The effects of moderate aerobic exercise training on influenza-immune response among the elderly population

by

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A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Exercise and Sport Science (Biological Basis of Physical Activity)

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2002

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INTRODUCTION

Aging is associated with functional decline in several components of the immune system. As a result of this age-associated decrement of the immune system, the elderly appear to be at an increased risk of contracting infectious diseases and also experience a greater incidence of autoimmune disorders. Upon infection, older adults often have higher mortality rates than the young. These changes in the immune responsiveness may compromise health status of older adults.

The functions of many immune cell types change with age, but the T-cell is most affected by the aging process. The T-cells show a decreased ability to proliferate when stimulated by mitogens. Within the T-cell subsets, both CD4+ and CD8+ cell numbers decrease with age, but the effect is more pronounced with respect to CD8+ T-cells, resulting in an age-related increase in the CD4:CD8 ratio. The production of an important cytokine, IL-2, also decreases with age. The activity of cytotoxic T-lymphocytes also declines with age. The consequence of these changes may be a reduced ability to respond to new antigens and clear infectious agents.

Changes in the aging immune system represent a permissive factor for the frequent occurrence and the severity of disease. Vaccine efficacy is also impaired in elderly populations. Efficient protection of elderly individuals through vaccination strategies is a matter of great importance. Therefore, any intervention that can improve the immune response to immunization may reduce the incidence of infection.

Interventions such as diet and/or exercise may influence immunity. Moderate exercise has been associated with enhanced immune responses to nonspecific immune

activators. Also, vitamin E has been targeted as enhancing the immune function in aged animals and elderly people.

The parameters that we researched within this study include antibody titer, lymphocyte proliferation, and cytokine production (interleukin-2, interleukin-10, and interferon gamma). Antibodies, secreted by B-cells, are proteins that specifically bind a wide variety of protein antigens. They are produced in response to either an infectious agent or vaccination. They elicit a response that is significant in antimicrobial defense. Antibodies are the mediators of circulating immunity and their presence on mucosal surfaces provides resistance to many infectious agents (1).

Lymphocytes are one of the main types of immune cells. The two main types of lymphocytes are the B and T cells. B cells are important for secreting antibodies. T cells work primarily by secreting cytokines. They direct and regulate the immune responses. Other T cells act as killer cells by attacking cells that are infected (1).

Cytokines are protein hormones produced during the activation and effector phases of innate and specific immunity. They serve to mediate and regulate immune and inflammatory responses. Cytokines are a means of intracellular communication. They are generally secreted by one cell to stimulate the activity of another cell (1).

Our study examined exercise as an intervention to improve the immune response to influenza vaccine. The effects of a long-term exercise intervention on immune response are essentially unknown. In this study, we will also look as the diet and the role that it may play in the immune response.

LITERATURE REVIEW

BASIC IMMUNE RESPONSE

The immune system is a network of cells and organs that work together to defend the body against attacks by foreign invaders. The immune system is amazingly complex. It can recognize millions of different enemies and produce secretions and cells to match up with and destroy each one of them. The immune system is involved in a communication network in which cells pass information back and forth among one another. Once the immune cells receive an alarm from an invader, they undergo changes and produce powerful chemicals.

Anything that can trigger an immune response it called an antigen. An antigen can be a germ such as a virus, or even part of a virus. Antigens carry marker molecules that identify themselves as foreign to the immune cells. An immune response can also be sparked by immunization with vaccines. Vaccines contain microorganisms, or parts of organisms, that have been treated so they will be able to provoke an immune response, but not a mature disease.

INFLUENZA IMMUNIZATION AND ANTIBODY TITER

Influenza is a significant cause of morbidity and mortality in the elderly populations. Estimates are that more than 80% of deaths from influenza occur in adults $\geq$ age 65 (2). Following immunization, protection against infection is also reduced in the elderly. For example, among the persons over age 65 hospitalized for influenza, 58-61% had been previously vaccinated (3,4). Vaccine efficacy estimated for the elderly ranges from 31% to 65% in preventing influenza (5). This is quite low, especially when compared to the vaccine efficacy seen in younger adults, which ranges from 70% to 90% (4).

Many vaccinated elderly demonstrate a decreased antibody response against influenza immunization which may account for the decreased vaccine efficacy. Serum antibody, mainly IgG, produced after vaccination, has the capability of neutralizing viral particles and thus protects against infection. Hemagglutination inhibition (HI) antibody titers ≥ 40 are considered protective in young people (6). The antibody titer is the primary predictor for an immune response, having the greatest correlation with protection from influenza. Several studies have shown that at least 25% of the elderly do not develop HI antibody titers ≥ 40 in response to vaccine (4,7,8).

Not all studies suggest an age-related decrease in humoral immune responsiveness to vaccination. However, in the elderly, antibody responses that are equal in magnitude to young people may not provide the same level of protection from influenza. There may be a large percentage of elderly with influenza infections who have post-vaccination titers > 40 and still suffer from influenza infection. For example, in one study Gravenstein and colleagues showed that of 72 vaccinated elderly who were later confirmed to have influenza infection, 60% had titers ≥ 40 and 31% had titers ≥ 640 four weeks after vaccination (9). This suggests that levels of antibody considered protective in the young are not necessarily protective in the elderly.

To determine the influence of aging as well as the priming histories on the antibody response to influenza vaccination, 43 healthy young subjects and 55 elderly subjects were compared. Remarque and others found that elderly were capable in mounting similar antibody responses to the influenza vaccine strains as healthy young subjects. For certain strains, antibody responses were lower in the elderly compared to young subjects. He

concluded that the strain-related difference in the antibody response may be due to a difference in priming histories, rather than a result of the aging immune system (12).

ELDERY AND INFLUENZA VACCINATION

The reduced capacity of elderly people to mount an effective immune response has been well documented (11). Influenza vaccines are only effective in 30% to 50% of the elderly population immunized (12), leaving the elderly population at a large risk for life-threatening illness. More than 80% of influenza-related deaths occur in people 65 years and older (13).

Strassburg and colleagues researched on influenza outbreaks in nursing homes during 1967 to 1982 and the effectiveness of the influenza vaccination. Compared with unvaccinated residents, the mean reduction of mortality in vaccinated residents was 67% (14). However, the reduction of morbidity was much lower (23%).

It is known that older people have a lower antibody titer response to influenza vaccination than younger people, but other factors, such as cellular immunity may influence resistance to influenza.

INFLUENZA AND CELL-MEDIATED IMMUNITY

In regards to cell-mediated immunity, age associated decrements of immune function, particularly T-lymphocyte function, likely contribute to the increased incidence and severity of influenza infection. Upon infection, cell-mediated responses, particularly cytotoxic T-lymphocytes, are significant in viral clearance (15). It is suggested that cell mediated immune responses to influenza vaccination are also impaired in the elderly, mostly related to a decline in T-cell function. Cytokines produced by T-cells drive cytotoxic effector function and antibody production, and therefore may be of great importance in terms of disease

outcome and the development of immune protection following vaccination. Young people produce greater amounts of the influenza specific interleukin-2 (IL-2) and interferon - *y* (IFN-*y*) post-vaccination than older adults (4), so it is possible that the age-associated decrease in cell-mediated immune effector function is related to decreased IL-2 and/or IFN-*y*.

Cell-mediated immune responses, particularly T-helper cells, are important in promoting antibody production by producing cytokines. Naïve and memory helper T-cells have different activation requirements *in vitro,* and differ in their lymphokine profiles. Thus, the altered composition of the helper T cell compartment may result in qualitative and quantitative differences in T-cell help delivered to B cells, which in turn can influences the antibody levels. Remarque and colleagues found that the number of helper T cells with memory phenotype are positively correlated with the IgG response to the influenza vaccination. However, age-related differences in the composition of the helper T cell compartment, such as a decline in naïve helper T-cells and increase in memory helper T-cells, did not explain age-related differences in antibody production (16).

STRATEGIES TO ENHANCE VACCINE EFFICACY

The post-vaccination antibody levels achieved are not as high in the old as in the younger populations. An approach to increasing the rate of vaccine protection has been the development of the cold adapted live attenuated vaccine by Maassab and colleagues (10). In this study, the use of the cold adapted live attenuated vaccine did not reduce infection rates, and antibody titers remained lower among the elderly (17). Cold adapted vaccine almost completely blocked infection and clinical illnesses among the young. It appears that cold adapted vaccines are more efficacious among the young, healthy subjects, but less effective among the elderly population.

A novel oil-in-water emulsion, MF59, has been tested on elderly people as a method of improving vaccine efficacy. MF59 has been known to stimulate immune response. When combined with the influenza vaccine, fewer deaths occurred among the recipients who received MF59. The MF59 emulsion was thought to stimulate immune functions that typically decrease with age (18).

Dehydroepiandrosterone (DHEA) treatment has also been tried as a method of improving influenza vaccine effectiveness in the elderly. Numerous animal studies have demonstrated beneficial effects of DHEA administration in preventing atherosclerosis, cancer, and immunosenescence. However, human studies with DHEA did not find a beneficial effect on antibody titer post-immunization (3).

EXERCISE AND IMMUNITY

It is possible that exercise may enhance immunity in the elderly population. There is growing belief that endurance exercise may slow the age-related decline in immune function (19). A few cross-sectional comparisons of immune status between physically fit elderly individuals and sedentary controls suggest that regular physical activity may enhance natural killer (NK) cell activity (20, 21, 22). Highly trained elderly individuals have enhanced lymphocyte proliferation and cytokine production in response to mitogen stimulation compared to sedentary elderly people (23,24), suggesting that exercise may also enhance T-cell function. Some recent results have demonstrated that anti-influenza antibody titer (IgG and IgM) were greater in active participants as compared to moderately active or sedentary participants (25). A couple of studies utilizing 12 weeks of exercise as an intervention failed to find an enhancement of mitogen-induced T-cell function or NK cell cytotoxicity (23,26). This may be because an exercise intervention of longer duration is necessary to elicit

significant changes in immune response in elderly. Six months of exercise in a separate controlled trial showed a trend towards increased mitogen-induced T-cell proliferation and increased NK activity (27). Perhaps an exercise intervention of a longer duration (> 6 months) may be necessary to elicit statistically significant changes in immune function.

DIET AND IMMUNITY

Dietary intakes can affect the function of immune system. The impact of diet and specific food groups on aging has been widely recognized in recent years. The free radical theory of aging suggest that there is a shift in the antioxidant/pro-oxidant balance that leads to increased oxidative stress and dysregulation of cellular function. Several epidemiological and clinical studies have revealed potential roles for dietary antioxidants in the age-associated decline of immune function. It has been reported that long term vitamin E supplementation enhances immune function in aged animals and elderly subjects (28). Evidence suggests that a level of vitamin E greater than the currently recommended level enhances certain clinically relevant in vivo indexes of T-cell-mediated function in the elderly population (29,30). Meydani and others found a six-fold increase in antibody titer to hepatitis and a significant increase in antibody titer to tetanus vaccine in elderly individuals who were supplemented with 200 mg/d of vitamin E compared to the placebo group (29). Han and colleagues compared the effect of vitamin E on the course of influenza infections with that of other antioxidants in mice. From this mouse study, they concluded that only vitamin E supplementation was effective in reducing pulmonary viral titers and preventing an influenza-mediated decrease in food intake and weight loss (31).

STATEMENT OF THE PROBLEM

An age-related decline in several components of the immune system puts the elderly population at a great risk for developing infections and diseases. The purpose of this study is to determine whether moderate to vigorous aerobic exercise training can enhance influenza-specific immune response among the elderly population. A second purpose is to determine if nutrient intake is associated with the immune response to influenza immunization. The hypothesis is that 10 months of supervised moderate to vigorous aerobic exercise will enhance influenza-specific immune response following immunization in individuals $\geq$ 65 years of age. A second hypothesis is that there will be an some association between nutrients and the immune response to the influenza vaccination in individuals $\geq$ 65 years of age.

EXPERIMENTAL DESIGN AND METHODOLOGY

In this study, the hypothesis tested was that 10 months of moderate to vigorous aerobic exercise will increase the influenza-specific immune responses following immunization in individuals $\geq$ 65 years of age. The immune outcome measures included are: influenza-specific lymphocyte proliferation, influenza-specific cytokine production (IL-2, IFNy, IL-10 measured in supernatants by ELISA), and anti-influenza antibody titer. Fourteen sedentary subjects (7 male, 7 female) were assigned to a 10-month aerobic exercise intervention. Preliminary studies have detected differences in the immune response to influenza immunization based on reported activity level within an average of 14 per group. Thirteen people (6 male, 7 female) served as control subjects. Originally there were fourteen controls, but a quarter of the way through the study one subject developed cancer and was required to terminate from the study. The control group members were instructed to continue with current activity levels. The control group either had not exercised on a regular basis and/or scored in the lowest quartile for aerobic fitness measured by a six-minute walk test. Each of the subjects signed an informed consent that was approved by the ISU Human Subjects in Research Committee.

SUBJECTS

Fourteen subjects, 7 male and 7 female, age $\geq$ 65, not currently participating in any type of consistent aerobic exercise were recruited from the local community to participate in he proposed research study. Thirteen subjects, 6 male and 7 female, aged $\geq$ 65, considered sedentary and/or low aerobic fitness were selected to serve as controls. Subjects were defined as healthy, but may not have met the rigid selection set forth in the SENIEUR protocol. Subject inclusion and exclusion was dependent on age, health

status, exercise history, medication use, influenza vaccination history, and ability to perform aerobic exercise on a regular basis. Reasoning for the lower age limit of 65 was based upon research suggesting that immune response to vaccination may be impaired as age increases. There was no set upper limit selected with regards to age. However, the upper age limit was affected by the ability of the subjects to perform the aerobic exercise intervention. Health status was determined by a detailed medical history. Exclusion from the study included any individuals suffering from any condition that may have altered the immune variables of interest (i.e., autoimmune disorder, malignancy, etc.). Also, any subject experiencing a disease or condition that may have impaired the ability to safely perform the exercise intervention was excluded from the study. Subjects assigned to the exercise group completed a maximal graded exercise test. The maximal graded exercise test screened potential subjects for the ability to perform the exercise intervention. An abnormal maximal exercise test resulted in exclusion from the study. However, none of the subjects were excluded for this reason. Medication use also limited participation in the study. Subjects treated with medications known to alter immune response were excluded from the study. Prior exercise history was another criterion for inclusion into the study. Subjects were included in the study if in the past two years they did not exercise or if they engaged in aerobic exercise $\leq 40\%$ of the heart rate reserve ≤ 3 times per week, or if their aerobic fitness was in the lowest quartile for that age and gender. If the exercise heart rate information was not available, subjects that regularly walk at a pace ≤ 2.0 miles per hour or did not report vigorous activity (defined as strenuous enough to cause large increases in breathing, heart rate, leg fatigue, or produce perspiration) were included in the study. Prior vaccination history allowed us to select

individuals with a similar antigenic history and therefore, subjects were included in the study if they received a flu vaccine in the previous five years.

TIME-LINE

The study began within two months prior to the first influenza season. Initial recruitment and testing of subjects was performed during this 2-month period. During the fall of 2000, a baseline blood sample (pre-immunization), a fitness assessment adapted for older adults, and a diet questionnaire were administered one month prior to immunization as part of the initial screening. A physician-supervised maximal treadmill test was performed on the subjects in the exercise group prior to the exercise intervention. All subjects received the 2000/2001 influenza vaccine (A/New Caledonia/20/99, A/Panama/2007/99, B/Yamanashi/166/98) in the fall of 2000. Venous blood was collected at 1, 4, and 12-weeks post-immunization. After 10 months of participation in either the aerobic exercise or control group, the same procedures were followed in terms of measurements taken. In the fall of 2002, subjects completed a fitness assessment, diet questionnaire, and a pre-immunization blood-sample was taken. Subjects were vaccinated with the 2001/2002 influenza vaccine (A/New Caledonia/20/99, A/Panama/2007/99, B/Victoria/504/2000). Blood was collected at 1, 4, and 12-weeks post-immunization for immune analyses. Subjects continued participation in either the exercise group or the control group until the final blood sample was taken. The following immune variables were assessed by the blood samples: influenza-specific lymphocyte proliferation, influenza-specific IL-2, IL-10, IFN-y, and anti-influenza antibody titer. The selection of immune measures to be assessed at these time points was based on data collected in our laboratory and other investigators. We followed the schedule below (Table 1).

Table 1. Schedule for blood draw immune analysis, fitness tests, and diet questionnaires.

Immunization #1 ↓ **Immunization #2** ↓

Blood Draw	Pre-Immuni-zation #1	Week 1 Post-vaccine	Week 4 Post-vaccine	Week 12 Post-vaccine	Pre-immuni-zation #2	Week 1 Post-vaccine2	Week 4 Post-vaccine2	Week 12 Post-vaccine2
Time in Weeks	-4 to -1	1	4	12	40	42	45	53
Antibody Titer	X	X	X	X	X	X	X	X
Cytokines	X	X	X	X	X	X	X	X
Prolif-Eration	X	X	X	X	X	X	X	X
Fitness Test	X				X			
Diet Questions	X				X			

ASSESSMENT OF DIET AND FITNESS

As part of the initial screening, all participants completed a dietary questionnaire (block 98 Dietary data systems, Berkeley, CA). Participants were asked to inform the investigators regarding any changes in diet or dietary supplement use.

Fitness tests appropriate for older adults were administered following the recommendations given at the recent ACSM Specialty Conference on Physical Activity Programming for the Older Adult (32). Physician's approval prior to fitness testing was required if the subject had ever had congestive heart failure, was told by a physician not to exercise, had pain in chest or joints that may be made worse by exercise, or had blood pressure > 160/90. The fitness tests included a 30 second chair stand, 30 second arm curl, 6 minute walk, 2 minute step in place, chair sit and reach, back scratch flexibility, 8 foot up and go. Height and weight were measured and body composition was assessed using

skinfold thickness and age-appropriate equations. Fitness tests were done to see if exercise program improved all aspect of fitness. However, our hypothesis is based only on improving aerobic fitness.

ASSESSMENT OF INFLUENZA INFECTION

All subjects were called bi-weekly during the influenza season (November-April) to answer questions regarding symptoms of illness. If necessary, subjects with symptoms of influenza at any time were referred to a physician for laboratory confirmation of influenza and appropriate antiviral treatment. This was not necessary in our study.

BLOOD COLLECTION AND IMMUNIZATION

All subjects received influenza immunization and blood samples (40 ml) were collected between 7-9 a.m. on all blood collection days. Collecting in the morning helped to control for the potential influence of daily levels of plasma hormones such as cortisol. All subjects received influenza immunizations by a registered nurse. Blood samples were collected 1-week pre-immunization and 1, 4, and 12-weeks post-immunization as described previously under the timeline.

EXERCISE OR CONTROL INTERVENTION

After initial measures were taken and the first immunization had been administered, exercise participants participated in a supervised aerobic exercise class three times per week. They used treadmills, stair-steppers, rowing machines, and cycle ergometers for the twelve-month intervention period. This period of time had been chosen based on cross-sectional comparisons as well as experimental studies suggesting that a longer period of time may be necessary to evoke alteration of the immune function. This is opposed to typical improvements in cardiorespiratory fitness that is observed after 8-12 weeks of exercise.

Initially, subjects exercised at an intensity corresponding to 40-60% of heart rate reserve (HRR) for 20 minutes/session and gradually progressed to an intensity corresponding to 65-75% of HRR for 25-30 minutes. The subject's heart rate was monitored by exercise leaders or individuals recorded the heart rate from the electronic device on the machine.

IMMUNE ANALYSES

Each time blood samples were taken, serum was used for anti-influenza antibody analysis and the cell fraction were used for the other immune assays. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood samples by centrifugation over Ficoll-Pague Plus gradient. Cells were washed, adjusted to an appropriate concentration, 5×10^5 to 2×10^7 cells/ml in AIM-V media. Live influenza virus was added to culture (50 HA per ml) to stimulate PBMC proliferation and cytokine production.

ASSAY FOR CYTOKINE PRODUCTION

Lymphocyte influenza-specific cytokine production including inerleukin-2 (IL-2), interferon-gamma (IFNy), and interleukin-10 (IL-10) was measured in cell supernatants using ELISA (enzyme-linked immunosorbent assay). Peripheral blood mononuclear cells (PBMC) were cultured with virus. Ninety-six well enhanced protein binding ELISA plates were coated overnight with purified IL-2, IFN-y or IL-10 capture mAb. Plates were then blocked with PBS-10% fetal bovine serum (FBS). Supernatant samples and standards were added to the plates. Biotinylated anti-cytokine detecting mAb were added as well as avidin-peroxidase in PBS-10% FBS. The substrate tetramethylbenzidine (TMB) was added and absorbance at 405 nm was read with the Bio-Rad Benchmark microplate reader.

HEMAGGLUTINATION INHIBITION (HI) ASSAY

Influenza-specific antibody production in serum was determined by the HI assay. Serum was treated with receptor destroying enzyme for 12-18 hours. The following day, 2.5 % of sodium citrate solution was added to serum to inactivate it. Treated samples were transferred to 96 well plates, PBS and 50% red blood cells (RBC's) were added also. Rooster blood cells were used for H1N1 and B virus, and turkey blood cells were used for H3N2 virus. The plate incubated for 30 minutes, then centrifuged for 10 minutes. To determine the virus working solution, we performed an HA titration. The last well containing an agglutinated mat was considered the end point of the virus titration. The working solution was calculated by 8 HA units. A back titration was performed to be certain that the working solution was adjusted correctly. PBS was added to new 96-well plates. The serum was added and then a serial 2-fold dilution was made down the plate. Working virus was added, incubated for 60 minutes, then .5% RBC's were added. Incubation for 45 minutes and then the well with the last button was considered the endpoint.

PROLIFERATION ASSAY

Influenza-specific lymphocyte proliferation was assessed with an MTT proliferation assay. With this colorimetric assay, PBMC were plated with or without influenza for 96 hours at 37 $^{\circ}$ in 5% CO_2. MTT (Sigma Chemical Co.) was added for the last 4 hours of incubation, followed by the addition of 0.04 N HCl in isopropanol. Absorbance was read at dual wavelength of 570 and 630 nm with an automated microplate reader. The absorbance in wells without virus was subtracted as background from the absorbance in wells with virus.

STATISTICS

A General Linear Model (GLM) Multivariate Analysis procedure (SPSS) was used to examine whether the factors of gender, exercise treatment, and time alter the immune response.

The potential association between nutrient intake and immunity were assessed with a test for correlation.

RESULTS

GENDER EFFECTS

There was no effect of gender on any of the immune parameters measured. Therefore, for all analysis, the data from males and females were combined.

WEIGHT AND BODY MASS INDEX

Table 1 summarizes the body weight and body mass index (BMI) of all subjects (control = 13, exercise = 14). Included are both the pre (2000) and post (2001) time points. The groups did not differ significantly in either weight or body mass index.

Table 1. Body mass index (BMI) and weight of groups at pre and post time periods.

Factor	Exercise group	Control group
Pre-BMI	28.5	27.5
Post-BMI	27.8	27.4
Pre-weight (pounds)	187	167.3
Post-weight (pounds)	181.7	168.3

SYMPTOM INCIDENCE

All participants were called bi-weekly concerning influenza signs and symptoms. Throughout the study, there was one individual within the control group who demonstrated symptoms of influenza one week.

AEROBIC FITNESS

After the ten-month exercise intervention, subjects in the exercise group improved their six-minute walk distance by 88 meters compared to only a 10-meter increase within the control group (Figure 1). This demonstrates that the exercise group's endurance program was successful at improving cardiovascular fitness. There was a significant difference at the 2001 time point between the two groups ($p = .016$).

INTERLEUKIN-2 PRODUCTION

The results from ANOVA suggest that physical activity did not influence IL-2 production for any of the influenza antigens (Type A H1N1, Type A H3N2, Type B). There was not a main effect of treatment or time and there was not a significant treatment by time interaction (Figures 2, 3, and 4).

INTERFERON GAMMA PRODUCTION

The results from ANOVA suggest that physical activity did not influence IFN-γ production for any of the influenza antigens (Type A H1N1, Type A H3N2, Type B). There was not a main effect of treatment of time and there was not a significant treatment by time interaction (Figures 5, 6, and 7).

INTERLEUKIN-10 PRODUCTION

The results of the ANOVA suggest that physical activity did not influence IL-10 production for any of the influenza antigens (Type A H1N1, Type A H3N2, Type B). There was not a main effect of treatment of time and there was not a significant treatment by time interaction (Figures 8, 9, and 10).

LYMPHOCYTE PROLIFERATION

Lymphocyte proliferation was not influenced by physical activity for any of the influenza antigens (Type A H1N1, Type A H3N2, Type B). There was not a main effect of treatment of time and there was not a significant treatment by time interaction (Figures 11, 12, and 13).

ANTI-INFLUENZA ANTIBODY TITER

The exercise group had a significantly greater change in antibody titer from pre-immunization to 4-week post immunization for Type A H1N1 ($p=.05$) and Type A H3N2

(p=.038) compared to the control groups (Figures 14, 15, and 16). The change in antibody titer from pre-immunization to 4-week post immunization in Type B was not significant (p=.311). The change in antibody titer from pre-immunization to 3-month post immunization was significant for Type A H1N1 (p=.04). The change in antibody titer from pre-immunization to 3-month post immunization was not significant for Type A H3N2 (p=.382) and Type B (p=.632). In Figure 14, it is shown that the control group's antibody titer actually decreased below baseline at the 3-month time point, whereas the exercise group increased significantly.

Table 2 shows the percentages of subjects with antibody titers greater than 1:40 for each antigen at two different time-points (4-week post, 3-month post). An antibody titer greater than 1:40 is considered at the protective level.

NUTRIENT INTAKE

There were no significant differences of nutrient intake among the control and exercise group for any of the nutrients measured (Table 3). There was a negative correlation between carbohydrate intake and IL-2 production (R^2=.324). There was a trend toward increased IL-2 production within subjects who consumed a vitamin C supplement (R^2=.331) and vitamin E supplement (R^2=.36) for some of the time points.

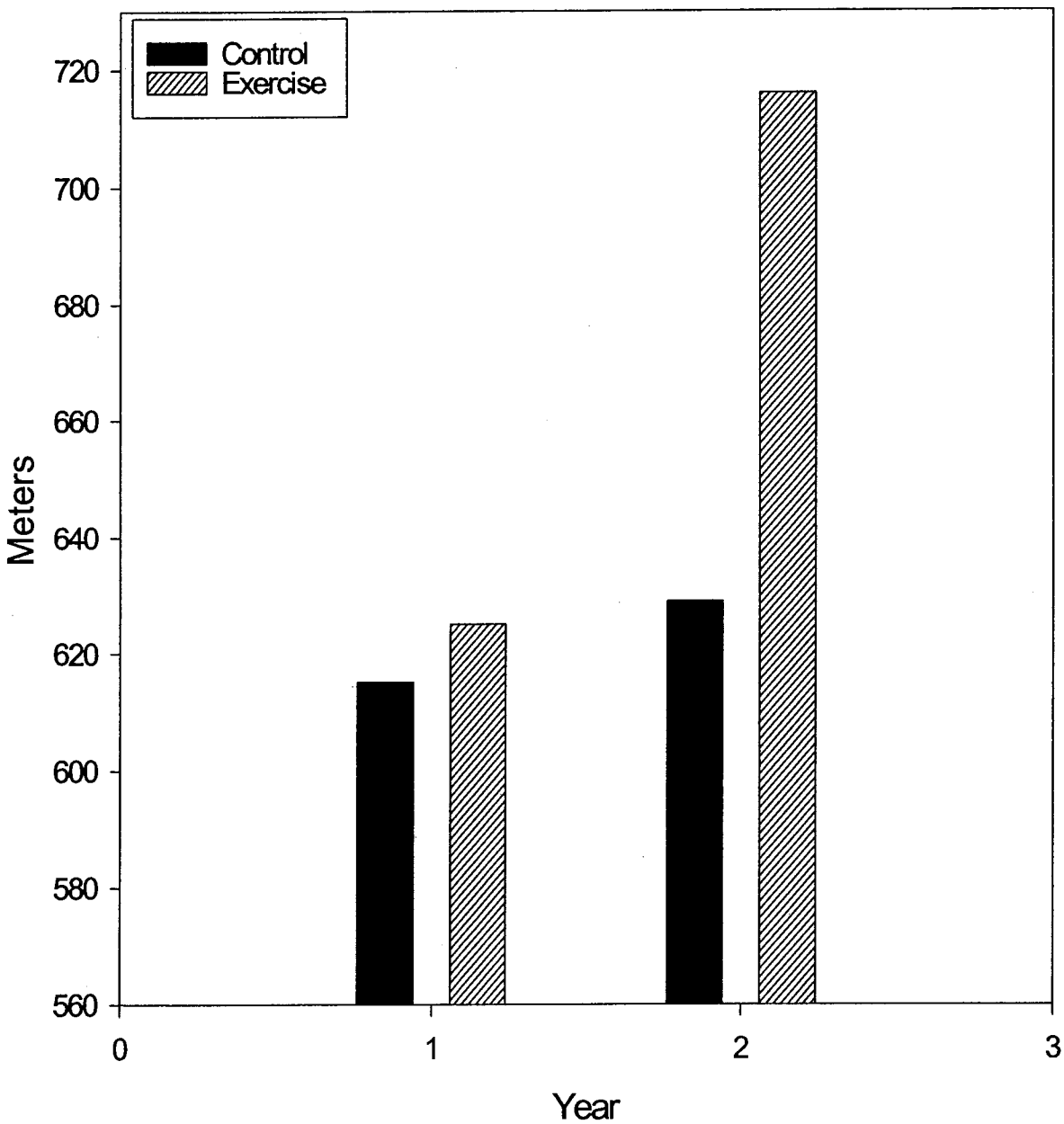

Figure 1. Difference in mean 6-minute walk times between the control and exercise group from 2000 (1) and 2002 (2). The 6-minute walk was measured in meters. The 6-minute walk test is a measure of aerobic fitness.

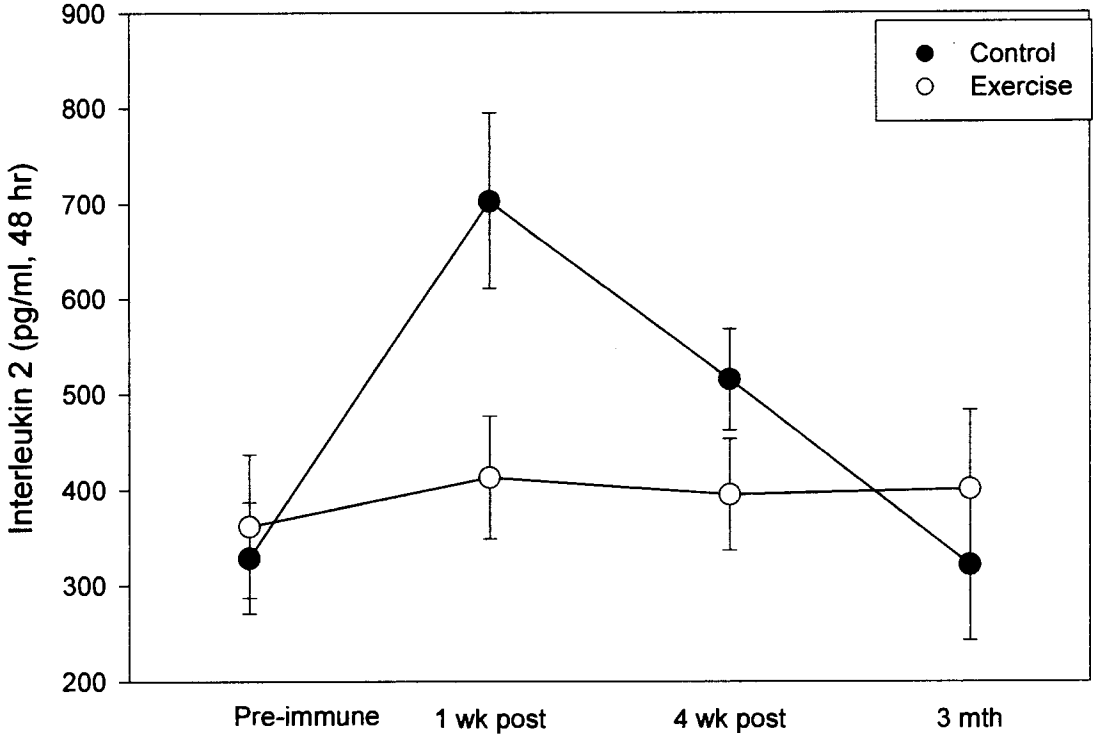

Figure 2. Effect of exercise on Interleukin-2 production at four time points in regards to influenza antigen Type A H1N1

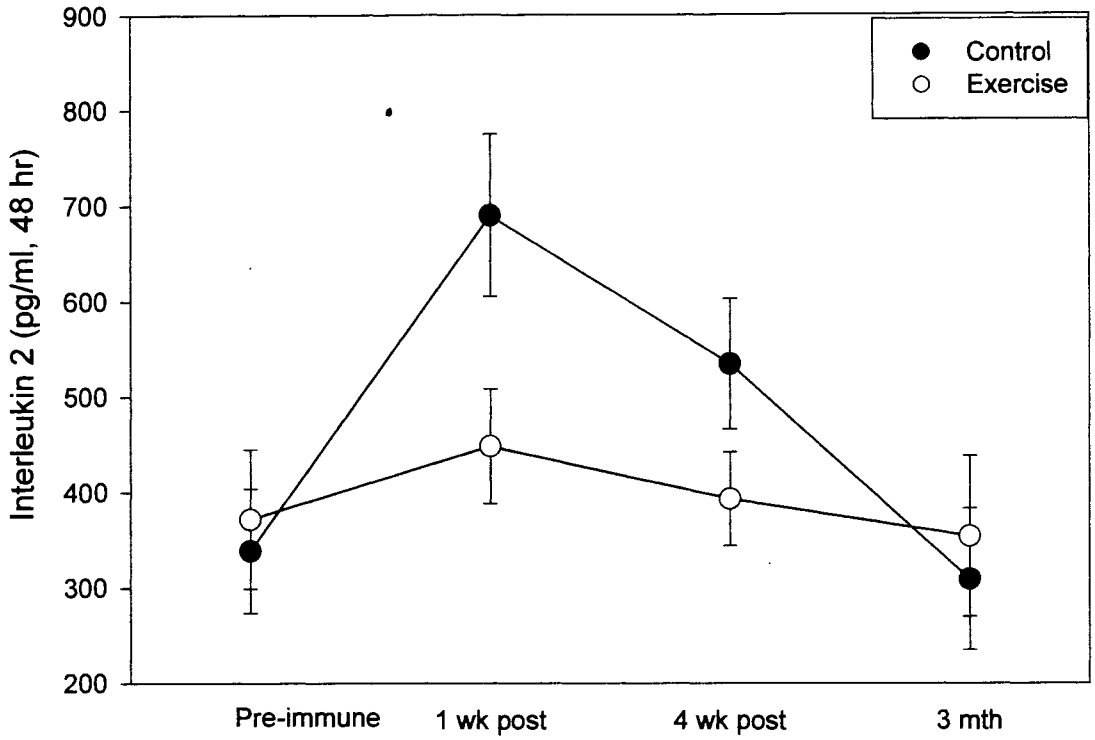

Figure 3. Effect of exercise on Interleukin-2 production at four times in regards to influenza antigen Type A-H3N2

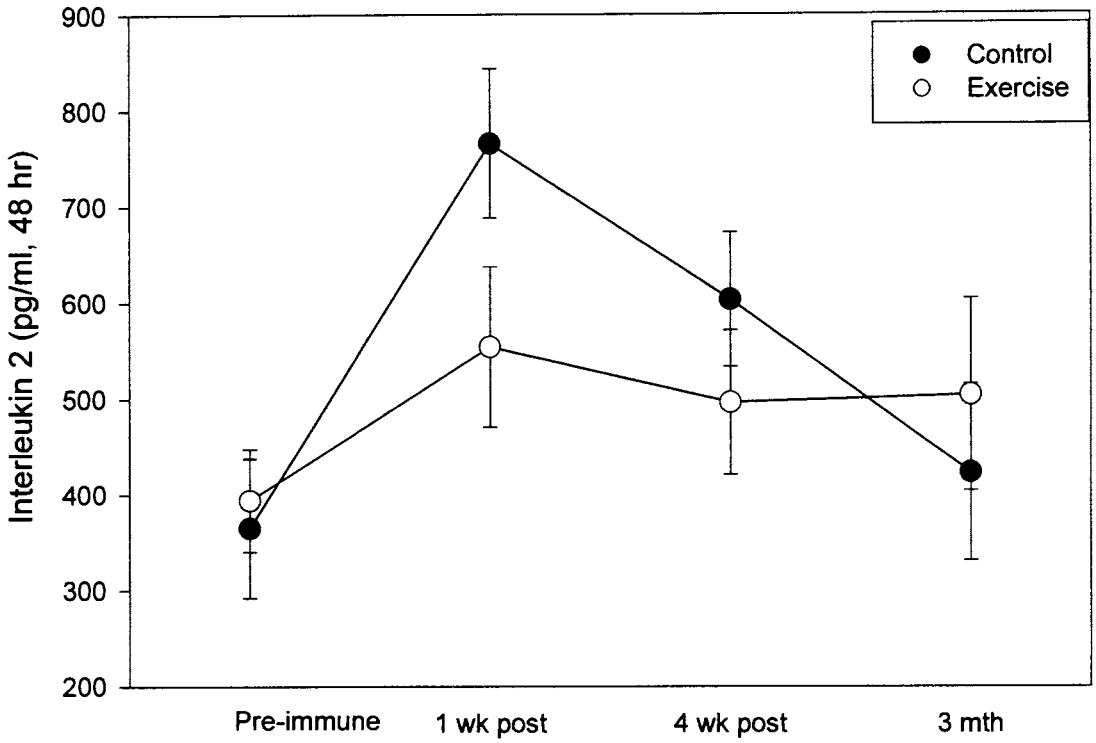

Figure 4. Effect of exercise on Interleukin-2 production at four time points in regards to influenza antigen Type B

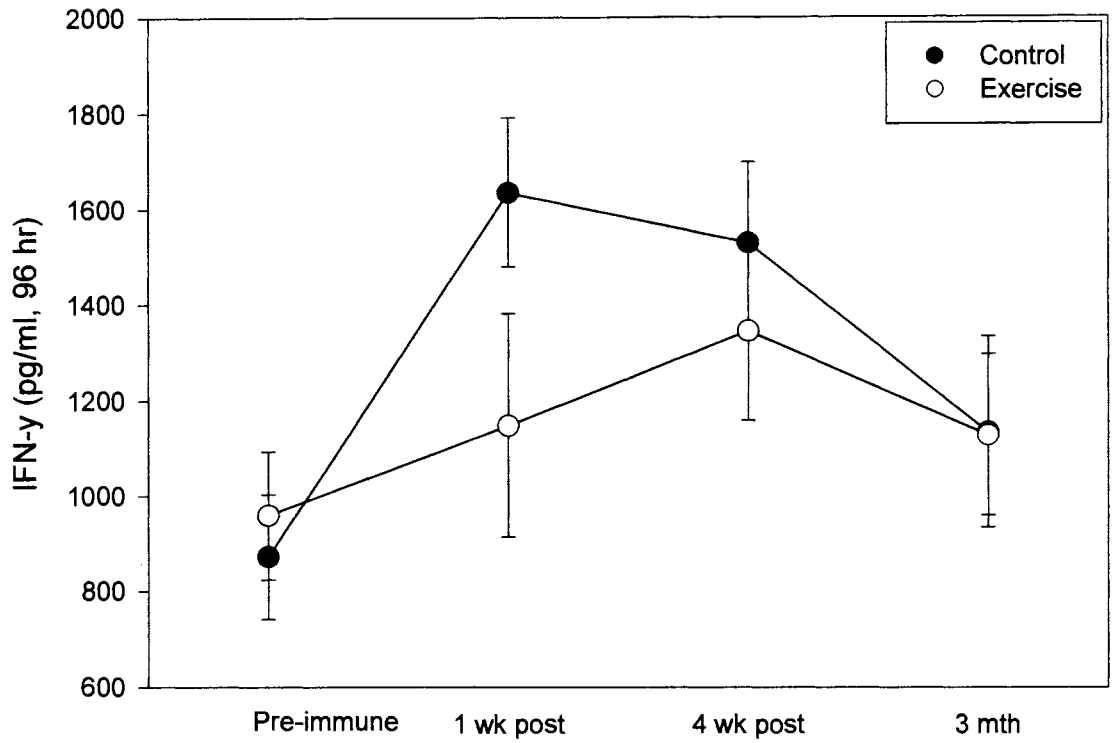

Figure 5. Effect of exercise on Interferon gamma production at four time points in regards to influenza antigen Type A H1N1

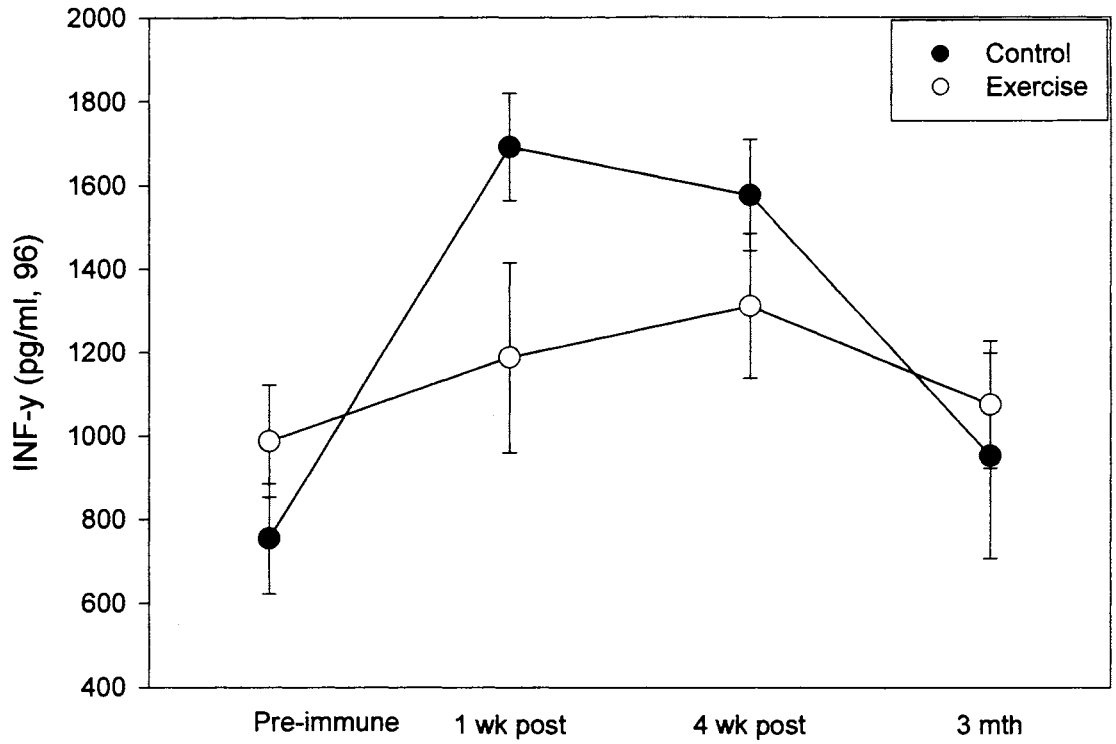

Figure 6. Effect of exercise on Interferon gamma production at four time points in regards to influenza antigen Type A H3N2

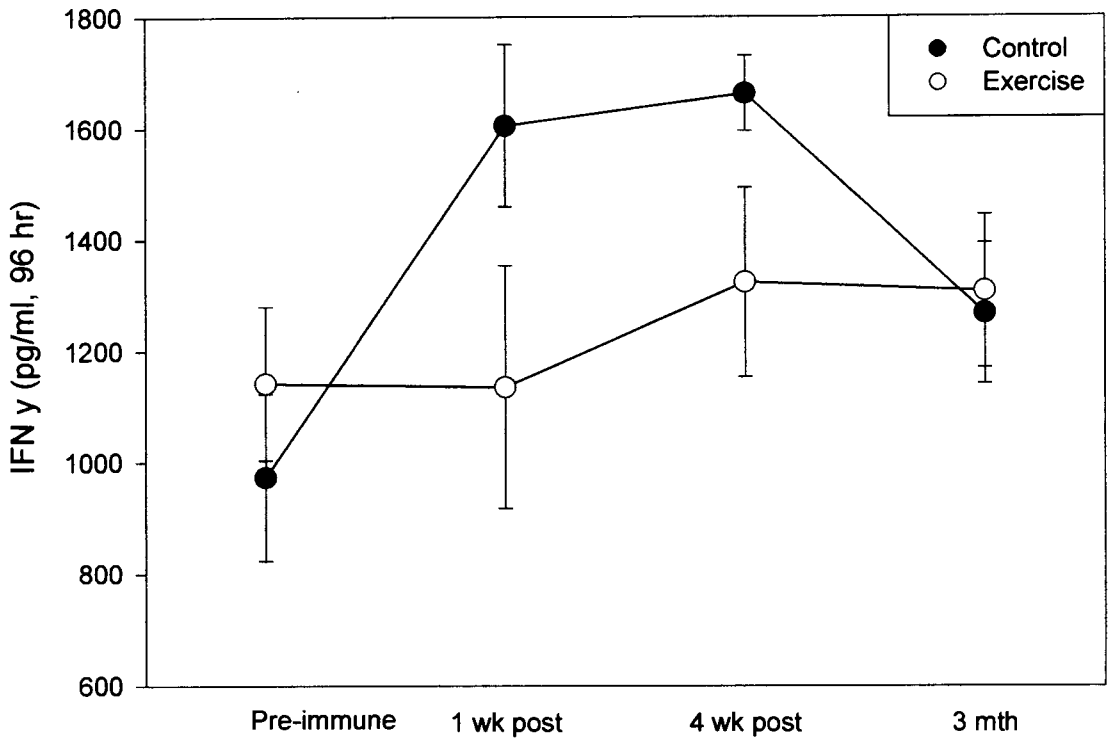

Figure 7. Effect of exercise on Interferon gamma production at four time points in regards to influenza antigen Type B

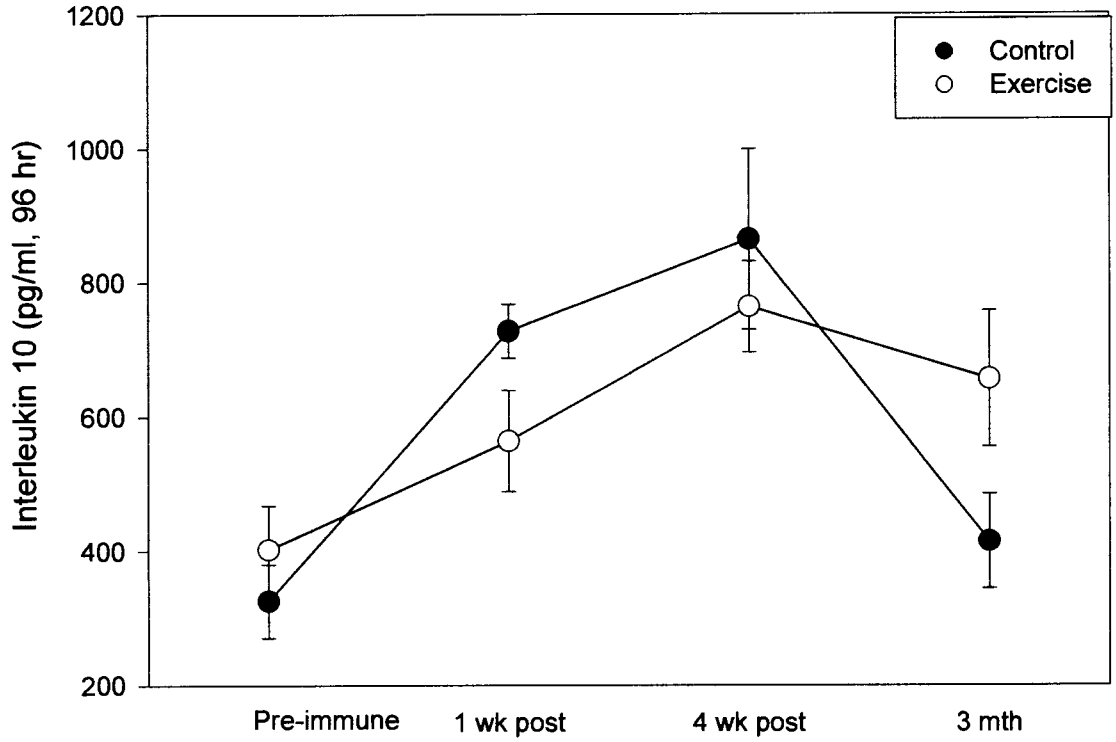

Figure 8. Effect of exercise on Interleukin 10 production at four time points in regards to influenza antigen Type A H1N1

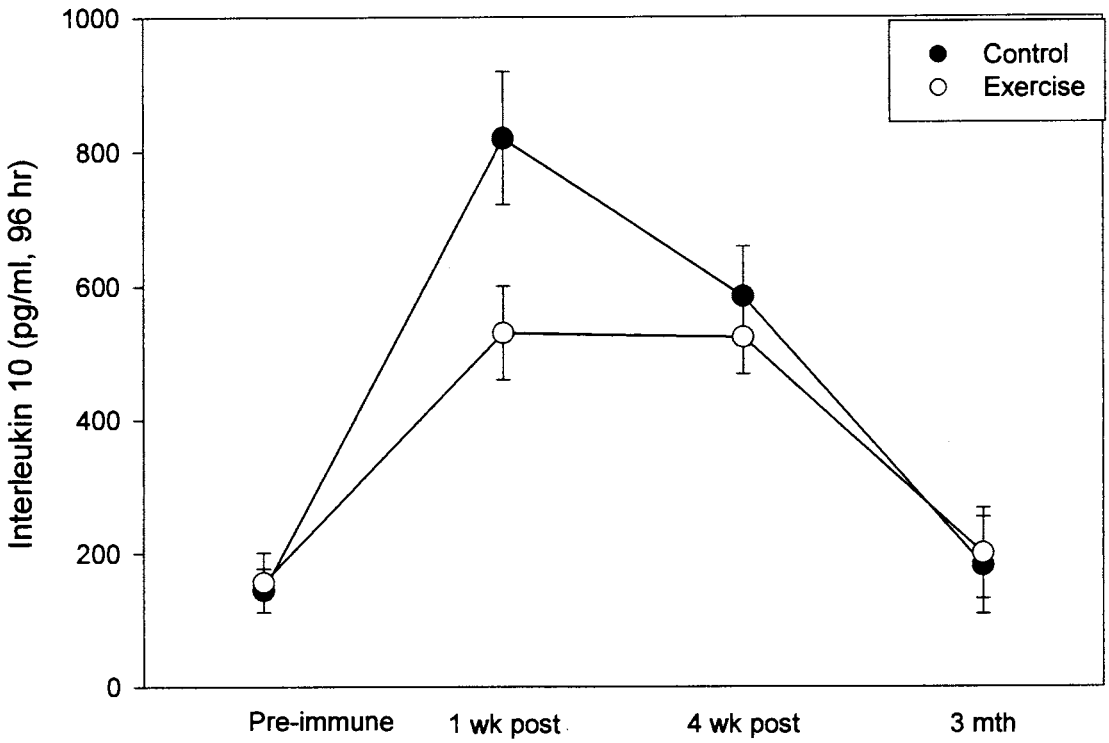

Figure 9. Effect of exercise on Interleukin 10 production at four time points in regards to influenza antigen Type A H3N2

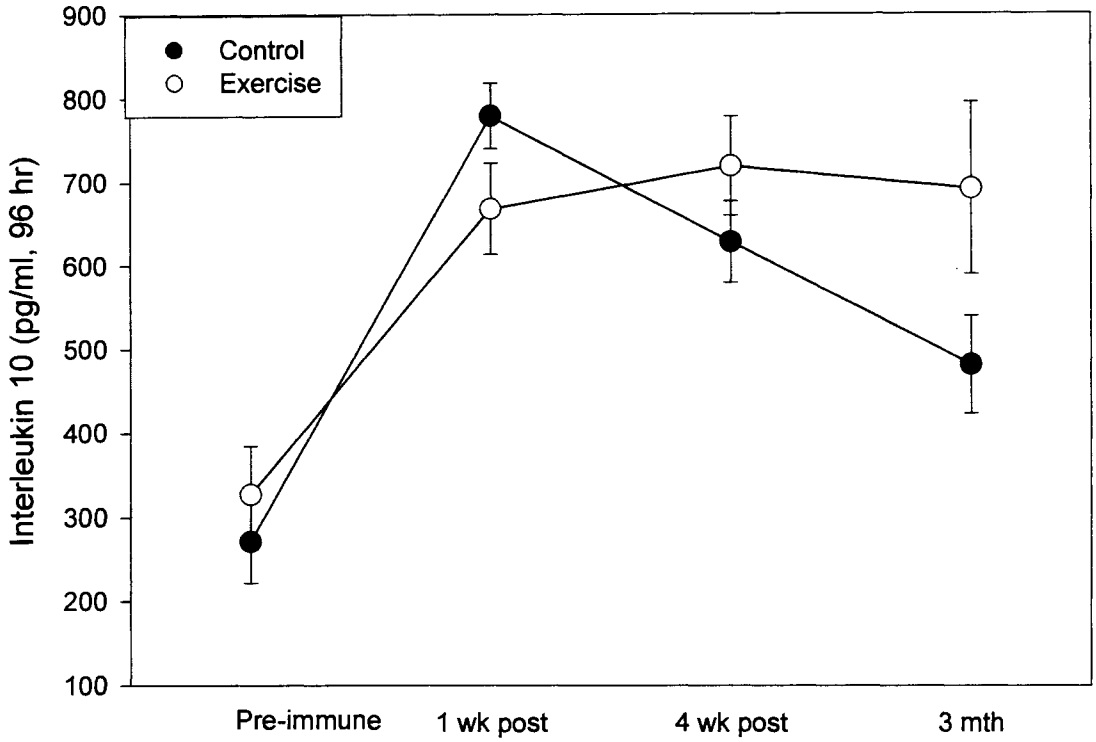

Figure 10. Effect of exercise on Interleukin 10 production at four time points in regards to influenza antigen Type B

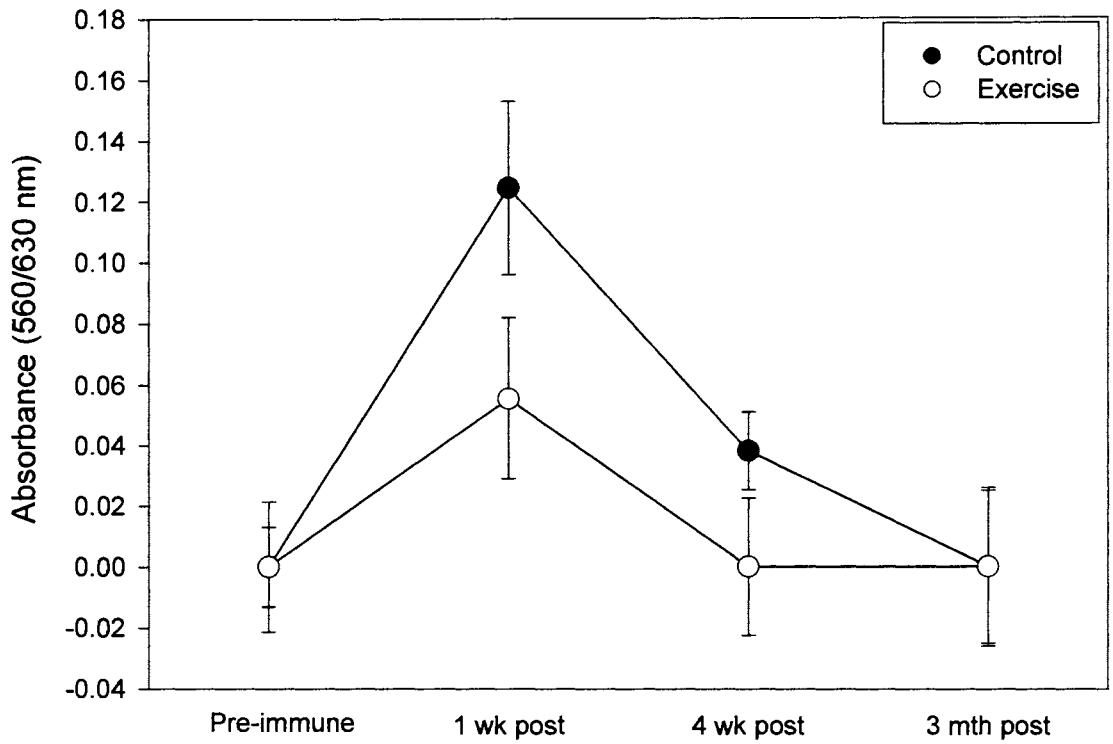

Figure 11. Effect of exercise on lymphocyte proliferation at four time points in regards to influenza antigen Type A H1N1

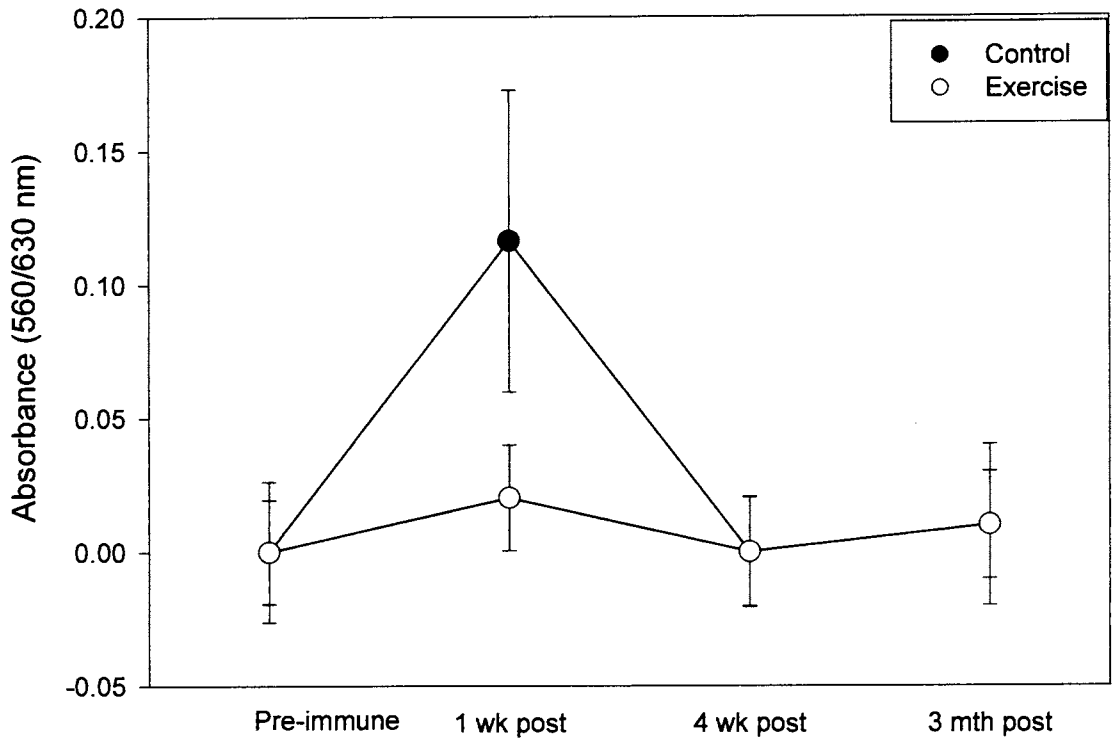

Figure 12. Effect of exercise on lymphocyte proliferation at four time points in regards to influenza antigen Type A H3N2

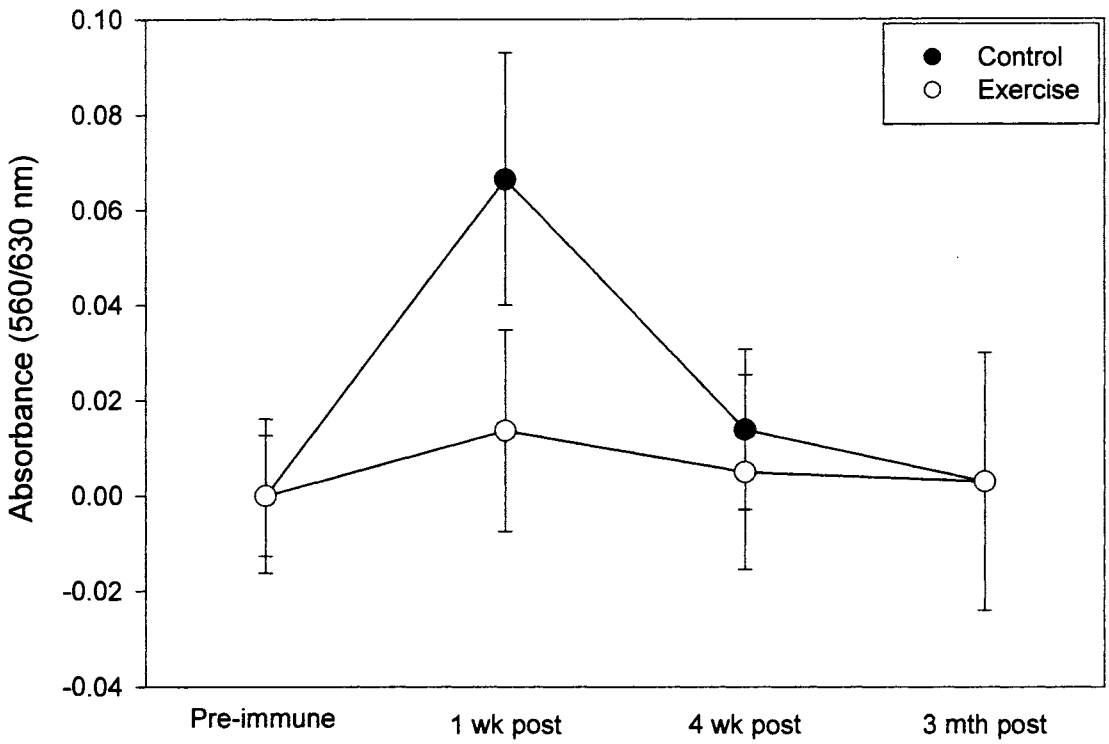

Figure 13. Effect of exercise on lymphocyte proliferation at four time points in regards to influenza antigen Type B

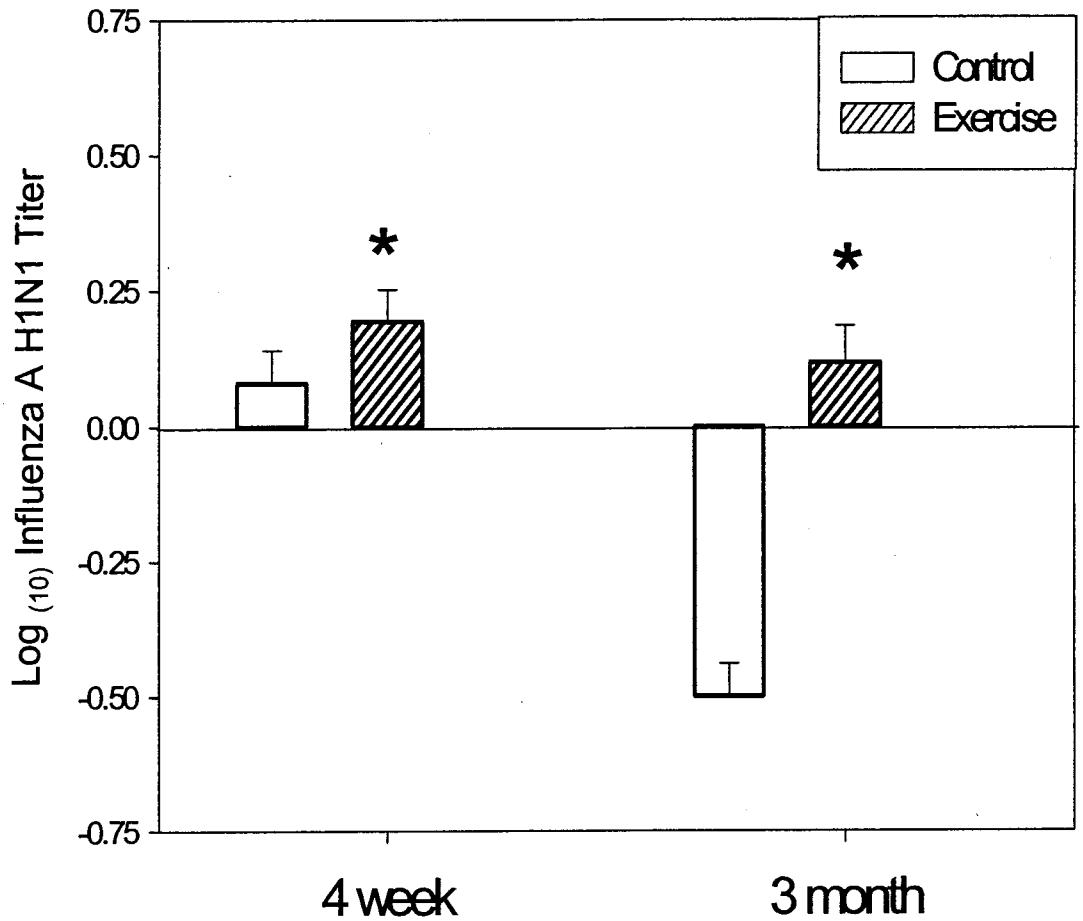

Figure 14. The change in antibody titer (Type A H1N1) from pre-immunization values are shown at 4 weeks post-immunization and 3 months post-immunization. Antibody titer was greater in exercise compared to control subjects at both time points (* $p<0.05$).

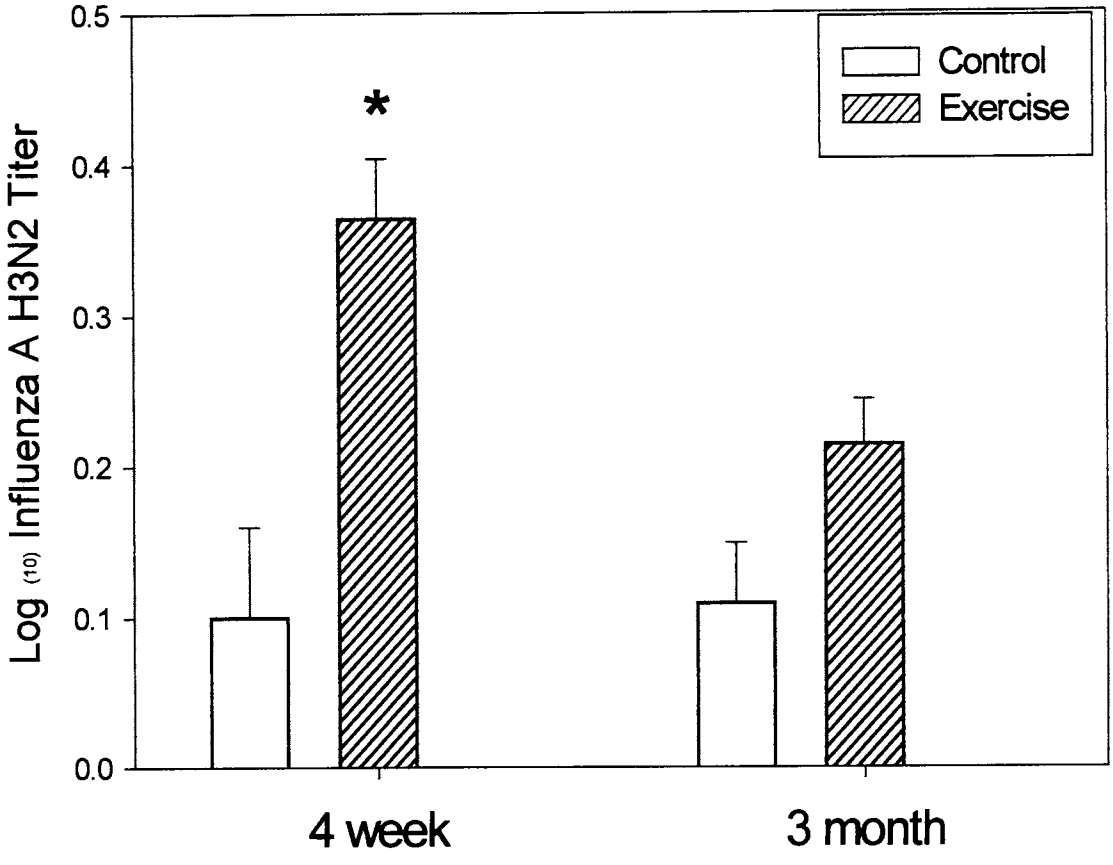

Figure 15. The change in antibody titer (Type A H3N2) from pre-immunization values are shown at 4 weeks post-immunization and 3 months post immunization. Antibody titer was greater in exercise compared to control subjects at both time points (* $p<0.05$).

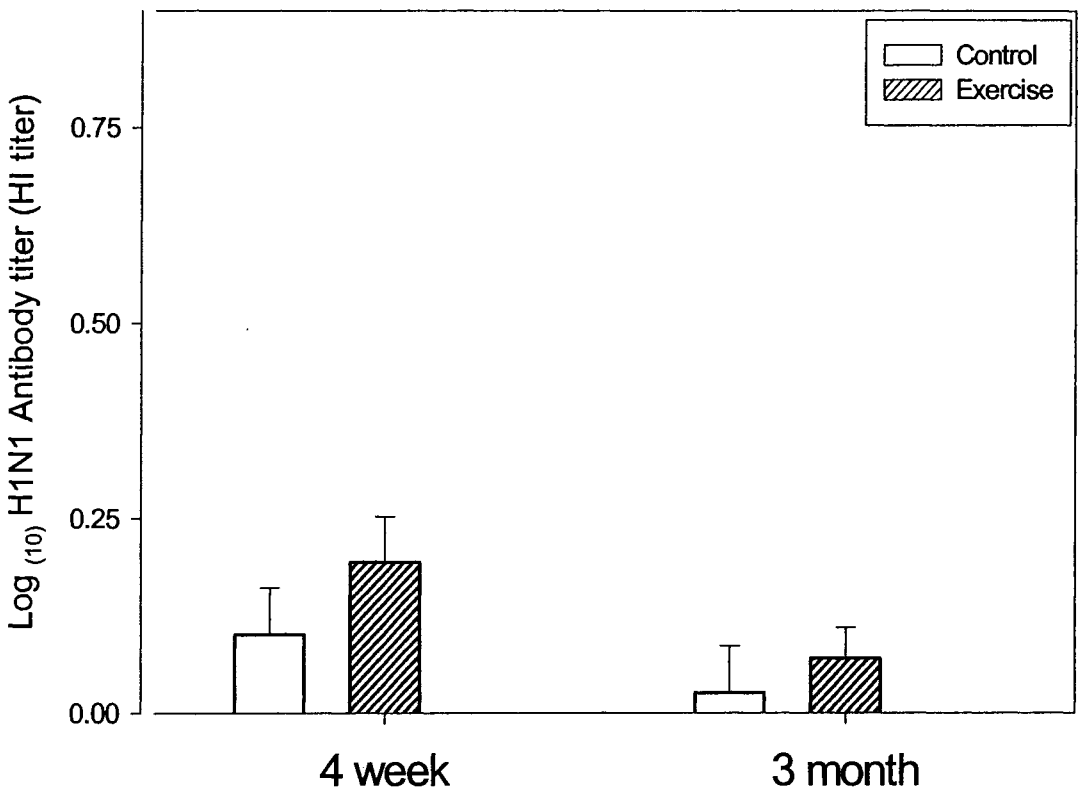

Figure 16. The change in antibody titer (Type B) from pre-immunization values are shown at 4 weeks post-immunization and 3 months post-immunization. Antibody titer showed a trend toward greater levels in exercise compared to control group at both time points.

Table 2. Percentages of each group at the 4-week and 3-month timepoints who have an antibody titer greater than 1:40 which is considered a protective level. There were no significant differences between the two groups.

Vaccine and time point	Exercise group	Control group
Type H1N1, 4 week	86%	43%
Type H1N1, 3 month	83%	50%
Type H3N2, 4 week	93%	69%
Type H3N2, 3 month	86%	69%
Type B, 4 week	31%	31%
Type B, 3 month	14%	33%

Table 3. Means and standard error of nutrient intakes among the exercise and control groups.

Nutrient	Exercise Group	S.E.	Control Group	S.E.
Vitamin E (milligrams)	102.7	35.3	183.2	64.1
Vitamin C (milligrams)	334.4	76.2	476.4	119
Zinc (milligrams)	65.6	40.4	29.5	5.22
Calories (kilocalories)	1817	219	1549	118
Carbohydrate (grams)	210.5	19.8	186.7	15.4
Fat (grams)	81.2	14.9	63.3	5.83
Protein (grams)	68.7	9.12	61.0	5.47

DISCUSSION

The findings from this study suggest that there is an association between physical activity and the immune response to influenza immunization in adults ≥ 65 years of age. We observed that 10 months of moderate to vigorous aerobic exercise increased anti-influenza antibody titer. The antibody titer is the primary predictor for an immune response and has the best correlation with protection from influenza. During the four-week post time point, the antibody titer acts as a gold standard to demonstrate if the vaccine is effective. During this four-week post period, a peak increase in antibody titer should be seen if the vaccine is effective. An increase in antibody titers suggests improved vaccine efficacy. Our research demonstrated a peak increase in antibody titer during the four-week post for the exercise group within all three of the influenza vaccines. This study is the first one that we are aware of to examine immune response to influenza vaccine following an exercise intervention in older adults. One other published study evaluated the association between physical fitness and anti-influenza antibody titer in young adults. However, no association between fitness and antibody titer was found. The other study that showed an association between physical activity and immune response identified a greater antibody titer to the influenza immunization (25).

Vaccine efficacy is reduced among older adults because of a decreased immune response. This puts the elderly at risk for serious morbidity and greater mortality rates from influenza infection than the younger population. Bernstein and colleagues found that a younger population had a higher antibody titer than an elderly population after vaccination (33). Although the elderly population has an increase in antibody titer from their pre-vaccination levels, the titer may not be high enough to provide protection again influenza.

Exercise may be one way to improve the efficacy of the influenza vaccine in the elderly. In our study, the exercise group had a greater antibody titer change from pre-vaccination than the control group.

In our study, older individuals aged $\geq$ 65 years of age participated in exercise for at least 20 minutes per session, three or more times per week at an intensity estimated to be approximately 65 to 75% of heart rate reserve for 10 months had a higher influenza- specific antibody titer response than the control group. Although antibody titer to influenza vaccine was not different between exercise and control before the intervention, after 10 months of aerobic exercise, exercise subjects had a significantly higher antibody titer to the Type AH1N1 and Type A H3N2 components of the influenza vaccine. However, cytokine production, more specifically interleukin-2, interleukin-10, and interferon gamma, did not appear to be associated with physical activity for the time points that were measured in the study. Also, lymphocyte proliferation of peripheral blood mononuclear cells did not appear to be affected by physical activity for the time points that were measured in this study. However, cytokine production and lymphocyte proliferation are not as great of indicators for a change in immune variables as the antibody titer. The antibody titer is the gold standard to monitor if there is a change in immune variables. Thus, there was an increase in antibody titer within the exercise group, suggesting that exercise does have a positive effect on antibody titer. These findings are similar to the first study mentioned above that had also shown an association between physical activity and greater antibody titer (25). However, within the first study, they also demonstrated that the more physically active individuals had a greater amount of influenza-specific lymphocyte proliferation than sedentary individuals. Previous studies have supported this theory of enhanced response in well-conditioned older

individuals compared to less active older individuals, although none of these studies have examined antigen-specific immune response (23). Other controlled trials have reported that exercise has little or no effect on immune function. However, these studies were conducted over shorter periods of time (12 weeks-6 months) and perhaps a longer period of exercise is necessary to observe enhanced immune function. Research, in general, suggests that people who trained for many years seemed to show an increase in immune response (21).

Looking at our findings, it would seem appropriate to conclude that the immune response to an influenza vaccine may be enhanced in older adults through exercise. Evidence shows that antibody titer in serum determined by hemagglutination inhibition, provides a great predictor of an individuals resistance to infection (34). A higher antibody titer provides greater protection from infection (35). In our study, the exercise subjects had a higher antibody titer suggesting a greater degree of protection from influenza. The incidence of infection was not significantly different between the exercise and control groups in our study. One control subject and no exercise subjects got influenza. A much larger sample size is necessary to detect differences in illness given that only a small number of individuals experience infection after receiving immunization. Antibody titer was higher in exercise group.

Cell-mediated responses to the influenza virus are essential for viral clearance. Also, cell-mediated responses to influenza virus may provide cross-reactivity from strains of influenza that may not have been included in the annual influenza vaccine (15). In terms of cell-mediated responses to the influenza vaccination, we did not observe increased lymphocyte proliferation among the exercise group. There was also not an association between exercise and cytokine production. Considering that IL-2 promotes cell proliferation,

it is not surprising that we did not observe an increase in IL-2 given that we had not seen an increase in cell proliferation. Another cytokine, interferon gamma, is also important in cell-mediated responses to viral infection. We did not observe an effect of exercise on influenza-induced interferon gamma production. Others reported in older adults that cytokine production varies by individual influenza strain (36). In general, cell-mediated responses did not appear to be altered by exercise. However, there was a lot of variability in cytokine production and lymphocyte proliferation and perhaps a larger sample is needed to detect meaningful changes in cell-mediated immune responses.

It was surprising that there was not an increase in IL-10 production among the exercise group. IL-10 is a Th2 cytokine that promotes antibody production. We observed an increase in antibody production among the exercise group, but not an increase in IL-10 production compared to the control group. It is possible that IL-10 was different at other time-points. It is also possible that the cells producing IL-10 in the peripheral blood do not reflect IL-10 production in other immune tissues (spleen, lymph nodes). Perhaps if we had been able to measure IL-10 in other tissues, we may have seen a difference between exercise and control.

The role of exercise in modulating antibody response to influenza immunization has been studied in college-aged individuals. However, no effect of exercise was found within this group (37). Perhaps, the immunomodulatory effects of exercise may be greater among the aged population compared to the younger populations (38). It could also be that different research techniques among young and old yield different results.

We observed a higher IL-2 production within individuals who consumed less carbohydrate. Also, we observed a trend toward increased IL-2 production within

individuals who consume greater vitamin E and vitamin C intakes. Given the small number of subjects, it is premature to make any dietary supplement recommendations, rather, it is important to consider nutrient intake as a factor when analyzing immune function in the elderly population, especially considering that malnutrition often exists in this population.

The primary mechanisms responsible for the exercise-induced enhancement of immune function are unknown. Neuroendocrine changes that occur during exercise may mediate the alteration of the immune response (Table 4). The possible neuroendocrine changes due to exercise include changes in a variety of neurohormones, such as glucocorticoids, catecholamines, β-endorphins, and growth hormones. All of these neurohormones have been shown to alter the immune response (39). It has been thought that during exercise glucocorticoids and catecholamines are the primary stressor hormones that affect the immune system (40). Perhaps the regular release of catecholamines and/or glucocorticoids improves immune responsiveness. A secondary mechanism for altered immune response due to exercise may include psychosocial factors. Immune functioning is compromised with high levels of psychosocial stress and depression. However, exercise may attenuate psychosocial stress and reduce depression, perhaps resulting in improved immune function.

Table 4. Neurotransmitters and hormones with immunomodulatory properties[a].

Factor	*Exercise result*	*Action*	*Effect*
Glucocorticoids	Increases	S/E	Antibody production, NK activity, cytokine production
Catecholamines	Increases	S/E	Lymphocyte proliferation to mitogen, cytokine production
β-endorphin	Increases	E/S	Antibody synthesis, macrophage activation, T-cell activation
Prolactin	Increases	E	Macrophage activation, IL-2 production
Growth hormone	Increases	E	Antibody synthesis, macrophage activation, IL-2 modulation
Vasopressin	Increases	E	T-cell proliferation
ACTH	Increases	E/S	Cytokine production, NK activity, antibody synthesis

[a]These represent general properties. In many cases, the action depends on the concentration, target cell and immune function studied. S: suppression, E: enhancement

CONCLUSION

In summary, numerous changes occur in immune responsiveness among the elderly population. Influenza is a significant cause of mortality among this population group. Vaccine efficacy is reduced among the elderly population. It is significant to identify specific lifestyle habits that may enhance vaccine efficacy. The findings from this study suggest that regular, vigorous exercise for 25-30 minutes $\geq$ 3 times per week for at least 10 months may enhance immune response to influenza immunization by increasing antibody titer in older adults. The antibody titer is the primary predictor for an immune response. Further research to clarify the mechanisms responsible for enhanced immune function are necessary. It would be relevant to investigate the effects of a long-term exercise program on older adults, in terms of the immune response to influenza vaccination; and to examine exercise-associated alterations in psychological state as potential mediators of the exercise-induced modulation of immunity. Perhaps the immunomodulatory effects of exercise are mediated by the binding of catecholamines released during exercise to lymphocyte beta-adrenergic receptors. A study that incorporates investigating the immune, psychological, and physiological responses will provide a basis for further study into the mechanisms mediating these relationships.

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