The effects of exercise on the immune system and stress hormones in sportswomen

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Abstract

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BACKGROUND: Despite the numerous studies controversial results exist in specific immune response to exercise. The aim of this study was to determining the differences in the humoral immune parameters, serum ACTH and cortisol levels existing between sportswomen and sedentary subjects and the effect of acute aerobic and anaerobic exercise on these parameters.

MATERIALS/METHODS: 40 sportswomen (Groups 1 and 2) and 20 sedentary women (Group 3) were enrolled and Group 1 performed aerobic exercise on a treadmill for 30 minutes while the Group 2 was subjected to the Wingate effort test for 30 seconds. Before exercising (at 8.30 a.m), immediately after the exercise, and 4 hours, days 2 and 5 days after exercising blood samples were obtained and the levels of IgA, IgG, IgM, complement 3 (C3) and complement 4 (C4) were determined turbidometrically.

RESULTS: Before exercise: the means of IgA and IgG values in the G1 and G2 groups were higher than the Group G3, and the mean cortisol levels in the sedentary group was significantly higher (p < 0.05).

After Exercise: Whereas the C3 and C4 levels decreased significantly at the end of the exercise in Group 1 and 2 (p < 0.05), the IgA, IgG (p < 0.05) and IgM levels (p < 0.01) at the 4th and 5th determinations were observed to be significantly higher in only the Group 1. The cortisol and ACTH levels were found to have increased significantly (p < 0.05) in the Group 1. In Group 2, no changes were observed in the IgA, IgG and IgM levels.

CONCLUSIONS: We conclude that regular and moderate exercise has favorable effects on the immune system by increasing immunoglobulines which are potent protective factors.

Introduction

Despite the numerous studies aimed at explaining the specific immune response to exercise, conflicts exist in the results obtained so far from such studies. Most of the studies examining the effects of exercise on immune functions have either focused on only one type of exercise or on the changes following exercise of short duration [3,15,30]. There have been a limited number of studies examining the effects of regular exercise in the long term on the immune system [3, 7, 40, 31]. Regular exercise has been reported to have several favorable effects on physiological, psychological, and immunological functions [8, 17, 29, 34], and increase in the resistance against infections [17, 27, 32, 33].Vigorous exercise, however, has been reported to have a negative effect on these functions [5, 15, 19, 24]. In elite sportswomen the effects of acute aerobic and anaerobic exercise on the immune and neuro-humoral system has not been fully investigated.

For this reason, our study was aimed to investigate the differences between the sportswomen and those leading sedentary lives by comparing their humoral immune parameters, serum ACTH and cortisol levels and the effects of acute aerobic and anaerobic exercise on these. In human, plasma cortisol level is approximately 13.2 µg/dL. The level of cortisol shows a circadian rhythm during the day, and it is regulated by corticotrophin Releasing Hormone (CRH) secreted by hypothalamus and ACTH released by pituitary gland [9].

Materials and Method

The ethical consent to study on human subjects was provided by The Ethical Committee of Firat University and Marmara University according to The Declaration of Helsinki.

Forty elite sportswomen who have been playing volleyball three times a week for 120 minutes each for at least 5 years and 20 healthy age-matched sedentary females were enrolled in this study. Exclusion criteria were history or finding of chronic cardiovascular, endocrine or immune diseases.

The sportswomen were randomly separated into two groups G1 and G2. G1 (n=20) performed aerobic exercise while G2 (n=20) performed anaerobic exercise. The control group (n=20) was the sedentary group. For all the groups, age, height, weight and laboratory parameters of IgA, IgG, IgM, C3 and C4 were measured with the exercise groups having the measurements before and after the exercise period. All the subjects were taken into the same diet program. During the study, menstrual cycle of the sportswomen were considered. We carefully selected the subjects out of this period.

For estimation of the max. VO_2 of the subjects, 20 m Shuttle run test was employed. The max VO₂ values were expressed in ml/kg/min from the results obtained [18].

The women in the group 1 were subjected to an aerobic exercise program. The running pace was adjusted to provide an exercise work load of approximately 60%–70% of their cardiac reserves. The work load was estimated from the Karvonen's protocol [12]. Based on the maximum oxygen consumption value (70%) calculated indirectly, exercise was conducted for 30 minutes on a treadmill (Star Trac Tr 900). The second group was subjected to the Wingate Test and made to exercise for 30 seconds by turning pedals and motivated from the side to do so as quickly as possible [1]. Resting Heart Rates of subjects were measured by physician by means of stethoscope.

Blood samples were taken once from subjects in Group 3 at 8.30 in the morning of the exercise, and five times in Groups 1 and 2; before exercise (at 8.30'), at the end of exercising, 4 hours after exercising. During the last days of experimental study, all the subjects followed their regular physical training. and on 2 and 5 days after exercising. The blood samples were transported to the laboratory and sera separated as soon as possible and stored at -80°C until analyzed.

IgA, IgG, IgM, C3 and C4 determinations were done turbidometrically using the Space model (Schiapperelli

Table 1: Demographic properties of the Sporting and Sedentary groups (Mean \pm STD).				
	G1	G2	G3	
	(n=20)	(n=20)	(n=20)	
Age (years)	21.60 ± 1.42	20.8 ± 1.13	20.10 ± 0.99	
Height (cm)	170.1 ± 5.91	169 ± 0.39	164.1 ± 6.36 *	
Body Weight (Kg)	56.50 ± 5.11	57.3 ± 6.32	61.4 ± 5.73	
Max VO ₂	45.2 ± 2.11	46.2 ± 1.15	33.5 ± 4.08 *a	
Sporting age (years)	5.5 ± 1.4	5.5 ± 1.1		
Resting Heart Rate	66.5 ± 3.2	65 ± 1.6	78.4 ± 5.7 *a	
BP-Systolic (mmHg)	139 ± 1.5	138 ± 2.1	146.5 ± 1.5	
BP-Diastolic (mmHg)	75.1 ± 1.8	76 ± 13.1	80 ± 2.7	

a: Between sporting (G1 and G2) and Sedentary groups (G3), *: p<0.05 G1- Aerobic group, G2 - Anaerobic group, G3 - Sedentary group

STD: standard deviation

Table 2: Comparison of the Immune System and Hormonal Parameters before exercising in the Sporting and Sedentary groups (Mean \pm STD).

	G1	G2	G3
	(n=20)	(n=20)	(n=20)
IgA (g/lt)	1.89 ± 0.13	1.81 ± 0.17	1.27 ± 0.12 *a
IgG (g/lt)	13.46 ± 0.76	13.59 ± 0.85	10.73 ± 0.87 *ª
IgM (g/lt)	1.03 ± 0.07	1.05 ± 0.05	1.09 ± 0.08
C3 (g/lt)	1.64 ± 0.12	1.65 ± 0.55	1.66 ± 0.12
C4 (g/lt)	0.25 ± 0.04	0.24 ± 0.04	0.29 ± 0.12
ACTH	35.74 ± 2.32	34.50 ± 2.02	36.50 ± 3.81
Cortisol	9.48 ± 1.54	9.44 ± 1.81	$12.36 \pm 0.46^{*a}$

a: Between sporting (G1 and G2) and Sedentary groups (G3), *: p<0.05

G1– Aerobic group, G2 – Anaerobic group, G3 – Sedentary group

STD: standard deviation

Biosystems, USA) specific protein analyzer. ACTH and cortisol determination was done using the IMMULYTE (DPC, Diagnostic Product Corporations, Losangeles USA) model hormone analyzer employing the chemiluminesence method.

For the statistical analysis, the SPSS (SPSS for Windows, version 11.0) program was used. For the statistical evaluation of data, the Kruskall Wallis Variance analysis was used for continuous data, whilst the Bonferroni's revised Mann Whitney U test was used for significance testing as non-parametric tests. For the analysis of repeating measurements in the G1 and G2 groups the Friedman's Variance analysis was employed whiled the Wilcoxon Rank test non-parametric was used for analyzing significant values with p < 0.05 considered significant.

Results

The demographic properties of the sporting (G1 and G2) and sedentary groups are presented in *Table 1*.

When we compared the immune system parameters and stress hormones, including ACTH and cortisol in sporting (G1 and G2) and sedentary group (G3), it was found that IgA and IgG approximately 30% lower and cortisol levels were elevated as 31% in G3 groups. Results are shown at *Table 2*.

For aerobic and anaerobic test groups, immunoglobulin and stress hormones before and after aerobic exercise (at the end, 4 hours, and 2 days, and 5 days after exercising) in sporting women of the groups G1 and G2 were evaluated and results are shown at *Table 3* and 4. In the group aerobic exercise, levels of cortisol and ACTH increased as approximately 36% in post-exercise immediate period. In the 2nd and the 5th days, IgA levels were observed to be elevated approximately 16%, and the elevations in IgG and IgM levels were respectively 11% and 100% in the group of aerobic exercise.

Discussion

Different results have been obtained in studies on the effects of exercise on the immune system [8, 20, 28]. In addition to factors like type, duration, intensity, and program of the exercise and the use of different subjects [29], various complex mechanisms including hormonal, metabolic and psychoneural stress are also known to have effects on the immune system [14, 32].

Changes in the immune functions due to acute exercise and training have been attributed to the increased secretion of cortisol, cathecholamine and the neuropeptides [3, 13, 37]. During exercise, when the max O_2 consumption exceeds 60% an increase in the epinephrine and cortisol concentrations occurs. Under any kind of stress vasopressin stimulates the release of corticotropin-relasing factor, which in turn leads to the release of ACTH [2]. Exercise increases the number of lymphocytes in the circulation by acting as a lymphocytic β 2-adrenergic agonist. Cortisol on the other hand blocks the entry of lymphocytes which would otherwise lead to strong neutrophilia in the circulation, thereby facilitating the passage of lymphocytes from the lymphoid compartments [4,16, 26, 37].

In our study, comparison of the IgA and IgG levels revealed significantly lower parameters in the sedentary group than in the groups G1 and G2 before exercise. We concluded that the elevated levels of immunoglobulines in the sporting groups may be caused by the chronic effect of regular exercise. While Mackinon and Smith [16, 36], reports of the acute and chronic effects of exercise on the immune system other investigators emphasized that no matter the duration of the exercise there is always an increase in the parameters of the immune system. In another study on the topic, the IgG, IgA, and IgM levels in male marathon runners at rest have been reported to be within clinically normal limits [25]. Nehlsen et al., reported that at 60% of max VO₂ moderate exercise results in transient increases in the IgG, IgA, and IgM levels [21]. In the same study, it was found that at the 6th week of the training exercise program with intensity of 60% max VO₂ similar increase in the basal immunoglobulin levels was noted. In a dif-

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Table 3: Comparison of the Immunoglobulin and Hormonal System Parameters before and after aerobic exercise (at the end, 4 hours, and 2 days, and 5 days after exercising) in Sporting women (Mean ± STD)

(n=20)	Pre-Exercise	Post-Exercise immediate	3rd Measurement 4 hrs after exer.	4th Measurement 2 days after exer.	5th Measurement 5 days after exer.
IgA (g/lt)	1.89 ± 0.13	1.73 ± 0.03	1.85 ± 0.20	2.13 ± 0.44 *b	2.29 ± 0.27 *b
IgG (g/lt)	13.46 ± 0.76	13.77 ± 1.38	13.70 ± 0.92	15.23 ± 1.11 *b	15.05 ± 0.46 *b
IgM (g/lt)	1.03 ± 0.07	1.08 ± 0.02	1.06 ± 0.31	2.07 ± 0.19 ** b	2.10 ± 0.09 **b
C3 (g/lt)	1.64 ± 0.03	1.06 ± 0.08 *a	1.68 ± 0.04	1.67 ± 0.05	1.67 ± 0.05
C4 (g/lt)	0.25 ± 0.04	0.11 ± 0.03 *a	0.28 ± 0.11	0.22 ± 0.09	0.21 ± 0.06
ACTH	35.74 ± 2.32	45.72 ± 2.90 *ª	36.30 ± 1.76	34.86 ± 2.65	34.53 ± 2.32
Cortisol	11.48 ± 1.54	15.06 ± 2.55 *a	10.56 ± 1.18	10.72 ± 1.16	11.27 ± 1.39

(a: Pre- and Post-Exercise, b: Pre-Exercise and 3rd, 4th and 5th measurements *:p<0.05**:p<0.01) STD: standard deviation

Table 4: Comparison of the immunoglobulin and hormonal parameters before and after anaerobic exercise (at the end, 4 hours, 2 days, and 5 days afterwards) in the sporting women (Mean ± STD)

(n=20)	Pre-Exercise	Post-Exercise immediate	3rd Measurement 4 hrs after exer.	4th Measurement 2 days after exer.	5th Measurement 5 days after exer.
IgA (g/l	1.81 ± 0.13	1.76 ± 0.03	1.80 ± 0.09	1.79 ± 0.17	1.83 ± 0.18
IgG (g/lt)	13.89 ± 0.85	13.55 ± 1.53	13.52 ± 1.47	13.84 ± 1.41	13.77 ± 1.68
IgM (g/lt)	1.05 ± 0.05	1.09 ± 0.03	1.02 ± 0.03	1.03 ± 0.04	1.07 ± 0.05
C3 (g/lt)	1.66 ± 0.05	1.14 ± 0.06 *a	1.68 ± 0.04	1.67 ± 0.05	1.64 ± 0.05
C4 (g/lt)	0.24 ± 0.04	$0.12 \pm 0.01^{**a}$	0.23 ± 0.10	0.22 ± 0.5	0.21 ± 0.07
ACTH	34.50 ± 2.02	33.14 ± 2.48	33.77 ± 1.98	35.02 ± 2.52	34.80 ± 2.09
Cortisol	12.44 ± 1.81	12.11 ± 1.91	12.17 ± 1.68	12.16 ± 1.5	11.36 ± 1.38

(a: Pre- and Post-Exercise, *:p<0.05**:p<0.01

STD: standard deviation

ferent study, it has been reported that the plasma immunoglobulin levels was increased by regular exercise of moderate intensity [40]. The results from our study in which the IgA, IgG, and IgM levels were increased by regular exercise are in agreement with these data and demonstrate the positive effects of exercise on the immune system [3, 11, 15, 23, 33].

While the IgA level at 4 hours after exercise was found to have returned to the pre-exercise levels, at 2 and 5 days post-exercise it was found to be higher than the pre-exercise level. In the studies reported in previous, in the measurements conducted after the aerobic and anaerobic exercise, the IgA level was found to have fallen. However, the fall was not statistically significant. This fall was thought to be probably due to the inflammation that results from the microtrauma in which the muscle tissue is subjected to during exercise [24, 32].

The IgG and IgM levels at 2 and 5 days after exercise were found to be statistically higher than that before exercise. In another study, the observation that no change in the IgA, IgG, and IgM levels occurred, was explained by the fact that the duration of exercise did not probably lead to any significant changes in the glutamine levels that would otherwise affect the function of lymphocytes and macrophages [39].

From studies conducted, it has been found that, though the measured resting state C3 and C4 levels in long distance runners is significantly lower than that in individuals leading sedentary lives, with aerobic exercise the level rises [6, 22, 23, 35]. It is known that short duration exercise leads to activation of the C3 and C4 levels [11, 23]. In a study conducted on experienced athletes, despite the small increase in the C3a and C4a levels between the pre-exercise and immediately after exercise, a corresponding fall in the C4H level was noted [22, 35]. In the study presented here, no difference was observed in the C3 and C4 levels before exercise between the sporting and sedentary groups. However, in the sporting groups (G1 and G2) the measurement after both aerobic and anaerobic exercise showed statistically significant falls in the and C4 levels after exercise. The fact that mild acidosis that occurs in the blood of these sporting subjects during anaerobic exercise leads to activation of the alternative pathway in the utilization of the C3 and C4 system together with the inflammation due to microtrauma of the muscles during exercise offers an explanation for this fall [6, 32].

In reports, a high correlation has been established between the cortisol level and the intensity of exercise [10, 11, 31]. The highest value of cortisol was reported in aerobic capacity exercises [4,38]. We observed significantly higher cortisol levels in the sedentary group than in the sporting groups (G1 and G2). The ACTH levels, however, showed no difference between the two groups. Whereas anaerobic exercise for a brief period led to no changes whatsoever in the sporting subjects, with aerobic exercise an increase in the cortisol and ACTH hormone secretion was observed. In studies conducted, while the ACTH increased after exercising a parallel increase in the level of cortisol which is secreted under the influence of ACTH has been reported [8, 10, 11, 38, 39]. However, these findings showed differences between individuals, with some studies demonstrating much higher increases in sporting individuals [37, 38]. Thuma and his colleagues (1995) found a positive relationship between the rise in cortisol concentration after exercise and the max VO₂. In the light of these findings the changes in ACTH and cortisol levels observed in our study can be said to agree with those of the literature. Aerobic exercise might lead to increased cortisol and decreased IgA and IgG, which could increase susceptibility to infections.

In conclusion, whereas the anaerobic exercise of short duration did not lead to an increase in the cortisol and ACTH levels in the sporting women, aerobic exercise was observed to have led to changes in immunoglobulines and caused to elevated levels of cortisol and ACTH hormone levels. According the data obtained from the study, we conclude that regular and moderate exercise has favorable effects on the immune system by increasing immunoglobulines which are potent protective factors.

REFERENCES

- 1 Adams GM. Physiology Laboratory Manual, (4th Ed.) Brown & Benchmark Publishers.Madison, Wisconsin-Dubuque, Iowa 2002.
- 2 Ahtiainen JP, Pakarinen A, Kraemer WJ, Hakkinen K. Acute hormonal responses to heavy resistance exercise in strength athletes versus nonathletes. Can J Appl Physiol. 2004; 29:527–543.
- 3 Bauer T, Weisser B. Effect of aerobic endurance exercise on immune function in elderly athletes. Schweiz Rundsch Med Prax 2002; 30: 91:153–158.
- 4 Brenner I, Shek PN, Zamecnik J, Shephard RJ. Stress hormones and the immunological responses to heat and exercise. Int J Sports Med 1998; 19:130–143.
- 5 Callow KA. Effect of specific humoral immunity and some nonspecific factors on resistance of volunteers to respiratory coronavirus infection. J Hyg 1985; **95**:173–189.
- 6 Cannon JG. Fiatarone M.A, Fieldinig RA, Evans WJ. Aging and stress induced changes in complement activation and neutrophil mobilization. J Appl Physiol 1994; 76:2616–2620.
- 7 Fahlman MM, Engels HJ, Morgan AL, Kolokouri I. Mucosal IgA response to repeated wingate tests in females. Int J Sports Med 2001; 22:127–131.
- 8 Filaire E, Bonis J, Lac G. Relationships between physiological and psychological stress and salivary immunoglobulin A among young female gymnasts. Percept Mot Skills 2004; 99:605–617
- 9 Ganong WF. Review of Medical Physiology. East Norwalk, Connecticut: Appleton & Lange; 1995. pp. 327–351.

- 10 Heitkamp H, Schulz, Röcker K, Dickhuth: Endurance traning in females: changes in β-endorphin and Acth. Int J Sports Med 1997; 19:260–264.
- 11 Karacabey K, Peker I, Saygin O, Ciloglu F, Ozmerdivenli R, Bulut V. Effects of acute aerobic and anaerobic exercise on humoral immune factors in elite athletes. Biotechnology& Biotechnological Equipment 2005; 19:175–180.
- 12 Karvonen MJ, Kentala E, Mustala O. The effects of training on heart rate: longitudinal study. Ann Med Exp Biol Fenn 1957; **35**:307–315.
- 13 Koppel M, Tvede N, Galbo H, Haahn M. Evidence that the effect of physical exercise on NK cell activity is mediated by epinephrine. J. Appl Physiol 1991; **70**:2530–2534.
- 14 La Parriere A, Ironson G, Anton M.H, Schneiderman N, Klisman N. Exercise and psychoneuroimmunology. Med Sci Sports Exerc 1994; 26:182–190.
- 15 Mackinnon LT. The effect of exercise on secretory and natural immunity. Adv Exp Med Biol 1987; **216**:869–876.
- 16 Mackinnon, LT. Effects of exercise on the immune system: overtraining effects on immunity and performance in athletes. Immunol Cell Biol 2000; 78:502–509.
- 17 Mackinnon, LT. Chronic exercise training effects on immune function. Med Sci Sports Exerc 2000; 32:369–S376.
- 18 Manios Y, Moschandreas J, Hatzis C, Kafatos A. Evaluation of a health and nutrition education program in primary school children of Crete over a three-year period. Preventive Medicine 1999; 28:149–159.
- 19 Moldoveanu AI, Shephard RJ, Shek PN. The cytokine response to physical activity and training. Sports Med Review 2001; **31**:115–144.
- 20 Nash MS. Exercise and immunology. Med Sci Sports Exerc 1994; 26:125–127.
- 21 Nehlsen–Canarella, SL, Nieman DC. Jensen J, Chang G. The effect of moderate exercise on lymphocle function and serum immunoglobin. Int J Sports Med 1991; 12:391–398.
- 22 Nielsen EW, Johansen HT, Gaudesen O, Osterud B, Olsen JO, Hogasen K, Hack CE, Mollnes TE. C3 is activated in hereditary angioedema, and C1/C1-inhibitor complexes rise during physical stress in untreated patients. Scand J Immunol 1995; 42:679–685.
- 23 Nieman DC, Tan SA, Lee JW, Berk LJ. Complement and immunglobulin levels in athletes and sedentary controls. Int J Sport Med 1989; **10**:124–128.
- 24 Nieman DC. Exercise infection and immunity. Int J Sports Med 1994; **15**:116–123.
- 25 Nilssen DE, Oktedalen O, Lygren I, Opstad PK, Brandtzaeg P. Intestinal Iga- and Igm-producing cells are not decreased in marathon runners. Int J Sports Med 1998; 19:425–431.
- 26 Perna FM, Schneiderman N, Laperriere A. Psychological stress, exercise and immunity. Int J Sports Med 1997; 18:78–83.
- 27 Peters EM. Exercise, immunology and upper respiratory tract infections. Int J Sports Med 1997; 18:69–77.
- 28 Pyne DB, Gleeson M, Mcdonald WA, Clancy RL, Perry C J, Fricker PA.Training strategies to maintain immunocompetence in athletes. Int J Sports Med 2000; 21:51–60.
- 29 Reid MR, Drummond PD, Mackinnon LT. The effect of moderate aerobic exercise and relaxation on secretory immunoglobulin A. Int J Sports Med 2001; 22: 132–137.
- 30 Rowbottom DG, Green KJ. Acute exercise effects on the immune system. Med Sci Sports Exerc 2001; 32:396–405.
- 31 Sage D, Maurel D, Bosler O. Involvement of the suprachiasmatic nucleus in diurnal ACTH and corticosterone responsiveness to stress. Am J Physiol Endocrinol Metab 2001; 280:260–269.
- 32 Sharp NC, Kouledakis Y. Sport and the overtraning syndrome. Immunological aspects. British Med. Bul 1992; **48**:518–533.
- 33 Shinkai S, Shore S, Shek PN, Shephard RJ. Acute exercise and immune function: Relationship between lymphocyte activity and changes in subset counts. Int J Sports Med 1992; 13:452–461.
- 34 Simonson SR. The immune response to resistance exercise. J Strength Cond Res 2001; 15:378–384.
- 35 Smith J, Chi D, Krish G, Reynolds S, Cambron G. Effect of exercise on complement activity. Ann Allergy 1990; **65**: 304–310.
- 36 Smith JA. Guidelines, standards and perspectives in exercise immunology. Med Sci Sports Exerc 1995; 27: 497–506.
- 37 Tabata, I, Ogita F, Miyachi M, Shibayama. Effect of low blood glucose on plasma crf, Acth, and cortisol during prolonged physical exercise. J Appl Physiol 1991; 71:1807–1812.

- 38Thuma JR, Gilders R,Verdun R, Loucks AB. Circadian rhythm of cortisol confounds cortisol responses to exercise: implications for future research. J Appl Physiol 1995; **78**:1657–1664.
- 39 Tilz GB, Domej W, Diez-Ruiz A, Weiss G, Brezincshek R, Brezincshek HP. Increased immune activation during and after physical exercise. Immunobiol 1993; **188**:194–202.
- 40 Tvede N, Pedersen BK, Hansen FR, Bendix T, Christensen LD, Galbo H. Halkjaen- Kristensen J. Effect of physical exercise on blood mononuclear cell subpopulations and in vitro proliferative responses. Scand J Immunol 1989; **29**:383–389.