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Comparison of Interleukin-8 Levels in Long-Distance Runners and Healthy Sedentary Non-Athletic Control Subjects

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We have previously demonstrated that the level of leukocytes and neutrophils significantly increased immediately and 30 min after exercise. Interleukin-8 (IL-8) is an inflammatory cytokine that acts as a chemokine on neutrophils. In the present study, we evaluated the correlation between the number of neutrophils and leukocytes, and between the number of neutrophils and plasma IL-8 level. Long-distance trained runners (TRs, n = 10) and untrained sedentary control subjects (SEDs, n = 10) ran for one hour at 70% of heart rate reserve. In the TR, the number of neutrophils correlated significantly with the number of leukocytes in the blood. However, there was no correlation between the number of neutrophils and the plasma IL-8 concentration in both groups. Expressions of IL-8 protein and mRNA were markedly higher in the TRs as compared to the SEDs at three time intervals (pre-exercise, immediately after exercise, and post exercise). In conclusion, our results show that 1) the neutrophil level was dependent on the level of leukocytes 2) there was no correlation between the neutrophils count and plasma IL-8 concentration and 3) a higher plasma IL-8 level in athletes may be a unique characteristic of intensive training.

Key Words: Interleukin-8, Leukocyte, Exercise, Neutrophil, Trained runner, Plasma

INTRODUCTION

Chemokines and their receptors play important roles in many processes including immunosurveillance as well as trafficking, recruitment and activation of specific cell populations during inflammation. The cytokines can be divided into five classes, depending on the arrangement of the amino acid cysteine in the amino-terminal region: CXC chemokines, CC chemokines, C chemokines, CX3C chemokines, and virus-encoded chemokines (Wells et al, 1999).

Interleukin-8 (IL-8), called neutrophil-activating peptide-1 or SCYB8, is a tissue-derived peptide secreted by several types of cells in response to inflammatory stimuli (Modi et al, 1989; Modi et al, 1990). IL-8 belongs to a subgroup of CXC chemokines, and this subgroup has the amino acid sequence glu-leu-arg (ELR) preceding the first conserved cysteine amino acid residue in the primary structure of these proteins (Baggiolini, 2001).

IL-8 is one of a family of 13 human CXC chemokines. These small basic heparan-binding proteins are proinflammatory and mediate primarily the activation and migration of neutrophils into tissue from peripheral blood. Radiation-hybrid mapping has indicated that 11 of the 13 genes for these proteins reside on 4q, spanning a region of approximately 2.75 Mb (Modi & Chen, 1998).

An increase in the levels of IL-8 has been associated with exercise (Simiti et al, 1998; Mucci et al, 2000; Akerstrom et al, 2005). However, it is unclear what kinds of differences in plasma IL-8 levels exist between well-trained athletes and non-athletic subjects at resting or after exercise. In addition, it is not clear whether there is any correlation between the number of neutrophils and plasma IL-8 concentration.

We previously demonstrated that the number of leukocytes and neutrophils was significantly increased immediately and 30 min after exercise (Shin et al, 2004). In this study, we evaluated the correlation between the number of neutrophils and plasma IL-8 concentration, and measured the expression of IL-8 protein and mRNA in the blood from two subject groups?long-distance trained runners (TRs) and untrained sedentary control subjects (SEDs).

METHODS

Subjects

Ten well-trained male athletes (TRs; mean age, 21.3 ± 1.1 yrs; mean height, 174.3 ± 7.1 cm; mean weight, 62.6 ± 3.9 kg) were recruited from the Korea National Sport University. Athletes lived in a dormitory and trained

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ABBREVIATIONS: HRR, heart rate reserve; IL-8, interleukin-8; MCP-1, monocyte chemoattractant protein-1; SEDs, untrained sedentary control subjects; TRs, long-distance trained runners.

regularly. They usually trained 3 h per day, 6 days per week throughout year. Ten healthy but sedentary age-matched male college students were selected as control subjects (SEDs; mean age, 22.4±1.5 yrs; mean height, 174.9± 5.3 cm; mean weight, 72.7±11.0 kg). The SEDs had not performed regular physical exercise for at least 3 years before the study commenced. All subjects did not have any physical activity during the last 3 days before the collection of basal blood samples. All of the subjects read and signed an informed consent regarding the purpose of the study and the procedures. Great attention was paid 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 into the experimental room, subjects wore light clothing and rested quietly for 60 min before the experiment commenced.

After lunch, on the experimental day, subjects arrived at the laboratory in the afternoon at 2-5 P.M. All subjects performed one bout of prolonged running on a treadmill (Quinton, Bothell, WA, USA). Subjects undertook running for 1 h at 70% intensity of heart rate reserve (HRR). Each target heart rate was calculated from the use of the Karvonen formula and was maintained during the run. Heart rates were monitored by the polar system (Finland) throughout the experiment. Body weight and oral temperature were measured immediately before and immediately after exercise, and 30 min post-exercise cessation. When the body weights were measured, the subjects wore only running pants.

Blood

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

RNA isolation and PCR

Total RNA was extracted from the blood using Trizol reagent (Invitrogen., Carlsbad, CA, USA), and the RNA was dissolved in diethylpyrocarbonate (DEPC, Sigma, St. Louis, MO, USA) treated water. The RNA concentration was quantified by measuring the optical density at 260 nm. cDNA was produced with reverse transcriptase from the RNA samples (1 μg) using a Ready-To-Go™ T-Primed First-Strand Kit (Pharmacia Biotech, Uppsala, Sweden) in a 33 μl volume according to the manufacturer's instructions. We performed the PCR reactions in a volume of 25 μl with 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 of primer and 0.1 units 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-8, 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 DNA products were analyzed after separation by 1% agarose gel electrophoresis and ethidium bromide staining. The amount of DNA product produced was quantified using a Flour-S® MultiImager (Bio-Rad, Richmond, CA, USA). All PCR products measured were normalized to the amount of GAPDH. The following primers were used: 5'-GGTGAA-GGTCGGAGTCAACG-3' and 5'-CAAAGTTGTCATGGATG-ACC-3' for GAPDH, 5'-ATGACTTCCAAGCTGGCCGTGGCT-3' and 5'-TCTCAGCCCTCTTCAAAAACTTCTC-3' for IL-8. The expected size of the PCR products was 465 bp for GAPDH and 162 bp for IL-8.

Measurement of interleukin-8 by ELISA

Plasma IL-8 was measured using a quantitative sandwich enzyme immunoassay kit (R & D Systems, San Diego, CA, USA). The lower limit of detection is 3.13 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%.

Statistical analysis

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

RESULTS

Correlation between the number of leukocytes and neutrophils, and plasma IL-8 protein concentration

In the TRs, the number of neutrophils in blood correlated significantly with the number of leukocytes ($p<0.01$, R= 0.724, Fig. 1A; $p<0.05$, R=0.705, Fig. 1B; $p<0.01$, R=0.763, Fig. 1C; $p<0.05$, R=0.614, Fig. 1E and $p<0.001$, R=0.936, Fig. 1F respectively). However, there was not a significant correlation for the SEDs (Fig. 1D). When the entire study population were examined, no correlation between the number of neutrophils and plasma IL-8 protein concentration was found ($p>0.05$, R=0.129).

IL-8 mRNA expression

IL-8 gene expression was significantly higher in the TRs than that with the SEDs at all three intervals examined ($p<0.01$, respectively). After a bout of exercise, there was no alteration of IL-8 mRNA expression in both groups (Fig. 2).

IL-8 protein production

As with IL-8 gene expression, the production of IL-8 protein was significantly higher in the TRs than that with the SEDs at all three intervals examined ($p<0.01$, respectively). After a bout of exercise, there was no alteration of IL-8 protein production in both groups (Fig. 3).

DISCUSSION

Exercise influences immune function, and the effect of exercise on immune function depends on the intensity and time duration of exercise. Regular moderate exercise enhances

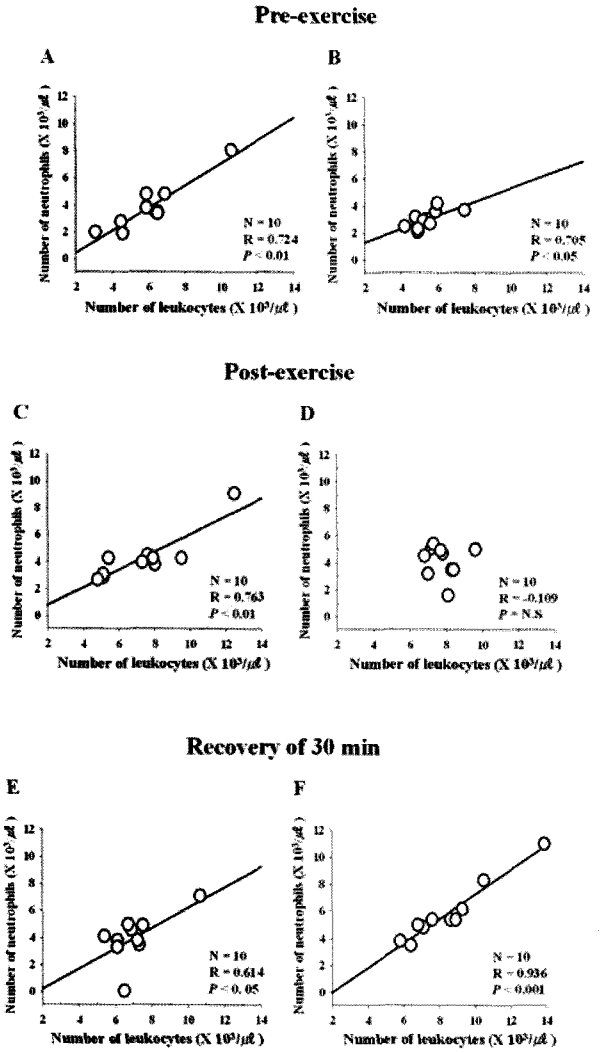

Fig. 1. Correlations between the number of neutrophils and leukocytes in blood from the TRs (n = 10) (A, C, E) and SEDs (n = 10) (B, D, F). Statistical analysis was performed using the Spearman's rank test. For the SEDs in (D), there is no significant (N.S.) difference from post-exercise.

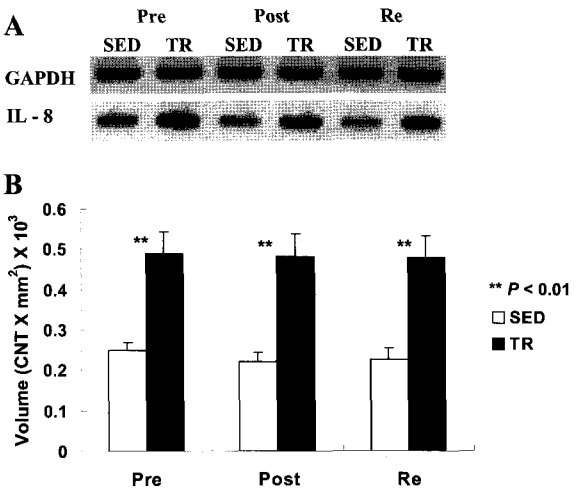

Fig. 2. Changes in IL-8 mRNA expression in plasma of TR and SED following exercise. (A) RT-PCR of the 289bp IL-8 DNA product detected in plasma. Pre: pre-exercise; Post: post-exercise; Re: recovery after 30 min; SED: untrained sedentary control subjects, TR: trained runners. (B) The gel separating the PCR products was scanned and then quantified with an image analyzer (mean ± SD). **p<0.001, indicates a significant difference between groups.

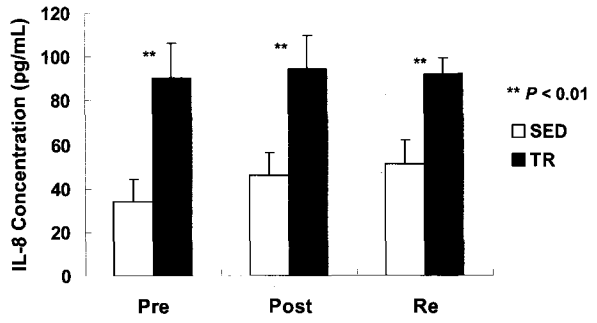

Fig. 3. Effect of exercise on plasma IL-8 protein level in SED and TR. Products of IL-8 protein detected in plasma. Pre: pre-exercise; Post: post-exercise; Re: recovery after of 30 min (mean ± SD). **p <0.001 indicates a significant difference between groups.

immune function, while intense exercise can suppress immune function. Generally, exercise perturbs the immune system and results in leukocytosis and neutrocytosis. Neutrophils represent 50~60% of the total circulating leukocyte pool. These cells are part of the innate immune system, essential for host defense, and involved in the pathology of various inflammation conditions.

We have previously showed that the number of leukocytes and neutrophils are significantly increased immediately and 30 min after exercise (Shin et al, 2004). The present results showed that the number of neutrophils was strongly correlated with the number of leukocytes (Fig. 1), suggesting that neutrophils are the predominant fraction of leukocytes at rest and during exercise.

Chemokines are cytokines with chemotactic activity, and IL-8 is the prototype of chemokines with a specific and potent chemotactic activity for neutrophils (Baggiolini & Clark-Lewis, 1992). Studies in humans (Geiser et al, 1993; Tan and Davidson, 1995), rats (Watanabe et al, 1991), and dogs (Thomsen et al, 1991) showed that their chemotactic activity is more powerful than other neutrophil chemotactic substances such as platelet activating factor and leukotriene B4 (Thomsen et al, 1991; Watanabe et al, 1991; Baggiolini & Clark-Lewis, 1992; Geiser et al, 1993; Tan & Davidson, 1995).

Exercise induces the expression of IL-8 and increases the number of neutrophils in plasma. However, in the present study there is no linear correlation between IL-8 concentration and the number of neutrophils in plasma after stimulation exercise by Peake et al. (2005) reported that, although there was a significant increase in the neutrophils count after downhill running, there was no change in neutrophil receptor expression and plasma IL-8 levels. Niess et al. (2003) demonstrated that 60 min treadmill running at 75% VO_2max

significantly increased the neutrophils count but not the level of IL-8, Suzuki et al. (2003) reported that both the plasma IL-8 level and number of neutrophils significantly increased after a marathon race. On the contrary, however, our present results, on the change in the number of neutrophils and the level of IL-8 after exercise was different from those above: Our results suggested that there might be a difference between the systemic plasma IL-8 level and number of blood neutrophils, but IL-8 alone did not induce neutorphil production.

It has been reported that many cytokines which recruit and prime neutrophils are secreted after exercise (Suzuki et al., 2000; Suzuki et al., 2002; Suzuki et al., 2003). As for humoral mediators of neutrophil mobilization, concentrations of growth hormone (GH), cortisol and granulocyte colony-stimulating factor (G-CSF) have been shown to increased after exercise. In addition, concentrations of neutrophil-priming substances (IL-6, IL-8, G-CSF, GH and prolactin) have also been reported to increase (Suzuki et al, 2003) after exercise. Therefore, we couldn't explain clearly why there was a discrepancy in alteration between plasma IL-8 level and the number of neutrophils. However, it is highly possible that intensity and types of exercise may contribute to that.

The plasma concentration of IL-8 increases in response to exhaustive running exercise, composed of eccentric and concentric components (Nieman et al, 2001; Ostrowski et al, 2001; Nieman et al, 2003; Suzuki et al, 2003). Incremental bicycle ergometer exercise to exhaustion resulted in a small increase in the plasma IL-8 concentration (Mucci et al, 2000), whereas the plasma IL-8 concentration remained unaltered during rowing or bicycle exercise for 1 or 2 h (Henson et al, 2000; Chan et al, 2004). These studies indicated that concentric exercise alone is not related to a marked increase in IL-8 plasma concentration (Akerstrom et al, 2005). Furthermore, there is disagreement about the expression of IL-8 between plasma and skeletal muscle. Skeletal muscle IL-8 mRNA increases in response to 1 h of bicycle exercise without affecting the IL-8 plasma concentration (Chan et al, 2004). Contracting skeletal muscle responds to a 3 h treadmill run by several fold increase of IL-8 mRNA, concomitantly with increased plasma levels of IL-8 (Nieman et al, 2003). As IL-8 is produced by most tissues, including skeletal muscle, monocytes, marcrophage, vascular tissue, and adipose tissue (Baggiolini et al, 1994; Bruun et al, 2001), and is partially released from these tissue or cells, it is a matter of course that there was no coincidence between expression of IL-8 in plasma and local tissue. A further study is needed to clarify this discrepancy.

Our present results showed that IL-8 mRNA levels were markedly higher in the TRs than those in the SEDs (Fig. 2). Moreover, IL-8 protein levels at pre-exercise, immediately after exercise, and post exercise were significantly higher in the TRs than those in the SEDs (Fig. 3). Overall, higher protein and mRNA levels of IL-8 protein levels were found in the TRs. These results are in good agreement with previous studies (Mucci et al., 2000; Nieman et al., 2006; Cox et al., 2007). Nieman et al. (2006) reported that mRNA and protein expression of IL-8 increased in athletes, and Mucci et al. (2000) showed that plasma levels of interleukin-1 beta (IL-1β) and IL-8 in arterial blood in highly trained athletes were higher at resting and during exercise, which was related to the increased histamine release. Releases of histamine, IL-8, and IL-1β during exercise reflect an inflammatory reaction. In our present study, we didn't analyze blood histamine levels, however, we found that plasma levels of IL-1β (Bae et al., 2006; another paper of ours) and IL-8 in trained athletes were up-regulated, as shown by Mucci et at. (2000). Cox et al. (2007) demonstrated that IL-8 concentration was higher in trained distance runner at resting and during exercise than that with illness-prone runners, suggesting enhanced inflammatory response to exercise stimulation. As one of indicators of inflammation, plasma IL-8 indicates immunologically responsive activation. Because higher IL-8 level means not only processing of inflammation but also representing the capacity of immune function in response to exercise, the higher level seems to imply adaptation to the exercise. Therefore, exercise training may be attributable to a unique characteristic of higher IL-8 level in athletes.

Increment of IL-8 in disease patients as well as athletes has been reported. However, there seems to be a difference between physiological adaptation and pathological phenomena. Epidemiological studies have shown that increasing levels of physical activity are associated with reduced inflammation in healthy subjects (Abramson & Vaccarino, 2002; Ford, 2002). On the other hand, in patients with heart failure and increased plasma levels of chemokines (Damas et al, 2000), modest exercise has been reported to reduce peripheral markers of inflammation, such as MCP-1 (monocyte chemoattractant protein-1) (Adamopoulos et al, 2001). Trøseid et al. (2004) demonstrated that exercise reduces plasma levels of MCP-1 and IL-8 in subjects with metabolic syndrome. However, these anti-inflammatory effects through exercise intervention seem to be not applicable to athletes.

In conclusion, our results showed that the number of neutrophils was dependent on the number of leukocytes, and that there was no correlation between the neutrophils count and level of plasma IL-8. A higher plasma IL-8 level in athletes may be a unique characteristic of intensive training.

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