Sports Medicine, Training and Rehabilitation 10: 235–246, 2001

1057-8315/01 $12.00 + .00

Taylor&Francis
healthsciences

Immune Function in Adolescent Tennis Athletes and Controls

DRU A. HENSON, DAVID C. NIEMAN, AND MICHAEL W. KERNODLE

Appalachian State University

GERALD SONNENFELD

Morehouse School of Medicine

D. MORTON

Carolinas Medical Center

MILLA M. THOMPSON

Appalachian State University

Resting immune function and infection incidence were compared between 20 (10 male, 10 female) elite teenage tennis athletes and 18 (9 male, 9 female) non-athletic, age-matched controls. Male and female athletes trained an average of 17.6 ± 0.8 hours per week and possessed $\dot{V}O_2$max values that were 15% and 22% higher, respectively, than controls. NK cell counts were 53% higher ($p = 0.015$) and neutrophil counts were 16% lower ($p = 0.030$) in the athletes; however, salivary IgA output, serum/plasma concentration of IL-6, Il-1ra, cortisol, and growth hormone, PHA-induced lymphocyte proliferation and IL-2 production, and the incidence of upper respiratory tract infections (URTI) during 2.5 months did not differ between groups. These data suggest

This work was funded by the United States Tennis Association.

We wish to acknowledge the assistance of the following individuals in this research project: Brian Schilling, Alan Jung, Max Shute, Marc Bolton, Cristiana Piccinni, and Lora McCoy.

Address correspondence to Dru A. Henson, Ph.D., Department of Biology, Appalachian State University, Boone, NC 28608. E-mail: hensonda@appstate.edu

that despite intensive training by adolescent tennis athletes, immune function and URTI incidence are normal. The NK cell elevation is consistent with previous studies in athletes that show an enhanced recirculation and activity of NK cells.

Keywords Natural killer cells, lymphocyte proliferation, salivary IgA, IL-6, IL-1ra

Introduction

Among athletes and coaches, a common perception is that athletes are at an increased risk of upper respiratory tract infection (URTI) during a period of intensive training or following a major competitive event (Mackinnon, 1997; Nieman, 1997; Peters, 1997). Most studies suggest that the adaptive immune system, as reflected in measures of T- and B-cell function, is not affected by prolonged intensive exercise (Nieman, Brendle, et al., 1995; Nieman, Buckley, et al., 1995; Tvede, Steensberg, Baslund, Kristensen, & Pedersen, 1991). However, several markers of innate immune function do appear to be differentially altered by the chronic stress of intensive exercise, including the augmentation of natural killer cell activity (NKCA) and the suppression of neutrophil function (Baj et al., 1994; Gleeson et al., 1995; Hack, Strobel, Weiss, & Weicker, 1994; Nieman, 1998; Nieman et al., 2000; Nieman, Buckley, et al., 1995; Pedersen et al., 1989; Pyne et al., 1995; Smith, 1997). Indicators of mucosal immunity also appear to be affected by prolonged, intense training. This has been suggested by a report of lower resting salivary IgA concentration in elite cross-country skiers compared with non-athletic controls (Tomasi, Trudeau, Czerwinski, & Erredge, 1982), and by several reports of a progressive decline in resting salivary IgA concentration in elite swimmers throughout their competitive season (Gleeson et al., 1995, 1999; Mackinnon & Hooper, 1994).

Despite the significant difference in immune function between athletes and non-athletes, investigators have had little success in linking this to an alteration in the incidence of infectious illness (Nieman & Pedersen, 1999; Peters, 1997; Pyne & Gleeson, 1998). To date, only salivary IgA has emerged as a potential marker for infection risk in athletes, however this has not been consistently observed. In a study of elite hockey and squash athletes, Mackinnon, Ginn, and Seymour (1993) provided suggestive evidence of a link between a decreased salivary IgA concentration and subsequent URTI. Similarly, Gleeson et al. (1999) demonstrated a significant correlation between suppression of salivary IgA level and infection rate in elite swimmers. However, in a study of elite female rowers, Nieman et al. (2000) reported that the number of days with URTI was not associated with salivary IgA concentration or other immune measure; Pyne et al. (1995) also found no significant difference in URTI incidence between elite swimmers and age- and sex-

matched secondary controls, despite a significant suppression in neutrophil oxidative activity.

Few data are currently available on the effect of sports participation on immune function in adolescents. Limited studies have indicated that adolescent sports participants have a suppressed resting bactericidal (Wolach et al., 1998) and natural killer (NK) cell activity (Boas et al., 1996), and a reduced number of circulating NK cells and granulocytes (Boas et al., 1996) compared with untrained controls. However, athletic participation does not appear to be associated with a higher incidence of respiratory infections (Österback & Qvarnberg, 1987). The purpose of this study was to compare various indicators of resting immune function in adolescent male and female tennis athletes with the same indicators in non-athletic controls. These data were then related to the history of URTI in the groups in an attempt to define the clinical importance of any immune data alteration.

Materials and Methods

Subjects

Subjects included 20 (10 male, 10 female) elite teenage tennis athletes (age range, 14 to 18 years) located at the Van Der Meer World Class Tennis Training Center in Hilton Head, South Carolina, and 18 (9 male, 9 female) non-athletic, age-matched controls from the surrounding community. Informed consent was obtained from each participant, and the experimental procedures were in accordance with the policy statements of the institutional review board of Appalachian State University.

Research Design

In this cross-sectional study, immune function, plasma hormone concentration, and two and one-half month upper respiratory trace infection histories were contrasted between male and female tennis players and controls. Blood samples were collected from all subjects in mid-May, a time at which the tennis players were undergoing intense training.

Cardiorespiratory Fitness Assessment

Maximal aerobic power and heart rate were measured during a graded cycle ergometer protocol (Storer, Davis, & Caiozzo, 1990), using the MedGraphics CPX Express metabolic system (MedGraphics Corporation, St. Paul, MN) and chest heart rate monitors (Polar Electro Inc., Woodbury, NY). Body composition was estimated from seven skinfolds using the equations of Jackson and Pollock (1985). An estimate of weekly exercise training duration was reported by each subject in a questionnaire response.

Blood Samples

Blood samples were collected from the athletes and controls at 6:30 a.m., after they had avoided food and exercise for nine hours. A 35-ml sample was drawn from an antecubital vein with the subject in the supine position. Routine complete blood counts (CBC) were performed by a clinical hematology laboratory (Lab Corp, Burlington, NC), which provided leukocyte subset counts, hemoglobin, and hematocrit values, respectively, for each subject.

Mitogen-induced lymphocyte proliferation and IL-2 production were determined in whole blood cultures using phytohemagglutinin (PHA) at optimal and suboptimal doses previously determined by titration experiments. Heparinized venous blood was diluted 1:10 with complete media (RPMI-1640 supplemented with 10% heat-inactivated fetal calf serum, penicillin, streptomycin, L-glutamine, and 2-mercaptoethanol). PHA was prepared in PRMI-1640 media, at a concentration of 1 mg/ml, and then further diluted with complete media to optimal and suboptimal working concentrations (5 μg/ml and 25 μg/ml). A 100 μl aliquot of diluted blood was dispensed into each of triplicate wells of a 96-well flat-bottom microtiter plate. To each of these was added 100 μl of the mitogens at the appropriate dose. Control wells received an aliquot of complete media instead of mitogens. After 72h incubation at 37° C, the cells were pulsed with 1 μCi of thymidine (methyl)-^{3}H (New England Nuclear, Boston) prepared with RPMI 1640. Before pulsing with thymidine (methyl)-^{3}H, a 100 μl aliquot was removed and frozen for interleukin-2 (IL-2) analysis. After pulsing, cells were incubated for an additional four hours before harvesting. Samples were harvested using a Filtermate Cell Harvester (Packard Instruments, Meridien, CT), onto a 96-well Unifilter GF/C plates (Packard). A small amount (25 μl) of scintillation fluid (Microscint 20, Packard) was added to each well and radionucleotide incorporation was assessed on a Topcount Microscintillation Counter (Packard). Background counts from control wells were subtracted from the mitogens-induced counts. The IL-2 concentration in each well was determined using a quantitative sandwich ELISA kit provided by R&D Systems, Inc. (Minneapolis, MN) with a sensitivity of < 7 pg/ml.

The proportions of T cells ($CD3^{+}$), B cells ($CD19^{+}$), and NK cells ($CD3^{-}$ $CD16^{+}CD56^{+}$) were determined in whole blood preparations and absolute numbers were calculated using CBC data to allow group comparison of blood concentration cells. Lymphocyte phenotyping was accomplished by two-color fluorescent labeling of cell surface antigens with mouse anti-human monoclonal antibodies conjugated to fluoresceinisothiocyanate (FITC) and phycoerythrin (PE) using Simultest monoclonal antibodies and isotype controls (Becton Dickinson, San Jose, CA). For immunophenotyping, 50 μl aliquots of heparinized whole blood from each sample were added to five wells of a 96 well plate. Five μg (diluted in 50 μl RPMI) of each antibody or isotype control were added to appropriate wells for 20 minutes on ice in the dark with orbital shaking (170 rmp). The cell suspension was then lysed using 200 μl FACSlyse solution (Becton Dickinson) for 10

minutes in the dark on ice with shaking. Plates were then centrifuged for five minutes (Beckman GS-R6 Centrifuge) at 1500 x g. Samples were kept at 4° C in the dark until analyzed by flow cytometry (FacsCalibur, Becton Dickinson).

Total serum concentration of interleukin-1 receptor antagonist (IL-1ra) and interleukin-6 (IL-6) were determined using quantitative sandwich ELISA kits provided by R&D Systems, Inc. (Minneapolis, MN) and Endogen, Inc. (Woburn, MA), respectively. A standard curve was constructed using standards provided in the kits and the cytokine concentration was determined from standard curves using linear regression analysis. The assays were a two step "sandwich" enzyme immunoassay in which samples and standards were incubated in a 96-well microtiter plate coated with monoclonal antibodies for IL-1ra or IL-6 as the capture antibody. Following the appropriate incubation time, the wells were washed and a second detection antibody conjugated to horseradish peroxidase was added. The plates were incubated and washed, and the amount of bound enzyme-labeled detection antibody was measured by adding a chromogenic substrate. The plates were then read at the appropriate wavelength (450 minus 570 nm for IL-1ra and 450 nm for IL-6). The minimum detectable concentration of IL-1ra was 14 pg/ml and IL-6 was <1 pg/ml.

Plasma cortisol was assayed using a competitive solid-phase ^{125}I radioimmunoassay (RIA) technique (Diagnostic Products Corporation, Los Angeles, CA). RIA kits were also used to determine the plasma concentration of growth hormone according to the manufacturer's instructions (Diagnostic Products Corporation, Los Angeles, CA).

Salivary IgA

Unstimulated saliva was collected for four minutes into 5 ml plastic, sterilized vials. Participants were urged to pass as much saliva as possible into the vials during the 4-minute timed session. The saliva was then frozen until further analysis. Saliva volume was measured to the nearest 0.1 ml, and saliva total protein was quantified using the Coomassie® protein assay reagent, a modification of the Bradford (1976) Coomassie® dye binding colorimetric method. Salivary IgA was measured by enzyme linked to immunosorbent assay according to the procedures of the Hunter Immunology Unit (Royal Newcastle Hospital, Newcastle, NSW, Australia) (personal communication, Dr. Maree Gleeson). The data were expressed as the concentration of salivary IgA relative to total protein (μg/mg) and salivary immunoglobulin secretion rate (μg/min).

Statistical Analysis

Statistical significance was set at the $p = 0.05$ and the values are expressed as mean ± SEM. Independent *t*-tests were performed, contrasting all measured variables for the athletes and controls.

Results

Table 1 summarizes the physical and training characteristics of the male and female tennis athletes and controls. Groups were similar in age (range, 14–18 years) and the athletes trained an average of 17.6 ± 0.8 hours per week. Compared to their non-athletic, sex-matched counterparts, male athletes were similar in height and body mass, but had a 32% lower body fat percentage, a 15% higher $\dot{V}O_{2max}$, and a 17% higher $\dot{V}E_{max}$. Likewise, female athletes were similar in height and body mass, and had a 22% higher $\dot{V}O_{2max}$ and a 37% higher $\dot{V}E_{max}$ than the non-athletic females. Since no gender influence was observed for the immune data, all male and female athlete data were combined for subsequent analysis.

As shown in Table 2 and Figure 1, the neutrophil count was 16% lower ($p = 0.030$) and the natural killer cell count was 53% higher ($p = 0.015$) in tennis athletes than in the controls. Other leukocyte and lymphocyte subset counts were not significantly different between groups (Table 2). No differences were observed between groups for PHA-induced lymphocyte proliferation, PHA-induced IL-2 production, salivary IgA concentration or secretion rate, or serum/plasma concentration of IL-6, IL-1ra, cortisol, and growth hormone (Table 3). A two and one-half month health log indicated that the upper respiratory tract infection (URTI) rates were similar between athletes and controls, with athletes exhibiting 4.2 ± 1.2 days and controls 6.6 ± 1.1 days with symptoms of upper respiratory tract infection ($p = 0.226$). In addition, athletes indicated a perception that they experienced the same, or fewer, number of sick days compared to their non-athletic peers.

Discussion

The present study compared immune function in elite adolescent tennis athletes and non-athletic controls. Despite a marked difference in $\dot{V}O_{2max}$ and physical activity, only circulating numbers of NK cells (higher in athletes) and to a lesser extent, neutrophils (lower in athletes), emerged as being significantly different between groups. PHA-induced lymphocyte proliferation and IL-2 production, salivary IgA concentration, serum concentration of IL-6 and IL1ra, and the incidence of URTI were similar between the athletes and controls. These data suggest that immune function in adolescent athletes and controls may be more similar than disparate.

A consistent finding among cross-sectional studies is an enhancement of NK cell activity (NKCA) in athletes compared to non-athletes, in both adult and elderly populations (Nieman et al., 1993, 2000; Nieman Buckley, et al., 1995; Pedersen et al., 1989; Tvede et al., 1991). The higher NKCA has been attributed to a greater concentration of circulating NK cells or to an enhanced cytotoxic activity of individual NK cells. Compared to sedentary controls, NKCA was 57% higher in experienced marathon runners (Nieman, Buckley, et al., 1995), 160% higher in elite rowers (Nieman et al., 2000), and 64% higher in elite cyclists during their high

Table 1
Characteristics of Male and Female Adolescent Tennis Athletes and Controls

	Males		Females	
	Athletes (N = 10)	Controls (N = 9)	Athletes (N = 10)	Controls (N = 9)
Age (yr)	15.9 ± 0.4	16.1 ± 0.5	15.9 ± 0.5	15.9 ± 0.3
Height (cm)	70.2 ± 0.7	69.4 ± 1.1	65.0 ± 0.9	64.6 ± 1.2
Body mass (kg)	69.5 ± 2.3	70.5 ± 2.7	61.8 ± 3.4	58.6 ± 2.4
Body mass index ($kg \cdot m^{-2}$)	21.8 ± 0.5	22.8 ± 1.0	22.5 ± 0.8	21.0 ± 0.6
Body fat (%)	9.5 ± 1.0*	13.9 ± 1.7	20.3 ± 1.8	21.8 ± 1.8
HR_{max} ($beats \cdot min^{-1}$)	201 ± 2	197 ± 4	190 ± 2	195 ± 4
VO_{2max} ($ml \cdot min^{-1} \cdot kg^{-1}$)	52.7 ± 0.9*	45.8 ± 2.0	43.3 ± 1.9*	35.5 ± 1.7
VE_{max} ($ln \cdot min^{-1}$)	131 ± 5*	112 ± 6	104.2 ± 2.5*	76.3 ± 5.9
RR_{max} ($breaths \cdot min^{-1}$)	54.5 ± 3.2	45.3 ± 3.0	55.5 ± 1.8*	45.6 ± 2.1
RER_{max}	1.16 ± 0.02	1.19 ± 0.02	1.22 ± 0.02	1.23 ± 0.02
Exercise training ($hr \cdot wk^{-1}$)	17.6 ± 1.4**	2.3 ± 0.6	17.7 ± 0.7**	2.72 ± 0.72

HR_{max} = maximal heart rate; VO_{2max} = maximal oxygen uptake; VE_{max} = maximal ventilation rate; RR_{max} = maximal respiratory rate; RER_{max} = maximal respiratory exchange ratio. Significant difference between athletes and sex-matched controls: * p = 0.05, ** p = p.001.

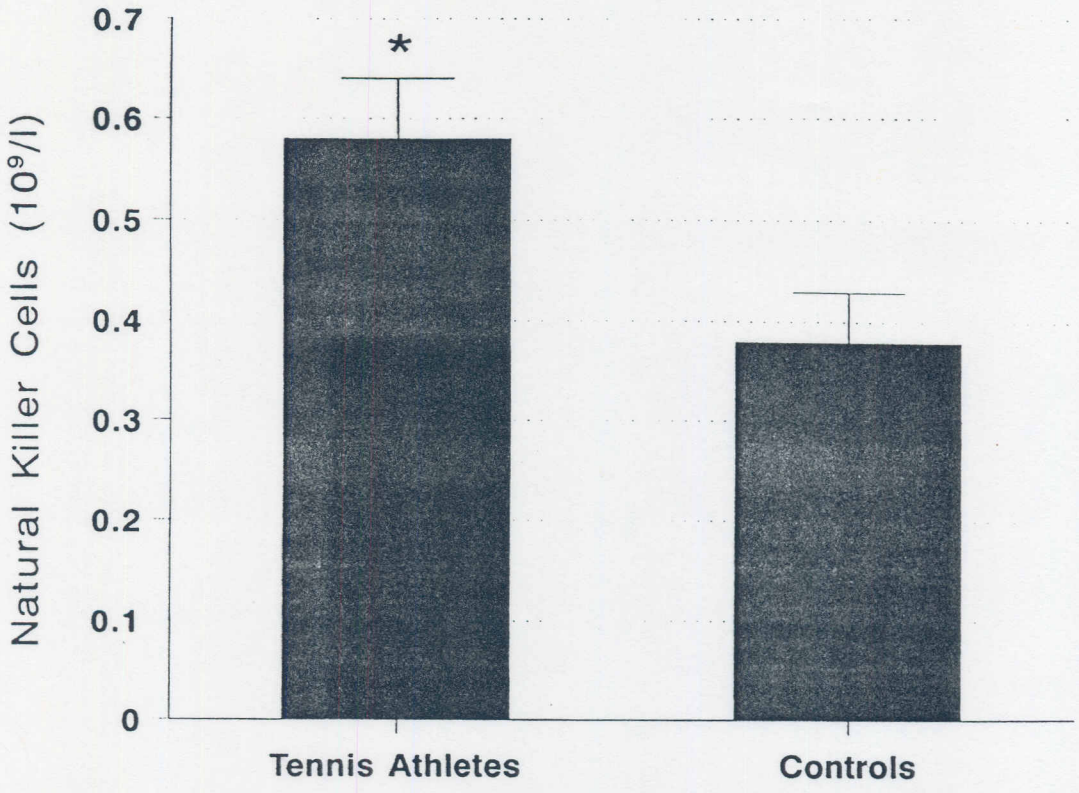

Figure 1. Natural killer cell concentrations in tennis athletes and controls. NK cell concentrations were 53% higher in tennis athletes (N = 20) versus non-athletic controls (N = 18). *$p \leq 0.05$.

intensity training season (Tvede et al., 1991). This increase occurred despite a similar NK cell concentration among the groups, indicative of an enhanced lytic capacity of the athletes' NK cells. In contrast, Pedersen et al. (1989) reported a higher NKCA value in elite cyclists during their low training season compared to sedentary controls, but attributed this to an increase in the percentage of circulating NK cells. Contrary to these alterations in adults, Boas et al. (1996) reported that resting NK cell numbers and cytolytic activity were reduced in a group of highly trained adolescent swimmers compared to untrained, non-swimmers. In the present study, we observed a 53% higher number of circulating NK cells in the tennis athletes compared to controls. Although NKCA was not measured, it is reasonable to assume that the elevated NK cell numbers is reflected by an enhanced cytolytic capacity of the blood compartment. The clinical significance of such an enhancement is unclear.

In contrast to the present authors' observation with NK cells, neutrophil concentration was slightly lower (16%) in the tennis athletes than in the controls. Although numerous studies have reported that circulating neutrophil numbers are

Table 2
Blood and Leukocyte Subset Counts in Adolescent Tennis Athletes and Controls

10^9 cells/l	Tennis Athletes (N = 20)	Controls (N = 18)
Total leukocytes	6.63 ± 0.26	7020 ± 0.24
Neutrophils	3.38 ± 0.17*	4.03 ± 0.24
Total lymphocytes	2.53 ± 0.13	2.37 ± 0.13
T-cells (CD3+)	1.35 ± 0.11	1.39 ± 0.12
B-cells (CD19+)	0.42 ± 0.04	0.42 ± 0.05
Neutrophil/lymphocyte ratio	1.39 ± 0.08*	1.77 ± 0.14
Monocytes	0.43 ± 0.04	0.51 ± 0.04
Eosinophils	0.27 ± 0.03	0.29 ± 0.03

Significant difference between athletes and controls: * p = 0.05.

not chronically altered in highly trained runners (Hack, Strobel, Rau, & Weicker, 1992; Nieman, Brendle, et al., 1995), cyclists (Lewicki, Tchórzewski, Denys, Kowalska, & Goliska, 1987), or rowers (Nieman et al., 2000), several studies have reported a lower neutrophil count in athletes (Blannin, Chatwin, Cave, & Gleeson, 1996; Boas et al., 1996; Nieman, Buckley, et al., 1991). Compared to sedentary controls, Blannin et al. (1996) reported a significantly lower resting neutrophil count in a small group of trained cyclists, and Nieman, Buckley, et al. (1995) reported a tendency for neutrophils to be lower in marathon runners. In addition, highly trained adolescent swimmers were reported to have a 19% lower resting granulocyte count than untrained, non-swimmers (Boas et al., 1996), a decrease similar in magnitude to the neutropenia observed in the present study. Since it has been reported that a high leukocyte, neutrophil, and monocyte count are risk factors for ischemic disease, the decreased neutrophil count observed in tennis players may be interpreted as consistent with a lowering of risk status (Ernst, Hammerschmidt, Bagge, Matrai, & Dormandy, 1987).

Several investigators have shown that intensive exercise training is associated with a decreased level of salivary IgA. Tomasi et al. (1982) reported a lower resting salivary concentration in elite cross-country skiers compared to non-athletic controls, and several studies have reported a significant, progressive decline in resting salivary IgA concentration in elite swimmers throughout their competitive season (Gleeson et al., 1995, 1999; Mackinnon & Hooper, 1994). To date, the suppression of salivary IgA is the only immune alteration that has been directly

Table 3
Immune Function and Hormone Concentration in Adolescent Tennis Athletes and Controls

	Tennis Athletes (N = 20)	Controls (N = 18)
Saliva IgA secretion rate (μg • min ^{1})	326 ± 28	295 ± 40
Saliva protein IgA concentration (μg • mg ^{1})	459 ± 39	480 ± 42
PHA-induced IL-s production (pg • ml ^{1})	37.4 ± 25.1	62 ± 32.8
PHA-induced lymphocyte proliferation		
PHA 5 μg • ml ^{1} (counts/min•10 ^{3})	16.4 ± 1.5	15.7 ± 1.6
PHA 25 μg • ml ^{1} (counts/min • 10 ^{3})	19.7 ± 1.5	19.7 ± 1.7
IL-6 (pg • ml ^{1})	1.00 ± 0.22	0.75 ± 0.18
IL-l receptor antagonist (pg • ml ^{1})	172 ± 12	172 ± 8
Cortisol (nmol • l ^{1})	776 ± 33	737 ± 37
Growth hormone (ng • ml ^{1})	2.14 ± 0.77	2.16 ± 1.00

associated with the appearance of URTI in athletes; however this association has not been consistently established (Mackinnon et al., 1994; Pyne & Gleeson, 1998). In the present study, we found no difference between salivary IgA concentration (expressed per total protein) and secretion rate in tennis athletes and controls, a finding consistent with the authors' previous observation on elite female rowers (Nieman et al., 2000).

No difference in either PHA-induced proliferation or IL-2 production was observed between tennis athletes and controls. This proliferation result is consistent with other cross-sectional reports comparing non-athletes and elite cyclists (Tvede et al., 1991) or collegiate and middle-aged runners (Nieman, Brendle, et al., 1995; Nieman, Buckley, et al., 1995). Similarly, serum pro- and anti-inflammatory cytokine concentration was not significantly different between groups, a finding consistent with previous findings from the author's laboratory in which plasma concentration of IL-6, Il-1ra, and tumor necrosis factor was similar in elite female rowers and non-athletes (Nieman et al., 2000).

These data indicate that despite physical training engaged in by elite adolescent tennis athletes, susceptibility to respiratory infection is not increased and there is no impairment of the immune system. This suggests that adolescent athletes are able to handle the physical and emotional challenge of their lifestyle without experiencing a detrimental effect on their health.

Pedersen, B. K., N. Tvede, L. D. Christensen, K. Klarlund, S. Kragbak, and Halkjær-Kristensen. 1989. *International Journal of Sports Medicine* 10: 129.
Peters, E. M. 1997. *International Journal of Sports Medicine* 18: S69.
Pyne, D. B., M. S. Baker, P. A. Fricker, W. A. McDonald, R. D. Telford, and M. J. Weideman. 1995. *Med. Sci Sports Exerc.* 27: 536.
Pyne, D. B., and M. Gleeson. 1998. *International Journal of Sports Medicine* 19: S183.
Smith, J. A. 1997. *International Journal of Sports Medicine* 18: S46.

Stofer, T. W., J. A. Davis, and V. J. Caiozzo. 1990. *Med. Sci. Sports Exerc.* 22: 764.
Tomasi, T. B., F. B. Trudeau, D. Czerwinski, and S. Erredge. 1982. *Journal of Clinical Immunology* 2: 173.
Tvede, N., J. Steensberg, B. Baslund, J. H. Kristensen, and B. K. Pedersen. 1991. *Scandinavian Journal of Sports Medicine* 1: 163.
Wolach, B., A. Eliakim, R. Gavrieli, E. Kodesh, Y. Yarom, M. Schlesinger, and B. Falk. 1998. *Scand. J. Med. Sci. Sports* 8: 91.