ORIGINAL ARTICLE

Change in the capability of reactive oxygen species production by neutrophils following weight reduction in female judoists

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Objective: Athletes undergoing weight reduction are recognised as being more prone to infection. Few studies exist for athletes on the weight reduction-mediated changes in neutrophil function and related activities such as reactive oxygen species (ROS) production capability, phagocytic activity (PA) and serum opsonic activity (SOA).

Methods: 16 Japanese female university judoists were examined in the early morning of the first day (pre-values) and the last day (post-values) of a 20-day pre-competition training period. Of the 16 subjects, 8 needed to reduce weight (WR group) and the other 8 did not (control group). The parameters assessed were the neutrophil count, serum immunoglobulins and complements, myogenic enzymes, ROS production capability, PA and SOA.

Results: Comparing the post-values with the pre-values, ROS production significantly increased in both groups ($p<0.01$ for both). PA significantly decreased in the WR group ($p<0.05$); it also decreased in the control group but the decrease was not significant. SOA significantly increased in the control group ($p<0.05$), but showed no significant change in the WR group.

Conclusions: The changes in the WR group were probably a direct consequence of the weight-reduction regimen coupled with the exercise regimen, suggesting that neutrophil parameters (ROS production, PA and SOA) had tended to deviate from their typical compensatory changes to maintain immune system homoeostasis.

Neutrophils are cellular factors in the host which have an important role as the first line of defence against invading foreign substances including microorganisms. Neutrophils engulf microorganisms (phagocytic activity, PA) and produce reactive oxygen species (ROS).[1 2] Serum opsonic activity (SOA) contributes to this microbicidal activity through opsonisation of microorganisms—that is, acceleration of adhesion of neutrophils to opsonised substances via IgG, C3 and others. Although appropriate levels of ROS from neutrophils can destroy invading microorganisms,[3 4] at higher levels, on the other hand, ROS can cause oxidative damage to normal body tissues and organs.[5 6]

There are many studies that have examined the effect of exercise and sports including judo on neutrophils and neutrophil-related functions. However, only three studies have reported on the relationship between weight reduction and SOA (one of the neutrophil-related functions) in university judoists.[7–9]

Mochida *et al*[10] reported that athletic training-induced changes in immune functional activities of neutrophils and related factors, such as ROS production, PA and SOA, may compensate for each other to maintain the overall integrity of the neutrophil immune function. A study examining the same three neutrophil-related components after a period of pre-competition compulsory weight reduction in judoists would therefore be of interest.

In this study, we simultaneously measured three major neutrophil functions—namely, ROS production capability, PA and SOA, in female university judoist during weight reduction.

SUBJECTS AND METHODS

Research object and period

The study subjects comprised 16 Japanese female judoists who were taking part in a competition in June 2000. Eight subjects who required weight reduction to meet their class requirements were defined as the weight reduction (WR) group, and eight subjects without a need for weight reduction were defined as the control group. The assessments were performed on the first (pre-values) and last (post-values) days of a 20-day period just before a competition, the second and final assessment being carried out on the morning of the day before the competition.

The physical characteristics of the16 subjects were as follows: mean (SD) height, 162.3 (8.3) cm; body weight, 65.3 (8.3) kg; percentage of body fat, 20.7% (3.2%); quantity of body fat, 13.8 (3.6) kg; and fat-free body mass (FFM), 51.4 (3.6) kg.

Approval for the study was obtained from the ethics committee of Hirosaki University School of Medicine, Aomori, Japan. The study protocol and purpose were explained to all subjects, and written informed consent was obtained from all of them before the study.

Research content

Measurement of the physical characterisitcs of all subjects (weight, body density, percentage of body fat, quantity of body fat and fat-free body mass) was performed on days 1 and 20 under fasting conditions early in the morning before training, and blood samples were collected for the blood biochemistry assay, including ROS and PA (measured by flow cytometry) and SOA (measured by the luminol-dependent chemiluminescence method). The dietary intake of the subjects during the research period was also investigated.

Abbreviations: FFM, fat-free body mass; FITC, fluorescein isothiocyanate; HBSS, Hank's balanced salt solution; HE, hydroethidine; OZ, opsonised zymosan; PA, phagocytic activity; ROS, reactive oxygen species; SOA, serum opsonic activity; WR, weight reduction

Table 1 Training programme per week during the research period

	Training period		
Day	**06:30–07:30**	**09:00–11:30**	**17:30–20:00**
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	Training D	Training D
Saturday	Training C	Rest	Training D
Sunday	Rest	Rest	Rest

Training A, interval training consisting of sprint running (800 m ×1, 400 m ×3, 200 m ×3, 100 m ×4) and jogging.
Training B, weight training.
Training C, distance running for 30 min and short sprint running (repeated 30 m/50 m sprint running).
Training D, judo training practice.
Rest, take a rest or attend lectures.

Weekly training programme during the research period

All subjects performed their usual weekly practice regimen during the research period, after having rested for 2 weeks beforehand. Table 1 shows the weekly regimen in detail. It consisted of 6 days of exercise, alternating running (distance and sprinting) and weight training in the mornings, judo practice in the afternoons, and 1 day of rest. The 2½ h judo practice, which is classed as intermittent anaerobic exercise, consisted of warm-up (stretching) for 15 min; ''uchikomi'' (the same technique practiced repeatedly, such as throw-down, push-down and hook-down) for 20 min; ''randori'' (exercise training in the form of mini-matches) for 100 min; and cool-down for 15 min.

Physical characteristics and energy intake

Body weight was measured using an A&D Company digital scale system (AD6205, Tokyo, Japan). All subjects had their body weights measured early in the morning before training.

Table 5 shows the changes in blood leucocyte counts, Igs and complements. No significant changes in these parameters were seen in either of the groups.

The body composition was measured with B-mode ultrasound equipment, as it is impossible to use underwater weighing for females over a research period of approximately 1 month because of problems associated with menstruation. Subcutaneous fat thickness was measured with B-mode ultrasound equipment (Echo Camera SSD-500, ALOKA, Tokyo, Japan) using 3.5 or 7.5 MHz at six sites on the right side: the biceps and triceps (on the anterior and posterior surface 60% distal between the lateral epicondyle of the humerus and the acromial process of the scapula); the abdomen (at an area 2–3 cm to the right of the umbilicus); the subscapular region (at an area 5 cm directly below the angulus inferior of the scapula); and the quadriceps and hamstrings (on the anterior and posterior surface at the midpoint between the lateral condyle of the femur and the greater trochanter). Body density was calculated using the total subcutaneous fat thickness of these six points from the equation of Abe *et al*,[11] and percentage fat, FFM and fat mass were calculated from measured body density using Brozek's equation.[12] All these measurements were performed by the same trained person. All sites were measured three times. Furthermore, Abe *et al*[11] previously reported that the results of the ultrasound technique correlated well with the results of the underwater weighing method.

Table 2 Physical characteristics and changes in anthropometric parameters

	Pre	End
Body weight (kg)		
WR	65.5 (12.0)	62.3 (12.0)**
Control	64.8 (8.4)	64.5 (8.1)
Weight loss rate (%)		
WR	0.0–0.0	4.9 (2.7)**†
Control	0.0–0.0	0.5 (1.1)
Relative body weight (%)		
WR	20.1 (1.2)	19.9 (1.0)
Control	21.3 (1.5)	20.6 (1.4)
Body fat (kg)		
WR	13.5 (1.7)	12.6 (1.5)*
Control	14.1 (1.6)	13.5 (1.4)
FFM (kg)		
WR	52.0 (2.7)	49.7 (2.9)*
Control	50.7 (1.5)	50.9 (1.5)

End, 1 day before the competition; FFM, fat-free mass; Pre, 20 days before the competition; WR, weight reduction.
WR group, n = 8; control group, n = 8.
*p<0.05, **p<0.001, compared with pre-value, †p<0.001, compared with control.
Results are expressed as mean (SD).

Dietary survey

The subjects recorded their meals and the weight of their food intake every day during the study. Daily nutrient intake and total energy intake were calculated using the fourth revision of the standard Tables of Food Composition.[13] The mean of total energy intake during the 3 days before each measurement was used to determine the total energy intake.

Haematological and biochemical assays

Blood samples were taken from the forearm vein to measure the following parameters: (i) white blood cell and leucocyte counts in whole blood (using a blood cell autoanalyser, MicroBiff-II; Coulter, Fullerton, California, USA); (ii) Igs (IgG, IgA and IgM, nephelometry method); and (iii) complements (C3 and C4, nephelometry method) in serum. Furthermore, serum samples were used to determine the concentrations of asparate aminotransferase, alanine aminotransferase, lactic acid, lactate dehydrogenase, and creatine kinase. These serum parameters were measured using the ultraviolet method.

Neutrophil oxidative burst and PA

Neutrophil oxidative burst (ROS production) and PA were measured with FACScan (Becton Dickinson, San Jose, California, USA) using two-colour flow cytometry. Hydroethidine (HE; 44.4 μmol/l; Polyscience, Warrington, Pennsylvania, USA) was used as an indicator for oxidative burst activity, and opsonised zymosan particles (Sigma Chemical Co, St Louis, Missouri, USA) labelled with fluorescein isothiocyanate (FITC; Sigma Chemical) were used as indicators

Table 3 Changes in energy intake per kg body weight

	Pre	End	Reduction rate (%)
Energy (kcal/kg)			
WR	37.97 (6.20)†	18.11 (5.41)*	52.30
Control	29.50 (7.20)	24.95 (8.94)	15.43
Protein (g/kg)			
WR	1.23 (0.21)	0.49 (0.15)*†	60.08
Control	1.02 (0.27)	0.78 (0.23)	23.40
Lipids (g/kg)			
WR	1.24 (0.21)	0.55 (0.17)*	55.48
Control	0.94 (0.30)	0.82 (0.39)	13.48
Carbohydrates (g/kg)			
WR	5.35 (1.13)	3.50 (1.27)*	33.77
Control	4.25 (1.05)	2.84 (1.00)	33.13

End, 1 day before the competition; Pre, 20 days before the competition; WR, weight reduction.
WR group, n=8; control group, n=8.
*p<0.05, compared with pre-value.
†p<0.05, compared with control.
Results are expressed as mean (SD).

for PA. In brief, 100 µl of heparinised whole blood was mixed with 22 µl of HE to a final concentration of 8 µM, and incubated at 37℃ for 35 min. After the addition of 25 µl of FITC-labelled opsonised zymosan (FITC-OZ; final concentration of 5 mg/ml), the sample was incubated at 37℃ for 35 min. The same amount of whole blood labelled with only HE was prepared to measure the basal oxidative burst activity. After incubation, Lyse and Fix (Immunotech, Marseille, France) was added for haemolysis of red blood cells and fixation of white blood cells. The sample was washed twice in phosphate-buffered saline with sodium azide. The fluorescence intensity in the activated neutrophils was measured with FACScan (Becton Dickinson). Extracellular fluorescence was quenched by adding 30 µl of trypan blue (0.25 mg/ml, pH 4.5) just before the assay to differentiate between attached and ingested FITC-OZ in the neutrophils.[14 15]

FACScan

Neutrophils were analysed on a standard flow cytometer (FACScan). In each sample, 10 000 neutrophils were analysed. Neutrophil oxidative burst activity (ROS production) and PA data were collected using a logarithmic amplifier and estimated as the mean fluorescence intensity channel number of activated neutrophils. The percentages of positive cells producing ROS and incorporating OZ were calculated.

Table 4 Changes in serum myogenic enzymes

	Pre	End*
CK (IU/l)		
WR	174.5 (44.5)	604.6 (993.6)†
Control	276.8 (108.8)	211.3 (44.2)
LDH (IU/l)		
WR	348.1 (28.2)	385.9 (88.6)†
Control	372.4 (25.5)	367.5 (25.4)
AST (IU/l)		
WR	18.5 (2.1)	40.4 (49.3)†
Control	20.1 (2.5)	18.0 (1.4)
ALT (IU/l)		
WR	13.9 (1.2)	25.0(22.1)†
Control	14.9 (2.0)	14.4 (1.0)

ALT, alanine aminotransferase; AST, asparate aminotransferase; CK, creatine kinase; End, 1 day before the competition; LDH, lactate dehydrogenase; Pre, 20 days before the competition; WR, weight reduction.
WR group, n=8; control group, n=8.
*The post-values were adjusted for dehydration by plasma volume.
†p<0.05, compared with pre-value.
Results are expressed as mean (SD).

Measurement of SOA

Chemiluminescence has been used as a method to detect ROS sensitively.[16 17] In this study, SOA was examined by measuring neutrophil ROS production using luminol-dependent chemiluminescence.

Zymosan from *Saccharomyces cerevisiae*, a well-known activator of the alternative pathway of the complement system, was employed for opsonised particles. Zymosan A (Sigma) was suspended in Hank's balanced salt solution (HBSS) at a concentration of 5 mg/ml and then opsonisation was performed by adding to the serum samples to a final concentration of 20% and incubating at 37℃ for 30 min. The particles were then washed twice with HBSS and resuspended in HBSS at a concentration of 5 mg/ml. Luminol was prepared by dissolving 5-amino-2,3-dihydro-1,4-phatalazinedione (Sigma) initially in 1 M NaOH to give a clear solution and then adjusted using HCl and HBSS to give a final concentration of 2 mM (pH 7.4). Normal pooled human neutrophils were obtained from the peripheral blood of a healthy adult male volunteer, whereby whole blood centrifuged through Mono-Poly resolving medium

Table 5 Changes in blood leucocytes, neutrophils, Igs and complements

	Pre	End
Total leucocytes (per µl)		
WR	6537.5 (911.7)	6245.3 (917.9)
Control	5975.0 (882.8)	6084.7 (979.7)
Neutrophils (per µl)		
WR	3931.4 (946.2)	3575.0 (847.4)
Control	3452.5 (467.7)	3588.2 9646.1)
IgG (mg/dl)		
WR	1246.0 (91.2)	1297.5 (42.5)
Control	1252.4 (123.6)	1244.8 (118.7)
IgA (mg/dl)		
WR	175.3 (19.0)	181.0 (44.8)
Control	261.8 (22.9)	261.9 (24.6)
IgM (mg/dl)		
WR	159.1 (18.5)	171.3 (5.9)
Control	207.9 (43.0)	197.4 (36.7)
C3 (mg/dl)		
WR	108.4 (2.7)	97.1 (4.6)
Control	111.4 (5.6)	95.0 (4.1)
C4 (mg/dl)		
WR	22.8 (1.7)	23.3 (22.0)
Control	22.0 (2.4)	20.0 (1.9)

End, 1 day before the competition; Pre, 20 days before the competition; WR, weight reduction.
WR group, n=8; control group, n=8.
Results are expressed as mean (SD).

Table 6 Changes in luminol-dependent serum opsonic activity

	Pre	End
LmCL-AUC ($\times10^5$)		
WR	315.9 (7.4)	325.4 (13.1)
Control	304.6 (9.6)	345.2 (7.1)*
LmCL-PH ($\times10^4$)		
WR	107.8 (1.5)	111.4 (3.0)†
Control	107.8 (3.1)	119.6 (1.5)*

AUC, area under the curve for 45 min; End, 1 day before the competition; LmCL, luminol-dependent chemiluminescence; PH, peak height; Pre, 20 days before the competition; WR, weight reduction.
WR group, n = 8; control group, n = 8.
*p<0.05, compared with pre-value.
†p<0.05, compared with control.
Results are expressed as mean (SD).

(Dainippon Pharmaceutical, Tokyo Japan) has been modified. The neutrophils were suspended to 3×10^6 cells/ml using an automatic blood cell counter (Coulter MD II, Coulter Co, Tokyo, Japan).

OZ suspension and chemiluminigenic probes prepared as 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).[18] All measurements were performed at 37℃. As peak height and area under the curve have been widely used and are reliable parameters,[19–21] the results were evaluated using the maximum light emission (peak height) and the area under the curve of chemiluminescence response. Each sample was run in duplicate and values were expressed as means.

Statistical analysis

Data were presented as mean (SD). The changes within each group before and after weight reduction were tested with the Wilcoxon's test, and the Mann–Whitney U test was used to analyse the differences in all parameters between the two groups. The differences were considered to be statistically significant at $p<0.05$.

RESULTS

Table 2 shows changes in body composition in the WR and control groups. In the WR group, body weight, body fat and FFM significantly decreased after weight reduction ($p<0.001$, $p<0.05$ and $p<0.05$, respectively). There was no significant change in the control group.

Table 3 shows the changes in nutritional intake per kg body weight during the trial period. In the WR group, intakes of energy, protein, lipid and carbohydrate decreased, with a reduction rate for all of >50%, with the exception of carbohydrates. On the other hand, no changes in these intakes were seen in the control group.

Table 4 shows the changes in serum myogenic enzymes: creatine kinase, lactate dehydrogenase, asparate aminotransferase and alanine aminotransferase levels had significantly increased on the last (post-values) day of the 20-day study period.

As for neutrophil and related functions such as the ROS (neutrophil oxidative burst activities) production capability, PA and SOA (tables 6–8), comparing the post-values with the pre-values, ROS production significantly increased in both groups ($p<0.01$ for both), PA significantly decreased in the WR group ($p<0.05$), and, although it decreased in the control group, the difference was not significant, and SOA significantly increased in the control group ($p<0.05$), although it showed no significant change in the WR group.

Table 7 Changes in neutrophil phagocytic rate and activity[9]

	Pre	End
Phagocytic rate (%)		
WR	88.2 (16.1)	94.4 (2.1)
Control	87.2 (17.3)	94.0 (1.3)
Phagocytic activity per cell (FI)		
WR	737.7 (96.5)	567.7 (74.6)*†
Control	824.4 (227)	658.7 (103.6)

End, 1 day before the competition; FI, fluorescence intensity; Pre, 20 days before the competition; WR, weight reduction.
WR group, n = 8; control group, n = 8.
*p<0.001, compared with pre-value.
†p Value compared with control.
Results are expressed as mean (SD).

Spearman's correlation coefficients among the changes from pre-value to post-value for ROS production, PA and SOA of Igs and complements are shown in table 9. No significant correlations were seen among these parameters.

DISCUSSION

Some studies have reported that weight reduction for athletes by a combination of dieting and exercise decreases muscle volume and body fat. In the present study, against their total weight reduction, subjects reduced their fat mass and FFM by 34% and 64%, respectively, suggesting severe weight reduction and, in fact, an inappropriate degree of weight reduction, considering the maintenance of performance levels in the competition.

The typical change in the three neutrophil functions examined after a single bout of normal exercise with no dietary restrictions is an increase in both ROS and SOA and decrease in PA,[22–24] and it has been reported that these three neutrophil functions compensate for each other. For example, when PA decreases, ROS and SOA increase, or when PA increases, ROS and SOA decrease.[10 25] Mochida *et al*[10] suggested that such compensatory changes allow a ''balancing act'' to take place among neutrophil activities and related factors, so that the overall integrity of the neutrophil immune function is maintained; however, it is not known which ones compensate for the others. However, under conditions of severe and prolonged exercise such as a full marathon and a training camp plus weight reduction, neutrophil parameters have tended to deviate from such typical compensatory changes. For example, although PA decreased, ROS also decreased.[26 27]

In the 20-day study period, the control group showed the following changes: ROS and SOA significantly increased and PA decreased, although it was not significant. These changes

Table 8 Changes in neutrophil oxidative burst rate and activity[9]

	Pre	End
Oxidative burst rate (%)		
WR	72.5 (18.9)	96.8 (2.7)*
Control	72.1 (18.5)	97.4 (2.5)*
Oxidative burst activity per cell (FI)		
WR	169.4 (15.3)	337.7 (21.5)**†
Control	171.0 (14.3)	307.5 (22.6)**

End, 1 day before the competition; FI, fluorescence intensity; Pre, 20 days before the competition; WR, weight reduction.
WR group, n = 8; control group, n = 8.
*p<0.05, **p<0.001, compared with pre-value.
†Compared with control.
Results are expressed as mean (SD).

Table 9 Spearman's correlation coefficients among measured parameters

	IgG	IgA	IgM	C3	C4
PA per cell	0.267	0.217	0.211	0.127	0.112
ROS production per cell	–0.222	0.167	0.200	0.100	0.023
AUC of SOA	0.198	–0.045	0.212	0.267	0.121

AUC, area under the curve; PA, phagocytic activity; ROS, reactive oxygen species; SOA serum opsonic activity.

are probably due to the effects of the daily training for this period. On the other hand, although PA significantly decreased and ROS production significantly increased in both WR and control groups, SOA did not show a significant increase in the WR group, unlike the control group.

In the control group, a change in the three major neutrophil functions was demonstrated, which is similar to that induced by normal exercise. The difference in the changes between the control and WR group is that the increase in SOA in the WR group was less than that in the control group, even though the decrease in PA was more notable in the WR than in the control group. These changes in the WR group were probably a direct consequence of the weight reduction regimen coupled with the exercise regimen, suggesting that neutrophil parameters tended to deviate from the typical compensatory changes reported previously.

As for weight reduction in athletes, an inhibitory association in male university judoists between SOA and weight reduction plus exercise has been reported only in the study by Ohta *et al*,[7] which is contrary to the results of the present study. The change in Ohta's study may have two explanations. First, the decrease in SOA was to compensate for an increase in PA. Second, the decrease in SOA was a deviation from the typical changes already mentioned above, even though PA had increased. It is impossible to state which of these are correct, because, firstly, PA was not measured in the study by Ohta *et al*, and secondly, we cannot evaluate whether the exercise/training and weight reduction in that study was normal or not. Furthermore, the difference in the changes between the current study and Ohta *et al*'s study may have been due to gender difference.

From the results of both the current study and that by Mochida *et al*,[10] the measurement of changes in the three major neutrophil functions should allow accurate evaluation of the relationship between exercise loading and the subjects' physical condition.

On the other hand, the values of Igs and complements, although they are recognised as affecting SOA, had no correlation with the changes in SOA levels. Therefore, factors other than Igs and complements may be involved in changes in the SOA in athletes undergoing a combined weight reduction and exercise regimen.

What is already known on this topic

The typical change in the three neutrophil functions (reactive oxygen species production capability, phagocytic activity and serum opsonic activity) examined following a single bout of normal exercise with no dietary restrictions is an increase in both reactive oxygen species production capability and serum opsonic activity, whereas phagocytic activity decreases, and it has been reported that these three neutrophil functions compensate for each other

What this study adds

The changes in the weight reduction group were probably a direct consequence of the weight reduction regimen coupled with the exercise regimen, suggesting that neutrophil parameters (reactive oxygen species production capability, phagocytic activity and serum opsonic activity) had tended to deviate from their typical compensatory changes to maintain immune system homoeostasis

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EDITORIAL BOARD MEMBER

Philip Conaghan

Philip Conaghan finished training in rheumatology and general medicine at the University of Melbourne in 1992 and moved to Sydney, learning about research: clinical trials, basic science and the perils of PhDs. In 1997 he moved to Leeds where he is currently a senior lecturer in rheumatology and an honorary NHS consultant.

His major research interests are in the overlapping areas of: MRI and arthritis; musculoskeletal medicine and osteoarthritis; outcome measurement.

He is academic liaison to the Leeds Musculoskeletal Service, the intermediary MSK service for the city of Leeds. He has over 70 publications (39 peer-reviewed, 20 reviews, 12 book chapters/books), has presented more than 110 abstracts at scientific meetings, and is often an invited speaker both nationally and internationally.

Figure 1 Philip Conaghan.