Immunological Impact of Taekwondo Competitions

Authors: Y. W. Lee[1,*], K. W. Shin[2,*], I.-Y. Paik[3], W. M. Jung[3], S.-Y. Cho[3], S. T. Choi[4], H. D. Kim[5], J. Y. Kim[6]

Affiliations: Affiliation addresses are listed at the end of the article

Key words
- B cells
- CD4/CD8 ratio
- reactive oxygen species
- Taekwondo
- T cells

Abstract

Immunological changes in elite adolescent female athletes during Taekwondo competitions were investigated on-field. 6 female athletes (16.7 ± 0.8 year-old) volunteered and performed 5 bouts of demonstration Taekwondo competitions simulating real tournaments in intensity, duration, and break-time intervals on the same day. Blood samples were taken before, after the competitions and during the recovery, respectively. Immunological changes and oxidative stress in peripheral blood mononuclear cells were evaluated by flow-cytometry. During the competitions, exercise intensity was 92.2 ± 3.8 % (86.1~95.7) of the maximal heart rate. Blood lactate increased immediately after the competitions ($p = 0.0165$) and decreased to baseline during recovery. Intracellular reactive oxygen species (ROS) in the peripheral blood increased continuously during recovery ($p < 0.05$, respectively). Natural killer cells increased immediately after the competitions ($p = 0.0006$), and decreased during recovery. B and T cells increased immediately after the competitions and remained elevated throughout recovery ($p < 0.05$, respectively). CD4/CD8 ratio after the competitions was decreased ($p = 0.0091$) and returned to baseline during recovery. These results suggest that the immunological function of the elite female adolescent athletes could be attenuated after Taekwondo competitions. Further large-scaled Taekwondo studies on immunologic and apoptotic changes related to oxidative stress should be performed for improving and protecting the health of adolescent athletes.

Introduction

Taekwondo is the national martial art and sport of Korea with a 5 000 year history, and has gained an international reputation as a global sport. After the World Taekwondo Federation (WTF) was admitted to the International Olympic Committee (IOC) in 1980, Taekwondo became a demonstration sport in the 1988 and 1992 Olympic games, and it was finally admitted as an official event in the Sydney 2000 Olympiad [7, 10].

Taekwondo is characterized by fast, high, and spinning kicks, as the name means "the art of kicking and punching". In international tournaments such as the Olympic Games or World Taekwondo Championships, Taekwondo competition is composed of 3 rounds that compete for 3 min and rest for 1 min between the competitions. For the finals, athletes have to compete more than 5 to 7 times during a single day [8, 48], and experience physiological changes including accumulation of hydrogen ion, phosphorus, ammonia and lactate, affecting muscle metabolism, local muscle fatigue, and neuromuscular activities [19, 38]. Such an excessive amount of either acute physical activity or intensive training can cause a temporary modulation of immune function [14, 27]. In response to acute exercise, lymphocyte subpopulations in vascular compartment, such as natural killer (NK) cells, B cells, and T cells increase and fall below pre-exercise values after exercise [16]. Furthermore, high intensity exercise, such as 45 min of 80 % VO_{2max} intensity treadmill exercise, is associated with a decrease in mitogen-stimulated lymphocyte proliferation immediately after exercise compared to resting values [28].

These strenuous physical activities can result in immune suppression and reduction of lymphocytes immediately after exercises, and this is associated with an increased susceptibility to viral infections [13, 26]. One of the possible explanations for immune suppression and loss of

accepted after revision
July 21, 2011

Bibliography
DOI http://dx.doi.org/10.1055/s-0031-1285926
Published online: November 23, 2011
Int J Sports Med 2012; 33: 58–66 © Georg Thieme Verlag KG Stuttgart · New York
ISSN 0172-4622

Correspondence
Joo Young Kim, PhD
Ewha Womans University
College of Pharmacy
Rm207 Pharmacy Bldg. B
Seoul
Republic of Korea
120–750
Tel.: +82/2/3277 3016
Fax: +82/2/3277 3051
jooyoungkim75@gmail.com

*The authors contributed equally.

lymphocytes during sports competitions is apoptotic cell death, whereas this type of programmed cell death does not occur in inflammation [16]. Reactive oxygen species (ROS) generation is one of the signals that induce apoptosis in lymphocytes [34].

Many studies have demonstrated that heavy exercise induces apoptotic cell death and intracellular ROS generation [17, 31, 50]. Vider et al. demonstrated that physical exercise with heavy intensity (80 % VO_{2max}, 1 h) increased oxidative stress and NF-κB activation in human peripheral blood lymphocytes [50]. In mouse studies, caspase 3, one of the downstream targets for intracellular signalling of apoptosis, activated thymocytes after a single intensive bout of treadmill running (26 m/min, 6 degrees slope, 90 min) [31], and annexin-V, a marker for early apoptosis, is increased in intraepithelial intestinal T cells 24 h after treadmill running (32 m/min, 8 degrees slope, 90 min) [17].

A temporary suppression of immune function and increased susceptibility to viral infection after various exhausting exercises has already been reported in many studies [42]. However, these investigations focused mainly on easily accessible physical activities categorized into intensity, duration, and type of exercise. Immunological changes during martial arts competitions including Taekwondo, which require more complex and dynamic physical activities, have not yet been fully investigated.

This study investigated the physiological and immunological changes experienced by female adolescent athletes during Taekwondo competitions by simulating the tournaments leading up to the final match. In addition, this approach could provide ideas for the improvement and protection of the health of female adolescent athletes.

Table 1 Physical characteristics of the subjects.

Characteristics	Values[1]
age (years)	16.7 ± 0.8
height (cm)	163.2 ± 5.3
weight (kg)	58.6 ± 12.8
body fat (%)	15.4 ± 7.4
VO_{2max} (ml/kg/min)	49.2 ± 4.8
heart rate maximum (beat/min)	188.3 ± 8. 9

[1]Values are mean ± SD of 6 independent subjects

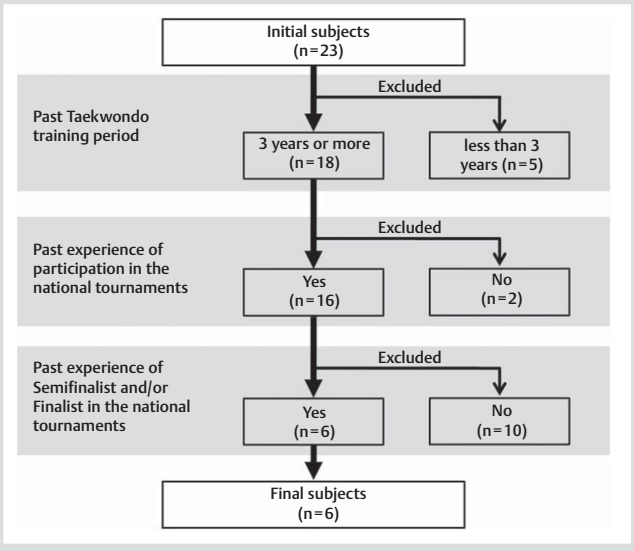

Fig. 1 A flow chart indicating subject selection process.

Materials and Methods

Subjects

23 female Taekwondo athletes of a high school located in the Republic of Korea were recruited. They were healthy and were not currently taking any medication. Among them, 6 athletes, who had similar technical skills, trained 20 h a week, and who had participated in the semifinals or final of the national Taekwondo tournaments for at least the past 3 years, were selected (**Fig. 1**). Their physical characteristics are shown in **Table 1**. Informed consent was obtained from all of the subjects and their parent(s)/guardian. This study was approved by the Institutional Review Board of Kwandong University College of Medicine, Myongji Hospital (Goyang, Republic of Korea) for the protection of human subjects and was performed in accordance with the ethical standards of the International Journal of Sports Medicine [15].

Scheme of study

Subjects underwent a pre-test for analysis of their physical characteristics, and demonstrated a Taekwondo tournament similar to a real-life tournament. To avoid any potential influence on subject's exercise performance, all subjects were requested to abstain from taking caffeine, vitamins, food and exercise during the 6 h preceding pre-test or demonstrated Taekwondo tournament. The pre-test and competitions were held between 14:00 and 16:00 h. Prior to the study, all subjects had tutorials to become familiarized with the equipment and protocols for this study.

Blood samples were collected prior to the tournament, after the end of the tournament, and after 0.5 and 24 h of the recovery phase, respectively (**Fig. 2a**). During the recovery phase, subjects were provided with the same restricted meals that satisfied essential nutrients requirement. This was standardized for all participants.

Preliminary measurement of baseline characteristics

Body composition (%body fat) was assessed by bioelectrical impedance analysis (BIA) and Body Composition Analyzer Model 310 (Biodynamics, Seattle, WA, USA), and heart rate (HR) during the tournament was transmitted by Polar T31 transmitter (Polar Electro, Lake Success, NY, USA) and detected by MetaMax 3B Receiver (Cortex, Leipzig, Germany).

To measure the maximal oxygen uptake (VO_2max), we had the subjects perform a maximal test on the Q65 treadmill (Quinton, Seattle, WA, USA) according to the Bruce protocol [2]. During this test, Borg's 15RPE scale was used as an indication of impending fatigue [4]. Oxygen consumption during exercise was measured by MetaMax 3B (Cortex). HR and oxygen consumption were analyzed by MetaSoft Ver. 3.9.5 (Cortex).

Demonstrated Taekwondo tournament

In order to satisfy conditions like those of a real tournament such as exercise intensity, the number of competitions, or break time between each competition, subjects performed 5 rounds of the Taekwondo competition [7]. Each competition was composed of 3 matches for 3 min and rest for 60 s between the matches. Break time between each competition was allowed from 40 min to 10 min in a gradually decreasing pattern (**Fig. 2b**).

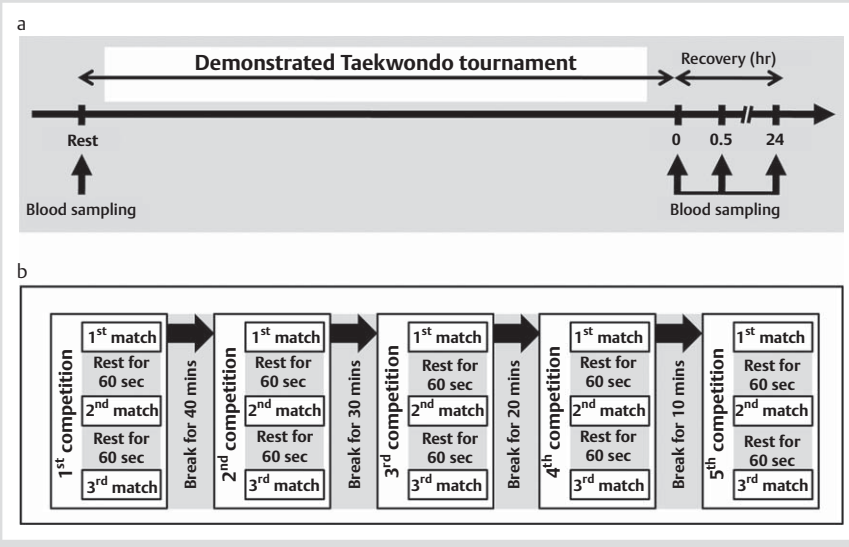

Fig. 2 Study design. **a** Blood samples were obtained at rest, and 0, 0.5 and 24 h after the demonstration Taekwondo tournament. **b** Scheme of the demonstration Taekwondo tournament. 5 rounds of competition composed of 3 matches for 3 min and rest for 60 s between the matches. Break time between each competition was offered from 40 min to 10 min in a gradually decreasing pattern.

HR was monitored for all subjects, and oxygen consumption was measured for one of the subjects in the first competition of the tournament. According to the manufacturer's instructions, the MetaMax 3B is a mobile, mains independent indoor and outdoor metabolic exercise testing system for various sports activities. The exercise intensity was calculated by the HR reserve (HRR) method [21] and VO_2reserve (VO_2R) method, respectively [24,53].

Blood lactate levels

For the determination of blood lactate concentration levels, serum samples were collected from each subject and analyzed by Ektachem DT 60II (Kodak, Rochester, NY, USA). Briefly, peripheral venous blood was collected from the antecubital vein of the subjects used by plain vacuum tube, and spun down at 1 000 × g, at 4 °C for 30 min. The serum was separated and immediately stored at −70 °C. The measurement of the blood lactate levels was duplicated.

Isolation of PBMCs

To isolate peripheral blood mononuclear cells (PBMCs), peripheral venous blood was collected from the antecubital vein of the subjects. Heparin-containing vacuum tubes were used to collect 10 ml of blood. Ficoll-Hypaque density centrifugation was performed as follows. The samples were mixed into the same amount of PBS (phosphate based saline). The diluted blood samples were overlaid on 12.5 ml of Ficoll and centrifuged at 800 × g continuously for 25 min. The interphase was then transferred to 30 ml of PBS and mixed by inverting the tubes, and then centrifuged at 500 × g continuously for an additional 10 min. The supernatant was discarded and the cell pellet was suspended in 20 ml of PBS. For all experiments, the viable cells were obtained as determined by trypan blue staining, and were used immediately for analysis.

Flow cytometry analysis

For the cytometric analysis of the ROS positive cells, natural killer (NK) cells, B cells and T cells, each cell was incubated with antibodies labelled with fluorescein isothiocyanate (FITC) and/or phycoerythrin (PE) in a FACS buffer (PBS, 1% FCS, 0.05% sodium-azide) for 30 min on ice, then washed twice with the FACS buffer. The data was acquired using a LSR II flow cytometer (BD Biosciences, San Jose, CA, USA) and analyzed using BD FACSDiva Software (BD Biosciences).

Flow cytometric analysis was performed in duplicate. The antibodies used for the cell staining and flow cytometry analysis were as follows: PE conjugated anti-CD19 mAb, FITC conjugated anti-CD3 mAb, FITC conjugated anti-CD4 mAb, PE conjugated anti-CD8 mAb, and PE conjugated anti-CD16+56 mAb (BD Pharmingen, SanDiego, CA, USA). The production of ROS was estimated by fluorescence using 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA; Molecular Probes, Eugene, OR, USA).

Statistical analysis

All data were expressed as the mean ± SD analysed by SPSS for windows version 18.0 (SPSS Inc., Chicago, IL, USA) and the statistical package R 2.13.1 using coin, multicomp, Bioconductor packages, and in-house scripts [12,36]. Considering the limited sample sizes (n = 6), nonparametric approaches were used for statistical analyses. The resting and post-exercise (0, 0.5, and 24 h) data were compared by using the non-parametric one-way repeated measures analysis (Friedman test). These results were followed up by post hoc analyses using both the Dunn's multiple comparison test (attributed to Bonferroni) and the Wilcoxon-Nemenyi-McDonald-Thompson test [18,51]. The results with a p-value <0.05 were considered significant (**Supplementary Table 1**). The intra- and inter-subject variabilities were calculated and expressed as the coefficient of variation: CV (%) = 100 × SD/mean value. Statistical power was calculated by PASS 11 (NCSS, Kaysville, UT, USA).

Results

The HR increased significantly after each competition compared with that in the resting stage (**Fig. 3a** and **Table 2**). The changes in HR and oxygen consumption were measured from one of the athletes in the first competition of the tournament. Exercise intensity set according to HRR was approximately equal to the percent value for the VO_2R (**Fig. 3c** and **Supplementary Table 2**).

Blood lactate level after the tournament increased significantly compared with that in the resting stage ($p = 0.0165$) and

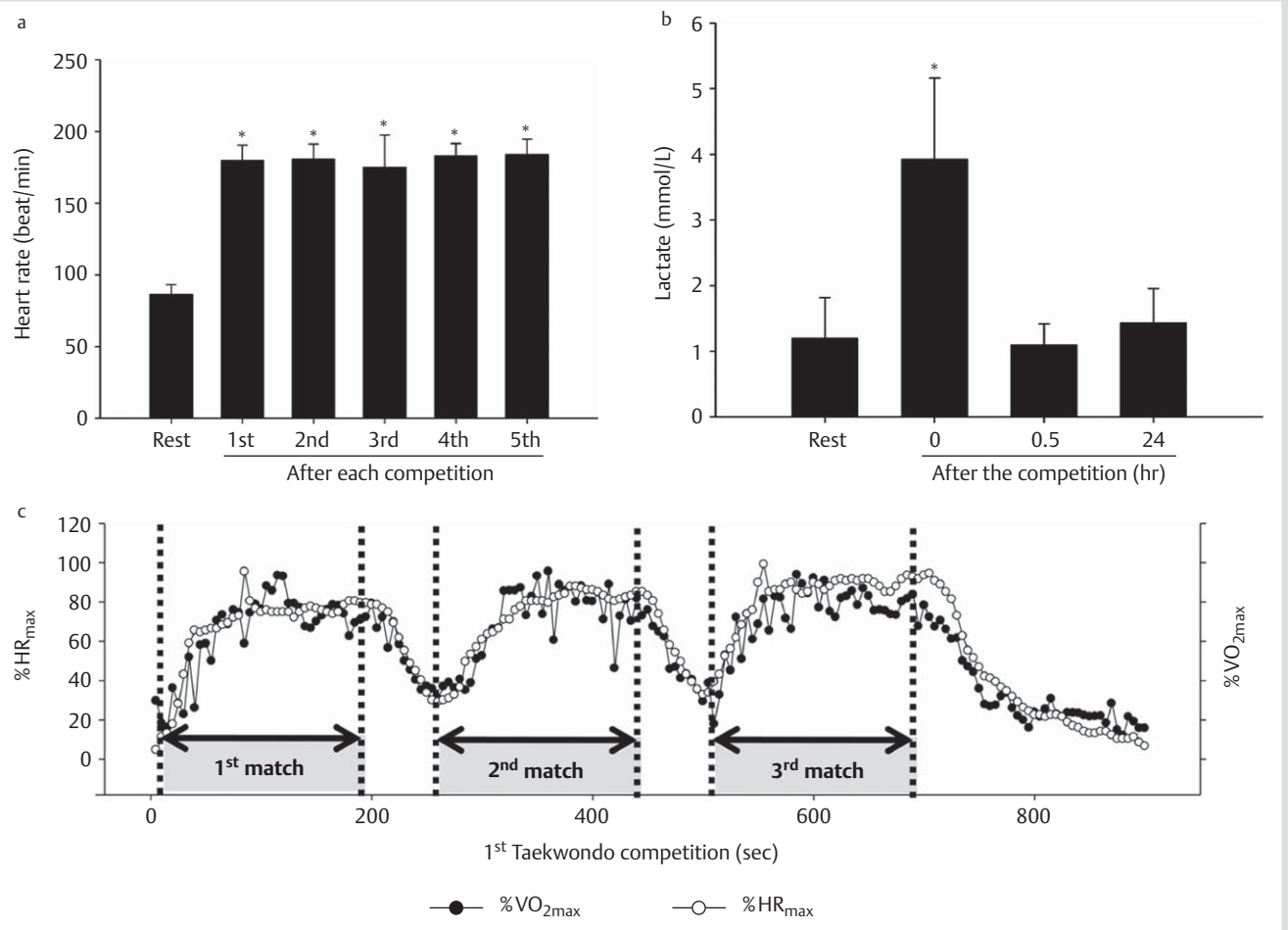

Fig. 3 Physiological changes in the demonstrated Taekwondo tournament. **a** Heart rate at rest and after each competition (n = 6). **b** Lactate concentration in the serum at rest, and 0, 0.5 and 24 h after the tournament (n = 6). Each value represents mean ± SD. *, Significant difference from "Rest" ($p < 0.05$). **c** Comparison of $\%HR_{max}$ and $\%VO_{2max}$ during the 1st competition of the tournament in the one individual.

Table 2 Changes of physiological and immunological factors during the demonstrated Taekwondo tournament.

	Rest	After each Taekwondo competition					Recovery phase	
		1st	2nd	3rd	4th	5th	0.5 h	24 h
heart rate (beat/min)	86.5 ± 6.7	180.0 ± 10.6*	180.8 ± 10.4*	175.0 ± 22.5*	183.0 ± 8.6*	183.8 ± 10.7*	–	–
%HRmax (%)	–	91.8 ± 4.5	92.7 ± 3.8	86.1 ± 16.7	94.9 ± 4.4	95.7 ± 7.8	–	–
lactate (mmol/L)	1.2 ± 0.6	–	–	–	–	3.9 ± 1.2*	1.1 ± 0.3	1.4 ± 0.5
NK cells (%)	5.2 ± 2.5	–	–	–	–	37.6 ± 5.7*	11.8 ± 3.5	19.1 ± 7.6
B cells (%)	3.5 ± 1.9	–	–	–	–	10.8 ± 3.4*	11.0 ± 3.3*	12.1 ± 2.6*
T cells (%)	19.9 ± 7.7	–	–	–	–	39.3 ± 7.9*	62.0 ± 9.0*	57.2 ± 7.2*
CD4 T cells (%)	13.3 ± 5.3	–	–	–	–	18.3 ± 5.5	33.5 ± 2.5*	31.6 ± 2.3*
CD8 T cells (%)	9.6 ± 4.1	–	–	–	–	29.9 ± 7.8*	26.6 ± 7.4*	28.6 ± 7.9*
CD4/CD8 ratio	1.2 ± 0.7	–	–	–	–	0.5 ± 0.4*	1.1 ± 0.6	1.0 ± 0.5
ROS (MFI)	61.1 ± 12.5	–	–	–	–	87.9 ± 9.9	178.4 ± 90.8*	166.6 ± 25.3*

All values are mean ± SD of 6 independent subjects; %HRmax, a percentage of the maximal heart rate; *, Significant difference from "Rest" ($p < 0.05$)

decreased at 0.5 and 24 h of the recovery phase, respectively (**Fig. 3b** and **Table 2**). The average value of intra-CV for duplicated lactate levels was 5.5%, and the average values of inter-CV between lactate levels of each subjects ranged from 24.6% to 47.0% at each time point.

The NK cell population in PBMCs after the tournaments showed a significant increase compared with that in the resting stage ($p = 0.0006$), and fell at 0.5 and 24 h after the tournament, respectively (**Fig. 4** and **Table 2**). The average value of intra-CV for duplicated NK cell population was 1.6%, and the average values of inter-CV between NK cell populations of each subject ranged from 15.2% to 48.1% at each time point.

The B cell and T cell populations in PBMCs after the tournament increased significantly compared with that in the resting stage and such increments were maintained through the recovery period, respectively ($p < 0.05$, respectively) (**Fig. 5** and **Table 2**). The average values of intra-CV duplicated were 2.7% for B cell populations and 0.6% for T cell populations. The

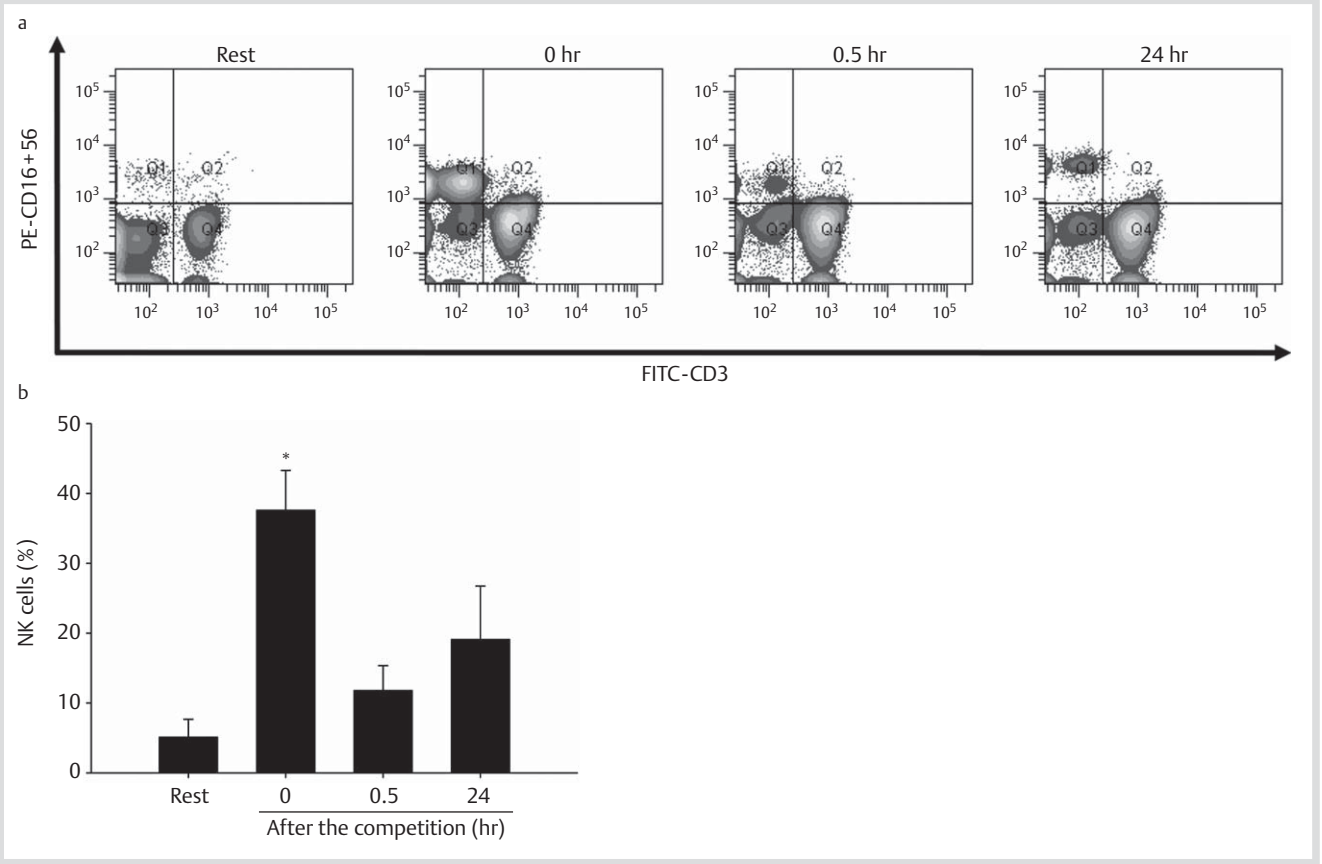

Fig. 4 Change of NK cell population during the tournament. **a** One representative data was performed by flow cytometry (FITC-CD3 and PE-CD16+56). **b** NK cell population in PBMCs at rest, and 0, 0.5 and 24 h after the tournament. *NK cells*, $CD16+56^{+}$ and $CD3^{-}$ cell population. Each value represents mean ± SD. *, Significant difference from "Rest" ($p < 0.05$).

average values of inter-CV between each subject ranged from 20.4% to 57.3% for B cell populations and ranged from 12.8% to 38.9% for T cell populations at each time point.

The $CD4^{+}$ T cell population in PBMCs after the tournament was similar to the resting stage. However, the $CD4^{+}$ T cell population increased significantly at 0.5 and 24 h after the tournament ($p = 0.0044$, and 0.0190, respectively) (**Fig. 6a,b**, and **Table 2**). The average value of intra-CV for duplicated $CD4^{+}$ T cell population was 0.9%, and the average values of inter-CV between $CD4^{+}$ T cell populations of each subject ranged from 5.9% to 32.5% at each time point.

The $CD8^{+}$ T cell population in PBMCs after the tournament increased significantly compared with that in the resting stage and such increments were maintained through the recovery period ($p = 0.0043$, and 0.0191, respectively) (**Fig. 6a,c** and **Table 2**). The average value of intra-CV for duplicated $CD8^{+}$ T cell population was 1.0%, and the average values of inter-CV between $CD8^{+}$ T cell populations of each subjects ranged from 25.8% to 41.7% at each time point.

The CD4/CD8 ratio after the tournament decreased significantly compared with that in the resting stage ($p = 0.0091$). The CD4/CD8 ratio returned to the resting level at 0.5 and 24 h after the tournament, respectively (**Fig. 6d** and **Table 2**). The average value of intra-CV for duplicated CD4/CD8 ratios was 8.4%, and the average value of inter-CV between CD4/CD8 ratios of each subject ranged from 37.1% to 53.9% at each time point.

The ROS level in PBMCs increased markedly at 0.5 and 24 h after the tournament ($p = 0.0040$, and 0.0091, respectively) (**Fig. 7** and **Table 2**). The average value of intra-CV for duplicated ROS level was 0.1%, and the average values of inter-CV between ROS levels of each subject ranged from 9.1% to 32.4% at each time point.

Discussion

Martial-arts including Taekwondo require intense competitions [5,7,23,35]. Chiodo, et al. reported that international-level Taekwondo competitions imposed a high exercise load (HR > 90% of individual HRmax) on 10 male and 6 female elite adolescent athletes independent of gender during mean 65.4% of the competition time [8]. It has already been reported that prolonged and strenuous physical activities like intense exercises could induce physical damages [43], immunosuppression [40,44], and increased susceptibility to illness and/or infection [43]. The immune system is temporarily impaired, as described in the 'open window' theory after intense exercise of long duration [25,47]. Such impairment is associated with an increased susceptibility to opportunistic infections, particularly upper respiratory tract infections [13].

Although common exercises, such as swimming, had been widely studied [22], Taekwondo has not yet been fully evaluated because of difficulties in its physiological quantification of the loads and efforts, especially in competition [7]. And there are only a few studies on the immunological impacts of Taekwondo competition on elite athletes [49].

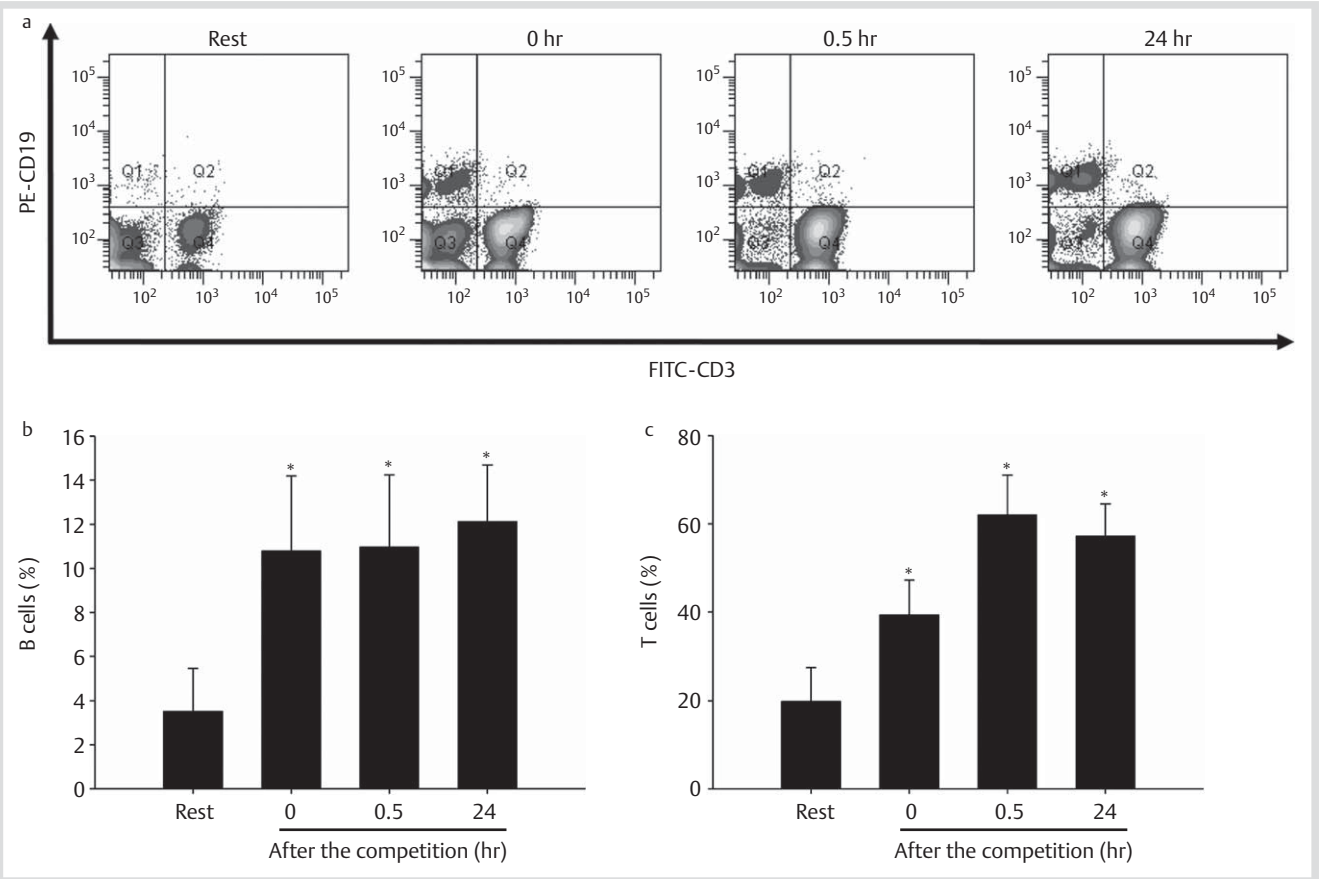

Fig. 5 Change of T cell and B cell populations during the tournament. **a** One representative data was performed by flow cytometry (FITC-CD3 and PE-CD19). **b** B cell population in PBMCs at rest, and 0, 0.5 and 24 h after the tournament. **c** T cell population in PBMCs at rest, and 0, 0.5 and 24 h after the tournament. Each value represents mean ± SD. *, Significant difference from "Rest" ($p < 0.05$).

To investigate immunological changes after a Taekwondo tournament, the teenage female athletes demonstrated Taekwondo competitions simulating real tournaments in exercise-intensity, competition times, and break-time intervals. During the competitions, HR of the athletes was remarkably increased (180.5 ± 12.9 bpm) and blood lactate concentrations varied above 3.9 mmol/L. In previous reports about HR and blood lactate concentrations during Taekwondo competitions, HR ranged from 183.0 ± 9.0 to 190.1 ± 8.1 bpm and blood lactate concentrations ranged from 3.3 to 7.5 mmol/L [5, 7, 23, 35]. However, one of the limitations of this study was insufficient psychological stress on the subjects imposed by the simulated competitions. In real sports tournaments, athletes usually experience psychological stress, which is also one of the influencing factors on their performance, and simulated situations could underestimate stress-related psychobiological responses of athletes which occur under highly competitive real conditions [6, 8, 11, 39, 46].

In the innate immune system the NK cell provides a preferential and expanded physical defense mechanism against virus-infected cells or certain types of tumour cells before the adaptive immune responses are activated [1, 43]. Previous studies reported that the NK cell population in peripheral blood was increased according to physical activity up to VO_2max, and the increased NK cell population of PBMCs immediately decreased in the recovery phase after the end of intense exercises [9, 41, 45]. They suggested that the endocrine system, such as catecholamines, affected the systemic immune response in the peripheral blood stream. In this study, the NK cell population in PBMCs of athletes was increased over 7-fold compared to the resting stage after the Taekwondo tournament, and decreased during the recovery phase. According to the severity of exercise intensity and duration at the tournament, this result was confirmed by the preceding works of Shephard and Shek [45].

Macrophages, immune cells mediated by innate immunity, intracellularly eliminate microbial antigens via phagocytosis [20]. A microbial endotoxin, a lipopolysaccharide, captured in a phagosome of macrophages is eliminated by nitric oxide (NO) and ROS produced by the macrophages [54]. If the removal of NO and ROS is inadequate, it will cause oxidative damage to the phagocytes [32]. A tight balance between production and removal (reduction and oxidation; redox) is critical. Excessive production of NO and ROS causes cellular apoptosis of the phagocytes themselves [3].

This study showed that ROS production of PBMCs gradually increased from the end of the tournament to 24 h of the recovery phase. Radak et al. suggested that intracellular ROS increased due to intense exercise could negatively affect cellular viability in spite of physical protection [37]. Such results were also supported by previous studies reporting that intracellular ROS accumulation was associated with the increase of cellular apoptotic markers including caspase 3 and annexin V [20].

In this study, the B cell and T cell population in PBMCs increased from the end of the tournament up to 24 h of the recovery stage. These results were different from the previous studies reporting that blood lymphocytes (NK cells, T cells, and B cells) increased

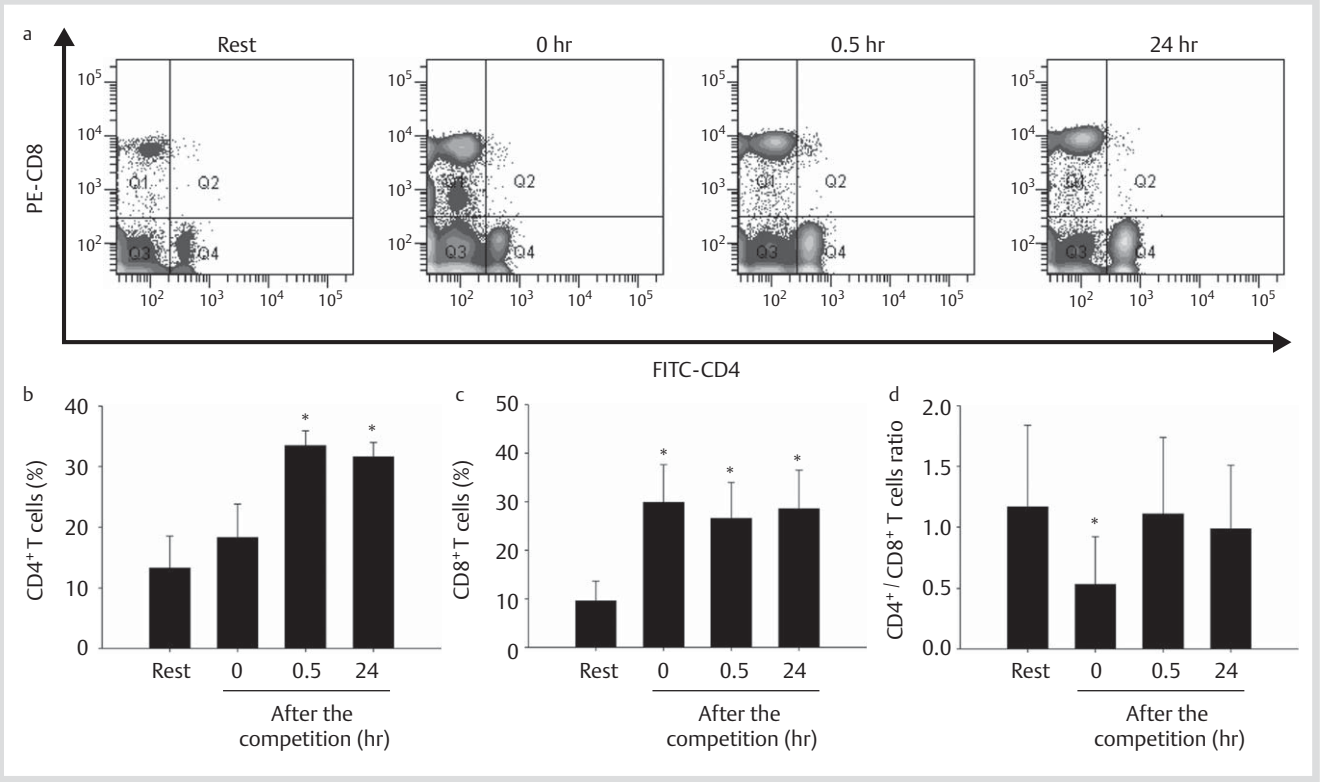

Fig. 6 Change of $CD4^+$ T cell and $CD8^+$ T cell population during the tournament. **a** One representative data was performed by flow cytometry (FITC-CD4 and PE-CD8). **b** $CD4^+$ T cell population in PBMCs at rest, and 0, 0.5 and 24 h after the tournament. **c** $CD8^+$ T cell population in PBMCs at rest, and 0, 0.5 and 24 h after the tournament. **d** CD4/CD8 ratio at rest, and 0, 0.5 and 24 h after the tournament. Each value represents mean ± SD. *, Statistical significance from "Rest" ($p < 0.05$).

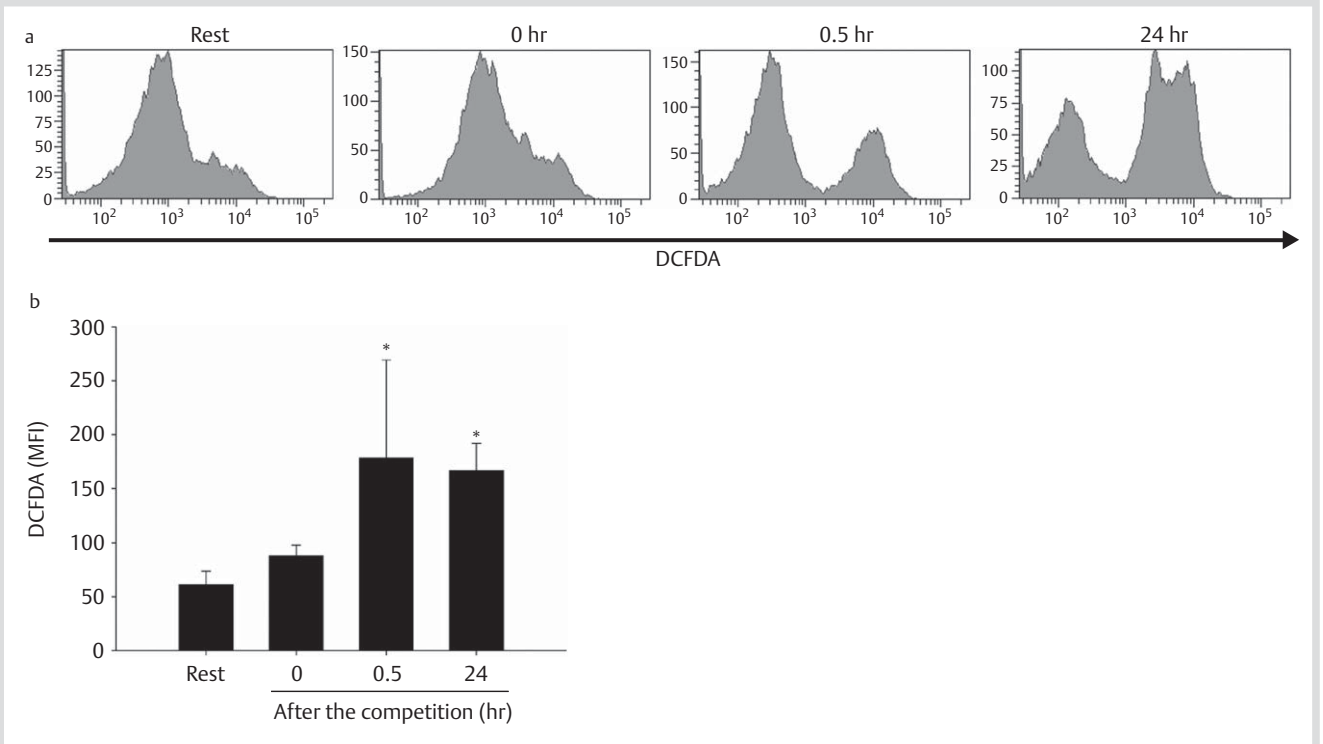

Fig. 7 Change of ROS^+ PBMCs population during the tournament. **a** One representative data was performed by flow cytometry (CM-H_2DCFDA). **b** ROS^+ PBMCs population at rest, and 0, 0.5 and 24 h after the tournament. *DCFDA*, 5-(and-6)-chloromethyl-2′, 7′-dichlorodihydro-fluorescein diacetate (CM-H_2DCFDA). Each value represents mean ± SD. *, Significant difference from "Rest" ($p < 0.05$).

during the exercises of approximate maximal intensity and decreased below the levels of resting stage after cessation of the exercises [52]. Furthermore, an insufficient number of previous studies on the changes of immune cells during Taekwondo competitions exists. In addition to quantifying the changes of lymphocytes, further functional evaluation including NK cell cytotoxic activity, lymphocyte activation markers, and cytokines release should be performed.

Recent evidence suggests that the CD4/CD8 ratio does not drop below 1.0 under normal conditions of healthy individuals [29]; however, the ratio decreases below 1.0 in stressful conditions such as intensified training in elite swimmers and HIV-1 patients [22,30]. The marked decrement of CD4/CD8 ratio shown in our study suggested that Taekwondo competitions could cause prominent changes of T cell-mediated immunity and might increase viral susceptibility in subjective athletes.

There are some limitations in this study. The small of only female study subjects might have attenuated the statistical power of data and led to biased results. However, several previous studies on Taekwondo competitions used a rationale similar to that in our study [7,23]. Statistical calculation based upon these previous studies showed that more than 5 subjects would be needed to yield a power of 80% (assuming α , 2-tailed, was set at 0.05) for the blood lactate level and HR between resting stage and cessation of Taekwondo competitions. The retrospective statistical calculation for this study also yielded a power of more than 80% for the blood lactate level, HR, CD4/CD8 ratio and ROS level between resting stage and cessation of the competitions.

The narrow age range of female-only subjects might make it possible to include more homogenous subjects and to reduce the inter-individual variability [8]. Most of the previous studies on Taekwondo athletes also had maximally narrowed parameters on sex, age, and athletic competency of subjects [5,7,8,23,35,48]. Lack of a control group and insufficient analyses confined to quantification of blood immune cells were also limitations of this study. In future, further controlled studies using molecular biological methods and subjects on a large scale should be performed for evaluating immunological changes including proliferation, activation, and functional differentiation of immune cells during Taekwondo competitions. It was suggested that hormonal responses including cortisol and catecholamine might have a mechanistic role in exercise-related immunological changes [33]. More detailed studies investigating the association between cell apoptosis and ROS production may also be needed.

In conclusion, after the Taekwondo competitions, CD4/CD8 ratio decreased and intracellular ROS levels increased continuously. These results suggest that the immunological functions of female adolescent athletes could be attenuated after Taekwondo competitions. B, T, and NK cells increased immediately after the Taekwondo competitions. During the recovery, B and T cells remained elevated and NK cells decreased. Further investigations on impacts of Taekwondo competitions on blood immune cells including functional and apoptotic changes induced by intracellular ROS production should be performed on a large scale for the improvement and protection of the health of adolescent athletes.

Affiliations

[1] Division of Allergy-Immunology and Rheumatology, Department of Internal Medicine, Kwandong University College of Medicine, Goyang, Republic of Korea

[2] Division of Physical Education, Silla University College of Medical and Life Sciences, Pusan, Republic of Korea

[3] Department of Physical Education, Yonsei University College of Sciences in Education, Seoul, Republic of Korea

[4] Division of Rheumatology, Department of Internal Medicine, Chung-Ang University College of Medicine, Seoul, Republic of Korea

[5] Department of Taekwondo, Dong-Eui University College of Sports Science, Pusan, Republic of Korea

[6] Ewha Womans University College of Pharmacy, Seoul, Republic of Korea

References

1 *Abbas AK*, *Lichtman AH*, *Pillai S*. Cellular and Molecular Immunology. (6th edition). Philadelphia, PA: WB Saunders Company, 2007; 566

2 *American College of Sports Medicine*: *Thompson WR*, *Gordon NF*, *Pescatello LS*. ACSM's Guidelines for Exercise Testing and Prescription. (8th edition). Philadelphia, PA: Lippincott Williams & Wilkins, 2010; 380

3 *Baran CP*, *Zeigler MM*, *Tridandapani S*, *Marsh CB*. The role of ROS and RNS in regulating life and death of blood monocytes. Curr Pharm Des 2004; 10: 855–866

4 *Borg GA*. Psychophysical bases of perceived exertion. Med Sci Sports Exerc 1982; 14: 377–381

5 *Bridge CA*, *Jones MA*, *Drust B*. Physiological responses and perceived exertion during international Taekwondo competition. Int J Sports Physiol Perform 2009; 4: 485–493

6 *Bridge CA*, *Jones MA*, *Hitchen P*, *Sanchez X*. Heart rate responses to Taekwondo training in experienced practitioners. J Strength Cond Res 2007; 21: 718–723

7 *Butios S*, *Tasika N*. Changes in heart rate and blood lactate concentration as intensity parameters during simulated Taekwondo competition. J Sports Med Phys Fitness 2007; 47: 179–185

8 *Chiodo S*, *Tessitore A*, *Cortis C*, *Cibelli G*, *Lupo C*, *Ammendolia A*, *De Rosas M*, *Capranica L*. Stress-related hormonal and psychological changes to official youth Taekwondo competitions. Scand J Med Sci Sports 2011; 21: 111–119

9 *Espersen GT*, *Elbaek A*, *Schmidt Olsen S*, *Ejlersen E*, *Varming K*, *Grunnet N*. Short-term changes in the immune system of elite swimmers under competition conditions. Different immunomodulation induced by various types of sport. Scand J Med Sci Sports 1996; 6: 156–163

10 *Feehan M*, *Waller AE*. Precompetition injury and subsequent tournament performance in full-contact taekwondo. Br J Sports Med 1995; 29: 258–262

11 *Filaire E*, *Alix D*, *Ferrand C*, *Verger M*. Psychophysiological stress in tennis players during the first single match of a tournament. Psychoneuroendocrinol 2009; 34: 150–157

12 *Gentleman RC*, *Carey VJ*, *Bates DM*, *Bolstad B*, *Dettling M*, *Dudoit S*, *Ellis B*, *Gautier L*, *Ge Y*, *Gentry J*, *Hornik K*, *Hothorn T*, *Huber W*, *Iacus S*, *Irizarry R*, *Leisch F*, *Li C*, *Maechler M*, *Rossini AJ*, *Sawitzki G*, *Smith C*, *Smyth G*, *Tierney L*, *Yang JY*, *Zhang J*. Bioconductor: open software development for computational biology and bioinformatics. Genome Biol 2004; 5: R80

13 *Gleeson M*. Can nutrition limit exercise-induced immunodepression? Nutr Rev 2006; 64: 119–131

14 *Gleeson M*, *Nieman DC*, *Pedersen BK*. Exercise, nutrition and immune function. J Sports Sci 2004; 22: 115–125

15 *Harriss DJ*, *Atkinson G*. Update – Ethical Standards in Sport and Exercise Science Research. Int J Sports Med 2011; 32: 819–821

16 *Hoffman-Goetz L*, *Pedersen BK*. Exercise and the immune system. In: Tipton CM. (ed.). ACSM's Advanced Exercise Physiology. Philadelphia, PA: Lippincott Williams & Wilkins, 2006; 482–500

17 *Hoffman Goetz L*, *Quadrilatero J*. Treadmill exercise in mice increases intestinal lymphocyte loss via apoptosis. Acta Physiol Scand 2003; 179: 289–297

18 *Hollander M*, *Wolfe DA*. Nonparametric Statistical Methods. (2nd edition). New York: Wiley, 1999; 295

19 *Iaia FM*, *Perez-Gomez J*, *Nordsborg N*, *Bangsbo J*. Effect of previous exhaustive exercise on metabolism and fatigue development during intense exercise in humans. Scand J Med Sci Sports 2010; 20: 619–629

20 *Janeway CA*, *Travers P*, *Walport M*, *Shlomchik MJ*. Immunobiology. (5th edition). New York: Garland Science, 2005; 887

21 *Karvonen MJ*, *Kentala E*, *Mustala O*. The effects of training on heart rate; a longitudinal study. Ann Med Exp Biol Fenn 1957; 35: 307–315
22 *Mackinnon LT*, *Hooper SL*, *Jones S*, *Gordon RD*, *Bachmann AW*. Hormonal, immunological, and hematological responses to intensified training in elite swimmers. Med Sci Sports Exerc 1997; 29: 1637–1645
23 *Matsushigue KA*, *Hartmann K*, *Franchini E*. Taekwondo: Physiological responses and match analysis. J Strength Cond Res 2009; 23: 1112–1117
24 *McArdle WD*, *Katch FI*, *Katch VL*. Exercise Physiology. (5th edition). Philadelphia: Lippincott Williams & Wilkins, 2001; 324
25 *Nieman DC*. Risk of upper respiratory tract infection in athletes: an epidemiologic and immunologic perspective. J Athl Train 1997; 32: 344–349
26 *Nieman DC*. Marathon training and immune function. Sports Med 2007; 37: 412–415
27 *Nieman DC*, *Pedersen BK*. Nutrition and Exercise Immunology. Boca Raton: CRC Press LLC, 2000
28 *Nieman DC*, *Miller AR*, *Henson DA*, *Warren BJ*, *Gusewitch G*, *Johnson RL*, *Davis JM*, *Butterworth DE*, *Herring JL*, *Nehlsen Cannarella SL*. Effect of high- versus moderate-intensity exercise on lymphocyte subpopulations and proliferative response. Int J Sports Med 1994; 15: 199–206
29 *Nigam PK*, *Patra PK*, *Khodiar PK*, *Gual J*. A study of blood CD3+, CD4+, and CD8+ T cell levels and CD4+:CD8+ ratio in vitiligo patients. Indian J Dermatol Venereol Leprol 2011; 77: 111
30 *O'Gorman MR*, *Zijenah LS*. CD4 T cell measurements in the management of antiretroviral therapy – A review with an emphasis on pediatric HIV-infected patients. Cytometry B Clin Cytom 2008; 74: (Suppl 1): S19–S26
31 *Patel H*, *Hoffman Goetz L*. Effects of oestrogen and exercise on caspase-3 activity in primary and secondary lymphoid compartments in ovariectomized mice. Acta Physiol Scand 2002; 176: 177–184
32 *Pawate S*, *Shen Q*, *Fan F*, *Bhat NR*. Redox regulation of glial inflammatory response to lipopolysaccharide and interferongamma. J Neurosci Res 2004; 77: 540–551
33 *Pedersen BK*, *Kappel M*, *Klokker M*, *Nielsen HB*, *Secher NH*. The immune system during exposure to extreme physiologic conditions. Int J Sports Med 1994; 15: (Suppl 3): S116–S121
34 *Phaneuf S*, *Leeuwenburgh C*. Apoptosis and exercise. Med Sci Sports Exerc 2001; 33: 393–396
35 *Pilz-Burstein R*, *Ashkenazi Y*, *Yaakobovitz Y*, *Cohen Y*, *Zigel L*, *Nemet D*, *Shamash N*, *Eliakim A*. Hormonal response to Taekwondo fighting simulation in elite adolescent athletes. Eur J Appl Physiol 2010; 110: 1283–1290
36 *R-Development-Core-Team*. R: A language and environment for statistical computing. Vienna, Austria R Foundation for Statistical Computing, ISBN 3-900051-07-0, URL http://wwwR-projectorg/ 2011
37 *Radak Z*, *Chung HY*, *Koltai E*, *Taylor AW*, *Goto S*. Exercise, oxidative stress and hormesis. Ageing Res Rev 2008; 7: 34–42
38 *Ravier G*, *Dugu B*, *Grappe F*, *Rouillon JD*. Maximal accumulated oxygen deficit and blood responses of ammonia, lactate and pH after anaerobic test: a comparison between international and national elite karate athletes. Int J Sports Med 2006; 27: 810–817
39 *Salvador A*, *Suay F*, *Gonzlez-Bono E*, *Serrano MA*. Anticipatory cortisol, testosterone and psychological responses to judo competition in young men. Psychoneuroendocrinol 2003; 28: 364–375
40 *Shek PN*, *Shephard RJ*. Physical exercise as a human model of limited inflammatory response. Can J Physiol Pharmacol 1998; 76: 589–597
41 *Shek PN*, *Sabiston BH*, *Buguet A*, *Radomski MW*. Strenuous exercise and immunological changes: a multiple-time-point analysis of leukocyte subsets, CD4/CD8 ratio, immunoglobulin production and NK cell response. Int J Sports Med 1995; 16: 466–474
42 *Shephard RJ*. Special feature for the Olympics: effects of exercise on the immune system: overview of the epidemiology of exercise immunology. Immunol Cell Biol 2000; 78: 485–495
43 *Shephard RJ*. Sepsis and mechanisms of inflammatory response: is exercise a good model? Br J Sports Med 2001; 35: 223–230
44 *Shephard RJ*, *Shek PN*. Immune responses to inflammation and trauma: a physical training model. Can J Physiol Pharmacol 1998; 76: 469–472
45 *Shephard RJ*, *Shek PN*. Effects of exercise and training on natural killer cell counts and cytolytic activity: a meta-analysis. Sports Med 1999; 28: 177–195
46 *Terry PC*, *Slade A*. Discriminant effectiveness of psychological state measures in predicting performance outcome in karate competition. Percept Mot Skills 1995; 81: 275–286
47 *Tipton CM*. ACSM's Advanced Exercise Physiology. Philadelphia, PA: Lippincott Williams & Wilkins, 2006; 684
48 *Tsai ML*, *Chou KM*, *Chang CK*, *Fang SH*. Changes of mucosal immunity and antioxidation activity in elite male Taiwanese taekwondo athletes associated with intensive training and rapid weight loss. Br J Sports Med 2011; 45: 729–734
49 *Tsai ML*, *Ko MH*, *Chang CK*, *Chou KM*, *Fang SH*. Impact of intense training and rapid weight changes on salivary parameters in elite female Taekwondo athletes. Scand J Med Sci Sports. 2010
50 *Vider J*, *Laaksonen DE*, *Kilk A*, *Atalay M*, *Lehtmaa J*, *Zilmer M*, *Sen CK*. Physical exercise induces activation of NF-kappaB in human peripheral blood lymphocytes. Antioxid Redox Signal 2001; 3: 1131–1137
51 *Viglietta V*, *Bourcier K*, *Buckle GJ*, *Healy B*, *Weiner HL*, *Hafler DA*, *Egorova S*, *Guttmann CR*, *Rusche JR*, *Khoury SJ*. CTLA4Ig treatment in patients with multiple sclerosis: an open-label, phase 1 clinical trial. Neurology 2008; 71: 917–924
52 *Walsh NP*, *Gleeson M*, *Shephard RJ*, *Woods JA*, *Bishop NC*, *Fleshner M*, *Green C*, *Pedersen BK*, *Hoffman-Goetz L*, *Rogers CJ*, *Northoff H*, *Abbasi A*, *Simon P*. Position statement. Part one: Immune function and exercise. Exerc Immunol Rev 2011; 17: 6–63
53 *Winett RA*, *Carpinelli RN*. The quantity and quality of exercise for healthy adults. Med Sci Sports Exerc 1999; 31: 916–920
54 *Wu F*, *Tyml K*, *Wilson JX*. iNOS expression requires NADPH oxidase-dependent redox signaling in microvascular endothelial cells. J Cell Physiol 2008; 217: 207–214