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ORIGINAL ARTICLE

Elevation of interleukin-6 and attenuation of tumor necrosis factor-α during wheelchair half marathon in athletes with cervical spinal cord injuries

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Study design: Nonrandomized study.
Objectives: The purpose of this study was to determine the effects of long and intensive exercise on interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in athletes with cervical spinal cord injuries (CSCI).
Setting: The 30th Oita International Wheelchair Marathon Race.
Methods: Blood samples from six athletes with CSCI and eight athletes with thoracic and lumber spinal cord injuries (SCI) participating in wheelchair half marathon race were collected before the race, immediately after the race and 2 h after the race. IL-6, TNF-α, adrenaline and blood cell counts were measured.
Results: Monocyte count remained stable throughout the study in the CSCI group but was significantly high at 2 h after the race in the SCI group. Plasma IL-6 concentrations were significantly elevated immediately after the race in both groups, although the levels in CSCI were significantly lower than in the SCI group. Plasma adrenaline was significantly elevated immediately after the race in the SCI group but recovered at 2 h after the race. In contrast, plasma adrenaline did not change in the CSCI group throughout the study and was significantly lower than in the SCI group. Plasma TNF-α did not change throughout the study in the SCI group compared with a significant decrease at 2 h after the race in the CSCI group.
Conclusion: Long and intensive exercise increased IL-6 in the CSCI group despite the small muscle mass and lack of sympathetic nervous system. The post-race fall in plasma TNF-α in the CSCI group could be related to the inhibitory effect of rising IL-6 in the presence of normal monocyte count and stable adrenaline level.
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INTRODUCTION

Pedersen and Febbraio[1] reported recently that cytokines and other peptides are produced, expressed and released by muscle fibers (collectively termed 'myokines') during exercise and that these compounds exert paracrine, autocrine or endocrine effects. Our studies also demonstrated the beneficial effects of exercise and/or sports activities in individuals with thoracic and lumber spinal cord injuries (SCI),[2] and suggested that the mechanisms of the beneficial effects seem to depend on skeletal muscle interleukin-6 (IL-6) production during exercise and/or sports activities in individuals with SCI. Whether skeletal muscle production of IL-6 translates into any increase in serum IL-6 remains to be determined.

Interestingly, Umemoto *et al.*[3] reported previously in a study of SCI individuals that 2-h arm crank ergometer exercise at 60% of maximum oxygen consumption (VO_2max) significantly increased plasma IL-6 but not plasma tumor necrosis factor (TNF). In addition, Sasaki *et al.*[4] demonstrated that wheelchair full and half marathon race in SCI athletes increased IL-6 but not TNF-α. These two studies suggest that upper arm exercise in SCI induces an increase in IL-6 but not in TNF-α. However, the physiological features of individuals with cervical spinal cord injury (CSCI) are quite different from SCI, because individuals with CSCI do not have complete skeletal muscles even in upper extremities and their sympathetic nervous system does not work well during exercise.[5] These differences could involve adrenaline and IL-6 response in the CSCI group.

In able-bodied individuals, the rise in IL-6 in contracting muscle during exercise is dependent on exercise intensity and duration.[1] Based on these two factors, we hypothesized that because of the relatively small muscle volume in the CSCI group, as compared with the SCI group, exercise is not likely to increase IL-6 during exercise. Actually, Kouda *et al.*[6] reported that 20-min arm exercise at 60% of VO_2max in individuals with CSCI did not increase IL-6.

However, long and intensive exercise might increase circulating IL-6 during and after exercise in individuals with CSCI. We have studied previously a few CSCI individuals and reported that some could not complete the half marathon race.[5] In this regard, a few international wheelchair events hold half marathon division for CSCI athletes. The wheelchair half marathon race is one of the most difficult sports and a well-organized event of all sports activity for individuals with SCI and CSCI. The Oita International Wheelchair Marathon Race holds half marathon division for wheelchair CSCI athletes in Japan.

The purpose of the present study was to determine the effects of long and intensive exercise on IL-6 in the presence of sympathetic

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nervous system dysfunction. Six individuals with CSCI and eight with SCI were studied during wheelchair half marathon race.

MATERIALS AND METHODS

Subjects

Six CSCI and eight SCI Japanese athletes were provided with details of the study protocol and possible risks, and they signed the informed consent form before the study and voluntarily participated in the present study. All subjects participated and completed the half marathon division of the 30th Oita International Wheelchair Marathon Race in Japan. All subjects were involved in a regular physical training program before the race. The subject characteristics are shown in Table 1 and there were no differences between CSCI and SCI groups with respect to age, height and weight. The selection criteria for the study were the following: (1) men; women were excluded because of possible effects of menstrual cycle-related hormonal changes on the cardiovascular, endocrine and fluid regulation systems; (2) more than 1 year after injury to avoid the potential effects of unstable mental, physical and medical condition; (3) American Spinal Injury Association (ASIA) Impairment Scale A, that is, complete spinal cord injury; and (4) all participants were free from acute infection and healthy except for SCI-related dysfunctions. Patients with CSCI, but not those with SCI, suffered paralysis of some upper extremity muscles. Thus, the mass of exercising muscles during wheelchair half marathon was, in general, smaller in CSCI than in SCI group. None took any medications that would affect the cardiovascular and endocrine responses during the study period.

Study protocol

Blood samples were collected from the antecubital vein using heparinized tubes and EDTA-2K-containing tubes in the morning before the warm-up time for the race, immediately after completion of the race (distance: 21.0975 km) and 2 h after the completion of race. The blood samples were taken in all subjects to measure IL-6, TNF-α, adrenaline and counts of blood cells. Total blood volume in each sampling period was 9 ml (3 ml for IL-6 and TNF-α, 3 ml for adrenaline and 3 ml for blood cell count).

Assays of IL-6

Blood samples for IL-6 measurement were drawn into glass tubes containing EDTA. The tubes were spun immediately at 3500 *g* for 15 min at 4 °C. The plasma was stored at −80 °C until analysis. High-sensitivity chemiluminescent enzyme immunoassay (CLEIA) kit (Fujirebio Co., Tokyo, Japan) was used for measurement of IL-6 concentration in plasma (sensitivity: 0.2 pg ml^{-1}). All measurements were performed in duplicate.

Other blood tests

Total blood cell counts were determined using a cell counter. Hematocrit was measured by centrifugation. Enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) was used to measure plasma TNF-α concentration. Catecholamines were extracted from plasma using alumina and measured by high-performance liquid chromatography using a modification of the procedure described by Hunter *et al.*[7]

Table 1 Anthropometric data

	SCI subjects	*CSCI subjects*	P-*value*
Number	8	6	
Age (years)	42.7 ± 3.2	35.8 ± 3.1	NS
Height (cm)	168.2 ± 2.9	175.3 ± 3.3	NS
Weight (kg)	58.5 ± 1.9	65.6 ± 8.2	NS
Spinal lesion	T4–L1	C6–C8	
ASIA Impairment Scale	A	A	
History of injury (months)	307.8 ± 58.5	162.3 ± 35.9	NS

Abbreviations: ASIA, American Spinal Injury Association; CSCI, cervical spinal cord injury; NS, not significant; SCI, spinal cord injury.
Data are mean ± s.e.m.

Statistical analysis and ethical considerations

Data were expressed as mean ± s.e.m. and analyzed using analysis of variance. When the results of analysis of variance tests were significant ($P<0.05$), we used Tukey's test to determine the differences between before and after race, and between before and 2 h after race. Differences between CSCI and SCI subjects were compared using Tukey's test. Correlation between plasma IL-6 and plasma adrenaline was calculated using Pearson's correlation coefficient. A *P*-value of <0.05 denoted the presence of a significant difference between two groups.

The present study was approved by the Human Ethic Committee of Wakayama Medical University.

RESULTS

The results of all analysis of variance tests in the present study were significant ($P<0.05$). Therefore, Tukey's test was performed to test the differences between before and after race, and between before race and 2 h after race.

The race time of the SCI group (1.06 ± 0.06 h) was significantly shorter than that of the CSCI group (1.44 ± 0.09 h, $P<0.01$). The mean wheelchair speed in the SCI group (20.2 km h^{-1}) was significantly faster than that in the CSCI group (14.8 km h^{-1}, $P<0.01$; Figure 1).

Hematocrit did not change throughout the study and there were no differences between the SCI and CSCI groups (Figure 2). Monocyte count remained constant through the study in the CSCI group compared with a significant increase ($P<0.05$) at 2 h after the race in the SCI group (Figure 3).

In SCI athletes, plasma IL-6 concentrations increased significantly ($P<0.01$) immediately after the race but returned to the baseline level at 2 h after the race. In comparison, plasma IL-6 concentrations of subjects with CSCI were significantly higher immediately after the race ($P<0.01$) and also at 2 h after the race ($P<0.05$), compared with the baseline. Furthermore, the mean plasma IL-6 level immediately after the race was significantly higher in SCI than in CSCI group ($P<0.05$; Figure 4).

At baseline, the mean plasma concentration of adrenaline was significantly higher in SCI (26.0 ± 5.2 pg ml^{-1}) than in CSCI (8.8 ± 0.8 pg ml^{-1}, $P<0.05$) athletes. In SCI athletes, plasma adrenaline was significantly higher immediately after the race ($P<0.01$) but returned to baseline at 2 h after the race. However, plasma adrenaline in CSCI athletes did not change throughout the study, and was significantly lower than in SCI athletes before the race, after the race and at 2 h after the race ($P<0.05$, each; Figure 5).

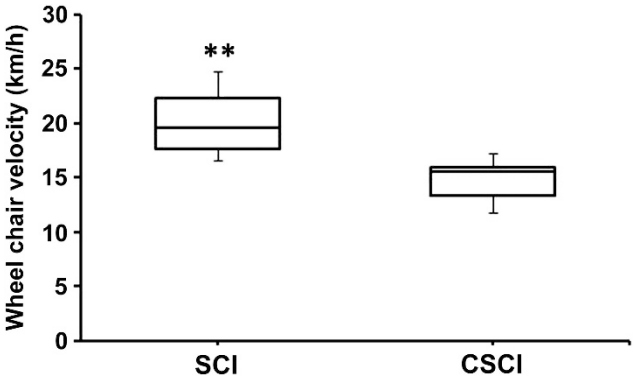

Figure 1 Box-and-whisker plots of wheelchair speed of the SCI and CSCI groups. In these plots, lines within the boxes represent median values; the upper and lower lines of the boxes represent the 25th and 75th percentiles, respectively; and the upper and lower bars outside the boxes represent the 90th and 10th percentiles, respectively. $^{**}P<0.01$ vs CSCI.

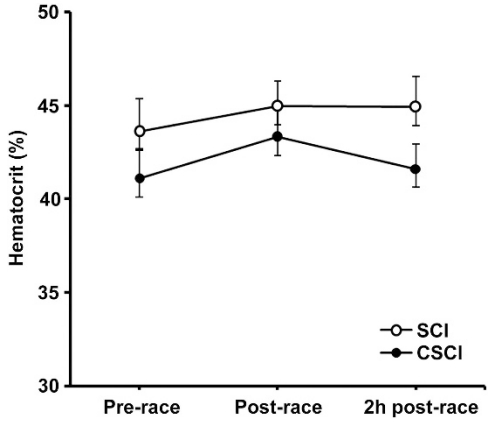

Figure 2 Hematocrit levels in patients with SCI and CSCI before the race (pre race), immediately after the race (post race) and 2 h after the race (2 h post race). Data are mean ± s.e.m.

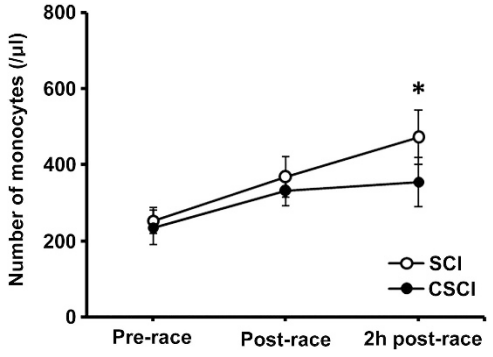

Figure 3 Monocyte counts in patients with SCI and CSCI before the race (pre race), immediately after the race (post race) and 2 h after the race (2 h post race). Data are mean ± s.e.m. $^{*}P<0.05$ vs before race in SCI.

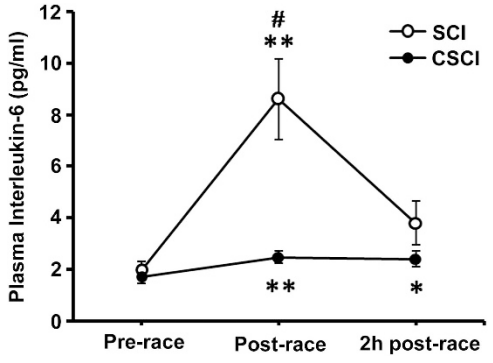

Figure 4 Plasma IL-6 concentrations in patients with SCI and CSCI before the race (pre race), immediately after the race (post race) and 2 h after the race (2 h post race). Data are mean ± s.e.m. $^{*}P<0.05$ vs before race in CSCI. $^{**}P<0.01$ vs before race in SCI and CSCI. $^{\#}P<0.05$ vs CSCI immediately after the race.

Plasma TNF-α did not change throughout the study in SCI group compared with a significant decrease at 2 h after the race in the CSCI group ($P<0.05$; Figure 6).

There was a significant relationship between plasma IL-6 and plasma adrenaline immediately after the race ($P<0.01$; Figure 7).

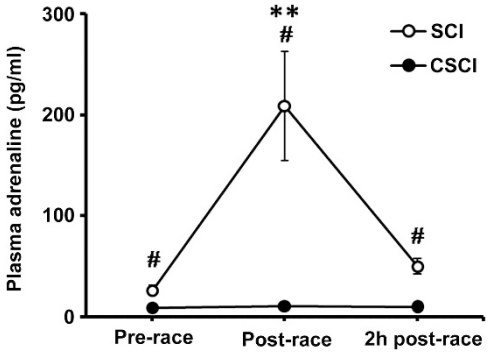

Figure 5 Plasma adrenaline t in patients with SCI and CSCI before the race (pre race), immediately after the race (post race) and 2 h after the race (2 h post race). Data are mean ± s.e.m. $^{**}P<0.01$ vs before race in SCI. $^{\#}P<0.05$ vs CSCI before the race, after the race and at 2 h after the race.

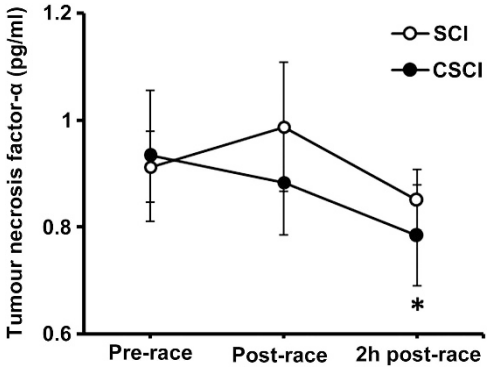

Figure 6 Plasma TNF-α in patients with SCI and CSCI before the race (pre race), immediately after the race (post race) and 2 h after the race (2 h post race). Data are mean ± s.e.m. $^{*}P<0.05$ vs before race in CSCI.

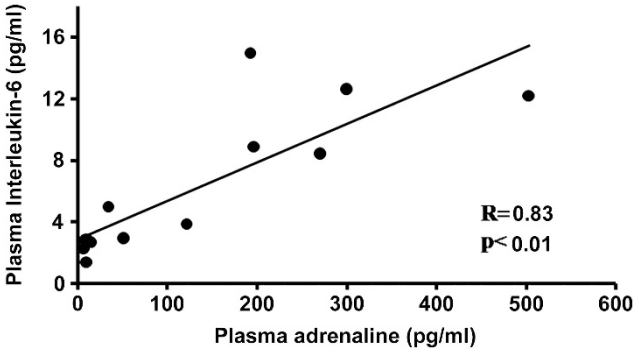

Figure 7 Relationship between plasma IL-6 and plasma adrenaline levels measured immediately after the race. Note the significant relationship between the two parameters ($P<0.01$).

On the other hand, there was no significant relationship between plasma IL-6 and wheelchair velocity, and between plasma IL-6 and monocyte count.

The above data suggest that circulating IL-6 concentrations increased during and after wheelchair half marathon race in SCI and CSCI groups but the magnitude of the increase was smaller in the CSCI than in the SCI group. Furthermore, the increase in plasma IL-6 correlated with the increase in plasma adrenaline concentration.

DISCUSSION

The present study demonstrated that strenuous upper arm exercise increased IL-6 and decreased TNF-α in the CSCI group. Other major findings of the present study were the following: (1) plasma IL-6 concentration of both SCI and CSCI groups significantly increased immediately after the race, (2) the magnitude of increase in plasma IL-6 was significantly lower in the CSCI than in the SCI group, (3) plasma adrenaline concentration was attenuated and did not change throughout the race in CSCI athletes and (4) plasma adrenaline concentrations of both groups correlated with those of IL-6.

Circulating TNF-α concentrations increase markedly and rapidly during systemic inflammation, followed by a similar increase IL-6. In contrast, during exercise, the marked increase in IL-6 is not preceded by an increase in TNF-α.[1] Keller *et al.*[8] indicated that the exercise-induced increase in IL-6 is mainly induced by IL-6 gene transcription in contracting skeletal muscles. IL-6 is produced within the contracting skeletal muscle cells and then released into the circulation and, in this respect, Febbraio and Pedersen[9] refer to IL-6 as a myokine. The present result of significant IL-6 increase immediately after the half marathon race in the SCI group was consistent with the previous studies of Sasaki *et al.*,[4] who suggested that the source of exercise-induced rise in IL-6 is the contracting muscles in SCI. The present study demonstrated that half marathon race also induced a significant increase in IL-6 in the CSCI group. Based on the above studies, we also suggest that the source of increased peripheral blood IL-6 level in the CSCI group is the contracting muscles of the upper arms.

However, the magnitude of increase in IL-6 in the CSCI group was much smaller than that seen in the SCI group immediately after the race. In able-bodied individuals, the magnitude of exercise-induced increase in plasma IL-6 is determined by the combination of mode, intensity and duration of running.[1] Exercise intensity indirectly represents the muscle mass involved in the contractile activity. Contracting skeletal muscles *per se* are an important source of IL-6 found in the plasma. In general, the higher the level of SCI, the more profound motor paresis should be below the level of the injury. Therefore, the mass of exercising muscles during wheelchair propulsion in individuals with chronic CSCI should be less than those with SCI. Furthermore, it has been reported that CSCI with long-standing injury have strikingly low muscle fiber area in the paralyzed area.[10] In other words, muscles of the trunk and lower extremities did not contract during the race in CSCI subjects, whereas the contracting muscles during the race in SCI athletes included not only those of the upper extremities but also the trunk. Therefore, the difference in plasma IL-6 concentrations in this race between SCI and CSCI groups probably reflects the difference in exercise intensity.

In addition, patients with high CSCI have sympathetic nervous system impairment because of transection of sympathetic neural pathway from the central nervous system to peripheral sympathetic nerves at the cervical spinal cord lesion, and thus catecholamine response to exercise is reduced compared with subjects with low-thoracic level SCI.[11] Actually, the present study demonstrated stable plasma adrenaline levels throughout the study in the CSCI group. Febbraio and Pedersen[9] suggested that adrenaline stimulates IL-6 gene transcription *via* β-adrenergic stimulation of protein kinase A. Therefore, impairment of the sympathetic nervous system is another mechanism responsible, at least in part, for the attenuated increase in IL-6 in the CSCI group.

Although adrenaline plays a minor role in exercise-induced increase in plasma IL-6,[12] the significant relationship between adrenaline and IL-6 levels immediately after the race suggested strong interaction with exercise-induced IL-6 production in the half-marathon race. It is also possible that sympathetic control is linked to contracting muscle mass, although such relationship needs to be confirmed in future studies.

Another new finding in this study was the significant decrease in plasma concentration of TNF-α in the CSCI group at 2 h after the race, compared with no change in the level in the SCI group. In previous studies, no significant change in TNF-α mRNA was observed in muscle samples and plasma TNF-α did not increase during exercise in healthy male subjects.[13] In fact, in most exercise studies, TNF-α is reported to remain stable during exercise.[1] Only highly strenuous, long exercise results in a small increase in plasma concentration of TNF-α.[1] However, TNF-α levels are markedly elevated in anti-IL-6-treated mice and in IL-6-deficient knockout mice, indicating that circulating IL-6 is involved in the regulation of TNF-α levels.[1] In addition, both recombinant human IL-6 infusion and exercise-induced increase inhibit endotoxin-induced increase in circulating levels of TNF-α in healthy humans.[14] To our knowledge, there are no studies that have reported inhibition of circulating levels of TNF-α by exercise-induced IL-6 in humans. One previous study showed that inhibition of TNF-α rise is caused by appearance of IL-1 receptor antagonist and the anti-inflammatory cytokine IL-10, which also increases in a manner similar to the increase in plasma IL-6.[1] Considered together with the above findings, it is possible that the attenuated TNF-α levels observed in individuals with CSCI are related to the appearance of IL-1 receptor antagonist and IL-10 following increase of IL-6 during exercise. However, as plasma TNF-α concentrations did not change throughout study in individuals with SCI, it is likely that some other mechanisms, for example, increased monocyte count, were responsible for the change seen in the CSCI group.

CONCLUSION

Plasma IL-6 concentrations of both SCI and CSCI groups increased significantly immediately after the race. Immediately after the race, plasma IL-6 levels were significantly lower in the CSCI as compared with the SCI group. The increase in plasma adrenaline and exercising muscles might explain the rise in IL-6 level. Plasma TNF-α levels were significantly lower at 2 h after the race in the CSCI group. The appearance of IL-1 receptor antagonist and IL-10 following the increase in IL-6 during muscular exercise may attenuate TNF-α in the CSCI group.

DATA ARCHIVING

There were no data to deposit.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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