Exercise training and energy restriction decrease neutrophil phagocytic activity in judoists

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ABSTRACT

KOWATARI, K., T. UMEDA, T. SHIMOYAMA, S. NAKAJI, Y. YAMAMOTO, and K. SUGAWARA. Exercise training and energy restriction decrease neutrophil phagocytic activity in judoists. *Med. Sci. Sports Exerc.*, Vol. 33, No. 4, 2001, pp. 519–524. **Purpose:** To investigate the effects of weight reduction as the result of exercise training and energy restriction on neutrophil function. **Methods:** Eighteen male competitive college judoists participated in the study. In a whole blood assay, oxidative burst activity, phagocytic activity, expressions of Fc gamma receptor 3 (CD16), and complement receptor 3 (CD11b) of neutrophils were measured on a per cell basis by flow cytometry at day 20, 5, and 1 before and at day 7 after the competition. **Results:** The rate of neutrophil producing reactive oxygen species decreased before the competition, whereas the oxidative burst activity per cell increased significantly in all subjects, which resulted in a significant increase of the total oxidative burst activity. However, there were no significant effect of energy restriction on oxidative burst activity. The rate of neutrophils incorporating opsonized zymosan decreased significantly with energy restriction. The total phagocytic activity of 10000 neutrophils and the phagocytic activity per cell also decreased significantly by severe energy restriction. The surface antigen expressions of CD11b and CD16 were unaffected by weight reduction. **Conclusions:** The results suggest that with respect to the management of health conditions, weight reduction for judoists should be composed of exercise training and energy restriction should be moderate. **Key Words:** ATHLETE, IMMUNOSUPPRESSION, OXIDATIVE BURST, FLOW CYTOMETRY

Management of health and weight are very important for athletes to compete successfully. In the sports classed by weight, few athletes maintain their daily body weight within the limits of their class, and most have to reduce their weight by 5–10% before competition (5). Athletes must reduce their weight without decreasing their lean body mass to avoid the decline of physical strength. Most athletes rely on rapid weight reduction with intensive training and energy restriction (18,36).

In general, the basis of weight reduction is mainly loss of body fat by energy restriction and exercise-induced energy consumption. A number of reports have shown that moderate exercise increases the body's defense against infection (4,15,33). However, there is a general perception among athletes, coaches, and team physicians that athletes are susceptible to infectious disease, primarily upper respiratory tract infection (URTI) due to chronic immunosuppression, during intense training and major competition (25,27,32). The risk of URTI appears to be related to the amount of training intensity (13,21,26). It is also well established that severe deprivation of energy and nutrients result in compromised immune system and hence a decreased resistance to infection (7).

Rapid weight reduction by energy restriction may have additional disadvantages on athletes who are in a chronic immunosuppressive state. Filteau et al. (11) have demonstrated that exercise training has a role in attenuating energy restriction-induced immunosuppression in rodents. In athletes, however, there are many reports about the effects of exercise on immunity, whereas no previous studies examined the effects of energy restriction on immunity. It is therefore important for athletes to investigate the effects of exercise training and energy restriction on immunity and weight reduction before competition.

Neutrophils play an important role in the immune system, forming the first defense against invading microorganisms (31) by phagocytosis and using toxins such as reactive oxygen species (ROS) and lysosomal enzymes (2). The attachment and ingestion of foreign bodies depends mainly on the expressions of Fc gamma receptor 3 (FcγR3) and complement receptor 3 (CR3) (9,38). Numerous attempts have been made to investigate the effects of exercise training or energy restriction on neutrophil functions. However, no studies have investigated the effects of exercise training and energy restriction on neutrophil oxidative burst or phagocytic activities, including the expressions of FcγR3 and CR3 in weight reduction before competition.

The purpose of this study was to investigate neutrophil functions during weight reduction in athletes. In addition,

0195-9131/01/3304-0519/$3.00/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

Received for publication April 2000.
Accepted for publication July 2000.

the differences in the effects between exercise training and energy restriction in judoists as a model were also examined.

MATERIALS AND METHODS

Subjects and study protocol. The subjects in this study were 22 male Japanese college judoists aged 18–21 yr, who took part in the selection at university level in May 1999 to participate in the Tokyo Men's College Judo Championship Classed by Weight. Fourteen subjects who required weight reduction were defined as the low energy intake (LEI) group, and eight subjects who did not need weight reduction served as the control group. Because the LEI group started weight reduction 20 d before the competition, anthropometric conditions and neutrophil functions were studied on day 20, 5, and 1 before and 7 d after the competition and results were expressed as the values of pre-, during-, end-, and post-weight reduction, respectively. Approval was obtained from the Ethics Committee of the Hirosaki University School of Medicine. The study protocol and purpose were explained, and consent was obtained from all subjects before the commencement of the study.

Anthropometry. Anthropometric status was examined calculating body weight (BW), lean body mass (LBM), body fat (BF), and relative body fat (%fat). Body density was measured by underwater weighing and %fat was calculated from measured body density using Brozek's equation (6). Residual lung volume was measured by oxygen rebreathing nitrogen dilution (39).

Exercise training and energy intake. All subjects performed running and weight training for 1 h and judo training for 2.5 h every day. Meals and food weights were recorded daily in a dietary survey table by each subject, and food intake weight per day calculated. The intake of nutrients per food weight was calculated and added to obtain the nutrient intake per day. The fourth revision of the food composition table (28) was used for calculating total energy intake (TEI). The mean TEI for 3 d before each measurement was calculated.

Blood leukocyte, neutrophil, and lymphocyte counts. Venous blood samples (10 mL) were collected from the forearm vein of seated subjects after overnight fasting. Total leukocyte, neutrophil and lymphocyte counts were measured using an automatic cell counter (Micro Diff-II, Coulter Co., Ltd., Fullerton, CA).

Neutrophil oxidative burst/phagocytosis assays. Neutrophil oxidative burst and phagocytic activities were measured using two-color flow cytometric methods. To determine the capacity of oxidative burst, Hydroethidine (HE, 44.4 μM, Polyscience Inc., Warrington, PA) was employed as an indicator for the generation of oxygen radicals. Opsonized zymosan particles labeled with fluorescein isothiocyanate (FITC, Sigma Chemical Co., St. Louis, MO) was employed as an indicator of phagocytosis.

Heparinized whole blood (100 μL) portions were mixed with 22 μL HE (final concentration (f.c.) was 8 μM). After incubation at 37°C for 5 min, 30.5 μL FITC-labeled opsonized zymosan (FITC-OZ) was added (f.c. 1 $mg \cdot mL^{-1}$) and incubated at 37°C for 40 min. Lyse and Fix 10Test (IMMUNOTECH, Marseille, France) was added to lyse the red blood cells and the samples fixed. The samples were washed twice in phosphate-buffered saline (PBS) with sodium azide (PBS+) for before flow cytometry. To determine the oxidative burst activities, neutrophils in a 100 μL whole blood labeled HE without FITC-OZ was prepared as a control (basal state). Immediately before flow cytometry, trypan blue (30 μL; 0.25 $mg \cdot mL^{-1}$, pH4.5) was added to differentiate attached and ingested FITC-OZ by using fluorescence quenching method (14,30).

Analysis of FcγR3 and CR3 expressions on neutrophil activity/function. Monoclonal antibodies to CD16 and CD11b (IMMUNOTECH) were used to analyze the expressions of FcγR3 (CD16) and CR3 (CD11b) on neutrophils by two-color flow cytometric methods. Briefly, heparinized whole blood samples (100 μL) were mixed with 100-μL monoclonal antibodies specific for CD16 and CD11b, and incubated at room temperature for 40 min. Lyse and Fix 10Test was added to lyse the red blood cells and fix the samples. The samples were washed twice in PBS+ and measured by flow cytometry.

Flow cytometry assay. Neutrophils were analyzed on a standard flow cytometer (FACScan, Becton Dickinson, San Jose, CA). For each sample, 10,000 neutrophils were analyzed. The mean channel number of fluorescence intensity of activated neutrophils (FI) was used as the index of oxidative burst, phagocytic activity, and the expressions of CD16 and CD11b. The percentage of positive cells was counted to express the rate of neutrophils producing ROS, incorporating OZ, and expressing CD16 or CD11b. The cumulative fluorescence intensity (CFI), which is the value of FI multiplied by the value of %, was used as a quantitative index of total oxidative burst and phagocytic activity.

Statistics. All values are presented as means ± SD. The difference in the degree of energy restriction was evaluated using a two-way analysis of variance with repeated measures on one factor (effect of treatment). The differences in each value of anthropometric parameters and energy intake were evaluated by the paired *t*-test. The difference of values in each neutrophil function was tested by Dunn's procedure as a multiple comparison procedure. The differences were considered to be statistically significant at $P < 0.05$.

RESULTS

Two subjects were excluded from each group because of injury during judo training before the competition. To investigate the degree of the effects of energy restriction on body composition and neutrophil functions, we divided the LEI group into two groups, using the median value of TEI before the competition, very-low energy intake group (VLEI), and low energy intake group (LEI).

Anthropometric parameters and energy intake. The anthropometry and energy intake are summarized in Table 1. Subjects in VLEI and LEI groups restricted energy intake significantly before the competition. BW decreased

TABLE 1. Changes in anthropometric parameters and energy intake.

	control ($N = 6$)	LEI ($N = 6$)	VLEI ($N = 6$)
Body weight (kg)			
Pre	75.0 ± 5.7	74.7 ± 5.6	74.3 ± 6.2
During	74.9 ± 5.6	73.7 ± 5.4 *	72.8 ± 6.5 *
End	74.3 ± 6.0 *	72.0 ± 6.3 **	71.0 ± 6.9 *
Post	74.4 ± 5.8 *	74.5 ± 4.9	73.3 ± 5.1
Lean body mass (kg)			
Pre	67.5 ± 2.4	66.6 ± 1.5	65.6 ± 4.4
During	66.8 ± 2.6	66.6 ± 2.3	64.5 ± 3.7
End	66.0 ± 2.2 **	65.1 ± 2.5	63.5 ± 4.0 *
Post	67.2 ± 2.4	67.5 ± 1.7	66.5 ± 3.5
Body fat (kg)			
Pre	7.4 ± 3.9	8.1 ± 4.6	8.7 ± 4.6
During	7.9 ± 3.8	7.1 ± 4.1	8.3 ± 4.6
End	8.2 ± 4.3	6.9 ± 4.0 **	7.5 ± 4.1
Post	7.2 ± 4.4	6.7 ± 3.8 *	7.0 ± 5.0 *
Relative body fat (%)			
Pre	9.6 ± 4.5	10.6 ± 5.2	11.5 ± 5.4
During	10.4 ± 4.3 *	9.4 ± 5.0	11.0 ± 5.6
End	10.8 ± 5.0	9.3 ± 4.6 **	10.2 ± 4.9
Post	9.3 ± 5.3	8.8 ± 4.5 *	9.2 ± 6.3
Energy intake (kcal)			
Pre	3272 ± 994	3182 ± 258	2530 ± 284
During	2877 ± 384	2579 ± 301*	2028 ± 322*†
End	3291 ± 361	2387 ± 385*†	1796 ± 478**††
Post	3285 ± 427	2974 ± 467	2537 ± 400††

Results were expressed as mean ± SD.
Pre, 20 d before the competition; during, 5 d before the competition; end, 1 d before the competition; post: 7 d after the competition.
* $P < 0.05$, ** $P < 0.01$ compared with prevalue.
† $P < 0.05$, †† $P < 0.01$ compared with the value of control group.

significantly before the competition in all groups. Subjects in VLEI and LEI groups attained to lose 3.3 ± 2.1 (4.4%) and 2.7 ± 1.2 (3.6%) kg, successfully. BF decreased significantly in VLEI group (1.26 ± 1.62 kg) and in LEI group (1.21 ± 0.69 kg). LBM decreased significantly before the competition in all groups, and the maximal decrease was seen in VLEI group (2.05 ± 1.82 kg). There was a significant difference in EI between control and energy restriction group, whereas there were no significant changes in any other parameters between each group.

Blood leukocyte, neutrophil, and lymphocyte counts. Changes in blood leukocyte, neutrophil, and lymphocyte counts are summarized in Table 2. There were no significant changes throughout the experimental period in all groups.

Neutrophil oxidative burst/phagocytic activities. Basal neutrophil oxidative burst was increased significantly at postweight reduction in all groups (Table 3). Total oxidative burst activity increased significantly at, during, and postweight reduction in comparison with prevalue, in all groups, showed a bimodal pattern. The rate of neutrophil producing ROS was decreased significantly at the end of weight reduction in control and LEI groups, whereas oxidative burst per cell increased significantly throughout the experimental period in all groups. There was no significant difference in these parameters between groups.

Total phagocytic activity decreased significantly during and at the end of weight reduction in the VLEI group only. The rate of neutrophils incorporating OZ decreased significantly at the end of weight reduction in LEI and VLEI groups. The phagocytic activity per cell decreased significantly during weight reduction in VLEI group.

CD16 and CD11b expressions on neutrophils. Changes in the surface antigen expressions of CD16 and CD11b were shown in Table 4. There were no significant

TABLE 2. Changes in blood leukocyte, neutrophil, and lymphocyte counts.

Cell counts ($10^9 \cdot L^{-1}$)	control ($N = 6$)	LEI ($N = 6$)	VLEI ($N = 6$)
Total leukocytes			
Pre	6.75 ± 2.24	5.72 ± 2.09	5.73 ± 2.31
During	6.85 ± 1.88	5.67 ± 1.19	6.17 ± 1.17
End	5.85 ± 1.83	5.50 ± 0.89	5.65 ± 0.71
Post	6.30 ± 1.72	5.27 ± 0.91	5.48 ± 0.77
Neutrophils			
Pre	3.45 ± 1.08	2.51 ± 0.77	2.82 ± 1.87
During	3.40 ± 1.60	2.43 ± 0.47	2.98 ± 1.16
End	2.72 ± 1.16	2.53 ± 0.55	2.73 ± 0.87
Post	2.84 ± 1.18	2.12 ± 0.40	2.44 ± 0.91
Lymphocytes			
Pre	2.76 ± 1.17	2.52 ± 1.10	2.28 ± 0.40
During	2.84 ± 1.24	2.57 ± 0.59	2.62 ± 0.59
End	2.69 ± 1.13	2.50 ± 0.67	2.48 ± 0.31
Post	2.91 ± 1.18	2.53 ± 0.48	2.59 ± 0.39

Results were expressed as mean ± SD.
There was no significant difference.

TABLE 3. Changes in neutrophil phagocytic activity and oxidative burst.

	Control (*N*=6)	LEI (*N*=6)	VLEI (*N*=6)
Basal oxidative burst (CFI)			
Pre	12.5 ± 1.5	12.0 ± 1.8	13.1 ± 1.1
During	16.9 ± 3.8	18.9 ± 4.4 **	17.7 ± 1.7
End	14.5 ± 1.6	15.0 ± 2.3	16.0 ± 2.0
Post	24.7 ± 4.3***	21.9 ± 4.6 ***	23.6 ± 4.7 ***
Total oxidative burst activity (CFI)			
Pre	97.8 ± 16.1	93.9 ± 17.6	101.0 ± 21.6
During	217.4 ± 35.5 ***	211.9 ± 43.6***	227.2 ± 30.3***
End	131.1 ± 18.7	130.5 ± 28.0	160.9 ± 41.0**
Post	199.0 ± 16.2 ***	196.0 ± 21.2***	222.3 ± 31.4***
Oxidative burst rate (%)			
Pre	72.6 ± 5.0	71.4 ± 2.7	69.2 ± 7.3
During	67.7 ± 8.4	63.8 ± 8.8	65.1 ± 6.9
End	56.8 ± 6.2 **	57.5 ± 6.6 ***	60.3 ± 6.3
Post	64.5 ± 9.6	62.6 ± 4.0	62.9 ± 6.1
Oxidative burst activity per cell (FI)			
Pre	134.5 ± 18.7	131.5 ± 23.3	145.6 ± 27.1
During	320.5 ± 29.2 ***	332.1 ± 51.3***	350.4 ± 42.9***
End	231.5 ± 27.1 ***	225.6 ± 28.3***	263.9 ± 45.1***
Post	311.7 ± 31.5 ***	314.8 ± 46.0***	352.0 ± 17.1***
Total phagocytic activity (CFI)			
Pre	255.8 ± 34.3	255.5 ± 43.9	309.4 ± 66.0
During	226.0 ± 66.7	247.4 ± 55.1	220.3 ± 32.9**
End	212.9 ± 99.1	210.4 ± 42.7	218.6 ± 31.5**
Post	242.1 ± 30.0	249.8 ± 47.4	270.2 ± 27.0
Phagocytic rate (%)			
Pre	84.1 ± 4.3	83.5 ± 3.5	86.1 ± 6.3
During	81.0 ± 10.6	79.0 ± 9.0	79.5 ± 7.0
End	70.4 ± 9.4	69.5 ± 6.2 ***	72.1 ± 6.8 **
Post	78.8 ± 6.9	77.8 ± 3.3	79.7 ± 5.1
Phagocytic activity per cell (FI)			
Pre	303.6 ± 28.4	304.7 ± 42.2	357.1 ± 54.8
During	274.6 ± 49.7	312.0 ± 55.6	276.4 ± 25.5**
End	307.5 ± 110.2	301.2 ± 41.0	302.8 ± 28.1
Post	309.5 ± 47.8	321.1 ± 60.5	338.9 ± 27.9

Results were expressed as mean ± SD.
Oxidative burst or phagocytic rate, the rate of neutrophil producing ROS or incorporating OZ; FI, the mean channel number of fluorescence intensity of activated neutrophil; CFI, the value of FI multiplied by the value of %.
* $P<0.05$, ** $P<0.01$, *** $P<0.001$ a significant difference compared with each prevalue.

changes in the rate of neutrophils expressing these receptors and the expression of these receptors per cell.

DISCUSSION

Subjects in VLEI and LEI groups who attempted weight reduction attained 4% of weight reduction compared with prebody weight. A significant difference was seen in the reduction of BW between the control group and the energy restriction group in spite of the same exercise intensity. These results suggest that combination of exercise and energy restriction decrease BW effectively. With respect to body composition, BF decreased effectively in the energy restriction group, whereas the maximal decrease of LBM

TABLE 4. Changes in Fcγ receptor 3 and C3 receptor expressions on neutrophil.

	control (*N* = 6)	LEI (*N* = 6)	VLEI (*N* = 6)
CD11b rate			
Pre	99.0 ± 0.6	99.0 ± 0.6	98.3 ± 1.3
During	97.6 ± 1.3	98.0 ± 1.6	98.3 ± 0.7
End	96.5 ± 4.6	97.9 ± 0.8	98.4 ± 0.7
Post	99.0 ± 0.6	98.8 ± 0.9	97.5 ± 3.0
CD11b per cell			
Pre	58.4 ± 15.6	60.5 ± 16.7	66.3 ± 25.4
During	59.7 ± 25.4	70.0 ± 19.5	80.2 ± 22.3
End	67.5 ± 40.1	66.9 ± 18.4	71.2 ± 19.4
Post	72.9 ± 36.2	74.2 ± 18.1	81.5 ± 21.5
CD16 rate			
Pre	94.9 ± 2.3	87.6 ± 6.4	89.2 ± 4.1
During	93.1 ± 4.1	83.5 ± 3.8	87.1 ± 4.1
End	91.5 ± 2.9	84.3 ± 6.7	87.3 ± 7.6
Post	93.9 ± 2.7	84.9 ± 5.8	86.1 ± 7.0
CD16 per cell			
Pre	749 ± 178	903 ± 290	991 ± 271
During	877 ± 142	1100 ± 368	1179 ± 329
End	947 ± 286	1134 ± 405	1178 ± 317
Post	981 ± 258	1155 ± 387	1221 ± 398

Results were expressed as mean ± SD.
CD11b rate, CD16 rate; percentage of neutrophil expressing CD11b or CD16, CD11b per cell, CD16 per cell; the mean fluorescence intensity of CD11b or CD16 per cell. There was no significant difference.

was seen in VLEI group. There have been many reports that exercise and energy restriction brought the collapse of protein resulting in a decrease in LBM (1,16). Therefore, a high degree of energy restriction which caused an enhanced decrease of LBM may not be suitable for weight reduction before competitions so that competitor can avoid the decrease of physical strength (17,23,29).

Total leukocyte, neutrophil and lymphocyte counts were not affected by both exercise training or energy restriction. It is well known that acute exercise induces an increase in total leukocyte and neutrophil counts (20). However in this study these parameters were not measured just before and immediately after the exercise. As increased total leukocyte and neutrophil counts decrease with time after exercise training (10,34,35), in this current study, it is suspected that these parameters might have decrease at the time of measurement. The results suggested that energy restriction might not affect the circulating leukocyte counts. Many studies have shown that weight reduction by restricting fluids intake induces dehydration and greater serum electrolyte loss (8). In this current study, fluids were withheld from subjects, which could induce hemoconcentration. However, because there were no significant increase in hemoglobin, hematocrit, and PT, we did not make any corrections on our results.

To date, there have been no studies that have examined the changes in neutrophil oxidative burst activity during weight reduction in athletes. Furthermore, the effects of energy restriction on neutrophil oxidative burst activity have not been compared with that of exercise training in athletes. In the present study, neutrophil oxidative burst activity was unaffected by energy restriction during weight reduction in judoists. Therefore, it is possible that the increase of oxidative burst activity reflected the effects of exercise training. Suzuki et al. (37) reported that the increase of neutrophil oxidative burst activity was seen in athletes after the exercise and remained elevated until 1 h postexercise. Another report also demonstrated that neutrophil oxidative burst activity increased during the sports season in basketball players (3). Increase of total oxidative burst activity was the result of the increase of oxidative burst activity per cell, which might be up-regulated by the decrease of neutrophils producing ROS.

The increase of basal oxidative burst at postweight reduction might be due to neutrophil priming phenomenon, probably induced by hard exercise training and the stress of competition (35). The neutrophil priming phenomenon might influence the increase of total oxidative burst activity at postweight reduction.

There have been several reports about the effects of exercise training on neutrophil phagocytosis in athletes. Most of them demonstrated unchanged or increased capacity of neutrophil phagocytosis after single bouts of various kinds of exercise (24,27,35). In contrast, after long-distance running, a decrease of neutrophil phagocytic activity in the nasal mucosa was observed (19). Gabriel et al. (12) reported that strenuous endurance exercise induced a decrease in neutrophil phagocytosis per cell. In this current study, neutrophil phagocytic activity was not affected by exercise training in judoists without energy restriction. We also examined the effects of energy restriction on neutrophil phagocytic activity. No studies have ever investigated the difference of the effects between exercise training and energy restriction on neutrophil phagocytic activity in athletes. Nieman et al. (22) reported that neutrophil phagocytosis was not affected by moderate weight reduction by both energy restriction alone and a combination of exercise training and energy restriction in obese women. The present results suggest that energy restriction decreases phagocytic activity and severe energy restriction induces an enhanced decrease of phagocytic activity per cell. We also examined the expressions of two important antigen binding receptors, FcγR3 and CR3. However, the decrease of phagocytic activity per cell was not due to the decrease of the expression of FcγR3 and CR3. As receptors were likely to increase with time and to be up-regulated in response to the decrease of phagocytic activity. Further explanations such as decreased avidity to the receptor, altered outside-in signaling, and inhibitory mechanisms on the intracellular level remain to be investigated.

In conclusion, we studied the effects of weight reduction by exercise training and energy restriction on body composition and neutrophil functions in judoists. With respect to body composition, energy restriction decreased BW and BF significantly, but severe energy restriction decreased LBM. With respect to neutrophil functions, energy restriction did not affect neutrophil oxidative burst activity but decreased phagocytic activity. In fact, in early reports, severe deprivation of energy and nutrient results in compromised immunocompetence and decreased resistance to infection (7). The findings suggest that weight reduction in the sports classed by weight, such as judo, wrestling, and boxing, should be composed of exercise training, and energy restriction should be moderate even if it is necessary.

The excellent technical assistance provided by Drs. Satoru Kudoh, Mutsuo Yamada, Kazuma Danjo, and Qiang Liu are gratefully acknowledged. Thanks also to Manabu Totsuka, Kazuo Saitoh, and Tomomi Katagiri for their support in the measurement of body composition and to students at Nippon Sport Science University for their participation in this survey. This work was supported, in part, by a Grant in Aid from the Ministry of Education, Science, and Culture (11470092), Japan.

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