[ORIGINAL ARTICLE]

Physical Condition in Female Judoists over 20-days Strengthening and Tapering Periods

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Keywords

1. female judoists
2. periodization
3. muscular fatigue
4. immune function

In order to assess the appropriateness of 20 days periodization for top female judoists, the changes in muscle fatigue and immune function were investigated in each training phase. The subjects were 10 female judoists who participated in the 2009 world judo championship. The investigation was conducted at 3 points - before and after the 6-day intensive training camp, which was held 30 days before the competition (pre-camp and post-camp) and 10 days before the competition day (tapering period). At each assessment point, body composition, neutrophil count, myogenic enzymes, superoxide dismutase (SOD) activity, reactive oxygen species production capability, phagocytic activity (PA) and so on, were measured. Subjects were asked to carry out the same 4-hour judo training on each assessment day, and all parameters were measured before and after the training (pre-practice and post-practice). The level of serum myogenic enzymes at pre-practice in the post-camp significantly increased compared with those in the pre-camp, and decreased significantly in the tapering period. The neutrophil count at pre-training in the tapering period was significantly lower compared with that in the pre-camp. PA significantly decreased at post-practice in the tapering period, whereas such a trend was not shown in the pre- and post-camp. SOD activity at pre-practice in the tapering period significantly decreased compared with those in the pre- and post-camp, showing the reduction of anti-oxidative function. The 10-day tapering after the training camp for subjects was considered adequate to allow for pre-competition recovery from muscle fatigue but was, however, insufficient for full recovery of reduced immune and anti-oxidative functions.

JPFNI 2014;24(1):10-20

Introduction

Many athletes carry out highly intensive and lengthy daily training sessions to win competitions or to exceed their personal best. Prior to important competitions, athletes schedule their training on a monthly basis by dividing a year into a preparation period, competition period and transition period, with set goals for each period [1,2]. They further divide this schedule on a weekly basis and implement their training menu by setting different goals for each period [1,2]. Such systematic planning of athletic training is known as periodization.

Unfortunately, inadequate periodization planned

and carried out by athletes is common, preventing them from performing their best at the competitions owing to their poor physical condition, suggested to be due to the overtraining syndrome induced by intensive training during the preparation period [3,4]. In other words, tapering after the intensive training was inadequate and/or insufficient for the body to recover from the overtraining syndrome, leaving the athletes no choice but to perform in less-than-peak physical condition.

According to the previous studies which investigated tapering after an intensive training period, performance was improved when training was reduced to 60 to 90% of the peak period over 7 to 21 days [5-8]. Other researchers stated that tapering carried out by marathon runners or cyclists increased and improved levels of oxidative enzymes and glycogen in muscle tissues [7,9]. Thus, the adequacy of periodization can be considered more important than improvement of skills or strengthening of body muscles for athletes in order to prepare themselves to perform their best in competitions.

Judo is a combat sport, involving direct contact between players throughout the match. As players must keep exerting high muscle force constantly and are exposed to strong physical impact by being thrown down on the tatami mat by their opponent, judo has been reported to have one of the highest risks of suffering sports disorders and injuries [10]. Previously, our research group found that transient judo training caused loss of electrolytes and dehydration in judoists, loss of energy source, reduction of renal function, changes in and damage to muscle tissues as well as increased stress and inflammatory reactions [11,12]. Moreover, our group has also reported that a highly intensive judo training camp caused physical fatigue and reduced physical function, leading to critical lowering of immune functions [13]. Thus, judoists are exposed to a high risk of injury due to the highly intensive, lengthy and exceedingly frequent daily training they carry out, making it very difficult to adequately maintain and manage their physical condition by strengthening and tapering to prepare themselves for the competition.

Such highly intensive training over a long period of time by athletes has been suggested to suppress the immune function of cells such as neutrophils and lymphocytes as well as increasing susceptibility to upper respiratory infections [14, 15]. Our previous research on professional soccer players also revealed that soccer matches and training sessions carried out over a 10-month period resulted in accumulation of chronic muscle fatigue as well as suppression of neutrophil function [16]. A number of studies that investigated the association between transient physical exercise and immune function also reported that transient, highly intensive training caused activation of reactive oxygen species (ROS), leading to increased oxidative stress and immunosuppression after the exercise [17,18]. Our previous studies on a variety of athletes also found that transient physical exercise accelerated neutrophil ROS production capability while reducing its phagocytic activity (PA) [12,19-24]. In addition, we revealed that athletes developed the overtraining syndrome and suffered from reductions in their immune function when they performed higher intensity and more lengthy training during the training camp compared to the normal training period [13, 25]. In other words, these results suggested that athletes, who have been constantly exercising without being allowed to fully recover from acute oxidative stress and/or immunosuppression caused by intensive transient exercise, and maintaining of such conditions over a long period of time may induce chronic immunosuppression as part of the overtraining syndrome.

In the present study, in order to assess the appropriateness of 20 days periodization for top female judoists, the changes in muscle fatigue and immune function were investigated in each training phase (strengthening and tapering periods).

Subjects and methods

Subjects and research period

The study subjects comprised 10 female judoists (included 3 reserves) who were the members of all Japan women team for the World Championship in August 2009 in Rotterdam. Two subjects competed in the under 48 kg class, 1 in the under 52 kg class, 1 in the under 57 kg class, 1 in the under 63 kg class, 2 in the under 70 kg class, 2 in the under 78 kg class, and 1 in the 78 kg or over. Reserves did same training as the representative members. The seven members who attended this Championship attained the following results: 3 were champions, 3 were in third position and one got beaten in the first round.

The final intensive training camp was held between 31st July and 5th August in 2009. Each subject went to their own team to have training or rest for 10 days, and returned to the National team on the 17th August (10 days before the match) and participated in the final conditioning training camp for 4 days.

In this study, assessments were performed at tree time points: in the afternoon of the second day of the training camp (pre-camp) and in the afternoon of the day before the end of training camp (post-camp), and in the afternoon of the first day of the conditioning training camp (tapering period). Measurements were done immediately before and after 4 hours of *randori* (free-style practice) in a standing position (2-3 hours after lunch).

The average age, height, weight, % fat and

Table 1. Subject demographics and changes in anthropometric parameters

	The pre-camp (1 Aug., 2009)	The post-camp (5 Aug., 2009)	Tapering period (17 Aug., 2009)
Age (Years)	22.6 ± 2.7	-	-
Height (cm)	164.0 ± 7.8	-	-
Weight (kg)			
Pre-practice	66.0 ± 12.3	65.3 ± 12.1 †	65.8 ± 13.3
Post-practice	64.7 ± 12.1 **	63.9 ± 11.9 **	64.7 ± 13.3 **
Relative body fat (%)	22.3 ± 5.7	21.2 ± 5.7 ††	22.2 ± 7.1
Lean body mass (kg)	50.6 ± 6.3	50.9 ± 6.3	50.4 ± 6.2 ‡

Subjects were 10 female judoists.
Values are shown as the mean ± standard deviation.
**: $p<0.01$, Significant difference from the pre-value.
†: $p<0.05$, ††: $p<0.01$, Significant difference from the value before the training camp.
‡: $p<0.05$, Significant difference from the value after the training camp.

fat-free mass of the subjects at the commencement of the this investigation were 22.6 ± 2.7 years old, 164.0 ± 7.8 cm, and 66.0 ± 12.3 kg, 22.3 ± 5.7 cm, and 50.6 ± 6.3 kg, respectively (Table 1).

Subjects did 7 hours practice in the intensive training camp with the following contents: 1 hour running training, 2 hours rest and breakfast, 2 hours *randori* (mainly *newaza* or grappling/pinning techniques) in the morning, followed by a 3-hour rest and lunch, then 4 hours of *randori* in a standing position in the afternoon. Furthermore, the daily training contents of each judoist's own team comprised 4-5 hours including running, weight training and judo practice.

Nine subjects of 10 members needed weight reduction to reach their match weight of 1.6 ± 2.2 kg (2.5 ± 3.4%), which was a smaller amount than cases in previous reports [26-28].

The study was approved by the Ethics Committee of Hirosaki University School of Medicine. The study protocol and purpose were thoroughly explained and written consent was obtained from all subjects prior to the investigation.

Body composition

After subjects' heights were measured, their body weight, body fat percentage and fat-free mass were measured using the multi-frequency body composition meter (MC-190, TANITA, Tokyo, Japan).

Blood biochemical parameters

Blood samples (10 ml) were taken from each subject 2-3 hours after lunch (pre-practice) and after the 4-hour practice (*randori* in the standing position). Two milliliters of the sample was used to analyze the blood cell components, and the remaining 8 ml was centrifuged at 3,000 rpm for 10 minutes to extract blood serum for further analysis.

For investigation of immune-related functions, leukocyte, neutrophil and lymphocyte counts were determined. Several myogenic enzymes including aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and creatine kinase (CK) as well as immunoglobulins (IgG, IgA and IgM) and complements (C3 and C4) were measured. In order to determine the anti-oxidative function, the activity of superoxide dismutase (SOD) was also measured.

White blood cell (WBC), neutrophil counts, hematocrit (Hct) and hemoglobin (Hb) were determined with a blood cell autoanalyzer (System XE-2100 and SE-9000, Kobe, Japan).

Levels of AST, ALT, LDH and CK were measured using the JSCC standardized method. For measurements of immunoglobulins and complements, the turbidimetric immunoassay (TIA) was used. The SOD activity was measured with the NBT reduction method.

As dehydration of the subjects was observed based on weight loss after the match and changes in the levels of hemoglobin and hematocrit, these items were measured using the plasma volume method after correcting for dehydration [29].

All measurements of blood biochemical items were consigned to an independent laboratory (Mitsubishi Chemical Medience Corporation).

Measurement method of neutrophil-related functions

Reactive oxygen species (ROS) generation and the phagocyte activity (PA) of peripheral blood neutrophils were determined with a FACScan system (Becton Dickinson, San Jose, CA) using two-color flow cytometry. Hydroethidine (HE; 44.4 μmol/L, Polyscience Inc., Warrington, PA) was used as an indicator for the ROS production capability, and opsonized zymosan (OZ) particles labelled with fluorescein isothiocyanate (FITC; Sigma Chemical Co., St. Louis, MO, USA) for assessment of PA. Zymosan was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Briefly, 100 μL heparinized whole blood was mixed with 22 μL HE (final concentration, f.c. 8μmol/

Table 2. Changes in serum myogenic enzymes

	The pre-camp (1 Aug., 2009)		The post-camp (5 Aug., 2009)		Tapering period (17 Aug., 2009)	
AST (IU/l)						
Pre-practice	28.9 ± 6.5		43.7 ± 18.0	†	21.5 ± 6.6	†, ‡‡
Post-practice[a]	35.4 ± 8.8	**	51.5 ± 23.3	**	25.5 ± 8.6	**
Change ratio (%)	22.1 ± 6.4		16.5 ± 7.4		18.1 ± 8.9	
ALT (IU/l)						
Pre-practice	17.2 ± 4.1		25.0 ± 9.0	††	17.0 ± 5.7	††
Post-practice[a]	19.4 ± 4.6	**	27.9 ± 11.1	**	18.3 ± 6.4	*
Change ratio (%)	12.9 ± 6.8		10.6 ± 6.2		7.3 ± 6.6	
CK (IU/l)						
Pre-practice	470.6 ± 180.8		919.3 ± 594.0	†	177.5 ± 120.3	††, ‡‡
Post-practice[a]	711.6 ± 291.4	**	1197.4 ± 815.0	**	272.8 ± 230.5	**
Change ratio (%)	51.0 ± 11.9		28.7 ± 10.5	††	47.5 ± 26.5	
LDH (IU/l)						
Pre-practice	262.3 ± 44.1		329.8 ± 65.8	††	208.3 ± 46.9	††, ‡‡
Post-practice[a]	308.7 ± 56.8	**	359.7 ± 93.8	*	250.4 ± 52.0	**
Change ratio (%)	17.6 ± 7.0		8.4 ± 11.3		20.9 ± 8.9	

Subjects were 10 female judoists.
Values are shown as the mean ± standard deviation.
a: Values of post-practice were adjusted for dehydration by plasma volume.
Change ratio= (post-value - pre-value)/the pre-value*100.
*: $p<0.05$, **: $p<0.01$, Significant different from the pre-value.
†: $p<0.05$, ††: $p<0.01$, Significant difference from the value before the training camp.
‡‡: $p<0.01$, Significant difference from the value after the training camp.

Table 3. Changes in leukocyte and neutrophil cell counts in peripheral blood

	The pre-camp (1 Aug., 2009)	The post-camp (5 Aug., 2009)	Tapering period (17 Aug., 2009)	
Blood leukocyte counts (/μl)				
Pre-practice	6830 ± 1216	6500 ± 1425	5830 ± 1333	
Post-practice[a]	7173 ± 1175	6714 ± 1575	7181 ± 1500	*
Change ratio (%)	5.9 ± 12.7	3.8 ± 12.0	26.5 ± 26.6	‡
Blood neutrophil counts (/μl)				
Pre-practice	4580 ± 1220	4114 ± 871	3437 ± 1032	†
Post-practice[a]	5001 ± 1188	4437 ± 1209	5344 ± 1136	*
Change ratio (%)	11.8 ± 21.1	7.4 ± 13.2	66.1 ± 49.8	†, ‡‡

Subjects were 10 female judoists.
Values are shown as the mean ± standard deviation.
a: Values of post-practice were adjusted for dehydration by plasma volume.
*: $p<0.05$, Significant difference from the pre-value.
†: $p<0.05$, Significant difference from the value before the training camp.
‡: $p<0.05$, ‡‡: $p<0.01$, Significant difference from the value after the training camp.

L) and incubated at 37°C for 5 min. After the addition of 25 μL FITC-labeled OZ (FITC-OZ; f.c. 5 mg/mL), the samples were incubated at 37°C for 35 min. Neutrophils labelled with only HE served as the control to measure nonstimulated neutrophil function, basal ROS generation. After incubation, Lyse and Fix (IMMUNOTECH, Marseille, France) was added to lyse the erythrocytes and to fix the samples. The samples were washed twice in phosphate-buffered saline with sodium azide, and the fluorescence intensity (FI) in activated neutrophils was measured with the FACScan system. 30 μL Trypan blue (0.25 mg/mL, pH 4.5) was added just before the assay to differentiate between attached and ingested FITC-OZ by fluorescence quenching [30, 31].

FI was measured as the value of neutrophils per 10,000 screened with forward and side scattering light for each sample. The accumulated FI (cumulative FI, CFI) was calculated by multiplying the intensity and the rate of fluorescence-positive cells. The FI was used as a quantitative index of the function per one activated neutrophil. The CFI was used as a quantitative index of neutrophil function.

SOA

SOA was measured with the lucigenin(Lg) and

Table 4. Changes in serum immunoglobulins and complements

	The pre-camp (1 Aug., 2009)	The post-camp (5 Aug., 2009)	Tapering period (17 Aug., 2009)
IgA (mg/dl)			
Pre-practice	188.3 ± 91.6	180.8 ± 95.7 †	194.9 ± 103.0
Post-practice[a]	193.5 ± 94.6 *	186.6 ± 99.8 *	199.3 ± 104.1 *
Change ratio (%)	2.8 ± 2.6	3.2 ± 4.0	2.7 ± 3.3
IgG (mg/dl)			
Pre-practice	1043 ± 142	1052 ± 127	1075 ± 169
Post-practice[a]	1082 ± 131 *	1097 ± 131 *	1113 ± 174 *
Change ratio (%)	4.0 ± 3.2	4.3 ± 4.2	3.7 ± 3.7
IgM (mg/dl)			
Pre-practice	136.7 ± 40.5	136.1 ± 40.4	139.3 ± 39.5
Post-practice[a]	137.6 ± 42.2	137.8 ± 40.5	140.5 ± 38.1
Change ratio (%)	0.5 ± 3.9	1.2 ± 2.6	1.2 ± 2.4
C3 (mg/dl)			
Pre-practice	81.4 ± 9.8	85.4 ± 8.5 ††	83.4 ± 7.8
Post-practice[a]	82.2 ± 10.1	88.5 ± 9.9	83.6 ± 9.1
Change ratio (%)	0.9 ± 2.7	3.6 ± 5.7	0.2 ± 4.5
C4 (mg/dl)			
Pre-practice	25.2 ± 14.6	25.3 ± 13.9	21.1 ± 6.7
Post-practice[a]	25.6 ± 14.6	25.9 ± 13.5	21.7 ± 6.9 **
Change ratio (%)	1.8 ± 2.1	3.2 ± 6.3	2.8 ± 2.4

Subjects were 10 female judoists.
Values are shown as the mean ± standard deviation.
a: Values of post-practice were adjusted for dehydration by plasma volume.
*: $p<0.05$, **: $p<0.01$, Significant difference from the pre-value.
†: $p<0.05$, ††: $p<0.01$, Significant difference from the value before the training camp.

luminol(Lm) chemiluminescence assay based on the ROS produced when standard neutrophils phagocytosed opsonized zymosan in serum from the subjects [32, 33]. Two chemiluminigenic probes, the Lg and Lm, were employed for the detection of ROS. The Lg 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) before use. The Lm was dissolved with 1 mol/L NaOH and the solution was adjusted to an isotonic state of 12.5 mmol/L at pH 7.4 by adding HCl, ultrapure water and NaCl. The Lm solution was diluted with HBSS to 0.5 mmol/L before use.

Zymosan A (Sigma, USA) was suspended in Hank's balanced salt solution (HBSS) at a concentration of 5 mg/ml and then opsonization was performed by adding the solution to the serum samples (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.

Standard neutrophils were obtained from the peripheral blood of a healthy adult male volunteer, whereby whole blood is modified by centrifugation through Mono-Poly resolving medium. The neutrophils were suspended to 3×10^6 cell/ml using an automatic blood cell counter (Coulter MD II, Coulter Co. Ltd., Tokyo, Japan).

Opsonized zymosan (OZ) suspension and chemiluminigenic probes prepared as described above were added to each well of black flat-bottom microplates (Greiner Japan, Tokyo, Japan), and 50 µl of standard neutrophils was added. The plates were automatically measured on the Auto Luminescence Analyzer, Alfa system (Tokken, Funabashi, Japan) [34].

All measurements were performed at 37 °C. The results were evaluated the area under the curve (AUC) of the chemiluminescence response [34, 35].

Statistical analysis

All values were presented as means ± standard deviation. Differences in mean values between before and after the practice were tested with the generalized Wilcoxon t-test. Differences in mean values between 3 investigation points were tested with the One-Way Repeated-Measures ANOVA or Bonferroni method was used. All values were considered to be statistically significant at $p<0.05$. SPSS ver.12.0J (SPSS Japan Inc., Tokyo, Japan) was used for analysis of the data.

Results

Table 1 shows the changes in the levels of body composition during the investigation period. Body weight decreased significantly at post-practice in all

Table 5. Changes in ROS production, PA, SOA and serum SOD activity

	The pre-camp (1 Aug., 2009)	The post-camp (5 Aug., 2009)	Tapering period (17 Aug., 2009)	
Total ROS production (CFI)				
Pre-practice	6.5 ± 6.2	13.3 ± 15.1	6.1 ± 10.5	
Post-practice	11.5 ± 11.1	18.4 ± 23.6	7.3 ± 5.0	
Change ratio (%)	205.3 ± 580.3	114.1 ± 280.5	170.2 ± 199.6	
Total PA (CFI)				
Pre-practice	4708 ± 1231	4290 ± 1370	7765 ± 2332	
Post-practice	4931 ± 786	4463 ± 704	5018 ± 872	**
Change ratio (%)	8.6 ± 22.3	10.5 ± 28.3	-31.4 ± 18.5	†, ‡‡
LgCL・AUC (cpm*sec)				
Pre-practice	524.0 ± 64.0	542.1 ± 54.2	547.4 ± 50.4	
Post-practice	514.4 ± 65.9	516.9 ± 49.2	508.3 ± 63.2	*
Change ratio (%)	-1.6 ± 8.6	-4.1 ± 10.7	-7.2 ± 6.7	
LmCL・AUC (cpm*sec)				
Pre-practice	6969 ± 706	7180 ± 450	7092 ± 554	
Post-practice	7188 ± 755	6965 ± 693	6995 ± 832	
Change ratio (%)	3.5 ± 10.3	-3.0 ± 7.6	-1.1 ± 11.0	
Serum SOD activity (%)				
Pre-practice	6.3 ± 0.9	6.0 ± 1.4	3.8 ± 1.3	††, ‡
Post-practice	5.8 ± 1.8	5.6 ± 0.9	4.4 ± 1.1	
Change ratio (%)	-0.5 ± 1.8	-0.4 ± 1.9	0.6 ± 1.2	

Subjects were 10 female judoists.
Values are shown as the mean ± standard deviation.
ROS production: reactive oxygen species production in neutrophils.
PA: phagocytic activity in neutrophils.
SOA: serum opsonic activity.
CFI: cumulative fluorescence intensity.
LgCL: lucigenin-dependent chemiluminescence response.
LmCL: luminol-dependent chemiluminescence response.
AUC: area under the curve for 45 min.
SOD: superoxide dismutase.
*: $p<0.05$, **: $p<0.01$, Significant difference from the pre-value.
†: $p<0.05$, ††: $p<0.01$, Significant difference from the value before the training camp.
‡: $p<0.05$, ‡‡: $p<0.01$, Significant difference from the value after the training camp.

three investigations compared with those at pre-practice ($p<0.01$ for all). Furthermore, body fat percentage significantly decreased in the post-camp compared with that in the pre-camp ($p<0.01$).

Fat-free mass significantly decreased in the tapering period compared with that in the post-camp ($p<0.05$).

Table 2 shows the changes in myogenic enzymes during the investigation period. All myogenic enzymes increased significantly at post-practice for all periods (AST : all $p<0.01$, ALT : $p<0.01$, $p<0.01$, $p<0.05$ for pre-camp, post-camp, tapering period, respectively, CK : all $p<0.01$, LDH : $p<0.01$, $p<0.05$, $p<0.01$ for the pre-camp, post-camp, tapering period, respectively).

Also, all myogenic enzymes increased significantly at pre-practice in the post-camp compared with those in the pre-camp (AST: $p<0.05$, ALT: $p<0.01$, CK: $p<0.05$, LDH: $p<0.01$). AST, CK and LDH decreased significantly at pre-practice in the tapering period compared with those in the pre- or post-camp (AST: $p<0.05$, $p<0.01$, respectively, CK and LDH: both $p<0.01$). ALT decreased significantly at pre-practice in the tapering period in comparison with that in the pre-camp ($p<0.01$).

Table 3 shows the changes in leukocyte and neutrophil counts during the investigation period. Neutrophil counts at pre-practice in the tapering period decreased significantly compared with that in the pre-camp ($p<0.05$). Leukocyte and neutrophil counts significantly increased at post-practice compared with those at pre-practice in the tapering period (both $p<0.05$), whereas such a trend was not shown in pre- and post-camp.

Table 4 shows the changes in levels of immunoglobulins and complements. IgA and IgG increased significantly at post-practice compared with those at pre-practice in all investigations (all $p<0.05$). C4 also increased significantly at post-practice compared with that at pre-practice in the tapering period ($p<0.01$). IgA decreased significantly at

pre-practice in the post-camp compared with that in the pre-camp ($p<0.05$). C3 increased significantly at pre-practice in the post-camp compared with that in the pre-camp ($p<0.01$).

Table 5 shows the changes in levels of ROS production capability, PA, SOA and serum SOD activity. PA and SOA decreased at post-practice in the tapering period compared with those at pre-practice (PA: $p<0.01$, LgCL · AUC: $p<0.05$).

Serum SOD activity at pre-practice in the tapering period significantly decreased compared with those in the pre- and post- camp ($p<0.01$, $p<0.05$, respectively).

Discussion

In the present study, significant weight loss was observed at all post-practice assessments, suggesting accelerated sweating and loss of water from the body [36]. Also, significant decreases in body weight and body fat percentage were suggested to be due to accelerated energy and lipid metabolism, caused by the dramatically increased amount of training during the training camp [37].

It has been reported that observation of serum myogenic enzymes is an effective method to understand the changes in and damage to muscle tissues caused by transient exercise and the accumulation of damage due to repetition of such conditions [38, 39]. In the present study, the serum myogenic enzyme levels increased significantly at all post-practice assessments. In other words, the intensive 4-hour judo training (practice) sessions performed by the subjects were intense enough to cause muscle tissue changes and damage. In terms of accumulation of muscle fatigue during the investigation period, the levels of serum myogenic enzymes at pre-practice were the highest after the training camp, and lowest at the tapering period. In other words, these results suggested that muscle fatigue accumulated during the camp and recovered adequately to lower levels with the subsequent tapering.

Neutrophils, members of the leukocyte family, are considered as the most essential cells associated with immune functions [40]. Many studies have reported that transient exercise increases the leukocyte count in proportion to the intensity of the exercise [40]. In addition, it has been suggested that such an increase in the number of neutrophils has been caused by accelerated stress reaction, which was directly stimulated by stress hormones including the catecholamines and cortisol [41, 42]. Thus, significant increases in the leukocyte count and neutrophil count after the practice at tapering period in the present study suggested that the 4-hour training sessions carried out by the subjects was intense enough to cause muscle tissue changes and damage, leading to an accelerated inflammatory reaction, and/or accelerated stress reaction mediated by the physical exercise itself.

On the other hand, it has been reported that when the overtraining syndrome occurs due to long-term training, secretion of catecholamines such as adrenaline and noradrenaline are reduced and blood circulation volumes of leukocytes and neutrophils also decrease, or show a reduction in their functions [43, 44]. Similarly, it has also been reported that when athletes develop the overtraining syndrome, secretion of cortisol increases, leading to reduced volumes of circulating blood and/or reduced leukocyte and neutrophil functions [45].

Therefore, a significant decrease of the pre-practice neutrophil count in the tapering period compared to those at pre-camp in the present study suggested that the intensive training performed by athletes during the training camp caused the overtraining syndrome, and led to reduced levels of catecholamines and increased levels of cortisol. Also in the present study, the effects of the overtraining syndrome on immune functions were observed not at post-camp, but approximately 10 days after the training camp during the tapering period, and this supported our previous findings [46].

Immunoglobulins and complements are blood constituents that are essential for immunity as the stress reaction brought about by physical exercise itself as well as inflammatory reactions to exercise-mediated adverse changes in and damage to muscle tissues [40, 41]. Activation of these reactions is also known to be associated with various stress hormones and inflammatory cytokines [40, 41]. Although a number of researchers have investigated the relationship between immunoglobulins and complements, conflicting results have shown that they either increased, decreased or stayed the same with physical exercise [47-50]. The research by Dufaux et al found that levels of C3 and C4 increased after 2.5 hours of running, suggesting that such increases/activation of complements were triggered by muscle damage caused from highly intensive physical exercise [51]. Thus, significant increases in levels of IgA and IgG at post-training compared to pre-training at all assessment points in the present study suggested that muscle tissues were changed and damaged as a result of the 4-hour judo training, which led to activation and acceleration of the inflammatory reaction. Unfortunately, we cannot assess their time-course changes, because each parameter had a different change in the time-course.

There are specific and non-specific types of defense mechanisms against infections. One of the non-specific mechanisms includes the function by

phagocytes, and neutrophil activity in particular is known to play important roles. Also, immunoglobulins and complements in blood opsonize foreign substances so that neutrophils are able to detect them efficiently [52]. Neutrophils then engulf the opsonized foreign substances more effectively through phagocytosis and process the substances by producing ROS. It is recognized that ROS produced by neutrophils is required for sterilization, however, as a downside, excess production of ROS causes oxidative damage to normal cells [53, 54]. As SOA reflects the amount of ROS produced in the body, determination of SOA is often used as an index for ROS production and opsonization within the body [32, 33]. The level of LgCL determined in the present study is a primary marker of ROS metabolism, and reflects the production of superoxide (O_2^-), which has relatively low toxicity [35, 55, 56]. The level of LmCL reflects the production of hydrochlorous acid (HOCl/OCl$^-$) which has relatively high toxicity. Levels of these substances have been suggested to have strong associations with several inflammatory cytokines and stress hormones such as the catecholamines and cortisol [40-42].

In previous studies on physical exercise and neutrophil function it has been variously reported that ROS production capability increases [57, 58] or decreases [59, 60] after acute physical exercise. Several studies on the relationship between physical exercise and PA conflictingly reported that transient physical exercise led PA to accelerate or remain unchanged [57, 59, 61]. In addition, Gabriel *et al.* reported that PA per neutrophil decreased after intensive physical exercise [62].

Moreover, in our past research which investigated ROS production capability and PA simultaneously, ROS production capability was found to increase after transient physical exercise, whereas PA decreased [12, 19-24]. According to studies on long-term training and neutrophil function, it was also demonstrated that neutrophil function was suppressed by continuing highly intensive training for a long period of time [60, 63, 64]. Similar results were also obtained in our previous investigation with professional soccer players [16].

SOD exists within the blood stream and has a scavenger function against ROS. When ROS is produced excessively within the body, SOD reacts against ROS through cytokines to destroy the ROS [65, 66]. The oxidative stress caused by ROS production is reduced and suppressed by antioxidative substances including SOD. It has been reported that acceleration of ROS production by transient exercise increases the serum SOD activity as an antioxidative reaction [67, 68].

Also, research on the association between long-term training and SOD activity reported that SOD activity is increased by continuous, long-term aerobic physical activity [65]. In the present study, serum SOD activity decreased significantly at the tapering period, suggesting that reduction of the antioxidative function was greater at the tapering period compared to the pre- and post-camp. Although the detailed mechanism of this phenomenon is unknown, one of the possibilities is that, between the end of the post-camp and the tapering period where subjects were expected to be experiencing severe muscle fatigue, ROS production was accelerated as a result of repairing and regenerating muscle tissues, resulting in the activation, consumption and depletion of serum SOD. As we have observed a significant decrease of PA at the tapering period, this suggested that chronic fatigue caused by the training camp resulted in production and reduced function of physiologically active substances such as cytokines that are associated with serum SOD activity.

Our results suggested that participation in the intensive training camp by top female judoists aiming to compete in the World Championship caused severe physical fatigue. On the other hand, the 10-day tapering they carried out after the training camp was initially considered sufficient to recover from the muscle fatigue that had accumulated during the training camp from the muscle function point of view.

However, our results clearly indicated that recovery with the 10-day tapering period from the reduced immune antioxidative functions was inadequate, suggesting that the training schedule was not necessarily appropriate from an immunological point of view. Our results therefore suggested that once immune function is decreased, it requires a certain period which is longer than that required to recover from muscle fatigue, and with tapering that includes different periods and contents. The results also proposed that the reduction of various bodily functions after physical exercise occurred at different times, and each of them required a different period for recovery by following different steps. Thus, these points should be carefully considered in order to maintain athletes' physical health and conditioning when carrying out strengthening and tapering prior to any competition. It is therefore important for coaches to understand athletes' physical condition objectively and thus, put extra thought into designing the contents of training programs that can be adapted to take into careful consideration the athletes' physical fatigue during the tapering period (intensity, frequency and period of training), method for resting (sleeping duration etc.) and the athletes' diet (types and amounts of nutrients they should be taking).

Acknowledgment

This study was partly supported by a Grant-in-Aid from the Ministry of Education, Culture,

Sports, Science and Technology, Japan (no. 23650427), and was based on the Iwaki Health Promotion Project as a project by Hirosaki University Graduate School of Medicine, in collaboration with Aomori Heath Evaluation and Promotion Center and Hirosaki City Office, Department of Health Promotion.
(Received Nov 5, 2013. Accepted Dec 10, 2013)

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