ORIGINAL ARTICLE

Effects of rugby sevens matches on human neutrophil-related non-specific immunity

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Aims: To evaluate the influences of the accumulative effect of two consecutive rugby sevens matches (Sevens) on aspects of human neutrophil-related non-specific immunity.
Methods: In seven players participating in the Japan Sevens, neutrophil reactive oxygen species (ROS) production capability and phagocytic activity were measured using flow cytometry, and serum opsonic activity (SOA) was assessed by measuring neutrophil ROS using the peak height of lucigenin-dependent chemiluminescence before and after two consecutive matches.
Results: ROS showed no change immediately after the first match, and had significantly ($P<0.05$) increased 4 h later, but showed a decrease after the second match. Phagocytic activity showed no change immediately after the first match, but had significantly ($P<0.01$) decreased 4 h later, and showed a further decrease after the second match, although it was not significant. SOA significantly ($P<0.01$) increased after the first match, and still maintained its high 4 h later, but decreased after the second match. ROS production capability, phagocytic activity and SOA significantly ($P<0.01$) decreased after the second match.
Conclusions: When rugby players play two consecutive Sevens matches, the exercise loading is thought to be hard, similar to that experienced during a marathon race and intensive or long training in a training camp, although the expected changes were not seen after the first match. Differences between after the first and the second matches may be due to the ''cumulative effect''.

Rugby is a competitive ball game with a long history, which usually has 15 players per team. The rugby sevens match (Sevens), played with seven players, was recently derived from the original game of rugby, with its own World Cup, and many competitions are held in and outside Japan.

Rugby is one of the most intense contact sports among competitive sports, and requires a high degree of physical fitness. The incidence of injuries during rugby matches is higher compared with other sports.[1] The basic rules of Sevens, including the size of the pitch, are the same as for an ordinary rugby match, except for a shorter match duration. As Sevens players must play on a full-sized pitch, it follows that they have a potentially higher exercise loading than under the conditions of a normal game. Usually, more than two games are held on the same day. It can thus be assumed that Sevens players experience high levels of physiological stress, and the incidence of injury will probably be higher than in the case of a 15-a-side game. However, to the best of our knowledge, no study on sports medicine concentrating on Sevens players has ever been carried out.

Some reports have shown that intense exercise can adversely affect the immune system. The incidence of upper respiratory tract infection among endurance athletes is notably high, and may be due to decreased neutrophil function.[2 3] In addition, decreases in neutrophil functions have been reported after a rugby match.[4]

Neutrophils are one of the cellular factors playing an important part in the first line of defence against foreign substances, including microorganisms. Neutrophils engulf microorganisms (phagocytic activity) and produce reactive oxygen species (ROS).[5 6] Serum opsonic activity (SOA) contributes to this microbicidal activity through opsonisation of microorganisms—that is, an acceleration of adhesion of neutrophils to opsonised substances via immunoglobulin (Ig) G, C3 and others. The expression of CD11b (complement receptor type 3; CR3) and CD16 (Fc γ receptor type 3; FcγR3) on the surface of neutrophils facilitates efficient phagocytosis of opsonised foreign bodies and consequent production of ROS.[7 8]

A single bout of exercise has been reported to change the neutrophil functions. Depending on the report one reads, ROS production increases[9 10] or decreases after acute exercise.[11–13] Phagocytic activity decreases after intense exercise[9 12 14] or increases or does not change after moderate exercise.[15–17] SOA does not change or increase after a long-distance race.[18 19] As changes in these functions are linked to the intensity and duration of exercise, measurements of these functions become interesting when the immune response to repeated bouts of exercise is assessed. The influence of repeated bouts of intense exercise on the same day, such as Sevens matches, on immune function has not been investigated. In addition, as recovery of neutrophil function needs >2 days,[4] repeated bouts of intense exercise with incomplete immunological recovery might increase the risk of infection.

In this study, we examined the influence of two consecutive Sevens games on neutrophil function (ROS and phagocytic activity) and neutrophil-related activity (SOA).

SUBJECTS AND METHODS

Study subjects and study protocol

The subjects were seven players of the Japan Sevens squad, with an average age (standard deviation (SD)) of 20.7 (1.3) years. The body weight and height of the subjects were 86.4 (8.4) kg and 179.3 (8.2) cm, respectively. This team played two games on the same day, and all subjects participated in both games. The interval between the two games was about 4 h. Peripheral blood samples were obtained immediately before the first match (pre-first), immediately after the end of

Abbreviations: ALT, alanine aminotransferase; AST, asparate aminotransferase; FITC, flouorescein isothiocyanate; LDH, lactate dehydrogenase; ROS, reactive oxygen species; SOA, serum opsonic activity

Table 1 Changes in the levels of serum enzymes

	Concentration (IU/l)			
Enzyme	Pre-first	Post-first	Pre-second	Post-second
CK	359.3 (130.5)	400.3 (147.8)	423.9 (139.9) †	508.8 (183.7) ††*
AST	23.0 (5.3)	26.1 (6.9)	28.0 (8.1) ††	28.1 (6.8)
ALT	19.1 (8.1)	19.7 (8.3)	21.1 (9.2)	20.4 (8.1)
LDH	199.1 (18.7)	224.5 (33.5) *	239.1 (30.5) ††	249.1 (35.0) ††
Lactic acid	11.2 (2.9)	71.7 (22.9) *	77.4 (27.8) ††	61.7 (26.5)

ALT, alanine aminotransferase; AST, asparate aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase. Values are mean (SD), n = 7.
*p<0.01, a significant difference compared with each pre-value. †p<0.05, ††p<0.01, a significant difference compared with the pre-first value.

the first match (post-first), immediately before the second match (pre-second) and immediately after the end of the second match (post-second).

This study was conducted after obtaining the approval of the Ethics Committee, Hirosaki University School of Medicine, Hirosaki, Aomori, Japan. In addition, before the study began, the objectives and requirements of the study were explained to all the subjects and written informed consent was obtained from them.

Sevens games

The 11th Japan Sevens took place in April 2003. The weather was cloudy, with intermittent rain. Mean (SD) ambient temperature and relative humidity were 16.5 (0.4)℃, and 66 (2.2)%, respectively. The mean (SD) participation times in each match were 10.3 (4.2) and 10.0 (4.3) min, respectively. Players were occasionally allowed to take drinks during the two matches and in the interval between them. The team won the first game against the Hosei University rugby team 19 to 17, but lost the second game against the Kobe Steel rugby club 17 to 26.

Blood biochemistry

Blood samples were taken from the forearm vein. Total leucocyte, neutrophil, immunoglobulin and complement counts were measured. The levels of asparate aminotransferase (AST), alanine aminotransferase (ALT), lactic acid, lactate dehydrogenase (LDH) and creatine kinase in serum were also measured.

We adjusted dehydration for the post-values with the plasma volume method using haematocrit and haemoglobin.[20]

Preparation for neutrophil oxidative burst and phagocytic activity

Hydroethidine (44.4 μmol/l; Polyscience, Warrington, Pennsylvania, USA) was used as an indicator for oxidative burst (ROS production), and opsonised zymosan particles (Sigma Chemical, St Louis, Missouri, USA) labelled with fluorescein isothiocyanate (FITC; Sigma) were used as an indicator for phagocytic activity.

A 100-μl whole-blood sample was mixed with 22 μl hydroethidine (8 μmol/l) and incubated. After the addition of 25 μl FITC-labelled opsonised zymosan (5 mg/ml), the sample was incubated. The same amount of whole blood labelled with only hydroethidine was prepared to measure the basal oxidative burst activity. Extracellular fluorescence was quenched by adding 30 μl trypan blue (0.25 mg/ml, pH 4.5) just before the assay to differentiate between attached and ingested FITC-labelled opsonised zymosan in the neutrophils.[21 22]

Preparation for CD11b and CD16 expression on the neutrophil surface

Monoclonal antibodies to CD11b and CD16 (Immunotech, Fullerton, California, USA) were used to measure the expression on neutrophils.

A 100-μl sample of heparinised whole blood was mixed with the monoclonal antibodies and incubated.

Flow cytometry

Neutrophils were analysed on FACScan (Becton Dickinson, San Jose, California, USA). ROS production, phagocytic activity and expression of CD11b and CD16 were estimated as the mean fluorescence intensity channel number of activated neutrophils. The percentages of activated neutrophils were calculated. The cumulative fluorescence intensity, the sum of the values of fluorescence intensity multiplied by the percentage of positive cells, was used as a quantitative index.

Measurement of SOA

The chemiluminigenic probe, lucigenin, was prepared by dissolving bis-N-methylacridinium nitrate (Sigma) in Hank's balanced salt solution to give a final concentration of 0.5 mmol/l (pH 7.4). Lucigenin mainly reacts with superoxide (O_2^-), which is the first substance in the metabolism of ROS.

Measurement of chemiluminescence

A suspension (5 mg/ml) of zymosan was opsonised by each serum sample at the final concentration of 20%. Each opsonised zymozan sample and lucigenin and 50 μl of the standard neutrophils obtained from a healthy volunteer were added and measured on the Auto Luminescence Analyzer, Alfa System (Tokken, Funabashi, Japan).[23] Peak heights of the chemiluminescence response were used in this study.[24 25]

Table 2 Changes in blood leucocyte counts

	Cell counts (10^3/μl)			
	Pre-first	Post-first	Pre-second	Post-second
Total leucocytes	5.7 (1.2)	8.78 (2.41)**	9.4 (2.98) ††	9.53 (2.59)
Neutrophils	2.85 (0.8)	3.84 (1.39)	4.4 (1.48)†	4.62 (1.29)

Values are mean (SD), n = 7.
**p<0.01, a significant difference compared with each pre-value.
† p<0.05, †† p<0.01, a significant difference compared with the pre-first value.

Table 3 Changes in the levels of immunoglobulins and complement components

	Concentration (mg/dl)			
	Pre-first	Post-first	Pre-second	Post-second
IgG	1257 (173)	1225 (171)	1308 (182)	1242 (149.4)
IgA	214.4 (46)	208 (42.5)	222.9 (49.9)	208.7 (38.9)*
IgM	119.9 (23)	114.6 (22.9)*	121.7 (21.4)	115.2 (25.5)**
C3	102 (15)	99.1 (15.5)	106.4 (21.1)	98.2 (15.2)**
C4	21.9 (5.2)	20.7 (5.2)	22.3 (6.6)	20.8 (4.9)*

Ig, immunoglobulin.
Values are mean (SD), n = 7.
*p<0.05, **p<0.01, a significant difference compared with each pre-value.

Statistical analysis

All data are presented as mean (SD), and analysed by one-way analysis of variance followed by Tukey's retrospective tests. Relationships between the rates of change in neutrophil oxidative burst activity, phagocytic activity and SOA were investigated by Spearman's correlation coefficient analysis. The differences were considered to be significant at $p<0.05$.

RESULTS

Serum parameters

The post-first values of creatine kinase and AST showed no change but had significantly increased 4 h later at pre-second ($p<0.05$; table 1). In creatine kinase, a further increase was seen at post-second ($p<0.01$). LDH increased significantly at post-first, and showed significantly higher values at pre-second and post-second compared with pre-first and post-first ($p<0.01$, table 1).

The post-first levels of lactic acid showed a significant increase ($p<0.01$), which was maintained at post-second.

Leucocyte and neutrophil counts (table 2)

Leucocyte counts significantly increased ($p<0.01$) at post-first and showed a tendency towards further increase at post-second. Post-first neutrophil counts showed a tendency to increase, with a significant increase ($p<0.05$) 4 h later at pre-second, and a further but not significant increase at post-second. Further, a positive correlation was found between the rate of change of the neutrophil and leucocyte counts in the first match 4 h later at pre-second ($r = 0.89$, $p = 0.007$; $r = 0.92$, $p = 0.003$).

Immunoglobulins and complements

Post-first IgM values decreased ($p<0.05$; table 3) significantly, with a tendency to decrease seen in IgG and IgA. At post-second, IgA and IgM values decreased significantly ($p<0.05$), with a tendency to decrease seen in IgG. However, there were no significant differences among these factors between their pre-first and pre-second values.

C3 and C4 showed no change at post-first, but had significantly decreased ($p<0.05$) at post-second. However, there were no significant differences between the pre-first and pre-second values.

ROS production

The proportion of neutrophils producing ROS significantly increased ($p<0.01$; table 4) at post-first, but showed no significant change at post-second. The amount of ROS produced and total oxidative burst activity per cell showed no significant change at post-first but both significantly decreased ($p<0.01$ for both) at post-second. Further, the values for the proportion of ROS-producing cells, the amount of ROS produced and the total oxidative burst activity were significantly higher ($p<0.05$ for all) at pre-second than at pre-first.

Phagocytic activity

The proportion of neutrophils incorporating opsonised zymozan significantly increased ($p<0.05$; table 5) at post-first, but showed no significant change at post-second. The amount of ingested opsonised zymozan per cell showed a tendency to decrease after both matches. The total phagocytic activity per cell also showed no significant change at post-second. Further, the amount of ingested opsonised zymozan and total amount of phagocytic activity at pre-second and post-second were significantly lower ($p<0.05$) than at pre-first and post-first.

CD11b and CD16 expression on neutrophils

We found no significant change in CD11b and CD16 expression after both matches (table 6). On the other hand, at pre-second (4 h after the end of the first match), the proportion of neutrophils expressing CD16 was higher than at pre-first ($p<0.01$). Thereafter, there was no significant change in total CD16 expression.

Table 4 Changes in neutrophil oxidative burst activity

	Pre-first	Post-first	Pre-second	Post-second
Oxidative burst proportion (%)	80.2 (5.3)	87.8 (5.1)**	90.8 (2.2)††	89.3 (4.8)
Oxidative burst activity per activated cell (FI)	282 (24)	260 (16.8)	318 (21.0)†	256 (38.2)**
Total oxidative burst activity per cell (CFI)	225.0 (27)	228 (14.6)	289 (17.6)†	228.0 (33.9)**

CFI, the value of FI multiplied by the percentage value of the oxidative burst proportion; FI, the mean channel number of fluorescence intensity of activated neutrophils.
Values are mean (SD), n = 7.
**p<0.01, a significant difference compared with each pre-value.
†p<0.05, ††p<0.01, a significant difference compared with the pre-first value.
Oxidative burst proportion: the proportion of neutrophils producing reactive oxygen species.

Table 5 Changes in neutrophil phagocytic activity

	Pre-first	Post-first	Pre-second	Post-second
Phagocytic proportion (%)	93.6 (1.2)	96 (1.3)**	95.5 (0.9)†	95 (1.8)
Phagocytic activity per activated cell (FI)	564 (99)	553 (88)	479 (62.5)†	435 (61.1)††
Total phagocytic activity per cell (CFI)	527 (87)	530 (79.3)	457 (55.0)†	413 (57.6)††

CFI, the value of FI multiplied by the percentage value of the phagocytic proportion; FI, the mean channel number of fluorescence intensity of activated neutrophils.
Values are mean (SD), n = 7.
**p<0.01, a significant difference compared with each pre-value.
†p<0.05, ††p<0.01, a significant difference compared with the pre-first value.
Phagocytic proportion: the proportion of neutrophils incorporating opsonised zymozan.

Serum opsonic activity

Table 7 shows the changes in the peak height of the luminescence. The peak height significantly increased ($p<0.01$ for both) at post-first, maintained these high levels 4 h later at pre-second, but significantly decreased ($p<0.05$ for both) at post-second.

Correlation among the major neutrophil function factors

Negative correlations were seen between ROS production and IgA at pre-second ($r = -0.85$, $p<0.05$; table 8), and positive correlations were seen between phagocytic activity and IgM in the second match. No significant correlations were seen between in any other pairs of variables.

DISCUSSION

Intense exercise liberates serum myogenic enzymes such as creatine kinase, AST, ALT and LDH into the blood owing to muscle inflammation and collapse of skeletal muscle or increase in the permeability of muscle cell membranes.[26 27] In this study, the values of creatine kinase, AST and LDH increased accumulatively from pre-first to post-second (table 1). On the other hand, lactic acid is well known as a carbohydrate metabolite associated with anaerobic exercise, and lactic acidosis leads to skeletal muscle fatigue.[28 29] In this study, lactic acid significantly increased at post-first, and high levels were still maintained at post-second compared with pre-first. Therefore, the exercise loading in this study was intense enough to induce muscle fatigue/collapse.

Experimental studies have shown an increase in the circulating neutrophil count after exercise, with the magnitude of the neutrophilia reflecting the intensity and duration of the workload.[30] In this study, neutrophil counts showed a tendency to increase after the first match, and had significantly increased 4 h later. However, total neutrophil count showed no significant change after the second match. This result might suggest that the first match had induced mobilisation of neutrophils from endothelial tissues and bone marrow before the second match. In addition, neutrophils might have been consumed by the inflammatory response, as a result of which neutrophil counts showed no significant change after the second match instead of an increase as a reaction to exercise. This result suggested that the repetition of intense exercise diminishes the neutrophil inflammatory reaction, and the recovery from physical damage may be delayed.

In this study, ROS production capability showed no significant change after the first match, but by 4 h after the end of that match it had increased significantly. On the other hand, it decreased after the second match. This finding, together with data from our previous studies, shows that ROS production capability is increased by most types of exercise loading,[9 10] but is decreased only after strenuous endurance exercise loading such as a marathon.[11–13] From these considerations, in this study, the exercise loading in the first match is similar to the first condition—that is, normal exercise loading—and in the second match it is similar to that induced by strenuous endurance loading. However, the absolute exercise loading may be similar in the first and second matches. The difference in the change in ROS between the first and second matches may be due to the different physical characteristics of the subjects, such as fatigue. In other words, the exercise loading in the first match was carried over to the second match. Therefore, the exercise loading of the second match was greater than that of the first match. This increasing effect could be called a ''cumulative effect''. As the exercise loading of a short term, such as a Sevens match, repeats itself, an effect similar to strenuous endurance exercise was seen in the neutrophil ROS production capability. The decreased ROS production after the second match seemed detrimental to the

Table 6 Changes in CD11b and CD16 expression on neutrophils

	Pre-first	Post-first	Pre-second	Post-second
CD11b				
Proportion (%)	94.8 (2.1)	96.3 (1.4)	96.2 (1.6)	95.4 (2.3)
Per positive cell (FI)	103 (11)	98.3 (10.2)	104 (14.8)	96.6 (14.1)
Per cell (CFI)	97.3 (11)	94.6 (9.1)	99.7 (13.4)	92.1 (12.2)
CD16				
Proportion (%)	90.3 (3.1)	92.3 (2.6)	94.9 (2.0)††	94.6 (2)
Per positive cell (FI)	962 (276)	974 (308)	866 (320)	933 (247.2)
Per cell (CFI)	866 (242)	897 (274)	819 (290)	880 (218)

CFI, the value of FI multiplied by the percentage proportion value; FI, the mean fluorescence intensity of CD11b or CD16 per cell.
Values are mean (SD), n = 7.
††p<0.01, a significant difference compared with the pre-first value.
CD11b, CD16 proportion: percentage of neutrophils expressing CD11b or CD16.

Table 7 Changes in peak height of lucigenin-dependent chemiluminescence response (serum opsonic activity)

	Pre-first	Post-first	Pre-second	Post-second
PH ($\times 10^4$)	94.6 (2.2)	106 (3.9)**	107 (4.8)††	98 (3.8)††**

PH, peak height is the maximum intensity of the chemiluminescence response of isolated neutrophils after stimulation with opsonised zymosan.
Values are mean (SD), n = 7.
**p<0.01, a significant difference compared with each pre-value.
††p<0.01, a significant difference compared with the pre-first value.

immune status and therefore the health of the subjects. As top athletes, such as the subjects in this study, usually participate in several matches in 1 day, any detrimental physiological disturbance induced as a result of heavy training—for example, accumulated fatigue and depressed ROS—should be removed in as short a time as possible.

The differences in the changes in phagocytic activity and SOA between the first and second matches in this study may also point to a cumulative effect. For example, although phagocytic activity showed no significant change after the first match, it decreased significantly after the second match. On the other hand, SOA significantly increased after the first match, showed no change until 4 h after the end of the first match and then decreased after the second match.

Our previous studies suggested that neutrophil ROS production capability and phagocytic activity, and SOA compensate for each other to maintain the overall integrity of the neutrophil immune function—that is, contributing to immune homoeostasis.[31] For example, in general, the increases in ROS and SOA compensate for decreased phagocytic activity under conditions of normal exercise loading.[9 10 19] The fact that the three factors significantly decreased together immediately after the second match suggests absolute depression of neutrophil functions in the subjects in this study. Thus, repeated exercise loading

Box 1: What is already known on this topic

The influence of repeated bouts of intense exercise on the same day on immune function has not been investigated.

Box 2: What this study adds

Repeated bouts of intense exercise with incomplete immunological recovery may increase risk of infection by the cumulative effect.

Table 8 Correlation among main variables

	ROS production per cell	PA per cell	PH of SOA
Changes 4 h later from the first match			
CK	−0.07	−0.71	0.29
AST	−0.33	−0.38	0.22
ALT	0.16	−0.51	0.12
LDH	0.18	−0.68	−0.11
LA	−0.39	−0.07	0
IgG	−0.57	0.04	0.25
IgA	−0.85*	0.09	0.54
IgM	−0.49	−0.09	0.18
C3	−0.56	0.02	0.23
C4	−0.49	−0.09	0.18
CD11b expression per cell	0.68	0	−0.18
CD16 expression per cell	0.5	0.07	−0.18
ROS production per cell	1.00	−0.39	−0.25
PA per cell	−0.39	1.00	−0.18
PH of SOA	−0.25	−0.18	1.00
Changes in the second match			
CK	0.57	0.14	−0.36
AST	0.36	0.57	0.57
ALT	0.21	0.32	0.68
LDH	0.39	0.46	0.54
LA	0.07	0.07	−0.43
IgG	0.75	0.61	0.29
IgA	0.36	0.5	0.57
IgM	0.68	0.82*	0.43
C3	0.57	0.71	0.21
C4	0.68	0.54	0.11
CD11b expression per cell	−0.57	−0.04	0.39
CD16 expression per cell	−0.71	−0.54	0
ROS production per cell	1.00	0.54	−0.11
PA per cell	0.54	1.00	0.43
PH of SOA	−0.11	0.43	1.00

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; Ig, immunoglobulin; LA, lactic acid; LDH, lactate dehydrogenase; PA, phagocytic activity; PH, peak height; ROS, reactive oxygen species; SOA, serum opsonic activity.
*p<0.05, significant correlation.

without complete recovery may cause depression of the athlete's immune function.

On the other hand, the rates of change in immunoglobulins and complement, which affect SOA, had no correlation with SOA rates of change. The rates of change in the neutrophil CD receptors, through which phagocytic activity is efficiently executed, had no correlation with those seen in the levels of phagocytic activity. Factors other than immunoglobulins and complement or CD receptors may therefore be involved in the levels of SOA and phagocytic activity. On the subject of correlation between IgA and ROS production per cell, Mashiko *et al*[32] reported that muscle injury due to intensive exercise had triggered the immune response of immunoglobulin and complement in serum, and induced the inflammatory reaction. In addition, neutrophil ROS production is thought to play a part in clearance of phagocyte-damaged host tissue, such as muscle tissue, by exercise.[33] Therefore, changes in immunoglobulin may influence neutrophil ROS production at the site of inflammation. However, the reason for the significant correlation between only IgA and ROS production, although IgG and IgM showed a similar change to IgA, remains unclear.

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REFERENCES

1 **Sparks JP**. Half a million hours of rugby football. The injuries. *Br J Sports Med* 1981;**15**:30–2.
2 **Mackinnon LT**. Chronic exercise training effects on immune function. *Med Sci Sports Exerc* 2000;**32**:S369–76.
3 **Nieman DC**. Current perspective on exercise immunology. *Curr Sports Med Rep* 2003;**2**:239–42.
4 **Suzuki M**, Umeda T, Nakaji S, *et al*. Effect of incorporating low intensity exercise into the recovery period after a rugby match. *Br J Sports Med* 2004;**38**:436–40.
5 **Benestad HB**, Laerum OD. The neutrophilic granulocyte. *Curr Top Pathol* 1989;**79**:7–36.
6 **Sawyer DW**, Donowitz GR, Mandell GL. Polymorphonuclear neutrophils: an effective antimicrobial force. *Rev Infect Dis* 1989;**11**:S1532–44.
7 **Ehlenberger AG**, Nussenzweig V. The role of membrane receptors for C3b and C3d in phagocytosis. *J Exp Med* 1977;**145**:357–71.
8 **Unkeless JC**, Wright SD. Structure and modulation of Fc and complement receptors. *Contemp Top Immunobiol* 1984;**14**:171–87.
9 **Chinda D**, Umeda T, Shimoyama T, *et al*. The acute response of neutrophil function to a bout of judo training. *Luminescence* 2003;**18**:278–82.
10 **Pedersen BK**, Bruunsgaard H. How physical exercise influences the establishment of infections. *Sports Med* 1995;**19**:393–400.
11 **Miyazaki H**, Oh-ishi S, Ookawara T, *et al*. Strenuous endurance training in humans reduces oxidative stress following exhausting exercise. *Eur J Appl Physiol* 2001;**84**:1–6.
12 **Chinda D**, Nakaji S, Umeda T, *et al*. A competitive marathon race decreases neutrophil functions in athletes. *Luminescence* 2003;**18**:324–9.
13 **Robson PJ**, Alston TD, Myburgh KH. Prolonged suppression of the innate immune system in the horse following an 80 km endurance race. *Equine Vet J* 2003;**35**:133–7.
14 **Muns G**. Effect of long-distance running on polymorphonuclear neutrophil phagocytic function of the upper airways. *Int J Sports Med* 1994;**15**:96–9.
15 **Scharhag J**, Meyer T, Gabriel HH, *et al*. Does prolonged cycling of moderate intensity affect immune cell function? *Br J Sports Med* 2005;**39**:171–7.
16 **Gabriel H**, Muller HJ, Kettler K, *et al*. Increased phagocytic capacity of the blood, but decreased phagocytic activity per individual circulating neutrophil after an ultradistance run. *Eur J Appl Physiol Occup Physiol* 1995;**71**:281–4.
17 **Ortega E**, Collazos ME, Maynar M, *et al*. Stimulation of the phagocytic function of neutrophils in sedentary men after acute moderate exercise. *Eur J Appl Physiol Occup Physiol* 1993;**66**:60–4.
18 **Sato H**, Abe T, Kikuchi T, *et al*. Changes in the production of reactive oxygen species from neutrophils following a 100-km marathon. *Nippon Eiseigaku Zasshi* 1996;**51**:612–16.
19 **Saito D**, Nakaji S, Umeda T, *et al*. Effects of long-distance running on serum opsonic activity measured by chemiluminescence. *Luminescence* 2003;**18**:122–4.
20 **Elkinton JR**, Danowski TS, Winkler AW. Hemodynamic changes in salt depletion and in dehydration. *J Clin Invest* 1946;**25**:120–9.
21 **Hed J**, Hallden G, Johansson SG, *et al*. The use of fluorescence quenching in flow cytofluorometry to measure the attachment and ingestion phases in phagocytosis in peripheral blood without prior cell separation. *J Immunol Methods* 1987;**101**:119–25.
22 **Sahlin S**, Hed J, Rundquist I. Differentiation between attached and ingested immune complexes by a fluorescence quenching cytofluorometric assay. *J Immunol Methods* 1983;**60**:115–24.
23 **Kumae T**. A study of the fluorescence measurement using a 96-well microplate by a remodelled parallel luminescent measuring system. *Luminescence* 1999;**14**:375–81.
24 **Allen RC**. Phagocytic leukocyte oxygenation activities and chemiluminescence: a kinetic approach to analysis. *Methods Enzymol* 1986;**133**:449–93.
25 **Hasegawa H**, Suzuki K, Nakaji S, *et al*. Analysis and assessment of the capacity of neutrophils to produce reactive oxygen species in a 96-well microplate format using lucigenin- and luminol-dependent chemiluminescence. *J Immunol Methods* 1997;**210**:1–10.
26 **Flynn MG**, Pizza FX, Boone JB Jr, *et al*. Indices of training stress during competitive running and swimming seasons. *Int J Sports Med* 1994;**15**:21–6.
27 **Koutedakis Y**, Raafat A, Sharp NC, *et al*. Serum enzyme activities in individuals with different levels of physical fitness. *J Sports Med Phys Fitness* 1993;**33**:252–7.
28 **Abdessemed D**, Duche P, Hautier C, *et al*. Effect of recovery duration on muscular power and blood lactate during the bench press exercise. *Int J Sports Med* 1999;**20**:368–73.
29 **Hultman E**, Del Canale S, Sjoholm H. Effect of induced metabolic acidosis on intracellular pH, buffer capacity and contraction force of human skeletal muscle. *Clin Sci (London)* 1985;**69**:505–10.
30 **Pyne DB**. Regulation of neutrophil function during exercise. *Sports Med* 1994;**17**:245–58.
31 **Mochida N**, Umeda T, Yamamoto Y, *et al*. The main neutrophil and neutrophil-related functions may compensate for each other following exercise – a finding from training in university judoists. *Luminescence*. In press.
32 **Mashiko T**, Umeda T, Nakaji S, *et al*. Position related analysis of the appearance of and relationship between post-match physical and mental fatigue in university rugby football players. *Br J Sports Med* 2004;**38**:617–21.
33 **Peake J**, Suzuki K. Neutrophil activation, antioxidant supplements and exercise-induced oxidative stress. *Exerc Immunol Rev* 2004;**10**:129–41.