Exercise, aging, stress, and the efficacy of the influenza vaccine

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Exercise, aging, stress, and the efficacy of the influenza vaccine

by

Megan Marie Cooper

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)

Major Professor: Marian Kohut

Iowa State University

Ames, Iowa

2000

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Graduate College
Iowa State University

This is to certify that the Master's thesis of

Megan Marie Cooper

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy
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INTRODUCTION

Stressful life events have been shown to suppress immune function. This decreased immunity may lead to an increased risk of infections. Results from previous epidemiological studies show that persons reporting more psychological stress have a higher incidence and greater severity of illness (Cohen, Doyle, & Skoner, 1999). For example, in one study subjects were experimentally infected with influenza A virus, and the results showed that higher levels of psychological stress were associated with a greater number of upper respiratory infectious illnesses. Other psychosocial factors, such as loneliness, optimism, depression, etc., may impact immune function. However, further research is necessary to elucidate the complex relationship between psychosocial state and immunity.

Aging has been associated with various declines in immune functioning. The largest age-related differences are in T-cells, which are responsible for cellular immunity. In aged people, declines in various aspects of protective immunity, such as hypersensitivity reactions to antigens and long-lasting memory responses after vaccination, result in poorer immune response. The elderly population (60+ yrs. old), whose immune systems are less resilient, are especially susceptible to stress and influenza-related deaths. Those with an increased vulnerability to infectious illness are more likely to experience age-related immunological declines (Glaser et al., 1998). In the past 20 years, the majority of the influenza-related deaths have occurred in the elderly. Reasons for their greater susceptibility to serious morbidity and greater mortality may be due to impaired host defenses, decreased immune response, and chronic medical conditions that often accompany aging (McGlone & Arden, 1987). The influenza virus can spread rapidly, and is of utmost concern in nursing homes
and group living situations. Up to 30 percent of all nursing home residents may die due to the influenza virus or related illnesses. In the elderly population living in the community, hospitalization rates during influenza epidemics have been shown to be almost three times higher than the rates for younger individuals (McGlone & Arden, 1987). Due to the severity of the influenza infection, vaccinations for each specific strain are often recommended. In a study by Bernstein et al (1999), in a healthy elderly population, the efficacy of the influenza vaccine was only 50%. This undoubtedly is related to the low rate of protective levels of humoral immunity following the vaccination.

Exercise has been shown to have a positive affect on immune function. Immune function after an acute bout of exercise appears to be enhanced in endurance trained athletes. There also is an increase in the number of natural killer cells after exercise. Few studies have been done in this area, and few of them have studied the effects of exercise on the immune system in an elderly population. The elderly are very susceptible to infectious diseases, therefore it would be important to know if exercise plays a role in immune responses (Mazzeo, 1994).

Older people differ from younger adults in the specific stressful situations and conditions they are likely to encounter. In addition, older people do not always have a strong social support system to help them deal with adversities. Thus, elderly people who experience stress may be more susceptible to infectious viruses, such as influenza, and they may experience a lower immune response to the influenza vaccination. These findings suggest that activity and psychosocial factors, independent of activity, influence immune response to influenza immunization in older adults.
LITERATURE REVIEW

Advancing age brings with it an increased vulnerability to infectious agents. Associated with advancing age is also a decline in immune function (immunosenescence), which may contribute to the high rates of influenza in the elderly. Miller (1996) indicated that aged people and rodents show declines in various aspects of protective immunity, such as a decline in T-cell proliferation, a decline in interleukin-2 (IL-2) production, an increase in IL-10 production, an increase in autoantibodies, and a decline in antibody hypermutation. Wick and Grubeck-Loebenstein (1997) reported in a recent review that the primary immune response change in the elderly is the age-dependent intrinsic decline of immune responsiveness. The authors suggest that the primary alterations that do occur are a decrease in CD4, a decrease in T-cell mitogen response, a decrease in T-cell antigen response, a decrease in IL-2 production, and an increase in autoantibodies. Secondary immune responses to aging are usually due to underlying disease and environmental factors, such as diet and physical activity level.

Influenza may result in higher morbidity and mortality rates, among people aged 65 and older. Barker and Mullooly (1980) conducted a cohort analysis of mortality and morbidity rates due to an influenza epidemic in an adult population (age 14+). Findings from this analysis suggested that the risk for contracting influenza increased with age. The elderly group was found to be the most susceptible to influenza mortality and morbidity. The numbers of influenza or pneumonia-related deaths were significantly greater in this 65+ age group. Total hospitalization and death rates for influenza-related morbidities during the epidemic periods were significantly increased over rates during the non-epidemic era. A
related study (Sprenger, Mulder, Beyer, VanStrik, & Masurel, 1993), found that every year, influenza mortality is present in the 60+ age group. This group, on average, experiences 82 deaths per 100,000 people per season/year. As a consequence, the majority of the total influenza deaths occurred in the elderly population.

The efficacy of the influenza vaccine, especially in the elderly, is still being researched. A large study (Govaert, Thijs, Masurel, Sprenger, Dinant & Knottherus, 1994) that included 1838 subjects aged 60+ years old who were not high risk found that less influenza-like illnesses were seen in the vaccinated group. Contrary to this, in the 70+ age group there was little difference seen between the vaccinated group and the placebo group. This study found that with an increase in age, vaccine efficacy decreased. In the elderly population, the vaccination may halve the incidence of clinical influenza. In an elderly population less than 70 years old, the influenza vaccine may reduce the incidence of influenza-like illnesses, but in a population greater than 70 years old, the vaccine may not be effective. Keren and associates (1988) sought to determine the prevaccination immunity titer in healthy nursing home residents and study the efficacy of revaccination in patients who did not respond strongly to the original influenza vaccination. Ninety-five residents were vaccinated once, while 32 residents were revaccinated 30 days following the original vaccination. The results of this study suggest that 30% of healthy elderly people remain unprotected after two influenza vaccinations separated by one month, and revaccination did not provide any change in their immune response.

Several studies have examined specific immune parameters following influenza immunization in older adults. The first study (Bernstein, Gardner, Abrutyn, Gross, & Murasko, 1998) compared healthy elderly to a healthy young population. A blood sample
was taken 4 weeks before the vaccination and all post-vaccination samples were taken before any influenza-like symptoms appeared. The results indicated that the younger subjects had greater immune cell proliferation than the elderly subjects did, both pre- and post-vaccination. After the vaccination, the elderly showed lower antibody titers and the young had a higher production of IFN-γ and IL-10. Results with IL-4 and IL-6 were inconclusive. After vaccination, both populations showed a significant increase in antibody titers, but the young group’s titer was dramatically higher than the elderly antibody titer.

In a related study by Bernstein et al. (1999), IFN-γ production increased after influenza vaccination in the elderly, but the increase was not statistically significant. The antibody titers did increase significantly after vaccination. This study showed that the vaccination of an elderly population is less efficacious than in their younger counterparts; however, vaccination did reduce the severity of the symptoms associated with influenza. It was hypothesized that the elderly population responds less to the influenza vaccination due to a decrease in humoral response. Similarly, deBruijn et al. (1997) measured antibody titer for four consecutive years of annually repeated influenza vaccination and found that the previously vaccinated subjects of all ages had higher pre-vaccinated titers than the unvaccinated subjects. In addition, both the pre-and post-vaccination titers were lower in the elderly than in the young.

Exercise may also have an affect on the elderly immune system. An acute bout of exercise is a stressor that can modulate immune function. The extent to which exercise affects the immune response depends on the intensity and duration of exercise and the training status of the individual. Previous research has found that the proportion of T-helper cells is decreased after an exhaustive exercise bout, while the percentage and number of
natural killer cells (NK) have increased. Mazzeo (1994) suggested that the relationship between long-term moderate exercise and immunity is not clear. Shinkai, Konishi, and Shephard (1998) report that the immune responses to acute exercise have not yet been studied extensively in the elderly. They found that the natural killer cell response to a single bout of exercise is normal in an elderly population, but immediately following the exercise, the elderly subjects show less suppression of lymphocyte proliferation than younger subjects. In contrast, strenuous exercise caused a more sustained postexercise suppression of cellular immunity in the older subjects compared to the younger subjects. This study concluded that the immune response to a single bout of exercise in an elderly population may depend on the type, intensity, and duration of exercise, as well as the fitness level of the individual.

Nieman et al. (1993) explored the relationship between exercise, immune function and upper respiratory tract infection (URTI) in elderly women. A highly conditioned group was compared to a sedentary group. This study found that exceptionally active, highly conditioned elderly women with an aerobic power 67% greater than sedentary elderly subjects, had greater NK and T-cell function. The incidence of URTI was lowest in the highly conditioned subjects and highest in the sedentary subjects. This study also reported that twelve weeks of moderate cardiorespiratory training in older women failed to improve immune function. Woods et al. (1999) studied the effects of six months of moderate aerobic exercise training on immune function in the elderly. The study used previously sedentary elderly individuals and randomly assigned them to two groups: a supervised, three times per week exercise intervention, or a flexibility/toning control group. The study found that post-intervention NK cell killing showed a trend towards an increase in the exercise group
compared to the control group. The authors conclude that 6 months of exercise training can lead to nominal increases on some immune responses while not affecting others.

Similar results were found in studies using rodents. Nasrullah and Mazzeo (1992) investigated the extent to which a fifteen-week endurance training program could influence the immune function in young, middle aged, and older rats. The study found that splenocyte proliferation, IL-2 production, and cytolytic activity declined significantly with age. Proliferation and IL-2 production was suppressed in younger animals, but improved these responses in older animals. Kohut, Boehm, and Moynihan (in press) found that in the older mice, exercise was associated with an enhanced production of HSV-1 specific Th1-associated cytokines, IL-2, and IFN-γ, with no effect on the Th2-associated cytokine IL-10 or IgM antibody. No effect of exercise was seen in young mice. In this study, the mice exercised at a moderate level on a treadmill for 8 weeks with a gradually increasing speed and duration. Perhaps exercise has an effect on some, but not all immune parameters.

Psychosocial factors also influence immunity. Stress response can differ among people and stress is associated with a decrease in immune response. Henry and Stephens (1977) proposed that different perceptions of stress have differing physiological consequences. Accordingly, the ability to control and predict the occurrence of the stressor may be more important than the actual physical nature of the stressor itself. Previous studies have concluded that positive social relationships have a buffering effect on the stress response. Cohen and colleagues (1997) found that the more types of social ties one has, the less susceptible they are to the common cold. This leads us to believe that the more diverse the social network in the elderly, the lesser the risk for upper respiratory infection. Russell and Cutrona (1991) found that the effect of stress on health-related issues varies depending
on the level of social support. They report that deficient social support may lead to depression in the elderly. In an elderly population, their social and personal relationships may be inconsistent, leading to an increased stress response. Stress has been shown in some studies to influence the immune system by decreasing the response of cellular immunity. For example, mitogen-stimulated T-cell function decreases after repeated exposure to bereavement stress (Sali, 1997).

Physical activity has been shown to lead to greater psychological well-being for many individuals. Numerous studies have been done in this area, with an emphasis on differentiating between physiological explanations to well-being, and psychosocial explanations to well-being. For example, Brown (1992) questioned if it is exercise that makes people feel better, or the social support that they receive that makes them feel better. Ruuskanen and Ruopila (1995) did a study on 1244 elderly Finlanders. They found a significant association between depression and lack of regular physical activity. Self-rated meaningfulness of life was also related to regular physical activity. The authors suggest that involvement in physical exercise may promote psychological well-being among the elderly. They also found that psychological well-being could be a predictor for staying physically active at advanced ages (Ruuskanen & Ruopila, 1995).

To date, results have been inconclusive on the effects of exercise training on psychological functioning among older adults. Some studies have found improvements in cognitive performance and mood with exercise, whereas others have failed to find any changes related to psychological well-being after exercise training. Blumenthal and colleagues (1999) did a study on the effects of exercise training on older patients with major depression. The study found that aerobic exercise was as effective in reducing depression as
antidepressant medication. Katula, Blissmer, and McAuley (1999) examined the effects of varying exercise intensity on anxiety levels in healthy, older adults. The results revealed that anxiety was reduced following a light-intensity activity, no change occurred following a moderate-intensity activity, and anxiety increased following a high-intensity activity (Katula, Blissmer, & McAuley, 1999). This suggests there is an exercise threshold to which psychological functioning and improvements may be seen.

Many of the changes in the immune functioning associated with stress are comparable to those of advancing age (Padgett, MacCullum & Sheridan, 1998). Padgett et al. (1998) tested whether stress-mediated anti-viral immunity would exacerbate age-related decreases in immune function. Mice (3 or 22 months old) were infected with .05 m. of influenza A/PR8 virus. The mice were placed in well-ventilated centrifuge tubes to induce restraint stress. The results showed that restraint stress had increased the rate of mortality in the 22-month old mice, but mortality rates in the 3-month old mice had not been altered. The cytokine production, interferon (IFN)-γ and interleukin (IL)-10 were all significantly lower in the older mice. Lastly, restraint stress further depressed the cytokine production in the 22-month old mice. The study concluded that the inability of older animals to respond to stress may further decrease the immune response of an already immuno-compromised animal.

In two related human studies, spousal caregivers of Alzheimer disease patients were used as subjects, along with control groups. All subjects in both studies received the appropriate influenza vaccine and had blood drawn both pre-vaccination and post-vaccination. The results indicated that caregivers responded less often to the vaccine than the control groups after vaccination and had a more rapid decline in a virus-specific T-cell response. Using spousal caregivers added a dimension of chronic stress, with the belief that
chronic stress also exacerbates immunosenescence. It was indicated that caregivers showed clear deficits relative to non-caregivers in both cellular and humoral response to the influenza vaccine, indicating that older adults who experienced chronic stress may be more vulnerable to immune-related health changes (Kiecolt-Glaser, Glaser, Gravenstein, Malarkey & Sheridan, 1996; Glaser, Kiecolt-Glaser, Malarkey, Sheridan, 1998). Vedhara and colleagues (1999) studied spousal caregivers of patients with dementia. The authors concluded that elderly caregivers had a poorer antibody response to the influenza vaccine. The data suggest that chronically stressed elderly individuals may be at higher risk from viral disease because of the inability to mount a “substantial” immune response.

Physiological consequences of chronic psychological stress may also be measured by alterations in lymphocyte proliferative responses. To assess T cell number, T helper and T cytotoxic/suppressor cells can be investigated. Pariante et al. (1997) found that caregivers of handicapped people had a significantly lower percentage of T cells, a significantly higher percentage of T suppressor/cytotoxic cells and a significantly lower T helper:suppressor ratio. When compared to younger, matched controls, the older caregivers had lower numbers of T cells and T helper cells. Also, the severity of stress reported by caregivers was significantly positively correlated with T suppressor/cytotoxic cells and negatively correlated with the T helper:suppressor ratio. This study concluded that age does play a fundamental role in modulating the effect of stress on the immune system.

Cytokine production is altered as an aging population experiences stress. Shinkai et al. (1995) found that elderly groups had a lower proliferative response and IL-2 production than their younger counterparts. Male elderly and young volunteers were used to investigate the extent of age-related changes in immune functions. Venous blood was drawn and
proliferative response and cytokine productions were analyzed using an ELISA kit. Although IL-2 production decreased, IFN-γ and IL-4 production remained unchanged.

In summary, the elderly population experiences a decline in their immune function when infected with the influenza virus. As these age-related declines in the immune system progress, the effects of influenza-like illnesses are more detrimental than what would be seen in a younger population. Chronic psychological stress also plays a role in decreasing the immune response. The elderly population has a greater sensitivity to stress-induced alterations. Elderly people who experience psychological stress may be more susceptible to infectious viruses, such as influenza, and they may experience a lower immune response to influenza vaccination. Elderly individuals who participate in regular exercise may have an increase in immune function (NK cells), but more research is needed in this area. The purpose of this study was to determine whether psychological stress and exercise are related to immunity in an elderly population (60+ yrs. old) and to determine the efficacy of the influenza vaccination. It is hypothesized that moderate exercise and improved psychosocial state, as measured by the Life Orientation Test, Perceived Stress Scale, and a social activity survey will be associated with enhanced immunity in the elderly population.
METHODS

Participants

Elderly male and female human subjects (greater than 60 years of age) who received an influenza immunization in the fall of 1999 were recruited to participate in the study (n=55; see Table 1). Because the study sought to examine varying levels of exercise, it was necessary to specifically recruit participants from different settings. Subjects were specifically recruited from the Iowa State University Exercise Clinic, the senior Mall Walkers Club, the Senior Center, and at the local hospital during vaccination sessions. Potential subjects consuming any medications that might alter immune function or psychosocial state (corticosteroids, antibiotics, nonsteroidal anti-inflammatory drugs, antidepressants) were excluded from the study. A medical history (Appendix A) was administered and individuals suffering from diseases that might alter immune function (cancer, autoimmune disease) were also excluded. Activity level was assessed by questionnaire and personal interview. Active individuals are classified as those participating in aerobic exercise at a minimum 60% VO2max (estimate based on heart rate as calculated from the Karvonen formula) for at least 20 minutes 3 or more times per week. Moderately active individuals are classified as somewhat active, but not at the level of the active participants. These individuals perform normal daily activities, including outdoor chores, but do not exercise at an intensity of >60% VO2max, and/or more than 3 times per week. The third level is those individuals who are sedentary and do not participate in any form of aerobic exercise, but perform the normal activities of daily living.
Table 1 Subjects

<table>
<thead>
<tr>
<th>Exercise Group</th>
<th>Average Age (yrs.)</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary</td>
<td>71</td>
<td>n=9</td>
<td>n=4</td>
</tr>
<tr>
<td>Moderately Active</td>
<td>71</td>
<td>n=17</td>
<td>n=8</td>
</tr>
<tr>
<td>Active</td>
<td>72</td>
<td>n=9</td>
<td>n=8</td>
</tr>
</tbody>
</table>

**Exercise survey instruments**

A physical activity questionnaire was used to initially assess the activity level of each participant. Following the questionnaire, a personal phone interview was used to confirm the activity level and answer any remaining questions (Appendix B). Questionnaire material was taken and modified from the Yale Physical Activity Survey. The Yale Physical Activity Survey was designed for a healthy, elderly population, with a correlation coefficient between two different administrations of the survey from 0.42 to 0.65 (Dipietro, Caspersen, Ostfeld, Nadel, 1993).

**Psychological survey instruments**

Two surveys were used to assess psychological stress. The Perceived Stress Scale is a 14-item scale that assesses the perception of stress (Appendix C). The scale has been shown to possess substantial reliability ($\alpha = 0.86$) and validity (Cohen, Kamarck, & Mermelstein, 1983). The reliability for this particular study is $\alpha = 0.86$. This scale does not measure psychological distress or symptomatology. The Life Orientation Test (LOT)
(Appendix D) assesses a generalized expectancy that things will turn out well related to health, and has a Cronbach's Alpha of 0.76 and test-retest reliability of 0.79 (Scheier & Carver, 1985). The reliability of the LOT in this sample was is $\alpha = 0.81$.

**Social activity survey**

Social activity was determined by a survey (Appendix E). The survey asked for the activity, frequency (days/week), time (min./session), and how many year of participation. Social activity levels were determined by calculating how many different social activities the subject participated in over a period of one month.

**Immunization**

Influenza vaccination was administered by Crystal Haglund, R.N. The trivalent Influenza Type A and B (Flushield®) was obtained from Wyeth Labs (Wyeth-Ayerst, PA). Each dose of the 1999-2000 vaccine contains 15 ug hemagglutination (HA) of A/Beijing/262/95 (H1N1), 15 ug HA of A/Sydney/5/97 (H3N2) and 15 ug HA of B/Yamanashi/166/98 (B/Beijing/184/93-like).

**Isolation of peripheral blood mononuclear cells**

Fifty ml of heparinized blood was collected from the subjects on day 14 following influenza immunization as determined by pilot data. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood samples by centrifugation over Ficoll Paque plus (Amersham Pharmacia Biotech, Piscataway, New Jersey) gradients. Cells were adjusted
to concentrations ranging from $5 \times 10^5$ to $2 \times 10^7$ cells/ml and incubated *in vitro* with the inactivated 1999 trivalent influenza virus at 0.18 ug/ml.

**Proliferation assay**

Virus specific lymphocyte proliferation was assessed with an MTT proliferation assay. In this colorimetric assay, PBMC was adjusted to $4 \times 10^6$ cells/ml plus 100 ul and plated in a 96 well plate with or without 0.18 ug/ml inactivated influenza virus for five days at 37 °C in 5% CO₂. 10 ml of 5 mg/ml 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 a dual wavelength of 570 and 630nm with an automated microplate reader (Kruszewska, Felten, & Moynihan, 1995).

**Assay for cytokine production**

Lymphocyte influenza-specific cytokine production (interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-10 (IL-10), interleukin-12 (IL-12) and interferon-gamma (IFN-γ)) was measured in cell supernatants using ELISA. PBMC was adjusted to $4 \times 10^6$ cells/ml plus 1 ml and incubated with 0.18 ug/ml inactivated influenza virus in vitro and cell supernatants were collected at 24, 48, 72, and 96 hours. An ELISA kit was purchased from Pharmingen (San Diego, CA) and manufacturers instructions were followed to assess IL-2, IL-4, IL-10, IL-12 and IFN-γ.
Anti-influenza antibody ELISA.

Influenza specific antibody production (IgG and IgM) in serum was determined by ELISA. Briefly, 96 well plates were coated overnight with inactivated influenza virus in carbonate coating buffer at various concentrations. Plates were incubated overnight and blocked for 1 hour with PBS-1% BSA. Sera was diluted at a range of dilutions (1:10 - 1:1000) in PBS-Tween containing 1M NaCl and incubated for several hours at 37 ° C. Alkaline-phosphatase-conjugated mouse anti-human IgM and IgG was added, followed by the addition of the substrate, p-nitrophenyl phosphate in a carbonate coating buffer. Absorbance at 405 nm was measured on a microplate reader. Sera from nonimmunized individuals served as a control.

Flow cytometry

In order to determine whether a change in cytokine production reflects a change in the number of cells producing cytokine or results from a functional decline in cytokine production on a per cell basis, flow cytometry was performed to determine the number of T helper cells in the PBMC population. Cells were prepared for flow cytometry in the laboratory of Dr. Joan Cunnick. Briefly, PBMC (100 µl of 5 X 10^6 cells/ml) were incubated with FITC-conjugated anti-CD4 (marker for human helper T cells) or FITC-conjugated mouse anti-human IgG1 (isotype control) diluted in PBS - 1% bovine serum albumin. Cells were washed and fixed in 1% paraformaldehyde. Cells were analyzed for fluorescent intensity on a Coulter XL flow cytometer at the Cell hybridoma facility at Iowa State University.
Analysis and interpretation

The independent variables were gender, activity level, and day of blood draw. A three-way ANCOVA (gender X activity X day) was used to assess differences for each of the dependent variables, (cytokines, antibody titer, and proliferation). The psychosocial variables (PSS, LOT, and social activity) were analyzed as covariates. A significant increase (p<0.05) in any of the immune parameters measured in the most active or moderately active elderly compared to the sedentary elderly would suggest that physical activity may enhance immunological response to immunization.

A regression analysis was performed to assess the significance of activity level net of psychosocial factors. Psychosocial factors were entered in Block 1, and activity was entered in Block 2. Exercise was converted to a continuous variable by multiplying the number of minutes per week spent exercising by an intensity factor. A test for interactions and a test for correlation were done between the activity level and each psychosocial factor.
RESULTS

The analyses for all the immune variables and psychosocial variables were conducted as an ANOVA (gender X activity X day of blood sample). In cases where there was no effect of “day of blood sample”, the ANOVA was run again without Day as a between subjects factor. In cases where there was no effect of gender, the ANOVA was run again without gender as a between subjects factor. In variables where there was more than one dilution (antibody titers) or time point (IFN-gamma 48 hours and 96 hours, IL-10 48 hours and 96 hours), dilution or time was included in the analysis as a within subjects factor.

After all the ANOVAs were run, the same ANOVA was run again, with the psychosocial variables (PSS, LOT, and social) included as covariates. Inclusion of psychosocial measures as covariates did not alter the significance of group (activity) differences for any immune variable, suggesting that the effect of activity extends beyond varying alterations in perceived stress.

A regression analysis was performed to assess the potential contribution of each factor (see Table 2). Perceived Stress Scale score, Life Orientation Test score, and social activity were all entered in Block 1. Activity was entered in Block 2. Exercise was converted to a continuous variable by multiplying the number of minutes per week spent exercising by an intensity factor. A test for interaction was completed by multiplying activity score by each psychosocial factor (Perceived Stress scale score, Life Orientation Test score, and social activity score). No interaction was found between any psychosocial factor and activity level.
Table 2. Regression table of various independent variables on antigen specific dependent variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>SE B</th>
<th>β</th>
<th>t</th>
<th>ΔR²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antigen Proliferation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Step 1</td>
<td></td>
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<tr>
<td>Activity level</td>
<td>0.003</td>
<td>0.016</td>
<td>0.286</td>
<td>2.217*</td>
<td>0.082</td>
</tr>
<tr>
<td><strong>IgG Antibody titer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>Step 1</td>
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<td></td>
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<tr>
<td>PSS score</td>
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<td>0.020</td>
<td>0.182</td>
<td>1.255</td>
<td>0.033</td>
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<tr>
<td>Step 2</td>
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<tr>
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<td>0.370</td>
<td>2.610*</td>
<td>0.127</td>
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<tr>
<td><strong>IgM Antibody titer</strong></td>
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<tr>
<td>PSS score</td>
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<td>0.029</td>
<td>-0.186</td>
<td>-1.286</td>
<td>0.035</td>
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<td>Step 2</td>
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<tr>
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<td>0.001</td>
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<td><strong>IFN-γ Production</strong></td>
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<tr>
<td>Step 1</td>
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<tr>
<td>PSS score</td>
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<td>1.440</td>
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<tr>
<td>Step 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity level</td>
<td>-0.098</td>
<td>0.238</td>
<td>-0.058</td>
<td>-0.410</td>
<td>0.003</td>
</tr>
</tbody>
</table>

* denotes significance at the 0.05 level

Table consists of nearing-significance and/or significant psychosocial predictors of each model.
Table 2. Continued

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>SE B</th>
<th>β</th>
<th>t</th>
<th>ΔR²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL-2 production</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Step 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PSS score</td>
<td>9.604</td>
<td>4.546</td>
<td>0.281</td>
<td>2.113*</td>
<td>0.115</td>
</tr>
<tr>
<td>Social</td>
<td>24.612</td>
<td>16.515</td>
<td>0.198</td>
<td>1.490</td>
<td></td>
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<tr>
<td>Step 2</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Activity</td>
<td>0.007</td>
<td>0.152</td>
<td>0.006</td>
<td>0.043</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>IL-10 production</strong></td>
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<tr>
<td>Step 1</td>
<td></td>
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<tr>
<td>LOT score</td>
<td>8.645</td>
<td>4.509</td>
<td>0.250</td>
<td>1.971</td>
<td></td>
</tr>
<tr>
<td>Social</td>
<td>40.705</td>
<td>19.044</td>
<td>0.278</td>
<td>2.137*</td>
<td>0.137</td>
</tr>
<tr>
<td>Step 2</td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>Activity</td>
<td>0.177</td>
<td>0.161</td>
<td>0.144</td>
<td>1.101</td>
<td>0.020</td>
</tr>
</tbody>
</table>

* denotes significance at the 0.05 level.
A test for correlation was done between activity level and the psychosocial factors (see Table 3). The Perceived Stress Scale was significantly negatively correlated with the Life Orientation Test \( (p = -0.481) \), suggesting that lower levels of perceived stress were correlated with greater levels of optimism, as measured by the Life Orientation Test. The Perceived Stress Scale was significantly negatively correlated with activity level \( (p = -0.299) \), suggesting that lower levels of perceived stress are correlated with greater activity levels.

**Table 3. Correlation matrix of psychosocial variables and activity level**

<table>
<thead>
<tr>
<th></th>
<th>Activity</th>
<th>LOT</th>
<th>PSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson LOT</td>
<td>0.030</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation PSS</td>
<td>-0.299*</td>
<td>-0.481*</td>
<td></td>
</tr>
<tr>
<td>Social</td>
<td>0.144</td>
<td>-0.026</td>
<td>-0.038</td>
</tr>
</tbody>
</table>

* denotes significance at the 0.05 level

**PBMC proliferation**

PBMC proliferation in response to *in vitro* restimulation with vaccine was influenced by physical activity. The results from an ANOVA indicated that the PBMC from sedentary subjects yielded significantly lower proliferation levels when compared to the moderate or active individuals \( (p=0.013; \) see Fig. 1). There was no significant effect of gender or gender by activity interaction. Results from regression analysis indicated that activity level accounted for 8.2% of the variance which was statistically significant \( (p=0.031) \).
Anti-influenza IgG antibody titer

The active individuals had a greater anti-influenza IgG antibody titer than individuals classified as sedentary or moderately active (p=0.030; see Fig. 2). Results from a regression analysis showed that lower perceived stress was associated with higher anti-influenza IgG antibody titer. Together, PSS and exercise accounted for 16.0% of the variance in anti-influenza IgG antibody titer. As individual factors, PSS was approaching significance (p = 0.054) and activity was a significant predictor of IgG antibody titer (p = 0.012). No interaction was found between PSS and activity.
Anti-influenza IgG antibody titer

Anti-influenza IgM antibody titer was also greater in the most active individuals when compared to moderately active or sedentary individuals (p = 0.042; see Fig. 3). The results from an ANOVA showed a significant main effect of activity and dilution. Both anti-influenza IgG and IgM antibody titers showed no effect of gender and no gender by activity interaction. Regression analysis results suggested that activity was a significant predictor (p = 0.042) of anti-influenza IgM antibody titer.
ANOVA results suggest that physical activity and psychosocial factors do not influence IFN-gamma production. The results for in vitro IFN-gamma production showed no effect of activity ($p = 0.228$) and there was no effect of gender ($p = 0.254$). As expected, a significant effect of time in culture was seen (within subjects factor) (see Fig. 4, 5). Significantly greater amounts of IFN-gamma were seen in the 96-hour culture when compared to the 48-hour ($p = 0.038$). The regression analysis showed that no factors were significant predictors, but gender approached significance ($p = 0.069$).
Figure 4. Interferon gamma (48 hour timepoint)

Figure 5. Interferon gamma (96 hour timepoint)
Anti-influenza IL-2 production

IL-2 production was not associated with physical activity. However, gender and psychosocial factors may impact IL-2 production. ANOVA results of IL-2 production, in vitro, showed no main effect of activity (p=0.865), but a significant main effect of gender (p=0.002; see Fig. 6). Across all exercise groups, females had greater amounts of IL-2 than males. Results of the regression analysis suggested that PSS and social together accounted for 11.5% of the variance, while PSS by itself was statistically significant (p=0.040). Less psychological stress was associated with greater levels of IL-2 production.

![Figure 6. Interleukin-2 production](image)

Figure 6. Interleukin-2 production
Anti-influenza IL-10 production

The results from the ANOVA suggest that physical activity and gender did not influence IL-10 production (Fig. 7). The results suggested that IL-10 showed no main effect of activity ($p=0.929$), no main effect of gender, and no gender by activity interaction. However, the results from the regression analysis showed that LOT scores and social interaction together accounted for 13.7% of the variance. Social, as an individual factor, was a significant predictor of IL-10 production ($p = 0.037$). Activity as a factor did not improve the model, suggesting that IL-10 is not influenced by exercise. IL-10 levels are greater in people with more social interaction and those who are more optimistic.

![Figure 7. Interleukin-10 production](image-url)
Anti-influenza IL-4 and IL-12 production

Anti-influenza IL-4 and IL-12 production by PBMC were below detectable limits of the ELISA assay.

Percentage of T-helper and T-cytotoxic cells

T-helper cell percentage (CD4+CD3+) was not significantly different across activity levels (p=0.398), as analyzed by ANOVA, but a significant main effect was seen for gender. Females had a greater percentage of T-helper cells compared to males (p=0.002; see Fig. 8).

Figure 8. T-helper cell percentage
With respect to T-cytotoxic cell percentage (CD8+CD3+), as analyzed by ANOVA, activity did not impact the percent of CD8+CD3+ cells (p=0.083). However, a significant main effect was seen between gender. Males had a significantly greater percentage of T-cytotoxic cells than females (p=0.001; see Fig. 9). There was no effect of activity for females, but males showed a trend such that sedentary individuals had a greater percentage of CD8+CD3+ cells than both moderate and active individuals (p=0.051). Regression analysis indicated that gender was a significant predictor of both T-helper (p = 0.001) and T-cytotoxic (p = 0.007) cell percentage.

![Figure 9. T-cytotoxic cell percentage](image)
DISCUSSION

This study was the first to suggest that there is an association between activity level and immune response to influenza vaccine in older adults. Older individuals participating in aerobic exercise for at least 20 minutes per session three or more days per week at an intensity estimated to be greater than or equal to 60% VO$_{2_{max}}$ had a higher influenza specific IgG and IgM antibody titer than sedentary and moderately active individuals. Activity was also associated with greater influenza specific PBMC proliferation; the PBMC proliferation in both active and moderately active individuals was greater than the PBMC proliferation for sedentary individuals. T-helper and T-cytotoxic cell percentages showed no effect of activity. This leads us to believe that exercise did not alter the cell percentage of T-helper and T-cytotoxic cells, and immune changes due to exercise are not due to T-helper and T-cytotoxic cells. Cytokine production did not appear to be associated with physical activity, at the one time point measured in this study.

Our findings suggest that immune response to an influenza vaccine may be enhanced in older adults by exercise. Previous studies have supported this theory of enhanced immune response in well conditioned older adults compared to less active older adults, although none of these studies have examined antigen-specific immune response (Nieman et al, 1993; Shinkai et al, 1995). Other controlled trials utilizing exercise as a factor have reported that exercise has no or little effect on immune function. We hypothesize that there may be a threshold effect with respect to the duration and/or intensity of exercise programs. Some previous research has looked at an exercise intervention for six weeks, others for three months, and still others for six months. Research has shown that people who have trained for
numerous years tend to show an increase in immune response. Woods et al (1999) showed that physical activity improved immunity, but not statistically, when they conducted a 6-month training study. Also, there may be a threshold effect on the intensity of the exercise. Taken together, this suggests that long-term interventions at a sufficient intensity may be necessary to detect any changes in immune functioning.

Vaccine efficacy is reduced among older adults (due to decreased immune response), which puts them at risk for serious morbidity and greater mortality rates than their younger counterparts. Several studies have supported this theory of compromised immune functioning in older adults (Bernstein et al, 1999; deBruijn et al, 1997; Keren et al, 1988). Bernstein and colleagues (1998) found that a younger population had a dramatically higher antibody titer than an elderly population after vaccination. Even though the elderly population has an increase in antibody titer from their pre-vaccination levels, they did not respond as positively as a younger population. Exercise may be one way to improve the efficacy of the influenza vaccine in an elderly population. In this study, the active group had greater antibody titer than both the moderate and sedentary groups after an influenza vaccination. Other research has shown a decrease in cell proliferation in an elderly population compared to a younger population. With respect to the influenza vaccination, the elderly population has shown lower levels of influenza specific cell proliferation. In our study, exercise (both moderate and active) enhanced PBMC proliferation. In an elderly population, exercise may enhance specific cell proliferation, which will in turn may enhance an immune response to the influenza vaccination.

Other psychosocial variables, independent of exercise, may also impact immune functioning. It has been commonly assumed that high levels of psychosocial stress result in
compromised immune function. Our study found that the Perceived Stress Scale, as a measure of psychosocial stress, was a significant predictor of immune response, specifically anti-influenza IgG antibody titer and IL-2 production. Cohen and colleagues (1998) found a linear increase in the relative risk for infection with increased duration of the stressor. Kiecolt-Glaser et al. (1996) measured IL-2 production 6-months post vaccine and found that those individuals with greater IL-2 production were less psychologically stressed. The previous studies, along with our study, provide additional evidence for the role of psychosocial stress in susceptibility to illness. Elderly people quite often are required to deal with chronic, psychosocial stressors, such as loneliness, depression, or spousal caregiving. Therefore, the elderly may be more susceptible to infection because the psychosocial stress that they experience in everyday living is compromising their immune functioning. Vedhara and colleagues (1999) found that spousal caregivers may be more vulnerable to infectious disease than a population of similar age.

Our results also showed that PSS levels were lower in the active group and moderately active group when compared to the sedentary individuals. Other research has shown that PSS is influenced by activity. Steptoe, Kimbell, and Basford (1998) found that on exercise days, persons perceived fewer events as stressful and had a better outlook on their day. This study suggests that exercise decreases the perceptions of stress, which would lead to a less stressful lifestyle. Krause and colleagues (1993) found that more frequent physical activity is associated with less psychological stress. They also found that some types of stressors tend to decrease the frequency of physical activity. We can not determine from our study if activity decreases psychological stress levels, or if those individuals with lower psychological stress are more apt to be physically active. Crews and Landers (1987)
conducted a meta-analytic review of exercise and reactivity to psychosocial stressors. The results indicated that aerobically fit individuals had a reduced psychosocial stress response. The authors suggest that exercise may act as a coping strategy or serve to more effectively respond to the intrusion of psychosocial stress. This suggestion correlates with our study, in which the active, elderly individuals reported less perceived stress. Perhaps the combination of exercise and decreased levels of stress would result in an enhanced immune response.

Other psychosocial factors impact immune response. The Life Orientation Test, a measure of optimism, and greater social activity were associated with higher levels of IL-10 production. Segerstrom and colleagues (1998) found that optimism has been associated with improved immune status among law students. Optimists cope differently with stressors and may have more adaptive health behaviors, which could lead to better immune status. Cohen and colleagues (1997) also suggest that an increased number of social ties may be associated with improved immunity and less susceptibility to infection.

We observed that gender appeared to influence immune response to vaccination in our elderly sample. Although our study measured different cytokines, we only found significance of gender in IL-2. IL-2 levels were highest in both sedentary groups, although females had higher levels than males. IL-2 is responsible for T-cell proliferation and is very central in the adaptive immune response. To the best of our knowledge, no other research has shown gender effects as related to immunity. The gender differences seen in our study may be due to the fact that our population was too small.

The primary mechanisms for why exercise enhances immune function seem to be related to neuroendocrine changes. The possible neuroendocrine changes due to exercise include changes in a variety of neurohormones, such as catecholamines, glucocorticoids, β-
endorphins, and growth hormones. All of these neurohormones have been shown to alter the immune response in one way or another (Guidi et al, 1998). It has been hypothesized that glucocorticoids and catecholamines are the primary stressor hormones that affect the immune system (Kusnecov, 1994). Moyna and colleagues (1998) suggest that exercise training at a sufficient intensity and/or duration may induce a nonspecific, generalized adaptation in the stress response. They also suggest that the immune alterations are partially mediated by altered activity of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system, although these mechanisms are not well understood. A secondary mechanism for altered immune response due to exercise may involve psychosocial factors. High levels of psychosocial stress compromise immune functioning, but exercise may attenuate psychosocial stress. These altering levels of psychosocial stress may have an effect on the immune response to exercise. Increased levels of psychosocial stress have been associated with increased cortisol levels. Also, chronic activation of the hypothalamic-pituitary-adrenal axis (which is common in an elderly population) has been associated with impaired antibody responses to influenza vaccination (Vedhara et al, 1999).

In conclusion, our findings demonstrate that moderate exercise enhances immune response to an influenza vaccine in an elderly population. Older individuals who were aerobically active had a higher influenza specific IgG and IgM antibody titer than their sedentary counterparts. Activity was also associated with greater PBMC proliferation in active and moderately active individuals when compared to sedentary individuals. Lower perceived stress was a predictor of high IgG antibody titer and IL-2 levels. Further research is needed in this area. A long-term training study on elderly individuals is warranted to see if there is any threshold effect of duration and/or intensity of exercise. Also, cytokines may be
measured at different timepoints to see whether the immune response is compromised or enhanced at different times. Finally, more work is needed on the neuroendocrine mechanisms for the immune response changes due to exercise. It is possible that psychosocial factors also modulate neuroendocrine-immune interaction in response to exercise. For example, cortisol has been shown to be high in stressed individuals, but it is not clear whether moderate exercise can alter psychosocial stress-induced changes in cortisol level. As more research is being done in this area, the relationships between immune function and exercise in the elderly may become clearer.
APPENDIX A: PHYSICAL ACTIVITY HISTORY

Today’s Date: __ / __ / ____

---

**Personal Information**

Name: ___________________________ Age: _____ Date of Birth: _____ / _____ / _____ Sex: _____

Address: ___________________________ Telephone No: (____)_________

Employer: ___________________________ Social Security No: _____-____-____

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**Emergency Information**

Personal Physician: ___________________________ Physician’s Telephone No: _________

Physician’s Address: ___________________________

Individual to be contacted in case of an emergency: ___________________________

Relationship to you: ___________________________

Home Address: ___________________________ Home Telephone No: _________

Work Address: ___________________________ Work Telephone No: _________

---

Do you have medical alert identification? _______ YES _______ NO

If YES, where is it located? ___________________________

---

**Current Medications (include ALL medications)**

<table>
<thead>
<tr>
<th>Name of Drug</th>
<th>Dosage; Times/day</th>
<th>Why are you on this drug?</th>
</tr>
</thead>
<tbody>
<tr>
<td>____________</td>
<td>_________________</td>
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<td>____________</td>
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<td>________________________</td>
</tr>
</tbody>
</table>
Hospitalizations
Please list the last five (5) times you have been ill (sick) enough to see a physician, been hospitalized or had surgery.

<table>
<thead>
<tr>
<th>When?</th>
<th>What was done (surgery, etc.)?</th>
<th>Why was this done?</th>
</tr>
</thead>
<tbody>
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Personal Medical History
Please describe your smoking history and indicate if you currently smoke _______.

Do you have any known allergies? ______ YES ______ NO If YES, please explain: ____________________________

Please check the following disease conditions that you had or currently have:

- High blood pressure
- High blood cholesterol
- High blood triglycerides
- Angina pectoris
- Heart attack
- Heart surgery (catheter, bypass)
- Heart failure
- Heart murmur
- Stroke/transient ischemia attacks
- Aneurysm
- Anemia
- Diabetes
- Jaundice
- Hepatitis
- Infectious mononucleosis
- Phlebitis
- Gout
- Kidney stones
- Abnormal chest X ray
- Asthma
- Emphysema
- Bronchitis
- Thyroid problems
- Hernia
- Cancer
- Epilepsy or seizures
- Prostate problem
Rheumatic fever  Urinary tract infections  Osteoporosis
Arteriosclerosis  Emotional disorder (depression, etc.)  Eating disorder
Other

Please provide dates and explanation to any of the above which you checked:


Recent Illness

Have you had any symptoms of illness within the last 3 weeks (such as runny nose, stuffy nose, sore throat, fever, aches, etc) ________ YES ________ NO  If YES, please list the symptoms that you experienced:


Have you taken any medication within the last 3 weeks for symptoms of illness or pain (aspirin, tylenol, ibuprofen, over the counter cold medications, etc) ________ YES ________ NO  If YES, please describe:


Prior Influenza Immunization

Have you received an influenza immunization before? ________ YES ________ NO

If yes, please circle the years that you received the vaccine 1999 1998 1997 1996 1995

Activity History

Please list any physical or recreational activities that you currently do or have done on a regular basis.

List each activity below  (ACTIVITIES MAY INCLUDE: walking, hiking, swimming, jogging, water exercise class, calisthenic exercises, badminton, basketball, exercise bicycle, bicycle outdoors, dancing – please list type of dance, farm work – please describe, gardening, golf, horseback riding,
skiing-downhill, skiing-cross country, tennis, racquetball, OR ANY OTHER ACTIVITY THAT CAUSES LARGE INCREASES IN HEART RATE, BREATHING, OR CAUSES YOU TO PERSPIRE.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Frequency (days/week)</th>
<th>Time (min/session)</th>
<th>How long (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</table>

**Vitamin and Nutritional Supplement Intake**

Please list below all of the vitamins, minerals, and nutritional supplements you consume

<table>
<thead>
<tr>
<th>NAME</th>
<th>AMOUNT</th>
<th>HOW OFTEN?</th>
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<tbody>
<tr>
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</table>

If you currently have, or have had in the past any medical condition you believe we should be aware of, that is not already listed on this form, please describe below

<p>| |</p>
<table>
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</table>
APPENDIX B: PHYSICAL ACTIVITY HISTORY

Please list any physical or recreational activities that you currently do or have done on a regular basis. List each activity below (ACTIVITIES MAY INCLUDE: walking, hiking, swimming, jogging, water exercise class, calisthenic exercises, badminton, basketball, exercise bicycle, bicycle outdoors, dancing – please list type of dance, farm work – please describe, gardening, golf, horseback riding, skiing-downhill, skiing-cross country, tennis, racquetball, OR ANY OTHER ACTIVITY THAT MAKE YOU BREATHE HARDER).

<table>
<thead>
<tr>
<th>Activity</th>
<th>Frequency (days/week)</th>
<th>Time (min/session)</th>
<th>How long (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</table>

A follow-up phone call was used to confirm the information listed in the Physical Activity Survey. Adjustments to the survey were made if necessary. The following question was asked to each subject during the phone call:

Do you know what your heart rate was during each activity?
   - If yes, write down the heart rate.
   - If no, ask if the activity was intense enough to cause large increases in the heart rate, breathing, and / or caused you to perspire?
     - If yes = active group
     - If no = moderately active group

After assessing the physical activity survey, and the follow-up phone call, the subjects were then placed in the appropriate exercise group.
APPENDIX C: PERCEIVED STRESS SCALE

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, you will be asked to indicate *how often* you felt or thought a certain way. Although some of the questions are similar, there are differences between them and you should treat each one as a separate question. The best approach is to answer each question fairly quickly. That is, don’t try to count up the number of times you felt a particular way, but rather indicate the alternative that seems like a reasonable estimate.

For each question, choose from the following alternatives:

- **0** = Never
- **1** = Almost Never
- **2** = Sometimes
- **3** = Fairly Often
- **4** = Very Often

1. In the last month, how often have you been upset because of something that happened unexpectedly?
   
   0 1 2 3 4

2. In the last month, how often have you felt that you were unable to control the important things in your life?
   
   0 1 2 3 4

3. In the last month, how often have you felt nervous and “stressed”?
   
   0 1 2 3 4

4. In the last month, how often have you dealt successfully with irritating life hassles?
   
   0 1 2 3 4

5. In the last month, how often have you felt that you were effectively coping with important changes that were occurring in your life?
   
   0 1 2 3 4

6. In the last month, how often have you felt confident about your ability to handle your personal problems?
   
   0 1 2 3 4

7. In the last month, how often have you felt that things were going your way?
   
   0 1 2 3 4

8. In the last month, how often have you found that you could not cope with all the things that you had to do?
   
   0 1 2 3 4
9. In the last month, how often have you been able to control irritations in your life?
   0  1  2  3  4

10. In the last month, how often have you felt that you were on top of things?
    0  1  2  3  4

11. In the last month, how often have you been angered because of things that happened that were outside of your control?
    0  1  2  3  4

12. In the last month, how often have you found yourself thinking about things that you have to accomplish?
    0  1  2  3  4

13. In the last month, how often have you been able to control the way you spend your time?
    0  1  2  3  4

14. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?
    0  1  2  3  4
APPENDIX D: LIFE ORIENTATION TEST

Below are several statements about how you feel about yourself. Please read each statement carefully. Indicate how much you agree with each statement by circling the appropriate letter below the statement.

Use any of the letters on this scale:

A = Strongly disagree
B = Disagree
C = Somewhat disagree
D = Neutral
E = Somewhat agree
F = Agree
G = Strongly agree

1. I feel that I'm a person of worth, at least on an equal level with (equal to) others.
   A B C D E F G

2. I feel that I have a number of good qualities.
   A B C D E F G

3. All in all, I'm inclined to feel that I'm a failure.
   A B C D E F G

4. I am able to do things as well as most other people.
   A B C D E F G

5. I feel I do not have much to be proud of.
   A B C D E F G

6. I take a positive attitude toward myself.
   A B C D E F G

7. On the whole, I am satisfied with myself.
   A B C D E F G

8. I wish I could have more respect for myself.
   A B C D E F G

9. I certainly feel useless at times.
   A B C D E F G

10. At times, I think I am no good at all.
    A B C D E F G
APPENDIX E: SOCIAL ACTIVITY QUESTIONNAIRE

Please list any social activities that you currently do or have done on a regular basis. (ACTIVITIES MAY INCLUDE: attending classes, playing cards, member of religious or non-religious social group, visiting family members, visiting friends, OR ANY OTHER ACTIVITIES THAT INVOLVE SOCIAL INTERACTION WITH OTHER PEOPLE.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Frequency (days/week)</th>
<th>Time (min/session)</th>
<th>How long (years)</th>
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REFERENCES CITED


