Increased proinflammatory and oxidant gene expression in circulating mononuclear cells in older adults: amelioration by habitual exercise

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Gano LB, Donato AJ, Pierce GL, Pasha HM, Magerko KA, Roeca C, Seals DR. Increased proinflammatory and oxidant gene expression in circulating mononuclear cells in older adults: amelioration by habitual exercise. *Physiol Genomics* 43: 895–902, 2011. First published May 24, 2011; doi:10.1152/physiolgenomics.00204.2010.—We tested the hypothesis that peripheral blood mononuclear cells (PBMC) of older adults demonstrate a proinflammatory/-oxidative gene expression profile that can be improved by regular aerobic exercise. PBMC were isolated from young (n = 25, 18–33 yr) and middle-aged/older (n = 40, 50–76 yr) healthy adults. The older adults had greater mRNA expression (real-time RT-PCR) of the proinflammatory/-oxidant transcription factor nuclear factor-κB (1.58-fold, P < 0.05) and receptor for advanced glycation end products receptor for advanced glycation end products (1.12-fold, P < 0.05), the proinflammatory cytokine tumor necrosis factor-α (1.90-fold, P < 0.05) and monocyte chemoattractant protein-1 (1.47-fold, P < 0.05), and the oxidant-producing enzymes nicotinamide adenine dinucleotide phosphate-oxidase (0.91-fold, P < 0.05) and inducible nitric oxide synthase (2.60-fold, P < 0.05). After 2 mo of aerobic exercise (brisk walking ~6 days/wk, 50 min/day, 70% of maximal heart rate), expression of interleukin-6 was not different with age or exercise intervention. Age group- and exercise intervention-related differences in gene expression were independent of other factors. PBMC of healthy older adults demonstrate increased expression of several genes associated with inflammation and oxidative stress, which is largely ameliorated by habitual aerobic exercise. This proinflammatory/-oxidative gene signature may represent a therapeutic target for lifestyle and pharmacological prevention and treatment strategies.

AGE IS THE MAJOR RISK FACTOR for cardiovascular diseases (CVD), largely as the result of vascular dysfunction (18). Chronic low-grade inflammation and oxidative stress are believed to play important roles in the development of vascular disorders with age (1, 29, 31). Plasma markers of inflammation and oxidative stress often are elevated in older adults (9, 11, 13). Proinflammatory changes to the intimal layer of arteries with aging have been reported on autopsy (34). Moreover, we recently found increased expression of the redox-sensitive proinflammatory nuclear transcription factor nuclear factor κB (NF-κB), the proinflammatory cytokines tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1), and the oxidant enzyme nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase in vascular endothelial cells obtained from older compared with young adults (9, 10). The proinflammatory/redox-sensitive proteins receptor for advanced glycation end products (RAGE) and inducible nitric oxide synthase (iNOS) also have been found to increase with age (2, 15).

Although evidence is accumulating for inflammation and oxidative stress in plasma and vascular cells/tissues with advancing age in humans, relatively little is known about the possibility of such changes in circulating leukocytes, particularly peripheral blood mononuclear cells (PBMC). PBMC interact constantly with vascular endothelial cells: rolling, adhering to, and, in some cases, infiltrating the endothelial layer of arteries (20). PBMC synthesize inflammatory proteins and reactive oxygen species that can cause damage and dysfunction to arteries and other tissues. It has been postulated that a proinflammatory/-oxidant gene expression profile in PBMC may contribute to increased risk of CVD (4). Consistent with this, mRNA expression of some inflammatory genes is altered in PBMC obtained from untreated patients with essential hypertension, an effect that is absent in patients undergoing successful antihypertensive therapy (3).

In the present study we tested the hypothesis that compared with young adults, PBMC obtained from middle-aged and older adult humans without clinical CVD demonstrate increased mRNA gene expression of the above-described nuclear transcription factors, cytokines, and enzymes associated with inflammation and oxidative stress. We then tested the hypothesis that habitual aerobic exercise, a treatment strategy with possible anti-inflammatory and antioxidative effects (17, 26), improves the altered gene expression of these factors in middle-aged/older adults.

**EXPERIMENTAL PROCEDURES**

**Subjects**

Data were obtained on 65 healthy men and women: 25 young (18–33 yr, 5 women and 20 men) and 40 middle-aged/older (50–76 yr, 15 women and 25 men). All subjects were free of CVD, diabetes, dyslipidemia, and other clinical disorders as assessed by medical history, physical examination, ankle brachial index, and blood chemistries. Middle-aged/older subjects also had negative electrocardiogram and blood pressure responses to incremental treadmill exercise performed to volitional exhaustion. Young and middle-aged/older subjects in the initial cross-sectional study and the subjects in the intervention study prior to exercise training were sedentary, defined as not having performed regular exercise (<30 min/day, <2 days/wk) for ≥2 yr. Subjects were nonsmokers and not taking anti-inflammatory agents or dietary supplements (including antioxidants). Subjects were not taking any prescription medications with the exception of two middle-aged/older adults in the cross-sectional analysis who were taking statins. All procedures were approved by the Human Research Committee of the University of Colorado at Boulder. The nature,
benefits, and risks of the study were explained to the volunteers, and their written informed consent was obtained prior to participation.

Study Design

The study consisted of two phases. First, to test the hypothesis that older adults demonstrate a more proinflammatory/oxidative PBMC gene expression profile than young adults, we compared 25 young (18–33 yr, 5 women and 20 men) and 29 middle-aged/older (50–76 yr, 9 women and 20 men) adults. Second, to test the hypothesis that regular aerobic exercise will improve the proinflammatory/oxidative PBMC gene expression profile in older adults, a separate group of 11 middle-aged/older adults (57–70 yr, 6 women and 5 men) completed a 2-mo aerobic exercise intervention. During the intervention period, subjects were asked to walk every day (7 day/wk) for 40–45 min at 70–75% of their individual maximal heart rate measured during the incremental treadmill exercise test. Adherence was documented with data downloaded every 2 wk from heart rate monitors worn during all exercise sessions and by physical activity diaries. All baseline measures were repeated in these subjects following the completion of the exercise intervention.

Study Procedures

All measurements were performed at the University of Colorado at Boulder Clinical Translational Research Center (CTRC) after an overnight fast and a 24-h abstinence from alcohol and physical activity.

Subject characteristics. Body mass index (BMI) was determined from measurement of height and body mass, and % body fat was measured by dual x-ray absorptiometry. Arterial blood pressure and resting heart rate were measured over the brachial artery during seated rest using a semiautomated device (Dynamap XL, Johnson and Johnson). Leisure time activity was estimated to provide a measure of total physical activity (12). Maximal oxygen consumption was assessed during incremental treadmill exercise performed to exhaustion and a plateau in oxygen consumption during maximal exercise was established in all subjects (8, 22). Diet composition and caloric intake were estimated from 3-day food intake records (The Food Processor 8.2, ESHA Research) analyzed by a CTRC nutritionist. Fasting circulating metabolic factors were determined by standard assays at the CTRC core laboratory. Plasma samples were analyzed for oxidized low-density lipoprotein (LDL), a marker of systemic oxidative stress, and serum samples were analyzed for total antioxidant status.

Mononuclear cell isolation. We collected 50 ml of blood into EDTA tubes from each subject, and NycoPrep 1.077 (Greiner Bio- determined by standard Coulter counter technique (Beckman Coulter Ac-T 5diff CP) (33).

PBMC Gene Expression in Young and Middle-Aged/Older Adults

RESULTS

PBMC Gene Expression in Young and Middle-Aged/Older Adults

Statistical analysis was completed with the SPSS statistical package (version 17.0; SPSS, Chicago, IL). Differences between the young and older groups were determined by an independent t-test. Differences between baseline and post-exercise values were determined using paired-samples t-test. For the cross-sectional age-group comparisons as well as for the exercise intervention study, relations between PBMC gene expression and subject characteristics that significantly differed between the young vs. older groups or pre- vs. postintervention, respectively, were determined using multiple linear regression analyses. Significance was set at $P < 0.05$. Results are presented as means ± SE.

PBMC Gene Expression Assays using reverse transcription-polymerase chain reaction (RT-PCR) included TNF-α (Hs00174128_m1), MCP-1 (Hs00234410_m1), IL-6 (Hs00174131_m1), NADPH oxidase (p47phox subunit) (Hs00417167_m1), iNOS (Hs00167257_m1), RAGE (Hs00153957_m1), and NF-κB (p65 subunit) (Hs01042012_g1).

...
Expression in Middle-Aged/Older Adults

Effects of Habitual Aerobic Exercise on PBMC Gene Expression in Middle-Aged/Older Adults

Adherence to the exercise intervention. Subjects walked for 6.0 ± 0.3 days/wk, 51.0 ± 2.1 min/day, at 71.0 ± 1.6% of maximal heart rate, for 9.0 ± 0.5 wk. Thus, the subjects were highly compliant to the exercise program.

Subject characteristics. General subject characteristics before and after the exercise intervention are shown in Table 2. Maximal aerobic exercise capacity was greater after the exercise intervention as indicated by greater maximal oxygen consumption and treadmill exercise time to exhaustion (P < 0.001). Waist-hip ratio, blood pressure, plasma HDL-cholesterol and triglycerides, and fasting blood glucose did not differ before and after the exercise intervention. There were small reductions in body mass, BMI, and % body fat (1–4%), and total and LDL-cholesterol (12%) after compared with before the exercise intervention. Compared with before, after the exercise intervention expression of iNOS was lower in four of the six subjects on whom we had data, but the mean difference was not significant (P = 0.21). Expression of IL-6, the PBMC of older subjects (all P < 0.05), whereas expression of IL-6 did not differ between groups. None of the subject characteristics that differed between the young and older groups were significant contributors to the differences in gene expression based on multiple linear regression analysis (all P < 0.05). Similarly, the age group differences in PBMC gene expression were not related to dietary macronutrients (all P > 0.05).

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Plasma markers of inflammation, oxidative stress, and antioxidant capacity. There were no significant differences in these markers before and after the exercise intervention (Table 2).

WBC. There were no significant differences in total WBC, lymphocytes, or monocytes (all P ≥ 0.14) before and after the exercise intervention (See Supplemental Table S1).

PBMC gene expression. Gene expression is shown in Figs. 3–5. Expression of RAGE, NADPH oxidase p47phox, MCP-1 (all P < 0.05), NF-κB p65 (P = 0.05), and TNF-α (P = 0.07) was or tended to be lower in PBMC after compared with before the exercise intervention. Compared with before, after the exercise intervention expression of iNOS was lower in four of the six subjects on whom we had data, but the mean difference was not significant (P = 0.21). Expression of IL-6,
which did not differ in young and middle-aged/older adults, was unaffected by the exercise intervention. None of the subject characteristics that differed before and after the exercise intervention were significant contributors to the changes in gene expression based on multiple linear regression analysis (all \( P < 0.05 \)). Moreover, the exercise-induced changes in PBMC gene expression were not associated with changes in dietary macronutrients (all \( P < 0.05 \)).

**DISCUSSION**

The two major findings from this study are as follows. First, compared with young adults, PBMC of middle-aged/older adults demonstrate increased expression of several genes associated with inflammation and oxidative stress. Second, habitual aerobic exercise largely ameliorates this proinflammatory-oxidative gene expression profile. Finally, these effects of age and habitual aerobic exercise on PBMC gene expression were independent of the influence of other factors.

**Expression of Proinflammatory/-oxidative Genes in PBMC of Middle-Aged/Older Adults**

In the present study, PBMC obtained from the middle-aged/older adults exhibited increased mRNA expression of the proinflammatory-oxidant factors NF-\( \kappa \)B (p65 subunit), TNF-\( \alpha \), MCP-1, iNOS, NADPH oxidase (p47phox subunit), and RAGE compared with young adults. Expression of the inflammatory cytokine IL-6 did not differ significantly between the groups. To our knowledge, these results represent the first systematic assessment of inflammation- and oxidant-related gene expression in PBMC from young and older adults without clinical disease. Our findings support the idea that PBMC of middle-aged/older adults demonstrate a shift in gene expression toward an increased proinflammatory-oxidant profile.

These observations generally are consistent with previous reports of increases in markers of inflammation and oxidative stress in plasma and vascular cells/intima in older adult humans (34) and whole arteries of old rodents (5–7). Of particular interest, we recently reported increases in protein expression of NF-\( \kappa \)B p65, TNF-\( \alpha \), MCP-1, and NADPH oxidase p47phox in vascular endothelial cells obtained from older compared with young healthy adults (9, 10). Previous data on expression of these factors in PBMC in humans are limited, although greater TNF-\( \alpha \) protein expression in T cells has been reported in older adults (25). Basal production of TNF-\( \alpha \) and IL-6 from PBMC culture in vitro is increased (28) or unchanged (25, 28) in older compared with young adults.

Several conventional and nonconventional risk factors for CVD change with age, even in healthy adults (9, 10, 14, 23), as was the case in the present study (Table 1). However, these factors were not significant predictors of the age-associated

**Table 2. Subject characteristics for exercise intervention study**

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass, kg</td>
<td>78.8±5.4</td>
<td>77.5±5.4*</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.2±1.0</td>
<td>25.5±1.1*</td>
</tr>
<tr>
<td>Total body fat, %</td>
<td>34.3±1.7</td>
<td>32.7±1.6*</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>0.87±0.04</td>
<td>0.86±0.03</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>117±3</td>
<td>116±4</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>72±2</td>
<td>70±2</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>216±8</td>
<td>190±9*</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>131±9</td>
<td>114±9*</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>61±4</td>
<td>58±3</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>119±17</td>
<td>92±9</td>
</tr>
<tr>
<td>Fasting blood glucose, mg/dl</td>
<td>89±1</td>
<td>90±2</td>
</tr>
<tr>
<td>Leisure physical activity, MET h/wk</td>
<td>17±5</td>
<td>NA</td>
</tr>
<tr>
<td>VO(_2) max, ml·kg(^{-1})·min(^{-1})</td>
<td>28±1</td>
<td>31±1*</td>
</tr>
<tr>
<td>Exercise test duration, min</td>
<td>9.6±0.3</td>
<td>11.4±0.4*</td>
</tr>
<tr>
<td>Oxidized LDL, IU/l</td>
<td>66±4</td>
<td>67±4</td>
</tr>
<tr>
<td>Total antioxidant status, mmol/l</td>
<td>1.2±0.1</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>C-reactive protein, mg/l</td>
<td>1.5±0.5</td>
<td>1.9±0.9</td>
</tr>
<tr>
<td>Plasma IL-6, pg/ml</td>
<td>1.3±0.3</td>
<td>1.3±0.4</td>
</tr>
<tr>
<td>Plasma TNF-( \alpha ), pg/ml</td>
<td>1.1±0.1</td>
<td>1.3±0.1</td>
</tr>
</tbody>
</table>

Data are means ± SE. VO\(_2\) max, maximal oxygen consumption; *\( P < 0.05 \) vs. before.

**Fig. 2. Expression of genes for proinflammatory cytokines.** mRNA expression of tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \), A), monocyte chemoattractant protein-1 (MCP-1, B), and interleukin-6 (IL-6, C) in peripheral blood mononuclear cells from sedentary (Sed) young and older adults (\( n = 8–14 \) group). Values are means ± SE. *\( P < 0.05 \) vs. young.
Fig. 3. Effects of aerobic exercise intervention on expression of genes for pro-oxidant factors. Changes in mRNA expression of iNOS and NADPH oxidase p47phox in the overall group and among individual subjects from before to after exercise intervention in peripheral blood mononuclear cells from middle-aged/older adults (n = 6–11/group). Values are means ± SE. Pre, before exercise; Post, after exercise; EX, exercise. *P < 0.05 vs. Pre-EX.

Fig. 4. Effects of aerobic exercise intervention on expression of genes for proinflammatory mediators. Changes in mRNA expression of RAGE and NF-κB p65 in the overall group and among individual subjects from before to after exercise intervention in peripheral blood mononuclear cells from middle-aged/older adults (n = 6–11/group). Values are means ± SE. *P < 0.05 vs. Pre-EX.
differences in PBMC gene expression in the present study. As such, our results suggest that differences in these factors with age do not account for the increased expression of proinflammatory and -oxidative genes in circulating PBMC of older adults.

As reported previously, circulating concentrations of oxidized LDL, CRP, and IL-6 were higher in the middle-aged/older subjects (9, 10, 23), whereas plasma total antioxidant status was lower (9, 23), and no difference was observed between the groups for TNF-α (9). As such, it is possible that increases in oxidative stress and inflammatory proteins and/or reductions in antioxidant status of the blood influenced the expression of genes in PBMC of the middle-aged/older adults. Alternatively, it is possible that the greater expression of pro-oxidant and -inflammatory genes in the PBMC of our middle-aged/older subjects contributed to increased synthesis and release of reactive oxygen species and inflammatory proteins in the blood. The direction of the cause-and-effect associations between circulating, PBMC, and vascular concentrations of these molecules is an important goal of future work in this area.

Finally, the proinflammatory/-oxidant gene expression profile of the middle-aged/older adults observed in our cross-sectional analysis could be explained by other chemical and/or physical/mechanical (e.g., shear stress, physical deformation) factors to which PBMC are exposed (4). In this context, it is possible that at least some of the mechanisms in question may be common to both PBMC and vascular endothelial cells of humans given the general similarities in expression of inflammatory and pro-oxidant factors in these cells with aging (9, 10).

**Amelioration with Habitual Aerobic Exercise**

In the present study, ~9 wk of daily brisk walking increased maximal aerobic exercise capacity as indicated by increases in both maximal oxygen consumption and duration of walking during incremental treadmill exercise. Most importantly, the exercise intervention reduced or tended to reduce PBMC mRNA gene expression of the proinflammatory/-oxidative factors that were elevated in middle-aged/older adults, namely NF-κB p65, TNF-α, MCP-1, NADPH oxidase p47phox, and RAGE, and lowered iNOS in four of the six subjects on whom expression of that gene was assessed. Expression of IL-6 was unaffected by age and habitual exercise. Thus, regular aerobic exercise may be an effective treatment for ameliorating the proinflammatory/-oxidative shift in gene expression in PBMC observed with age. Our findings are consistent with recent observations of reduced basal production of TNF-α by CD14+ monocytes after a program of endurance and resistance exercise training in healthy older adults (30).

As confirmed in the present study, the exercise intervention employed (brisk daily walking) results in only small or no changes in subject characteristics, including conventional and nonconventional risk factors for CVD (8, 22). Multiple regression analysis revealed that none of the small changes in subject characteristics observed were significant predictors of the changes in PBMC gene expression in response to the exercise intervention.

The results of these analyses are consistent with the overall dissociations between body composition and other subject characteristics and PBMC gene expression in the two parts of the study. For example, the differences in PBMC gene expression across age (range of 0.91–2.60) generally were similar in magnitude to those observed before and after the exercise intervention (range of 0.56–2.73), whereas the differences in body composition in the older vs. younger subjects (body mass, 4.73 kg; BMI, 2.91 kg/m2; % body fat, 8.65%; waist-hip, 0.08) were much greater than those observed before vs. after the exercise intervention (mass, 1.28 kg; BMI, 0.72 kg/m2; % body fat, 1.58%; waist-hip, 0.01). That is, following the exercise intervention the middle-aged/older subjects demonstrated a
PBMC gene expression profile similar to the young controls, despite continued differences in body composition. Collectively, our results suggest that the effects of habitual aerobic exercise on expression of pro-oxidative and -inflammatory genes in PBMC of middle-aged/older adults are not dependent on changes in body composition or other subject characteristics.

In agreement with previous reports on middle-aged/older adults without clinical disease (16, 21, 24, 30), the exercise intervention did not alter plasma markers of inflammation, nor were markers of oxidative stress or antioxidant capacity affected. These observations indicate that modulation of expression of proinflammatory-/oxidant genes by regular aerobic exercise is not associated with changes in these markers of the oxidative and inflammatory properties of the blood.

One intriguing possibility is that physical/mechanical stimuli altered by habitual aerobic exercise such as intravascular shear forces could play a role. Sustained, daily increases in laminar shear exert anti-inflammatory and -oxidative effects on vascular cells and tissues and are thought to mediate much of the beneficial effects of regular aerobic exercise (19). The fact that PBMC obtained from sheared whole blood demonstrate enhanced synthesis of anti-inflammatory, but not proinflammatory, cytokines (27) suggests that the almost daily increases in shear associated with our moderate aerobic exercise intervention may have contributed to the anti-inflammatory-/oxidative effects on gene expression observed here.

Conclusions and Significance

The findings of the present investigation demonstrate that in the absence of clinical disease, middle-aged/older adults exhibit a proinflammatory-/oxidative gene expression profile in PBMC when compared with healthy young adults. Importantly, our results show that 2 mo of regular, moderate-intensity aerobic exercise (brisk walking) reverses this profile, reducing the expression of these genes in PBMC. The present data also indicate that these effects of age and habitual exercise on PBMC gene expression are not dependent on differences in other subject characteristics.

PBMC interact with and modulate the function and health of the vasculature. An aberrant proinflammatory-/oxidative gene expression profile in PBMC is manifest in untreated patients with essential hypertension and is hypothesized to contribute to increased risk of CVD in such populations (4). Older age and regular aerobic exercise are associated with increased and decreased risk of CVD, respectively, which have been attributed at least in part to corresponding differences in inflammation and oxidative stress. As such, our findings here may provide insight into a novel mechanism by which older age and habitual exercise exert their opposing influences on CVD risk. A proinflammatory-/oxidative gene expression profile in PBMC may be a useful marker and/or therapeutic target of age-associated risk of CVD. Future investigations should assess this possibility and determine the efficacy of lifestyle and pharmaceutical strategies for the prevention and treatment of this age-associated gene signature in PBMC.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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