, Sweden) in 35 μl volume by following the manufacturer's instructions. PCR was carried out in 25 μl volume at a final concentration of reaction buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl; 1.5 mM $MgCl_2$), 0.2 mM each of dNTP, 0.5 μM each primer and 0.1 unit of Taq DNA polymerase (Bioneer, Seoul, Korea). The amplification procedure consisted of 35 cycles (denaturation at 95°C for 1 min, annealing at 56°C for 45 sec, elongation at 72°C for 45 sec) for IL-1β, and 35 cycles (denaturation at 94°C for 1 min, annealing at 56°C for 30 sec, elongation at 72°C for 30 sec) for GAPDH. The PCR products were analyzed by 1% agarose gel electrophoresis and ethidium bromide staining. The amount produced was quantified using a Flour-S® MultiImager (Bio-Rad, U.S.A). (All PCR products measured were normalized to the amount of GAPDH). The following primers were used; 5'-GGTGAAGGTCGGAGTCAACG and 5'-CAAAGTTGTCATG-GATGACC for GAPDH, 5'-GGATATGGAGCAACAAGTGG and 5'-ATG TACCAGTTGGGGAACTG for IL-1β. The expected size of the PCR products was 465 bp for GAPDH and 263 bp for IL-1β.

Measurement of interleukin-1beta (IL-1β) by ELISA

Serum IL-1β was measured by using quantitative sandwich enzyme immunoassay kit (R&D, San Diego, CA). The lower limit of detection was 7.8 pg/ml. Values below this limit were assumed to be zero for the statistical analysis. The inter- and intra-assay coefficients of variance were below 10%.

Statistics

All data are presented as means±SD. Repeated-measurement analysis of variance was employed using commercially available computer software. When appropriate, t-test and repeated contrast were performed for *post hoc* comparison. Spearman's rank correlation was calculated to assess correlations between data. Differences were considered to be significant when the probability was less than 0.05.

RERULTS

Comparison of total sweat volume and oral temperature between TR and SED

Total sweat volume was significantly higher in TR subjects (1,250±105 g) than SED (990±71 g) after exercise (Fig. 1, $p<0.001$). And the oral temperature in post exercise and recovery of 30 min increased significantly in both groups, compared with pre-exercise (Fig. 2, TR, $p<0.05$; SED, $p<0.01$). However, the ratio of oral temperature between TR and SED subjects from pre-exercise was trendily higher in SED than TR subjects.

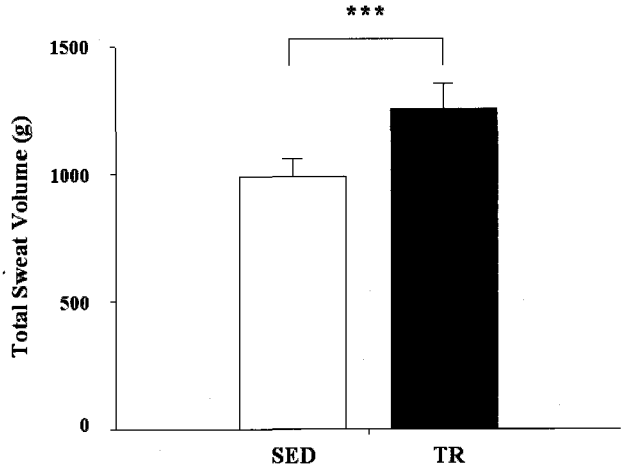

Fig. 1. Total sweat volume. SED, sedentary controls; TR, trained runners (mean±SD, n=10). ***$p<0.001$, means significant difference at post-exercise.

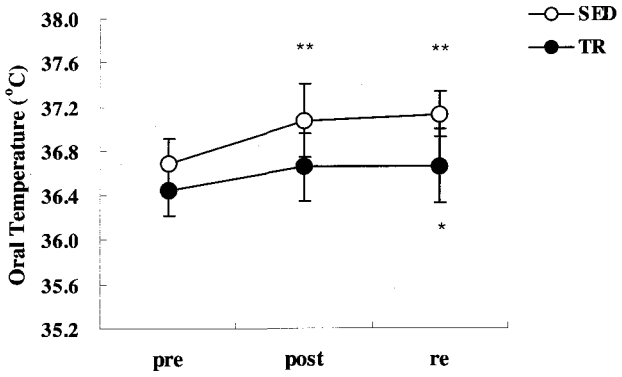

Fig. 2. Measurement of oral temperature. pre, pre-exercise; post, post-exercise; re, recovery of 30 min. And SED, untrained sedentary control subjects; TR, trained runners (mean±SD, n=10). *$p<0.05$, **$p<0.01$, means significant difference from pre-exercise.

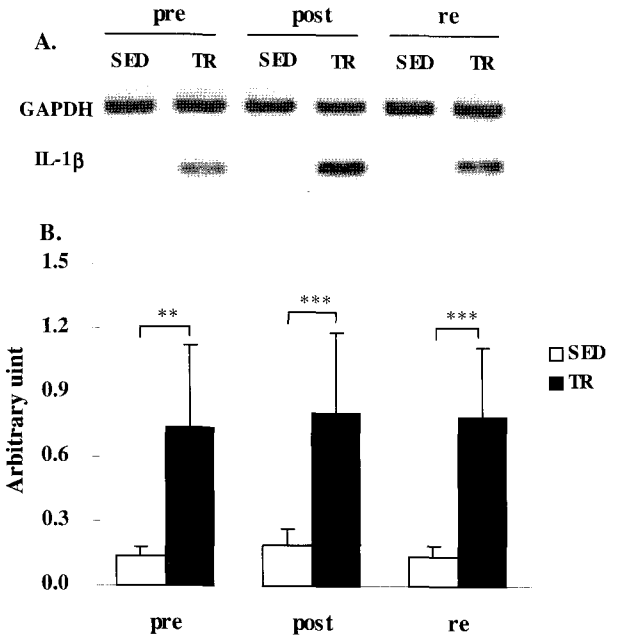

Fig. 3. RT-PCR of IL-1 β in blood. A. Gel electrophoresis of IL-1 β and GADHP-amplified products. Total RNA was isolated from blood. pre, pre-exercise; post, post-exercise; re, recovery of 30 min. And SED, untrained sedentary control subjects; TR, trained runners. B. The PCR products were scanned and then quantified with an image analyzer (quantitative data, with results expressed in arbitrary units relative to GAPDH expression, are shown, mean±SD, n=10). **$p<0.01$ and ***$p<0.001$, means significant difference between groups.

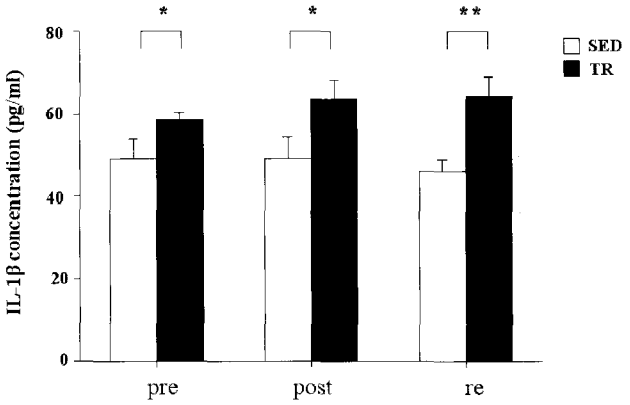

Fig. 4. Products of IL-1 β protein in serum. pre, pre-exercise; post, post-exercise; re, recovery of 30 min. And SED, untrained sedentary control subjects; TR, trained runners (mean±SD, n=10). *$p<0.05$, **$p<0.001$, means significant difference between groups.

IL-1 β mRNA and protein expression

IL-1 β gene expressions were significantly higher in TR than those in SED at pre-exercise (0.73±0.01 vs. 0.14±0.01 arbitrary unit), post-exercise (0.8±0.11 vs. 0.18±0.02 arbitrary unit) and recover of 30 min (0.78±0.12 vs. 0.13±0.01 arbitrary unit). It should be noted that the levels of mRNA are shown as arbitrary units normalized to GAPDH expression (Fig. 3, $p<0.01$ and 0.001, respectively). Furthermore, IL-1 β protein expressions were also significantly higher in TR than those in SED at pre-exercise (58.48±1.9 vs. 49.13±4.82 pg/ml), post-exercise (63.57±4.66 vs. 49.03±5.34 pg/ml) and recover of 30 min (64.26±4.75 vs. 45.97±3.00 pg/ml) (Fig. 4, $p<0.05$ and 0.01, respectively).

DISCUSSION

The physiological adaptations of heat acclimation include improved cardiac output with lowered heart rate, together with increased stroke volume, sweat rate and blood plasma volume, and decreased core temperature and mean skin temperature (Wyndham et al, 1976), rectal temperature at rest (Buono et al, 1998) and oxygen consumption at a given work rate, earlier onset of sweating during exercise and decreased sodium chloride losses in sweat and urine (Armstrong & Maresh, 1991).

In the present study, we employed physiological stress of moderate-intensity exercise to induce sweating, and the results showed that total sweat volume after exercise was higher in TR subjects than SED (Fig. 1). And the ratio of oral temperature in both groups increased in post exercise and recovery of 30 min than pre-exercise (Fig. 2). The data suggest that the oral temperature of TR subjects was lower than with SED subjects and this is caused by increased sweat in TR subjects more than SED subjects after exercise. Therefore, total sweat volume was inversely correlated with oral temperature.

Rhind et al (2001) reported that blood monocytes are the source of IL-1 β, IL-1ra, IL-6, and TNF- α production after acute strenuous exercise. Although numerous sources of cytokines have been identified *in vitro*, relatively few studies have been attempted to identify the origin of cytokines *in vivo* after exercise (Bagby et al, 1996; Pedersen et al, 1998).

In our study, IL-1 β gene transcripts and protein expression were significantly higher in TR than in SED at all 3 intervals examined (Fig. 3 and 4). Previous research has been limited to either culture supernatants (Rivier et al, 1994; Drenth et al, 1995; DeRijk et al, 1997) or urine (Sprenger et al, 1992; Weinstock et al, 1997). Citing a few, cytokine production was assessed at a cellular level in a heterogeneous cell population using a flow cytometric whole blood intracellular cytokine assay, and strong stimuli were used to assess the potential of leucocyte subsets to produce cytokines. Isolation of PBMCs or single cell lines eliminates other sources of cytokines, such as platelets and granulocytes, which have been shown to express an array of cytokines (Bordin et al, 1994; Wadhwa et al, 1996). Furthermore, some studies used bioassay techniques, which may not be specific for a single cytokine (Thorpe et al, 1992). Immunoassays of supernatants of bulk culture are of limited sensitivity, since a minor cell population produces the cytokine of interest.

The results presented herein indicate that exercise/lipopolysaccharide (LPS)-induced fever (body temperature rises) may act as a negative feedback control on the macrophage's activity. It has been suggested that hsp70 may play a role in this fever (heat) response. The release of IL-1 β from macrophage is an important step, which triggers a series of LPS-fever (heat) response. A variety of inducing signals suggests that negative regulatory mechanisms must exist to limit IL-1 β biosynthesis, however, these are as yet poorly understood. One possibility is that negative feedback

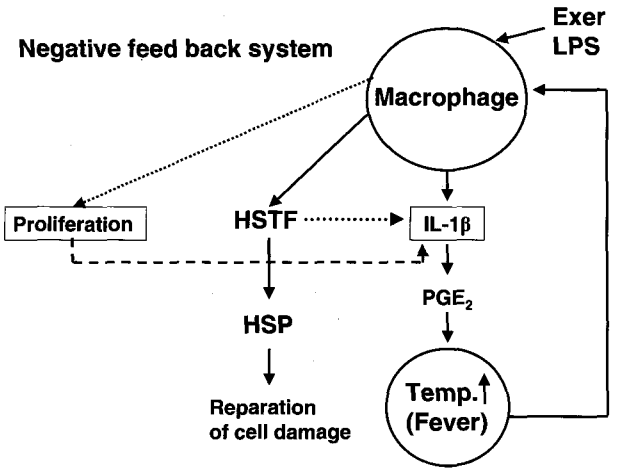

Fig. 5. A model of negative feedback system for thermoregulation with LPS, exercise and IL-1 β in macrophage. LPS, lipopolysaccharide; Exer, exercise; HSTF, heat shock transcription factor; HSP, heat shock protein; PGE_2, prostaglandin E_2; Temp., temperature.

loops exist where the IL-2 bioactivity act back on production. Such a role has been postulated for PGE_2 in response to IL-1 α and β (Knudsen et al, 1986). Schimidt and Abdulla (1988) reported that the synthesis of exercise/LPS induced IL-1 β precursor protein, p35 (and its mRNA), was down-regulated by heat shock, compared with control cells at 37°C. The above study also identified that not only the biosynthesis of pre-IL-1, p35, but also the release of mature IL-1 β was inhibited by heat shock. During heat stress, the redistribution of heat-shock transcription factor (HSTF) to heat shock element (HSE) (Wu et al, 1990) is accompanied by binding to many additional chromosomal sites and may act as a repressor of normal gene activity (Westwood et al, 1991).

Recently, several studies indicate that IL-1 β in human sweat is functionally active and derived from the eccrine sweat gland. Therefore, the existence of negative feed back loop (Fig. 5) has been suggested as one possible explanation of a self-limiting mechanism of lower temperature of exercise by long distance trained runners: exposed repeatedly (every day training) to incident of various infectious diseases, which are closely related to immune responses, and adapted to every day exercise environment.

In conclusion, our results showed that the sweating due to exercise was associated with increase of IL-1 β and correlated with decrease of oral temperature.

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