Available online at www.sciencedirect.com
SCIENCE DIRECT®
Brain, Behavior, and Immunity 19 (2005) 357–366
BRAIN, BEHAVIOR, and IMMUNITY
www.elsevier.com/locate/ybrbi

The exercise-induced enhancement of influenza immunity is mediated in part by improvements in psychosocial factors in older adults

M.L. Kohut*, W. Lee, A. Martin, B. Arnston, D.W. Russell, P. Ekkekakis, K.J. Yoon, A. Bishop, J.E. Cunnick

Department of Health and Human Performance, Immunobiology, Gerontology, Animal Science, Veterinary Diagnostic and Production Animal Medicine, Iowa State University, USA

Received 8 March 2004; received in revised form 6 July 2004; accepted 2 December 2004
Available online 4 February 2005

Abstract

The primary goal of this study was to determine whether exercise-associated improvements of the immune response to influenza vaccination were mediated by improvements in psychosocial factors in older adults. At baseline, prior to the exercise intervention, older adult participants were immunized with influenza vaccine. Blood samples collected pre-immunization, 1, 4, and 12 weeks post-immunization were analyzed for anti-influenza antibody, whereas influenza-specific cytokine (IFNγ) was evaluated at 1 week post-immunization. Depression and sense of coherence were measured pre-immunization. Four weeks post-immunization, participants were randomly assigned to either an aerobic exercise group ($n = 14$) or a control group ($n = 14$). After a 10-month exercise intervention, the immunization, blood collections, and psychosocial measures were repeated. At the post-intervention evaluation, exercise participants had improved scores on depression and sense of coherence. Also post-intervention, exercise participants had a greater increase in antibody and IFNγ production. After controlling for the effect of both psychosocial measures, the exercise treatment remained significant with respect to antibody titer suggesting that the increases in antibody were not mediated by improvement in the psychosocial factors. In contrast, the enhancement of IFNγ appeared to be mediated at least in part by the psychosocial factors. After controlling for psychosocial factors, exercise treatment was no longer significantly related to the change in IFNγ. Taken together, our findings may suggest that the mechanism(s) of exercise-induced improvement in immunocompetence involve both physiological and psychological pathways.

1. Introduction

Older adults experience a greater mortality rate from influenza infection and generally exhibit reduced vaccine efficacy (Keren et al., 1988; Lui and Kendal, 1987; Sprenger et al., 1993). Among persons over age 65 hospitalized for influenza, 58–61% had been previously vaccinated (Betts and Treanor, 2000; Falsey et al., 1995). Vaccine efficacy estimates for older adults range from 31 to 65% in preventing influenza (Gross et al., 1995), whereas vaccine efficacy ranges from 68 to 88% in younger adults (Demicheli et al., 2000; Wilde et al., 1999).

A significant number of older adults may not develop protective antibody titers (defined as hemagglutination inhibition HI $\geqslant 40$) after immunization (Davis and Grillis, 1989; Keren et al., 1988). Upon infection, cytotoxic T cells (CTL) are important in viral clearance (Bender et al., 1991; McMichael et al., 1983). However, CTL response to influenza viral infection is also impaired among older adults (Mbawuike et al., 1993; Powers, 1993). Age-related changes in cytokine balance may impact resistance to infection (Shearer, 1997), and younger adults produce greater amounts of interleukin-2 (IL-2) and interferon-γ (IFNγ) post-vaccination than

* Corresponding author. Fax: +1 515 294 8740.
E-mail address: mkohut@iastate.edu (M.L. Kohut).

0889-1591/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbi.2004.12.002

older adults (Bernstein et al., 1998; McElhaney et al., 1990, 1998). Ultimately, optimal defense against viral infection in older adults may be dependent on several factors: (1) the ability to mount an effective CTL response, (2) generation of antibody, and (3) production of appropriate cytokines (e.g., IFNγ to enhance CTL; Yamada et al., 1986). Therefore, the immune parameters measured in this investigation included antibody titer, as a measure of vaccine efficacy, and influenza-specific IFNγ, a cytokine important in driving CTL.

One of the strategies to improve immunocompetence among older adults is moderate exercise (Kohut et al., 2002; Shinkai et al., 1995; Woods et al., 1999). Exercise may enhance immunity through the release of neuroendocrine factors that bind to and alter immune cell function (Pedersen and Hoffman-Goetz, 2000). Alternatively, exercise may enhance immunity indirectly by reducing depression or improving mood, thereby attenuating the negative influence of psychosocial factors on immunocompetence. Exercise reduces depression, anxiety, and alters mood (Arent et al., 2000; Blumenthal et al., 1999; Dunn et al., 2001). Associations between stress, depression, and anxiety with immune responsiveness are also well documented (Kiecolt-Glaser et al., 2002; Zorrilla et al., 2001), and therefore it is plausible that an exercise-induced alteration of psychosocial variables may influence immunocompetence. Depression is measured in this study as a factor that may negatively impact immunity, whereas sense of coherence is measured as a factor that may positively affect immune response.

The purpose of this study was to determine whether improvements in immune response resulting from a long-term (10 month) exercise intervention are mediated by improvements in psychosocial factors in older adults. We hypothesized that an exercise-induced increase in immune response (antibody titer, IFNγ) would be mediated by improvements in psychosocial factors (depression, sense of coherence). A better understanding of how exercise attenuates the age-related decrement of immune function will provide us with insights concerning how to advise the elderly on enhancing resistance to infection via psychological or physiological means.

2. Materials and methods

2.1. Participant screening, enrollment, and randomization

Older adults were recruited from the local Ames, IA community through the placement of advertisements. All procedures involving human subjects were approved by the Institutional Review Board for Human Subjects at Iowa State University. Participant inclusion and exclusion were dependent on age, health status, independent living status, medication and supplement use, influenza vaccine history, and ability to perform aerobic exercise. Health status was determined by a detailed medical history, physical examination, and maximal graded exercise test. Any participant experiencing a condition that may have impaired his or her ability to safely perform the exercise intervention was excluded from the study. Participants with a condition that may have altered the immune variables of interest were also excluded from the study (e.g., cancer within past 5 years, autoimmune disorders, transplant recipients, etc.). Participants with chronic disorders were included in the study if the condition was controlled with appropriate medication and/or if the participant could safely perform the exercise intervention (see Table 1) based on data suggesting that several chronic conditions do not significantly impair the immune response to influenza immunization (McElhaney et al., 1996; McElhaney et al., 2004). Participants treated with medications known to alter the immune response to vaccination (e.g., oral corticosteroids), taking supplements thought to alter immunity (e.g., Echinacea), or treated with medication for depression were excluded from the study. All participants had been immunized with influenza vaccine for a minimum of three previous years since the antibody titer is influenced by previous vaccine history (Gardner et al., 2001). Participants were excluded if they could not attend three supervised sessions of aerobic exercise per week for 10 months. Participants were included if: (1) they had not exercised during the 2 previous years, (2) they participated in aerobic exercise $\leqslant 3$ times per week at $\leqslant 40\%$ heart rate reserve, or (3) their aerobic fitness level was in the lowest quartile for age/sex as assessed by the Senior 6 min walk test (Rikli and Jones, 2001).

Twenty-eight of 41 possible participants (68%) met requirements for participation in the study (Fig. 1). Fourteen participants were randomly assigned to the exercise group with the remaining 14 participants being assigned to the control group. Sample size was based on our previous work in this area (Kohut et al., 2002). Initial screening and enrollment of potential participants was performed

Table 1
Baseline subject characteristics

	Control group	Exercise group
Age (mean ± Std. Dev.)	70.25 ± 5.57	73.07 ± 5.59
Males	6	7
Females	7	7
# subjects heart disease history	1	3
# subjects hypertension (controlled)	4	5
# subjects Type II diabetes (controlled)	1	1
# subjects previous TIA	1	1
# subjects high cholesterol	5	4
# females estrogen replacement	2	1
# subjects thyroid medication	2	2
Body weight, kg (mean ± Std. Dev.)	76.05 ± 18.5	84.6 ± 17.8
BMI (kg/m^2)	27.5 ± 6.0	28.5 ± 4.8

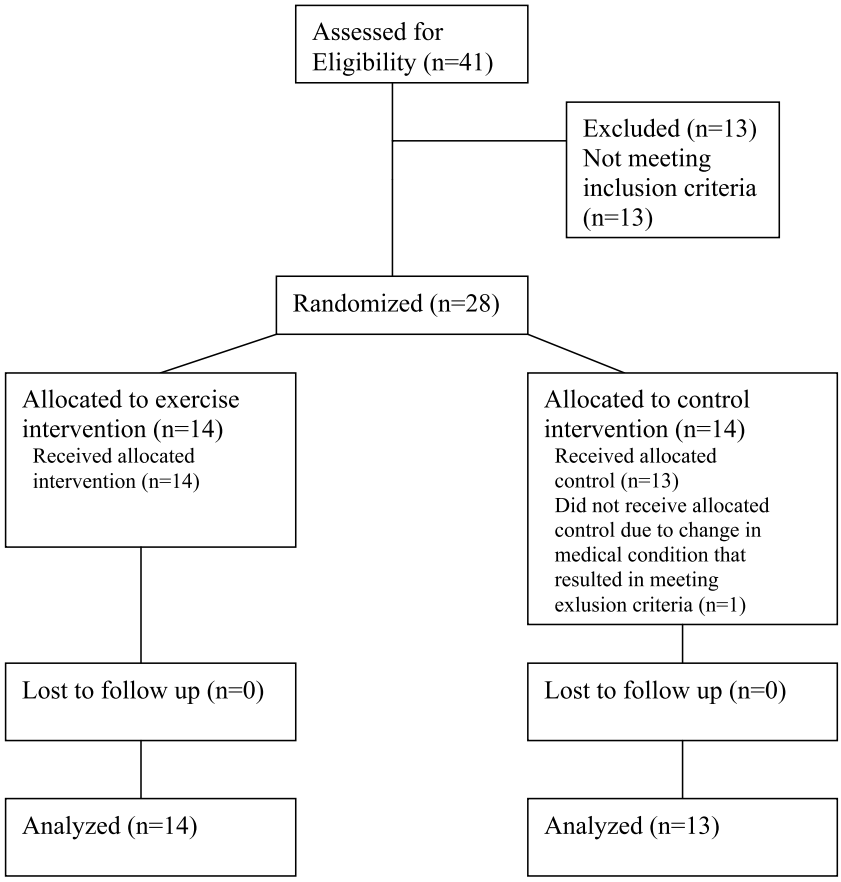

Fig. 1. Subject flow and allocation to intervention or control condition.

by the study coordinator, whereas randomization by subject number was performed by a separate individual. The personnel who administered the exercise intervention were not blind to group assignment, since it would have been impossible for them to lead the exercise sessions without knowledge of treatment condition. However, lab personnel who conducted the assays were blinded to treatment group. During the intervention, one participant in the control group was diagnosed with cancer and was therefore excluded from the study. All other participants successfully completed the exercise or control intervention.

2.2. Schedule of measurements and intervention

The study took place over two influenza seasons. Baseline immune, fitness, and psychosocial measures were assessed in Fall 2000 (prior to the intervention) to determine whether any baseline immune differences in response to influenza vaccine existed between the exercise and control groups. Blood samples were collected pre-immunization and at 1, 4, and 12 weeks post-immunization. The exercise intervention began 1 month after immunization (in Fall 2000) and continued for 10 months. The same variables were assessed in Fall 2001 (post-intervention) to determine whether the intervention altered the psychosocial variables and immune responses to the vaccine. Antigens contained in the vaccine may change from one year to the next. However, in this study, the two antigens that were studied pre-intervention and post-intervention did remain the same in Fall 2000 and Fall 2001 (i.e., Type A/New Caledonia/20/99 H1N1; Type A/Panama/2007/99 H3N2). Within 1 month prior to the pre-intervention immunization in Fall 2000, a baseline pre-immunization blood sample was collected, all subjects participated in the Senior Fitness Test (Rikli and Jones, 2001), completed psychosocial surveys, and filled out a diet questionnaire (Block 1998 Food Frequency). The same procedures were followed within 1 month prior to the post-intervention immunization in Fall 2001.

Psychosocial surveys of interest in this study were those measures on which participants in the exercise group as compared to the control group changed following the intervention as relected by a statistically significant treatment group by time interaction. These surveys were the Geriatric Depression Scale developed by Yesavage et al. (1983) and the sense of coherence inventory developed by Antonovsky (1993). Results for other psychosocial measures can be obtained from the authors. If nutrient intake differed between the treatment and control groups, this information would have been used as a covariate in the immune analyses. However, no significant nutrient differences between the groups were found.

In Fall 2000 all participants were immunized with the 2000/2001 trivalent influenza Fluzone vaccine (A/New Caledonia/20/99 H1N1 [**A1**]; A/Panama/2007/99 H3N2 [**A2**]; B/Yamanashi/166/98) manufactured by Aventis Pasteur, Swiftwater, PA. After 10 months of participation in the exercise or control group (Fall 2001), all participants were immunized with the 2001/2002 trivalent influenza Fluzone vaccine (A/New Caledonia/20/99 H1N1 [**A1**]; A/Panama/2007/99 H3N2 [**A2**]; B/Victoria/504/2000). For both immunizations, blood samples were collected pre-immunization and at 1, 4, and 12 weeks post-immunization. These samples were used for immune analyses of antibody titer and influenza-induced IFNγ production. Participants continued participation in either the exercise or control treatment until the final blood samples were taken 12 weeks post-immunization (Fall 2001). Therefore, the participants in the exercise treatment had been exposed to the exercise intervention for 10 months at the time of influenza immunization (Fall 2001), and continued to participate in the exercise program until 12 weeks post-immunization for a total time period of 13 months.

Participants in the control group were instructed to continue their current level of physical activity (i.e., low intensity exercise such as walking or no exercise). Experimental exercise participants attended supervised exercise sessions 3 times per week, initially exercising at an intensity corresponding to 40–60% of heart rate reserve (HRR) for 20 min, gradually progressing to 65–75% of HRR for 25–30 min. Participants exercised on aerobic equipment such as treadmills or cycle ergometers, and heart rate was monitored by exercise leaders and/or recorded from an electronic device on the exercise machine. All participants attended at least 80% of scheduled exercise sessions. The average exercise performance over the first 3 months was 22.4 ± 4.2 min (mean ± SD) at an average intensity of 52% HRR, and the performance over the last 7 months was 29.3 ± 3.3 min per session at an average intensity of 71% HRR.

2.3. Cytokine induced by influenza virus and measured by ELISA

IFNγ was measured in the 1 week post-immunization blood sample (previously determined to be the time point at which IFNγ is increasing). Peripheral blood mononuclear cells (PBMC) were isolated by centrifugation over Ficoll Paque plus (Amersham–Pharmacia Biotech, Piscataway, NJ), adjusted to 2.5×10^6 cells/ml, plated in 24-well plates at 1 ml/well, and cultured at 37 °C, 5% CO_2. The cytokine IFNγ was to be measured by cells responding to influenza virus. Therefore, IFNγ was measured in wells containing PBMC plus virus rather than measuring cytokine in serum (non-antigen specific cytokine). To elicit influenza-induced cytokine production, the cells were cultured with one of the following viruses: A/New Caledonia/20/99, H1N1 [**A1**]; A/Panama/2007/99, H3N2 [**A2**] at 50 HA U/ml (McElhaney et al., 2001); or media alone. Supernatant was collected 48 h later and frozen at −20 °C until analysis by ELISA. As expected, the amount of cytokine produced in wells containing media alone was low or not detectable, and this "background" level of cytokine was subtracted from the total cytokine produced in virus-stimulated wells. ELISA kits for human IFNγ were used to measure cytokine (Pharmingen, San Diego, CA). IL-10 was also measured under the same conditions. However, no differences were found as a result of the exercise intervention.

2.4. Anti-influenza antibody assay

Sera were collected from participants, centrifuged for 20 min, and then frozen in aliquots at −20 °C until analysis. The hemagglutination inhibition (HI) assay was performed using a standard microtiter procedure (Couch and Kasel, 1995). The influenza viruses used to analyze the HI titer in 2000–2001 were provided by Dr. Janet McElhaney, whereas the viruses used to analyze the HI titer in 2001–2002 were obtained from the Center for Influenza at the CDC in Atlanta, Georgia. All influenza viruses were propagated in 9- or 10-day-old embryonated chicken eggs by inoculation via allantoic–amniotic route. The titer was converted to a $\log_2$ transformed HI titer. To calculate a mean fold increase (MFI) in HI titer, the pre-immunization $\log_2$ transformed HI titer was subtracted from the post-immunization $\log_2$ transformed HI titer. The figures display antibody titer as MFI, whereas the actual $\log_2$ transformed HI titer is shown in Table 2.

2.5. Statistical analyses

Differences between the intervention and control groups on the measures taken prior to the intervention were tested using independent-sample t tests. Repeated measures analyses of variance (ANOVA) were employed to tests for differences between the treatment and control groups on the measures that were assessed over time. Analyses of covariance (ANCOVA) were employed to test the mediation effects of the psychosocial variables in the changes over time that occurred on the measures of immunocompetence. Finally, Pearson correlations were computed between the two psychosocial variables and the IFNγ measures.

3. Results

3.1. Effect of exercise intervention on health, fitness, and psychosocial variables

Prior to the intervention, differences did not exist on the health/fitness variables between the exercise and con-

Table 2
Health, fitness, psychosocial, and immune variables (results shown as means ± Std. Dev.; influenza results shown as $\log_2$ HI titer means ± SEM and % subjects with "protective" titer defined as HI > 40)

Variable	Control pre-intervention	Exercise pre-intervention	Control post-intervention	Exercise post-intervention
Weight (kg)	76.05 ± 18.5	84.6 ± 17.8	76.4 ± 18.0	82.6 ± 17.4
BMI (kg/m^2)	27.5 ± 6.0	28.5 ± 4.8	27.4 ± 5.3	27.8 ± 5.1
Systolic BP	137.1 ± 16.0	141.5 ± 17.7	135.9 ± 15.4	138.5 ± 10.3
Diastolic BP	81.2 ± 10.8	83.4 ± 8.0	79.0 ± 7.1*	77.6 ± 10.3*
6 min walk (yards)	615 ± 109	636 ± 58	632 ± 109	716 ± 78**
Sense of coherence	75.9 ± 8.5	70.3 ± 8.9	72.1 ± 10.8	72.8 ± 7.2**
Depression	2.8 ± 3.1	3.8 ± 2.0	4.2 ± 3.0	3.1 ± 2.8**
Influenza A H1N1 pre-immune	5.7 ± 0.22	6.1 ± 0.29	5.9 ± 0.28	6.4 ± 0.47
Influenza A H1N1	5.7 ± 0.32	5.2 ± 0.30	6.0 ± 0.32	7.3 ± 0.51
Week 4 post	85%	86%	58%	85%
Influenza A H1N1	6.5 ± 0.32	6.6 ± 0.29	5.8 ± 0.33	7.0 ± 0.54
Week 12 post	77%	65%	35%	79%
Influenza A H3N2 pre-immune	5.6 ± 0.32	5.7 ± 0.31	6.2 ± 0.32	5.7 ± 0.30
Influenza A H3N2	7.2 ± 0.40	5.7 ± 0.43	6.5 ± 0.34	6.7 ± 0.22
Week 4 post	77%	65%	69%	85%
Influenza A H3N2	6.7 ± 0.39	5.7 ± 0.26	6.5 ± 0.32	6.5 ± 0.25
Week 12 post	77%	65%	69%	85%

* Main effect of time (change occurred in both exercise and control groups, $p < .05$).
** Treatment by time interaction (improvement in exercise group > control group, $p < .05$).

trol group (see Table 2). After the 10-month exercise intervention, a significant main effect of time ($p < .05$) was observed with respect to diastolic blood pressure, $F(1,25) = 4.64$, $p = .041$, and 6 min walk distance, $F(1,25) = 35.81$, $p < .001$. However, a significant time by treatment interaction [$F(1,25) = 13.49$, $p = .001$] was also observed with respect to the 6 min walk distance, such that the exercise group showed a greater improvement in walk distance (aerobic fitness) than the control group. The walk distance findings indicated that the exercise intervention was successful in terms of improved cardiorespiratory fitness. Data on other fitness variables measured by the Senior Fitness Tests (Rikli and Jones, 2001) are not shown, although both groups improved with respect to leg strength [$F(1,25) = 37.66$, $p < .001$] and upper body flexibility [$F(1,25) = 5.06$, $p = .036$]. No changes were found on lower body flexibility or agility. A significant time by treatment interaction [$F(1,25) = 6.45$, $p = .018$] was observed with respect to body weight, with the results indicating that the change in weight was significantly different between the exercise group (loss of 3.6 lbs) and the control group (gain of 0.8 lbs). A significant time by treatment interaction [$F(1,26) = 5.20$, $p = .03$] was observed with respect to depression, such that depression scores decreased in the exercise group (−0.71) and increased in the control group (1.36). Similarly, a significant time by treatment interaction [$F(1,26) = 4.86$, $p = .037$] was observed with respect to sense of coherence, with the results indicating that the change in scores was significantly different between the exercise group (increase of 1.0) and the control group (decrease of 3.2). No serious adverse events among participants were observed throughout the duration of the study.

3.2. Effect of the exercise intervention on immunocompetence

Prior to the intervention, potential differences between the experimental and control group with respect to A1 and A2 antibody titer were evaluated to determine whether antibody response to influenza immunization differed initially between the two groups. As indicated in Table 2, antibody titer was not significantly different between the experimental and control groups prior to the intervention (4 weeks and 12 weeks post-immunization, Fall 2000). After the intervention, the first set of analyses tested for differences between the experimental and control groups on the A1 and A2 antibody measures that were assessed 1, 4, and 12 weeks following immunization in Fall, 2001. The pre-immunization, post-intervention antibody values were not significantly different between the experimental and control groups (see Table 2). (For detailed values regarding antibody titer in response to all antigens contained in the vaccine, and how these values compare to young individuals, see Kohut et al., 2004a.) Changes on these measures from the levels of the A1 and A2 antibody measures that were assessed prior to immunization were computed in order to control for individual differences in levels of these antibodies. The results for change on the A1 antibody measures over time for the two groups of participants are shown in Fig. 2A. Although there was not a significant main effect for treatment group, $F(1,25) = 2.35$, $p = .14$, there was a statistically significant treatment by time interaction, $F(2,50) = 5.95$, $p < .01$. As can be seen in Fig. 2A, there was no difference between the two groups at the 1 week assessment, $F(1,25) = 0.03$, whereas there was a significant difference between the groups at the 4

week assessment, $F(1,25) = 5.13$, $p < .05$, and a marginally significant difference at the 12 week assessment $F(1,25) = 3.11$, $p = .09$.

Results for change on the A2 antibody measure over time for the two groups of participants are shown in Fig. 2B. In contrast to the previous results, there was a significant main effect of treatment group, $F(1,25) = 10.67$, $p < .01$, with neither the time, $F(2,50) = 0.69$, nor the time by treatment interaction, $F(2,50) = 2.91$, being statistically significant. Across the three assessments, members of the exercise group were found to demonstrate an increase in the average number of antibodies to the A2 virus ($M = 1.02$, 95% CI = 0.57–1.47), whereas members of the control group showed no change in the average number of antibodies to the A2 virus ($M = 0.00$, 95% CI = −0.46 to 0.46).

The next set of analyses examined differences between the groups on the two IFNγ measures that were assessed prior to and following the intervention. One issue that arises in conducting these analyses concerns differences in the scale of measurement associated with pre-intervention and post-intervention IFNγ measures. For example, scores on the A1 IFNγ measure from prior to the intervention ($M = 446.94 \pm 175.83$) were much lower than scores on the A1 IFNγ measure taken following the intervention ($M = 1355.67 \pm 489.29$). Similarly, scores on the A2 IFNγ measure taken prior to the intervention ($M = 338.26 \pm 171.54$) were also much lower than scores on the A2 IFNγ measure taken following the intervention ($M = 1193.64 \pm 511.64$). This difference in the measures was due to changes in the virus that was employed in conducting the assessments during the first and second years of the study. Virus was obtained from two different sources in Fall 2000 and Fall 2001 and, although the same range of HAU per milliliter was added to the cultures each year, there was variability in the response. This does, of course, create problems in evaluating the impact of the treatment program on these

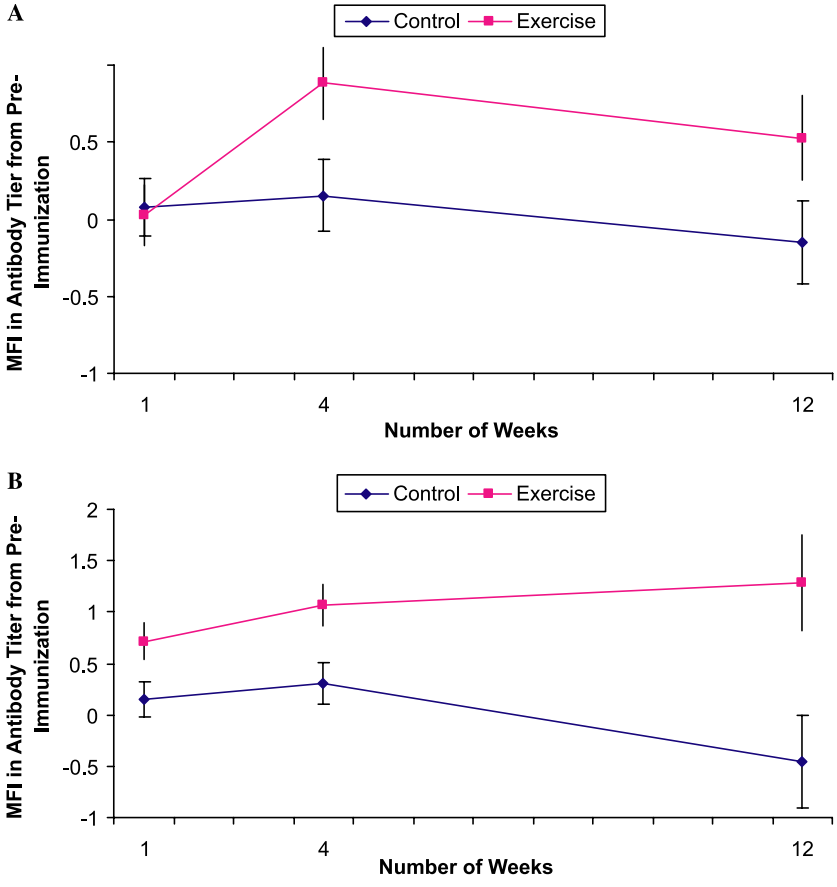

Fig. 2. (A) Plots of mean fold increase (MFI) in Type A H1N1 antibody titer (post-immunization minus pre-immunization) are shown on the *y*-axis and time post-immunization Fall 2001 are shown on the *x*-axis. The mean fold increase is calculated as the $\log_2$ transformed HI titer at post-immunizations time points minus the $\log_2$ transformed HI titer at pre-immunization. A statistically significant treatment by time interaction, $F(2,50) = 5.95$, $p < .01$. There was no difference between the two groups at the 1 week assessment, $F(1,25) = 0.03$, whereas there was a significant difference between the groups at the 4 week assessment, $F(1,25) = 5.13$, $p < .05$, and a marginally significant difference at the12 week assessment $F(1,25) = 3.11$, $p = .09$. (B) Plots of mean fold increase (MFI) in Type A H3N2 antibody titer (post-immunization minus pre-immunization) are shown on the *y*-axis and time post-immunization Fall 2001 are shown on the *x*-axis. The mean fold increase is calculated as the $\log_2$ transformed HI titer at post-immunizations time points minus the $\log_2$ transformed HI titer at pre-immunization. There was a significant main effect of treatment group, $F(1,25) = 10.67$, $p < .01$, with neither the time, $F(2,50) = 0.69$, nor the time by treatment interaction, $F(2,50) = 2.91$, being statistically significant.

measures over time. To control for this difference in the scale of the dependent measures over time, we first standardized scores on all four IFNγ measures relative to the sample's mean and standard deviation on each measure, so that the mean for each of these standardized scores was zero and the standard deviation was one. We then created change scores for each participant by subtracting their standardized score on the pre-intervention IFNγ measure from their standardized score on the post-intervention IFNγ measure. These change scores were then employed in testing for differences between the exercise and control groups.

Differences on the standardized change on the A1 IFNγ measure are shown in Fig. 3A. Members of the experimental group showed an average positive increase on the standardized IFNγ measure over time ($M = 0.57$) in contrast to the average decline by members of the control group on the standardized IFNγ measure over time ($M = -0.45$), a difference which was marginally significant, $F(1,19) = 3.07$, $p = .10$. Differences in change on the standardized A2 IFNγ measure are shown in Fig. 3B. Once again, members of the experimental group showed an average positive increase on the standardized IFNγ measure over time ($M = 0.62$) in contrast to the average decline by members of the control group on the standardized IFNγ measure over time ($M = -0.60$), a difference which was statistically significant, $F(1,20) = 4.66$, $p < .05$.

3.3. Tests of mediation

These results indicate that the exercise intervention had positive effects on several aspects of immunocompe-

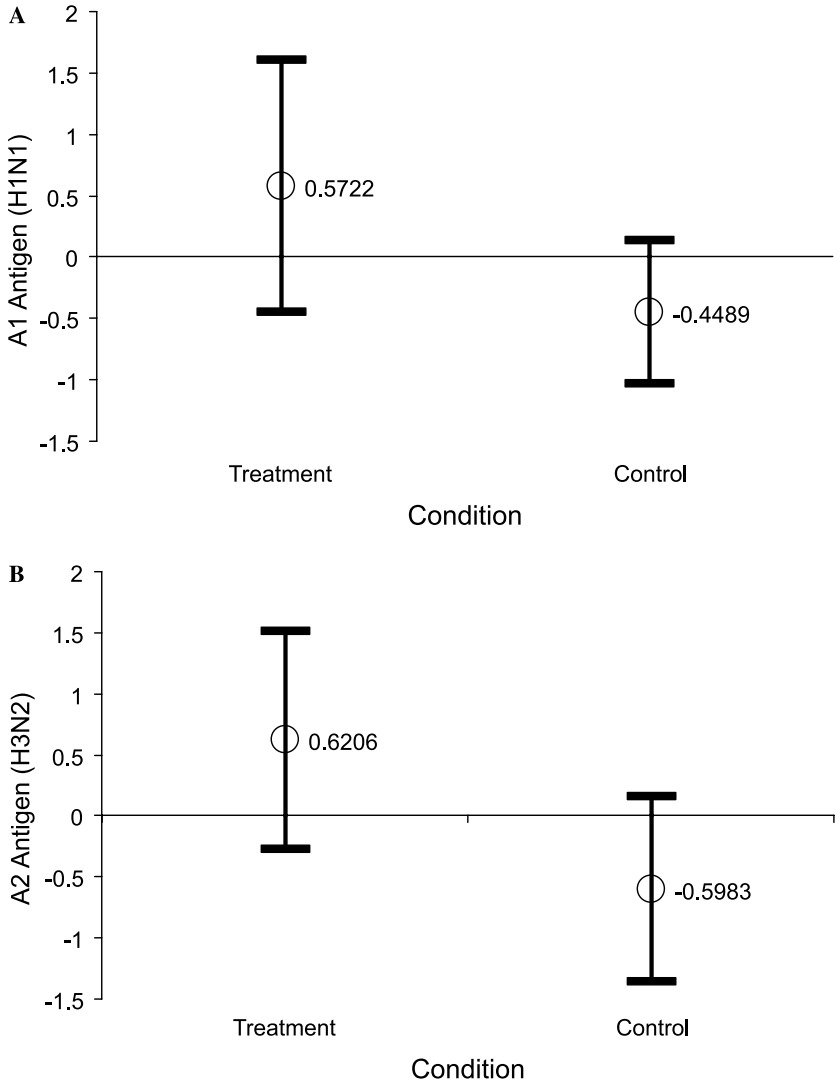

Fig. 3. (A) Standardized IFNγ measure over time in response to Type A H1N1 (A1). Over time members of the exercise group showed an increase over time in contrast to the average decline by members of the control group. The difference was marginally significant, $F(1,19) = 3.07$, $p = .10$. (B) Standardized IFNγ measure over time in response to Type A H3N2 (A2). The members of the experimental group showed an average positive increase on the standardized IFNγ measure over time ($M = 0.62$) in contrast to the average decline by members of the control group on the standardized IFNγ measure over time ($M = -0.60$), a difference which was statistically significant, $F(1,20) = 4.66$, $p < .05$.

tence. It also appears that the intervention had positive effects on two aspects of psychosocial functioning, involving change in depression and sense of coherence. The next set of analyses was designed to test whether or not changes on these two psychosocial variables may be responsible for the changes that were observed on the measures of immunocompetence (i.e., antibodies to the A1 and A2 virus and the A1 and A2 IFNγ measures). To control for any effects of these two psychosocial variables, we examined change on both variables simultaneously. (Additional analyses testing for mediation by each of the psychosocial variables were similar to the results reported below for both of the variables.) Results for the antibodies to the A1 virus indicated that none of the covariates or the interactions between the covariates and time were statistically significant. Furthermore, the time by treatment interaction remained statistically significant, $F(2,44) = 4.90$, $p = .01$, after controlling for the influence of the psychosocial variables on the A1 antibody measure. Results for the antibodies to the A2 virus indicated that one covariate, involving depression scores following completion of the exercise program, was significantly related to the number of antibodies, $F(1,22) = 10.12$, $p < .01$. However, despite this significant relationship, the impact of treatment group on the number of A2 antibodies remained statistically significant, $F(1,22) = 24.33$, $p < .001$. Furthermore, participants in the experimental treatment continued to have higher average change scores on the A2 antibody measure ($M = 1.33$, 95% CI = 0.90–1.76) than members of the control group ($M = -0.23$, 95% CI = −0.66 to 0.20) after controlling for the influence of the two psychosocial measures on the A2 antibody measure.

Analyses were also conducted examining the impact of the psychosocial measures on the relationship between treatment condition and change on the two standardized IFN measures. Neither of the psychosocial measures were found to be significantly related to either the change in the A1 IFNγ measure or the changes in the A2 IFNγ measure. However, once we controlled for the effect of the psychosocial measures on these two outcome variables, treatment condition was no longer significantly related to change on the A1 IFNγ measure, $F(1,16) = 1.31$, $p = .27$, or to change on the A2 IFNγ measure, $F(1,17) = 1.76$, $p = .20$.

4. Discussion

The purpose of this study was to determine whether exercise-induced alterations in the immune response to influenza immunization were mediated by improvements in psychosocial factors. The exercise treatment condition was associated with a greater increase in antibody titer to the influenza vaccine. However, the improvement in depression scores and sense of coherence *did not* mediate this increase in antibody titer. In contrast, the improvements in depression scores and sense of coherence *did* mediate at least some of the exercise-associated change in IFNγ. After controlling for the effects of the psychosocial measures, exercise treatment remained a significant predictor of A1 and A2 antibody titers. However, the effect of exercise on IFNγ was no longer significant. Taken together, our findings suggest that the mechanism of exercise-induced improvement in immunocompetence may involve both physiological and psychological pathways.

Other investigators have shown that psychosocial factors, such as the stress associated with caring for a person with dementia, may influence the immune response to influenza vaccine among older adults (Kiecolt-Glaser et al., 1996; Vedhara et al., 1999). We also observed that psychosocial factors can impact immunity to influenza vaccine. For example, IFNγ to A2 was correlated with depression scores ($r = -.42$, $p = .04$), and improvements in depression and sense of coherence accounted for some of the exercise-associated increase in IFNγ. Numerous studies have reported psychosocial-immunity relationships. Our results extend the previous work in this area by demonstrating that an intervention (exercise) may alter immunity via improvements in psychosocial status as well as via other pathways. However, these "other pathways" remain to be identified. One limitation to our study design was the possibility that the social interaction associated with regular exercise influenced immune outcomes. It is possible that the physiological alterations in the neuroendocrine milieu associated with exercise training influence immune function. Lymphocytes and macrophages have receptors for the neuroendocrine factors, and the concentration of many stress hormones in the blood (e.g., epinephrine, norepinephrine, growth hormone, β-endorphins, and cortisol) increase during acute exercise (Pedersen and Hoffman-Goetz, 2000). With regular exercise training, cells of the immune system may adapt to the neuroendocrine outflow in such a way that immune responses are improved. Existing evidence suggests that training-induced alterations in immunity involve neuroendocrine factors. For example, exercise training-induced modulation of the immune response to viral infection is mediated, in part, by catecholamines (Kohut et al., 2004b), and others have shown that endogenous opioids may also play a role in mediating the exercise training-associated changes in antibody (Kapasi et al., 2001). It is also possible that central nervous system responses are altered by exercise training, resulting in modulation of neuroendocrine–immune interaction. In support of this possibility, it appears that exercise training attenuates the sympathetic outflow to the spleen during stress, by diminishing the stress-induced activation of brain regions involved in sympathetic regulation (Greenwood et al., 2003). One could extrapolate from these findings that the immunosuppressive effects of stress may be blunted in

trained individuals. In summary, it is possible that the neuroendocrine–immune alterations that occur as a result of exercise training involve both cellular adaptations as well as central adaptations (e.g., brain activation).

Immune benefits associated with exercise and improved psychosocial status has been reported by others, although other studies did not assess the role of psychosocial factors as mediators of exercise-associated immunological changes. LaPerriere et al. (1990, 1991) showed that participants in a 10 week exercise program reported less anxiety and depression than non-exercisers, pending notification of HIV-1 status. Also, HIV-1 seropositive exercisers had a slight increase in $CD4^+$ cell count and no change in NK cell number compared to seropositive non-exercisers who did have a decline in NK cells (LaPerriere et al., 1990, 1991). A separate study demonstrated that a 7-month exercise intervention in cancer patients improved NK cell activity and life satisfaction (at 5 weeks of exercise) and reduced discomfort (at 7 months of exercise; Peters et al., 1994). Although the mechanisms by which exercise may alter psychological function have not been elucidated, exercise training appears to decrease risk of cognitive impairment and dementia among the aged, suggesting that exercise can impact brain function (Laurin et al., 2001). In microarray studies, exercise has been shown to upregulate genes involved in signal transduction pathways, neuronal activity, synaptic structure, and neuronal plasticity, particularly brain-derived neurotropic factors (Cotman and Berchtold, 2002; Molteni et al., 2002; Tong et al., 2001). Therefore, current literature supports the possibility that exercise may alter brain function and psychosocial factors, potentially leading to alterations of immune responsiveness.

In light of the findings presented here and related work on this topic (Kohut et al., 2001, 2004a), exercise shows promise as a positive immunomodulator in older adults. One would expect that the exercise-associated increase in antibody titer provides better protection from influenza infection (Davis and Grillis, 1989). Also, exercise-induced increase in IFNγ production may be beneficial in terms of viral clearance during actual influenza infection. The results of this study suggest that depression and sense of coherence as well as other undefined mechanisms mediate the improvement in immune response to influenza resulting from a long-term (10 month) exercise intervention. Current studies are underway to evaluate the potential role of neuroendocrine factors as mediators of exercise-induced enhancement of immunity in older adults.

Acknowledgment

This work was supported in part by a grant from NIAID, No. R01 AI49956.

References

Antonovsky, A., 1993. The structure and properties of the sense of coherence scale. Soc. Sci. Med. 36, 725–733.

Arent, S.M., Landers, D.M., Etnier, J.L., 2000. The effects of exercise on mood in the elderly: a meta-analytic review. J. Aging Phys. Activity 8, 407–430.

Bernstein, E.D., Gardner, E.M., Abrutyn, E., Gross, P., Murasko, D.M., 1998. Cytokine production after influenza vaccination in a healthy elderly population. Vaccine 16, 1722–1731.

Bender, B.S., Johnson, M.P., Small, P.A., 1991. Influenza in senescent mice: impaired cytotoxic T-lymphocyte activity is correlated with prolonged infection. Immunology 72, 514–519.

Betts, R.F., Treanor, J.J., 2000. Approaches to improved influenza vaccination. Vaccine 18, 690–695.

Blumenthal, J.A., Babyak, M.A., Moore, K.A., Craighead, W.E., Herman, S., Khatri, P., Waugh, R., Napolitano, M.A., Forman, L.M., Appelbaum, M., Doraiswamy, P.M., Krishnan, K.R., 1999. Effects of exercise training on older patients with minor depression. Arch. Intern. Med. 159, 2349–2356.

Cotman, C.W., Berchtold, N.C., 2002. Exercise: a behavioral intervention to enhance brain health and plasticity. Trends Neurosci. 25, 295–301.

Couch, R.B., Kasel, J.A., 1995. Influenza. In: Lennette, E.H., Lennette, D.A., Lennette, E.T. (Eds.), Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. American Public Health Association, Washington, DC, pp. 431–446.

Davis, J.R., Grillis, E.A., 1989. Natural or vaccine-induced immunity as a predictor of immunity in the face of natural challenge with influenza virus. Epidemiol. Inf. 102, 323–325.

Demicheli, V., Jefferson, T., Rivetti, D., Deeks, J., 2000. Prevention and early treatment of influenza in young adults. Vaccine 18, 957–1030.

Dunn, A.L., Trivedi, M.H., O'Neal, H.A., 2001. Physical activity dose–response effects on outcomes of depression and anxiety. Med. Sci. Sports Exerc. 33, S587–S597.

Falsey, A.R., Cunningham, C.K., Barker, W.H., 1995. Respiratory syncytial virus and Influenza A infections in the hospitalized elderly. J. Infect. Dis. 172, 389–394.

Gardner, E.M., Bernstein, E.D., Dran, S., Munk, G., Gross, P., Abrutyn, E., Murasko, D.M., 2001. Characterization of antibody responses to annual influenza vaccination over 4 years in a healthy elderly population. Vaccine 19, 4610–4617.

Greenwood, B.N., Kennedy, S., Smith, T.P., Campeau, S., Day, H.E., Fleshner, M., 2003. Voluntary freewheel running selectively modulates catecholamine content in peripheral tissue and c-Fos expression in the central sympathetic circuit following exposure to uncontrollable stress in rats. Neuroscience 120, 269–281.

Gross, P.A., Hermogenes, A.W., Sacks, H.S., Lau, J., Levandowski, R.A., 1995. The efficacy of influenza vaccine in elderly persons. Ann. Intern. Med. 123, 518–527.

Kapasi, Z.F., Catlin, P.A., Beck, J., Roehling, T., Smith, K., 2001. The role of endogenous opioids in moderate exercise training-induced enhancement of the secondary antibody response in mice. Phys. Ther. 81, 1801–1809.

Keren, G., Segev, S., Morag, A., Zakay-Rones, Z., Barzilai, A., Rubenstein, E., 1988. Failure of influenza vaccination in the aged. J. Med. Virol. 25, 85–89.

Kiecolt-Glaser, J.K., Glaser, R., Gravenstein, S., Malarkey, W.B., Sheridan, J., 1996. Chronic stress alters the immune response to influenza virus vaccine in older adults. Proc. Natl. Acad. Sci. USA 93, 3043–3047.

Kiecolt-Glaser, J.K., McGuire, L., Robles, T., Glaser, R., 2002. Psychoneuroimmunology and psychosomatic medicine: back to the future. Psychosom. Med. 64, 15–28.

Kohut, M.L., Cooper, M.M., Nickolaus, M.S., Russell, D.R., Cunnick, J.E., 2002. Exercise and psychosocial factors modulate immunity to

influenza vaccine in elderly individuals. J. Gerontol. Med. Sci. A 57, M557–M562.

Kohut, M.L., Boehm, G.W., Moynihan, J.A., 2001. Moderate exercise is associated with enhanced antigen-specific cytokine, but not IgM antibody production in aged mice. Mech. Ageing Dev. 122, 1135–1150.

Kohut, M.L., Arnston, B.A., Lee, W., Rozeboom, K., Yoon, K.J., Cunnick, J.E., McElhaney, J.E., 2004a. Moderate exercise improves antibody response to influenza immunization in older adults. Vaccine 22, 2298–2306.

Kohut, M.L., Thompson, J.R., Lee, W., Cunnick, J.E., 2004b. Exercise training-induced adaptations of immune response are mediated by beta-adrenergic receptors in aged, but not young mice. J. Appl. Physiol. 96, 1312–1322.

LaPerriere, A., Fletcher, M.A., Antoni, M.H., Klimas, G., Ironson, G., Schneiderman, N., 1991. Aerobic exercise training in an AIDS risk group. Int. J. Sports Med. 12, S53–S57.

LaPerriere, A.R., Antoni, M.H., Schneiderman, N., Ironson, G., Klimas, N., Caralis, P., Fletcher, M.A., 1990. Exercise intervention attenuates emotional distress and natural killer cell decrements following notification of positive serologic status for HIV-1. Biofeedback Self-Regulat. 15, 229–242.

Laurin, D., Verreault, R., Lindsay, J., MacPherson, K., Rockwood, K., 2001. Physical activity and risk of cognitive impairment and dementia in older adults. Arch. Neurol. 58, 498–504.

Lui, K.J., Kendal, A.P., 1987. Impact of influenza epidemics on mortality in the United States from October 1972 to May 1985. Am. J. Pub. Health 1977, 712–716.

Mbawuike, I.N., Lange, A.R., Couch, R.B., 1993. Diminished influenza A virus-specific MHC class I-restricted cytotoxic T lymphocyte activity among elderly persons. Viral Immunol. 6, 55–64.

McElhaney, J.E., Beattie, B.L., Devine, R., Grynoch, R., Toth, E.L., Bleackley, R.C., 1990. Age-related decline in interleukin 2 production in response to influenza vaccine. J. Am. Geriatr. Soc. 38, 652–658.

McElhaney, J.E., Pinkoski, M.J., Upshaw, C., Bleackley, R.C., 1996. The cell-mediated cytotoxic response to influenza vaccination using an assay for granzyme B activity. J. Immunol. Meth. 190, 11–26.

McElhaney, J.E., Upshaw, C.M., Hooton, J.W., Lechelt, K.E., Meneilly, G.S., 1998. Responses to influenza vaccination in different T-cell subsets: a comparison of healthy young and older adults. Vaccine 16, 1742–1747.

McElhaney, J.E., Gravenstein, S., Upshaw, C.M., Hooton, J.W., Krause, P., Drinka, M.P., Bleackley, R.C., 2001. Granzyme B; a marker of risk for influenza in institutionalized older adults. Vaccine 19, 3744–3751.

McElhaney, J.E., Herre, J.M., Lawson, M.L., Cole, S.K., Burke, B.L., Hooton, J.W., 2004. Effect of congestive heart failure on humoral and ex vivo cellular immune response to influenza vaccination in older adults. Vaccine 26, 681–688.

McMichael, A.J., Gotch, F.M., Noble, G.R., Beare, P.A., 1983. Cytotoxic T-cell immunity to influenza. New Engl. J. Med. 309, 13–17.

Molteni, R., Ying, Z., Gomez-Pinilla, F., 2002. Differential effects of acute and chronic exercise on plasticity-related genes in the rat hippocampus revealed by microarray. Eur. J. Neurosci. 16, 1107–1116.

Pedersen, B.K., Hoffman-Goetz, L., 2000. Exercise and the immune system: regulation, integration, and adaptation. Physiol. Rev. 80, 1055–1081.

Peters, C., Lotzerich, H., Niemeier, B., Schule, K., Uhlenbruck, G., 1994. Influence of a moderate exercise training on natural killer cytotoxicity and personality traits in cancer patients. Anticancer Res. 14, 1033–1036.

Powers, D.C., 1993. Influenza A virus specific cytotoxic T lymphocyte activity declines with advancing age. J. Am. Geriatr. Soc. 41, 1–5.

Rikli, Jones, 2001. Senior Fitness Test Manual, Human Kinetics, Champaign, IL.

Shearer, G.M., 1997. T_H1/T_H2 changes in aging. Mech. Ageing Dev. 94, 1–5.

Shinkai, S., Kohno, H., Kimura, K., 1995. Physical activity and immune senescence in men. Med. Sci. Sports Exerc. 27, 1516–1526.

Sprenger, M.J., Mulder, P.G., Beyer, W.E., Van Strik, R., Masurel, N., 1993. Impact of influenza on mortality in relation to age and underlying disease, 1967–1989. Int. J. Epidemiol. 192, 334–340.

Tong, L., Shen, H., Perreau, V.M., Balazs, R., Cotman, C.W., 2001 Effects of exercise on gene-expression profile in the rat hippocampus. Neurobiol. Dis., 8, 1046–1056.

Vedhara, K., Cox, N.K., Wilcock, G.K., Perks, P., Hunt, M., Anderson, S., Lightman, S.L., Shanks, N.M., 1999. Chronic stress in elderly carers of dementia patients and antibody response to influenza immunization. Lancet 353, 627–631.

Wilde, J.A., McMillan, J.A., Serwint, J., Butta, J., O'Riordan, M.A., Steinhoff, M.A., 1999. Effectiveness of influenza vaccine in health care professionals: randomized trial. JAMA 28, 908–913.

Woods, J.A., Ceddia, M.A., Wolters, V.W., Evans, L.K., McAuley, E., 1999. Effects of 6 months of moderate aerobic exercise training on immune function in the elderly. Mech. Ageing Dev. 109, 1–19.

Yamada, A., Meager, A., Yamada, O., Ennis, F.A., 1986. Human interferon alpha and gamma production by lymphocytes during the generation of influenza virus-specific cytotoxic T lymphocytes. J. Gen. Virol. 67, 2325–2334.

Yesavage, J.A., Brink, T.L., Rose, T.L., Lum, O., Huang, V., Adey, M., Leirer, V.P., 1983. Development and validation of a geriatric depression screening scale: a preliminary report. J. Psychiatr. Res. 17, 37–49.

Zorrilla, E.P., Luborsky, L., McKay, J.R., Rosenthal, R., Houldin, A., Tax, A., McCorkle, R., Seligman, D.A., Schmidt, K., 2001. The relationship of depression and stressors to immunological assays: a meta-analytic review. Brain Behav. Immun. 15, 199–226.