

ORIGINAL ARTICLE

## EFFECT OF INITIAL BLOOD GLUCOSE LEVEL ON TRANSIENT PHYSICAL STRESS

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**Abstract** We evaluated the effect of blood glucose level before transient physical exercise on an athlete's physical condition and neutrophil functions. The changes and associations between blood glucose level immediately before training and neutrophil function at rest were investigated in female long-distance runners. Seventeen females in a university track team were divided into 2 groups by medial blood glucose levels: 9 subjects were in the high blood glucose group (HBG group) and 8 subjects in the low blood glucose group (LBG group). Myogenic enzymes, immunoglobulines, complements and neutrophil function such as reactive oxygen species (ROS) production capability were measured. The post-practice rate of change in ALT and C3 levels were significantly higher in the LBG group compared to the HBG group. At pre-practice, total ROS production capability was significantly lower in the HBG group compared to LBG group. Total ROS production capability increased significantly post-practice ("normal pattern") in the HBG group, though it tended to decrease ("abnormal pattern") in the LBG group. In conclusion, an adequate blood glucose level in athletes prior to exercise is suggested to be effective not only to supply energy to the body, but also to maintain normal immune function which is potentially suppressed during exercise.

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**Key words:** Glucose; exercise; neutrophil function; long-distance runner.

原 著

### 運動実施前の血糖値レベルが運動実施による一過性の 身体ストレスに及ぼす影響

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**抄録** 大学女子長距離選手17名を対象に、練習開始直前の血糖値が安静時・運動負荷時の好中球機能に及ぼす影響を検討した。練習開始前の血糖値の中央値で低血糖群8名と高血糖群9名の2群に分けた。対象者に2時間15分のトレーニングを実施させ、その前後に、白血球数、好中球数、肝機能項目(Alanine aminotransferase, ALTなど)、好中球活性酸素種(reactive oxygen species: ROS)産生能及び食食能を測定した。その結果、ALTは低血糖群のみで有意に上昇し、その練習前後の変化率は高血糖群に比べ低血糖群で有意に大きかった。また、練習前(安静時)のROS産生量が、高血糖群で低血糖群より有意に少なく、練習前後の変化率は有意に大きかった。以上より、低血糖群に比較し、高血糖群では酸化ストレスの影響が小さい。かつ、運動負荷に対し好中球機能が正常に機能した可能性が示唆された。

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**キーワード:** 糖質; 運動; 好中球機能; 長距離ランナー。

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## Introduction

Many long-distance runners practice running over long distances on a daily basis, trying to beat their personal best time, and previous studies have pointed out the potential danger of the high physical and mental burdens on athletes causing chronic sports disorders. Long-distance runners in particular are at high risk of the overuse syndrome such as fatigue fractures of the shinbones and splint bones, shin splints and inflammation in the iliotibial band as well as the overtraining syndrome including sports anemia<sup>1,2)</sup>.

For many athletes including long-distance runners, the availability of glucose in the bloodstream is known to be the most important nutrient in the body as a main energy source. When an athlete carries out highly intensive exercise that requires anaerobic energy, glucose metabolism is accelerated in active muscles which in turn increases the demand for more glucose. Unfortunately, the amount of sugar which can be stored in the body is limited and thus it is considered important for athletes to build up their glucose levels as much as possible before performing anaerobic/aerobic exercise<sup>3, 4)</sup>. Moreover, some researchers reported that sufficient storage of sugar in the body can prevent and reduce the occurrence of over-reaching and/or over-training<sup>5,6)</sup>.

In other previous studies, immunosuppression was observed in athletes who carried out highly-intensive, highly-frequent as well as extended periods of physical exercise. Also, they were reported to be at high risk of infections including upper respiratory tract infection<sup>7,8)</sup>. Our research group also investigated the effect of highly-intensive transient exercise on immune functions such as neutrophil function of athletes including long-distance runners, and have found that exercise can cause immunosuppression and oxidative stress in athletes<sup>9-15)</sup>. We have also suggested that monitoring neutrophil functions

over short or long period of time can be useful as a conditioning index and an effective method to manage athletes' health<sup>16,17)</sup>.

Previous studies, which investigated the relationship between blood glucose and immune function in athletes, showed that glucose depletes after extended periods of exercise the production of IL-6 is increased, IL-6 being an inflammatory cytokine<sup>18, 19)</sup>. Also, other researchers have reported that glucose supplementation during extended training can suppress IL-6 and IL-10 synthesis, which are activated by physical exercise, while inhibiting increased of neutrophil counts and decreased T-cell function<sup>20-24)</sup>. However, in spite of the associations between blood glucose and immune function reported previously, detailed mechanisms as well as their relationship still remain unclear. Furthermore, no other researchers have reported on the relationship between blood glucose and neutrophil function, which is known to be the first line of defense in the immune system.

The present study therefore investigated the changes in and associations between blood glucose levels immediately before a training session and neutrophil function at rest in female long-distance runners based on changes in their blood biochemical parameters, reactive oxygen species (ROS) production capability and phagocytic activity (PA). In other words, the present study investigated the effect of blood glucose availability before transient physical exercise and its effects on changes in neutrophil functions during transient exercise.

## Subjects and Methods

### 1. Subjects and research period

The study subjects were 17 female long-distance runners in a university track team. They were divided into 2 groups based on their median blood glucose level immediately before the training: 9 subjects were assigned to the

**Table 1** Physical characteristics and the changes in body weight after the training session in the low and high blood glucose groups

	Low blood glucose group (n=8)	High blood glucose group (n=9)
Age (Years)	19.6 ± 0.9	19.8 ± 0.8
Height (cm)	160.7 ± 4.8	159.2 ± 5.1
Body weight (kg)		
Pre	50.2 ± 3.6	48.8 ± 6.1
Post	49.0 ± 3.5 *	48.1 ± 5.9 **
Relative body fat (%)	18.3 ± 3.7	17.7 ± 4.6
Fat-free mass (kg)	40.9 ± 2.8	40.0 ± 3.7
Pre-value of blood glucose	84.8 ± 4.8	95.1 ± 3.5 † †

Values are shown as the mean ± standard deviation.

Pre: pre-training. Post: post-training.

\*:  $p < 0.05$ , \*\*:  $p < 0.01$ , significant difference from the pre-value

† †:  $p < 0.01$ , significant difference from the value in the low blood glucose group

**Table 2** Contents of the training program performed by subjects

Warm-up	5 minutes
Distance running	70 minutes
Running with a slope of 500 m * 5	10 minutes
Weight training	30 minutes
100 m sprint running * 5	5 minutes
Jogging for cool-down	10 minutes
Cool-down	5 minutes
Total training time	2 hours 15 minutes

high blood glucose group (HBG group) and 8 to the low blood glucose group (LBG group). The average blood glucose levels before the practice in the LBG and HBG groups were  $84.8 \pm 4.8$  mg/dl and  $95.8 \pm 3.5$  mg/dl, respectively (Table 1).

The physical characteristics of the study subjects were shown in Table 1. The investigation was conducted on in March of 2009. All subjects trained for their normal 2 hours and 15 minutes and a number of parameters were measured. The training menu was shown in the Table 2. Although the rehydration regimen was not investigated in detail the present study, they were asked to drink plenty of water before the training.

The study was approved by the Ethics Committee of Hirosaki University School of Medicine. The study protocol and purpose were thoroughly explained to, and written consent

was obtained from, all subjects prior to the investigation.

## 2. Body compositions

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

## 3. Blood biochemical parameters

Blood samples (15 ml) were taken when subjects were at rest before practice (pre-practice) and immediately after 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. 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 activities of superoxide dismutase (SOD) and thiobarbituric acid reactive substance (TBARS) were 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 and TBARS activity were measured with the NBT reduction method.

As dehydration of the subjects was observed based on the weight loss after the race and changes in the levels of hemoglobin and hematocrit, these items were measured using the plasma volume method after correcting for dehydration<sup>25)</sup>. All measurements of blood biochemical items were consigned to an independent laboratory (Mitsubishi Chemical Medience).

#### **4. Measurement method of neutrophil ROS production capability and phagocytic capability**

Leukocyte and neutrophil counts were measured with an automated blood cell analyzer (Sysmex XE-2100 SE-9000, Kobe, Japan). ROS generation and the 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  $\mu\text{mol/L}$ , Polyscience Inc., Warrington, PA) was used as an indicator for the ROS production capability, and opsonized zymosan (OZ) particles

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

Briefly, 100  $\mu\text{L}$  of heparinized whole blood was mixed with 22  $\mu\text{L}$  of HE (final concentration, f.c. 8  $\mu\text{mol/L}$ ) and incubated at 37°C for 5 min. After the addition of 25  $\mu\text{L}$  of FITC-labeled OZ (FITC-OZ; f.c. 5 mg/mL), the samples were incubated at 37°C for 35 min. Neutrophils labeled with only HE served as the control to measure non-stimulated neutrophil function and 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  $\mu\text{L}$  of 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<sup>26, 27)</sup>.

#### **5. Statistical analysis**

All values were presented as means  $\pm$  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  $p < 0.05$ .

## **Results**

Table 1 shows the physical characteristics of subjects and changes in body weight from pre-practice to post-practice. There was no significant difference in height, body fat percentage or fat-free mass between the two groups. Their body weight decreased significantly at post-practice

**Table 3** Changes in serum myogenic enzyme values between the pre- and post-training assessments in the low and high blood glucose groups

	Low blood glucose group (n=8)	High blood glucose group (n=9)
AST (IU/l)		
Pre	32.1 ± 6.2	29.6 ± 13.8
Post <sup>a</sup>	35.4 ± 7.9 *	32.1 ± 12.9 *
Change ratio (%)	9.9 ± 5.8	11.2 ± 8.5
ALT (IU/l)		
Pre	25.9 ± 8.3	24.2 ± 11.3
Post <sup>a</sup>	27.2 ± 8.1 *	24.2 ± 11.3
Change ratio (%)	5.7 ± 5.7	-0.6 ± 4.6 †
CK (IU/l)		
Pre	290.1 ± 117.3	266.1 ± 144.7
Post <sup>a</sup>	329.1 ± 137.2 *	293.8 ± 138.1 *
Change ratio (%)	14.5 ± 10.0	15.8 ± 13.7
LDH (IU/l)		
Pre	217.5 ± 32.6	216.1 ± 59.8
Post <sup>a</sup>	239.7 ± 35.9 *	247.3 ± 60.4 *
Change ratio (%)	10.6 ± 7.9	15.8 ± 13.9

Values are shown as the mean ± standard deviation.

Pre: pre-training. Post: post-training.

Change ratio = (pre-value - post-value)/pre-value\*100

a: pre-values were adjusted for dehydration by plasma volume.

\*: p<0.05, significant difference from pre-value

†: p<0.05, significant difference from the value in the low blood glucose group

**Table 4** Changes in the values of blood leukocyte and neutrophil cell counts between the pre- and post-training assessments in the low and high blood glucose groups

	Low blood glucose group (n=8)	High blood glucose group (n=9)
Leukocyte counts (/μl)		
Pre	5000 ± 1374	5689 ± 1315
Post <sup>a</sup>	4840 ± 1129	5510 ± 1384
Change ratio (%)	-1.7 ± 10.4	-3.1 ± 10.6
Neutrophil counts (/μl)		
Pre	2964 ± 1013	3480 ± 997
Post <sup>a</sup>	3002 ± 842	3646 ± 1189
Change ratio (%)	4.1 ± 17.5	5.1 ± 20.9

Values are shown as the mean ± standard deviation.

Pre: pre-training. Post: post-training.

a: pre-values were adjusted for dehydration by plasma volume.

Change ratio = (pre-value - post-value)/pre-value\*100

in both groups (p<0.05 for the LBG group and p<0.01 for the HBG group).

Changes in myogenic enzymes after the practice are shown in Table 3. For both groups, levels of AST, CK and LDH increased significantly post-practice (p<0.05 for all). However, a

significant increase in ALT was only observed in the LBG group (p<0.05). The post-practice rate of change in ALT levels was significantly higher in the LBG group compared to the HBG group (p<0.05).

Table 4 shows the changes in leukocyte and

**Table 5** Changes in serum immunoglobulins and complements values between the pre- and post-training assessments in the low and high blood glucose groups

	Low blood glucose group (n=8)	High blood glucose group (n=9)
IgA(mg/dl)		
Pre	131.9 ± 45.8	142.1 ± 27.8
Post <sup>a</sup>	134.4 ± 45.1	140.6 ± 32.1
Change ratio (%)	2.1 ± 6.1	-1.7 ± 5.4
IgG(mg/dl)		
Pre	922 ± 157	1082 ± 231
Post <sup>a</sup>	954 ± 142 *	1081 ± 207
Change ratio (%)	3.8 ± 3.6	0.3 ± 3.8
IgM(mg/dl)		
Pre	100.6 ± 8.4	162.7 ± 58.0 †
Post <sup>a</sup>	102.3 ± 12.3	159.6 ± 59.7 †
Change ratio (%)	1.5 ± 5.5	-2.4 ± 2.7
C3(mg/dl)		
Pre	86.1 ± 15.2	88.8 ± 6.7
Post <sup>a</sup>	87.6 ± 16.3	86.5 ± 8.1
Change ratio (%)	1.6 ± 4.0	-2.6 ± 3.2 †
C4(mg/dl)		
Pre	18.4 ± 4.8	21.4 ± 3.0
Post <sup>a</sup>	18.6 ± 5.4	20.5 ± 3.7
Change ratio (%)	0.8 ± 6.8	-4.7 ± 6.2

Values are shown as the mean ± standard deviation.

Pre: pre-training. Post: post-training.

a: pre-values were adjusted for dehydration by plasma volume.

Change ratio = (pre-value - post-value)/pre-value\*100

\*: p<0.05, significant difference from pre-value

†: p<0.05, significant difference from values in the low blood glucose group

neutrophil counts after the practice. There were no significant differences in either component between pre- and post-practice in both groups.

Table 5 shows the difference in changes of immunoglobulins and complements levels between pre- and post-practice in both groups. The level of IgM in the LBG group at both pre- and post-practice was significantly higher compared with the HBG group (p<0.05 for both). Also, a significant increase in IgG levels was observed post-practice in the LBG group (p<0.05) but not in the HBG group. The rate of change in the C3 level was significantly low in the HBG group compared to the LBG group (p<0.05).

Differences in neutrophil functions between pre- and post-practice are shown in Table 6. At pre-practice, total ROS production capability was

significantly lower in the HBG group compared to LBG group (p<0.01). Total ROS production capability and total PA increased significantly post-practice ("normal pattern") in the HBG group (p<0.05 for both), though total ROS production capability tended to decrease and total PA increased significantly (p<0.05) post-practice ("abnormal pattern") in the LBG group.

Table 7 shows the changes in serum SOD activity and TBARS between pre- and post-practice. No significant differences were observed in pre-practice SOD activity or TBARS between the two groups. A significant decrease in SOD activity at post-practice was observed in HBG group (p<0.05), but not in the LBG group.

**Table 6** Changes in neutrophil function values between the pre- and post-training assessments in the low and high blood glucose groups

	Low blood glucose group (n=8)	High blood glucose group (n=9)
ROS production per cell (FI)		
Pre	42.6 ± 14.9	33.1 ± 10.5
Post	38.0 ± 4.5	42.1 ± 12.2
Change ratio (%)	-7.5 ± 46.5	21.8 ± 53.0
Total ROS production (CFI)		
Pre	3722 ± 3523	894 ± 480 † †
Post	2821 ± 1173	2562 ± 1406 *
Change ratio (%)	-60.1 ± 26.0	126.6 ± 317.9 † †
PA per cell (FI)		
Pre	41.4 ± 6.5	40.4 ± 4.2
Post	100.3 ± 35.2 *	81.6 ± 22.4 **
Change ratio (%)	-0.8 ± 15.1	-17.0 ± 31.1
Total PA (CFI)		
Pre	154707 ± 105725	135006 ± 29940
Post	327482 ± 142486 *	234116 ± 82532 **
Change ratio (%)	7.9 ± 45.4	0.5 ± 51.3

Values are shown as the mean ± standard deviation.

Pre: pre-training. Post: post-training.

ROS production: reactive oxygen species production in neutrophils.

Change ratio = (pre-value - post-value)/pre-value\*100

PA: phagocytic activity in neutrophils.

\*: p<0.05, \*\*: p<0.01, significant difference from pre-values

† †: p<0.01, significant difference from the value in the low blood glucose group

**Table 7** Changes in serum SOD and TBARS values between the pre- and post-training assessments in the low and high blood glucose groups

	Low blood glucose group (n=8)	High blood glucose group (n=9)
SOD (%)		
Pre	2.9 ± 0.7	3.6 ± 1.5
Post <sup>a</sup>	2.0 ± 1.0	2.4 ± 1.4 *
Change ratio (%)	-1.0 ± 1.3	-1.2 ± 1.4
TBARS(nmol/mL)		
Pre	4.4 ± 0.8	4.9 ± 1.1
Post <sup>a</sup>	4.4 ± 1.0	5.1 ± 1.5
Change ratio (%)	0.7 ± 14.4	6.1 ± 27.1

Values are shown as the mean ± standard deviation.

Pre: pre-training. Post: post-training.

a: pre-values were adjusted for dehydration by plasma volume.

Change ratio = (pre-value - post-value)/pre-value\*100

SOD: superoxide dismutase

TBARS: thiobarbituric acid reactive substance

\*: p<0.05, significant difference from pre-value

## Discussion

A number of researchers have reported that muscle contractions accompanied by intensive

exercise can damage/change muscle tissues and accelerates the permeability of the fascia, causing the release of serum myogenic enzymes into the bloodstream<sup>28, 29)</sup>. Additionally, levels of

myogenic enzymes including AST, ALT, CK and LDH have been shown to be effective indices for muscle fatigue in athletes, as they reflect the extent of the changes in and damage to muscle tissues and their activities<sup>30)</sup>.

In the present study, the post-practice levels of AST, CK and LDH increased significantly in both groups, suggesting the extent of the usual training carried out by athletes was considered high enough to cause changes in and damage to muscle tissues. In terms of changes in those levels, the rate of change in ALT was the only one that was observed to be significantly high in the LBG group compared to the HBG group, and none of the other myogenic enzymes changed significantly. Thus, the difference in blood glucose level before the practice was not considered to have much effect on muscle damage or changes, or protein catabolism accompanied by the accelerated energy metabolism during exercise<sup>31, 32)</sup>.

Many researchers have reported an increased leukocyte count after transient physical exercise, the extent of which depending on exercise intensity<sup>33)</sup>. Also, physical exercise is known to cause an increase in leukocytes, such as lymphocytes and neutrophils, and these increases are part of the inflammatory reaction driven by muscle tissue damage. Certain stress hormones such as catecholamine and cortisol are also known to be associated with such reactions<sup>33-35)</sup>. In addition, other studies showed that physical exercise not only accelerates the inflammatory reaction that is induced secondarily by increases in these hormones, but also directly stimulates the secretion of growth hormones and stress hormones including adrenaline and noradrenaline<sup>35)</sup>.

The present results indicated no significant differences in the pre-practice leukocyte / neutrophil counts in the rates of change between pre-and post-practice. Thus, blood sugar levels before the training was not considered to have

any effects on leukocyte / neutrophil count in the present study.

Immunoglobulins and complements are important blood components responsible for immunity<sup>33)</sup>. However, the results of previous studies that have investigated the association between these components and physical exercise have been inconsistent<sup>36-39)</sup>.

Dufaux and Order reported that levels of C3 and C4 increased after 2.5 hours of running, and muscle damage caused by highly intensive exercise was found to trigger the activation of complements<sup>40)</sup>. A study by Mashiko and colleagues observed significant muscle damage-mediated decreases in immunoglobulins and complements after a rugby match, as well as an accelerated inflammatory reaction leading to reduced blood circulation<sup>41)</sup>.

In this study, the level of IgM was significantly higher in the HBG group compared to the LBG group. Although not statistically significant, other immunoglobulin levels also tended to be higher in the HBG group than the LBG group. The reason for such difference may be due to the higher consumption of immunoglobulins against ROS in the LBG group compared to the BG group, which was evident from the greater total ROS production capability in the LBG than in the HBG group.

Neutrophils have an important immune function including opsonization of both endogenous and exogenous foreign substances to effectively sterilize them through phagocytosis<sup>42)</sup>. Neutrophils produce ROS to sterilize foreign substances, however, ROS overproduction can cause damage to normal cells through oxidative stress<sup>43, 44)</sup>. Thus, a significant increase in ROS production capability per neutrophil seen post-practice in both groups in the present study was probably due to an accelerated stress reaction caused by practice, and/or an inflammatory reaction induced by morphological changes in and/or damage to muscles.



Our research group has investigated the roles of neutrophil functions under various conditions including different degrees of exercise intensity (physical intensity and period of training), different environmental conditions (under conditions of normal training, intensive training, weight-controlling and tournaments or matches) and under different health conditions (normal conditions or physical fatigue)<sup>9-17, 45-48</sup>. As a result, we have found that neutrophil function exerts its power through a complex mechanism. Although its mechanism may seem unpredictable, it was found to function following a certain rule.

In our past studies, we have observed increased ROS production capability and decreased PA after physical exercise during a normal training period, and this combination was considered as the “normal pattern” of neutrophils<sup>9-12, 17, 45</sup>. On the other hand, physical exercise with high intensity or sports performed for an extended period such as marathon races or rugby matches can exceed the capability of the neutrophil function even in well-conditioned athletes, causing decreases in both neutrophil function and PA, and this was referred to as the “abnormal pattern” of neutrophil functions<sup>13-15</sup>. As for these two patterns, the results in our previous studies are shown in Table 8.

From this point of view, the results obtained in this study in the HBG group were considered to follow the “normal pattern” of neutrophil function, where PA or total PA per neutrophil decreased and ROS production capability increased significantly. On the other hand, the LBG group showed a different result from the HBG group, where increased PA and decreased ROS production capability were observed, and thus was considered to follow the “abnormal pattern” of neutrophil function.

Considering the different response of neutrophil function after the same amount of physical exercise, subjects in the HBG group was thought to be better-conditioned compared to LBG group

members. These findings also suggested that the neutrophil function of those who had sufficient and appropriate glucose levels at the beginning of the practice session were able to maintain normal neutrophil functions.

In the present study, ROS production capability per neutrophil in the LBG group was significantly high at rest compared to the HBG group, suggesting that a higher level of ROS was being produced in the LBG group before the day of the study than in the HBG group. In other words, subjects in the LBG group were thought to be much more affected by oxidative stress at rest compared to the HBG group. This result did not coincide with the results in previous studies. Previous studies investigating the relationship between basal ROS production and blood glucose levels in individuals with diabetes reported that hyperglycemia causes oxidative stress through basal ROS production by neutrophils<sup>49, 50</sup>. It was suggested that hyperglycemia enhances NADPH oxidase activity during neutrophil ROS production<sup>51, 52</sup>. According to such differences, the subjects in the current study may be considered to have normal blood glucose levels.

SOD is present in blood, and has the scavenger function of deleting excessive amounts of ROS<sup>53</sup>. Also, transient exercise has been reported to accelerate ROS production, leading to increased serum SOD activity as an anti-oxidative reaction<sup>54, 55</sup>. In the present research, SOD activity was found to be reduced significantly only in the HBG group. The possible reason is as follows. Total ROS production increased after exercise in the HBG group, and the amount of SOD was reduced as these subjects were used to lower ROS levels. Also, the reason why accelerated SOD activity was observed immediately after exercise in previous studies and not in this study was considered to be due to the difference in the physical exercise intensity, time duration and/or timing of blood collection etc...

**Table 8** Relationships between exercise and neutrophil immune functions from our previous studies

Author (year)	Reference number	Participants	Exercise or others	ROS	PA	
○ Usual training period						
Chinda <i>et al.</i> (2003)	(9)	37 male university judoists	2 h judo training session	Increase	Decrease	
Umeda <i>et al.</i> (2008)	(10)	22 male university judoists	2 h judo training session	Increase	No change	
Umeda <i>et al.</i> (2008)	(11)	17 male university sumo wrestlers	2.5 h sumo training session	Increase	Decrease	
Yamamoto <i>et al.</i> (2008)	(45)	24 male university judoists who had stopped judo training for 6 months and then restarted their training	2 h judo training session	Pre-training (restarted training) 2-month point 4-month point 6-month point	No change Increase Increase Increase	Decrease Decrease No change No change
Kojima <i>et al.</i> (2009)	(12)	17 male university sumo wrestlers	2.5 h sumo training session	—	—	
○ Game or race						
Chinda <i>et al.</i> (2003)	(13)	36 male marathon runners	42.195 km running	Decrease	Decrease	
Suzuki <i>et al.</i> (2004)	(14)	15 male college rugby football players	match: 80 min with an interval of 10 min	Decrease	Decrease	
Takahashi <i>et al.</i> (2007)	(15)	7 male rugby players of the Japan Sevens	Two consecutive Sevens games/ after the second match	Decrease	Decrease	
○ Training camp period						
Mochida <i>et al.</i> (2007)	(46)	13 female university judoists	After the 2 h exercise loading · During the 64-day period: 3.5 h ordinary training · During the 6day camp: 6.5 h intensifying training	Before 64-day training period Before the camp After the camp	Increase Increase Decrease	Decrease Decrease No change
○ Weight reduction period						
Kowatari <i>et al.</i> (2001)	(47)	18 male university judoists	Research period for 20 days · All subjects trained for 3.5 h every day during the research period · The weight reduction groups reduced energy intake for 20 days · The results compared the values before and after the weight reduction	Low energy intake group (n=6) Very low energy intake group (n=6) Control group (n=6)	Increase Increase Increase	Decrease Decrease No change
Yaegaki <i>et al.</i> (2007)	(16)	16 female university judoists	Research period for 20 days · All subjects trained for 3.5 h every day during the research period · The weight reduction groups reduced energy intake for 20 days · The results compared the values before and after the weight reduction	Weight reduction group (n=8) Control group (n=8)	Increase Increase	Decrease No change

ROS: reactive oxygen species, PA: phagocytic activity, SOA: serum opsonic activity.

Therefore, having a high blood glucose concentration prior to exercise can maintain the neutrophil function even with the induction of exercise-mediated stress. Also, adequate blood glucose levels in athletes prior to exercise is suggested to be effective not only to supply energy to the body, but also to maintain a normal immune function which is considered to be suppressed during exercise.

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