Exercise training-induced lowering of inflammatory (CD14+CD16+) monocytes: a role in the anti-inflammatory influence of exercise?

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Abstract: Exercise training or higher levels of physical activity are known to exert anti-inflammatory effects. CD14+CD16+ monocytes are potent producers of inflammatory proteins, and elevated levels of these "inflammatory" monocytes have been implicated in disease development. Little is known about the influence of exercise training on this cell population. On the basis of their physical activity pattern, male and female subjects, 65–80 years old, were assigned to a physically active (PA; $n=15$) or inactive (PI; $n=15$) group. The PI group performed 12 weeks (3 days/week) of endurance (20 min at 70–80% heart-rate reserve) and resistance exercise training (eight exercises, two sets at 70–80% of one repetition maximum). Subjects in the PA group maintained their habitual activity level. Flow cytometry was used to determine monocyte phenotype and monocyte TLR4 expression. ELISAs were used to measure whole blood, LPS-stimulated TNF-α production, and serum C-reactive protein (CRP). At baseline, the PA group had a lower percentage of CD14+CD16+ monocytes and lower unstimulated production of TNF-α than the PI group. CD14+CD16+ monocyte percentage and 1 ng/ml LPS-stimulated TNF-α production were reduced after the PI group underwent 12 weeks of exercise training. PI subjects also had higher TLR4 expression on classical monocytes, but there were no significant exercise training-induced changes in monocyte TLR4 expression. The PA group had significantly lower serum CRP than the PI group. Physical activity was associated with lower CD14+CD16+ monocyte percentage and LPS-stimulated TNF-α production. Exercise training-induced reductions in CD14+CD16+ monocytes may contribute to the anti-inflammatory effects of exercise training. *J. Leukoc. Biol.* 84: 1271–1278; 2008.

Key Words: *inflammation · TLR4 · aging*

INTRODUCTION

Aging is frequently characterized by an increase in systemic inflammation [1, 2] distinguished by elevated levels of proinflammatory cytokines such as TNF-α and IL1-β and the acute-phase reactant, C-reactive protein (CRP) [3]. Increased levels of these inflammatory markers have been implicated in the pathogenesis of several diseases, including atherosclerosis, type 2 diabetes, and rheumatoid arthritis (RA) [1, 4–7].

Regular physical activity and/or exercise training are reported to decrease inflammation [8–11], reduce the risk of disease development [12–14], and ameliorate the symptoms of active disease [15–17]. In fact, in recent studies from our lab group, physical activity exerted a more potent influence on inflammatory markers than age [11, 18]. The mechanisms responsible for the anti-inflammatory effects of exercise are not clearly defined but could be linked to shifts in monocyte phenotype [9, 11, 18, 19].

Twelve weeks of combined endurance and resistance exercise training [11] or resistance training alone [19] reduced LPS-stimulated cytokine production in RPMI-diluted whole blood cultures from previously inactive young and old subjects. In subsequent experiments, we demonstrated that exercise training reduced monocyte cell-surface expression of TLR4, the LPS signaling receptor, in conjunction with decreased, LPS-stimulated cytokine production [11, 18, 19]. Exercise training-induced reductions in monocyte TLR4 expression could explain the attenuated, inflammatory response to LPS observed following exercise training (see ref. [20] for review).

Skinner et al. [21] reported that the two main populations of monocytes, classical (CD14++) and inflammatory (CD14+CD16+), differentially express cell-surface TLR4, and inflammatory monocytes express 2.5 times more cell-surface TLR4. Despite constituting only 10% of the total monocyte population, inflammatory monocytes contribute significantly to the inflammatory potential of the monocyte pool as a whole [22, 23]. Researchers discovered recently that the circulating, inflammatory monocyte percentage was elevated in patients with RA [24] and cardiovascular disease [7] and also in a mouse model of diabetes [25]. Moreover, compared with classical monocytes, inflammatory monocytes adhere more avidly to activated endothelial cells and may be precursors to the

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CD16+ macrophages found readily distributed throughout atherosclerotic lesions [26–28]. Based on these data, researchers believe that inflammatory monocytes play a significant role in the pathogenesis of several diseases linked to inflammation.

Only two research groups have examined the influence of exercise on inflammatory monocytes; in both cases, there was a transient increase in the inflammatory monocyte percentage after a single, acute bout of intense exercise [29, 30]. This transient increase in circulating inflammatory monocytes was followed by a rapid return to baseline during recovery. To our knowledge, no researchers have examined the influence of regular exercise training on inflammatory monocytes. Consequently, the primary purpose of the present study was to determine the effect of 12 weeks of combined endurance and resistance exercise training in subjects aged 65–80 years on the percentage of inflammatory monocytes. A secondary purpose was to measure TLR4 expression on inflammatory and classical monocytes and to confirm our previous findings that exercise training reduces LPS-stimulated cytokine production and lowers monocyte TLR4 expression. Given the anti-inflammatory nature of exercise training, it was hypothesized that exercise training would reduce the percentage of inflammatory monocytes in circulation, decrease LPS-stimulated TNF-α production, and decrease monocyte TLR4 expression.

MATERIALS AND METHODS

Subjects

Physically active (PA; seven females and eight males) and physically inactive (PI; 11 females and four males) subjects, aged 65–80 years, were recruited for this study. All subjects were apparently healthy and free of disease. The Committee on the Use of Human Research Subjects at Purdue University (West Lafayette, IN, USA; Approval Number 0510003116) approved this study.

Screening and preliminary testing

Subjects underwent a preliminary health screening and were required to obtain approval from their personal physician before being enrolled in the study. After physician consent was granted and at least 2 weeks prior to baseline measurements, subjects reported to the laboratory and completed a medical history questionnaire. Subjects were excluded if they were taking hormone replacements, had a body mass index (BMI) >35 kg/m^2, showed signs of acute illness or infection, or were currently taking any prescription medication known to influence leukocyte function or other aspects of the immune system (e.g., corticosteroids). Other exclusion criteria included the use of selective estrogen receptor modulators, bis-phosphonates, or other "bone active" agents, over-the-counter medications or supplements within the previous 2 months, and smokers or smokeless tobacco users. Subjects reporting medical conditions (chronic or acute) that would preclude them from participating in regular exercise were excluded from the study. Additionally, during the preliminary screening and post-training, subjects' height, weight, BMI, and body composition were measured. Body composition was approximated using a three-site skin-fold method [31].

A physical activity questionnaire [32] and modified Balke submaximal test, designed to predict maximal oxygen uptake [33], were used to assign subjects to the appropriate physical activity group. Those who reported exercising at least 3 days per week for the last 6 months and who had a "good" to "excellent" maximal oxygen uptake ($VO_{2\ max}$; males: >35 ml·kg^{-1}·min^{-1}; females: >28 ml·kg^{-1}·min^{-1}) were assigned to the PA group. Those reporting no regular physical activity over the past 6 months and who had a "fair" to "very poor" $VO_{2\ max}$ (males: <26 ml·kg^{-1}·min^{-1}; females: <23 ml·kg^{-1}·min^{-1}) were assigned to the PI group. After preliminary screening, a one-repetition maximum (1RM) was measured on chest press, leg press, and leg curl for PA subjects. Those assigned to the PA group were asked to maintain their PA lifestyle, and the PI group underwent 12 weeks of combined endurance and resistance exercise.

Acclimation and exercise training

PI subjects completed three acclimation sessions prior to beginning the exercise-training program. On Acclimation Day 1, subjects were instructed in proper lifting technique and use of exercise equipment. Their 8RM was assessed for each of the following exercises: leg press, chest press, seated row, leg extension, leg curl, leg abduction, leg adduction, and "lat" pull-down (Keiser Equipment, Fresno, CA, USA). For each exercise, the 1RM was estimated for subjects using the measured 8RM value. On Acclimation Day 2, subjects performed two sets of each exercise at 50% of their estimated 1RM. The first set consisted of eight repetitions, and the subjects were asked to continue until fatigue during the second set. During the 3rd acclimation day, subjects' 1RM for three exercises (leg extension, leg curl, and chest press) was determined for baseline comparison with the PA group. All acclimation exercises were preceded by 5–10 min of treadmill walking.

PI subjects completed 12 weeks of combined endurance and resistive exercise training (3 days/week). Endurance training consisted of 20 min of treadmill walking at 60–70% of heart-rate reserve. Following endurance training, the subjects performed two sets of eight resistance exercises (three upper-body and five lower-body). The approximate duration of each resistance-training session was 30 min, and subjects were monitored individually during each session to ensure that they exercised at the appropriate intensity and completed all of the required sets. During the 1st week of training, subjects completed exercises at 70% of estimated or measured 1RM and 80% of 1RM thereafter. For the first set, subjects completed eight repetitions, and for the second set, they performed the exercise until "momentary muscular failure" or until they completed 15 repetitions. If subjects were able to perform more than 12 repetitions in the second set on Friday of each week, the resistance was increased the following week. Additionally, subjects' heart rates were checked periodically at rest and during treadmill walking to ensure that they were still exercising at the specified intensity. Following the 12th week of training, subjects were reassessed for 8RMs, estimated $VO_{2\ max}$, and body composition. 1RM for chest press, leg extension, and leg curl was reassessed in PI and PA subjects.

Blood collection

Blood samples were collected at baseline and after 12 weeks of training for the PI group. For comparison, blood samples were taken at baseline and after 12 weeks of habitual activity in the PA group. To control for macronutrient intake, subjects followed a suggested eucaloric diet, which was based on the American Diabetes Association Dietary Exchange Program. The 1-day control diet was designed to contain ~50% carbohydrate, 35% fat, and 15% protein. The diet was consumed on the day preceding blood sampling at baseline and after 12 weeks in both groups. Following an overnight fast and at least 72 h removed from their final training session, subjects reported to the laboratory for blood sampling between 6:00 a.m. and 8:00 a.m. Once in the lab, subjects rested quietly for 20 min prior to venipuncture. Blood samples (9 mL) were drawn into SST™ serum tubes and sodium heparin tubes (Becton Dickinson, Franklin Lakes, NJ, USA).

Leukocyte counts

Leukocyte counts were made using an automated method (Coulter Counter, Beckman Coulter, Miami, FL, USA). CD14+ monocyte count was determined by multiplying the percent of CD14+ monocytes, measured using flow cytometry, by the total leukocyte count. These data were used to normalize LPS-stimulated TNF-α production and to determine the concentration of inflammatory monocytes (10^4 cells·ml^{-1} whole blood).

LPS-stimulated TNF-α production

For whole blood cultures, 100 μl aliquots of heparanized whole blood were added to 1800 μl-supplemented (2 mmol/ml L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin) RPMI 1640, treated with 1 ng/ml LPS (*Salmonella enteriditis*; Sigma-Aldrich, St. Louis, MO, USA), 1 ng/ml LPS + 100 μg/ml polymyxin B (PMB; Sigma-Aldrich), 25 μg/ml LPS, 25 μg/ml LPS +

100 μg/ml PMB, or no LPS (0 LPS). The LPS and PMB were added to the supplemented RPMI and incubated for 15 min (37°C, 5% CO_2, humidified environment) prior to the addition of whole blood, thus allowing adequate time for PMB to bind LPS. PMB binds to the lipid A portion of LPS and prevents LPS from signaling through TLR4. PMB was used to determine that it was, in fact, the interaction of LPS, presumably through TLR4 signaling, which resulted in increased TNF-α production. The treated whole blood suspensions were subsequently incubated for 24 h (37°C, 5% CO_2, humidified environment) in 24-well culture plates. After 24 h, the plates were centrifuged (800 *g* for 10 min), the supernatant was removed and filtered (0.2 μm syringe filter), and the filtered supernatant was stored at −80°C until further analysis.

Supernatant concentration of TNF-α was measured using a commercially available ELISA (OptEIA, BD PharMingen, San Diego, CA, USA). Supernatant from all LPS-stimulated wells was diluted 1:10 in assay diluent immediately prior to analysis. The supernatant from the unstimulated well was undiluted. Data were expressed as TNF-α concentration per CD14+ monocyte (relative TNF-α production) and as absolute TNF-α concentration.

CRP

Serum concentration of CRP was measured using a commercially available sandwich enzyme immunoassay kit (Alpco Diagnostics, Windham, NH, USA). Serum samples were diluted 1:100 in assay diluent, and the assay was performed according to the manufacturer's specifications.

Monocyte subpopulation and TLR4 expression

Conjugated antibodies specific for human CD14 (FITC, BioLegend, San Diego, CA, USA), CD16 (Pe-Cy5, BioLegend), and TLR4 (PE, Beckman Coulter) were added to 100 μl heparanized whole blood in 12 × 75 mm polystyrene tubes. The optimum concentration for each antibody was determined prior to the study. Appropriate isotype controls were added to 100 μl heparanized whole blood in a separate tube. Following a 30-min incubation in the dark at room temperature, the samples were hemolyzed and fixed using an automated method (Q-prep, Beckman Coulter). Samples were stored at 4°C in the dark and analyzed within 24 h. The samples were analyzed using a FC500 flow cytometer (Beckman Coulter). Briefly, a primary gate was established for the monocyte subpopulation based on the forward- and side-scatter light properties of total leukocytes (**Fig. 1**). A secondary gate was then established selecting for CD14+ cells within the primary gate. Inflammatory (CD14+CD16+) and classical monocyte (CD14+CD16−) populations were quantified based on CD14 and CD16 expression within the secondary gate (**Fig. 2**). TLR4 mean fluorescence intensity (MFI) was determined for the monocyte gate and for CD14+ cells as a whole. Negative gates were established using the appropriate isotype control prior to running each sample.

Statistical analysis

Data were reported as mean ± SE. Baseline differences between PA and PI were analyzed using one-way ANOVA. For the analysis of the effects related to the training intervention, a 2 × 2 factorial design with group (PI, PA) and time (baseline, post) was used with repeated measures on time. Prior to statistical analysis, all data were tested for the assumptions of normality, equality of variance, independence, and outliers. If warranted, appropriate transformations were made. To satisfy the assumption of normality, log transformations were made to the following datasets: TLR4 MFI on CD14+ monocytes and TNF-α production per CD14+ monocyte. Additionally, as a result of BMI differences and unequal male/female representation between the PI and PA groups, the analyses were run with BMI and sex as covariates. Statistical analyses were performed using PC SAS (Cary, NC, USA), specifically the linear model procedure, PROC GLM. The level of significance was set at 0.05, and Bonferroni post-hoc analysis was used to determine the location of significance.

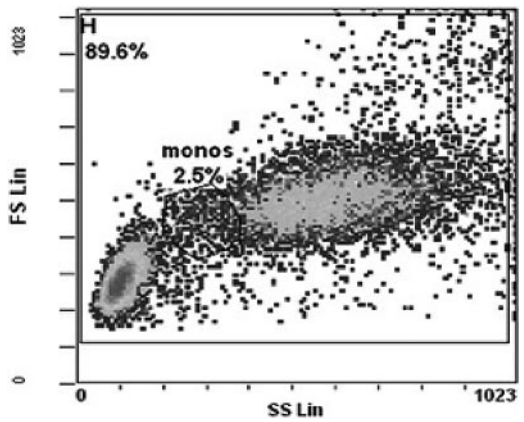

Fig. 1. Forward- and side-scatter (FS Lin and SS Lin, respectively) plot for leukocytes from a representative subject. A primary gate was established around the monocyte populations (monos). Lin, Linear scale.

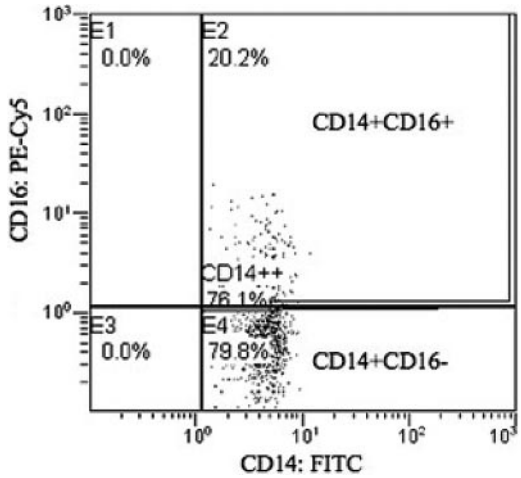

Fig. 2. Representative scatter plot for CD14:FITC and CD16:PE-Cy5 was used to determine the percentage of inflammatory (CD14+CD16+) and classical (CD14+CD16−) monocytes staining positive for CD14 within the primary gate.

RESULTS

Subject descriptive data

The PA group had significantly lower BMI (26.3±3.25 kg·m^2 vs. 28.1±4.98 kg·m^2; $P<0.0001$), body mass (71.7±10.50 kg vs. 78.9±19.01 kg; $P<0.0001$), and body fat percentage compared with the PI group (36.7±2.86% vs. 43.9±2.13%; $P<0.0001$; **Table 1**). The training intervention had no significant effect on these variables. During the course of the study, only one PI subject withdrew. The remaining PI subjects completed all 36 exercise sessions.

Training-induced changes in $VO_{2\ max}$ and 1RM

At baseline, the PA group had significantly higher $VO_{2\ max}$, leg press 1RM (**Table 2**; $P<0.05$) and chest press 1RM (Table 2; $P<0.001$) when compared with the PI group. There were no significant differences between the groups for leg curl 1RM at baseline ($P=0.42$). There were significant group × time effects, such that the PI group had significant increases in estimated $VO_{2\ max}$ (baseline: 20.13±4.22 ml·kg^{-1}·min^{-1}; post: 38.81±12.93 ml·kg^{-1}·min^{-1}; $P<0.001$), leg press 1RM ($P<0.05$), leg curl 1RM ($P<0.001$), and chest press 1RM ($P<0.0001$) following the training intervention (Table 2).

TABLE 1. Descriptive Data for PI and PA Subjects before (Pre) and after (Post) the 12 Weeks of Exercise Training or Continued Physical Activity

				Body mass (kg)		BMI (kg/m^2)		% Body fat	
Group	*n*	Age (yr)	Height (cm)	Pre	Post	Pre	Post	Pre	Post
PI	15	71 ± 5.74	168.3 ± 4.43	78.9 ± 19.0[a]	79.7 ± 19.7[a]	28.1 ± 4.98[a]	28.4 ± 5.35[a]	43.8 ± 8.24[a]	42.9 ± 10.37[a]
PA	15	70.9 ± 4.56	166.6 ± 8.24	71.7 ± 10.5	71.2 ± 8.4	26.3 ± 3.25	26.3 ± 2.83	36.7 ± 8.40	36.6 ± 9.47

Values are expressed as mean ± SD. [a] Significant group effect; PI significantly higher than PA ($P<0.05$).

There were no significant differences between PA and PI subjects for any of these variables at the end of the study.

LPS-stimulated TNF-α production

There was a significant group effect for unstimulated (0 LPS) TNF-α production per CD14+ monocyte at baseline ($P<0.05$), such that the PA group had lower, unstimulated, relative (fg/CD14+ monocyte) TNF-α production than the PI group (**Fig. 3A**). There was a significant group × time effect, such that the PI group had a significant decrease in unstimulated and 1 ng/ml LPS-stimulated ($P<0.05$), relative TNF-α production following the training intervention. There was a time effect for 25 μg/ml LPS-stimulated TNF-α production per CD14+ monocyte. Specifically, compared with baseline, both groups had lower TNF-α production per CD14+ monocyte at 25 μg/ml LPS stimulation at the end of the study. As expected, the addition of PMB, which binds to LPS and prevents its interaction with TLR4, significantly blunted cytokine production at both concentrations of LPS stimulation ($P<0.05$; Fig. 3). Additionally, the PI group had a significant reduction in unstimulated and 1 ng/ml LPS-stimulated, absolute (pg/ml) TNF-α production following the training intervention ($P<0.05$; Fig. 3B).

Monocyte subpopulations and TLR4 expression

At baseline, the PA group had a lower percentage of inflammatory monocytes than the PI group ($P<0.05$; **Fig. 4**). There was also a significant group × time effect, such that there was a 64% decrease in the percentage of inflammatory monocytes in PI subjects following the training intervention ($P<0.05$; Fig. 4). Additionally, the concentration of inflammatory monocytes decreased by 52% in the PI group following the training, but failed to reach statistical significance as a result of a significant contribution of BMI to the variance (**Fig. 5**).

TLR4 MFI was significantly higher on inflammatory monocytes compared with classical monocytes ($P<0.05$; **Fig. 6**). Additionally, TLR4 MFI was higher on classical monocytes from PI compared with PA (1.83±.34 vs. 1.58±.22; $P<0.05$) subjects at baseline. However, there were no baseline differences between PA and PI groups for TLR4 MFI on CD14+ monocytes as a whole (**Fig. 7**). There were also no training effects for TLR4 MFI on inflammatory monocytes, classical monocytes, or monocytes as a whole (Fig. 7).

When PA and PI groups were combined, there were significant correlations between pre-post change in inflammatory monocyte percentage and each of the following: pre-post change in 1 ng/ml LPS-stimulated TNF-α production (r=0.380; $P<0.05$), pre-post change in 25 μg/ml LPS-stimulated TNF-α production (r=0.50; $P<0.01$), and pre-post change in unstimulated TNF-α production (r=0.470; $P<0.05$).

Serum CRP

There was a group effect for CRP, such that the PA group had significantly lower serum CRP than PI subjects ($P<0.05$) at baseline. There was no exercise training effect for CRP (**Fig. 8**). Furthermore, for PI and PA combined, there was a significant correlation between BMI and CRP at baseline (r=0.582, $P<0.05$).

DISCUSSION

Inflammatory monocytes (CD14+CD16+) were first reported by Passlick et al. [34] to be a distinct subpopulation of CD14+ monocytes. In addition to being phenotypically different from classical monocytes, inflammatory monocytes display substantial, functional differences. Specifically, in comparison with classical monocytes, they are highly "proinflammatory", as

TABLE 2. 1RM Values for PI and PA Subjects before and after the 12 Weeks of Exercise Training or Continued Physical Activity

Exercise	PI		PA	
1RM	Pre	Post	Pre	Post
Leg press (kg)	155.1 ± 11.65	187.5 ± 12.12[a]	177.9 ± 13.28[b]	179.04 ± 13.21
Leg curl (kg)	38.48 ± 4.04	49.61 ± 4.45[a]	47.0 ± 5.00	42.5 ± 4.71
Chest press (kg)	26.21 ± 2.95	35.28 ± 3.74[a]	34.01 ± 4.07[b]	31.08 ± 4.48
VO_{2max} (ml·kg^{-1}·min^{-1})	20.13 ± 4.22	38.81 ± 12.93[a]	35.67 ± 15.87[b]	39.72 ± 15.84

Values are expressed as mean ± SE. [a] Significant group × time effect ($P<0.05$); PI-post higher than PI-pre. [b] Group effect ($P<0.05$); PA higher than PI at baseline.

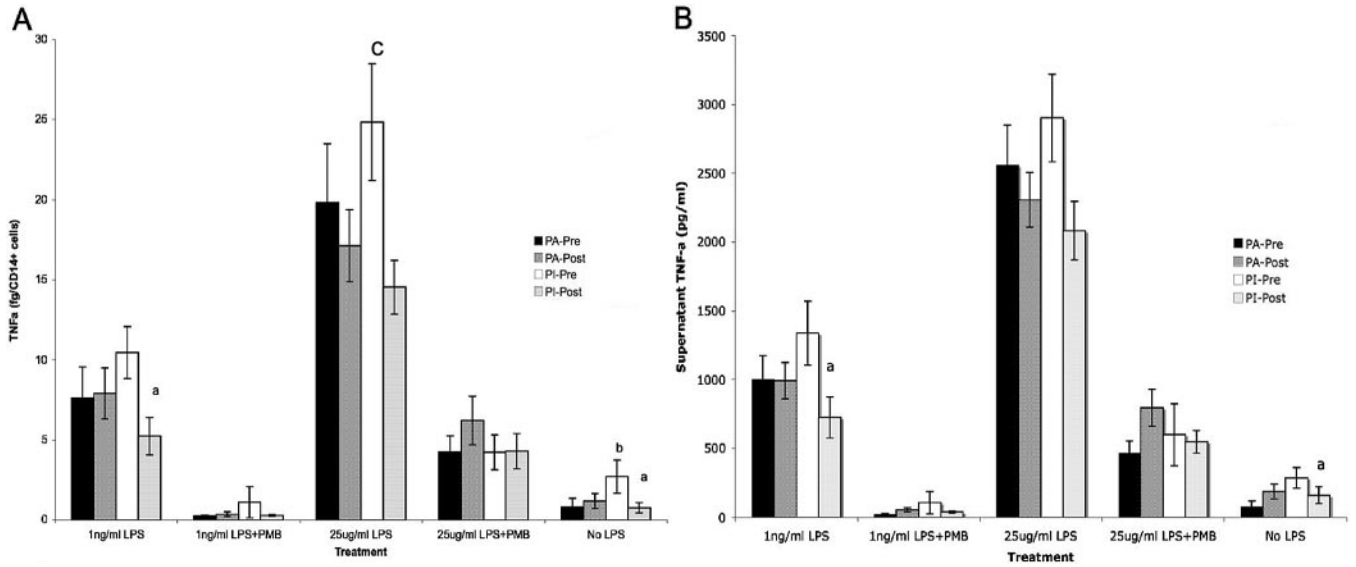

Fig. 3. TNF-α production. (A) fg/CD14+ monocyte; (B) pg/ml for PI and PA subjects at baseline (Pre) and following the 12 weeks of exercise training or continued physical activity (Post). a, Significant group × time effect ($P<0.05$); PI-Post, lower than PI-Pre; b, baseline group effect ($P<0.05$); PA-Pre, lower than PI-Pre; c, significant time effect ($P<0.05$).

illustrated by their proclivity for producing large amounts of inflammatory cytokines and relatively little anti-inflammatory cytokines [22]. In classical monocytes, a stimulus (e.g., LPS) to produce inflammatory cytokines, such as TNF-α and IL-6, is negatively regulated by the subsequent production of the anti-inflammatory cytokine, IL-10. In contrast, inflammatory monocytes produce virtually no IL-10 [23], while producing substantially more inflammatory cytokines than classical monocytes. Based on their proinflammatory nature and reports that their percentage is elevated in certain diseases [7, 24, 25], inflammatory monocytes are believed to be sensitive markers of the inflammatory milieu or significant contributors to the development and progression of diseases such as atherosclerosis and arthritis [35].

The results of the present study indicate that healthy physically inactive, 65- to 80-year-old men and women have a significantly higher percentage of circulating, inflammatory monocytes compared with an age-matched PA comparison group (13.25±2.8% vs. 7.5±2.1%). Twelve weeks of exercise training significantly reduced the percentage of inflammatory monocytes in the PI group by 64%. In fact, following just 12 weeks of exercise training, inflammatory monocyte percentage was equivalent for the trained PI and PA comparison group (PA: 6.47±0.79% vs. PI: 4.75±0.45%). Thus, in contrast to acute exercise, which has been shown to transiently increase the percentage of circulating inflammatory monocytes [29, 30], exercise training in older adults previously untrained reduced the percentage of inflammatory monocytes in circulation. Based on previous reports that glucocorticoid therapy selectively depletes CD14+CD16+ monocytes [36], it is interesting

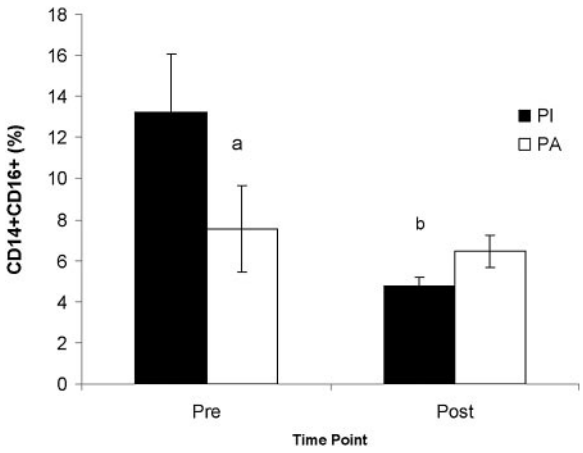

Fig. 4. CD14+CD16+ percentage in PI and PA subjects before and after the 12 weeks of exercise training or continued physical activity. a, Significant group × time effect ($P<0.05$); b, baseline group effect ($P<0.05$).

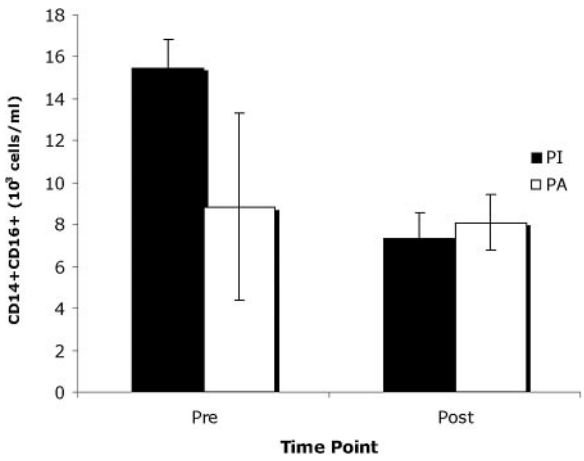

Fig. 5. CD14+CD16+ concentration (10^3 cells/ml) in PI and PA subjects before and after the 12 weeks of exercise training or continued physical activity. Despite a 52% decrease in the concentration of CD14+CD16+ cells, statistical significance was not achieved as a result of a significant contribution of BMI to the variance.

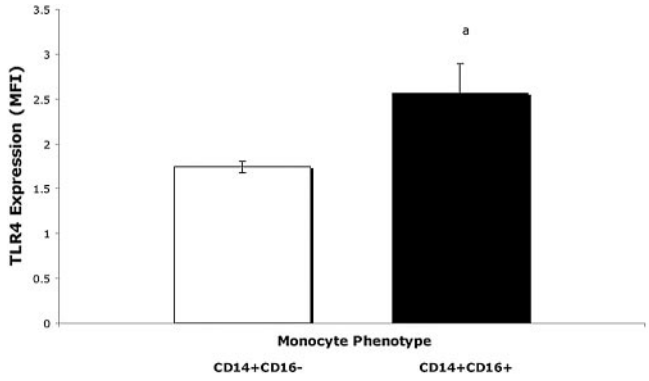

Fig. 6. Classical (CD14+CD16–) and inflammatory (CD14+CD16+) monocyte TLR4 MFI. Data were pooled for PA and PI groups. a, TLR4 on CD14+CD16+ significantly higher than on CD14+CD16– ($P<0.05$).

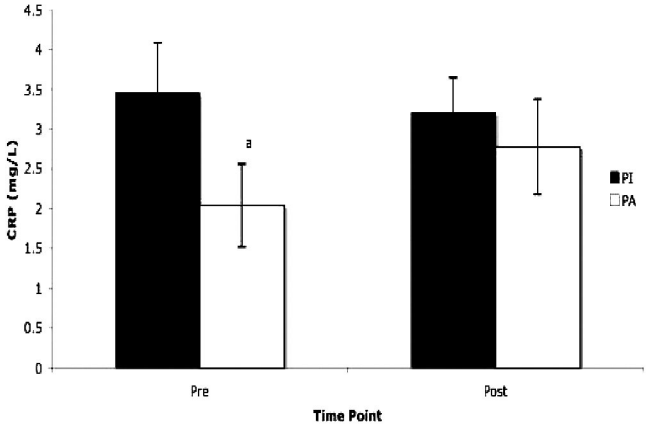

Fig. 8. Serum CRP in PI and PA subjects before and after the 12 weeks of exercise training or continued physical activity. a, PA subjects had significantly lower CRP in comparison with PI subjects at baseline ($P<0.05$).

to speculate that exercise-induced transient spikes in endogenous glucocorticoids (i.e., cortisol) may have played a role in reducing CD14+CD16+ monocytes in the PI group. Although we did not measure cortisol in these subjects, exercise bouts of a similar intensity and duration have been reported to transiently elevate cortisol levels in young and old subjects [37].

In addition to a reduction in CD14+CD16+ monocytes, unstimulated and 1 ng/ml LPS-stimulated TNF-α (absolute and relative) production was reduced significantly in PI subjects following the training intervention. At 25 μg/ml LPS stimulation, there was a trend for a training effect ($P=0.08$) and a significant time effect driven by a 41% decrease in TNF-α production in PI subjects. Myrianthefs et al. [38] reported that normal, healthy subjects experienced a decline in LPS-stimulated TNF-α production from early summer to early fall. As this was roughly the time-span for the present study, it is possible that the 13% decrease measured in PA subjects at this level of LPS stimulation resulted from normal, seasonal variation. Also of note in regards to TNF-α production was the finding that pre-post change in LPS-stimulated production of TNF-α was correlated significantly with the change in inflammatory monocytes. This makes it possible to conjecture that an exercise training-induced down-regulation of inflammatory monocytes could have a significant impact on in vivo inflammatory capacity. Further potential impact is recognized when the ability of inflammatory monocytes to strongly adhere and contribute to the damage of activated endothelium is considered [26].

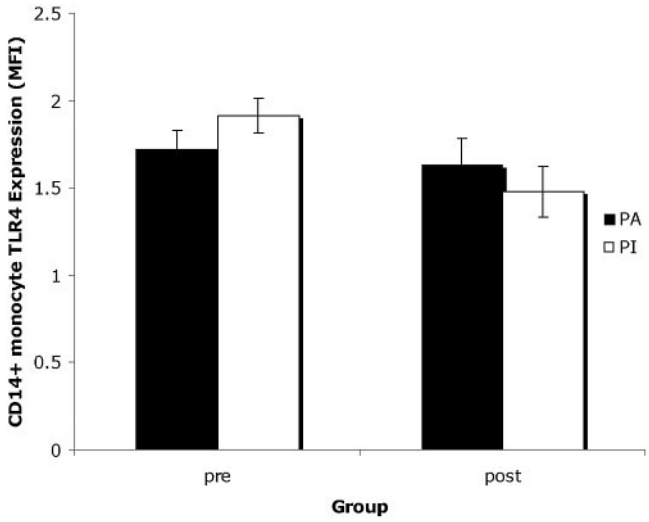

Fig. 7. CD14+ monocyte TLR4 MFI for PI and PA subjects before and after 12 weeks of exercise training or continued physical activity.

CRP levels were lower for PA compared with PI subjects at baseline, but contrary to a previous study from our lab [39] and others [40], there was no significant training effect. In our previous study, we used an almost identical exercise training protocol to the present study, and in the study by Okita et al. [40], subjects participated in a twice-weekly, 80-min aerobic dance workout followed by 30–60 min of bicycle or treadmill exercise at 60–80% of their peak heart rate. In our previous study and the study by Okita et al. [40], exercise training was associated with weight loss. Thus, a possible explanation for the discrepancy in post-intervention CRP changes is that the PI subjects in the present study did not lose any weight. In fact, Okita et al. [40] suggested that exercise training concomitant with weight loss may be necessary to observe reductions in CRP. Despite the lack of a training effect on CRP, the reduction in LPS-stimulated and basal TNF-α production reported here is in accord with previous studies demonstrating an anti-inflammatory effect of exercise training [8, 9, 11]. Although the training intervention failed to reduce CRP, the levels of this inflammatory mediator were significantly lower in the PA group. Additionally, consistent with the reports of others [41, 42], CRP was positively correlated with BMI.

We reported previously that exercise training-induced reductions in monocyte TLR4 may partially explain the anti-inflammatory effects of regular exercise training [9, 11, 19]. In the present study, exercise training did not significantly influence monocyte TLR4 expression ($P=0.11$). In agreement with previous reports [21], inflammatory monocytes expressed significantly more TLR4 than classical monocytes (Fig. 7), indicative of their inflammatory potential. Possible explanations for the lack of training-related reductions in TLR4 may be that compared with our previous studies, there were fewer subjects

in the present study and a higher ratio of women to men. Furthermore, there was a tendency ($P=0.11$) for the PI group to express lower monocyte TLR4 following exercise training. Post-hoc power analysis revealed that 21 subjects would have been required in each group to have 80% power to detect a difference in TLR4 expression following exercise training. We have previously detected a significant difference with as few as 16 subjects per group [11].

In conclusion, physical inactivity was associated with a higher percentage of inflammatory monocytes, and exercise training by previously inactive, healthy, elderly subjects markedly reduced the percentage and concentration of these proinflammatory cells in circulation. Together with the reduction of basal and LPS-stimulated TNF-α production, these data provide valuable insight into the relationship among inflammation, physical activity status, and exercise training. Finally, as inflammation is implicated in the pathogenesis of numerous diseases, these results are potentially, clinically relevant. Studies need to be conducted in clinical populations to determine if similar changes to inflammatory monocyte percentage occur and if these changes are correlated with improvements in the symptoms and progression of disease.

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