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ORIGINAL ARTICLE

EFFECTS OF L-GLUTAMINE INTAKE ON MUSCLE FATIGUE AND NEUTROPHIL FUNCTIONS DURING A JUDO TRAINING CAMP

Tadahiro Nomura[1, 2], Takashi Umeda[1], Ippei Takahashi[1], Kaori Iwane[1], Noriyuki Okubo[1], Yoshinobu Chiba[3], Ryosuke Miyake[3], Hiroyuki Konishi[1, 4], Itoyo Tokuda[1], Miho Komatsu[5], and Shigeyuki Nakaji[1]

Abstract We assessed the effects of L-glutamine intake on muscle tissues and neutrophil functions of judoists after intensive training. Thirty-five male judoists of university student were divided into two groups; glutamine group (18 subjects) and placebo group (17 subjects). During the 7-day intensive judo training camp, a total of 6 g of L-glutamine or placebo per day was given to each subject. Myogenic enzymes, reactive oxygen species (ROS) production capability and phagocyte activity (PA) of neutrophils were measured before and after the 2-hour intensive judo practice on the day before and after the training camp. As a result, at pre-practice, levels of all myogenic enzymes tended to be increased from pre-camp to post-camp in the control group ($p<0.05$ for CK; $p<0.01$ for AST, ALT and LDH), whereas such trend was not seen in the glutamine group. Further, the changing rate (before and after the practice) of ROS and PA tended to be lower in the glutamine group than in the control group after the training camp ($p<0.05$ for PA; $p=0.10$ for ROS), however there were no significant differences in those between two groups before the training camp ($p=0.25$ for PA; $p=0.28$ for ROS). In conclusion, intake of glutamine during an intensive judo training camp had a protective effect against damaged muscle functions and immune functions.

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Key words: Glutamine; judoist; camp training; neutrophil function; myogenic enzyme.

原　著

強化合宿中のL-グルタミンの摂取が柔道選手の筋組織，好中球機能に及ぼす影響について

野村忠宏[1, 2]　梅田　孝[1]　高橋一平[1]　岩根かほり[1]
大久保礼由[1]　千葉義信[1]　三宅良輔[3]　小西裕之[1, 4]
徳田糸代[1]　小松美穂[5]　中路重之[1]

抄録　大学男子柔道選手35名を対象に合宿中にL-グルタミンを摂取させ，筋組織，免疫機能への影響を検討した．対象者を，無作為にL-グルタミン6 g/日を摂取させる18名（グルタミン摂取群）とプラセボを摂取させた17名（対照群）に区分した．7日間の強化合宿を行い，合宿前日と終了翌日に調査した．また，両調査日に2時間の同一の柔道練習を実施し，その直前と直後に，白血球数，筋逸脱酵素等，好中球機能等の測定を行った．対照群でのみ筋逸脱酵素の練習前値は，合宿前から合宿後にかけて上昇する傾向にあった（CKは$p<0.05$，その他はすべて$p<0.01$）．一方，合宿前において，両群の好中球の活性酸素種産生能と貪食能の練習による変化率に差はみられなかったが，合宿後には，グルタミン群が対照群より小さい傾向にあった（貪食能のみ$p<0.05$）．以上より，合宿中のグルタミン摂取は，一過性の運動により発現する筋組織の変性・損傷や免疫機能の低下を抑制させる可能性が示唆された．

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キーワード：グルタミン；柔道選手；強化合宿；好中球機能；筋逸脱酵素．

[1] Department of Social Medicine, Hirosaki University Graduate School of Medicine
[2] Miki House Co. Ltd.
[3] Nippon Sport Science University
[4] Sendai University
[5] Healthcare Products Development Center, KHOWA HAKKO BIO CO. LTD.
Correspondence: S. Nakaji
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[1] 弘前大学大学院医学研究科社会医学講座
[2] ミキハウス（株）
[3] 日本体育大学
[4] 仙台大学
[5] 協和発酵バイオ株式会社・ヘルスケア商品開発センター
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Introduction

Judo is a sport that can be classified as both a competitive and a contact sport. It involves a player (judoist) coming into direct contact with their opponent through repeated movements involving frequent falls to the mat while attempting to throw each other down or pin each other to the mat. Strenuous muscular power is constantly required while participating in a judo bout, as the judoists must continuously attempt to takedown and subdue their opponent. Thus, judoists are more prone to injuries compared to athletes from other sports[1].

Previously, our research group has found that approximately 2 hours of routine judo practice in university judoists led to dehydration, loss of serum electrolytes, renal failure, muscular damage, as well as accelerated stress and inflammatory reactions[2-4]. In other words, these studies suggest the difficulty in maintaining and managing the health and physical condition of athletes, especially for judoists who are constantly exposed to a high risk of injury. Thus, adequate and appropriate recovery from fatigue and reduced physical function after training is essential for judoists and other athletes, in order to prevent chronic accumulation of fatigue which may lead to the overuse syndrome and/or overtraining syndrome. It is also important for the long-term management of athletes' health and conditioning.

Furthermore, athletes often participate in training camps during the off-season period to improve their physical strength and skills. The duration of these camps and the season in which they are held can vary, however, many of them include long hours of practice. Previous studies have reported that these training camps can cause extensive physical and mental fatigue in athletes[5-8].

According to previous studies on rugby players, a highly intensive training camp in summer was found to cause extensive nutrition consumption, damage in muscle tissues, accumulation of muscle fatigue, reduction in renal function and loss of electrolytes[9]. Moreover, in a 7-day training camp for female judoists, extensive consumption of energy, accumulation of muscle fatigue as well as immunodepression were all demonstrated[10, 11].

Glutamine is one of the amino acids most abundant in the human body, though there is no criterion for the daily recommended allowance[12]. Glutamine is synthesized in skeletal muscle cells, and it is necessary for immune function, energy production and nucleic acid synthesis[13]. Decreases of plasma glutamine concentration are correlated with immune suppression in patients with infection and fever[14], and athletes in overtraining and participation in training camps[15, 16]. According to these studies, immune function may be maintained by administering glutamine supplementation to increase its plasma concentration[17, 18]. However, there have been only a few studies that reported the positive effect of glutamine supplementation on immune function[17, 19, 20]. Furthermore, its association with neutrophils has not yet been found[21].

In the present controlled study, male university judoists were asked to take glutamine versus a placebo during a highly intensive training camp where physical fatigue was likely to accumulate, and the effects of glutamine on muscle tissues and immune functions were investigated. We focused on the relationship between glutamine intake and immune function, which to the best of our knowledge has not yet been thoroughly investigated before.

Subjects and Methods

1. Subjects and Research Period

The study subjects comprised 35 male

Table 1 Weekly training program during the research period.

	a.m. 6:30-a.m.7:30	a.m. 9:00-a.m. 11:30	p.m. 5:30-p.m. 8:00
Monday	Training A	Rest	Training D
Tuesday	Training B	Rest	Training D
Wednesday	Training C	Rest	Training D
Thursday	Training A	Rest	Training D
Friday	Training B	Rest	Training D
Saturday	Training C	Training D	Rest
Sunday	Rest	Rest	Rest

Training A: Interval training consisted of sprint running (800m*1, 400m*3, 200m*3, 100m*4) and jogging
Training B: Weight training
Training C: Distance running for 30 minutes and short sprint running (repeating 30-50m sprint running during training time)
Training D: Judo practice
Rest: Resting or attending lectures

university judoists who were the members of a university judo club. They were classified into different weight groups, and randomly divided into the glutamine group, who received glutamine tablets (18 subjects) and the control group, who received placebo tablets (17 subjects), subjects being unaware of which group they had been assigned to. The average age, height, body weight, lean body fat percentage were 18.4 ± 0.6 years old, 167.4 ± 6.9 cm, 74.7 ± 16.7 kg, 14.0 ± 7.6% and 63.1 ± 7.8 kg for the glutamine group, and 19.5 ± 1.1 years old, 171.2 ± 5.0 cm, 78.3 ± 14.9 kg, 14.5 ± 6.5% and 66.1 ± 7.5 kg for the control group. There was no significant difference in these parameters between the two groups.

The investigation was conducted over nine days in August 2007, when the subjects were participating in an intensive training camp. The measurements were taken on the day before the one-week training camp (pre-camp) and the day after the training camp (post-camp). Subjects carried out a 2-hour judo practice on those days and the measurements were taken immediately before (pre-practice) and after (post-practice) the practice. The 2-hour practice included 15 minutes of warm-up, 20 minutes of *uchikomi* (practice of the same technique repeatedly such as throw-down, push-down and hook-down), 70 minutes of *randori* (exercise training in the form of a match) and 15 minutes of cool-down.

Before the training camp, the weekly training menu carried out by subjects included 2 and a half hours of judo practice, 1 hour of running or weight training for 6 days with 1 day of rest (Table 1). During the training camp, judo practice was conducted for 3 and a half hours in the morning and 2 hours of running (combination of long-distance and short sprint) as well as 1 hour of weight training. Thus, the amount of exercise subjects carried out during the training camp was approximately twice as much as the usual practice.

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.

2. Intake method and amount of L-glutamine

Six grams of L-glutamine tablet (Kyowa Hakko Ltd.) was given to subjects in the glutamine group per day (1 tablet containing 1.5 g of glutamine was given 4 times a day). For the control group, a similar-looking tablet

without any active ingredients was given to subjects in the control group. As subjects had one training session in the morning and one in the afternoon, one tablet was given to each subject immediately before and after each practice. On the day before and after the training camp, judo practice was carried out only in the morning. Thus, their glutamic intakes on those days were 3.0 g in total on those days (1.5 g before and after the morning practice).

3. Body composition

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

4. Nutritional intake

All subjects pursued the same chart and wrote down what and how much they ate on a daily basis, accompanied by a picture of each meal. Based to the 5th version of the Standard Tables of Food Composition in Japan[22], the total energy intake, protein intake, fat intake and carbohydrate intake were calculated. In the present study, the average nutrition intake per day was derived from the food intake during the investigation period.

5. Blood biochemical parameters

Blood samples (15 ml) were taken from each subject one hour after breakfast (pre-practice) and immediately after the 2-hour practice (post-practice). Five milliliters of the sample was used to analyze the blood cell components, and the remaining 10 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 of blood serum, the activity of superoxide dismutase (SOD) was also measured.

All blood cell components were measured using an Automated Blood Cell Analyzer (System XE-2100 and SE-9000 Kobe, Japan), and 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[23]. All measurements of blood biochemical items were consigned to an independent laboratory (Mitsubishi Chemical Medience).

6. 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

Table 2 Characteristics of study subjects and their anthropometric parameters during the training camp

	Glutamine group (n=18)				Control group (n=17)			
	Pre-camp		Post-camp		Pre-camp		Post-camp	
Age (years)	18.4 ± 0.6		-		19.5 ± 1.1		-	
Height (cm)	167.4 ± 6.9		-		171.2 ± 5.0		-	
Weight (kg)								
Pre-practice	74.7 ± 16.7		75.5 ± 17.3	††	78.3 ± 14.8		79.4 ± 15.0	††
Post-practice	74.3 ± 16.6	**	75.0 ± 17.3	**	77.7 ± 14.9	**	78.6 ± 15.2	**
Relative body fat (%)	14.0 ± 7.6		14.5 ± 8.3		14.5 ± 6.5		15.2 ± 6.6	
Lean body mass (kg)	63.1 ± 7.8		63.3 ± 7.6		66.1 ± 7.5		66.5 ± 7.4	

Mean ± standard deviation
Glutamine group: 1.5 g of L-glutamine was given to subjects four times a day (total of 6 g/day). These were taken before and after the judo practice in the morning and afternoon
Control group: 1.5 g of placebo was given to subjects four times a day (total of 6 g/day). These were taken before and after the judo practice in the morning and afternoon
Pre: Pre-practice. Post: Post-practice.
**: $p<0.01$, Significant difference from the pre-value in the group
††: $p<0.01$, Significant difference from the pre-camp value in the group

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 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 mL 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[24, 25].

For each sample, 10,000 neutrophils were analyzed. Neutrophil ROS production capability and PA were recorded using a logarithmic scale and were estimated as the mean channel value of the FI per single activated neutrophil.

7. Statistical analysis

All values were presented as means ± standard deviation. Differences in mean values between before and after the practice in each group were tested with the generalized Wilcoxon t-test. Also, the Mann-Whitney U-test was used to analyze the change rates of mean values before and after the practice. 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 2 shows the changes in the levels of body composition during the investigation period. Body weight decreased significantly at post-practice of pre- and post-camp for both groups ($p<0.01$ for all).

The subjects' nutritional intake during the training camp is shown in Table 3. There was no significant difference in total energy intake, protein, fat or carbohydrate intakes between the two groups.

Table 4 shows the changes in myogenic enzymes during the investigation period. All myogenic enzymes increased significantly at post-practice for both groups ($p<0.01$ for all).

Table 3 Nutritional intakes during the training camp

	Glutamine group (n=18)	Control group (n=17)
Total energy intake(kcal)	4021.8 ± 431.4	4219.3 ± 316.6
Protein intake (g)	150.3 ± 7.1	154.5 ± 9.8
Lipid intake (g)	109.3 ± 8.6	112.1 ± 8.1
Carbohydrates intake (g)	594.1 ± 90.6	628.2 ± 61.3

Mean ± standard deviation
Glutamine group: 1.5 g of L-glutamine was given to subjects four times a day (total of 6 g/day), taken before and after the judo practice in the morning and afternoon
Control group: 1.5 g of placebo was given to subjects four times a day (total of 6 g/day), taken before and after the judo practice in the morning and afternoon

Table 4 Changes of serum myogenic enzymes during the training camp

	Glutamine group (n=18)				Control group (n=17)			
	Pre-camp		Post-camp		Pre-camp		Post-camp	
AST(IU/l)								
Pre-practice	25.2 ± 4.7		26.4 ± 5.5		23.3 ± 3.5		30.0 ± 6.9	††
Post[a]-practice	27.7 ± 5.8	**	29.9 ± 5.8	**	26.4 ± 4.5	**	33.5 ± 8.1	**††
Change ratio before the training (%)	10.1 ± 9.1		13.4 ± 6.7		13.3 ± 6.9		11.7 ± 6.1	
ALT(IU/l)								
Pre-practice	24.1 ± 14.3		26.4 ± 11.9		17.9 ± 4.5		29.4 ± 10.5	††‡
Post[a]-practice	25.5 ± 14.5	**	28.5 ± 12.8	**	19.8 ± 4.6	**	31.7 ± 12.1	**††
Change ratio before the training (%)	7.1 ± 6.7		7.9 ± 4.7		10.9 ± 6.9		7.2 ± 5.1	
CK(IU/l)								
Pre-practice	325.1 ± 178.0		391.5 ± 196.2		340.4 ± 162.5		463.6 ± 164.4	†
Post[a]-practice	375.4 ± 204.5	**	455.2 ± 215.7	**	410.5 ± 200.2	**	534.3 ± 177.9	**
Change ratio before the training (%)	17.3 ± 9.7		17.2 ± 7.9		20.6 ± 5.5		16.1 ± 9.2	
LDH(IU/l)								
Pre-practice	247.9 ± 54.1		270.8 ± 40.5	††	243.1 ± 33.5		287.7 ± 45.0	††
Post[a]-practice	275.2 ± 36.2	**	308.9 ± 39.2	**††	279.3 ± 43.5	**	319.9 ± 47.4	**††
Change ratio before the training (%)	12.9 ± 12.4		14.6 ± 8.5		14.8 ± 5.9		11.5 ± 6.1	

Mean ± standard deviation
Glutamine group: 1.5 g of L-glutamine was given to subjects four times a day (total of 6 g/day), taken before and after the judo practice in the morning and afternoon
Control group: 1.5 g of placebo was given to subjects four times a day (total of 6 g/day), taken before and after the judo practice in the morning and afternoon
a: Values after practice were adjusted for dehydration by plasma volume
**: $p<0.01$, Significant difference from the pre-value in the group
†: $p<0.05$, ††: $p<0.01$, Significant difference from the pre-camp value in the group
‡: $p<0.05$, Comparison of the values between the two groups

In the glutamine group, the post-camp level of LDH at pre- and post-practice was significantly higher ($p<0.05$ for all) compared with pre-camp levels. In the control group, the post-camp levels of AST, ALT, LDH ($p<0.01$ for all) and CK ($p<0.05$) at pre- and post-practice were significantly higher compared with pre-camp. In the end, at pre-practice, levels of all myogenic enzymes were increased significantly from pre-camp to post-camp in the control group, whereas those was not seen in the glutamine group.

Table 5 shows the changes in leukocyte and neutrophil counts during the investigation

Table 5 Changes in blood leukocyte and neutrophil cells during the training camp

	Glutamin group (n=18)				Control group (n=17)			
	Pre-camp		Post-camp		Pre-camp		Post-camp	
Blood leukocyte cell counts (/μl)								
Pre-practice	5744 ± 1231		5578 ± 1262		5912 ± 1104		5724 ± 1139	
Post[a]-practice	6656 ± 1618	**	6494 ± 1214	**	6261 ± 1249		6214 ± 1486	**
Change ratio before the practice (%)	16.6 ± 18.0		18.6 ± 16.1		6.4 ± 13.6		8.8 ± 17.4	‡‡
Blood neutrophil cell counts (/μl)								
Pre-practice	2912 ± 817.0		2838 ± 1007		3250 ± 826		3215 ± 997	
Post[a]-practice	4073 ± 1585	**	4015 ± 1174	**	3862 ± 980	**	3843 ± 1194	**
Change ratio before the practice (%)	39.8 ± 36.0		50.8 ± 40.8		20.4 ± 23.3		21.9 ± 25.1	‡‡

Mean ± standard deviation
Glutamine intake group: 1.5 g of L-glutamine was given to subjects four times a day (total of 6 g/day), taken before and after the judo practice in the morning and afternoon
Control group: 1.5 g of placebo was given to subjects four times a day (total of 6 g/day), taken before and after the judo practice in the morning and afternoon
a: Values after the practice were adjusted for dehydration by plasma volume
**: $p<0.01$, Significant difference from the pre-value in the group
‡‡: $p<0.01$, Comparison of the values between the two groups

period. These levels increased significantly at post-practice in both groups ($p<0.01$ for all). However, the rate of increase was less in the control group compared to glutamine group at post-camp ($p<0.01$ for both), and not at pre-camp.

Table 6 shows the changes in levels of immunoglobulins and complements. These parameters increased significantly at post-practice both pre- and post-camp in both groups ($p<0.05$), without any significant differences between the two groups.

Table 7 shows the changes in ROS production capability, PA and SOD activity. In the glutamine group, the pre-camp level of ROS production capability at post-practice decreased significantly whereas PA increased significantly ($p<0.01$ for both). Also, ROS production capability in the same group decreased significantly at the post-camp post-practice ($p<0.01$), whereas PA showed no significant change.

In the control group, pre-camp level of PA at post-practice increased significantly ($p<0.01$), and the post-camp level of ROS production capability at post-practice decreased significantly ($p<0.01$).

Changes in the ratio of PA post-camp was smaller than pre-camp ($p<0.05$, $p<0.01$, respectively), with this tendency being more noted in the glutamine group than in the control group.

Post-camp levels of SOD were lower at pre- and post-practice in the glutamine group compared with pre-camp, but not in the control group.

Discussion

In the present study, the body weight of subjects decreased significantly at post-practice in both groups, suggesting the accelerated perspiration and loss of water due to physical exercise[26]. There was no significant difference in body weight loss between the two groups, and thus glutamine intake was considered to have no effects on water loss during exercise.

In previous studies, monitoring the changes in myogenic enzyme levels has been recognized as an effective method to assess the accumulated muscle fatigue caused by repeated changes in and damage to muscle tissues during transient exercise[27, 28]. Glutamine has

Table 6 Changes in serum immunoglobulins and complements during the training camp

	Glutamine group (n=18)		Control group (n=17)	
	Pre-camp	Post-camp	Pre-camp	Post-camp
IgA (mg/dl)				
Pre-practice	186.0 ± 62.8	181.8 ± 64.4	158.5 ± 69.2	154.6 ± 63.4
Post[a]-practice	190.9 ± 63.5	189.0 ± 67.2 **††	162.3 ± 69.1	159.7 ± 63.7 **
Change ratio before the practice (%)	3.7 ± 8.3	4.1 ± 4.1	3.1 ± 7.0	3.8 ± 4.4
IgG (mg/dl)				
Pre-practice	1135.7 ± 193.5	1113.3 ± 208.9	1080.4 ± 194.8	1065.0 ± 187.9
Post[a]-practice	1153.2 ± 175.7	1174.2 ± 217.2 **	1119.2 ± 202.5 **	1101.8 ± 182.3 **
Change ratio before the practice (%)	1.9 ± 5.0	5.6 ± 4.4	3.8 ± 5.1	3.7 ± 5.3
IgM (mg/dl)				
Pre-practice	93.9 ± 24.0	90.2 ± 24.5	96.4 ± 32.9	92.2 ± 32.3 †
Post[a]-practice	96.0 ± 26.3 *	92.5 ± 26.0 *	98.1 ± 35.9 *	95.0 ± 33.8
Change ratio before the practice (%)	1.9 ± 3.7	2.3 ± 4.6	1.3 ± 5.8	3.1 ± 5.8
C3 (mg/dl)				
Pre-practice	109.2 ± 25.5	106.8 ± 20.7	105.0 ± 18.5	103.2 ± 13.9
Post[a]-practice	107.9 ± 23.6	109.6 ± 20.7 *	105.4 ± 15.7	105.1 ± 13.9
Change ratio before the practice (%)	−0.9 ± 5.3	2.7 ± 3.2 †	0.9 ± 6.4	1.9 ± 5.1
C4 (mg/dl)				
Pre-practice	24.0 ± 6.7	24.2 ± 5.9	25.4 ± 8.3	26.2 ± 9.8
Post[a]-practice	24.5 ± 6.1	25.3 ± 6.3 **	26.2 ± 8.4	27.4 ± 10.7 *
Change ratio before the practice (%)	2.9 ± 5.2	4.8 ± 4.0	3.2 ± 3.9	4.9 ± 5.3

Mean ± standard deviation
Glutamine intake group: 1.5g of L-glutamine was given to subjects four times a day (total of 6 g/day), taken before and after the judo practice in the morning and afternoon
Control group: 1.5g of placebo was given to subjects four times a day (total of 6 g/day), taken before and after the judo practice in the morning and afternoon
a: Values after the training were adjusted for dehydration by plasma volume
*: $p<0.05$, **: $p<0.01$, Significant difference from the pre-value in the group
†: $p<0.05$, ††: $p<0.01$, Significant difference from the pre-camp value in the group

also been reported in a number of studies to be an important nutrient as a part of the muscle component[29].

In our study, significant increases in myogenic enzymes were observed after practice in both groups, suggesting the high intensity of the 2-hour judo practice caused damage to the muscles and brought about structural changes. In terms of accumulation of muscle fatigue caused by repeated training during the training camp, the post-camp level of myogenic enzymes at pre-practice was higher in control group compared to the glutamine group. Thus, accumulation of muscle fatigue was more notable in the control than in the glutamine group. Also, glutamine may inhibit the dissimilation of body protein by accelerated energy metabolism during exercise[30].

Neutrophils are a member of the leukocyte family that are considered important especially for maintaining immune functions[31]. Various studies have reported that acute exercise increased the number of neutrophils and the extent of this increase depends on the intensity of the physical exercise[31]. These increases were found to be caused by inflammation through inflammatory cytokines produced due to changes in and damage to muscle tissues during exercise[32]. In addition, these changes are directly stimulated by several stress hormones

Table 7 Changes in ROS production, PA and serum SOD during the training camp

	Glutamine group (n=18)				Control group (n=17)			
	Pre-camp		Post-camp		Pre-camp		Post-camp	
ROS production per cell (FI)								
Pre-practice	26.7 ± 7.8		25.8 ± 7.5		24.3 ± 5.0		27.3 ± 6.7	
Post-practice	19.6 ± 4.5	**	20.7 ± 5.0	**	24.0 ± 11.9		17.8 ± 4.1	**
Change ratio before the practice (%)	−22.1 ± 24.5		−15.9 ± 21.0	†	−0.4 ± 45.8		−32.2 ± 19.8	†‡
PA per cell (FI)								
Pre-practice	80.9 ± 31.5		92.4 ± 22.2		86.0 ± 29.2		84.0 ± 16.9	
Post-practice	159.6 ± 75.6	**	91.8 ± 51.4	†	164.8 ± 78.2	**	106.1 ± 59.1	
Change ratio before the practice (%)	107.9 ± 91.9		3.7 ± 60.1	††	104.7 ± 110.2		33.4 ± 79.5	††
Serum SOD activity (%)								
Pre-practice	6.8 ± 2.3		5.4 ± 1.3	††	5.9 ± 1.5		5.2 ± 1.1	
Post[a]-practice	6.6 ± 1.4		5.3 ± 1.1	††	5.7 ± 1.8		4.9 ± 1.1	
Change ratio before the practice (%)	−0.3 ± 1.9		0.0 ± 1.2		−0.2 ± 1.7		−0.2 ± 1.0	

Mean ± standard deviation
Glutamine intake group: 1.5 g of L-glutamine was given to subjects four times a day (total of 6 g/day), taken before and after the judo practice in the morning and afternoon
Control group: 1.5 g of placebo was given to subjects four times a day (total of 6 g/day), taken before and after the judo practice in the morning and afternoon
ROS production: reactive oxygen species production in neutrophils
PA: phagocytic activity in neutrophils
SOD: superoxide dismutase
**: $p<0.01$, Significant difference from the pre-value in the group
†: $p<0.05$, ††: $p<0.01$, Significant difference from the pre-camp value camp in the group
‡: $p<0.05$, Comparison of the values between the two groups

such as catecholamine and / or cortisol which then accelerate the stress reaction within the body[33]. Thus, significant increases in the neutrophil and leukocyte counts in both groups observed in our study suggested the accelerated inflammatory reaction and stress reactions caused by intensive exercise.

When long-term training causes the overtraining syndrome, excretion of adrenaline and noradrenaline decreases, leading to reduced functions of leukocytes and neutrophils and their counts[34, 35]. Also, it was reported to increase cortisol, which can then affect functions of neutrophils and leukocytes as well as their counts[36]. In our study, the post-camp rate of increase in these cell counts tended to be significantly lower in the control group than in glutamine group. Although more signs of muscle damage were noted in the control group than in the glutamine group as mentioned above, the neutrophil count showed a strong increase in the glutamine group compared with the control group. These findings suggest that glutamine intake might keep neutrophils / leukocytes function and its counts as well as increasing muscle function.

Immunoglobulins and complements are important blood components responsible for immunity[31]. However, previous studies that have investigated the association between immunoglobulins and complements and physical exercise have been inconsistent[37-40]. Dufaux reported that levels of C3 and C4 increased after 2-and-a-half hours of running, and muscle damage caused by highly intensive exercise was found to trigger the activation of complements[41]. Mashiko et al. reported that the leukocyte count significantly increased and immunoglobulins and complements significantly decreased after a rugby match, caused by muscle damage and

structural changes during the match as well as an accelerated inflammatory reaction[42]. Thus, the significant increases of immunoglobulins and complements in both groups observed in our study suggested that exercise-induced muscle damage/change might be linked to the induction of immunoglobulins and complements through an inflammatory reaction. In our study, difference in these changes between the two groups was not significant.

Neutrophils have an important immune function including opsonization of endogenous or exogenous foreign substances to effectively inactivate them through phagocytosis[43]. Neutrophils produce ROS to inactivate foreign substances, however, ROS overproduction can cause damages in normal cells through induction of oxidative stress[44, 45].

Previously our group sowed the changes in neutrophil function including their ROS production capability and PA in a variety of athletes for different types and periods of loading as follows: a single bout (short term intensive exercise) or long-term training, e.g., the usual training term; for a match / race; during training camp; with or without dietary restriction, and so on[2, 3, 10, 11, 46-52]. In the end, we advanced the theory that the two neutrophil functions, *i.e.*, ROS and PA, as part of the defense mechanism, *e.g.*, against invading pathogens, would be preserved as result of compensation by each function for other immune functions.

In our study, ROS production capability at post-practice decreased significantly and PA increased significantly in both groups pre- and post- camp. Thus, if we assume the compensative relationship between ROS production capability and PA as mentioned earlier[11], the present result could be considered to be similar to the "normal (adapted) pattern" rather than the abnormal one, because we were able to evaluate that subjects had a compensatory balance of their neutrophil function; ROS production capability decreased and PA increased.

Glutamine is not only the part of the component for muscle tissues, but is also an important energy source for immune system cells[12, 13]. Many researches reported that acceleration of energy metabolism during exercise consumes and reduces glutamine levels in blood[13-19]. Moreover, when blood glutamine levels are reduced during exercise or through some debilitating disease, this leads to immune function suppression[15, 16, 53], and immune function could be maintained in these cases with glutamine supplement.

Various studies related to the association between glutamine and immune function focused on relationship of glutamine with lymphatic function rather than neutrophil function. Castell et al reported that a fast recovery of neutrophil count after a marathon race was observed by administering glutamine[54]. Previous studies reported that glutamine intake strengthened ROS production capability and PA by neutrophils[55]. The mechanism is as follows. Glutamine synthesized in muscle tissues was released into the circulation. Glutamine is utilized at high rates by neutrophils. Partially, glutamine is converted into adenosine triphosphate (ATP) as energy source and be used to activate nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase, a membrane-bound enzyme complex, which accelerates neutrophil ROS production capability when the body requires it[56, 57].

Although post-camp ROS production capability decreased significantly at post-practice in both groups in the present study, the rate of reduction was significantly higher in the control group compared to the glutamine group. This finding suggested that ROS reduction by accumulation of chronic physical fatigue was alleviated by taking glutamine. Also, glutamine

might be used efficiently as an energy source and/or by activating NADPH-oxidase.

Previous studies showed that SOD have been reported to have an important role as an antioxidative enzyme to eliminate ROS[58]. Transient physical exercise to accelerate ROS production causing an increase in SOD activity as an antioxidant reaction[59, 60]. One study reported that SOD activity adapts to a long-term and highly-frequent training that constantly accelerates ROS the production capability[61]. In our study, post-camp level of SOD was lower at the pre- and post-practice in glutamine group compared with the pre-camp, but not in the control group. Thus, neutrophil functions were maintained better in the glutamine group than the control group, suggesting that, in the glutamine group, the ROS production might accelerate SOD production as a countermeasure.

There were several limitations in this study. Firstly, we did not measure the subjects' blood glutamine concentration. Many previous studies showed an increased blood glutamine level after the administration of glutamine[54]. Also, the meals and dietary intake during the training camp were controlled by our research team so that meals provided for all subjects throughout the camp were the same. As a result, there was no significant difference in total energy intake or intakes of protein, fat and sugar between the two groups (Table 3). Thus, the present result was considered to be led purely from the difference in glutamine intake.

Another limitation was that we did not measure inflammatory cytokines or stress hormone. Although these mechanisms are closely related to changes in neutrophil function and the overtraining syndrome, we could not measure them due to the limited amount of budget and availabilities of subjects, equipment and facilities. Thus, these factors need to be considered for the future investigation.

In conclusion, intake of glutamine during an intensive judo training camp had protective effect against damaged muscle function and immune function.

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