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ORIGINAL ARTICLE

Attenuation of natural killer cell activity during 2-h exercise in individuals with spinal cord injuries

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Design: Non-randomized study.
Objective: To determine natural killer cell cytotoxic activity (NKCA) to 2-h arm ergometer exercise in persons with spinal cord injuries (SCI) and the underlying mechanism of such response.
Setting: University of Occupational and Environmental Health, Japan.
Methods: We examined NKCA response to 2-h arm crank ergometer exercise at 60% of maximum oxygen consumption (VO_{2max}) in SCI and able-bodied persons. NKCA and plasma concentrations of prostaglandin E_2 (PGE_2), adrenaline and cortisol were measured before, during and immediately after the exercise. The study included seven subjects with SCI between Th11 and L4 and six able-bodied persons.
Results: NKCA in able-bodied subjects increased ($P<0.05$) at 60 min of exercise and immediately after the exercise, and remained elevated up to 2 h after exercise. However, NKCA in SCI decreased ($P<0.05$) immediately after exercise but recovered at 2 h after exercise. Plasma adrenaline in both groups increased significantly ($P<0.05$) immediately after exercise and returned to baseline level 2 h after the exercise. Plasma cortisol in both groups remained constant throughout the study. In SCI, PGE_2 significantly increased immediately after 2 h exercise and returned to the baseline level 2 h after exercise; however, it remained unchanged during the test in able-bodied subjects.
Conclusion: Our results suggested that increase of PGE_2 in SCI partially contributes to NKCA.
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Keywords: adrenaline; cortisol; prostaglandin; immune system; exercise; spinal cord injuries

Introduction

Several studies have investigated the relationship between exercise and natural killer cell cytotoxic activity (NKCA) in able-bodied persons, since cytotoxicity of NK cells represents an important mechanism of natural defense against viral infections and the spread of malignant diseases. NKCA during exercise in able-bodied subjects is modulated by intensity and duration of exercise.[1,2] Severe or prolonged exercise suppresses immunological functions, while moderate or short-duration exercise activates immune functions.[3,4] Exercise in physically disabled persons contributes to the overall improvement of physical fitness and social interaction, and sports activities are highly recommended for individuals with spinal cord injury (SCI). It is widely known that persons with SCI easily develop infections of the urinary tract, respiratory tract and skin, because they seem to have weak host defense mechanisms.[5,6] In two separate studies,[7,8] we reported that a high-intensity exercise (wheelchair full marathon race) induced a transient suppression of NKCA in competitive wheelchair racers with SCI between T5 and L1, while wheelchair half marathon race induced activation of NK cell function in recreational athletes with SCI between T7 and L1. To our knowledge, there are no studies that examined NKCA during exercise of well-controlled duration and intensity in SCI subjects.

In able-bodied persons, the main factors that modulate NKCA during exercise are adrenaline, prostaglandin E_2 (PGE_2), cortisol[3,9–11] and various cytokines.[12,13] NKCA is stimulated by adrenaline,[3] suppressed by cortisol[10] and inhibited by prostaglandins.[9,11] The purpose of this study was to investigate the pattern of NKCA during exercise in SCI persons and to determine the mechanism(s) of any exercise-induced change in NKCA, including the effects of adrenaline, PGE_2 and cortisol. For this purpose, we examined NKCA response to 2-h arm ergometer exercise at 60% of maximum oxygen consumption (VO_{2max}) in SCI and able-bodied persons. NKCA and plasma concentrations of PGE_2,

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adrenaline and cortisol were measured before, during and immediately after the exercise.

Materials and methods

Subjects

The study included seven persons with SCI who were involved in a regular physical training program. We also included six able-bodied subjects as control subjects. The subject's characteristics for each group are presented in Table 1. There were no differences between SCI and able-bodied subjects with respect to age, height, weight and VO_2max. The selection criteria were the following: (1) men (women were not included in this study in order to exclude the possible influence of menstrual cycle-related hormone changes on the immune system), (2) lack of SCI other than those between Th11 and L4 so that upper limbs and excretions were intact, and (3) excellent current health and no medications that would affect the immune and endocrine responses. Informed consent was obtained from all participants and the study protocol was approved in advance by the Human Research Committee of our hospital.

Study protocol

Two weeks before the scheduled start of the study, subjects performed a progressive VO_{2max} test on an arm crank ergometer (818E, Hand ergometer, Monark, Sweden). The test protocol required the subjects to maintain a target cadence of 60 r.p.m. After a 15-min rest period, subjects performed unloaded exercise for 3 min. This was followed by increasing the power output by 10 W every minute. The test was terminated when the subjects reached exhaustion, or if the cadence fell below 60 r.p.m. O_2 uptake and ventilation were measured with respiratory metabolic cart (WLCU-5207A, Westron, Japan). Electrocardiogram (EKG) was monitored with EKG monitor (BP unit, PB14-136, NEC, Japan). Throughout the experiment, all subjects were allowed to drink water freely.

All subjects indicated that they had avoided intensive exercise for at least 24 h before the test and they were healthy and free of symptoms associated with respiratory and urinary tract infections. The subjects had their regular breakfast before 0900 and then refrained from eating but were allowed to drink tap water *ad libitum* until the start of experiment. They reported to the Human Performance Laboratory at 1000 and were outfitted with electrodes for EKG recording.

Table 1 Anthropometric data of participating subjects

	SCI subjects	*Able-bodied subjects*
Number	7	6
Age (years)	34.3±7.1	28.8±7.7
Height (cm)	169±10	172±2.7
Weight (kg)	60.6±11.5	65.2±5.6
VO_2max (ml/kg/min)	27.9±3.0	25.7±4.1
Spinal lesion	T11–L4	

Abbreviations: SCI, spinal cord injury; VO_2max, maximum oxygen consumption. Data are mean±s.e.m.

After resting in a quiet room for 30 min, subjects started to exercise on an arm crank ergometer for 2 h at intensities of 60% VO_{2max}; the power output was increased progressively from 0 W to the desired level over 3 min.

Blood samples were collected from the antecubital vein using heparinized tubes and ethylenediaminetetraacetic acid (EDTA)-2K-containing tubes before exercise, at 60 min of exercise, immediately after exercise and 2 h following completion of exercise. Total blood volume in each sampling period was 21 ml (10 ml for NKCA, 3 ml for CD16, 2 ml for counts of blood cells, 3 ml for adrenaline and cortisol, and 3 ml for PGE_2). Unfortunately, we did not obtain sufficient amount of blood samples from one SCI subject and his NKCA was not measured throughout the experiment. Six able-bodied subjects performed the same experiment as a control group.

Assays of NKCA

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized peripheral blood using a lymphocyte separation medium (Litton Bionetics, Kensington, MD, USA). The recovered cells were washed with 10 mM phosphate-buffered saline (PBS), pH 7.2, and resuspended at a concentration of 8×10^5/ml in Roswell Park Memorial Institute 1640 medium (Nissui, Tokyo, Japan) containing 10% fetal bovine serum (FBS, GIBCO, Grand Island, NY, USA). NKCA was assayed using sodium ^{51}chromate (^{51}Cr, Amersham International, Buckinghamshire, UK)-labeled T-cell leukemia cell line (MT2, donated by Dr Kimitaka Sagawa, Kurume University, Kurume, Japan), as target cells. ^{51}Cr-labeled MT2 cells (1×10^4/ml) were cultured with PBMC (8×10^5/ml) in microtiter plates (Costar, Cambridge, MA, USA) for 4.5 h at 37°C. After incubation, the culture supernatant was harvested and radioactivity was measured in an autowell gamma system (ARC-2000, Aloka, Tokyo). Spontaneous release was determined by incubating the same amount of cells in medium alone and maximum release was determined by treating the same amount of the target cells in the presence of 10% Triton X-100 (Sigma, St Louis, MO, USA). The percentage of cell lysis was calculated from the formula %lysis = [(experimental release−spontaneous release)/(maximum release−spontaneous release)] × 100, and averaged using triplicate readings.

Cell-surface marker analysis by flow cytometry

Staining of subsets of the obtained PBMC and flow cytometric analysis were performed according to the standard procedures as described previously.[3] Briefly, PMBC (1×10^6) were incubated with or without fluorescent CD16 monoclonal antibody Leu-11 (Becton Dickinson, Mountain View, CA, USA) in PBS containing 1% FBS and 0.2% NaN_3 (Sigma) for 45 min at 4°C. After washing the cells twice with the medium, cell staining was detected using a fluorescence-activated cell sorter/analyzer (FACScan, Becton-Dickinson). Amplification of the antibody binding was performed with a three-decade logarithmic amplifier. Results are expressed as the percentage of fluorescence-positive cells.

Other blood tests

Total blood cell counts were determined using a cell counter. Hematocrit (Hct) was measured by centrifugation. EDTA-2K blood samples were prepared on slides and stained using the Wright–Giemsa method to determine the percentage of neutrophils, total lymphocytes and monocytes. 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*.[14] Plasma cortisol levels were assayed using a competitive solid phase ^{125}I radioimmunoassay technique (Dainabot Lab., Tokyo). Mononuclear cells (10^6 cells/ml) were incubated at 37°C for 3 h, centrifuged and the concentration of PGE_2 in the supernatant was determined by radioimmunoassay (PGE2 Ria kit Dupnt).[15]

Statistical analysis

Data were expressed as mean±s.e.m. and analyzed using a 2×4 repeated measures analysis of variance (ANOVA). When the results of ANOVA tests were significant ($P<0.05$), we used the Sheffe's test to determine differences between pre-exercise and each time period, and between two groups (control and exercise). A *P*-value less than 0.05 denoted the presence of a significant difference between two groups.

Results

Changes in blood cell counts

Table 2 lists the results of blood cell counts of participants on four different time intervals: before exercise, during 60 min of exercise, immediately after exercise and 2 h after exercise. Red blood cell (RBC) counts, hemoglobin (Hb) levels and Hct were similar at all time intervals in SCI and able-bodied subjects. In SCI subjects, the absolute number of leukocytes markedly increased immediately after the exercise, compared with pre-exercise counts, and remained high until 2 h after the exercise. In able-bodied subjects, the absolute number of leukocytes markedly increased immediately after the exercise compared with pre-exercise count and the number remained high until 2 h after the exercise. In SCI and able-bodied subjects, the absolute number of peripheral neutrophils was markedly augmented immediately after the exercise and 2 h after the exercise. The absolute number of peripheral neutrophils increased immediately after exercise ($P<0.05$) and returned to the pre-exercise level after 2 h of exercise.

Changes in NK cell count and NKCA

Figure 1 displays the number of peripheral NK cells at the aforementioned four different time intervals in SCI and able-bodied subjects. The absolute numbers of NK cells before exercise, immediately after exercise and 2 h after exercise were significantly lower in SCI subjects than in able-bodied subjects. In SCI subjects, the absolute number of NK cells did not change throughout the experiment.

The results of NKCA (Figure 2) did not correlate with those of the absolute number of NK cells (Figure 1). At baseline, NKCA was significantly higher in SCI subjects than in able-bodied subjects. In able-bodied subjects, NKCA increased ($P<0.05$) at 60 min of exercise and immediately after the exercise and remained high 2 h after exercise. However,

Table 2 Changes in blood cell count, hemoglobin, hematocrit and leukocyte subpopulations in SCI and AB during 2-h arm ergometer exercise at 60% VO_2max

	Before exercise	*During 60 min of exercise*	*Immediately after exercise*	*Recovery (2 h after exercise)*	P-*value*
RBC ($\times 10^{10}$/l)					
SCI	497±50	524±58	518±53	499±53	NS
AB	510±23	524±27	540±34	506±17	NS
Hemoglobin ($\times$ 10 g/l)					
SCI	14.9±1.4	15.6±1.7	15.5±1.3	14.8±1.3	NS
AB	15.8±0.8	16.3±0.8	16.6±0.9	15.7±0.6	NS
Hematocrit (%)					
SCI	47.6±3.4	49.8±3.2	49.3±3.3	47.2±3.2	NS
AB	53.3±2.3	54.9±2.4	56.2±3.0	53.0±1.6	NS
Leukocytes ($\times 10^9$/l)					
SCI	6.6±1.5	7.6±1.3	11.5±4.1#	10.0±2.3*	
AB	5.8±0.7	5.6±1.1	8.9±2.9*	8.7±2.2*	
Lymphocytes ($\times 10^9$/l)					
SCI	1.6±0.6	1.9±0.5	3.0±1.8*	2.2±0.7	
AB	2.2±0.4	1.9±0.4	2.3±0.4	2.4±0.3	
Monocytes ($\times 10^6$/l)					
SCI	281±117	344±228	502±325	468±372	
AB	240±64	287±117	349±165	250±88	
Neutrophils ($\times 10^9$/l)					
SCI	4.5±1.6	5.3±1.6	7.9±2.9#	7.2±2.0*	
AB	3.1±0.7	3.2±0.7	6.0±2.6*	5.8±2.3*	

Abbreviations: AB, able-bodied subjects; NS: no significant differences between values at all phases; RBC, red blood cells; SCI, spinal cord injury; VO_2max, maximum oxygen consumption.
Values are mean±s.e.m. *P*-value is for time × group interaction.
*$P<0.05$, #$P<0.01$, compared with baseline.

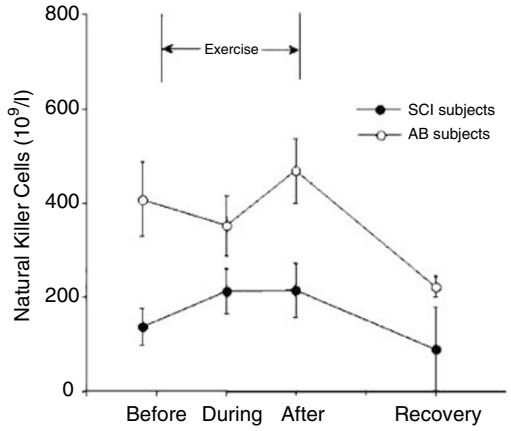

Figure 1 Number of NK cells during physical exercise (arm ergometer, 60% of VO_{2max}, 60 min) in SCI subjects and able-bodied subjects (AB). Data are mean ± s.e.m.

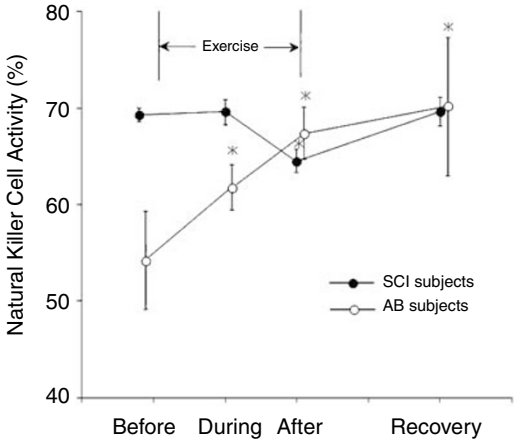

Figure 2 NK cells activity response to acute physical exercise (arm ergometer, 60% of VO_{2max}, 60 min) in SCI subjects and able-bodied subjects (AB). *$P<0.05$, compared with baseline (before exercise). Data are mean ± s.e.m.

NKCA in SCI subjects decreased ($P<0.05$) immediately after the exercise and recovered to the baseline level.

Adrenaline, cortisol and PGE_2

At baseline, there were no differences between able-bodied and SCI subjects with regard to the mean concentrations of cortisol (Figure 3), adrenaline (Figure 4) and PGE_2 (Figure 5). In both groups, plasma adrenaline concentrations significantly increased ($P<0.05$) immediately after exercise and returned to the baseline level 2 h after the exercise. On the other hand, the plasma concentration of cortisol did not change throughout the study in both groups. PGE_2 demonstrated different behavior immediately after 2-h ergometer exercise between able-bodied and SCI subjects (Figure 5). In SCI subjects, PGE_2 significantly increased immediately after exercise and returned to the baseline level at 2 h after exercise (Figure 5). In contrast, PGE_2 in able-bodied subjects did not change after exercise.

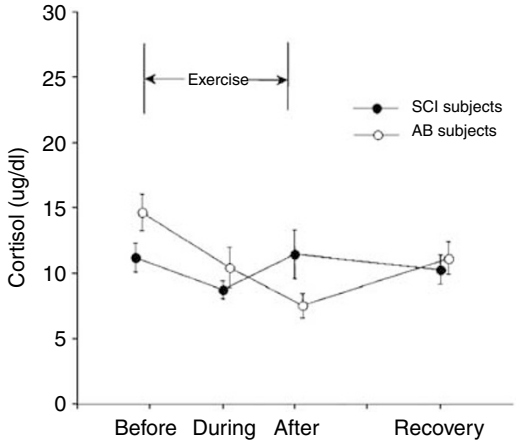

Figure 3 Plasma cortisol response to acute physical exercise (arm ergometer, 60% of VO_{2max}, 60 min) in SCI subjects and able-bodied subjects (AB). Data are mean ± s.e.m.

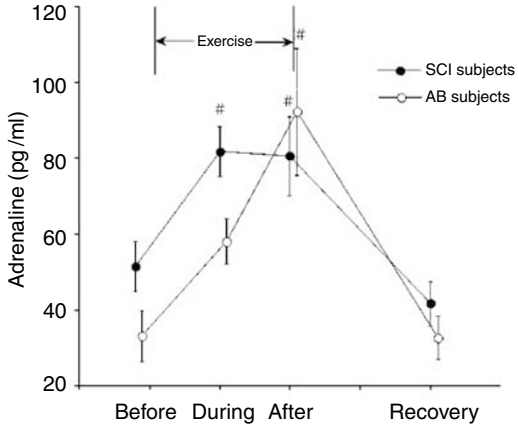

Figure 4 Plasma adrenaline response to acute physical exercise (arm ergometer, 60% of VO_{2max}, 60 min) in SCI subjects (SCI) and able-bodied subjects (AB). #$P<0.01$, compared with baseline (before exercise). Data are mean ± s.e.m.

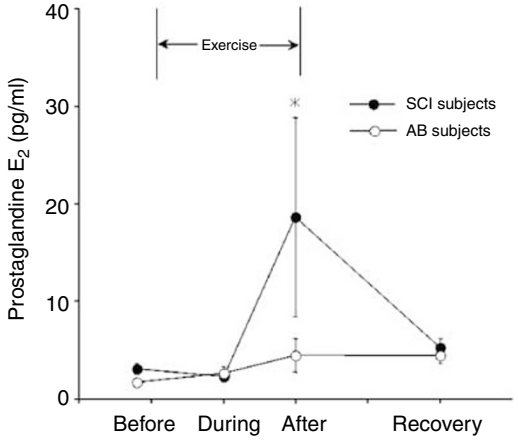

Figure 5 PGE_2 response to acute physical exercise (arm ergometer, 60% of VO_{2max}, 60 min) in SCI subjects and able-bodied subjects (AB). *$P<0.05$, compared with baseline (before exercise). Data are mean ± s.e.m.

Discussion

The major finding of the present study was that in SCI subjects, NKCA significantly decreased after 2-h arm crank ergometer exercise at 60% VO_{2max}, compared with a stable NKCA in able-bodied subjects after the exercise. However, the suppression of NKCA during exercise in SCI subjects returned to the baseline level at 2 h after post-exercise rest. This result adds support to our findings in a previous study,[7] in which NK cells and NKCA decreased immediately after a wheelchair full marathon in competitive wheelchair racers with SCI between T5 and L1, and then recovered to baseline values after just one night of rest. Considered together, our findings suggest that activation of certain immunosuppressive factors and/or attenuation of immunostimulants resulted in reduced NKCA immediately after exercise in SCI subjects and that such factors are not operational in able-bodied subjects.

In agreement with the findings of other groups, the number of NK cells increased in parallel with a rise in adrenaline following exercise.[2,3,15,16] Kappel *et al*.[16] reported that adrenaline administration in eight healthy individuals increased NKCA and such rise correlated with plasma adrenaline concentrations. Thus, increased concentrations of adrenaline during exercise resulted in significant increase of NKCA.[2,3,15,16] In the present study, plasma adrenaline concentrations increased significantly ($P<0.05$) immediately after exercise in both SCI and able-bodied subjects. However, NKCA increased after 2-h exercise in able-bodied subjects while it decreased in SCI subjects. This finding suggests that certain suppressive factors directed against NKCA could eliminate the adrenaline-induced activation of NKCA and result in a decrease of NKCA in SCI subjects.

Nieman *et al*.[17] showed that NKCA correlated negatively with serum cortisol levels, and hence a large exercise-induced surge in cortisol secretion could contribute to the suppression of NKCA. However, plasma cortisol in both SCI subjects and able-bodied persons did not increase throughout our study. Had we utilized resting controls, a drop in cortisol could have been observed, compared with exercising subjects. Thus, the higher levels of cortisol might have exerted some influence in our study. In this regard, several studies have shown that NKCA is higher in highly fit subjects.[18,19]

Previous studies showed that prostaglandins from activated monocytes and neutrophils that have migrated into damaged tissue might contribute to the reduction of NKCA.[9,11] In the present study, prostaglandins significantly increased after exercise in SCI subjects but not in able-bodied persons. Since the intensity of exercise in SCI subjects was similar to that of able-bodied subjects, we suggest that arm cranking during ergometer exercise in SCI subjects could result in compression of the tissue in paralyzed trunk, hip and lower limbs and induce distress and/or microdamage in the paralyzed area. The extent of the damaged tissue during exercise in SCI subjects might be greater on the paralyzed area compared with the normal part. Thus, we suggest that increased prostaglandin concentrations after exercise in SCI subjects contributed to the post-exercise fall of NKCA.

The reduction of NKCA closely parallels the drop in blood NK cell count, implying that each NK cell retains normal function.[16] In the present study, NKCA in SCI subjects decreased immediately after 2 h exercise; however, the number of NK cell at that period was stable and similar to that before exercise. Thus, the cytotoxic function of NK cells might be suppressed only during the exercise. We considered that the number of NK cells in peripheral blood that had been mobilized from the lymphatic system during exercise was equal to the number of NK cells moved to damaged tissue from peripheral blood in SCI subjects. Moyna *et al*.[20] reported that since NK cells from the spleen and bone marrow during exercise were immature, their cytotoxic activity must be lower than that of circulating NK. Therefore, 2-h arm ergometer exercise in SCI subjects reduced NKCA although the number of NK cells did not decrease after exercise.

Our results showed that under resting conditions (before exercise), the number of NK cells in SCI subjects was significantly lower ($P<0.001$) than in able-bodied subjects, while NKCA in SCI subjects before exercise was significantly higher than in able-bodied subjects. Kliesch *et al*.[21] reported the presence of a depressed immune state in individuals with SCI, compared with neurologically intact controls. The present finding of activated NKCA at rest in SCI subjects was not consistent with the above report. Our SCI subjects were well trained and their VO_{2max} was similar to that of able-bodied subjects, suggesting that the high daily activity was responsible for the increased cytotoxic activity per cell. Schmid *et al*.[22] reported that plasma adrenaline concentrations in most SCI patients are attenuated; however, plasma adrenaline concentrations in SCI subjects before exercise were similar to those of able-bodied subjects. Therefore, adrenaline in SCI subjects already activated before exercise and the relative elevation of adrenaline might contribute to increase the NKCA in SCI subjects before exercise. The increased cytotoxic activity per each cell in SCI subjects might reflect adaptation to reduced cell number of NK cells and compensation for the decreased number of NK cell in SCI subjects.

Nieman *et al*.[23] reported that NKCA was not different between endurance athletes and non-athletic subjects, and found no relationship between VO_{2max} and NKCA. However, it has been reported that persons with high VO_{2max} have a constantly elevated NKCA.[19] Our study design eliminated any possibility for different VO_{2max} to alter the results, and the average VO_{2max} of upper limbs exercise in able-bodied persons was similar to that of SCI subjects. Therefore, differences in VO_{2max} and exercise intensity between SCI and able-bodied subjects cannot explain the aforementioned findings in NKCA.

Loading of upper extremities in SCI subjects is usual in their daily use of wheelchair and the cranking arm ergometer is unusual for able-bodied persons. Before the present study, we were concerned that 60% VO_{2max} workload of arm ergometer in able-bodied subjects was much harder than in SCI subjects. However, all able-bodied subjects completed 2-h arm ergometer exercise in this study; thus, the present work load of upper limbs was optimal in order

to study NKCA during arm exercise in SCI and able-bodied subjects.

It should be noted that Hct, Hb and RBC remained unchanged throughout the study in both groups. These results suggest that dehydration did not occur during the exercise, a finding that was probably explained by the study design in which subjects were allowed to drink water freely during the exercise. The increased in leukocytes after 2-h exercise in both groups was mainly attributable to both neutrophilia and lymphocytosis. These results were similar to those described in previous studies.[4]

Study limitation

Subject numbers were marginal in the present experiment. However, several studies have examined exercise immunology in able-bodied subjects, and the subject numbers in some of these well-controlled studies were less than 10 persons.[4,6,15] Furthermore, it was difficult to recruit individuals with SCIs who met the strict inclusion criteria of the present study including completion of the 2-h arm crank ergometer exercise.

All subjects started the exercise without having breakfast and had a meal after the 2-h exercise. In a preliminary study, we studied few subjects using the same protocol but the results showed that all subjects suffered stress of hunger. Fasting affects immune variables such as T-cell subsets and NK cell activity.[24] Thus, we were afraid that this kind of stress might affect NKCA, and thus the design of our study was modified by asking all subjects to have a meal after the exercise. We thought that having meal might influence the immune response but starvation must be a heavy stress for the subjects; therefore, all results determined after 2 h of recovery should bear the influence of meal in both SCI and able-bodied subjects.

Recently, a large number of studies on exercise and cytokines have been published.[12,13] However, in our project, the amount of blood that could be collected from SCI subjects was limited, forcing us to focus on only adrenalin, cortisol and PGE2 but not cytokines.

Conclusion

NKCA in SCI subjects is suppressed by 2-h arm crank ergometer exercise at 60% VO_{2max} and an increase of PGE_2 was observed during exercise only in SCI subjects. These findings suggest that the exercise induces increases in PGE2, which resulted in reduction of NKCA in SCI subjects.

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References

1 Jonsdottir IH. Exercise immunology: neuroendocrine regulation of NK-cells. *Int J Sports Med* 2000; **21** (Suppl 1): S20–S23.
2 Nieman DC, Nehlsen-Cannarella SL. The immune response to exercise. *Semin Hematol* 1994; **31**: 166–179.
3 Pedersen BK, Ullum H. NK cell response to physical activity: possible mechanisms of action. *Med Sci Sports Exerc* 1994; **26**: 140–146.
4 Tvede N, Kappel M, Halkjaer-Kristensen J, Galbo H, Pedersen BK. The effect of light, moderate and severe bicycle exercise on lymphocyte subsets, natural and lymphokine activated killer cells, lymphocyte proliferative response and interleukin 2 production. *Int J Sports Med* 1993; **14**: 275–282.
5 Bhatt K, Cid E, Maiman D. Bacteremia in the spinal cord injury population. *J Am Paraplegia Soc* 1987; **10**: 11–14.
6 Kappel M, Tvede N, Galbo H, Haahr PM, Kjaer M, Linstow M *et al*. Evidence that the effect of physical exercise on NK cell activity is mediated by epinephrine. *J Appl Physiol* 1991; **70**: 2530–2534.
7 Furusawa K, Tajima F, Tanaka Y, Ide M, Ogata H. Short-term attenuation of natural killer cell cytotoxic activity in paraplegic athletes during wheelchair marathon. *Arch Phys Med Rehabil* 1998; **79**: 1116–1121.
8 Furusawa K, Tajima F, Umezu Y, Ueta M, Ide M, Mizushima T *et al*. Activation of natural killer cell function in recreational athletes with paraplegia during wheelchair half marathon race. *Arch Phys Med Rehabil* 2003; **84**: 706–711.
9 Pedersen BK, Kappel M, Klokker M, Nielsen HB, Secher NH. The immune system during exposure to extreme physiologic conditions. *Int J Sports Med* 1994; **15**: S116–S121.
10 Shinkai S, Watanabe S, Asai H, Shek PN. Cortisol response to exercise and post-exercise suppression of blood lymphocyte subset counts. *Int J Sports Med* 1996; **17**: 597–603.
11 Tvede N, Kappel M, Klarlund K, Duhn S, Halkjaer-Kristensen J, Kjaer M *et al*. Evidence that the effect of bicycle exercise on blood mononuclear cell proliferative responses and subsets is mediated by epinephrine. *Int J Sports Med* 1994; **15**: 100–104.
12 Pedersen BK, Steensberg A, Fischer C, Keller C, Ostrowski K, Schjerling P. Exercise and cytokines with particular focus on muscle-derived IL-6. *Exer Immunol Rev* 2001; **7**: 18–31.
13 Suzuki K, Nakaji S, Yamada M, Totsuka M, Sato K, Sugawara K. Systemic inflammatory response to exhaustive exercise. Cytokine kinetics. *Exer Immunol Rev* 2002; **8**: 6–48.
14 Hunter LW, Rorie DK, Yaksh TL, Tyce GM. Concurrent separation of catecholamines, dihydroxyphenylglycol, vasoactive intestinal peptide, and neuropeptide Y in superfusate and tissue extract. *Anal Biochem* 1988; **173**: 340–352.
15 Pedersen BK, Tvede N, Klarlund K, Christensen LD, Hansen FR, Galbo H *et al*. Indomethacin *in vitro* and *in vivo* abolishes post-exercise suppression of natural killer cell activity in peripheral blood. *Int J Sports Med* 1990; **11**: 127–131.
16 Kappel M, Tvede N, Galbo H, Haahr PM, Kjaer M, Linstow M *et al*. Evidence that the effect of physical exercise on NK cell activity is mediated by epinephrine. *J Appl Physiol* 1991; **70**: 2530–2534.
17 Nieman DC, Henson DA, Johnson R, Lebeck L, Davis JM, Nehlsen-Cannarella SL. Effects of brief, heavy exertion on circulating lymphocyte subpopulations and proliferative response. *Med Sci Sports Exerc* 1992; **24**: 1339–1345.
18 Nieman DC. Exercise immunology: practical applications. *Int J Sports Med* 1997; **18**: S91–S100.
19 Pedersen BK, Tvede N, Christensen LD, Klarlund K, Kragbzk S, Halkjzr-Kristensen J. Natural killer cell activity in peripheral blood of highly trained and untrained persons. *Int J Sports Med* 1989; **10**: 129–131.
20 Moyna NM, Acker GR, Weber KM, Fulton JR, Robertson RJ, Goss FL *et al*. Exercise-induced alterations in natural killer cell number and function. *Eur J Appl Physiol* 1996; **74**: 227–233.

21 Kliesch WF, Cruse JM, Lewis RE, Bishop GR, Brackin B, Lampton JA. Restoration of depressed immune function in spinal cord injury patients receiving rehabilitation therapy. *Paraplegia* 1996; **34**: 82–90.
22 Schmid A, Huonker M, Barturen JM, Stahl F, Schmidt-Trucksass A, Konig D *et al*. Catecholamines, heart rate, and oxygen uptake during exercise in persons with spinal cord injury. *J Appl Physiol* 1998; **85**: 635–641.
23 Nieman DC, Brendle D, Henson DA, Suttles J, Cook VD, Warren BJ *et al*. Immune function in athletes versus nonathletes. *Int J Sports Med* 1995; **16**: 329–333.
24 Komaki G, Kanazawa F, Sogawa H, Mine K, Tamai H, Okamura S *et al*. Alterations in lymphocyte subsets and pituitary-adrenal gland-related hormones during fasting. *Am J Clin Nutr* 1997; **66**: 147–152.