

The relationship between muscle damage and reactive oxygen species production capability after judo exercise

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柔道稽古後の筋損傷と好中球活性酸素種産生能の関連について

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Running title

Physical fatigue and neutrophil immune function in judoists

柔道選手における身体的疲労と好中球機能

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Abstract

The relationship between neutrophil function and the muscles damage before and after exercise in judoists was examined. The changes in the body composition, biochemical parameters (neutrophil counts and myogenic enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) etc. and the major neutrophil immune functions such as oxidative burst activity (OBA) and phagocytic activity (PA) were measured in 39 male university judoists before (pre value) and after 2 hours of unified exercise loading. Increases in myogenic enzymes were confirmed after the exercise, which suggested that the muscular tissue damage and degeneration was caused by the exercise itself. Neutrophil count and OBA increased and PA decreased after exercise. The amount of change (post value – pre value) of OBA showed a negative correlation with LDH and AST ($P < 0.01$ all) and that of PA displayed a positive relationship with LDH and AST ($P < 0.05$ all). Following the muscle damage, neutrophil PA may have increased due to increased biological detritus by phagocytosis such as muscle fragments. The reason for OBA movement against muscle damage (negatively correlated) may be due to the results of compensating OBA and PA e.g. increased OBA and decreased PA, or decreased OBA and increased PA.

Key words. muscle damage , judo , neutrophil function , reactive oxygen species (ROS)
, exercise

抄録

柔道選手を対象として稽古前後の好中球機能と筋損傷の関係を調査した。

39人の男子大学柔道選手において、普段の2時間の稽古前後に体組成と生化学指標（好中球数、好中球機能（活性酸素産生能（OBA）、貪食能（PA））、筋逸脱酵素（LDH（乳酸脱水素酵素）、アスパラギン酸アミノトランスフェラーゼ（AST）、アラニンアミノトランスフェラーゼ（ALT）など）を測定した。

稽古後に筋逸脱酵素は増加し、稽古に伴う筋組織の損傷と変性によると考えられた。また、稽古後に好中球数とOBAは増加し、逆にPAは低下した。一方、OBAの変化量はLDHおよびASTの変化量と負の相関関係を示し（ $p < 0.01$ ）、PAの変化量はLDH、ASTの変化量と正の相関関係を示した（ $p < 0.05$ ）。

筋損傷に対するPAの変動（正の相関）の要因としては、筋損傷に伴う筋肉の断片のような生理学的異物に対して好中球の異物反応が亢進した可能性が考えられた。また、筋損傷に対するOBAの変動（負の相関）の要因としては、好中球の各機能間における補完機構がOBAとPAの間で生じた可能性が考えられた。

Introduction

Judo is a martial art originated from Japan and has become an international competitive sport since the Tokyo Olympics, and is a competitive sport that can also be classified as a contact sport ¹⁾.

Although the duration of each judo match is short, the burden on the body and the risk of injury are both high ^{2,3)}. It is also thought that judo practice has a large effect on the body, especially the muscle tissue.

Exercise has been reported to elevate the levels of serum enzymes such as creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) through the mechanisms of muscular inflammation, increased permeability of muscle cell membranes^{4,5,6)}, anoxic or hypoxic damage to muscles ^{7,8)} or production of toxic free radicals ⁹⁾.

On the other hand, the number of neutrophils and the neutrophil function, i.e. neutrophil reactive oxygen species (ROS) production capability, has been reported to increase after high intensity exercise^{10,11,12,13,14,15)}. Neutrophils represent one of the cellular factors that play an important role in the first line of defense against invading pathogens, including microorganisms. Neutrophils engulf microorganisms through phagocytic activity (PA) and destroy them through the production of cytotoxic reactive oxygen species (ROS) ^{16,17)}. However, the meaning behind the changes in these neutrophil functions remains unclear. For example, ROS can destroy invading microorganisms ^{18,19)}, however, they can cause oxidative damage to normal body tissues and organs under different circumstances^{20,21)}. Unfortunately, the borderline between the beneficial and detrimental effects of ROS production remains unclear.

In recent studies, modified muscle use such as exercise or injury can produce an

inflammatory response in which neutrophils rapidly invade muscle tissue, followed by macrophages, which coincide with muscle repair, regeneration, and growth, and which involve the activation and proliferation of satellite cells, followed by their terminal differentiation^{22,23}).

Furthermore, recent studies have contributed to painting a complex picture in which inflammatory cells including neutrophils and macrophages promote both muscle injury and repair, through the combined actions of free radicals such as ROS, growth factors and chemokines²²). It has become clear that a role has emerged for neutrophils in promoting muscle damage soon after muscle injury or modified use^{24,25}). Unfortunately, no direct evidence is yet available to show that neutrophils play a beneficial role in muscle repair or regeneration, although few studies have evaluated the amount of neutrophil ROS production and its relationship with muscular tissue damage²⁶).

We focused our attention on the relationship between muscular tissue damage and neutrophil ROS production following judo exercise in this study. The characteristics of this study used two neutrophil function such as ROS and phagocytic activity (PA) (which generally show opposite movement following exercise).

Subjects and Methods

1. Subjects

The 39 male subjects of this study belonged to the Nippon Sport Science University judo team that participated in a summer training camp. The athletes' demographics and anthropometrics are shown in Table 1.

This study was approved by the Ethics Review Committee of Hirosaki University School of Medicine. Each participant was adequately informed of the goal, methods, and potential

risks, as well as the right to abstain from participating in this study. Freely given informed consent was obtained in writing from all participants.

2. Methods

We measured the changes in the body composition, biochemical parameters and the major neutrophil immune functions in 39 male university judoists before (pre value) and after a 2-hour unified exercise loading (UEL)(post value).

1. Training program

The 2-hour UEL consisted of a warm-up for 15 min; “*uchicomi*” (the same technique practiced repeatedly, such as throw down, push down and hook down) for 20 min; “*randori*” (exercise training in the form of a match) for 70 min; and cool-down for 15 min. This training was classified as irregular-interval training, and is a model of acute exercise. The mean heart rate during the training was 128.8 ± 12.0 bpm and the maximum heart rate was 180.5 ± 14.0 bpm, recorded in the *randori* phase. The heart rate was measured and recorded at intervals of 15 seconds using VANTAGE XL (POLAR ELECTLIC Inc. , Finland)²⁷, and was analyzed at intervals of 1 minute after the end of the measurement using POLAR HR ANALYSIS SOFTWARE VER. 4 (POLAR ELECTLIC Inc., Finland).

2. Anthropometry

The anthropometric parameters included body weight, relative body fat, and fat-free mass. An apparatus employing the impedance method (TBF-110, TANITA, Tokyo, Japan) was used in their measurement.

3. Blood chemistry parameters

Twenty milliliter samples of blood were collected from the forearm vein before and after the training. The collected blood samples were split up as described and allocated for measurement with the following methods. Five milliliters were used to analyze the hemocyte elements, 10 ml were used to analyze the serum elements and 5 ml were used to analyze the neutrophil function. Leukocyte and neutrophil counts, hemoglobin (Hb), hematocrit (Ht) were determined from venous blood treated with ethylenediaminetetraacetic acid using an automatic blood cell counter (Sysmex K-2000, Kobe, Japan). Serum samples were separated from blood samples by centrifugation for 10 min at 3,000 rpm and kept frozen at -30°C until used for measurement at a later date. The measurement items of the serum elements were AST, ALT, CK and LDH which were measured with the ultraviolet method. These values after the exercise were adjusted by the plasma volume calculated by the levels of hematocrit (Ht) and hemoglobin before and after the exercise²⁸⁾ because dehydration was observed in the changes of weight and Ht before and after the training.

4. Neutrophil oxidative burst activity (OBA) and phagocytic activity (PA)

These activities were measured with a FACScan system (Becton Dickinson, San Jose, CA) using two-color flow cytometry. Hydroethidine (HE: 44.4 µM, Polyscience Inc., Warrington, PA) was used as an indicator for OBA (representing the ROS production capability), and opsonized zymosan particles (Sigma Chemical Co., St. Louis, MO) labeled with fluorescein isothiocyanate (FITC, Sigma) were used as indicators for PA.

In brief, 100 µl of heparinized whole blood was mixed with 22 µl of HE (final concentration; f.c. 8 µM), and incubated at 37°C for 5 min. After the addition of 25 µl of

FITC-labeled opsonized zymosan (FITC-OZ, f.c. 5 mg/ml), the samples were incubated at 37°C for 35 min. To measure basal oxidative burst activity, neutrophils labeled with only HE served as controls. After the incubation, Lyse and Fix (IMMUNOTECH, Marseille, France) were added to the red blood cells to fix the 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 FACScan. Just before the assay, 30 µl of trypan blue (0.25 mg/ml, pH 4.5) was added to differentiate the attached from the ingested FITC-OZ in the samples following measurement by fluorescence quenching^{29,30}. The intensity of neutrophils expressing FITC-OZ and HE fluorescence was computed. Neutrophil OBA and PA were estimated as the mean channel number of the FI of activated neutrophils.

5. Statistical analysis

Data are presented as the mean \pm standard deviation. The differences between before and after exercise were tested with the paired *t*-test. The correlation between muscle tissue and ROS was tested with Spearman's coefficient. The differences were considered to be statistically significant at $p < 0.05$. All parameters were tested for normal distribution with the Shapiro-Wilkes test for normality before further analysis. The statistical analysis was carried out using the PC version of the statistical package SPSS/PC.

Results

Body weight significantly decreased after the exercise ($P < 0.01$) (Table 1).

Leukocyte counts did not show any significant change after the exercise. On the other

Table.1 挿入

hand, neutrophil counts showed a significant rise after the exercise ($P < 0.01$) (Table 2).

Table.2
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As for myogenic enzymes, LDH and AST increased significantly after the exercise ($P < 0.01$ for both) (Table 3).

Table.3
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As for neutrophil function, OBA increased significantly after the exercise ($P < 0.01$).

On the other hand, PA significantly decreased after the exercise ($p < 0.01$) (Figure 1).

Figure.1
挿入

The amount of change (post value – pre value) of OBA showed a negative correlation

with LDH and AST ($P < 0.01$ for both) and that of PA displayed a positive relationship with

LDH and AST ($P < 0.05$ for both) (Figure 2).

Figure.2
挿入

Discussion

Exercise has been reported to elevate the levels of serum myogenic enzymes including those mentioned in the Introduction section. Therefore changes in these enzyme levels may be effective markers for monitoring overtraining in athletes³¹⁾. Evans et al. have reported that CK levels increased more significantly in non-exercising subjects than in habitually exercising subjects under the same exercise load, and that habitual exercise might increase the capability of muscular function and tissue structure to perform more intense exercise without any exercise-related muscle injury⁴⁾.

Koutedakis Y. et al. have also reported that intense and high quality physical training in Olympic rowers failed to produce any significant change in the above-stated enzyme levels, leading to the speculation that an inevitable adaptation occurs in such elite competitors, i.e., a blunted incremental response to exercise³³⁾. This study confirmed the previous findings^{1,2,10,30,33)} that exercise increases the levels of serum CK, AST, ALT, and LDH.

The relationship between leukocytes and exercise has been well-reported: leukocyte and neutrophil counts increase with transient exercise, the degree of increase being dependent on

the intensity of the exercise ³³⁾. This study confirmed the previous findings that exercise increases the neutrophil count ^{8,11,12,13,15)}. Furthermore, the increases in the levels of these components 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 ^{6,7)}. On the other hand, another study has suggested that the elevated white blood cell 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 ⁶⁾.

Eccentric exercise commonly results in muscle damage. The primary sequence of events leading to exercise-induced muscle damage is believed to involve initial mechanical disruption of sarcomeres, followed by impaired excitation-contraction coupling and calcium signaling, and finally, activation of calcium-sensitive degradation pathways ^{28,34,35)}. Muscle damage is characterized by ultrastructural changes to muscle architecture, increased muscle proteins and enzymes in the bloodstream, loss of muscular strength and range of motion, and muscle soreness. The inflammatory response to exercise-induced muscle damage is characterized by leukocyte infiltration and production of pro-inflammatory cytokines within damaged muscle tissue, systemic release of leukocytes and cytokines, in addition to alterations in leukocyte receptor expression and functional activity. Current evidence suggests that inflammatory responses to muscle damage are dependent on the type of eccentric exercise, previous eccentric loading (repeated bouts), age and gender ^{36,37,38)}. Circulating neutrophil counts and systemic cytokine responses are greater after eccentric exercise using a large muscle mass than after other types of eccentric exercise involving a smaller muscle mass ^{39,40)}. After an initial bout of eccentric exercise, circulating leukocyte counts and cell surface receptor expression are attenuated ⁴¹⁾. However, such relationship is seen between neutrophil function such as OBA and PA and the exercise-induced muscle damage ^{22,42)}.

In the present study, a novel finding is shown as follows. The amount of change in OBA was negatively correlated with the amount of change in AST and LDH, though positive correlation was seen between the amount of change in PA and AST/ LDH.

As for PA, this result suggested that neutrophil PA increased in order to phagocytose the increased biologic detritus, like as muscle fragments following muscle damage.

To explain the relationship between the changes in OBA and myogenic enzyme, e.g. greater in degree of muscle damage, greater in inhibition OBA production, is difficult.

Mochida et al ¹¹⁾ suggested that such varied findings showed that these neutrophil immune functions may compensate for each other to maintain the overall integrity of the neutrophil immune function, e.g. increase OBA, decrease PA, or decrease OBA, increase PA, depending on the exercise loading and subject's physical condition, such as the degree of fatigue. Furthermore, in order to clarify the relationship between exercise and neutrophil immune function, we should study the change in these functions under various conditions of exercise loading and in subjects under different environmental conditions and degrees of physical fitness. Therefore, with myogenic enzymes, OBA may have changed by UEL opposite to PA.

However, these parameters have been well-recognized as showing a time trend dependant on sampling timing, subject conditions and so on ⁴³⁾. For example, Peake et al. reported a different result from the current result, that OBA remained unchanged after treadmill downhill running (immediately after, 1 h, and 24 h after), despite increases in plasma markers of muscle damage (plasma Mb concentration and CK activity) ⁴²⁾. Therefore, more detailed studies are required in order to clarify this.

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Table 1. Physical characteristics and changes in anthropometric parameters

	Before exercise	After exercise	
Body weight (kg)	79.4 ± 12.8	78.9 ± 12.8	††
Weight loss rate (%)	-	-0.58 ± 0.51	
Relative body fat (%)	13.9 ± 3.4	-	
Body fat (kg)	11.4 ± 5.2	-	
Fat free mass (kg)	67.9 ± 7.9	-	

Results were expressed as mean ± standard deviation .

††: p<0.01 compared with pre value.

Table 2. Changes in blood leukocyte/neutrophil counts

	Before exercise	After exercise *	
Leukocyte (cells/mm ³)	6469.2 ± 1268.9	6634.4 ± 1420.2	
Neutrophil (cells/mm ³)	3455.4 ± 1089.4	4119.3 ± 1370.3	††

Results were expressed as mean ± standard deviation.

††: p<0.01 compared with pre value.

*: The post values were adjusted for dehydration by plasma volume.

Table 3. Changes in serum myogenic enzymes

	Before exercise	After exercise *	
CK (IU/l)	539.1 ± 352.3	556.5 ± 343.7	
LDH (IU/l)	231.9 ± 33.9	246.6 ± 34.6	††
AST (IU/l)	26.6 ± 8.2	27.9 ± 8.8	††
ALT (IU/l)	21.6 ± 9.3	22.0 ± 9.5	

Results were expressed as mean ± standard deviation.

††: p<0.01 compared with pre value.

*: The post values were adjusted for dehydration by plasma volume.

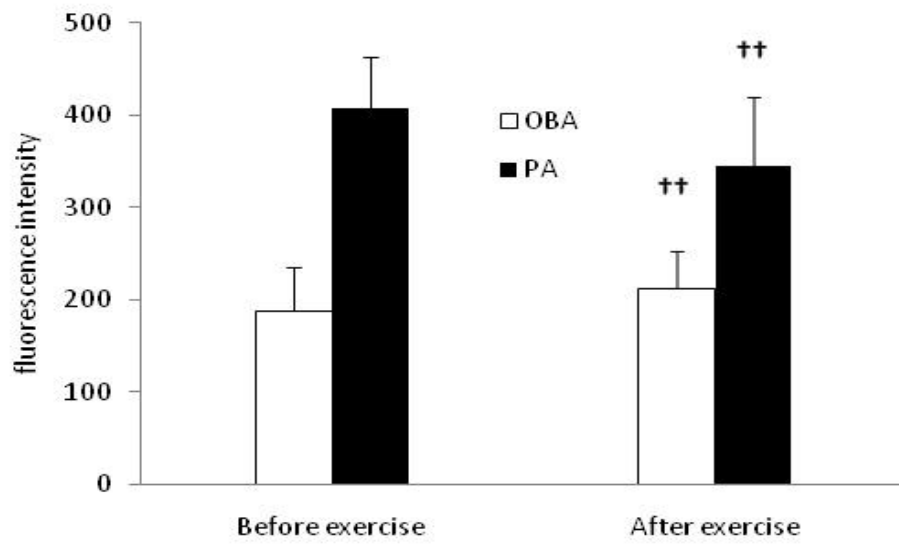


Figure1. Change in OBA and PA.

Results were expressed as mean \pm standard deviation. OBA: oxidative burst activity, PA: phagocytic activity. ††: $p < 0.01$ compared with pre value.

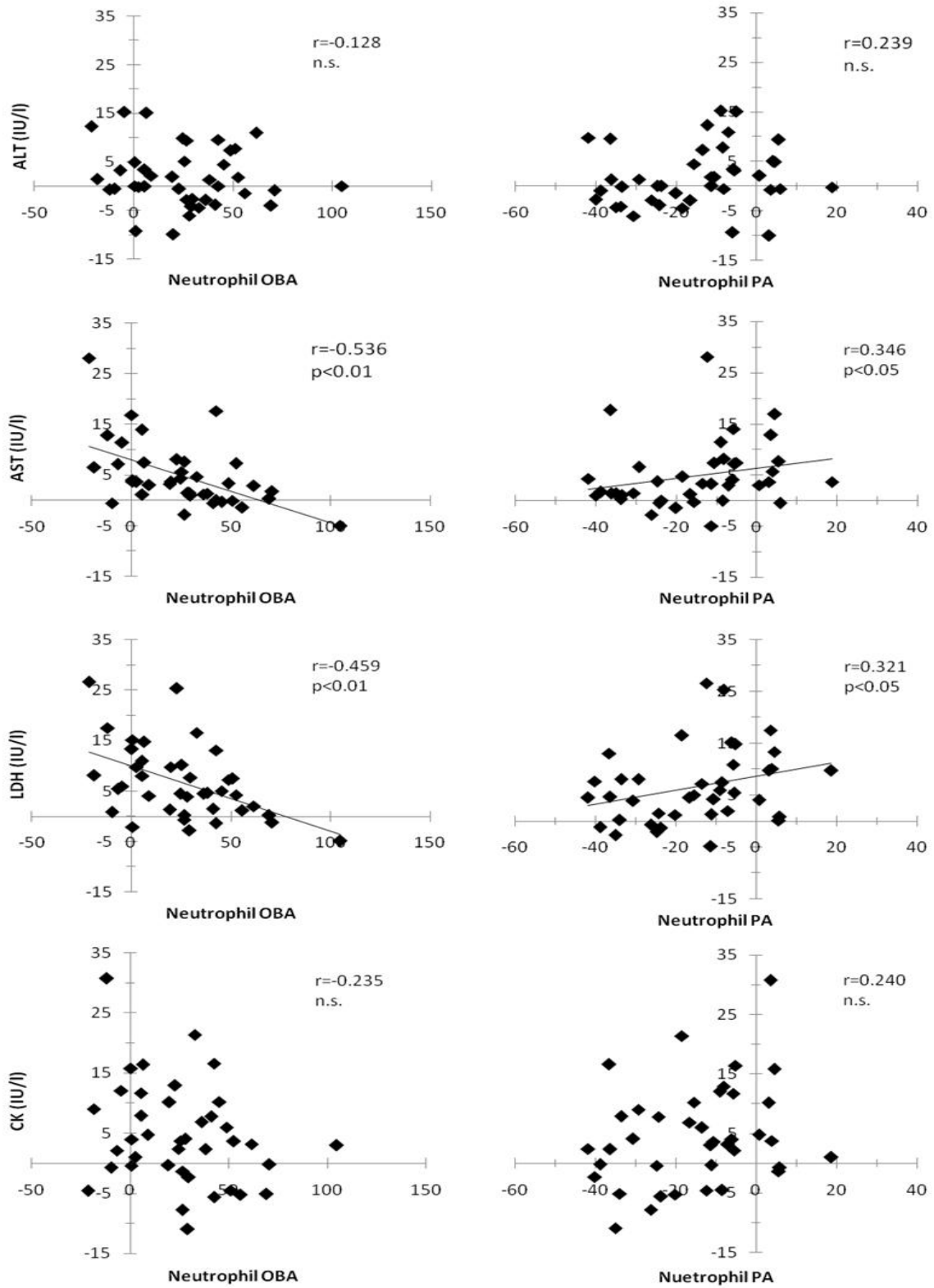


Figure 2. Correlation between the change rates of neutrophil PA and OBA, and serum myogenic enzymes.

OBA: oxidative burst activity, PA: phagocytic activity. n.s.: not significant.