
Title	Effects of training status and different treadmill exercises on the activity of complement receptor type 1 of erythrocytes
Author(s)	Q. Hu, M. Chia, G. Schmidt and S. Mochhala
Source	<i>Biology of Sport</i> , 25(4), 321-338
Published by	Institute of Sport (Warsaw, Poland)

This document may be used for private study or research purpose only. This document or any part of it may not be duplicated and/or distributed without permission of the copyright owner.

The Singapore Copyright Act applies to the use of this document.

EFFECTS OF TRAINING STATUS AND DIFFERENT TREADMILL EXERCISES ON THE ACTIVITY OF COMPLEMENT RECEPTOR TYPE 1 OF ERYTHROCYTES

Q. Hu¹, M. Chia², G. Schmidt³, S. Moochhala⁴

¹School of Medicine, University of North Carolina at Chapel Hill, USA; ²National Institute of Education, Nanyang Technological University, Singapore; ³Exercise and Movement Science, William Paterson University, New Jersey, USA; ⁴Defence Medical Research Institute, Singapore

Abstract. The aims of this study were to investigate the effects of training status, different intensities, durations and modes of exercises on the activity of complement receptor type 1 (CR1) of erythrocytes. Fifteen sedentary male adults and 15 male adult endurance athletes performed five separate treadmill exercise trials: $\dot{V}O_{2\max}$ test (T1), exercise at 40% $\dot{V}O_{2\max}$ for 30 min (T2), 80% $\dot{V}O_{2\max}$ for 30 min (T3) and for 60 min (T4) and downhill running (-10% gradient) at 60% $\dot{V}O_{2\max}$ for 30 min (T5). Blood samples were taken before exercise, immediately, one h, two h and 24 h after each exercise trial to assay the activity of erythrocyte CR1. The results showed that there was no significant difference between trained and untrained participants in erythrocyte-tumor cell rosette (ETCR) formations at rest ($p>0.05$). ETCR was significantly decreased after five exercise trials ($p<0.05$). Changes in ETCR were more obvious after T1, T3 and T4. ETCR 24 h after T5 was significantly lower than that after uphill running. Greater reductions and slower recoveries in ETCR were found in the untrained group than in the trained group. The results indicated that erythrocyte CR1 activity at rest was not affected by training status, but was significantly inhibited by acute exercise. Exercise at higher intensities and longer durations resulted in a greater suppression in the activity of erythrocyte CR1. The suppression was more marked in the untrained participants than in the trained participants. Downhill running induced a longer delay in recovery in erythrocyte CR1 activity compared to uphill running.

(Biol.Sport 25:321-338, 2008)

Key words: Erythrocyte - CR1 - Immune adherence - Training status - Treadmill exercise

Reprint request to: Dr Michael Chia, Physical Education and Sports Science, National Institute of Education, Nanyang Technological University, 1 Nanyang Walk, Singapore 637616, Singapore. Tel: (65) 6790 3690, E-mail: michael.chia@nie.edu.sg

Introduction

Many studies have demonstrated that erythrocytes have an immune function as well as a respiratory one since Siegel *et al.* put forward a new concept called 'the red-cell immune system' in 1981 [27]. One of the main immune functions of erythrocytes is immune adherence: that is erythrocyte CR1 (complement receptor type 1, CD35) bind to complement-opsonized substrates such as IC (immune complexes or antigen-antibody-complement complexes) [1]. CR1 in human erythrocytes has a high affinity for complement component C3b [1,2,11]. IC, incorporating C3b, binds to CR1 on erythrocytes and is then transported to the liver and/or spleen where IC may be removed and degraded by cells of the macrophage system. Thereafter, erythrocytes returned to the circulation, apparently able to bind further IC [1]. The first study of the relationship between erythrocyte CR1 and physical exercise was reported by Thomsen and his colleagues [33]. They found that there was no difference between elite athletes and untrained males in resting levels of erythrocyte CR1. Moreover, a 60-min bicycle exercise at 75% $\dot{V}O_{2\max}$ did not induce an alteration in the level of erythrocyte CR1 in untrained subjects. Since then, some studies have shown that different chronic and acute exercise programs lead to an increase [18,36], or a decrease, or no change [14,15,30] in immune function of erythrocytes. However, valid data in this area are still notably lacking.

It is well known that eccentric contractions of muscles are more likely than concentric contractions to cause muscle damage, which can burden the immune system by concurrently eliciting a local inflammatory response [4,22]. Camus *et al.* [6] and Smith *et al.* [29] reported that eccentric exercise elicited significant increases in total leukocyte count, plasma concentrations of myeloperoxidase and elastase compared to concentric exercise. However, there are apparently no published studies that examined the effect of eccentric exercise on the immune function of erythrocytes.

The present study was designed to observe the change in the activity of erythrocyte CR1 in trained and untrained participants at rest, immediately after and up to 24 h after different types of treadmill running. Specifically, the study objectives were to (i) examine the effect of training status on the immune function of erythrocytes and the acute erythrocyte immune responses to exercise stimuli according to exercise training status, (ii) explain the effects of different exercise intensities and durations of treadmill running on the activity of erythrocyte CR1 and (iii) determine the effects of uphill and downhill treadmill running on the immune adherence of erythrocytes.

Materials and Methods

Participants: The study was approved by the Human Subjects Ethics Committee of Physical Education and Sports Science, National Institute of Education, Nanyang Technological University in Singapore. All participants gave written informed consent to undertake the study. Fifteen healthy university male students represented the untrained group, while 15 male athletes comprising of long distance runners (n=7), biathletes (n=3) and triathletes (n=5) in Singapore represented the trained group. Participants of the untrained group had not received any sports training and had also not engaged in exercise regularly for at least the previous 24 months. Conversely, participants in the trained group had participated in regular sport training for at least the previous two years (i.e. 18.38 ± 1.15 h/week).

Testing sequence: All participants underwent five exercise tests on the same treadmill. The tests were maximal oxygen uptake ($\dot{V}O_{2\max}$) test (T1), walking at 40% $\dot{V}O_{2\max}$ for 30 min (T2), running at 80% $\dot{V}O_{2\max}$ for 30 min (T3), running at 80% $\dot{V}O_{2\max}$ for 60 min (T4) and downhill running on a -10% gradient at 60% $\dot{V}O_{2\max}$ for 30 min (T5). There was an interval of five to seven days between each exercise session. The room temperature of the laboratory was in the range of 22°-24°C with a relative humidity of 62-65%. The trained participants abstained from all forms of vigorous training two weeks before the exercise tests and throughout the weeks of data collection. And all participants were instructed to refrain from any form of vigorous physical activity for 48 h prior to testing and not to participants in any exercise for 24 h before each exercise test.

Maximal oxygen uptake was determined using a modified Bruce protocol [3], multi-staged progressive $\dot{V}O_{2\max}$ test, on a treadmill (Quinton Series 90, Quinton Instrument Company, USA). The participant began the stage at 4 km/h at a 12% gradient. The treadmill speed and gradient were increased every three min according to the established protocol. The participant continued running on the treadmill to volitional exhaustion. The expired gases of the participant during the test were analyzed by ORCA™ Cardiopulmonary Testing System (Quinton Instrument company, USA), which was calibrated with a gas mixture (Scott Medical Products, USA) before the exercise tests. Fifteen second heart rate average of the participant was measured continuously throughout the test using a heart rate monitor (Polar Accurex Plus™, Polar Electro Oy, Finland). During the test, the criteria of plateau in $\dot{V}O_2$ (failure to increase oxygen uptake by 150 ml/min) with increasing exercise intensity, a respiratory exchange ratio greater than 1.15, and an exercise heart rate of within 10 beats/min of the age-predicted maximum or failure

of heart rate to increase with further increases in exercise intensity were used to ensure that $\dot{V}O_{2\max}$ had been attained [8,9].

Blood sample collection: Venous blood samples were collected before and immediately after every exercise test, and at one h, two h, and 24 h of recovery. The samples were stored at 2-4°C until the analysis for erythrocyte CR1 activity, which was done within six h after the exercise tests.

The activity of erythrocyte CR1 assay: The activity of erythrocyte CR1 was assayed by observing the phenomenon of immune adherence of erythrocytes using a modified erythrocyte-tumor cell (Hela cell) binding test [12,34]. Two milliliters of blood were taken in vacutainers, containing 3.2% sodium citrate (Becton Dickinson Vacutainer Systems, France). The tube was centrifuged at 2000 rpm for 30 min. One hundred microliters of erythrocytes were pipetted into a clean centrifuge tube. The cells were washed three times with Hanks' balanced salt solution and were adjusted to a concentration of 1×10^8 /ml (erythrocyte suspension). Hela cell line (American Type Culture Collection, USA) was used to test the activity of erythrocyte CR1. Hela cells were washed three times with Hanks' balanced salt solution and were adjusted to a concentration of 1×10^6 /ml (tumor cell suspension). One hundred microliters of the tumor cell suspension, 50- μ l of autologous plasma of the participant and 50- μ l of erythrocyte suspension were added into a 5-ml test-tube and mixed gently. The cells were incubated in a water bath at 37°C for 30 min. After the incubation, 100- μ l of 0.9% sodium chloride solution was added and mixed with cells gently. Fifty microliters of 0.25% glutaraldehyde (Sigma-Aldrich, USA) were added and mixed with the cells gently to fix the cells for five min. After the fixation, 150- μ l of the cells was spread on a microscope slide, and cells were stained by Wright's Stain Solution (Sigma-Aldrich, USA). Three or more than three erythrocytes binding to a Hela cell was counted as one rosette. The number of erythrocyte-tumor cell rosette (ETCR) formation in 100 Hela cells (%) was calculated.

Statistical analyses: Participant information and data of the activity of erythrocyte CR1 are presented as means \pm standard errors. SPSS 11.0 statistical software was used for data analyses. Differences in key variables of interest between trained and untrained groups were analyzed by independent-samples t-test. Alterations in the activity of erythrocyte CR1 after the different exercise tests were analyzed using repeated measures analysis of variance. Statistical significance level was set at $p < 0.05$.

Results

Participants' characteristics: Table 1 shows the characteristics of the trained and untrained participants. The age, stature, body mass and resting blood pressure (BP) of the trained group were not significantly different from those of the untrained group ($p>0.05$). However, $\dot{V}O_{2\max}$ and VT in the trained group was significantly higher ($p<0.05$), and the tissue percentage of whole body fat and heart rates at rest (HR_{rest}) were significantly lower ($p<0.05$) in the trained group than those in the untrained group.

Table 1

Characteristics of the participants

	Untrained	Trained
Sample size (n)	15	15
Age (years)	23.0±0.70	24.7±0.82
Stature (cm)	172.7±1.73	173.4±1.84
Body mass (kg)	66.33±2.86	62.90±1.89
Body fat (%)	18.3±1.55	11.0±1.11*
HR_{rest} (beats/min)	78±2.59	59±1.75*
BP_{rest} (mmHg) (Systolic/Diastolic)	113/75±2.31/1.27	112/74±1.84/1.70
$\dot{V}O_{2\max}$ ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	44.15±1.81	64.19±1.29*
VT (% $\dot{V}O_{2\max}$)	54.02±1.48	61.87±2.17*

Values are means±SE; Significant difference between two groups: * $p<0.05$;

Body fat (%): the percentage of whole body fat; HR_{rest} : resting heart rate;

BP_{rest} : resting blood pressure; $\dot{V}O_{2\max}$: maximal oxygen uptake;

VT: ventilatory threshold presented in percentage of $\dot{V}O_{2\max}$.

Changes in activity of erythrocyte CR1 after different treadmill exercises: Table 2 shows that the change in activity of erythrocyte CR1 after five exercise tests in the trained group. ETCR was significantly reduced after T1 and returned to the pre-exercise test value at 24 h after the test. Significantly lower ETCR occurred immediately after and one h after T1 compared to the pre-exercise and 24 h post-test values ($p<0.05$). ETCR at two h after T1 was reduced compared to the pre-exercise value ($p<0.05$), but was significantly higher than the values immediately after and one h after the test ($p<0.05$). After T2, ETCR was significantly decreased

immediately post-test and recovered to the pre-exercise value one h after exercise. ETCR was significantly suppressed immediately, one h and two h after T3 and T4 compared to the pre-exercise values ($p < 0.05$), and returned to the pre-test values 24 h after the tests. After T5, ETCR was significantly reduced immediately after, one h and two h till 24 h after exercise ($p < 0.05$) when compared to the pre-test value.

Table 2

Number of ETCR (%) in trained group (n=15)

Exercise tests	Pre-test	Immediately post-test	1 h post-test	2 h post-test	24 h post-test
$\dot{V}O_{2\max}$ test	40.87 ± 0.94	28.47 $\pm 0.98^a$	30.93 $\pm 1.25^a$	37.13 $\pm 1.66^{a\ b\ c}$	40.07 $\pm 1.12^{b\ c}$
40% $\dot{V}O_{2\max}$ (30min)	38.33 ± 1.16	34.20 $\pm 1.14^{a\ A}$	38.00 $\pm 1.40^A$	39.53 $\pm 1.42^{b\ D\ E}$	41.4 7 ± 1.59^b
80% $\dot{V}O_{2\max}$ (30min)	38.53 ± 1.17	30.47 $\pm 1.37^{a\ B}$	32.13 $\pm 1.46^{a\ B}$	34.53 $\pm 1.64^{a\ b\ B}$	40.53 $\pm 1.20^{b\ c\ d}$
80% $\dot{V}O_{2\max}$ (60min)	40.67 ± 1.19	29.60 $\pm 1.15^{a\ B}$	26.47 $\pm 0.98^{a\ A\ B\ C}$	26.87 $\pm 1.24^{a\ A\ C}$	39.53 $\pm 0.97^{b\ c\ d}$
Downhill running (-10% gradient) at 60% $\dot{V}O_{2\max}$ (30min)	41.27 ± 1.16	32.33 $\pm 1.52^{a\ A}$	30.47 $\pm 1.67^{a\ B\ D}$	31.73 $\pm 1.50^{a\ A\ D}$	37.07 $\pm 0.98^{a\ b\ c\ d\ B\ C}$

Values are means \pm SE;

ETCR: erythrocyte-tumor cell rosette formation in 100 tumor cells

Significantly different from the values pre-test: ^a $p < 0.05$;

Significantly different from the values immediately post-test: ^b $p < 0.05$;

Significantly different from the values at one h post-test: ^c $p < 0.05$;

Significantly different from the values at two h post-test: ^d $p < 0.05$;

Significantly different from the values at 24 h post-test: ^e $p < 0.05$;

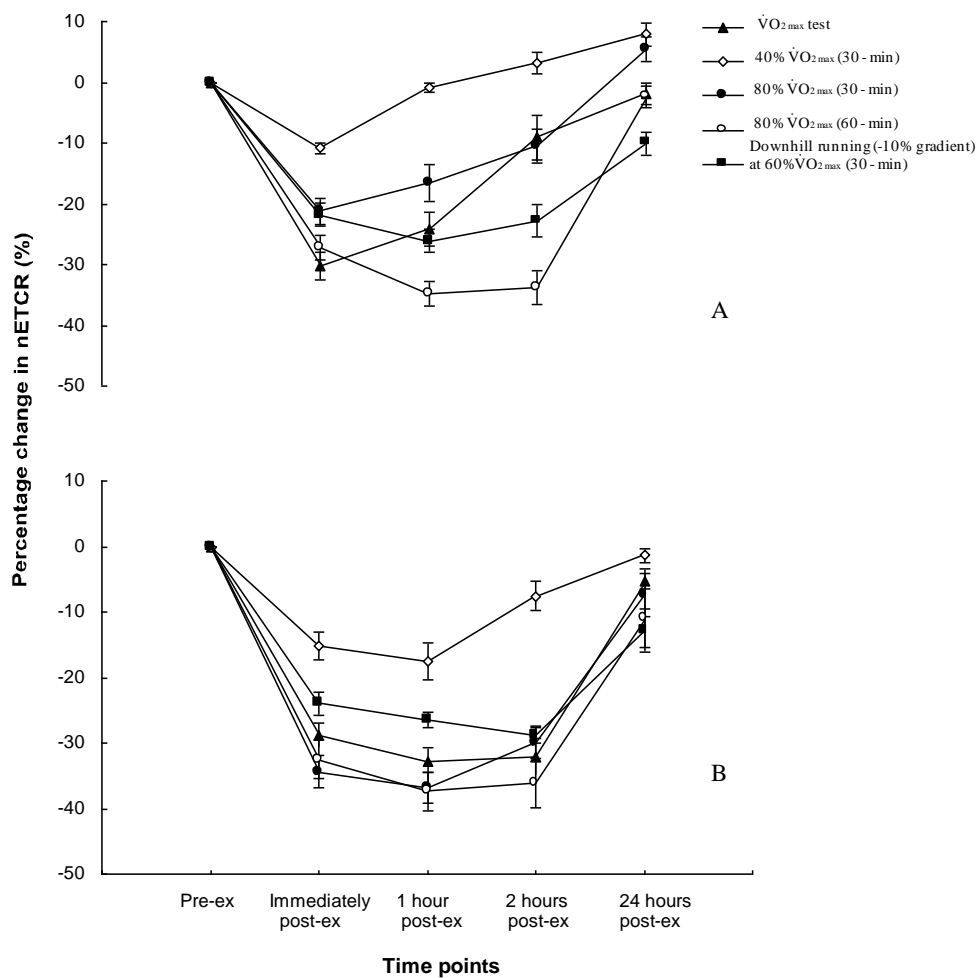
Significantly different from the values for $\dot{V}O_{2\max}$ test: ^A $p < 0.05$;

Significantly different from the values for 40% $\dot{V}O_{2\max}$ test: ^B $p < 0.05$;

Significantly different from the values for 80% $\dot{V}O_{2\max}$ (30-min) test: ^C $p < 0.05$;

Significantly different from the values for 80% $\dot{V}O_{2\max}$ (60-min) test: ^D $p < 0.05$;

Significantly different from the values for downhill running (-10% gradient) at 60% $\dot{V}O_{2\max}$ (30-min) test: ^E $p < 0.05$

**Fig. 1**

Percentage changes (compared to pre-exercise values) in nETCR after the different exercise tests in trained (n=15, A) and untrained groups (n=15, B)

Based on the data of Table 2 and Table 3; Values are means \pm SE;

nETCR: the number of erythrocyte-tumor cell rosette formation in 100 tumor cells;

Ex: exercise test

Table 3

Number of ETCR (%) in untrained group (n=15)

Exercise tests	Pre-test	Immediately post-test	1 h post-test	2 h post-test	24 h post-test
$\dot{V}O_{2\max}$ test	38.53 ±0.77	27.36 ±0.80 ^a	25.87 ±1.04 ^a	26.20 ±1.24 ^a	36.60 ±1.19 ^{bcd}
40% $\dot{V}O_{2\max}$ (30min)	40.27 ±0.71	34.13 ±0.88 ^{aA}	33.13 ±1.19 ^{aA}	37.20 ±1.06 ^{abcA}	39.73 ±0.83 ^{bc}
80% $\dot{V}O_{2\max}$ (30min)	40.07 ±0.68	26.33 ±1.15 ^{aB}	25.27 ±0.90 ^{aB}	27.87 ±0.94 ^{aB}	37.07 ±1.42 ^{abcd}
80% $\dot{V}O_{2\max}$ (60min)	38.33 ±0.73	25.93 ±1.25 ^{aB}	24.07 ±1.24 ^{aB}	24.60 ±1.63 ^{aBC}	33.93 ±1.50 ^{abcdB}
Downhill running (-10% gradient) at 60% $\dot{V}O_{2\max}$ (30min)	38.67 ±0.75	29.47 ±0.98 ^{aBCD}	28.47 ±0.82 ^{aBCD}	27.47 ±0.58 ^{aB}	33.93 ±1.12 ^{abcdB}

Values are means±SE; ETCR: erythrocyte-tumor cell rosette formation in 100 tumor cells; Caption: see Table 2

At rest, there was no significant difference between the different tests in ETCR ($p>0.05$) in trained participants. However, there were different changes in ETCR after the exercise tests (Table 2, Fig. 1A). Immediately after exercise, the greatest alteration in ETCR was found after T1 compared to the percentage changes in ETCR from the pre-exercise values immediately after the other four exercise tests. The greatest decreases in ETCR were found at one h and two h after T4. The changes in ETCR immediately after, one h and two h after T2 were smaller than the other exercise tests. Twenty four h after the exercise tests, ETCR showed a greater percentage decrease from the rest value after T5 than the percentage changes 24 h after the other four tests (Fig. 1A). Table 3 shows changes in immune adherence of erythrocytes after the five exercise trials in the untrained group. ETCR was significantly reduced immediately after, one h and two h after T1 and T2 compared to the pre-exercise values ($p<0.05$). Full recovery of ETCR to the pre-test values occurred by 24 h after the two tests. Similar trends in changes in ETCR were observed after T3 and T4. ETCR was significantly reduced immediately after, one h, two h after T3 and T4 ($p<0.05$) and remained reduced at 24 h after both tests compared to the pre-test values ($p<0.05$). However, ETCR 24 h after T3 and T4 was significantly higher ($p<0.05$) than the values immediately

after, one h and two h after the two tests. In comparison to the value before the exercise test, ETCR were significantly decreased immediately after T5 and up to 24 h after the test ($p < 0.05$). However, ETCR at 24 h after T5 was higher ($p < 0.05$) than ETCR at time points immediately after, one h and two h post-test.

ETCR at rest was also not significantly different ($p > 0.05$) between the different test conditions in the untrained participants. However, various immune responses of erythrocytes to different exercise tests were found (Table 3, Fig. 1B). T2 induced smaller alterations in ETCR immediately after, one h, two h and 24 h after the exercise compared to the percentage changes in ETCR from the pre-exercise values after the other four exercise tests. The percentage decreases in ETCR from the rest values were greater immediately after up to two h after T3 and T4, and 24 h after T5 (Fig. 1B).

The comparison of the activity of erythrocyte CR1 between trained and untrained groups: At rest before each exercise trial, ETCR in the trained group was not significantly different from the ETCR values in the untrained group ($p > 0.05$, Fig. 2). ETCR was not significantly different between two groups immediately after T1 ($p > 0.05$), but were significantly lower ($p < 0.05$) in the untrained group at one h and two h after the exercise test (Fig. 2A). The percentage changes in ETCR from the pre-exercise values showed that greater reductions occurred in the untrained group at time points one h and two h after T1 (Fig. 3A). After T2, ETCR was significantly reduced in the untrained group at one h after exercise compared to that of the trained group ($p < 0.05$). No significant difference ($p > 0.05$) between two groups in the same variable was found at immediately after, two h and 24 h after T2 (Fig. 2B). However, higher percentage decreases in ETCR from the pre-exercise values in the untrained groups were found at one h and two h after T2 (Fig. 3B). In the untrained group, ETCR was significantly lower than that in the trained group at immediately after, one h and two h after T3 ($p < 0.05$, Fig. 2C). The percentage decreases in ETCR from the pre-exercise value in the untrained group were greater than the changes in the trained group immediately post-test up to 24 h post-test (Fig. 3C). Significantly lower ETCR in the untrained group was observed at time points immediately after and 24 h ($p < 0.05$) after T4 compared to the values in the trained group. There was no significant difference ($p > 0.05$) between two groups in ETCR at one h and two h after T4 (Fig. 2D). Percentage change in ETCR from the pre-exercise value was greater in the untrained group 24 h after the test (Fig. 3D). After T5, significant differences ($p < 0.05$) between trained and untrained groups in ETCR were found until the time points two h and 24 h after the test, although no significant difference between two groups in ETCR immediately after and one h after T5 (Figs 2E, 3E).

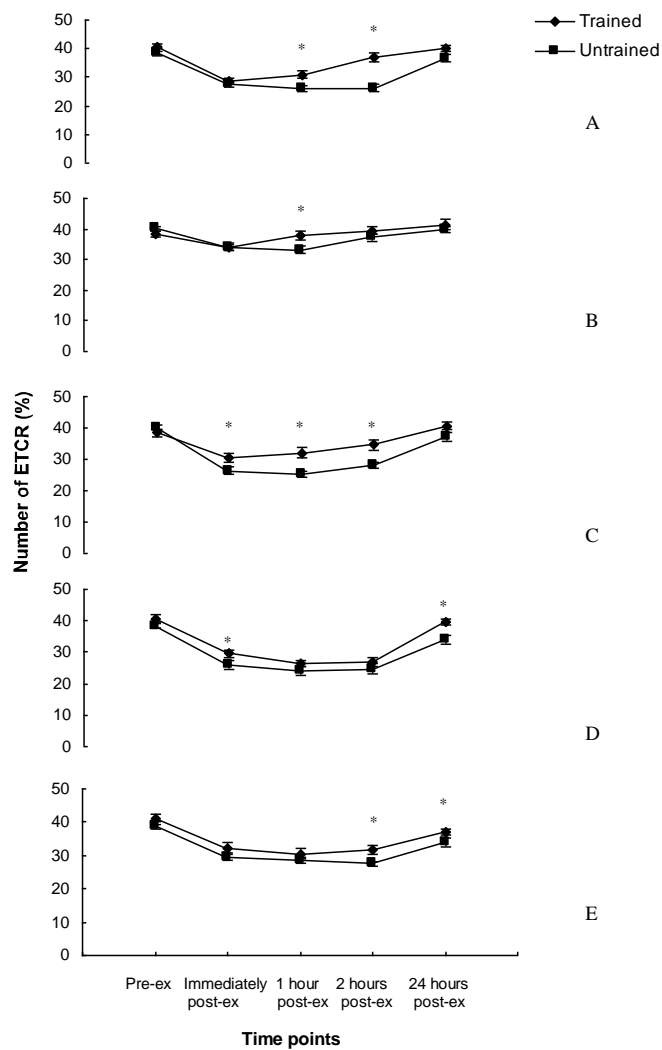
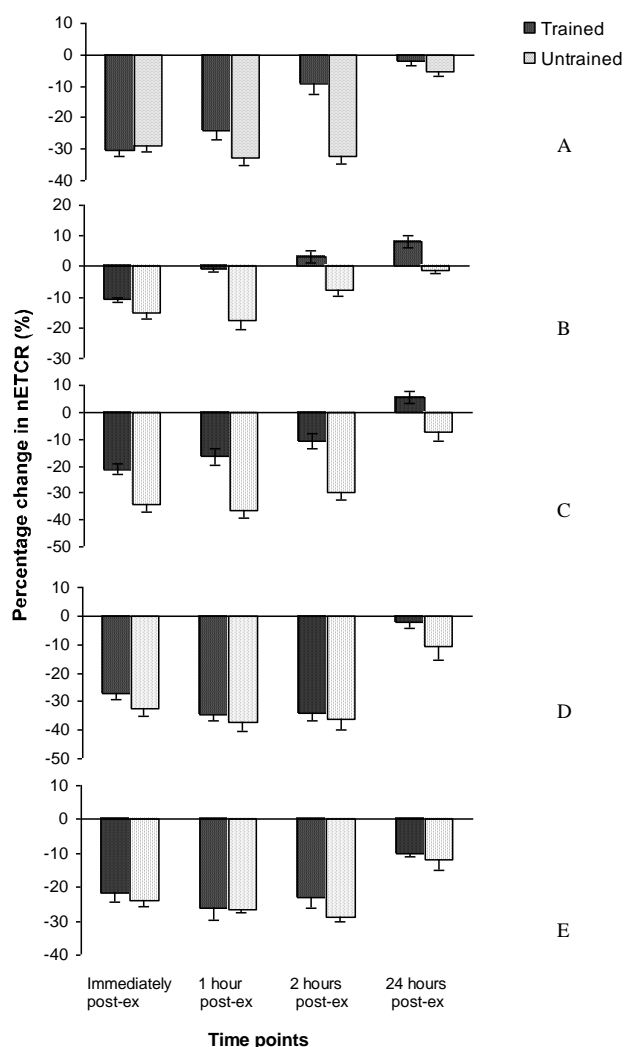


Fig. 2 Changes in ETCR in trained (n=15) and untrained (n=15) groups after maximal oxygen uptake test (A), exercise at 40% $\dot{V}O_{2\max}$ for 30 min (B), 80% $\dot{V}O_{2\max}$ for 30 min (C), 80% $\dot{V}O_{2\max}$ for 60 min (D) and downhill running (-10% gradient) at 60% $\dot{V}O_{2\max}$ for 30 min (E)

Values are means±SE; Significant difference between two groups: *p<0.05

ETCR: erythrocyte-tumor cell rosette formation in 100 tumor cells;

Ex: exercise test

**Fig. 3**

Percentage changes (compared to pre-exercise values) in nETCR in trained (n=15) and untrained (n=15) groups after maximal oxygen uptake test (A), exercise at 40% $\dot{V}O_{2\max}$ for 30 min (B), 80% $\dot{V}O_{2\max}$ for 30 min (C), 80% $\dot{V}O_{2\max}$ for 60 min (D) and downhill running (-10% gradient) at 60% $\dot{V}O_{2\max}$ for 30 min (E)

Based on the data of Figure 2. Values are means \pm SE;

nETCR: the number of erythrocyte-tumor cell rosette formation in 100 tumor cells;

Ex: exercise test

Discussion

Effects of training status on resting activity of erythrocyte CR1: The present results showed that there was no significant difference between the trained and untrained participants in ETCR at rest (Fig. 2). In other words, the activity of erythrocyte CR1 in the trained participants was not altered although the participants had engaged in regular endurance training for more than two years. The results were consistent with the findings of Thomsen and his colleagues [33], who first reported that the resting level of CR1 on erythrocytes in elite racing cyclists did not significantly differ from that in untrained males. The data were in agreement with those of previous studies as well [15,18,20]. For example, no obvious effect of chronic exercise on the resting activities of CR1 on erythrocytes was observed in young middle and long distance runners (male) and female athletes [6,9]. There was no change in the resting values of immune adherence of erythrocytes in 60-76 year old males who had practiced 'Taijiquan' for more than 10 years compared to age-matched controls [20]. The results from the present study indicated that human immune adherent function of erythrocytes at rest remained unaffected by endurance training lasting at least two years.

However, Qigong, a special Chinese exercise to build health, has been shown to enhance erythrocyte immune function. Masters of Qigong possessed a markedly higher erythrocyte immune adherence at rest compared to healthy controls [36]. Contrarily, animal research suggests that 4-8 weeks of heavy exercise training on a treadmill could induce a significant suppression in the immune function of erythrocytes in Sprague Dawley rats [24,30]. Therefore, it remains possible that there may be factors that could influence resting erythrocyte immune function due to exercise training styles. More research in this area is needed.

Effects of different acute exercises on the activity of erythrocyte CR1: The data from the present study have demonstrated that ETCR was significantly reduced in both trained and untrained participants after all five exercise tests (Tables 2, 3, Fig. 1), which meant that the activity of erythrocyte CR1 was significantly decreased and the immune function of erythrocytes was inhibited. These results supported findings of previous studies that reported that the activity of erythrocyte CR1 in both middle and long distance runners and untrained subjects significantly diminished immediately after two exercise tests on a cycle ergometer at 45% $\dot{V}O_{2\max}$ and 85% $\dot{V}O_{2\max}$, respectively, for 10 min [15]. Three step exercises at different durations (i.e. 15 min, 30 min and 45 min) but at the same intensity (700 kg·m·min⁻¹) produced significant inhibitions in immune adherence of erythrocytes in healthy young males immediately after and three h after exercises

[14]. These recovered to pre-exercise levels 15 h after exercises. Moreover, a negative relationship between the activity of erythrocyte CR1 and exercise durations was found.

The mechanisms for the change in activity of CR1 on erythrocytes due to physical exercise are not clear. The damage to the erythrocyte membrane, increase in oxidative stress, activation of the complement system, changes in the body internal environment, leukocyte immune function and the neuroendocrine system during exercise may all affect the results. It has been reported that physical exercise caused changes in blood lactate concentration and a negative relationship between immune adherence of erythrocytes and blood lactate concentration was established [16]. Inhibiting and enhancing factors for erythrocyte immune adherence in serum have been proposed. Both chronic and acute exercises resulted in changes in serum factors, which might be one explanation for the change in erythrocyte immune function due to exercise [18]. Soong *et al.* reported that an eight weeks of heavy training on treadmill reduced immune functions of erythrocytes and leukocytes in rats, and there was a positive correlation between the activity of erythrocyte CR1 and the immune function of T lymphocytes [30]. It has been demonstrated that there were hormone receptors and binding sites such as opiate, insulin, adrenergic and steroid binding sites in human erythrocyte membranes [7,10,28,32]. Some studies indicated that there was a biphasic endorphin-regulatory effect on the immune function of erythrocytes [19,31]. Animal research has shown that resting erythrocyte CR1 activity was significantly lower in trained rats than in control rats after an 8-week heavy training program on a treadmill. Meanwhile, plasma ACTH and β -endorphin levels decreased in trained animals. Physical exercise has been found to induce the response of the neuroendocrine system. The change in immune adherence of erythrocytes might be mediated by exercise-induced alterations in stress hormones [17,30]. Therefore, the factors that influence the immune function of erythrocytes in exercise are multifactorial.

There are apparently no published studies that examined the effect of eccentric exercise on the immune function of erythrocytes. The interesting finding in the present study was that the reductions in the activities of erythrocyte CR1 due to downhill running on a -10% gradient at $60\% \dot{V}O_{2\max}$ for 30 min were still marked up to 24 h of the recovery period in the participants. Moreover, both trained and untrained participants showed greater decreases in immune function of erythrocytes at 24 h after eccentric exercise compared to the other exercise tests, albeit the changes were smaller immediately after downhill running (Tables 2, 3, Fig. 1). The mechanisms for such changes in the activity of erythrocyte CR1 due to downhill running are unclear. These changes may be related to unaccustomed

eccentric contractions during exercise, which are more likely than concentric contractions to cause delayed onset of muscle soreness and muscle damage [4,5,21].

The data on immune adherence of erythrocytes in the present study showed that the suppression in immune function of erythrocytes was more apparent and the recovery in immune function of erythrocytes after the exercise tests was slower in the untrained participants than in the trained participants. After light exercise at 40% $\dot{V}O_{2\max}$ for 30 min, a significant reduction in activity of erythrocyte CR1 in the trained participants was only found immediately after exercise and recovered to the pre-exercise value within one h after exercise. Furthermore, ETCR increased (around 8%) from the pre-exercise value 24 h after the exercise. However, the immune adherence of erythrocytes in the untrained participants was significantly depressed immediately after exercise at 40% $\dot{V}O_{2\max}$ for 30 min up to two h after the same exercise and returned to the pre-exercise value 24 h after the test (Tables 2, 3, and Figs. 2B, 3B). After exercise at 80% $\dot{V}O_{2\max}$ for 30 min, the decrease in activity of erythrocyte CR1 was much greater in the untrained participants compared to that in the trained participants (Tables 2, 3, Figs. 2C, 3C). These results were consistent with a previous study, in which an acute exercise at 85% $\dot{V}O_{2\max}$ significantly reduced the activities of erythrocyte CR1 in both middle and long distance runners and untrained young males. Meanwhile, the untrained males showed greater changes in the ability of immune adherence of erythrocytes after exercise [15]. Physiological adaptations in the body to exercise training may explain this difference. Endurance training causes physiological adaptations in all the systems of the body in trained participants [25,35]. Therefore, exercise at the same workload is a relatively smaller stimulus to the body so that the change in the internal environment of the body is lower, and smaller effects on the immune function may be expected during exercise in trained participants.

However, it should be noted that conflicting results have been reported. For instance, Thomsen *et al.* reported that the level of erythrocyte CR1 in untrained people did not change during a 60-min bicycle exercise at 75% $\dot{V}O_{2\max}$, two h and 24 h after exercise, respectively [33]. Another study showed that a set of Wu style Taijiquan (20 min) did not cause alteration in the activity of erythrocyte CR1 immediately after exercise in older males (60-76 years), but elevated the activity at two h after exercise [20]. Contrarily, an increase in immune adherence of erythrocytes in young female participants was found after a 15-min treadmill running at 8 km/h on a level gradient. And the activity of erythrocyte CR1 returned to the pre-exercise level 24 h after the exercise [18].

Some of these conflicting results may be associated with the different techniques used for immune function analysis. For example, Thomsen detected

erythrocyte CR1 using enzyme linked immunosorbent assay (ELISA) that mainly measured the levels or numbers of CR1 on erythrocytes based on the principles of antigen-antibody reaction [33]. In the present study, the method used was an effective technique to assay the activity of erythrocyte CR1. During exercise, hemorheological and microcirculatory parameters were significantly influenced. The fragility and rigidity of erythrocytes were changed due to muscle contraction, extrusion, vascular friction and lipid peroxidation, resulting in impairment of erythrocytes and even hemolysis [13,23,26]. This suggested that the membrane of erythrocytes was impaired due to exercise. The CR1 molecule is a large transmembrane glycoprotein. The injured structure of the erythrocyte membrane due to exercise may affect the activity of CR1. However, the antigenicity of CR1 on erythrocytes may still exist. Therefore, it is assumed that no change in the number of CR1 may be detected using the experimental approach by antigen-antibody reaction, ELISA. These could have contributed to the conflicting results obtained.

Apart from differences in detection methods, differences in the intensities and durations of exercise, sex and fitness level of participants, the time of blood sampling, the duration of the recovery period may also affect the results. Thus, direct comparisons across different studies are difficult to accommodate.

In conclusion, ETCR at rest in the trained and untrained participants was similar in the present study. This indicated that at rest, the activity of erythrocyte CR1 was not affected by training status. Following acute exercise at $\dot{V}O_{2\max}$ intensity, 30 min of treadmill walking at 40% $\dot{V}O_{2\max}$, 30 min of treadmill running at 80% $\dot{V}O_{2\max}$ and 60 min of treadmill running at 80% $\dot{V}O_{2\max}$, ETCR were significantly reduced from resting values in trained and untrained participants. Exercise at $\dot{V}O_{2\max}$ intensity, 80% $\dot{V}O_{2\max}$ for 30 min and 60 min resulted in greater suppressions in ETCR in trained and untrained participants. However, the changes were more marked in the untrained participants than in the trained participants. Additionally, downhill running on treadmill at -10% gradient at 60% $\dot{V}O_{2\max}$ for 30 min induced significant changes in ETCR from resting values in trained and untrained participants up to 24 h after exercise. The decrease in ETCR at 24 h after downhill running on -10% gradient at 60% $\dot{V}O_{2\max}$ for 30 min was significantly greater than that induced by normal treadmill running, suggesting that eccentric type exercise induced a delayed recovery in activity of erythrocyte CR1 compared to concentric type exercise.

References

1. Birmingham D.J., L.A.Hebert (2001) CR1 and CR1-like: the primate immune adherence receptors. *Immunol.Rev.* 180:100-111
2. Birmingham D.J., W.Chen, G.Liang, H.C.Schmitt, K.Gavit, H.N.Nagaraja et al. (2003) A CR1 polymorphism associated with constitutive erythrocyte CR1 levels affects binding to C4b but not C3b. *Immunology* 108:531-538
3. Bruce R.A., F.Kusumi, D.Husmer (1973) Maximal oxygen intake and nomographic assessment of functional aerobic impairment in cardiovascular disease. *Am.Heart J.* 85:546-562
4. Bruunsgaard H., H.Galbo, J.Halkjaer-Kristensen, T.L.Johansen, D.A.MacLean, B.K.Pedersen (1997) Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. *J.Physiol.* 499(Pt 3):833-841
5. Bryner R.W., D.A.Donley, R.G.Cutlip, O.Wirth, S.E.Always (2004) Effects of downhill treadmill running on uncoupling protein 3 mRNA expression. *Int.J.Sports Med.* 25:433-437
6. Camus G., J.Pincemail, M.Ledent, A.Juchmes-Ferir, M.Lamy, G.Deby-Dupont et al. (1992) Plasma levels of polymorphonuclear elastase and myeloperoxidase after uphill walking and downhill running at similar energy cost. *Int.J.Sports Med.* 13:443-446
7. Ferreira A., A.Rivera, J.R.Romero (2004) Na⁺/Mg²⁺ exchange is functionally coupled to the insulin receptor. *J.Cell Physiol.* 199:434-440
8. Franklin B.A., M.H.Whaley, E.T.Howley (2000) ACSM's Guidelines for Exercise Testing and Prescription. 6th Ed. Lippincott Williams & Wilkins, Philadelphia, Pennsylvania
9. Gibson S.C.A, M.I.Lambert, J.A.Hawley, S.A.Broomhead, T.D.Noakes (1999) Measurement of maximal oxygen uptake from two different laboratory protocols in runners and squash players. *Med.Sci.Sports Exerc.* 31:1226-1229
10. Golden G.A., R.P.Mason, T.N.Tulenko, G.S.Zubenko, R.T.Rubin (1999) Rapid and opposite effects of cortisol and estradiol on human erythrocyte Na⁺,K⁺-ATPase activity: relationship to steroid intercalation into the cell membrane. *Life Sci.* 65:1247-1255
11. Guo F. (2005) Road map of hemaimmune reaction. *Shenzhen J. of Integrated Traditional Chinese and Western Med.* 15:1-4 (in Chinese, English abstract)
12. Guo F., R.L.Zeng, Y.H.Sun (1999) Enhancement of red cells adhering to tumour cells by Cordyceps Sinensis in vitro. *Clin.Immunol.* 90:444-445
13. Gurcan N., D.Erbas, E.Ergen, A.Bilgehan, S.Dundar, A.Aricioglu et al. (1998) Changes in blood haemorheological parameters after submaximal exercise in trained and untrained subjects. *Physiol.Res.* 47:23-27
14. Hao M., Y.J.Song (2001) The effects of different exercise volumes on the immune function of erythrocytes. *Shenzhen J. Integrated Traditional Chinese Western Med.* 11:339-341 (in Chinese, English abstract).

15. Hu Q.C., F.Guo, Y.Q.Weng, G.H.Xu (1993) Effects of training and exercise on immune function of erythrocytes. In: F.Guo and Y.Z.Luo (eds.) Advance of Red Cell Immunology Research. Nanjing University Publisher, Nanjing, pp. 98-101 (in Chinese, English abstract)
16. Hu Q.C., F.Guo, Y.Q.Weng, G.H.Xu (1995) The effect of change in blood lactate concentration on immune function of erythrocyte in exercise. *China J.Immunol.* 11(Suppl):211-213 (in Chinese, English abstract)
17. Hu Q.C., P.Soong, R.Karch, R.X.Huang (2002) Effects of exercise and herbs on beta-endorphin and ACTH levels in rats. *Med.Sci.Sports Exerc.* 34(Suppl.):70
18. Huang H., X.M.Hao, X.Y.Hu, D.Q.Quan (1999) Study on response of erythrocyte immune function to submaximal exercise. *J.China Sports Med.* 18:375-377 (in Chinese, English abstract)
19. Huang S.D., F.Guo (1991) The enhancement of β -endorphin to red-cell immune adherence. *China J.Immunol.* 7 (Suppl):10-11 (in Chinese, English abstract)
20. Li Z.Q., F.Guo (1993) The effects of practising Wu's Taijiquan on immune function of red cells and activity of NK cells in older people. In: F.Guo and Y.Z.Luo (eds.) Advance of Red Cell Immunology Research. Nanjing University Publisher, Nanjing, pp. 94-97 (in Chinese, English abstract)
21. Malm C., R.Lenkei, B.Sjodin (1999) Effects of eccentric exercise on the immune system in men. *J.Appl.Physiol.* 86:461-468
22. Miles M.P., R.J.Naikam, A.C.Hackney, P.M.Clarkson (1999) Blood leukocyte and glutamine fluctuations after eccentric exercise. *Int.J.Sports Med.* 20:322-327
23. Nageswari K., R.Banerjee, R.V.Gupte, R.R.Puniyani (2000) Effects of exercise on rheological and microcirculatory parameters. *Clin.Hemorheol.Microcirc.* 23:243-247
24. Pei X.Z., W.H.Chen, F.Guo (2001) The effects of heavy exercise training and oral amino acids on the immune function of erythrocytes. *Shenzhen J. of Integrated Traditional Chinese and Western Med.* 11:336-337 (in Chinese, English abstract)
25. Plowman S.A., D.L.Smith (2002) Exercise Physiology for Health, Fitness, and Performance. Benjamin Cummings, San Francisco
26. Senturk U.K., O.Yalcin, F.Gunduz, O.Kuru, H.J.Meiselman, O.K.Baskurt (2005) Effect of antioxidant vitamin treatment on the time course of hematological and hemorheological alterations after an exhausting exercise episode in human subjects. *J.Appl.Physiol.* 98:1272-1279
27. Siegel I., T.L.Liu, N.Gleicher (1981) The red-cell immune system. *Lancet* 2:556-559
28. Simpkins C.O., B.P.Chenet, Y.H.Kang, D.L.Mazorow, D.B.Millar, V.W.Hollis Jr (1989) Human erythrocytes have binding sites for beta-endorphin. *J.Natl.Med.Assoc.* 81:1149-1153
29. Smith L.L., M.McCammon, S.Smith, M.Chamness, R.G.Israel, K.F.O'Brien (1989) Leukocyte response to uphill walking and downhill jogging at similar metabolic loads. *Eur.J.Appl.Physiol.* 58:833-837
30. Soong P., Q.C.Hu, R.X.Huang (2003) The change in immune function of erythrocytes in rats after an 8-week heavy exercise training. *J.Exerc.Physiologyonline* 6:1-8

31. Sun Z.Y., F.Guo, Z.N.Guo, M.R.Zhao, Z.J.Yue (1993) Study of the regulation of β -endorphin to immune function of red cells in normal and healthy people. In: F.Guo and Y.Z.Luo (eds.) *Advance of Red Cell Immunology Research*. Nanjing University Publisher, Nanjing, pp. 34-37 (in Chinese, English abstract)
32. Susanni E.E., F.P.Ross, D.R.Scriven, C.Rosendorff (1985) Baboon erythrocyte ghosts contain beta-adrenergic receptors. *Am.J.Physiol.* 249:C15-19
33. Thomsen B.S., A.Rodgaard, N.Tvede, F.R.Hansen, J.Steensberg, J.Halkjaer Kristensen, et al. (1992) Levels of complement receptor type one (CR1, CD35) on erythrocytes, circulating immune complexes and complement C3 split products C3d and C3c are not changed by short-term physical exercise or training. *Int.J.Sports Med.* 13:172-175
34. Wang H.B., J.P.Zhang, F.Guo (1999) Establishment of methods for measurement of molecular quantity and activity of CR1 on red cells. *Shenzhen J. Integrated Traditional Chinese Western Med.* 9:13-17 (in Chinese, English abstract)
35. Wilmore J.H., D.L.Costill (2004) *Physiology of Sport and Exercise*. 3rd Ed. Human Kinetics, Champaign
36. Zhang Y.H., B.W.Yuan, Q.J.Gong, R.P.Liu, J.Zhou, S.H.Dai et al. (1993) Effect of Zhineng Qigong on red cell immune function of human. In: F.Guo and Y.Z.Luo (eds.) *Advance of Red Cell Immunology Research*. Nanjing University Publisher, Nanjing, pp. 104-107 (in Chinese, English abstract)

Accepted for publication 19.12.2006

Acknowledgement

The research was supported by the Singapore MINDEF–NTU Joint Fund and NIE Fund. The authors wish to acknowledge the data collection assistance of Darren Lim and Han Chow Koh