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The Acute Response of the Immune System to Tennis Drills in Adolescent Athletes

David C. Nieman, Michael W. Kernodle, Dru A. Henson, Gerald Sonnenfeld, and Darla S. Morton

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Many components of the immune system exhibit change after intensive and prolonged exertion, including neutrophilia (high blood neutrophil counts), lymphopenia (low blood lymphocyte counts), decrease in natural killer cell cytotoxic activity, decrease in mitogen-induced lymphocyte proliferation (a measure of T cell function), increase in plasma concentrations of pro- and anti-inflammatory cytokines, for example, interleukin-6 (IL-6), interleukin-10 (IL-10), and interleukin-1 receptor antagonist (IL-1ra), decrease in *ex vivo* production of cytokines in response to mitogens, and decrease in nasal and salivary IgA concentration (see reviews by Gabriel & Kindermann, 1997; Mackinnon, 1999; Nieman, 1997; Nieman & Nehlsen-Cannarella, 1994). Depending on the immune measure, these changes take from 3 hr to 3 days to return to pre-exercise levels. Together, these data suggest that the immune system is suppressed and stressed, albeit transiently, following prolonged endurance exercise.

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David C. Nieman, Michael W. Kernodle, and Dru A. Henson are with the Department of Health and Exercise Science at Appalachian State University. Gerald Sonnenfeld is with the Department of Microbiology and Immunology at Morehouse School of Medicine. Darla S. Morton is with the Department of General Surgery Research at Carolinas Medical Center.

Whether these acute immune changes occur following extended bouts of sports drills, play, or competition is largely unknown (Karpovich, 1935; Oyster, 1980; Tharp, 1991; Wake, Graham, & McGrath, 1953). Nearly all acute exercise immunology studies have measured responses to cycling, running, swimming, rowing, cross-country skiing, and other modes of endurance exercise (Nieman, 1997). The response of the immune system to 1–3 hr of endurance exercise may differ from sports participation that incorporates an element of play, a discontinuous mixture of aerobic and anaerobic exertion, and lower postexercise blood concentrations of stress hormones (Bishop, Blannin, Robson, Walsh, & Gleeson, 1999).

Österback & Qvarnberg (1987) reported that the incidence of respiratory tract infections was not elevated in young participants actively engaged in sports compared to controls. These data imply that youth can participate in sports for several hours daily without negative changes in immunosurveillance. Few data are currently available on exercise-induced changes in immune function among adolescents, which in general appears similar to adults (Boas et al., 1996; Tharp, 1991; Wolach et al., 1998).

In this study, the response of the immune system to a 2-hr intensive bout of tennis drills was measured in adolescent male and female athletes. We hypothesized that perturbations in immunity following intensive tennis drills would be of lower magnitude than previously measured in response to continuous, endurance exercise (Bishop et al., 1999; Nieman & Pedersen, 1999). Participants ingested carbohydrate beverages during the tennis drills according recommendations from sports nutrition profes-

sionals. Previous research has shown that carbohydrate compared to placebo ingestion has little or no influence on the immune response to sports drills (Bishop et al., 1999; Nieman et al., 1999).

Methods

Participants

Participants included 10 male and 10 female elite teenage tennis athletes (age range: 14–18 years) from the Van Der Meer World Class Tennis Training Center in Hilton Head, South Carolina. 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. The athletes reported an estimate of weekly exercise training duration in a questionnaire. 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 & Pollock (1985).

Blood Sampling and Exercise Paradigm

The athletes reported to the tennis facility at 6:30 a.m., having avoided food and exercise for 9 hr. A 35-ml blood sample was drawn from each participant. At 7:30 a.m., after ingesting 600 ml of a 6% carbohydrate beverage (Gatorade, Gatorade Sports Science Institute, Barrington, IL), the athletes began an intensive 2-hr bout of tennis drills. They took rest breaks of 4–5 min every 15 min to ingest the carbohydrate beverage (600 ml/hr) and measure heart rate (chest heart rate monitors) and rate of perceived exertion (RPE; 6–20 scale; Borg, 1982). The coaching staff directed the 20 athletes through a series of dynamic drills (cross-court rallies, overhead recovery, approach shot, and drop shot drills, etc.) on five separate tennis courts. During each 15-min drill session, the coaches kept the athletes moving as intensely as possible.

Immediately after exercise (9:30 a.m.), another 35-ml blood sample was taken, followed by a 1-hr postexercise sample at 10:30 a.m. Body mass and ambient temperature and humidity were measured pre- and postexercise. Participants drank 600 ml of the carbohydrate beverage during the 1 hr of recovery.

Assays for Immune Function

All blood samples were obtained from an antecubital vein from the athletes while in the supine position. Saliva and plasma samples (hormones and cytokines) were immediately frozen and stored at -80°C until analysis. Leukocyte and lymphocyte subset counts, mitogen-induced lymphocyte proliferation, and IL-2 production assays were conducted on the same day of sample collection. Routine complete blood counts (CBC) were performed by a clinical hematology laboratory (Lab Corp, Burlington, NC), and provided leukocyte subset counts, hemoglobin, and hematocrit.

PHA-Induced Lymphocyte Proliferation and IL-2 Production. The mitogenic response of lymphocytes in whole blood culture was determined 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 RPMI 1640 media at a concentration of 1 mg/ml and further diluted with complete media to the optimal and suboptimal working concentrations (5 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 mitogen at the appropriate dose. Control wells received complete media instead of mitogen. After a 72-hr incubation at 37°C, the cells were pulsed with 1 µCi of thymidine (methyl)-^{3}H (New England Nuclear, Boston, MA) prepared with RPMI 1640. Before pulsing with thymidine (methyl)-^{3}H, a 100µl aliquot was removed and frozen for IL-2 analysis. After pulsing, cells were incubated for an additional 4 hr before harvesting. Samples were harvested using a Filtermate Cell Harvester (Packard Instruments, Meridien, CT) onto 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 mitogen-induced counts. IL-2 was determined using a quantitative sandwich ELISA kit provided by R&D Systems, Inc. (Minneapolis, MN).

Lymphocyte Subsets. 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 comparisons on blood concentrations of cells. Lymphocyte phenotyping was accomplished by two-color fluorescent labeling of cell surface antigens with mouse antihuman monoclonal antibodies conjugated to fluoresceinisothiocyanate and phycoerythrin using Simultest monoclonal antibodies and isotype controls (Becton Dickinson, San Jose, CA). For immunophenotyping, 50µl aliquots of heparinized whole blood from each sample were added to 5 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 min in the dark on ice with orbital shak-

ing (170 rpm). The cell suspension was then lysed using a 200-µl FACSlyse solution (Becton Dickinson) for 10 min in the dark on ice with shaking. Plates were then centrifuged for 5 min (Beckman GS-R6 Centrifuge) at 1,500 x g. Samples were kept at 4°C in the dark until analyzed by flow cytometry (FacsCalibur, Becton Dickinson).

Salivary IgA. Unstimulated saliva was collected for 4 min into 5-ml plastic, sterilized vials. Participants were urged to pass as much saliva as possible during the 4-min timed session. Saliva volume was measured to the nearest 0.1 ml and then frozen until analysis. 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 immunosorbent assay according to the procedures of the Hunter Immunology Unit (Royal Newcastle Hospital, Newcastle, New South Wales, Australia; personal communication, Maree Gleeson). The data were expressed as concentration of sIgA ($\mu g \cdot ml^{-1}$), concentration of sIgA relative to total protein concentration ($\mu g \cdot mg^{-1}$), and salivary immunoglobulin secretion rate ($\mu g \cdot min^{-1}$).

Plasma Cytokines. Total serum concentrations 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 concentrations were determined from the 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 - 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.

Hormones, Lactate, and Plasma Volume. 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 plasma concentrations of growth hormone according to manufacturers instructions (Diagnostic Products Corporation, Los Angeles CA). Lactate was measured from finger stick blood samples using a lactate analyzer (YSI 2300 Stat Plus analyzer, YSI Incorporated, Yellow Springs, OH). The finger stick blood samples were taken simultaneously with blood sample collection from the antecubital vein. Plasma volume changes were estimated from blood hematocrit and hemoglobin values using the method of Dill and Costill (1974).

Statistical Analysis

Immune and hormone values were analyzed using repeated measures analysis of variance (ANOVA). If the overall time effect from the repeated measures ANOVA was significant ($p < .05$), changes from pre-exercise to immediate and 1-hr postexercise values were tested for significance using paired t tests ($p < .01$ due to repeated tests).

Results

Participant characteristics are summarized in Table 1. Body fat percentage and maximal oxygen consumption values for the male and female adolescent tennis athletes were within the ranges published for adult tennis athletes (Wilmore, 1989). The athletes trained at a pace that elicited a heart rate of 159 ± 4 beats/min (81 ± 2.4% maximum heart rate) and a RPE of 12.8 ± 0.8 or "somewhat hard." Blood lactate levels rose slightly from 0.86 ± 0.07 to 2.06 ± 0.39 mmol/l immediately postexercise. Temperature during the 2-hr bout of tennis drills was 18°C at a relative humidity of 78%. Each participant ingested 2.4 l of carbohydrate fluid between 6:30 and 10:30 a.m. Body mass and plasma volume did not change significantly from pre-exercise levels.

Immune and hormonal responses to the bout of tennis drills are summarized in Tables 2 and 3. The patterns of change shown by male and female athletes did not differ significantly. Thus, the data are presented for all 20 athletes combined. Significant overall time effects from the repeated measures ANOVA were measured for each leukocyte and lymphocyte subset listed in Table 2. The neutrophil and monocyte counts increased 77% and 26%, respectively, immediately postexercise ($p < .01$ compared to pre-exercise), with the lymphocyte count (primarily natural killer cells) falling 19% by 1 hr postexercise ($p <$

Table 1. Characteristics of the adolescent tennis athletes

Variable	Boys ($n = 10$)		Girls ($n = 10$)	
	M	*SD*	*M*	*SD*
Age (years)	15.9	0.4	15.9	0.5
Height (m)	1.78	0.02	1.65	0.02
Body mass (kg)	69.5	2.3	61.8	3.4
Body fat (%)	9.5	1.0	20.3	1.8
Exercise training ($hr \cdot wk^{-1}$)	17.6	1.4	17.7	0.7
HRmax ($beats \cdot min^{-1}$)	201	2	190	2
VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	52.7	0.9	43.3	1.9
RERmax	1.16	0.02	1.22	0.02

Note. *M* = mean; *SD* = standard deviation; HRmax = heart rate; VO_2max = maximal oxygen uptake; RERmax = maximal respiratory exchange ratio.

.01; see Table 2). The neutrophil-lymphocyte ratio increased 96% by 1 hr postexercise ($p < .01$). Eosinophils fell 33–41% postexercise ($p < .010$).

Significant overall time effects from the repeated measures ANOVA were measured for saliva IgA secretion rate and protein IgA concentration, PHA-induced lymphocyte proliferation (lower concentration only), IL-1ra, cortisol, and growth hormone (see Table 3). The salivary IgA excretion rate decreased 30% immediately postexercise ($p < .01$) but returned to near pre-exercise levels by 1 hr postexercise (see Table 3). When the concentration of salivary IgA was adjusted for protein concentration, a slight decrease (not significant at any single time) was measured after exercise. PHA-induced IL-2 production and lymphocyte proliferation (at a PHA concentration of 25 µg/ml) were unchanged following exercise but fell 25% at the lower PHA concentration of 5 µg/ml ($p < .01$; see Table 3). IL-6 did not increase significantly following exercise, with IL-1ra climbing 23% ($p < .01$, immediately postexercise; see Table 3). The pattern of change in cortisol followed a normal diurnal decrease ($p < .01$; Nehlsen-Cannarella et al., 1997), with growth hormone increasing slightly immediately postexercise (see Table 3).

Discussion

In this study, an intermittent, 2-hr session of tennis drills performed by elite adolescent tennis athletes resulted in mild perturbations of blood hormone levels, immune cell counts, and immune function. Conversely, prolonged, continuous, and intensive bouts of running and cycling resulted in much greater alterations in immunity (Nieman, 1997). The neutrophil-lymphocyte ratio is often used as an index of physiological stress to the im-

Table 2. Blood leukocyte and lymphocyte subset changes in response to an intermittent, 2-hr bout of tennis drills in adolescent athletes ($N = 20$)

Cell counts, 10^9 cells·l^{-1}	Pre-exercise		Postexercise		1 hr postexercise		p value time effect from ANOVA
	M	*SD*	*M*	*SD*	*M*	*SD*	
Neutrophils	3.38	0.17	5.77	0.43*	5.11	0.37*	< .001
Total lymphocytes	2.53	0.13	2.44	0.15	2.05	0.11*	.005
Neutrophil-lymphocyte ratio	1.39	0.08	2.54	0.27*	2.73	0.33*	< .001
Monocytes	0.43	0.04	0.54	0.04*	0.41	0.03	.001
Eosinophils	0.27	0.03	0.18	0.03*	0.16	0.03*	< .001
T cells ($CD3^+$)	1.35	0.11	1.51	0.09	1.28	0.09	.034
NK cells ($CD3^-CD16^+CD56^+$)	0.58	0.06	0.47	0.06	0.26	0.04*	< .001
B cells ($CD19^+$)	0.42	0.04	0.34	0.03	0.41	0.03	.010

Note. *M* = mean; *SD* = standard deviation; ANOVA = analysis of variance.
*$p < .01$, change from pre-exercise using paired *t* tests.

Table 3. Immune and hormonal changes in response to an intermittent, 2-hr bout of tennis drills in adolescent athletes ($N = 20$)

	Pre-exercise		Postexercise		1 hr postexercise		p value time effect from ANOVA
	M	*SD*	*M*	*SD*	*M*	*SD*	
Saliva IgA secretion rate (µg·min^{-1})	275	24	192	21*	241	22	.001
Saliva protein IgA concentration (µg·mg^{-1})	459	39	346	44	345	43	.014
PHA-IL-2 production (pg·ml^{-1})	37.4	25.1	44.0	21.9	40.5	28.9	.431
PHA-induced lymphocyte proliferation							
PHA 5 µg/ml (counts·min^{-1}·10^{-3})	16.5	1.5	12.4	1.4*	12.4	1.3*	< .001
PHA 25 µg/ml (counts·min^{-1}·10^{-3})	19.8	1.5	19.4	1.5	17.7	1.4	.071
IL-6 (pg·ml^{-1})	1.01	0.22	2.57	0.86	1.78	0.46	.055
IL-1 receptor antagonist (pg·ml^{-1})	173	12	212	13*	201	13	.011
Cortisol (nmol·l^{-1})	776	33	448	53*	414	41*	< .001
Growth hormone (ng·ml^{-1})	2.14	0.77	3.66	0.90	0.98	0.09	.006

Note. *M* = mean; *SD* = standard deviation; ANOVA = analysis of variance.
*$p < .01$, change from pre-exercise using paired *t* tests.

mune system because of its high correlation with exercise-induced changes in plasma cortisol levels (Nieman et al., 1998). In the tennis athletes, this ratio rose to 2.73, far lower than the ratio of 10–20 measured in athletes following 2.5-hr training bouts of running, cycling, or marathon race events (Haq, al Hussein, Lee, & al Sedairy, 1993; Nieman, 1997). Despite the 2-hr session of tennis drills, plasma cortisol levels decreased, staying at a concentration slightly above the typical diurnal pattern. As a result, the neutrophil-lymphocyte ratio stayed close to pre-exercise levels. In previous research, we have shown that prolonged and intensive exercise starting early in the morning causes the plasma cortisol to stay near pre-exercise levels, and this contrast with the normal diurnal decrease causes an increase in the neutrophil-lymphocyte ratio (Nehlsen-Cannarella et al., 1997; Nieman, 1997).

The NK cell count fell 55% from pre-exercise levels 1 hr after exercise, with no increase measured immediately postexercise, a pattern similar to that seen after exercise bouts lasting longer than 90 min (Nieman, 1997). The NK cell count rises immediately after intense and short bouts of exercise but not after prolonged exercise (Nieman & Nehlsen-Cannarella, 1994).

The secretory immune system of the mucosal tissues of the upper respiratory tract is considered the first barrier to colonization by pathogens, with IgA the major effector of host defense. The bout of tennis drills was associated with a small and transient decrease in salivary IgA output, again of lower magnitude than measured following intensive running or cycling sessions (Nieman, 1997; Steerenberg et al., 1997).

Mitogen-induced lymphocyte proliferative responses are the typical in vitro laboratory test for T and B cell function. The bout of tennis drills was associated with a 19% reduction in mitogen-induced proliferation rate (suboptimal concentration only), lower than the 30–40% reduction after heavy and prolonged exertion (Eskola et al., 1978; Nieman et al., 1995).

The tennis drills also caused little or no increase in plasma cytokines, and the normal diurnal variation in plasma cortisol concentrations was unaltered (Nehlsen-Cannarella, et al., 1997). Plasma levels of the proinflammatory cytokine, IL-6 often rise to 50–100 pg/ml, and the anti-inflammatory cytokine, IL-1ra, 1,000-5,000 pg/ml, following intensive running sessions of 2.5 hr and longer (Nehlsen-Cannarella, et al., 1997; Ostrowski, Rohde, Asp, Schjerling, & Pedersen, 1999). The low cytokine response to tennis drills suggests a low level of inflammation due to injury of muscle cells (Nieman & Pedersen, 1999).

The immune changes in adolescent athletes after 2 hr of tennis drills were comparable to what we had measured after female adult rowers performed 2 hr of rowing drills (Nieman et al., 1999). Both groups rested every 15 min to ingest fluid and for measurements to be taken. Although we did not make a direct comparison, these data suggest that the immune systems of adolescents and adults respond similarly to prolonged and intermittent sessions of sports drills. Our data also indicate that exercise-induced alterations in immunity are similar between male and female adolescent athletes.

This study had several limitations. A resting control group would have strengthened our analysis of repeated measures over time, especially for cortisol, which steadily decreases during the morning hours. We included resting controls in previous studies and measured very little, if any, change in immune measures during the time of this study (6:30–10:30 am; Nehlsen-Cannarella et al., 1997; Nieman, 1997). We also had the tennis athletes ingest 600 ml/hr of a 6% carbohydrate beverage. Although carbohydrate does attenuate many immune changes following prolonged and intensive exercise such as running and cycling, two studies have determined that carbohydrate has little, if any, influence on immune changes following exercise bouts such as sports drills, when perturbations in stress hormones and cytokines are low (Bishop et al., 1999; Nieman et al., 1999).

Conclusions

The male and female tennis athletes in this study engaged in a 2-hr bout of intensive tennis drills, with 4–5-min rest periods every 15 min. The tennis drills were comparable to those the athletes experienced on a near daily basis and represented a significant component of their 17–18-hr per week of training. The immune changes following the tennis drills were of low magnitude and much smaller than those measured after 2–3-hr bouts of intensive running and cycling. This disparity may be due to several factors, including the rest periods taken by the tennis athletes, the mixed aerobic and anaerobic exertion demands of tennis, and the element of play. There is no evidence from this study that an intensive and prolonged bout of tennis drills imposes undue physiological stress to the immune systems of male and female adolescent tennis athletes.

References

Bishop, N. C., Blannin, A. K., Robson, P. J., Walsh, N. P., & Gleeson, M. (1999). The effects of carbohydrate supplementation on immune responses to a soccer-specific exercise protocol. *Journal of Sports Science, 17,* 787–796.

Boas, S. R., Joswiak, M. L., Nixon, P. A., Kurland, G., O'Connor, M. J., Bufalino, K., Orenstein, D. M., & Whiteside, T. L. (1996). Effects of anaerobic exercise on the immune system in eight-to-seventeen-year-old trained and untrained boys. *Journal of Pediatrics, 129,* 846–855.

Borg, G. A. V. (1982). Psychosocial bases of perceived exertion. *Medicine and Science in Sports and Exercise, 14,* 377–381.

Bradford, M. M. (1976). A rapid and sensitive method for the quantification of microgram quantities protein utilizing the

principle of protein-dye binding. *Analytical Biochemistry, 72,* 248–254.

Dill, D. B., & Costill, D. L. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *Journal of Applied Physiology, 37,* 247–248.

Eskola, J., Ruuskanen, O., Soppi, E., Viljanen, M. K., Järvinen, M., Toivonen, H., & Kouvalainen, K. (1978). Effect of sport stress on lymphocyte transformation and antibody formation. *Clinical and Experimental Immunology, 32,* 339–345.

Gabriel, H., & Kindermann, W. (1997). The acute immune response to exercise: What does it mean? *International Journal of Sports Medicine, 18*(Suppl 1), S28–S45.

Haq, A., al Hussein, K., Lee, J., & al Sedairy, S. (1993). Changes in peripheral blood lymphocyte subsets associated with marathon running. *Medicine and Science in Sports and Exercise, 25,* 186–190.

Jackson, A. S., & Pollock, M. L. (1985). Practical assessment of body composition. *Physician and Sportsmedicine, 13,* 76–90.

Karpovich, P. V (1935). The effect of basketball, wrestling, and swimming upon white blood corpuscles. *Research Quarterly, 6,* 42–48.

Mackinnon, L. T. (1999). *Advances in exercise immunology.* Champaign, IL: Human Kinetics.

Nehlsen-Cannarella, S. L., Fagoaga, O. R., Nieman, D. C., Henson, D. A., Butterworth, D. E., Bailey, E., Warren, B. J., & Davis, J. M. (1997). Carbohydrate and the cytokine response to 2.5 hours of running. *Journal of Applied Physiology, 82,* 1662–1667.

Nieman, D. C. (1997). Immune response to heavy exertion. *Journal of Applied Physiology, 82,* 1385–1394.

Nieman, D. C., & Nehlsen-Cannarella, S. L. (1994). The immune response to exercise. *Seminars in Hematology, 31,* 166–179.

Nieman, D. C., Nehlsen-Cannarella, S. L., Fagoaga, O. R., Henson, D. A., Shannon, M., Davis, J. M., Austin, M. D., Hjertman, J. M. E., Bolton, M. R., Schilling, B. K., Hisey, C., & Holbeck, J. (1999). Immune response to two hours of rowing in female elite rowers. *International Journal of Sports Medicine, 20,* 476–481

Nieman, D. C., Nehlsen-Cannarella, S. L., Fagoaga, O. R., Henson, D. A., Utter, A., Davis, J. M., Williams, F., & Butterworth, D. E. (1998). Influence of mode and carbohydrate on the cytokine response to heavy exertion. *Medicine and Science in Sports and Exercise, 30,* 671–678.

Nieman, D. C., & Pedersen, B. K. (1999). Exercise and immune function: Recent developments. *Sports Medicine, 2,* 73–80.

Nieman, D. C., Simandle, S., Henson, D. A., Warren, B. J., Suttles, J., Davis, J. M., Buckley, K. S., Ahle, J. C., Butterworth, D. E., Fagoaga, O. R., & Nehlsen-Cannarella, S. L. (1995). Lymphocyte proliferative response to 2.5 hours of running. *International Journal of Sports Medicine, 16,* 406–410.

Österback, L., & Qvarnberg, Y. (1987). A prospective study of respiratory infections in 12-year-old children actively engaged in sports. *Acta Paediatrica Scandinavia, 76,* 944–949.

Ostrowski, K., Rohde, T., Asp, S., Schjerling, P., & Pedersen, B. K. (1999). Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *Journal of Physiology* [London], *515*(Pt 1), 287–291.

Oyster, N. (1980). Changes in plasma eosinophils and cortisol of women in competition. *Medicine and Science in Sports and Exercise, 12,* 148–152.

Steerenberg, P. A., van Asperen, I. A., van Nieuw Amerongen, A., Biewenga, A., Mol, D., & Medema, G. J. (1997). Salivary levels of immunoglobulin A in triathletes. *European Journal of Oral Science, 105,* 305–309.

Storer, T. W., Davis, J. A., & Caiozzo, V. J. (1990). Accurate prediction of VO_2max in cycle ergometry. *Medicine and Science in Sports and Exercise, 22,* 704–712.

Tharp, G. D. (1991). Basketball exercise and secretory immunoglobulin A. *European Journal of Applied Physiology, 63,* 312–314.

Wake, R. F., Graham, B. F., & McGrath, S. D. (1953). A study of eosinophil response to exercise in man. *Aviation Medicine, 24,* 127–130.

Wilmore, J. H. (1989). Design issues and alternatives in assessing physical fitness among apparently healthy adults in a health examination survey of the general population. In National Center for Health Statistics (Ed.), *Assessing physical fitness and physical activity in population-based surveys* (DHHS Pub. No. PHS 89-1253, pp. 107–140). Washington, DC: U.S. Government Printing Office.

Wolach, B., Eliakim, A., Gavrieli, R., Kodesh, E., Yarom, Y., Schlesinger, M., & Falk, B. (1998). Aspects of leukocyte function and the complement system following aerobic exercise in young female gymnasts. *Scandinavian Journal of Medicine and Science in Sports, 8,* 91–97.

Authors' Notes

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E-mail: niemandc@appstate.edu