

Effects of an Exercise Intervention on Immunologic Parameters in Frail Elderly Nursing Home Residents

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Background. Aging is associated with decline in both cell-mediated and humoral immunity and may contribute to increased incidence and severity of infections in frail elderly. Exercise, depending on intensity, has significant effects on the immune system. We conducted a randomized, controlled clinical trial of a 32-week functionally oriented exercise program in frail elderly living in nursing homes and determined whether the exercise intervention was associated with a change in immune parameters in this frail elderly nursing home population.

Methods. Nursing home residents were randomly assigned to an intervention ($n = 94$) and control group ($n = 96$). The intervention consisted of a functionally oriented endurance and resistance exercise training that was provided every 2 hours from 8:00 AM to 4:00 PM for 5 days a week for 8 months. Lymphocyte subpopulations, including activation markers (CD28, CD25, HLA-DR), in vitro proliferation, and soluble products of cytokine activity (neopterin and sTNF-RII) in serum were measured by taking blood samples at baseline and after 8 weeks and 32 weeks of the intervention.

Results. Exercise training did not induce changes in lymphocyte subpopulations, activation markers (CD28, CD25, HLA-DR), in vitro proliferation, and soluble products of cytokine activity (neopterin and sTNF-RII) in serum.

Conclusions. A 32-week exercise intervention did not bring about beneficial or detrimental effects on immune parameters in the frail elderly nursing home population and may explain why the intervention was not associated with a change in the incidence of infections in the intervention group compared with the control group.

IMPAIRED mobility and urinary incontinence are common comorbidities among nursing home (NH) residents (1). These conditions are associated with physical inactivity and with numerous related health problems, including pressure ulcers, urinary and respiratory tract infections, falls, and constipation (2-5). Immobility and incontinence have also been associated with acute hospitalization and mortality in this population (6).

Previous studies have demonstrated the feasibility and beneficial effects of a functionally oriented exercise program and incontinence care on several outcomes in NH residents, including continence, physical activity, and mobility endurance, but not on overall health (7-9). A recent randomized, controlled clinical trial of a functionally oriented exercise program and incontinence care showed significant positive effects on measures of upper body strength, urinary and fecal incontinence, physical activity, and mobility endurance (8). However, these effects were not associated with a reduction in the incidence and costs of selected acute health conditions (9).

The body's response to acute health conditions such as pneumonia, acute bronchitis, and urinary tract infection, among others monitored as part of this trial, involves proper

function of the immune system. Aging is associated with decline in both cell-mediated and humoral immunity (10) and may contribute to increased incidence and severity of infections in frail elderly (11,12). Exercise, depending on its intensity, has a significant effect on the immune system. It has been hypothesized that moderate levels of exercise improves—whereas strenuous exercise or overtraining suppresses—various immune function measures (13). NH residents have numerous comorbidities that are associated with physical inactivity (1) and impaired immune response (14). Thus, an exercise intervention that enhances immune function could be beneficial in this population. To date, 8 prospective studies of exercise (aerobic or resistance training) and immune response in the elderly have been conducted (15-22). Seven of the 8 studies examined the effects of exercise on immune function in healthy elderly subjects (15-20,22). Only 1 study investigated the effects of a 17-week moderate exercise training on the immune response of independently living, frail elderly subjects (79.2 ± 5.9 years) (21). In contrast to the healthy and independently living frail elderly subjects, we conducted a randomized, controlled clinical trial of a 32-week

functionally oriented exercise program in frail elderly living in NHs. During this study, we collected blood samples from frail elderly to measure immune parameters at different time points in the control and intervention groups.

METHODS

Setting and Participants

Residents were recruited from 3 proprietary NHs and 1 nonprofit NH. Residents met the inclusion criteria if they were not on postacute skilled care units or terminally ill and were incontinent of urine, free of a catheter, and able to follow a 1-step instruction (e.g., move your hand). The protocol was approved by the University of California, Los Angeles, Human Subjects Protection Committee. Research staff obtained consents directly from those residents who could pass a brief test documenting their understanding of the consent form, and for all other eligible residents, consents were obtained from their designated proxies. In all cases, assent was obtained from the residents for their participation in the trial. Details of the procedure to describe the characteristics of the participants and to assess resident's preferences to key components of the intervention have been described elsewhere (8).

Overview of the Study Design and Intervention

Residents were randomized into intervention and control groups by a computerized randomization program after baseline assessments were completed. The intervention group was then maintained in the intervention for 32 weeks. Postintervention assessments were completed at 8 weeks (Post-1) and 32 weeks (Post-2) in both the intervention and control groups. The intervention was implemented every 2 hours, 5 days a week, from approximately 8:00 AM to 4:30 PM, for a possible total of 4 care episodes per day. During each episode of care provided by research staff, residents were prompted to toilet and were changed if they were wet. No effort was made to influence the incontinence care practices of NH staff during hours when the resident was not being provided care by research staff. Either before or after this incontinence care, staff encouraged residents either to walk or, if they were nonambulatory, to wheel their chairs, and to repeat sit-to-stands up to 8 times using the minimum level of human assistance possible. During 1 episode per day, each resident, usually while he or she was in bed, was given upper body resistance training (arm curls or arm raises). Initial exercise goals were set at 75% of the residents' maximum distance walked or wheeled during the baseline assessment, or 75% of the highest weight lifted in 1 baseline trial. If residents achieved these goals on 90% of their weekly care episodes, then goals were increased by approximately 1 minute for mobility exercise and 2 stands per trial, or 1 pound per resistance training session. The maximum goal established for any resident was 10 minutes for walking or wheeling and 8 stands per trial. There was no maximum for resistance training.

The degree to which the intervention succeeded in increasing physical activity was assessed in 2 ways. First, behavioral observations were conducted every 15 minutes for 8 hours per day during a 2-day baseline period and on 2

days during the intervention period for both intervention and control residents. For these observations, research staff located each resident every 15 minutes and noted the resident's physical activity (e.g., walking, standing, lying, or sitting). During these same 2-day baseline and intervention assessments, all residents wore motion sensors (Caltracs, Hemo-Kinetics, Inc., Madison, WI) that provided a continuous record of their movements. Data accuracy for all measures was assured by conducting interobserver agreement checks throughout all phases of the trial and by blinding observers to group assignment whenever possible. For the major outcome measures (physical performance assessments), the agreement between a blinded observer and a second observer was documented on 100% of the observations. Blinding was accomplished by training people to conduct the assessment who had no responsibility for randomization or for the implementation of the trial on a daily basis. Blood was drawn in early morning and before any intervention from all subjects during the baseline and intervention periods and occurred at least 12 hours after the last exercise session to ensure that changes detected in immune parameters reflected the cumulative effect of multiple bouts of exercise (training) and minimized the measuring of acute changes in immune parameters in response to the last exercise session. All subjects began exercise between the months of May and July and ended between December and February, with the 8-week assessment occurring between the months of July and September, and the study was spread over 4 years (1996–2000). Radial pulse was taken immediately before and after the exercise intervention and was measured in sitting position.

Endurance

Standing, walking, and wheelchair endurance were assessed for both intervention and control residents using a standardized protocol on 3 separate occasions: at baseline and again at 8 and 32 weeks postbaseline. On each occasion, 8 trials were conducted on 2 separate days between 8 AM and 4:30 PM, with trials separated by approximately 2 hours. For each trial, residents were first asked to stand using the minimum level of human assistance possible, though they were allowed to use either their chair arms or wall rails if they could not otherwise stand without human assistance. This modification to the standing test was made because so few residents (2%) were capable of standing without using their arms during the baseline assessments. During these endurance tests, each resident was encouraged to walk or wheel their wheelchair for up to 10 minutes, with one 60-second rest stop allowed. For each 2-day assessment period, the number of trials that a resident actually attempted to walk or wheel, and the average distance walked or wheeled over all 8 trials, were calculated. The difference between these 2 methods of calculation did not result in different conclusions, and we therefore report the distance values averaged over all 8 trials, as well as the maximum distance that a resident walked or wheeled on any 1 of the 8 trials. During the second trial on each of the 2 days of assessment, the resident was asked to stand as many times as possible in 30 seconds. We report the average number of stands that

a resident completed over these 2 trials as well as the maximum number of stands they completed on their best effort during the 2 days. These data are reported only for residents who could stand with a level of assistance of 3 or less (e.g., no physical lift).

Strength

Upper body strength assessments were completed for both intervention and control residents on 2 separate trials on different days than the endurance assessments. A 1-repetition maximum lift was used to evaluate strength and took place at baseline and at 8 and 32 weeks postbaseline. Residents were positioned at 45° in bed and asked to complete an arm raise or arm curl with each arm. The arm raising and arm curl exercises were conducted on separate days. No weight was used initially in order to determine each resident's full range of motion. Residents were then asked to repeat this motion using a 1-lb handheld weight. The weight was gradually increased by 1-lb increments until the resident could no longer complete his or her full range of motion. Residents attempted each exercise using alternating arms in order to allow for a rest between each lift.

Lymphocyte Enumeration and Characterization

White blood cell and differential counts were performed by standard procedures using a Coulter model MD-16 (Coulter Diagnostics, Hialeah, FL) counter. Cell staining and cytofluorometric analyses were carried out on whole peripheral blood collected in EDTA anticoagulant. Triple combinations of monoclonal antibodies were used. The flow cytometric procedures employed in our laboratory have been described previously (23,24), and the reagents used are outlined in Table 1.

Lymphocyte Proliferation Assay

For the lymphocyte proliferation assay, peripheral blood mononuclear cells (PBMCs) from heparinized whole blood were separated by density gradient centrifugation on ficoll hypaque. The PBMCs (10^5 /well) were incubated in RPMI media with 10% human AB serum for 3 days at 37°C in triplicate wells with 10 or 1 µg/ml doses of PHA. Tritiated thymidine [3 H] TdR was added on day 3 (1 µCi/well), and cells were harvested on the fourth day. [3 H] TdR incorporation was measured in a beta scintillation counter, and the mean cpm of the triplicate samples was recorded.

Cytokine and Soluble Activation Markers in Plasma

Plasma level of neopterin was measured by using an EIA kit (BRAHMS Diagnostics GMBH, Berlin, Germany). Plasma level of sTNF-RII were measured with an EIA kit (Hycult Biotechnology, Uden, The Netherlands) using standard procedures (25).

STATISTICAL ANALYSIS

For each of the groups, the unadjusted mean, median, and standard deviation (*SD*) for the heart rate and immune parameters were calculated at each time point of measurement (Baseline, 8, and 32 weeks; Figure 1 and Tables 3–5). There were not enough cells in certain subjects to run an analysis on all the immune parameters. Consequently, in the

Table 1. Monoclonal Antibody Combinations Used for Lymphocyte Phenotype Analyses by 3-Color Flow Cytometry

MoAb1 (FITC)*	MoAb2 (PE)*	MoAb3 (PerCP)*
Anti-CD3 (pan T cells)	Anti-CD56+16 (NK cells)	Anti-CD19 (B cells)
Anti-CD4 (T helper cells)	Anti-CD25 (IL-2 receptor α)	Anti-CD8
Anti-HLA-DR (activated cell)	Anti-CD8	Anti-CD3
Anti-CD28 (2nd receptor activation)	Anti-CD45RO (memory cell)	Anti-CD8
Anti-CD28	Anti-CD45RO	Anti-CD4
Anti-CD28	Anti-CD25	Anti-CD3

*Fluorochrome label.

tables describing the results, we have reported the number of subjects on whom we could perform the analysis of immune parameters. Prior to statistical analysis, normality of distribution and homogeneity of variance were tested for each dependent variable within each group using the Shapiro-Wilk's test and the Bartlett's test, respectively. The data for comparison of the heart rate and immune parameters were normal and homogeneous; therefore, a 2-way mixed-model, repeated measures analysis of variance (ANOVA) was used (SAS, 2001, SAS Version 8.2, SAS Institute, Inc., Cary, NC). The factors used were the exercise condition (control and intervention groups) and time of measurement (baseline, 8 weeks, and 32 weeks); time of measurement was the repeated measure. Tukey's Honestly Significant Difference test was performed on least-squares-adjusted means, as indicated, for significant ANOVA findings. All statistical analyses were 2-tailed, and a criterion significance level (α) of $p < .05$ was used.

RESULTS

Six hundred and thirty-three residents occupied long-stay beds in the 4 participating NHs. Of these residents, 452 (69%) were incontinent, and 330 (73%) met inclusion criteria. Informed consent was obtained for 257 (79%) of these 330 residents. Baseline assessments were successfully completed on 190 (81%) of the 233 consented residents. Reasons for incomplete assessments included resident transfers, death, or refusal to cooperate and withdrawal of consent. The 190 residents were then randomized into an intervention group (94 residents) and a control group (96 residents). A total of 172 (91%) of the residents (85 intervention and 87 control) completed the 8-week (Post-1) assessments, and 148 (77%) of the residents (74 intervention and 74 control) completed the 32-week (Post-2) assessments. Attrition after the baseline assessments was primarily the result of death (28 residents) or a prolonged illness that prevented the resident from participating in any component of the 2-day functionally oriented exercise program outcome assessments (10 residents). There were no differences between intervention and control residents in terms of mortality (14 residents per group died postbaseline). Table 2 presents descriptive data for the intervention and control residents on the variables most relevant to this study. There were no significant differences between groups in terms of any of these descriptive variables.

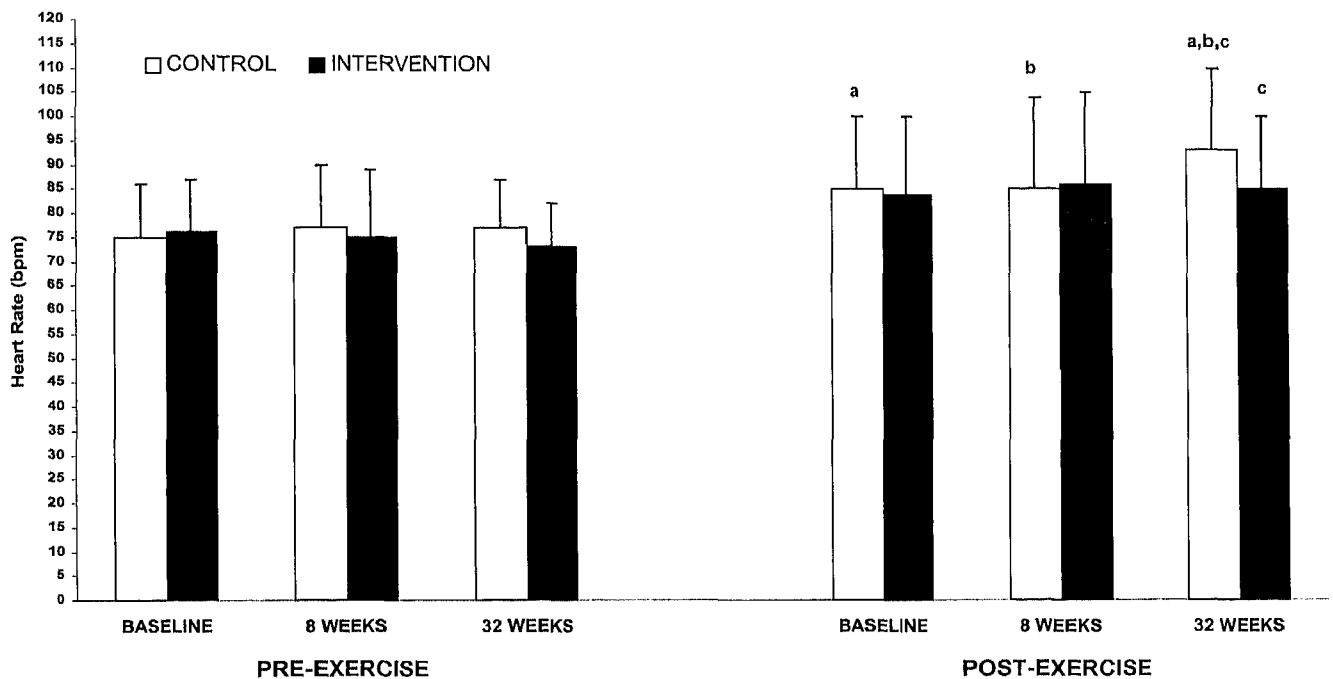


Figure 1. Heart rates (unadjusted mean \pm SD) pre- and postexercise at baseline, 8 weeks, and 32 weeks in control and intervention groups. Bars sharing lowercase letters are different ($p < .05$) according to repeated measures analysis of variance followed by Tukey's Honestly Significant Difference test.

Physical Performance Measurements

As reported elsewhere (8), there was a large increase in intervention residents' tolerance for exercise with more than a 100% increase in distance walked or wheeled from week 1 to week 32 during daily functionally oriented exercise sessions. For intervention residents, the average distance that residents walked or wheeled during daily sessions (not during the 2-day Post-1 or Post-2 assessments) increased from 135.4 m/week in week 1 to 295 m/week in week 32. This increase in exercise tolerance was independently documented by both the motion sensor and behavioral observational measures of activity. Intervention and control residents were both observed walking or standing on 3% of the observations in baseline and 6% or 3% of the time, respectively, during days when the intervention was being

implemented. The motion sensor data indicated that the estimated K-CALS per hour for the intervention and control residents measured 1.8 and 2.1 in baseline and 4.8 and 1.2, respectively, during the days the intervention was being implemented. Both measures of physical activity showed significant group \times time differences between intervention and control groups over the 32 weeks of the intervention ($F = 6.3$ for behavioral observation measures and 13.9 for motion sensor data respectively; $p < .001$ for both measures).

As reported elsewhere (8), significant group \times time interactions between intervention and control groups were observed on most functional measures at 32 weeks (8). These included upper body strength, physical activity, and mobility endurance. In all cases, the intervention group performed significantly better on these measures than did the control group. The significant differences between groups in mobility endurance occurred because the intervention group maintained endurance while the control group showed a significant decline.

Heart Rate Measurements

Significant group \times time interaction effects ($p = .0357$) and main effect of time ($p = .019$) were seen for the postexercise heart rate. Post hoc analyses showed that the postexercise heart rate at baseline was not different between the control and intervention groups but increased significantly over 32 weeks in the control group compared to the intervention group, which suggests a decline in cardiac function in the control group when compared with the exercise group after exposure to an exercise intervention (Figure 1). The increase in the postexercise heart rate in the control group when compared to intervention group at 32 weeks is all the more

Table 2. Resident Characteristics

	Intervention ($N = 94$)	Control ($N = 96$)
Age (years)	87 (8)	88 (7)
Length of Residency (months)	26 (31)	29 (32)
Mini-Mental State Exam*	12 (8)	14 (7)
Female (%)	81	86
Ambulatory (%)	60	63
Cumulative Illness Rating Scale (CIRS) (total score)**	19.9 (6.3)	19.8 (5.2)
Number of diagnoses	9.3 (4.1)	9.8 (5.4)
Medications prescribed		
Total number (routine plus prn)	10.1 (4.8)	9.1 (4.4)
Routine	7.1 (3.6)	6.6 (3.9)

*Possible scoring range from 0 to 30.

**CIRS total score is the sum of severity scores (ranging from 0 = no problem to 4 = extremely severe) for 14 organ system categories.

Numbers in parentheses () are standard deviations.

Table 3. Lymphocyte Subset Levels in Control and Intervention Group of Frail Elderly (unadjusted mean \pm SD)

	Control Group (n = 63-83)			Intervention Group (n = 66-81)			p Value Group \times Time
	Baseline	8 weeks	32 weeks	Baseline	8 weeks	32 weeks	
Lymphocytes (cells/ μ l)	2097 \pm 711	1985 \pm 629	2051 \pm 732	2042 \pm 629	2152 \pm 796	1982 \pm 737	.011*
CD3+ (cells/ μ l)	1582 \pm 565	1478 \pm 565	1605 \pm 632	1553 \pm 555	1615 \pm 655	1507 \pm 637	.009*
CD4+ (cells/ μ l)	1004 \pm 402	938 \pm 410	986 \pm 421	964 \pm 380	1019 \pm 455	961 \pm 449	.027*
CD8+ (cells/ μ l)	578 \pm 311	548 \pm 300	627 \pm 387	595 \pm 367	623 \pm 360	555 \pm 355	.003*
CD56 & 16+ (cells/ μ l)	240 \pm 139	299 \pm 197	239 \pm 150	274 \pm 145	336 \pm 232	268 \pm 154	.549
CD19+ (cells/ μ l)	163 \pm 112	161 \pm 123	146 \pm 96	157 \pm 101	165 \pm 113	153 \pm 103	.384
% Lymphocytes	31 \pm 9	30 \pm 9	31 \pm 9	30 \pm 8	31 \pm 8	30 \pm 10	.042*
CD3% T cells	76 \pm 8	74 \pm 11	77 \pm 8	75 \pm 8	74 \pm 8	75 \pm 9	.147
CD4% T cells	48 \pm 10	47 \pm 11	48 \pm 11	47 \pm 11	47 \pm 10	48 \pm 11	.208
CD8% T cells	28 \pm 12	28 \pm 12	30 \pm 12	28 \pm 12	29 \pm 12	27 \pm 12	.188
CD56+16% NK cells	12 \pm 7	16 \pm 9	12 \pm 6	14 \pm 6	16 \pm 7	14 \pm 7	.492
CD19% B cells	8 \pm 5	8 \pm 5	7 \pm 4	8 \pm 5	8 \pm 4	8 \pm 6	.810
Ratio CD4:CD8	2.32 \pm 1.9	2.20 \pm 1.6	2.17 \pm 1.8	2.11 \pm 1.3	2.04 \pm 1.1	2.18 \pm 1.2	.312

* $p \leq .05$.

significant because the exercise capacity of the control group had declined significantly in comparison to the exercise capacity of the intervention group by 32 weeks, and therefore, the control group exercised for a shorter duration when compared to the intervention group during the assessment trial session (8).

Immune Measurements

Previously we have reported the changes that take place in several immune parameters in a group of frail elderly subjects ($n = 116$, age 70-103 years, median 86 years) when compared to a healthy group of young subjects ($n = 21$, age 22-49 years, median 38 years) (14). Here we report the effects of an exercise intervention on immune parameters of the frail elderly.

Lymphocyte subpopulations.—Significant group \times time interaction effects were seen in the numbers and percentage of lymphocytes with no significant main effects for group or time (Table 3). Post-hoc analyses showed a slight decrease of 8% in numbers and 3% in percentage of lymphocytes in the intervention group from week 8 to week 32 of measurement.

T lymphocytes.—Although no significant group \times time interaction effects were seen in the percentages of T cells and their subpopulations, significant group \times time interaction effects were seen for the numbers of total T cells (CD3+), Helper T cells (CD4+), and Cytotoxic/Suppressor T cells (CD8+) (Table 3). The number of T cells and their subpopulations did not show significant main effects for group or time, and post-hoc analyses showed a slight decrease (between 6 and 11%) in the numbers of all T cells (CD3+, CD4+, and CD8+) in the intervention group from week 8 to week 32 of measurement. Additionally, the number of CD8+ T cells also showed a slight increase of 5% from baseline to week 8 of measurement, with no difference between baseline and week 32 of measurement in the intervention group.

The changes in the numbers/percentages of lymphocytes and T cells seen in the intervention group between different time points are well within ranges previously reported for

another group of frail elderly (14) and were not different from the control group at any time point of measurement.

Finally, exercise had no impact on the CD4:CD8 ratio. The group \times time interaction effect and the main effect of group was not significant (Table 3); however, the main effect of time was significant ($p = .0276$) and was attributable to a 5% decrease in the ratio from baseline to week 8 in the control group.

B lymphocytes.—No significant main or interaction effects were seen for group or time in the numbers and percentage of B cells (CD19+) (Table 3). Thus, significant differences seen in the numbers and percentage of total lymphocytes were mainly the result of changes in the T cell compartment.

NK cells.—Exercise has a significant effect on NK cell numbers and function (13). Immediately following acute bouts of maximal physical exertion, healthy elderly subjects (age 71 \pm 1 years) showed increased numbers and activity of NK cells (26). However, following endurance training of 10 or 12 weeks, elderly subjects have shown a decrease (22) or no change (19), respectively, in NK cell numbers at rest. Following an exercise intervention of 32 weeks, we observed only the main effect of time was significant for numbers ($p < .0001$) and percentage ($p < .0001$) of NK cells (CD56+ and/or CD16+/CD3-), with no significant main effect of group and interaction effect of group \times time (Table 3). The numbers and percentage of NK cells were higher at week 8 compared to levels observed at baseline and week 32, with no difference in NK cell levels between baseline and week 32 for both the control and intervention groups. NK cells increased in numbers by 23-25% and in percentage by 14-33% from baseline to week 8 and decreased in numbers by 20% and in percentage by 13-25% from week 8 to week 32 in both groups.

Memory T cells.—The group \times time interaction effect and the main effect of group was not significant (Table 4); however, the main effect of time was significant ($p < .0001$) for memory CD4 T cells (CD4+CD45RO+ T cells). Memory CD4 T cells showed a 3-4% increase from week 8 to week 32 for both groups. Memory CD8 T cells (CD8+CD45RO+

Table 4. Changes in Phenotypic Parameters in Control and Intervention Group of Frail Elderly (unadjusted mean \pm SD)

	Control Group (n = 41-82)			Intervention Group (n = 41-79)			p Value Group \times Time
	Baseline	8 weeks	32 weeks	Baseline	8 weeks	32 weeks	
%CD45RO in CD4	81 \pm 15	79 \pm 12	82 \pm 10	81 \pm 15	81 \pm 11	83 \pm 9	.549
%CD45RO in CD8	58 \pm 17	58 \pm 15	60 \pm 15	59 \pm 16	56 \pm 17	59 \pm 16	.431
%CD28 in CD3	74 \pm 14	73 \pm 16	73 \pm 15	75 \pm 15	76 \pm 15	76 \pm 14	.333
%CD28 in CD4	90 \pm 10	91 \pm 11	91 \pm 9	90 \pm 13	91 \pm 8	90 \pm 8	.321
%CD28 in CD8	37 \pm 12	38 \pm 15	38 \pm 16	39 \pm 15	38 \pm 15	40 \pm 15	.431
%CD25 in CD3	36 \pm 16	38 \pm 16	36 \pm 14	35 \pm 17	36 \pm 15	35 \pm 13	.886
%CD25 in CD4	36 \pm 21	35 \pm 20	28 \pm 17	34 \pm 20	37 \pm 20	30 \pm 16	.211
%CD25 in CD8	15 \pm 18	11 \pm 12	7 \pm 6	10 \pm 8	9 \pm 11	8 \pm 8	.144
%HLA-DR in CD3	21 \pm 14	23 \pm 15	21 \pm 13	20 \pm 12	23 \pm 13	22 \pm 12	.286
%HLA-DR in CD8	33 \pm 18	31 \pm 16	32 \pm 16	30 \pm 17	33 \pm 17	39 \pm 16	.059

T cells) showed no main effects for group or time or interaction effect between group and time (Table 4).

CD28 expression on T cells.—The CD28 glycoprotein is a costimulatory molecule that provides the second signal for proliferative stimulation, and CD8+ T cells show a marked reduction in CD28 expression with aging (27). Increased percentages of CD28- T cells (CD4+ and CD8+) in peripheral blood following acute bouts of exercise have been demonstrated in both young and elderly humans, suggesting recruitment of previously activated cells (28). We observed no significant main or interaction effects for group or time in the percentage of CD3+, CD4+, and CD8+ T cells expressing CD28 (Table 4), suggesting that exercise training, in contrast to acute exercise, does not increase the recruitment of CD28- T cells in circulation in this population.

CD25 expression on T cells.—CD25 is an α -chain receptor for a key cytokine, IL-2, involved in T-cell proliferation. CD25 expression decreases on T cells with aging (14). No significant main or interaction effects were seen for group or time in the percentage of CD3+ T cells expressing CD25 (Table 4). However, the group \times time interaction effect and the main effect of group was not significant (Table 4), but the main effect of time was significant ($p < .0001$) for CD4+ T cells ($p < .0001$) and CD8+ T cells ($p = .019$) expressing CD25. CD25 expression decreased on CD4+ T cells by 22% in the control group and by 12% in the intervention group from baseline to week 32, whereas CD25 expression decreased on CD8+ T cells by 53% in the control group only during the same time period (baseline to week 32). Again, no differences between control and intervention groups were seen in the expression of CD25 on CD4+ or CD8+ T cells.

HLA-DR expression on T cells.—HLA-DR is an essential component of the Class II major histocompatibility molecules and is expressed on activated T cells and constitutively on B cells and many other tissues. HLA-DR expression on T cells is characteristically increased with immune activation and chronic viral infections and is increased on T cells with aging (14). No significant main or interaction effects were observed for group or time in the percentage of CD3+ and CD8+ T cells expressing HLA-DR (Table 4).

Proliferative function.—Functional changes in immune cells were measured by proliferative response following stimulation with 10 (high dose) and 1 μ g/ml (low dose) of phytohemagglutinin (PHA) (Table 5). Proliferative response to PHA in PBMCs was not significant for the main effects of group or interaction effect of group \times time. However, they were significant for the main effects of time for the high dose ($p < .0001$) and low dose ($p < .0001$) of PHA. At 10 μ g/ml dose of PHA, both the control and intervention groups showed a 16–19% increase in proliferation of PBMCs by week 8 from baseline and an increase of 21–25% in proliferation by week 32 from the baseline. In contrast to the high dose, at 1 μ g/ml dose, both the control and intervention groups showed a 29–32% decrease in proliferation of PBMCs by week 8 from baseline and a decrease of 24–32% in proliferation by week 32 from the baseline.

Soluble products of immune activation.—Immune activation throughout all lymphoid tissues of the body is reflected in plasma levels of soluble products of cytokine activity. Neopterin reflects interferon- γ activity and sTNF-RII reflects TNF α activity (14,25). No significant main or interaction effects were seen for group or time in plasma neopterin (Table 5). Plasma sTNF-RII levels were not significant for the main effects of group or interaction effect of group \times time; however, they were significant for the main effects of time. Plasma sTNF-RII levels in the intervention group showed a 19% increase by week 32 from baseline. The control group did not show significant differences in plasma sTNF-RII levels by week 8 or 32 from baseline.

DISCUSSION

We are aware of 8 prospective studies of exercise (aerobic or resistance training) and immune response in the elderly (15–22). In 3 studies (15–17), a resistance training intervention lasting from 8 to 12 weeks in a group of healthy elderly (between 65–84 years of age) showed no beneficial or detrimental effect on various measures of immune function (lymphocytic counts, proliferation, etc.) The remaining 5 studies (18–22), which investigated the effects of endurance training (between 10–24 weeks) at moderate intensity in elderly subjects (between 65–87 years of age) on immune function, also reported no substantial beneficial or detrimental effects. The current study differs from all previous studies in several important aspects. This is the

Table 5. Functional Parameters and Immune Activation Markers in Plasma in Control and Intervention Group of Frail Elderly (unadjusted mean \pm SD)

	Control Group (<i>n</i> = 64-84)			Intervention Group (<i>n</i> = 66-82)			<i>p</i> Value Group \times Time
	Baseline	8 weeks	32 weeks	Baseline	8 weeks	32 weeks	
Proliferation ($\times 10^3$ cpm)							
PHA 10 μ g/ml	92 \pm 39	109 \pm 50	115 \pm 66	92 \pm 37	107 \pm 47	111 \pm 45	.787
PHA 1 μ g/ml	34 \pm 24	23 \pm 17	26 \pm 22	34 \pm 20	24 \pm 26	23 \pm 19	.796
PHA 0 μ g/ml	1.6 \pm 0.9	1.4 \pm 1	1.4 \pm 1.1	1.9 \pm 1.3	1.2 \pm 0.9	1.7 \pm 1.2	.095
Plasma Products							
Neopterin nm/l	15.3 \pm 9	14.8 \pm 8	14.6 \pm 9	15.4 \pm 8	13.8 \pm 6	16.1 \pm 11	.186
sTNF-RII ng/ml	4.0 \pm 1.9	4.1 \pm 1.7	4.2 \pm 1.6	3.7 \pm 1.4	3.8 \pm 1.6	4.4 \pm 2.4	.084

first study that combines both resistance and endurance training in a group of frail NH residents, in contrast to the majority of studies that have utilized either resistance or endurance training, but not both, performed in a largely healthy elderly subject population. Secondly, a period of 32 weeks (8 months) is the longest exercise intervention to investigate the effects of exercise training on immune function. Thirdly, the incidence of infection in the subjects was monitored during the clinical trial by weekly medical record reviews and interviews of nursing staff by trained research nurses and physicians who were masked to group assignment (9). Of all the 8 previous studies (15-22), only 2 studies (19,22) monitored the incidence and severity of infection (upper respiratory tract infection only), and in both cases, a self-report was used to monitor infection rather than clinical diagnosis, which was accomplished in the current clinical trial (9). Finally, the number of subjects that participated in this study has been the largest for all of the prospective studies that have examined the effects of exercise intervention on immune function.

As has been shown in healthy elderly subjects (15-20,22), 32 weeks of exercise did not bring about beneficial or detrimental effects on immune parameters in frail elderly NH population. Subjects in intervention and control groups showed changes in some immune parameters between baseline and follow-up, changes that for the most part reflected a 3-25% difference. These may have been secondary to seasonal and psychosocial factors and individual variability rather than an effect of the exercise. Also, the immune parameters in the intervention and control groups of frail elderly during baseline and follow-up are comparable to active, healthy elderly who met the clinical criteria for Senieur status (29,30). Moreover, the minor changes in immune parameters, as reported elsewhere, was not associated with any impact on the incidence of infections in the exercise group (9). For example, the incidence rates (incidence per 1000 resident weeks of monitoring) (9) of pneumonia and acute bronchitis were 6 and 4, respectively, in the control group and 5 and 4, respectively, in the intervention group during the baseline period. During the follow-up period, the incidence rates of pneumonia and acute bronchitis were 9 and 6, respectively, in the control group and 4 and 5, respectively, in the intervention group (9).

Apart from exercise, immune function also is influenced by psychosocial (31) and nutritional (32,33) factors. Supportive personal relationships have a positive effect on immune function (31), and the lack of socialization from the

research staff in the control group in this trial could have negatively affected immune function. Alternatively, in addition to exercise, socialization with the research staff would be an additional factor in affecting immune function in the intervention group. The lack of any difference in immune measures or infection episodes between the 2 groups indicates that socialization was not a factor in influencing immune function in this study. Finally, we controlled for normal circadian changes in levels of immune cells by drawing blood at the same time of day from all subjects. Apart from circadian changes, seasonal variation exists in immune function, including lymphocyte proliferation (34). Short day lengths enhance several aspects of immune function in laboratory studies, and melatonin appears to mediate many of the enhanced immunological effects of photoperiod. In contrast to laboratory studies, field studies generally report compromised immune function during the short days of autumn and winter (34). Our 32-week intervention ended during the winter months of December to February and thus may have led to an underestimation of immune function for both the control and intervention groups.

With regard to the effects of nutrition on immune function, diets that are vitamin E supplemented (32) or calorically restricted (33) can improve several parameters of immune function that decline with aging. Both groups in our clinical trial were provided similar diets. In addition, when a subset of subjects in both the groups were compared, there was no change in the average total percent food and fluid intake during mealtime for either control or intervention group (Simmons SF, Smith T, Cooper CB, Alessi CA, Schnelle JF. Unpublished observations).

It is possible that our exercise intervention was not intense enough to change the immune parameters we measured. However, the 8-month intervention was delivered at sufficient intensity to produce significant improvements on mobility, physical activity, and strength. Moreover, subjects were offered 4 opportunities per day to exercise, and on average they completed 3.2 of them (9). The primary reason for lack of compliance was fatigue (9), which suggests that increasing the exercise intensity would be difficult in this frail elderly population. Based on fatigue, one could argue that the exercise intervention was intense and resulted in maintenance of cardiorespiratory fitness (as measured by heart rate immediately following the exercise intervention) in the intervention group, with a decline in the control group. Intense exercise causes suppression of immune

parameters in young subjects (13). However, based on this study and data from aged animals (35), intense exercise has no detrimental effect on immune function in the elderly. Thus, relatively intense exercise programs may be prescribed that could maximize cardiopulmonary and musculoskeletal function without impairing immune function in frail elderly NH residents.

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