

# Long-Term Physical Activity and Inflammatory Biomarkers in Older Adults

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<sup>1</sup>Section on Gerontology and Geriatric Medicine, J. Paul Sticht Center on Aging, Department of Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, NC; <sup>2</sup>Department of Biostatistical Sciences, Wake Forest University School of Medicine, Winston-Salem, NC; <sup>3</sup>Preventive Medicine Research Laboratory, Pennington Biomedical Research Center, Baton Rouge, LA; <sup>4</sup>Department of Medicine, University of Pittsburgh, Pittsburgh, PA; and <sup>5</sup>Department of Aging and Geriatric Research, University of Florida, Gainesville, FL

## ABSTRACT

BEAVERS, K. M., F.-C. HSU, S. ISOM, S. B. KRITCHEVSKY, T. CHURCH, B. GOODPASTER, M. PAHOR, and B. J. NICKLAS. Long-Term Physical Activity and Inflammatory Biomarkers in Older Adults. *Med. Sci. Sports Exerc.*, Vol. 42, No. 12, pp. 2189–2196, 2010. **Purpose:** The purpose of this study was to determine the effects of a 12-month physical activity (PA) intervention on inflammatory biomarkers in elderly men and women. **Methods:** Four hundred and twenty-four elderly (age = 70–89 yr), nondisabled, community-dwelling men and women at risk for physical disability were enrolled in a multicenter, single-blind, randomized controlled trial. Participants were randomized to participate in either a 12-month moderate-intensity PA intervention or a successful aging health education intervention. Biomarkers of inflammation (interleukin (IL)-6sR, IL-1sRII, soluble tumor necrosis factor receptors 1 and 2 (sTNFRI, sTNFRII), IL-8, IL-15, adiponectin, IL-1ra, IL-2sR $\alpha$ , and TNF $\alpha$ ) were measured at baseline, at 6 months, and at 12 months. **Results:** A baseline blood sample was successfully collected from 368 participants. After adjustment for gender, clinic site, diabetes status, and baseline outcome measure, IL-8 was the only inflammatory biomarker affected by the PA intervention ( $P = 0.03$ ). The adjusted mean IL-8 at month 12 was 9.9% ( $0.87 \text{ pg}\cdot\text{mL}^{-1}$ ) lower in the PA compared with the successful aging group. Secondary interaction analyses between baseline biomarker status and treatment showed one significant interaction ( $P = 0.02$ ) such that the PA intervention reduced IL-15 concentrations in participants with a baseline IL-15 above the median value of  $1.67 \text{ pg}\cdot\text{mL}^{-1}$ . However, these associations were no longer significant after consideration for multiple comparisons. **Conclusions:** Overall, this study does not provide definitive evidence for an effect of regular exercise for altering systemic concentrations of the measured inflammatory biomarkers in older adults. **Key Words:** EXERCISE, AGING, INFLAMMATION, CYTOKINES, SOLUBLE RECEPTORS

Inflammation, the body's complex biological reaction to damaging stimuli, is a necessary response of the adaptive immune system. This response is typically acute, resulting in increases in proinflammatory cytokines and acute phase proteins that are rapidly released into the circulation. However, a prolonged inflammatory state has detrimental health effects and predisposes to a wide variety of chronic diseases, especially those that are more prevalent with advanced age, such as cardiovascular disease and diabetes (19). Chronic inflammation is also a strong predictor of both disability and mortality in the elderly—even in the absence of clinical disease (16,30). Moreover, higher con-

centrations of proinflammatory cytokines and acute phase reactants are often seen in older adults compared with middle-aged or younger adults (10,34). This observation, coupled with the disproportionate adverse consequences of a prolonged inflammatory state in the elderly, points to the inflammation pathway as a potential target for interventions to reduce aging-related disease and disability. However, at present, there are no known definitive therapies for treating chronic inflammation in the elderly.

Although anti-inflammatory medications will reduce inflammation acutely, their clinical application for the ongoing treatment of chronic inflammation is limited, indicating the need to identify nonpharmacologic treatments. Notably, regular physical activity (PA) is associated with lower risk of aging-related chronic disease and disability, even in those who begin to exercise later in life (31). However, the mechanism responsible for this protective adaptation is not completely understood. Numerous studies report the beneficial effects of habitual PA on more traditional disease risk factors such as hypertension, insulin insensitivity, altered lipid profiles, and obesity (29), but less is known about its effect on inflammation. Since a single bout of exercise induces an inflammatory response that is similar to that

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induced by infection or trauma (28), it is questionable whether long-term PA may be effective for reducing chronic inflammation, especially in the elderly.

Data from numerous smaller studies suggest that regular PA has the potential to reduce circulating levels of several inflammatory biomarkers (24). Yet to date, there are very limited data from randomized controlled trials to definitively conclude that long-term regular exercise training reduces chronic inflammation. We previously showed, in elderly men and women, that 12 months of moderate-intensity PA lowers systemic concentrations of interleukin-6 (IL-6) but not C-reactive protein (CRP) relative to a nonexercise control intervention (25). However, because of the complexity of the immune system and the interrelatedness of inflammatory biomarkers (23), it is unlikely that a single biomarker reflects all health risk. Therefore, the purpose of this study was to expand our previous research by determining whether this 12-month exercise intervention would affect multiple biomarkers of inflammation. Specifically, we chose to focus on adiponectin (a protein with anti-inflammatory properties) and on the cytokines tumor necrosis factor alpha (TNF- $\alpha$ ), IL-8, and IL-15 as well as various cytokine receptors (IL-6sR, IL-1ra, IL-1sRII, sTNFRI, sTNFRII, and IL-2sR $\alpha$ ), which may be more representative of the inflammatory response as a whole because of the short half-life of many cytokines (1).

## METHODS

### Study Design

This study was conducted as an ancillary study to the Lifestyle Interventions and Independence for Elders Pilot (LIFE-P) trial, a four-site, single-blind, randomized controlled clinical trial comparing a 12-month PA intervention with a successful aging (SA) intervention in 424 elderly, nondisabled, community-dwelling men and women at risk for physical disability. The study design and the main findings on physical function of the LIFE-P study (27,33) as well as IL-6 and CRP outcome data (primary inflammatory biomarkers of interest) (25) are published. The local institutional review boards at the clinical sites (Wake Forest University, Cooper Institute, University of Pittsburgh, and Stanford University) approved the study, and all study participants gave written informed consent to participate. The guidelines of the Consolidated Standards of Reporting Trials were followed.

### Study Participants

Detailed randomization, inclusion and exclusion criteria, and a flow diagram of specific numbers of individuals screened and reasons for exclusion are published (27,33). Briefly, the major inclusion criteria were age of 70–89 yr, low functional performance on the basis of a Short Physical Performance Battery (SPPB) score of less than 10 (on a scale of 0 (worst) to 12 (best)), sedentary lifestyle, ability to

complete a 400-m walk test within 15 min without sitting and without using an assistive device, completion of a behavioral run-in that required tracking and logging of healthy behaviors, and willingness to be randomized to either treatment group. The major exclusion criteria were living in a nursing home, self-reported inability to walk 1 mile, significant cognitive impairment (Mini-Mental State Examination score < 21), severe hearing or visual impairment, and severe cardiac, pulmonary, neurological, orthopedic, renal, or psychiatric disease.

## Interventions

Interventions have been described in detail elsewhere (33). Briefly, the PA intervention consisted of a combination of aerobic, strength, balance, and flexibility exercises and was divided into three phases. For the first 2 months (adoption phase), three center-based exercise sessions (40–60 min) per week were conducted in a supervised setting. During the next 4 months (transition phase), the number of center-based sessions was reduced (two times per week), and home-based exercises (three times per week or more) were started. The subsequent maintenance phase (week 25 to trial end) consisted of the home-based intervention, optional one to two times per week center-based sessions, and monthly telephone contacts.

In addition, the PA intervention included group-based behavioral counseling sessions (once a week for the first 10 wk) that focused on PA participation. The intervention focused on walking as the primary mode of exercise, and the goal was to engage in walking for at least 150 min·wk<sup>-1</sup>. A brief warm-up preceded each session, and a brief cool-down period followed. Participants also completed lower extremity strengthening exercises followed by lower extremity stretching exercises. The intensity of training was gradually increased over the first 2–3 wk. Perceived exertion was quantified using the Borg scale and used to regulate the intensity of exercise. Participants were asked to walk at a target intensity of 12–13 (somewhat hard), and they were discouraged from exercising at levels of 15 or higher (hard) or 11 or less (fairly light). Strengthening exercises were performed at a perceived exertion of 15–16.

An SA health education intervention was used as the active control. Participants met in groups weekly for the first 26 wk and monthly for the remaining weeks. Sessions included health topics relevant to older adults such as nutrition, medications, foot care, and recommended preventive health care. At the end of each session, a short instructor-led intervention (5–10 min) of gentle upper extremity stretching was delivered. Phone calls were made after each missed session to encourage regular participation, and participants received a monthly newsletter.

## Measurements

Participants were enrolled between April 2004 and February 2005. Baseline assessments included personal interview, anthropometric measures, physical examination,

electrocardiogram, and physician evaluation. Follow-up visits in the clinic occurred at 6 and 12 months. Prevalence of clinical conditions was determined using self-reported physician-diagnosed disease information.

**Short Physical Performance Battery.** A global measure of physical function was performed to characterize functional status of the participants. The SPPB is based on a timed short-distance walk, repeated chair stands, and balance test (15). Each of the performance measures is assigned a score ranging from 0 to 4, with 4 indicating the highest level of performance and 0 indicating the inability to complete the test. The categories computed for walking speed and chair stands are derived from cut points on the basis of quartiles of time to perform each task assessed in the Established Populations for Epidemiologic Study of the Elderly. A summary score ranging from 0 (worst) to 12 (best) is calculated by summing all scores.

**Inflammatory biomarkers.** All blood samples were collected from the LIFE study participants in the early morning (between 7 and 9 a.m.) after a 12-h fast at the baseline and at the 6- and 12-month assessment visits. The 6- and 12-month blood samples were collected at least 24 h after the last acute bout of exercise, and blood sampling was postponed (1–2 wk after recovery of all symptoms) in the event of an acute respiratory, urinary tract, or other infection. All blood was collected, processed, divided into aliquots, and stored locally at  $-80^{\circ}\text{C}$  until shipment to the Biological Specimen Repository at Wake Forest University, where samples were placed for long-term storage at  $-80^{\circ}\text{C}$  until later analysis.

All of the 424 randomized participants consented to the baseline blood draw, and a sufficient blood sample was successfully collected from 368 (87%) participants. Blood samples were available from 345 participants (81% of randomized participants) at the 6-month follow-up and from 334 participants (79%) at the 12-month follow-up. Of the 79 participants with missing 6-month data, 2 had died, 22 withdrew consent or dropped from the study, and 55 did not have a blood sample drawn because of technical difficulties. Of the 90 participants with missing 12-month data, 4 had died, 25 withdrew consent or dropped from the study, and 61 did not have a blood sample drawn because of technical difficulties.

All biomarker assays were run using Quantikine enzyme-linked immunosorbent kits from R&D systems (Minneapolis, MN), with the exception of IL-8 and IL-15, which were run using the QuantiGlo chemiluminescent enzyme-linked immunosorbent assay kits from R&D systems, and CRP, which was determined using an automated immunoanalyzer (IMMULITE; Diagnostics Products Corporation, Los Angeles, CA). Sensitivities, detection and reference ranges, and inter- and intra-assay coefficients of variation for each biomarker assessed are presented in Table 1. All samples were measured in duplicate, and the average of the two values was used for data analyses. Duplicate samples that did not provide a coefficient of variation of less than 30% for TNF- $\alpha$ , 25% for sTNFR1, sTNFR2, IL-2sR $\alpha$ , IL-6sR, IL-1sR1, IL-8, and IL-15, and 20% for adiponectin, IL-6, and CRP were reanalyzed. All values were averaged for data analyses.

### Statistical Analysis

All statistical analyses were performed using SAS software, version 9.2 (SAS Institute, Inc., Cary, NC), and a probability level of  $<0.05$  was adopted throughout. Sample mean and SD values were computed for the continuous descriptive characteristics, and count and proportions were calculated for the discrete descriptive characteristics according to intervention groups. Estimation of missing data was done using maximum likelihood techniques (8). Mean values between groups were compared using independent *t*-tests to assess the balance achieved via randomization for the continuous descriptive characteristics (after transformation to approximate the normality assumption if necessary) and using chi-squared tests for the discrete descriptive characteristics. Relationships among all baseline inflammatory biomarkers were assessed using Spearman correlation coefficients.

For the primary statistical analysis, to minimize the heterogeneity of variance and to best approximate the conditional normality assumption, sTNFR1, sTNFR2, IL-8, IL-15, adiponectin, IL-1ra, IL-2sR $\alpha$ , and TNF- $\alpha$  were log-transformed. IL-6sR and IL-1sR1 were normally distributed and therefore not transformed. CPR and IL-6 data were previously reported (24); in addition, although presented in the

TABLE 1. Relevant assay details for each biomarker.

Biomarker	Sensitivity	Standard Range	Interassay Variability (%)	Intra-assay Variability (%)	Reference Range <sup>a</sup>
CRP (mg·L <sup>-1</sup> )	0.1	0–250	6.7	3.5	1.4–11.0
IL-6 (pg·mL <sup>-1</sup> )	0.1	0–10	9.8	3.0	3.12–12.5
IL-6sR (pg·mL <sup>-1</sup> )	6.5	31.2–2000	12.6	2.4	17,000–46,000
IL-1sR1 (pg·mL <sup>-1</sup> )	<10	31.2–2000	14.8	5.5	6000–18,000
sTNFR1 (pg·mL <sup>-1</sup> )	<3.0	7.8–500	11.7	3.0	484–1407
sTNFR2 (pg·mL <sup>-1</sup> )	0.6	7.8–500	8.4	5.3	829–2262
IL-8 (pg·mL <sup>-1</sup> )	0.03	1.6–5000	12.1	4.2	1.75–7.74
IL-15 (pg·mL <sup>-1</sup> )	0.11	1.03–750	10.9	5.1	0.98–3.23
Adiponectin ( $\mu\text{g}\cdot\text{mL}^{-1}$ )	0.25	3.9–250	13.9	7.1	1.20–19.97
IL-1ra (pg·mL <sup>-1</sup> )	6.26	31.2–2000	10.4	2.6	105–1062
IL-2sR $\alpha$ (pg·mL <sup>-1</sup> )	<10	78–5000	9.6	2.7	410–2623
TNF- $\alpha$ (pg·mL <sup>-1</sup> )	0.11	0.5–32	15.6	5.2	0–2.14

All visits from one sample were run on the same plate.

<sup>a</sup> Values obtained from manufacturer kit insert,  $n < 100$  per biomarker.

TABLE 2. Baseline descriptive characteristics according to treatment group.

Characteristics	PA (n = 182)	SA (n = 186)
Age (yr)	76.4 ± 4.1	77.0 ± 4.4
Female, n (%)	126 (69.2)	125 (67.2)
White, n (%)	140 (76.9)	141 (75.8)
BMI (kg·m <sup>-2</sup> )	30.7 ± 6.0	29.8 ± 5.5
Smoking, n (%)		
Never	147 (80.8)	157 (84.4)
Former	28 (15.4)	25 (13.4)
Current	7 (3.9)	4 (2.15)
Mini-Mental State Examination score	27.0 ± 2.3	27.5 ± 2.1
Prevalent comorbidities, n (%)		
Hypertension	126 (69.6)	129 (69.4)
Diabetes mellitus	52 (28.6)	32 (17.2)*
Cancer	28 (15.4)	32 (17.2)
Myocardial infarction	21 (11.6)	12 (6.5)
Stroke	8 (4.4)	12 (6.5)
Congestive heart failure	11 (6.1)	12 (6.5)
Chronic obstructive pulmonary disorder	26 (14.4)	27 (14.6)
SPPB score	7.60 ± 1.46	7.46 ± 1.41

Data are presented as mean ± SD or %.

\*  $P < 0.01$  between groups.

tables for completeness, these data were not included in the primary statistical analysis for this article. Raw values for each intervention group at each time point are reported as mean and SD. Differences in mean values of each biomarker between treatment groups were estimated using repeated-measures ANCOVA, with baseline outcome measure, sex (stratifying variable for randomization), clinic site, diabetes status, intervention assignment, visit, and intervention × visit interaction included in the model. Hypothesis tests for intervention effects at the 6- and 12-month assessment visits were performed using contrasts of the 6- and 12-month intervention means. Between-group differences in biomarker changes over time, adjusted for baseline value, are reported as least squares mean and 95% confidence interval (CI). Overall comparisons between groups across follow-up visits were obtained using a contrast to compare average effects across both follow-up visits. In addition, a Bonferroni correction method was used to correct for the problem of multiple comparisons. Lastly, interactions between baseline biomarker status (<median vs ≥median) and intervention group were also examined.

## RESULTS

**Baseline characteristics and relationships between inflammatory biomarkers.** Baseline characteristics of the study sample are shown in Table 2. The two treatment groups were similar concerning all baseline characteristics except prevalence of diabetes, which was greater in the PA group ( $P < 0.01$ ). In addition, no differences were noted between groups at baseline concerning any inflammatory biomarker (all  $P$  values  $> 0.40$ ). We examined the degree of interrelatedness between biomarkers, and these descriptive data are presented as Spearman correlation coefficients in Table 3. The strongest relationships were seen between the soluble receptors. Furthermore, all significant correlations between biomarkers were positive, except for the correlation between adiponectin and IL-1ra and the correlation between IL-15 and TNF- $\alpha$ , which were negative.

**Effects of PA intervention on inflammatory biomarkers.** Adherence to the PA and SA interventions was previously reported (27). Briefly, in the PA group, attendance during the adoption and transition phases averaged 71% and 61%, respectively. During the maintenance phase, participants engaged in an average of 3.7 walking sessions per week and walked an average of  $138 \pm 149$  min·wk<sup>-1</sup> (median =  $119$  min·wk<sup>-1</sup>, interquartile range =  $123$  min·wk<sup>-1</sup>). Attendance at the SA group sessions averaged 70% for weeks 1–26 and 73% for weeks 27–52. The estimated calories expended engaging in moderate PA were similar in the two groups at baseline ( $P = 0.98$ ) and significantly higher in the PA group during follow-up ( $P < 0.01$  at 6 and 12 months) (27). There were no changes in body weight as a result of either intervention. Likewise, in the subset of participants with measures of body composition (32), there were no changes in fat mass or lean mass in either group.

Table 4 shows the unadjusted mean and SD of the individual biomarkers for each time point × treatment group. The between-group differences in the 6- and 12-month changes from baseline (adjusted for baseline value) are also shown in Table 4 as least squares mean and 95% CI. IL-6, IL-8, and sTNFRI were the only biomarkers that decreased

TABLE 3. Spearman correlation analysis among inflammatory biomarkers.

	CRP	IL-6	IL-6sR	IL-1sRII	sTNFRI	sTNFRII	IL-8	IL-15	Adiponectin	IL-1ra	IL-2sR $\alpha$	TNF- $\alpha$
CRP	1	0.39***	0.04	0.05	0.17	0.17	-0.08	0.08	-0.09	0.16**	0.12*	0.00
IL-6		1	0.04	0.05	0.30***	0.25***	0.08	0.08	-0.03	0.21***	0.07	0.13*
IL-6sR			1	0.10*	0.20***	0.16**	0.07	-0.06	0.09	0.10	0.23***	0.02
IL-1sRII				1	-0.02	-0.04	0.04	-0.00	-0.01	0.03	0.00	0.12*
sTNFRI					1	0.73***	0.13*	0.17**	-0.03	0.26***	0.55***	0.16**
sTNFRII						1	0.15**	0.19***	0.00	0.29***	0.58***	0.21***
IL-8							1	-0.04	0.04	0.05	0.12*	0.14**
IL-15								1	-0.00	0.08	0.04	-0.12*
Adiponectin									1	-0.26***	0.12*	0.01
IL-1ra										1	0.13**	0.11*
IL-2sR $\alpha$											1	0.17**
TNF- $\alpha$												1

Spearman correlation coefficients ( $r$ ) presented.\*  $P < 0.05$ .\*\*  $P < 0.01$ .\*\*\*  $P < 0.001$ .



TABLE 4. Plasma cytokine concentrations according to treatment group at baseline, at 6 months, and at 12 months as well as the between-group difference in change.

Biomarker	Baseline (Mean $\pm$ SD)	6 Months (Mean $\pm$ SD)	12 Months (Mean $\pm$ SD)
CRP (mg·L <sup>-1</sup> )			
PA	5.63 $\pm$ 11.4	3.57 $\pm$ 4.15	4.23 $\pm$ 5.54
SA	4.38 $\pm$ 5.29	4.85 $\pm$ 8.96	4.08 $\pm$ 4.89
Between-group difference in change; least squares mean (95% CI)		1.70 (0.14 to 3.25)	0.00 (-1.18 to 1.17)
IL-6 (pg·mL <sup>-1</sup> )			
PA	3.38 $\pm$ 4.04	3.26 $\pm$ 3.59	2.98 $\pm$ 1.91*
SA	3.36 $\pm$ 4.01	3.75 $\pm$ 5.15	3.59 $\pm$ 4.65
Between-group difference in change; least squares mean (95% CI)		0.80 (-0.08 to 1.68)	0.81 (0.10 to 1.51)
IL-6sR (pg·mL <sup>-1</sup> )			
PA	36,849 $\pm$ 13,720	36,692 $\pm$ 15,446	35,371 $\pm$ 13,238
SA	36,772 $\pm$ 14,315	37,920 $\pm$ 14,180	36,338 $\pm$ 12,675
Between-group difference in change; least squares mean (95% CI)		585 (-1531 to 2701)	-138 (-2066 to 1789)
IL-1sRII (pg·mL <sup>-1</sup> )			
PA	10,262 $\pm$ 3580	10,334 $\pm$ 3429	9971 $\pm$ 3348
SA	10,231 $\pm$ 3482	9833 $\pm$ 2912	9812 $\pm$ 2936
Between-group difference in change; least squares mean (95% CI)		-605 (-1049, -160)	-361 (-854 to 132)
sTNFR1 (pg·mL <sup>-1</sup> )			
PA	1626 $\pm$ 1034	1644 $\pm$ 1069	1620 $\pm$ 903*
SA	1654 $\pm$ 1104	1639 $\pm$ 895	1752 $\pm$ 1256
Between-group difference in change; least squares mean (95% CI)		106 (21 to 191)	103 (-20 to 225)
sTNFR2 (pg·mL <sup>-1</sup> )			
PA	2990 $\pm$ 1045	3032 $\pm$ 1234	2950 $\pm$ 995
SA	3029 $\pm$ 1208	2979 $\pm$ 1044	3098 $\pm$ 1217
Between-group difference in change; least squares mean (95% CI)		-5 (-161 to 152)	141 (2 to 280)
IL-8 (pg·mL <sup>-1</sup> )			
PA	7.50 $\pm$ 4.73	7.96 $\pm$ 7.80	7.77 $\pm$ 4.67*
SA	7.73 $\pm$ 10.37	8.33 $\pm$ 13.44	8.81 $\pm$ 12.15
Between-group difference in change; least squares mean (95% CI)		0.32 (-0.61 to 1.26)	0.8 (0.06 to 1.55)
IL-15 (pg·mL <sup>-1</sup> )			
PA	1.77 $\pm$ 0.56	1.72 $\pm$ 0.40	1.72 $\pm$ 0.42
SA	1.76 $\pm$ 0.42	1.74 $\pm$ 0.45	1.80 $\pm$ 0.54
Between-group difference in change; least squares mean (95% CI)		0.05 (-0.02 to 0.11)	0.08 (0 to 0.16)
Adiponectin ( $\mu$ g·mL <sup>-1</sup> )			
PA	11.27 $\pm$ 7.77	12.32 $\pm$ 10.19	11.25 $\pm$ 8.03
SA	12.12 $\pm$ 8.93	12.45 $\pm$ 10.24	12.76 $\pm$ 10.21
Between-group difference in change; least squares mean (95% CI)		-0.23 (-1.57 to 1.10)	1.05 (-0.16 to 2.26)
IL-1ra (pg·mL <sup>-1</sup> )			
PA	366 $\pm$ 287	334 $\pm$ 204	359 $\pm$ 249
SA	349 $\pm$ 295	389 $\pm$ 425	350 $\pm$ 373
Between-group difference in change; least squares mean (95% CI)		66 (7 to 126)	7 (-46 to 60)
IL-2sR $\alpha$ (pg·mL <sup>-1</sup> )			
PA	1060 $\pm$ 456	1105 $\pm$ 509	1076 $\pm$ 501
SA	1088 $\pm$ 621	1084 $\pm$ 511	1110 $\pm$ 601
Between-group difference in change; least squares mean (95% CI)		23 (-35 to 81)	48 (-19 to 114)
TNF- $\alpha$ (pg·mL <sup>-1</sup> )			
PA	2.68 $\pm$ 4.26	2.60 $\pm$ 3.93	2.43 $\pm$ 3.54
SA	2.58 $\pm$ 3.35	2.62 $\pm$ 4.46	2.68 $\pm$ 4.26
Between-group difference in change; least squares mean (95% CI)		0.23 (-0.45 to 0.90)	0.31 (-0.26 to 0.89)

All biomarker group mean  $\pm$  SD values are unadjusted. Between-group differences in change are reported as change from baseline (month 6–baseline; month 12–baseline) and between groups (SA–PA) and are adjusted for the baseline value. The *n* for PA group  $\times$  time point: 182 at baseline, 173 at 6 months, and 172 at 12 months for all biomarkers except for IL-8 (*n* = 170) and IL-15 and adiponectin (*n* = 171) at 12 months. The *n* for SA group  $\times$  time point: 186 at baseline, 172 at 6 months, and 162 at 12 months for all biomarkers except for CRP at all time points (*n* = 185, 171, and 161, respectively), IL-6 (*n* = 171) at 6 months, and sTNFR1 (*n* = 161) and sTNFR2 (*n* = 160) at 12 months. All missing data were due to insufficient quantity of sample (*n* = 6) or values below the lower limit of detection (*n* = 4).

\* Unadjusted statistical significance (*P* < 0.05).

more at 12 months in the PA versus SA group. Linear regression modeling, adjusting for baseline outcome measure, sex, clinical site, diabetes mellitus, intervention assignment, visit, and intervention  $\times$  visit interaction, showed that the PA intervention resulted in significantly lower levels of IL-8 than the SA intervention (*P* = 0.03). Adjusted mean IL-8 at

12 months was 9.9% (0.87 pg·mL<sup>-1</sup>) higher for the SA group than the PA group. Marginally significant effects of the PA intervention were also seen for IL-1sRII (*P* = 0.06) and sTNFR1 (*P* = 0.06). IL-1sRII values were significantly higher in the PA group at 6 months (SA = 9919  $\pm$  158 pg·mL<sup>-1</sup> vs PA = 10,419  $\pm$  161 pg·mL<sup>-1</sup>; *P* = 0.03);

however, by 12 months, values between groups were not significantly different (SA =  $9900 \pm 175$  pg·mL<sup>-1</sup> vs PA =  $10,188 \pm 176$  pg·mL<sup>-1</sup>;  $P = 0.25$ ), rendering the overall ANCOVA model not significant. sTNFR1 tended to be lower in the PA group at 6 months (SA =  $1693 \pm 30$  pg·mL<sup>-1</sup> vs PA =  $1592 \pm 30$  pg·mL<sup>-1</sup>;  $P = 0.05$ ) and at 12 months (SA =  $1740 \pm 43$  pg·mL<sup>-1</sup> vs PA =  $1639 \pm 43$  pg·mL<sup>-1</sup>;  $P = 0.17$ ). However, after adjustment for multiple comparisons, there were no significant effects of the PA intervention on any inflammatory biomarker.

Further analyses (using the same covariates as above in the main model) were performed to determine whether any effect of the PA intervention on inflammatory biomarker concentration was contingent on baseline biomarker level. Stratification of each baseline biomarker into less than the median value or greater than or equal to the median value yielded only one significant result, with the PA intervention reducing IL-15 only in those participants with higher IL-15 at baseline ( $P = 0.02$ ).

## DISCUSSION

Overall, the findings from this randomized controlled trial do not provide definitive evidence that a 1-yr PA intervention is successful at mediating systemic biomarkers of inflammation in older adults at risk for disability. Although the exercise intervention did lower systemic concentrations of IL-8 and reduce IL-15 in individuals with elevated IL-15 at baseline, these associations were no longer significant after adjustment for multiple comparisons. In addition, correlation analyses performed in this study agree with previously published results (36), suggesting that inflammatory cytokines and their receptors work in tandem and that levels are intercorrelated.

Because of the inverse relationship that exists between muscle mass, muscle strength, and other measures of physical function with inflammation in the elderly (4,38) as well as the role of inflammation in predisposing to various chronic diseases (3,18), identification of successful interventions to attenuate inflammation is a major goal of studies on healthy human aging. Although our study results do not strongly support the notion that long-term exercise can affect the panel of inflammatory biomarkers measured in this study, adoption of regular exercise does have many other health benefits for older adults (2,22,37). Indeed, the primary aim of the LIFE-P trial was to examine the effect of a structured PA intervention on physical functioning in the elderly, with published results (27) showing improved physical performance (as measured by the SPPB and the 400-m walk speed) in the PA group. Moreover, we previously demonstrated that the LIFE PA intervention reduced circulating levels of IL-6, likely the strongest systemic biomarker of inflammation in an aging population, especially in those with functional limitations and elevated IL-6 at baseline (25). Thus, findings from this study do not refute the general recommendation for exercise in older adults.

When comparing the results of this study with that of prior related research, many discrepancies exist. Evidence from epidemiologic studies in older adults show that greater levels of PA or fitness are associated with lower circulating levels of several inflammatory biomarkers, including IL-6, TNF- $\alpha$ , and CRP (5,38), although these correlations do not prove a causal mechanism. Data from several uncontrolled exercise training studies also contradict the present findings, at least concerning certain inflammatory biomarkers. For instance, in patients with chronic heart failure, 12 wk of aerobic exercise reduced TNF- $\alpha$  concentrations (20), 6 wk of cycle ergometry reduced sTNFR2 concentrations (21), and 16 wk of combined aerobic and resistance exercise training decreased both TNF receptors (but not TNF- $\alpha$  itself) (6). Importantly, all of these studies had a small number of participants, were conducted in populations with elevated inflammatory markers (i.e., patients with congestive heart failure or obesity), and had relatively short durations. At the time of this writing, only a few randomized controlled trials have been published that report the effects of a structured exercise intervention on inflammatory biomarkers. In agreement with our findings, 12 wk of high-intensity progressive resistance strength training did not affect IL-1, IL-2, TNF- $\alpha$ , or IL-6 in healthy elderly individuals (32). Similarly, in frail, elderly, nursing home residents, 32 wk of exercise did not induce changes in soluble receptors of cytokine activity (neopterin and sTNFR2) in the serum (17).

Given the inconsistencies between epidemiologic and randomized controlled trial data, it has been recently suggested that the reduction in body weight, specifically body fatness, could be a potential confounding mechanism through which PA may reduce systemic inflammation. The cytokines IL-6, TNF- $\alpha$ , TNF- $\alpha$  receptors, IL-8, and IL-1ra are elevated in obese people and are reduced with weight loss achieved through dietary restriction (26). In a recent epidemiologic study by Elosua et al. (7), authors report that intermediate and higher levels of self-reported PA and physical performance (as assessed by a 400-m walk test) were inversely associated with IL-6, CRP, and IL-1ra. However, after adjustment for body mass index (BMI), the association between PA and CRP and IL-1ra were not statistically significant, suggesting that only exercise interventions that reduce body weight may be effective in lowering systemic inflammation. Results from the Cardiovascular Health Study showed that greater PA was significantly associated with a lower inflammatory state as well as lower BMI and waist-to-hip ratio (11). This suggests that central obesity is connected with an elevated inflammatory state and that lesser degrees of central obesity associated with exercise could also be linked to inflammation. Finally, a large randomized controlled trial showed that a 2-yr diet and exercise counseling intervention that reduced BMI by  $4.2$  kg·m<sup>-2</sup> also reduced serum concentrations of CRP, IL-6, and IL-18 and increased levels of adiponectin (9). In our study, body weight and composition were unchanged by the intervention. Therefore, it may be that exercise interventions

are effective for improving chronic inflammation only if there is concomitant weight loss.

The current findings extend those of previous studies in several ways. First, the data provide evidence from a randomized controlled study, which is the definitive research design for examining effects of a treatment. Second, to our knowledge, this is the first large-scale exercise trial to include such a diverse panel of inflammatory markers, including several pro- and anti-inflammatory cytokines and their receptors. Each biomarker was chosen specifically for its known stimulation of the inflammatory response as well as age-related increased expression (10,12,14,35). Finally, the study design controlled for seasonal, postprandial, and diurnal variation in measured biomarkers, and the assay variability was acceptable.

Despite the strengths of this study, some limitations are worthy of note. First, cytokine expression is very variable, and given the precision of our estimates, a larger sample size may have yielded different results. Second, although the PA intervention was able to improve physical functioning in the elderly, attenuated adherence rates during the adoption and transition phases of the PA intervention may have compromised the ability to detect a significant effect of the intervention on the inflammatory biomarkers. Third, systemic measures were only obtained at one time during the day at each session, and no information was collected on local inflammation. In a recent study by Gielen et al. (13), the authors reported that exercise training did not reduce serum inflammatory markers in male patients with congestive heart failure but did reduce TNF- $\alpha$ , IL-6, and IL-1 $\beta$  gene expression in local skeletal muscle. This suggests that exercise may elicit local anti-inflammatory effects that may not

be evident in the systemic circulation but could be very important to clinical outcomes such as sarcopenia. Lastly, results from this study can only be generalized to populations of similar demographics, including baseline inflammatory status.

Given that there is no known treatment for age-related inflammation, exploration in this area of study is still warranted. Future research should aim to determine whether type, intensity, and duration of exercise are important to intervention success. Researchers should also be careful to note study details, such as the specific parameters being studied, body weight status, and baseline inflammatory status, because variations in such characteristics may yield conflicting results and inherent confusion in the literature. Moreover, it is important to recognize that although elevated inflammatory biomarkers are associated with poorer health outcomes, inflammation remains an essential component of immune defense. Even IL-6, the cytokine most strongly associated with advancing age, morbidity, and mortality, possesses both proinflammatory (39) and anti-inflammatory (40) properties. Thus, more data are needed to fully understand the role of inflammation in disease and aging.

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