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ORIGINAL ARTICLE

EFFECT OF VITAMIN C SUPPLEMENTATION ON NEUTROPHIL FUNCTION IN MALE JUDOISTS DURING A TRAINING CAMP

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Abstract We examined the effects of vitamin C supplementation on neutrophil function during exercise loading. Neutrophil functions, namely the reactive oxygen species (ROS) production capability, neutrophil phagocytic activity (PA) and serum opsonic activity (SOA) were measured before and after a 2-hour unified loading exercise (ULE) both before and after a 7-day intensified training camp for 22 male judoists. The parameters were assessed neutrophil count, myogenic enzymes, vitamin C in serum, SOA, PA, ROS production capability, body composition, and so on. Subjects were randomly assigned to two groups; the VC group (daily diet supplementation of 1,500 mg vitamin C) and the Control group (no vitamin C supplementation). The post-camp pre-ULE vitamin C level was higher in the VC group than in the Control group, though no such difference was seen at pre-camp. As for neutrophil function, although the typical changes seen following a single bout of normal exercise, namely an increase in SOA and ROS, and a decrease in PA, were recorded following the pre- and post-camp ULEs in both groups, significant difference in change rates were not seen between both groups. In conclusion, vitamin C supplementation had no significant influence on changes in neutrophil function.

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Key words: Vitamin C; neutrophil; reactive oxygen species; judo; exercise.

原　著

男子柔道選手の強化合宿におけるビタミンCサプリメントが好中球機能に及ぼす影響

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抄録　本研究では運動負荷時におけるビタミンCサプリメントの好中球機能に対する影響を検討した．22人の男子柔道選手を対象として，無作為にVC群（毎日1,500 mgのビタミンCサプリメントを摂取する群）と対照群に割付けた．一週間の強化合宿前後での2時間の練習前後における好中球機能，すなわち活性酸素種（ROS）産生能，貪食能（PA）および血清オプソニン化活性（SOA）を測定した．両群において強化合宿前の血清ビタミンC濃度に差はみられなかったが，合宿後練習前での血清ビタミンC濃度は対照群と比較してVC群が高かった．好中球機能では，両群とも合宿前後ではSOAとROS産生能の上昇やPAの低下といった一過性の運動負荷後として典型的な変化を認めたが，両群間で有意差を認めなかった．したがって，ビタミンCサプリメントの単独摂取では，好中球機能の変化には有意な影響を及ぼさないことが明らかとなった．

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キーワード：ビタミンC；好中球；活性酸素種；柔道選手；運動．

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Introduction

For many competitive sports, a special intensive training session (the so-called "training camp") is held to reinforce physical strength and skill, where, in general, the physical load level is set at a very much higher intensity and/or for longer periods than during ordinary quotidian training. The potential effects of exercise or training on the immune system have recently attracted attention. There has been evidence that exercise causes some indefinite but significant changes in the distribution and function of immune factors, either cellular or humoral[1, 2].

Neutrophils represent one of the cellular factors playing an important role in the first line of defense against invading pathogens, including microorganisms. Neutrophils engulf microorganisms (through phagocytic activity (PA)) and kill them through the production of cytotoxic reactive oxygen species (ROS)[3, 4]. Serum opsonic activity (SOA) contributes to this microbicidal activity through opsonization of the target microorganisms, *i.e.*, acceleration of the adhesion of neutrophils to opsonized substances via IgG, C3 and others. However, the meaning behind the changes in these neutrophil functions remains unclear. For example, ROS can destroy invading microorganisms, but under different circumstances they can cause oxidative damage to normal body tissues and organs[5-8]. Furthermore, the borderline of ROS production between these beneficial and detrimental effects remains unclear.

Vitamin C has generated a great deal of interest for its potential influence on immune function and host defense[9]. Vitamin C is widely recognized for its antioxidant function and its ability to neutralize oxygen radicals. However, some studies have examined the effects of vitamin C supplementation on neutrophil function e.g. ROS production capability and PA during exercise, with a variety of results as follows.

Nieman et al. supplemented the daily diet in runners with 1,000 mg vitamin C for eight days prior to a 2.5-h run at 75-80% VO_{2max}[10]. There appeared to be a small non-significant trend towards reduced *in vitro* neutrophil ROS production below pre-exercise values in the hours after exercise. Krause et al. reported that 2,000 mg vitamin C supplementation taken for one week before intense running and cycling activity did not affect the changes in PA and ROS production capability[11]. On the other hand, Robson et al. reported that a combination of vitamins A, C and E given to athletes for seven days before 2-h running at 65% VO_{2max} enhanced the capability of neutrophils to produce ROS *in vitro* after exercise[12]. As has mentioned above, a definite conclusion still remains unclear.

Furthermore, it has been recognized that neutrophil and neutrophil-related functions can vary depending on the host condition/exercise loading based on their tendency to compensate for each other as has been mentioned above[13, 14]. Bearing this in mind, the influence of vitamin C supplementation on changes in the three major neutrophil functions must be examined at different assessment points under differing host condition/exercise loading to elucidate the potential relationship between vitamin C supplementation and neutrophil function.

In order to clarify the effects of vitamin C supplementation on neutrophil function under exercise loading, we simultaneously measured three major neutrophil and neutrophil-related functions (ROS production capability, PA, and SOA) before and after a unified loading exercise (ULE) both before (pre-camp) and after a 7-day intensified training camp (post-camp) for male judoists.

Subjects and Methods

Twenty-two male university judoists participated in this study. All subjects were assigned

Table 1 Characteristics of subjects and changes in the anthropometric parameters during the training camp

	Vitamin C group (n=12)		Control group (n=10)	
	Before the camp	After the camp	Before the camp	After the camp
Age (years old)				
Pre-ULE	19.1 ± 0.8	–	19.0 ± 0.8	–
Height (cm)				
Pre-ULE	170.9 ± 6.8	–	172.4 ± 7.4	–
Weight (kg)				
Pre-ULE	76.1 ± 12.5	77.2 ± 12.7††	80.6 ± 13.6	82.0 ± 13.6††
Post-ULE	75.2 ± 12.6**	75.7 ± 12.4**	80.0 ± 13.7*	80.3 ± 13.4**
Change ratio (%)	-1.3 ± 0.7	-2.0 ± 0.6†	-0.8 ± 0.9	-2.2 ± 0.9
Relative body fat (%)				
Pre-ULE	12.7 ± 4.0	12.8 ± 4.1	14.2 ± 3.9	14.3 ± 3.6
Post-ULE	–	–	–	–
Fat-free mass (kg)				
Pre-ULE	66.0 ± 7.6	66.9 ± 7.6	68.7 ± 8.5	69.9 ± 8.6
Post-ULE	–	–	–	–

Values are mean ± standard deviation.
ULE: unified loading exercise
Change ratio=(Pre-value - Post-value)/Pre-value*100
*: $p<0.05$, **: $p<0.01$, significantly different from the pre-values.
†: $p<0.05$, ††: $p<0.01$, significantly different from the values before the trainig camp.

to one of two groups: the VC group (daily diet supplementation with vitamin C 1,500 mg) and the Control group (no vitamin C supplementation). No subject required weight reduction in this study period.

Two students competed in the under 60 kg class (both in the VC group), 7 in the under 66 kg class (4 in the VC group and 3 in the Control group), 6 in the under 73 kg class (3 each in both groups), 2 in the under 86 kg class(1 each in both groups), 3 in the under 90 kg class (1 in the VC group and 2 in the Control group), and 2 in the 100 kg class (1 each in both groups). The average age, height, and weight of the athletes at the commencement of this investigation were 19.0 ± 0.8 years old, 171.6 ± 7.0 cm, and 78.2 ± 12.9 kg, respectively.

1. Study Protocol

All subjects completed a training camp between August 11th and 17th. Measurement and blood sampling were performed before and after a 2-hour ULE in the early morning under fasting conditions both one day before (pre-camp) and after (post-camp) a 7-day intensified training camp. Blood work-ups were executed before and after the loading. The mean values of ambient temperature and humidity were 28.2 ± 0.4 °C and 70.2 ± 3.5 % on August 10th, and 23.7 ± 0.5 °C and 85.8 ± 4.8 % on August 18th.

Both the study aim and protocol were approved by the Ethical Review Committee of the Hirosaki University School of Medicine. All subjects were adequately informed of the aim, methods and potential risks as well as the right to abstain from participation in the study. Freely-given informed consent in writing was obtained from all of them.

2. Physical load in a 2-hour ULE and training camp

The ULE comprised warming up and cooling down for 15 minutes each, "*uchikomi*" (repetitive practice of a technique) for 20 minutes, and "*randori*" (freestyle practice) for 70 minutes. Blood samples were taken from each participant, and body composition, blood biochemistry, neutrophil PA, and ROS production capabilities were measured before and after the

Table 2 The training program during the training camp for 7 days

Time	Training program
a.m. 8:30-p.m. 0:00	Judo training for practice
p.m. 0:00-p.m. 2:00	Rest
p.m. 2:00-p.m. 4:00	Distance running for 30 minutes and short sprint running (repeated 30-50 m sprint running during training time)
p.m. 4:00-p.m. 5:00	Weight training

reinforcement training camp and before and after both ULE sessions.

The average heart rate was 130 bpm with a maximum average heart rate of 160 bpm during ULE sessions. The heart rate was measured and recorded using VANTAGE XL (Polar Electric Inc., Finland), and was analyzed at intervals of 1 minute after the end of the measurement using POLAR HR ANALYSIS SOFTWARE VER. 4 (Polar Electric Inc., Finland).

On the other hand, the daily training menu in the training camp was as follows: judo practice for 3 hr 30 min. in the morning, long-distance running and short sprints for 2 h, followed by weight training for 1 hr in the afternoon, for a total of 6 hr 30 min. of training per day for 1 week (Table 2). The normal training regimen outwith the training camp consisted of 1 hr running or weight training and 2 h 30 min. judo training, with total training lasting about 3 h 30 min.. Accordingly, the daily training camp regimen was almost twice as long as normal training. No dietary intervention or weight control was carried out during this study.

3. Measurements of body composition

The anthropometric parameters; body weight (BW), relative body fat (% fat), and fat-free mass (FFM) were measured with the impedance method (TBF-110, TANITA, Tokyo, Japan).

4. Blood biochemical examination

Blood samples were taken from the forearm vein before and after the exercise loading session at both pre- and post-camp. The serum was separated from the blood by centrifugation for 10 min at 3,000 rpm and kept frozen at -30°C until analysis.

As for white blood cell (WBC), neutrophil counts, hematocrit (Hct), and hemoglobin (Hb) were determined with a blood cell autoanalyzer (Micro Biff-II, Coulter Co. Ltd., CA, USA). As for serum enzymes, serum creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured with a biochemical assay kit (OLYMPUS AU-5232, Tokyo, Japan). Immunoglobulins (IgG, IgA, and IgM) and complements (C3, C4) were assayed with a turbidimetric immunoassay kit (Nittobo Medical Co. Ltd., Tokyo, Japan). As for serum vitamin C level, the serum level of vitamin C was also measured using High Performance Liquid Chromatography (HPLC). The post-exercise values were adjusted by the serum volume calculated by Hct and Hb levels before and after the exercise, because dehydration was observed after the ULE based on changes in weight and Hct[15].

5. Neutrophil function

1) ROS production capability and PA

These neutrophil activities were determined with a FACScan system (Becton Dickinson, San Jose, CA, USA) using two-color flow cytometry. Hydroethidine (HE 44.4 μmol/L; Polyscience Inc., Warrington, PA, USA) was used as an indicator for oxidative burst activities (OBA) (mostly reflects superoxide 'O_2^-'), and opsonized

zymosan particles labeled with fluorescein isothiocyanate (FITC; Sigma Chemical Co., St Louis, MO, USA) were used as an indicator for PA.

The measurement of ROS production capability and PA was carried out as previously described[16]. Briefly, 100 μL of heparinized whole blood sample was mixed with 22 μL HE (final concentration, f.c. 8 μmol/L) and incubated at 37 °C for 5 min. After the addition of 25 μL FITC-labeled opsonized zymosan (FITC-OZ; f.c. 5 mg/mL), samples were incubated at 37° C for 35 min. 100 μL of whole blood labeled with only HE was served as a control to measure basal OBA (basal state). After the incubation, Lyse and Fix (IMMUNOTECH, Marseille, France) was added for lyse the red blood cells and to fix samples. The samples were washed twice in phosphate buffered saline with sodium azide (PBS+), and the fluorescence intensity (FI) in the activated neutrophils was measured with the FACScan system. Just before the assay, 30 μL of Trypan blue (0.25 mg/mL, pH 4.5) was added to differentiate attached and ingested FITC-OZ in the samples by fluorescence quenching[17, 18].

Total of 10,000 neutrophils were analyzed for each sample. ROS production capability and PA values were collected using a logarithmic amplifier (scale) and estimated as the mean channel number of FI of activated per cell in neutrophils.

2) Measurement of SOA

The serum samples for measuring SOA were stored frozen at -80° C and were rapidly thawed at 37° C just before analysis. In this study, OZ was manufactured in the following method. Firstly, Zymosan A (Sigma, USA) was suspended in Hank's balanced salt solution (HBSS) at a concentration of 5 mg/mL. The opsonization was performed by adding it to the serum samples to a final concentration of 20 % and incubating at 37° C for 30 min. The particles were then washed twice with HBSS and resuspended in HBSS at a concentration of 5 mg/mL. Normal pooled human neutrophils were obtained from the peripheral blood of a healthy adult male volunteer and isolated by using the Mono-Poly Resolving Medium (Dainippon Pharmaceutical, Tokyo, Japan). Neutrophil suspensions were prepared by adjusting neutrophil counts to 3.0×10^3 cells/μL through dilution with HBSS.

The chemiluminigenic probe, lucigenin, was prepared by dissolving bis-*N*-methylacridinium nitrate (Sigma, USA) in HBSS to give a final concentration of 0.5 mmol/L (pH 7.4). Lucigenin mainly reacts with superoxide, which is the first substance in the metabolism of ROS[19, 20].

The lucigenin-dependent chemiluminescence (LgCL) response of each sample were studied in 96-well microplate (well capacity of 400 μL, Greiner Japan, Tokyo, Japan) simultaneously, and 50 μL of neutrophil suspension, 50 μL of OZ, 50 μL of lucigenin solution, and 100 μL of HBSS were added to each well of a microplate. The final concentration of lucigenin was 0.1 mmol/L, and total dose was 250 μL.

LgCL was measured continuously for 45 min at 37° C using the Alfa system Auto Luminescence Analyzer (Tokken, Inc., Funabashi, Japan). The results were evaluated by determining the area under the curve (AUC), which was integrated with the area under the luminescence curve for 45 min[21, 22].

6. Dietary survey

The subjects recorded their meals and the weight of their food intake every day during the training camp. Daily nutrient intake and total energy intake were calculated using the fifth revision of the Food Composition Table[23].

7. Statistical analysis

Data were presented as the mean ± standard deviation. The differences in each parameter

Table 3 Nutritional intake per day during the training camp

	Vitamin C group (n=12)	Control group (n=10)	
Total energy intake (kcal)	3905.6 ± 466.3	4111.8 ± 475.9	ns
Protein intake (g)	127.4 ± 6.5	129.8 ± 9.8	ns
Lipids intake (g)	109.0 ± 8.2	108.8 ± 6.6	ns
Carbohydrates intake (g)	584.9 ± 103.2	632.4 ± 96.5	ns
Vitamin C intake (mg)	219.1 ± 106.1	155.2 ± 68.7	ns

Values are mean ± standard deviation.
Each nutritional intakes per day calculated the means by quantities of food and drink taken in during the training camp.
Vitamin C intake was taken by the food and drink except the supplement.
ns: not significant.

Table 4 Change of serum vitamin C during the training camp

	Vitamin C group (n=12)		Control group (n=10)	
	Before the camp	After the camp	Before the camp	After the camp
Serum vitamin C (μg/dl)				
Pre-ULE	8.6 ± 2.6	12.2 ± 2.9††‡‡	7.8 ± 2.4	8.2 ± 2.0
Post-ULE[a]	11.6 ± 3.1**	22.8 ± 8.0**	10.8 ± 3.5**	12.8 ± 5.1**
Change ratio (%)	37.5 ± 20.3	88.8 ± 48.6	40.3 ± 35.7	53.4 ± 24.9

Values are mean ± standard deviation.
ULE: unified loading exercise Change ratio=(Pre-value - Post-value)/Pre-value*100
a: values after the training were adjusted by the plasma volume for dehydration
**: $p<0.01$, significantly different from the pre-values.
††: $p<0.01$, significantly different from the values before the trainig camp.
‡‡: $p<0.01$, significantly different from the values in the Control group.

between the pre- and post-values of ULE, the values in the VC group and the Control group, and the values before and after the camp were analyzed using a three-way ANOVA. The differences were considered to be statistically significant at $p<0.05$. The statistical analysis was carried out using the PC version of the statistical package SPSS/PC.

Results

1. Body composition

BW significantly decreased after ULE ($p<0.05$-0.01) at both pre- and post-camp in both groups. The pre-ULE values of %fat and FFM did not change during the training camp (Table 1).

2. Nutritional intake

No significant differences in intakes of energy, protein, fat, carbohydrates and vitamin C (exception of the VC group who supplemented their daily intake with vitamin C 1,500mg), were seen in this period (Table 3).

3. Serum vitamin C level

The post-camp pre-ULE serum vitamin C level (12.2 ± 2.9) was higher in the VC group than in the Control group (8.2 ± 2.0) ($p<0.01$), though no such difference was seen at pre-camp. The serum vitamin C levels significantly increased following ULE in the two assessment points (pre- and post-camp) in both groups ($p<0.01$ all), but no significant differences in change rates were seen between the two groups (Table 4).

Table 5 Change in serum enzymes during the training camp

	Vitamin C group (n=12)		Control group (n=10)	
	Before the camp	After the camp	Before the camp	After the camp
AST (IU/l)				
Pre-ULE	24.5 ± 5.1	32.0 ± 7.7†	21.0 ± 3.9	33.0 ± 9.0
Post-ULE[a]	27.7 ± 7.0**	34.9 ± 8.7*	24.8 ± 5.3**	37.2 ± 10.1**
Change ratio (%)	12.7 ± 9.2	9.1 ± 5.9	18.2 ± 11.1	12.9 ± 5.2
ALT (IU/l)				
Pre-ULE	23.5 ± 13.1	27.8 ± 12.2†	23.2 ± 13.5	28.4 ± 13.0†
Post-ULE[a]	25.3 ± 15.0*	30.5 ± 12.0**	25.8 ± 15.3**	32.1 ± 14.0**
Change ratio (%)	7.3 ± 9.7	11.4 ± 7.8	12.0 ± 7.4	14.6 ± 8.3
CK (IU/l)				
Pre-ULE	260.1 ± 128.8	531.4 ± 295.4††	212.2 ± 93.2	676.6 ± 340.1††
Post-ULE[a]	310.6 ± 137.7**	611.5 ± 332.9**	267.0 ± 80.9**	802.3 ± 386.7**
Change ratio (%)	22.5 ± 12.0	15.8 ± 6.9†	32.5 ± 20.4	20.4 ± 7.2
LDH (IU/l)				
Pre-ULE	237.2 ± 28.3	276.9 ± 43.8††	205.8 ± 29.4	260.0 ± 45.5††
Post-ULE[a]	274.2 ± 42.1**	307.0 ± 51.9**	252.6 ± 40.7**	302.5 ± 50.7**
Change ratio (%)	15.4 ± 8.0	10.9 ± 6.4	23.3 ± 12.8	16.6 ± 5.7

Values are mean ± standard deviation.
ULE: unified loading exercise Change ratio=(Pre-value - Post-value)/Pre-value*100
a: values after the training were adjusted by the plasma volume for dehydration
*: $p<0.05$, **: $p<0.01$, significantly different from the pre-values.
†: $p<0.05$, ††: $p<0.01$, significantly different from the values before the trainig camp.

Table 6 Change in blood leukocyte and neutrophil cell counts during the training camp

	Vitamin C group (n=12)		Control group (n=10)	
	Before the camp	After the camp	Before the camp	After the camp
Blood leukocyte cell counts (/μl)				
Pre-ULE	6825 ± 1114	5783 ± 1156††	7000 ± 1350	6090 ± 823†
Post-ULE[a]	6550 ± 1085	6066 ± 1196	6760 ± 1187	6340 ± 1323
Change ratio (%)	-3.0 ± 14.9	5.9 ± 10.8	-2.1 ± 16.3	3.7 ± 15.2
Blood neutrophil cell counts (/μl)				
Pre-ULE	3207 ± 727	3308 ± 707	3352 ± 552	3528 ± 663
Post-ULE[a]	3943 ± 1045*	3322 ± 726	3880 ± 896	3492 ± 906
Change ratio (%)	25.8 ± 33.5	1.2 ± 12.5†	17.5 ± 31.0	-1.2 ± 18.1†

Values are mean ± standard deviation.
ULE: unified loading exercise Change ratio=(Pre-value - Post-value)/Pre-value*100
a: values after the training were adjusted by the plasma volume for dehydration
*: $p<0.05$, **: $p<0.01$, significantly different from the pre-values.
†: $p<0.05$, ††: $p<0.01$, significantly different from the values before the trainig camp.

4. Serum enzymes

AST, ALT, CK, and LDH significantly increased after ULE in both groups at both pre- and post-camp assessments ($p<0.05$-0.01), with no significant differences in change rates by ULE between the two groups (Table 5).

5. Leukocyte and neutrophil counts

Neutrophil counts was significantly increased at only pre-camp assessment point in the VC group ($p<0.05$), with no significant differences in change rates by ULE between the two groups (Table 6).

Table 7 Change in serum immunoglobulins and complements during the training camp

	Vitamin C group (n=12)		Control group (n=10)	
	Before the camp	After the camp	Before the camp	After the camp
IgG (mg/dl)				
Pre-ULE	1184 ± 231	1125 ± 228†	1154 ± 200	1118 ± 206
Post-ULE[a]	1225 ± 246*	1185 ± 253**	1188 ± 203	1182 ± 215**
Change ratio (%)	3.5 ± 4.7	5.1 ± 3.4	3.0 ± 4.3	5.8 ± 2.5
IgA (mg/dl)				
Pre-ULE	252.8 ± 98.5	238.0 ± 96.8††	229.9 ± 98.4	216.4 ± 97.9†
Post-ULE[a]	258.6 ± 99.5	244.5 ± 96.9*	239.7 ± 113.1	227.6 ± 107.9*
Change ratio (%)	2.3 ± 5.7	3.1 ± 4.0	2.9 ± 5.4	4.2 ± 4.0
IgM (mg/dl)				
Pre-ULE	103.9 ± 36.1	94.4 ± 30.4††	115.9 ± 33.2	107.6 ± 31.3†
Post-ULE[a]	106.8 ± 36.8	95.5 ± 29.7	117.6 ± 36.5	111.1 ± 33.4*
Change ratio (%)	2.7 ± 6.2	1.7 ± 4.0	1.1 ± 5.5	3.1 ± 3.4
C3 (mg/dl)				
Pre-ULE	102.4 ± 21.6	104.3 ± 18.4	107.6 ± 21.4	103.5 ± 17.6†
Post-ULE[a]	104.7 ± 22.7	107.6 ± 19.3**	108.8 ± 21.4	107.9 ± 17.9**
Change ratio (%)	2.2 ± 4.7	3.2 ± 3.2	1.3 ± 5.1	4.4 ± 3.3
C4 (mg/dl)				
Pre-ULE	20.4 ± 5.9	23.2 ± 4.7	25.3 ± 9.3	25.3 ± 6.4
Post-ULE[a]	20.8 ± 6.3	23.7 ± 5.2	25.4 ± 8.6	26.4 ± 7.1*
Change ratio (%)	1.4 ± 5.7	0.6 ± 1.0	0.8 ± 4.5	1.1 ± 1.1

Values are mean ± standard deviation.
ULE: unified loading exercise Change ratio=(Pre-value - Post-value)/Pre-value*100
a: values after the training were adjusted by the plasma volume for dehydration
*: $p<0.05$, **: $p<0.01$, significantly different from the pre-values.
†: $p<0.05$, ††: $p<0.01$, significantly different from the values before the trainig camp.

6. Immunoglobulins and complements

All parameters for immunoglobulins and complements increased following the ULE at the pre- and post-camp assessment points in both groups (some parameters showed significant change), with no significant differences in change rates by ULE between the two groups (Table 7).

7. ROS production capability, PA and SOA

ULE-mediated ROS production capability and SOA increased at the pre- and post-camp assessment points in both groups ($p<0.01$ all for ROS and $p<0.05$ at pre-camp in the VC group for SOA), with no significant differences in change rates by ULE between the two groups (Table 8). On the other hand, PA decreased following ULE at in both groups ($p<0.05$ at post-camp in both groups), with no significant differences in change rates by ULE between the two groups (Table 8).

Discussion

It has already been clarified that intense exercise causes damage to the muscular tissue, releasing serum enzymes from the damaged tissue into the blood[24, 25]. In our results, although serum myogenic enzymes also significantly increased following the ULE in both group at the pre- and post-camp assessments ($p<0.05$-0.01), no significant differences in the rate of increase were seen between the VC and Control groups.

The relationship between leukocytes and exercise has been well-reported: leukocyte

Table 8 Change in neutrophil ROS production capacity, neutrophil PA, and SOA during the training camp

	Vitamin C group (n=12)		Control group (n=10)	
	Before the camp	After the camp	Before the camp	After the camp
ROS production per cell (FI)				
Pre-ULE	204.4 ± 22.9	192.1 ± 36.8	195.3 ± 18.6	196.5 ± 50.3
Post-ULE	264.1 ± 36.7**	293.5 ± 56.1**	258.6 ± 35.6**	292.2 ± 72.3**
Change ratio (%)	29.3 ± 12.4	56.2 ± 36.3†	32.2 ± 11.4	54.0 ± 41.8
PA per cell (FI)				
Pre-ULE	405.1 ± 83.7	448.8 ± 127.5	401.2 ± 57.1	450.4 ± 120.2
Post-ULE	372.6 ± 60.6	400.0 ± 99.0*	372.6 ± 51.7	366.5 ± 68.2*
Change ratio (%)	-5.0 ± 22.8	-9.6 ± 10.0	-6.3 ± 12.1	-15.8 ± 16.8†
SOA (cpm*sec)				
Pre-ULE	327.2 ± 21.5	345.8 ± 38.2	335.2 ± 43.5	327.9 ± 28.4
Post-ULE	375.2 ± 38.5*	368.5 ± 54.7	371.5 ± 53.5	342.3 ± 52.3
Change ratio (%)	15.0 ± 12.5	6.6 ± 12.1†	12.0 ± 19.3	4.4 ± 13.5

Values are mean ± standard deviation.
ROS: reactive oxgen species, PA: phagocytic activity, SOA: serum opsonic activity, ULE: unified loading exercise
FI: the mean channel number of fluorescence intensity of activated neutrophils. cpm: counts per minutes
Change ratio=(Pre-value - Post-value)/Pre-value*100
*: $p<0.05$, **: $p<0.01$, significantly different from the pre-values.
†: $p<0.05$, significantly different from the values before the trainig camp.

and neutrophil counts increase with transient exercise, the degree of increase being dependent on the intensity of the exercise. Neutrophil counts which comprise part of the total leukocyte count, also become elevated with exercise[26]. Furthermore, these elevations are recognized as an anti-inflammatory reaction to the degeneration of and injury to muscle tissue caused by exercise, and it is possible that inflammatory cytokines participate in this reaction[26, 27]. On the other hand, another study has suggested that the elevated WBC counts are not only due to the anti-inflammatory reaction, but possibly exercise itself may become the stressor which stimulates the production and release of growth hormones, adrenaline, and noradrenaline[28]. In this study, neutrophil count increased following the pre-camp ULE in the VC group, but no significant differences were seen in the rate of increase between the VC and Control groups.

Some confusion is apparent in the literature regarding the post-exercise serum concentrations of immunoglobulins and complements which have been variously reported to remain unchanged, to decrease, or to increase[29-32]. In the present study, the majority of immunoglobulins and complements significantly increased following the pre- and post-camp ULEs, but as with the neutrophil count, no significant differences were seen in the rate of increase between the VC and Control groups.

Mochida et al. suggested that neutrophil and neutrophil-related immune functions such as ROS production capability, PA and SOA may compensate for each other to maintain the overall integrity of the neutrophil immune function, depending on the exercise loading and subject's physical condition, such as the degree of fatigue[14]. In previous studies including Mochida's report, the typical change in ROS production capability and PA by a single bout of normal exercise has been an increase in ROS whereas PA decreased[13, 14, 33]. However, under the conditions of severe and prolonged exercise such as a full marathon and a training camp plus weight reduction, neutrophil parameters have tended to deviate from such typical

compensatory changes. For example, in some reports both PA and ROS decreased[34, 35].

In this study, ROS production capability, PA and SOA showed a typical response at both pre- and post-camp assessments in both group. Therefore, it is thought that the degree of the training camp ULEs in the present study was acceptable as far as immune homeostasis was concerned, and neutrophil function was maintained by balancing ROS increase with decreasing PA and increasing SOA. The present study is an important report which assessed the effect of vitamin C on three major neutrophil functions (SOA, ROS and PA) after ULE.

Vitamin C and, especially, vitamin E have been shown to decrease the exercise-induced increase in the rate of lipid peroxidation[36]. In the present study, vitamin C supplementation did not affect ULE-related neutrophil function at both pre- and post-camp, and no significant differences in the rate of change were seen between the VC and Control groups. There are two possible reasons for this discrepancy: firstly, vitamin C requires collaborative activity with other substances such as vitamin E, niacin, zinc, copper, manganese, selenium, *et cetera*, to work effectively[12]; secondly, the findings at the two assessment points *i.e.*, pre- and post-camp, were similar to each other with the well-compensative relationship in the three major neutrophil functions being maintained. Therefore, further study (for example, when vitamin C is coadministered with multivitamins/multiminerals, or when deviating from the typical compensatory changes) is required to clarify in detail the relationship between vitamin C supplementation and neutrophil function under conditions of exercise loading.

This study has one limitation in that we did not measure the intensity (e.g. % maximal oxygen consumption; $\%VO_{2max}$) of the judo exercise and the physical fitness of the subjects, although heart rate was measured. In this study, the total average heart rate was 130 bpm and maximum average heart rate was 160 bpm. Thus, the exercise intensity in this study was suggested to be 50-70% VO_2max, referring to reports of Banbsbo and Girard et al[37, 38]. The intensity of ULE was similar to the previous studies, in which the vitamin C uptake did not affect the change of neutrophil function[10, 12, 39].

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