

Neutrophil function response to aerobic and anaerobic exercise in female judoka and untrained subjects

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Abstract

Objectives—Recent studies have indicated reduced immunity in trained athletes.

Aim—To assess the effects of aerobic and anaerobic exercise on the phagocytic process in 18–26 year old trained female judoka (n = 8) and untrained controls (n = 7).

Methods—Each subject participated randomly in two different testing sessions (aerobic, 20 minutes of treadmill running at 70–80% of maximal heart rate; anaerobic, Wingate anaerobic test). Venous blood samples were drawn before, immediately after, and 24 hours after each session.

Results—There were no significant differences in basal values of net chemotaxis (chemotaxis – random migration), bactericidal activity, and superoxide anion release between the judoka and the untrained women. There was a significant decrease in net chemotaxis 24 hours after the aerobic exercise in both the judoka (from 64 (19) to 39 (13) cells/field, $p < 0.02$) and the untrained controls (from 60 (7) to 47 (12) cells/field, $p < 0.05$). Bactericidal activity and superoxide anion release did not change significantly after aerobic exercise in either group. There were no significant changes in net chemotaxis, bactericidal activity, and superoxide anion release after anaerobic exercise in either the judoka or untrained women.

Conclusions—The decrease in net chemotaxis after aerobic, but not after anaerobic, exercise, suggests that net chemotaxis is affected by the combination of exercise intensity and duration, and not by the exercise intensity itself. Similar effects of both exercise sessions in the judoka and the untrained women suggest that training had no effect on neutrophil function response to aerobic and anaerobic exercises.

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Keywords: exercise; aerobic; anaerobic; neutrophil function; female athlete

In recent years studies have indicated that intense exercise affects the immune system and may increase susceptibility to infection.^{1–3} Moreover, training during the incubation period of infectious diseases can aggravate the clinical course of the developing disease.^{4,5}

The importance of a normal phagocytic arm for immunological function has been well established.^{6,7} However, there is little information on the effect of exercise on neutrophil phagocytic activity, especially at different intensities and duration. Studies from our own labo-

ratory have recently shown reduced neutrophil function in prepubertal female gymnasts after aerobic exercise compared with sedentary controls, reflecting a possible effect of training level.⁸ However, the contribution of the poor nutritional status that characterised the gymnasts and may affect immune function^{9–11} was not examined. Thus, it is possible that the observed differences in the phagocytic response to aerobic exercise between the gymnasts and untrained girls were partially related to nutritional factors.

The aim of this study was to assess the effects of aerobic exercise on the phagocytic process in well nourished highly trained female judoka in comparison with age and weight matched untrained women. In addition, judo training emphasises anaerobic exercise; therefore, to test a possible training effect after anaerobic exercise, we also assessed neutrophil function after an intense anaerobic exercise task in the two groups.

Based on our previous findings in young girls,⁸ we hypothesised that training level would affect the neutrophil response to exercise, and that an impaired neutrophil response would be more apparent after the aerobic than after the anaerobic exercise task. To the best of our knowledge, the present report describes for the first time the effect of both aerobic and anaerobic exercise in the same subjects and is one of the first studies of highly trained female athletes.

Materials and methods

Eight healthy postpubertal trained judoka and seven matched healthy controls participated in the study. Puberty was assessed by the criteria developed by Tanner.¹² The age range of the study participants was 18–26 years. The judoka (weight 64.1 (8.9) kg; height 165 (8) cm; percentage fat 25.1 (5.6); age 19.9 (1.1)) trained an average of 22 hours a week and participated in national and international level competitions. The control subjects (weight 61.7 (3.4) kg; height 162 (4) cm; percentage fat 29.5 (4.8), age 22.5 (1.5)) were physically active but did not train regularly in any sport. None of the participants had suffered from any illness for at least two weeks before the study. The purpose of the study was explained to the subjects and all gave their informed consent. The study was approved by the human studies committee at the Meir General Hospital, Kfar Saba, Israel.

In a preliminary visit body height and weight were measured using standard techniques. Body fat was estimated using the sum of four skinfolds (biceps, triceps, subscapular, and suprailiac) as described by Durnin and Womersley.¹³

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Each subject participated in two different testing sessions (aerobic and anaerobic). The order of the sessions was randomised, and the exercise tests were separated by at least 14 days. Both tests took place in the morning (between 7 00 and 8 30 am). Venous blood (10 ml) was withdrawn from an antecubital vein before, immediately after, and 24 hours after each exercise task. In addition, blood was also withdrawn at similar times from a non-exercising control. The judoka trained regularly before the exercise sessions. However, subjects from both groups—that is, controls and judoka—were instructed not to participate in any physical activity during the day before the study, and between the second and third blood withdrawals.

We did not assess the dietary intake of the judoka and untrained subjects in the present study. However, we assume on the basis of recent observations of some of the judoka from this study, as well as of a different group living and training under similar conditions, that there was no apparent deficiency in macronutrients or micronutrients in their diet.

AEROBIC EXERCISE

Exercise consisted of 20 minutes of treadmill running at a heart rate of 170–180 beats/min. This heart rate was chosen as an estimate of 70–80% of maximal age predicted heart rate. Heart rate was monitored using a Polar Sports Tester (Polar Electro Oy, Finland). Some of the untrained subjects were unable to run for 20 minutes at a heart rate of 170–180 beats/min. Therefore, in order for them to complete 20 minutes of exercise, the velocity of the treadmill was reduced during exercise, and they performed a fast walk. Thus untrained girls walked/ran at a velocity of 6–8 km/h with a slight slope (1.5%), while the judoka ran at a velocity of 10.6–11.3 km/h with the same slope (1.5%).

ANAEROBIC EXERCISE

All subjects performed a Wingate anaerobic test on a Fleisch Metabo (Switzerland) cycle ergometer. Resistance was applied according to body mass, with a load of 0.049 kg/kg. A four to five minute warm up preceded the test, during which time subjects were familiarised with the test procedures and performed two to three maximal unresisted accelerations. The maximal unloaded revolutions per minute were recorded. The Wingate anaerobic test was performed three to four minutes after the warm up. Resistance was applied when subjects reached 95% of their maximal revolutions per minute. Mean power was the average power attained during the entire 30 second test. The Wingate anaerobic test is known to be a highly reliable and valid test of anaerobic power.¹⁴

ISOLATION OF POLYMORPHONUCLEAR LEUCOCYTES (PMNs)

Human purified PMNs (98%) were isolated from heparinised venous blood. After sedimentation with 3% dextran (molecular weight 250 kDa; Sigma, Rehovot, Israel), the leucocyte enriched plasma was layered on to a Ficoll-

Hypaque gradient (Lymphoprep; Nycomed Pharma AS, Oslo, Norway) and centrifuged at 400 *g* for 30 minutes, as previously described.¹⁵ The supernatant was discarded and the pellet subjected to hypotonic lysis for 20 seconds to free the PMNs of contaminating red cells. The PMNs were resuspended for chemotaxis in M199 medium (Earle's salt with L-glutamine; Biological industries, Kibbutz Beth Haemek, Israel). For superoxide anion release, PMNs were suspended in Hanks buffered salt solution (Sigma) and in phosphate buffered saline with 0.2% glucose and 1% bovine serum albumin (PBS/GA) for bactericidal activity. Neutrophil functions were assessed in our laboratory as previously reported.⁶

CHEMOTAXIS ASSAY

A 48 well chemotactic microchamber (Neuro Probe Inc, Bethesda, Maryland, USA) was used to determine random migration and chemotaxis.¹⁶ Either the chemoattractant *N*-formylmethionyl-leucyl-phenylalanine (FMLP; Sigma) at a concentration of 10⁻⁶ M or the M199 medium was added to the bottom wells. A polycarbonate membrane filter, PVP free, with 3 μm pores (Nucleopore Corp, Pleasanton, California, USA) was placed on top of the wells in the bottom plate. The gasket and the top plate were affixed, and 50 μl of 10⁶ PMNs/ml was added to the upper wells. The assembly was incubated for 60 min in humidified air. After incubation the filter was wiped off and stained with May-Grunwald-Giemsa; the number of cells was determined under light microscopy with a 20 × objective and an optical grid at 10 × magnification. The net chemotaxis was calculated by subtracting the random migration from the chemotactic activity. Cell counting was performed blindly and in duplicate.

BACTERICIDAL ACTIVITY

Maximal bactericidal activity was quantified as previously reported.¹⁷ Bacteria (*Staphylococcus aureus*; American Type Culture Collection) were freshly grown before each experiment and allowed to enter an early stationary phase (four hours shaking at 37°C). The final concentration of bacteria was determined spectrophotometrically at 640 nm. A suspension of PMNs at a final concentration of 10⁶/ml was incubated with bacteria at a ratio of 1 to 3, in the presence of autologous, or a pool of homologous, AB serum at 37°C for 90 minutes, with continuous shaking. The PMNs were then lysed with 5 volumes of distilled water, and the suspensions were diluted and plated (30 μl) in triplicate in broth agar plates, for 18 hours at 37°C. Each experimental set up included two controls comprising PBS/GA and bacteria or autologous serum and bacteria. In these control systems we observed none or less than 0.02 exponential decrease in bactericidal activity respectively (n>100). The colonies were counted and the exponential decrease was calculated and compared with that of the control counts.

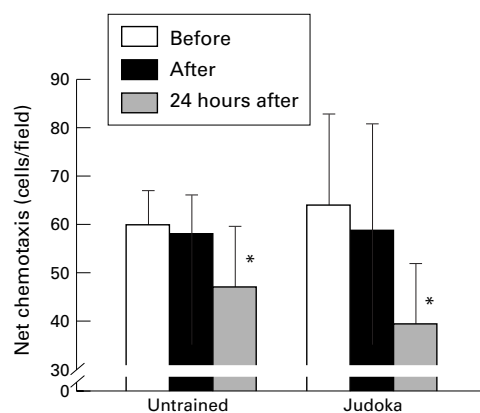


Figure 1 The effect of aerobic exercise on net chemotaxis in judoka and untrained women. There was a significant decrease ($*p < 0.05$) in net chemotaxis in both the judoka and untrained women 24 hours after the aerobic exercise.

SUPEROXIDE ANION RELEASE

This assay was performed as previously reported.¹⁸ The PMNs were suspended in Hanks buffered salt solution at a final density of 5×10^6 cells/ml and incubated at 37°C with $60 \mu\text{M}$ ferricytochrome c (Sigma), with or without 214 U superoxide dismutase (Sigma), in a total volume of 1 ml. To initiate the reaction, $1 \mu\text{g}$ phorbol 12-myristate 13-acetate (PMA; Sigma) or $0.1 \mu\text{M}$ FMLP (Sigma) was added for 10 minutes in a shaking water bath. The reaction was stopped by placing the tubes in ice and the cells were spun down in a chilled centrifuge. The absorbance of the supernatant was measured at 550 nm. The results of triplicate tests were averaged, and superoxide anion release was calculated using the Massey absorption coefficient of $2.1 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$.

STATISTICAL ANALYSIS

An unpaired *t* test was performed to assess differences in neutrophil function between the groups before the exercise tests. Variables measured repeatedly—for example,

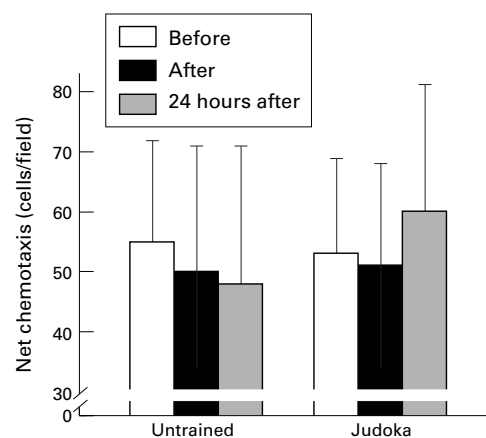


Figure 2 The effect of anaerobic exercise on net chemotaxis in judoka and untrained women. Anaerobic exercise had no effect on net chemotaxis in either.

chemotaxis—were analysed using an analysis of variance for repeated measures, with time serving as the within subject factor and training level as the between subject factor. Post hoc analysis was performed using the Bonferroni procedure.¹⁹ Statistical significance was set at $p = 0.05$ for all statistical analyses. Data are presented as means (SD).

Results

AEROBIC EXERCISE

Figure 1 and table 1 summarise the effect of aerobic exercise on neutrophil function. There was a significant decrease in net chemotaxis 24 hours after the aerobic exercise in both judoka and untrained subjects (fig 1). It is important to emphasise that the decrease in net chemotaxis 24 hours after exercise occurred in all the 15 subjects. There were no significant changes in random migration, bactericidal activity with both autologous and homologous serum, and in the PMA and FMLP stimulated superoxide anion release by neutrophils after exercise in either the judoka or untrained controls (table

Table 1 Effect of aerobic exercise on neutrophil function in judoka and untrained women

	Judoka (n=8)			Untrained (n=7)		
	Before	After	24 h after	Before	After	24 h after
Bactericidal activity- autologous serum	1.26 (0.2)	1.26 (0.3)	1.17 (0.2)	1.39 (0.3)	1.4 (0.1)	1.27 (0.2)
Bactericidal activity- homologous serum	1.15 (0.3)	1.1 (0.3)	0.96 (0.1)	1.23 (0.1)	1.18 (0.2)	1.14 (0.3)
PMA induced superoxide anion release (mmol $\text{O}_2^-/10^6$ PMNs/min)	3.29 (0.8)	3.14 (1.0)	3.72 (0.4)	3.71 (0.4)	3.74 (0.3)	3.39 (0.5)
FMLP induced superoxide anion release (mmol $\text{O}_2^-/10^6$ PMNs/min)	1.43 (0.4)	1.58 (0.5)	2.3 (1.3)	1.72 (0.9)	1.54 (0.5)	1.64 (0.3)

Values are mean (SD). Bactericidal activity is expressed as exponential decrease in number of colonies. PMA, phorbol 12-myristate 13-acetate; FMLP, N-formylmethionyl-leucyl-phenylalanine; PMN, polymorphonuclear leucocyte.

Table 2 Effect of anaerobic exercise on neutrophil function in judoka and untrained women

	Judoka (n=8)			Untrained (n=7)		
	Before	After	24 h after	Before	After	24 h after
Bactericidal activity- autologous serum	1.08 (0.2)	1.13 (0.3)	1.2 (0.3)	1.07 (0.1)	1.1 (0.2)	1.1 (0.2)
Bactericidal activity- homologous serum	1.08 (0.2)	1.08 (0.2)	1.04 (0.3)	1.0 (0.2)	1.2 (0.4)	1.04 (0.1)
PMA induced superoxide anion release (mmol $\text{O}_2^-/10^6$ PMNs/min)	3.78 (0.5)	3.82 (0.3)	3.23 (0.8)	3.68 (0.4)	3.57 (0.59)	3.46 (0.5)
FMLP induced superoxide anion release (mmol $\text{O}_2^-/10^6$ PMNs/min)	2.06 (0.6)	1.82 (0.6)	2.1 (1.3)	1.41 (0.8)	1.32 (0.8)	1.55 (1.1)

Values are mean (SD). Bactericidal activity is expressed as exponential decrease in number of colonies. PMA, phorbol 12-myristate 13-acetate; FMLP, N-formylmethionyl-leucyl-phenylalanine; PMN, polymorphonuclear leucocytes.

1). At any time measured there were no significant differences in chemotactic activity, bactericidal activity, and superoxide anion release between the judoka and untrained women.

ANAEROBIC EXERCISE

Figure 2 and table 2 summarise the effect of anaerobic exercise on neutrophil function. There were no significant changes in net chemotaxis (fig 2), bactericidal activity (with both autologous and homologous serum), and superoxide anion release (of both PMA and FMLP stimulated neutrophils) after exercise in either the judoka or untrained controls (table 2). There were no significant differences in these variables between groups.

Discussion

This study examined the effect of an aerobic exercise task compared with a supramaximal anaerobic exercise task on neutrophil function in highly trained judoka and untrained women. At 24 hours after the aerobic exercise there was a significant decrease in neutrophil net chemotaxis in both groups, but not in the neutrophil random migration, bactericidal activity, and superoxide anion release. In contrast, there were no significant changes in neutrophil function after the anaerobic exercise, in either the judoka or untrained controls. The decrease in net chemotaxis after aerobic, but not after anaerobic, exercise suggests that net chemotaxis is affected by the combination of exercise duration and intensity, and not only by the exercise intensity. However, further combinations of exercise intensity and duration need to be examined. In contrast with our hypothesis, similar effects of both exercise sessions in the judoka and untrained women indicate that training had no effect on neutrophil function response to aerobic and anaerobic exercises.

It should be noted that the aerobic exercise was aimed to be an intensity eliciting 70–80% of the age predicted maximal heart rate. In view of the fact that some of the untrained subjects were unable to run for the full 20 minutes and continued at a fast walk, it is possible that the anaerobic component in this task was higher among this group than in the judokas. Nevertheless, the neutrophil function response after the 20 minute run/walk was similar in both groups.

The decrease in chemotactic activity 24 hours after exercise, which occurred in all the study participants, is consistent with our previous observation of decreased chemotactic activity 24 hours after aerobic exercise in prepubertal female gymnasts.⁸ In contrast, several investigations^{20–22} reported increased chemotactic activity after moderate cycling and treadmill running. Rodriguez *et al*²³ observed no change in chemotaxis among untrained subjects after a short period of running at a similar intensity to that used in this study. This discrepancy suggests that the intensity and duration of exercise may influence the exercise associated chemotactic response. Moreover, the subjects studied in two of the reports^{20 21}

were patients with exercise induced asthma. Therefore the effect of other inflammatory mediators on chemotaxis cannot be ruled out.

The mechanism for the reduced chemotactic activity after exercise is not clearly understood but may involve exercise induced intrinsic changes in the cell itself or may be related to humoral factors which influence chemotaxis. As the circulation half life of PMNs is about seven hours, the reduced chemotaxis found 24 hours after the exercise probably reflects an effect on bone marrow PMNs, presumably of humoral origin. Muns *et al*²⁴ showed increased neutrophil chemotactic activity of PMNs obtained from nasal lavage of amateur runners immediately after a marathon race. This increased local chemotactic activity may reflect a local immune adaptation to the increased exposure to respiratory pathogens during a long competitive race. Whether this increase in local chemotactic activity contributes to the decrease in systemic chemotactic activity shown in the present study is still speculative.

We did not assess chemotactic activity more than 24 hours after exercise and therefore do not know when it recovers or returns to values found before exercise. However, the normal resting values before the beginning of the physical effort, and the fact that there were no differences in values found before exercise between the groups, suggests complete recovery with time and indicates only transient changes after exercise. The clinical implications of the transient decrease in net chemotaxis after aerobic exercise are still not clear. However, the possibility that this transient decrease in net chemotaxis reflects an “open window” in which the host defence mechanism is decreased and the risk of infection is increased^{25 26} has to be considered.

Bactericidal activity and superoxide anion release were not significantly affected by aerobic or anaerobic exercise in the present study. This is consistent with reports of Benoni *et al*²⁷ who showed no effect of 10 minutes of cycle ergometry on cellular adhesion, bactericidal activity, and superoxide anion production in active and inactive subjects. We previously reported, however, a decrease in PMA induced superoxide anion release after the same aerobic exercise protocol in prepubertal female gymnasts compared with untrained girls.⁸ The apparent inconsistency between the present study and our previous study with the gymnasts may be related, at least partially, to the poor nutritional status of the gymnasts^{11 28} (as mentioned above, the gymnasts had significantly lower body weight and their energy intake was low). In the present study, the untrained girls had a somewhat higher percentage body fat (insignificant) compared with the judoka, and may be considered overweight. While it is known that malnutrition may affect neutrophil function, the possible effect of being overweight is unknown. Nevertheless, in this study, no differences were observed between the two groups. In addition, the differences in the response to aerobic exercise between

prepubertal gymnasts and postpubertal judoka may be related to maturational factors.

In contrast, other studies²⁹⁻³¹ have shown a decrease in bactericidal activity after longer exercise duration—for example, 50 mile walking race²⁹ and 100 km run³⁰—, after graded treadmill exercise to exhaustion,³² and during periods of intense training.³³ This suggests that extensive aerobic exercise and/or intensive training can result in alterations in immune function. The inconsistency in results may reflect differences in exercise intensity, fitness level, or training status of the subjects, as well as differences in gender and nutritional status. Interestingly, the previously reported changes in bactericidal activity after extensive aerobic exercise and/or intensive training correlated with elevated levels of cortisol and ketone bodies²⁹ and with levels of adrenaline (epinephrine).³³ These variables were not assessed in this study.

There were no significant differences in neutrophil function between the judoka and untrained women before the exercise sessions, indicating that intensive training that emphasizes mainly anaerobic exercise does not chronically affect neutrophil function. Several studies, however, showed decreased bactericidal activity among elite prepubertal female gymnasts⁸ and trained adult cyclists³⁴⁻³⁵ compared with untrained controls. Smith *et al.*³⁶ suggested that partial suppression of neutrophil activity in athletes serves to reduce the inflammatory response to low level tissue damage which may occur during regular training. In addition, the reduced bactericidal activity among elite athletes may reflect reduced immunity resulting from intense physiological and psychological stress. In contrast, Benoni *et al.*³⁷ reported increased bactericidal activity and superoxide anion release in professional basketball players during the season. The mechanism of these differences is not clearly understood, but may be attributable to differences in age, maturation, gender, nutritional factors, different training methods—for example, aerobic *v* anaerobic—the initial fitness level of the subjects studied, as well as different methodological procedures.

In summary, in this study aerobic exercise induced a chemotactic activity in young adult trained and untrained women. This observation supports the concept that exercise may adversely affect the immune system.³⁸ Although some studies—for example, that of Pyne *et al.*³⁹—failed to find an association between reduced neutrophil function and increased incidence of upper respiratory tract infection during training, the interrelation between exercise related immune deficits and the development of infection should be further evaluated. In addition, the practical value of this knowledge for monitoring the health and training programmes of elite athletes requires further clarification.

Contributors: B W participated in formulating the hypothesis and design of the study, interpretation of the results, and writing of the paper. B F participated in formulating the design of the study and data collection, performed the statistical analysis, and participated in writing the paper. R G performed data collection and analysis of the neutrophil function. E K participated in formulating the study design, recruiting subjects, and execution of

the study. A E initiated and coordinated the study hypothesis, participated in designing the study, collecting the data, interpreting the results, and writing the paper.

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Take home message

Aerobic exercise decreases net chemotactic activity in young trained and untrained women. This supports the concept that exercise may adversely affect the immune system, and awareness of this should be increased among sports physicians.

Fighting spirit

Fencing is addictive, probably more so than many sports. I started at the age of 10, with bamboo sticks at school in Singapore. At 14, in my first competition, the British Junior Schoolgirls, I reached the semifinal but won the style prize—a cup that outclassed the winner's trophy. That was it, and for the next 13 years I trained hard: technical lessons from my coach, fighting practice at the club, footwork sessions, circuit training for strength, running for stamina and the competition circuit for tactical experience, and those oh so satisfying victories, with a few medals thrown in along the way plus a great social life.

I enjoy the fights: permission to be aggressive in a controlled and safe way against all sorts of male and female opponents. I love the unique combination of physical skill, mental agility, fitness, and competitive toughness that produces winners, and the excitement of defeating a difficult adversary through speed and guile. Individual lessons, with their infinite variety of moves involving hand, arm, feet, body, and brain, demand concentration and hard work from both the fencer and the coach. This special relationship is vital to success in a way that is unique to fencing.

Now, having enjoyed my first international career while a student, first of biochemistry then of medicine, I am competing again with the British Veterans Team, so it's back to the running and the gym. My stamina and reactions gratifyingly improve as I train, and beating the younger fencers annoys them no end.

This time around, although the fencing is serious, the events are sociable, without the emotional extremes and stress of Olympic sport. It's becoming addictive again!

CLARE HALSTED
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