Mechanism of Beneficial Effects of Physical Activity on Atherosclerosis and Coronary Heart Disease

Effects of acute and chronic endurance exercise on intracellular nitric oxide and superoxide in circulating CD34\(^+\) and CD34\(^-\) cells

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Jenkins NT, Landers RQ, Prior SJ, Soni N, Spangenberg EE, Hagberg JM. Effects of acute and chronic endurance exercise on intracellular nitric oxide and superoxide in circulating CD34\(^+\) and CD34\(^-\) cells. J Appl Physiol 111: 929–937, 2011. First published June 23, 2011; doi:10.1152/japplphysiol.00541.2011.—We investigated the influence of acute and chronic endurance exercise on levels of intracellular nitric oxide (NO), superoxide (O\(_2\)\(^-\)), and expression of genes regulating the balance between these free radicals in CD34\(^+\) and CD34\(^-\) peripheral blood mononuclear cells (PBMCs; isolated by immunomagnetic cell separation). Blood samples were obtained from age- and body mass index (BMI)-matched endurance-trained (n = 10) and sedentary (n = 10) men before and after 30 min of exercise at 75% maximal oxygen uptake (VO\(_{2\text{max}}\)). Baseline levels of intracellular NO (measured by DAF-FM diacetate) and O\(_2\)\(^-\) (measured by dihydroethidium) were 26% (P < 0.05) and 10% (P < 0.05) higher, respectively, in CD34\(^+\) PBMCs from the sedentary group compared with the endurance-trained group. CD34\(^+\) PBMCs from the sedentary group at baseline had twofold greater inducible nitric oxide synthase (iNOS) mRNA and 50% lower endothelial NOS (eNOS) mRNA levels compared with the trained group (P < 0.05). The baseline group difference in O\(_2\)\(^-\) was eliminated by acute exercise. Experiments with apocynin indicated that the training-related difference in O\(_2\)\(^-\) levels was explained by increased NADPH oxidase activity in the sedentary state. mRNA levels of additional angiogenic and antioxidant genes were consistent with a more angiogenic profile in CD34\(^+\) cells of trained subjects. CD34\(^+\) PBMCs, examined for exploratory purposes, also displayed a more angiogenic mRNA profile in trained subjects, with vascular endothelial growth factor (VEGF) and eNOS being more highly expressed in trained subjects. Overall, our data suggest an association between the sedentary state and increased nitro-oxidative stress in CD34\(^-\) cells.

CD34\(^+\) PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCs) are stem/progenitor cell precursors for hematopoietic and endothelial cells (2). Given appropriate environmental cues, CD34\(^+\) PBMCs can perform beneficial angiogenic functions (2, 7), and subsets of CD34\(^+\) cells coexpressing endothelial antigens have endothelial progenitor cell (EPC) characteristics. However, controversy over precise definitions of EPCs and other angiogenic cells has slowed progress in the field (9, 17), making it difficult to reach consensus on which cells should be targeted for investigation of exercise-induced effects on cell-based endothelial repair mechanisms. It is generally agreed upon that CD34\(^+\) progenitor cells, whether through actions of particular endothelially directed subsets or by acting as a pool of generic progenitors, perform proangiogenic actions that contribute to the maintenance of vascular endothelial integrity (10, 11). CD34\(^+\) cell numbers predict cardiovascular (CV) disease risk and are functionally susceptible to adverse effects resulting from lifestyle-related metabolic and CV disease in vivo environment (8, 10, 11). In addition, CD34\(^+\) PBMCs have recently received a great deal of attention in the literature for cell therapy applications, with a recent study finding a reduction in myocardial infarct size following coronary infusion of CD34\(^+\) bone marrow cells (26). However, no studies have investigated the effects of acute or chronic exercise on functional aspects of CD34\(^+\) cells.

There is growing evidence that acute and chronic endurance exercise increase the functional capacity of circulating angiogenic cells (18, 20, 30, 36–38), but the mechanisms underlying the functional adaptations to exercise training are not clear. The balance between nitric oxide (NO) and superoxide (O\(_2\)\(^-\)) production is a critical mechanistic aspect of the angiogenic functions of cultured progenitor cells (12, 34), and there is a link between CV/metabolic disease and reduced NO/increased O\(_2\)\(^-\) production in EPCs (31, 33). There have been some recent investigations into whether exercise training favorably alters the balance between NO and O\(_2\)\(^-\) levels in circulating angiogenic cells. For example, we reported that increased activity of the prooxidant enzyme NADPH oxidase is a mechanistic mediator of lower intracellular NO levels in cultured EPCs of sedentary compared with trained men (20). Additionally, a recent study found that exercise training in metabolic syndrome patients increases NO production and reduced O\(_2\)\(^-\) production in cultured EPCs, in conjunction with increased endothelial repair capacity in vivo (31). However, the effects of exercise training on intracellular NO and O\(_2\)\(^-\) need to be examined in CD34\(^+\) cells, given their angiogenic properties discussed above. Furthermore, no exercise-based EPC studies have tested the assumption that the phenotype of cells generated after several days in culture under powerful angiogenic.
conditions accurately reflects the in vivo phenotype, and thus there is a need to examine cells in their freshly isolated state.

Another issue that has not received adequate attention is whether acute exercise alters oxidant status of circulating angiogenic cell types. Acute exercise increases NO production (20) and the migratory capacity (36) of cultured EPCs. However, no studies have directly assessed the effects of acute exercise on O$_2^-$ production in any angiogenic cell type. Given the known role for acute exercise-induced oxidative stress as a stimulus for beneficial adaptive responses in the form of increased NO bioavailability and upregulation of antioxidant defense systems in muscle (21) and the vasculature (13), there is a need to investigate the effects of acute exercise on circulating cells with angiogenic function.

Therefore, we tested the hypothesis that acute and chronic endurance exercise would favorably influence the balance between NO and O$_2^-$ in freshly isolated CD34+ PBMCs, and elevated NADPH oxidase activity in cells of the sedentary group would be a causal mechanism underlying these effects. We also hypothesized that the expression of genes involved in the regulation of intracellular NO and O$_2^-$ levels would be consistent with greater antioxidant capacity and angiogenic function in the trained state. Finally, although there are angiogenic monocyte and T-cell subpopulations within the CD34+ PBMC fraction (4, 6, 15, 22, 39), no studies have examined the effects of acute or chronic endurance exercise on their functional properties. Thus, we also explored the effects of acute and chronic exercise on NO, O$_2^-$, and gene expression in CD34+ PBMCs.

METHODS

Screening

All potential participants completed medical and physical activity history questionnaires that we have used previously (20). All participants were nonsmoking men age 18–35 yr with no history and currently free of CV disease and diabetes. Subjects were normotensive and were not on cholesterol, antihypertensive, or antihyperglycemic medications. Subjects were not on cholesterol, antihypertensive, or antihyperglycemic medications. Participants were nonsmoking men age 18–35 yr with no history and were currently free of CV disease and diabetes. Subjects were normotensive and were not on cholesterol, antihypertensive, or antihyperglycemic agents. Endurance-trained individuals (n = 10) performed at least 4 h/wk of endurance exercise, and sedentary individuals (n = 10) reported engaging in exercise for <20 min/day on <2 days/wk. Groups were matched for age and body mass index. The study was approved by the University of Maryland College Park Institutional Review Board, and all participants provided written informed consent.

Maximal Graded Exercise Test and Body Composition

All testing occurred in the morning after an overnight fast. Participants refrained from alcohol, vitamins, and caffeine for 12 h and antihistamines or NSAIDs for 24 h prior to testing. Body composition was estimated using the seven-site skinfold procedure (19). Maximal oxygen uptake (VO$_{2\text{max}}$) was assessed using a constant-speed treadmill protocol (20) and the respiratory exchange ratio >1.10, a rating of perceived exertion >18, and a peak heart rate within 10 beats/min of the age-predicted maximum. Heart rate was measured during the test using heart rate monitors (Polar).

Submaximal Exercise Test

Subjects reported to the laboratory 2–7 days following their VO$_{2\text{max}}$ test. The endurance-trained subjects performed one of their usual exercise training sessions 16–24 h before this test. Seated blood pressure was measured and blood samples were obtained before and after exercise for assessment of conventional CV risk factors (baseline sample only), hematoctrit, hemoglobin, and isolation of circulating CD34+ and CD34- cells. The exercise consisted of 30 min of treadmill running at 75% of the subject's VO$_{2\text{max}}$. The treadmill speed was the same as that used for the maximal test, and %incline was adjusted to elicit the appropriate intensity according to the ACSM equation for VO$_2$ during treadmill running (1). Intensity was verified using the heart rate reserve method.

Isolation of CD34+ Cells

PBMCs were isolated from 30 ml EDTA-anticoagulated blood samples using density gradient centrifugation. CD34+ -enriched PBMCs were obtained using immunomagnetic selection according to the manufacturer's instructions (Stemcell Technologies). Briefly, the final PBMC pool was resuspended in PBS + 2% FBS at a density of 2 × 10^6 cells/ml. The CD34+ selection antibody cocktail was added (100 µl/ml) to the PBMC suspension and incubated at room temperature for 15 min. Magnetic nanoparticles were then added (100 µl/ml) and incubated for 10 min, and PBS + 2% FBS was added to bring suspensions to a final volume of 2.5 ml. Cells were then incubated in the selection magnet for 5 min. CD34+ cells remain attached to the side of the tube, and CD34- cells were poured off in the supernatant. The magnetic incubation step was repeated, and CD34+ and CD34- cell fractions were counted by hemocytometer. Flow cytometry analysis of the immunomagnetically purified CD34+ and CD34- cell fractions indicated 59% purity in the positively selected fraction. Importantly, we detected no CD34+ cells in the CD34-depleted fraction. These values are comparable to or better than previously published purity values for immunomagnetically selected CD34+ cells from unsterilized adult peripheral blood. For example, the purity of CD34+ cells reported by Ashara et al. (3) was only 16%, and Schattman et al. have reported CD34+ cell purity values ranging from 20 to 50% (15, 27, 28). Finally, the percent yield of immunomagnetically purified CD34+ was not affected by acute exercise (~1% of total PBMCs both before and after exercise). In addition, PCR confirmed high expression of CD34 mRNA in CD34+ -enriched cells, with only faint expression detected in CD34- cells.

Detection of Intracellular Free Radicals and Inhibition of NADPH Oxidase Activity

NO measurements were performed in duplicate as we have described previously (20), with modifications to examine freshly isolated cells in a 96-well format. Briefly, 1.5 × 10^5 cells stained with 10 µM DAF-FM diacetate for determination of NO levels and 10 µM dihydroethidium (DHE) for determination of O$_2^-$ levels (Molecular Probes). Cells were also incubated with or without 1 mM apocynin (a pharmacological NADPH oxidase inhibitor) to determine the mechanistic role of NADPH oxidase underlying the effects of acute exercise or differences between trained and sedentary groups. Cells were incubated with DAF-FM, DHE, and drug or vehicle treatments in a final volume of 150 µl serum-free PBS for 60 min at 37°C. Excess DAF-FM and DHE were removed by centrifugation at 500 g for 5 min, and cells were resuspended in 150 µl PBS. NO fluorescence was quantified using a fluorescent plate reader (Wallac Victor 1400, Perkin Elmer) using excitation and emission filters of 488 and 535 nm, respectively. O$_2^-$ fluorescence was measured using excitation and emission filters of 543 and 620 nm, respectively. NO and O$_2^-$...
fluorescence values were normalized to cell number and are expressed relative to the mean for the endurance-trained group’s baseline CD34⁺ cells. Intra-assay coefficients of variation for NO and O₂⁻ were 3.6% and 2.6%, respectively. Because each assay was performed on different days for each subject, interassay coefficients of variation were determined on blank samples (PBS only) to document the day-to-day variability in arbitrary/background fluorescence. The interassay coefficients of variation were 5.0% and 2.7% for NO and O₂⁻ assays, respectively, indicating good day-to-day reliability of the assays. For validation of the fluorescent probes, unfractonated PBMCs and in CD34⁺-enriched cells were treated with a NOS inhibitor (l-NAME, 300 μM) and a SOD mimetic (Tempol), which reduced the DAF and DHE fluorescence signals, respectively, to 10–20% of basal levels (n = 3 in pilot experiments). Conversely, for positive controls, treatment of cells with NO and O₂⁻ donor 3-morpholino-sydnonimine dose-dependently increased the DAF and DHE signals in by ~1,200–1,800 fold compared with basal levels.

**Semiquantitative Reverse-Transcriptase PCR**

mRNA levels of endothelial nitric oxide synthase (eNOS), NADPH oxidase subunits gp91phox and p47phox, superoxide dismutases (SOD; SOD1 and SOD2) and glutathione peroxidase-1 (GPX-1) were measured exactly as described previously (20). Additionally, cells were examined for expression of CD34 (forward primer: TGAAAAAGCTGGAGATTTGA, reverse primer: TCCTCAGCTTGACAGATCCG, vascular endothelial growth factor (VEGF) (forward primer: AAGGAGGAGCCGAGAATCCT, reverse primer: ATCTGCATGGTGATGTTGGA), and inducible NOS (iNOS; forward primer: GGGCGCAGAACTGACCTCA, reverse primer: CTCTAAAACAGCCTGCCATTCCTCAGAA). PCR products were electrophoresed on 1.5% agarose gels and visualized under UV light. Band intensities were quantified using imageJ and normalized to the signal for 18S as a reference gene. Data are expressed relative to the endurance-trained group’s normalized baseline value (set at 100%).

**Statistics**

Data were analyzed using a two-factor [group (trained or sedentary) × time (baseline and after exercise)] repeated-measures ANOVA. Assumptions of normality and homoscedasticity were verified for all data. The criterion for statistical significance was P ≤ 0.05. Data are presented as means ± SE.

**RESULTS**

**Subject Characteristics**

Endurance-trained and sedentary groups were successfully matched for age and BMI, but differed substantially (~33%; P < 0.05) in terms of VO₂max (Table 1). For one subject in the endurance-trained group, only baseline data were available (thus n = 9 for endurance-trained subjects after exercise).

**Intracellular NO and O₂⁻**

CD34⁺ cells. Sedentary subjects had 26% higher baseline NO levels compared with endurance-trained subjects (P < 0.05, Fig. 1A) and NO levels in the trained group tended to increase with acute exercise (P = 0.055; Fig. 1A). Apocynin treatments had no effect on NO levels of CD34⁺ cells in either group (P > 0.05). Analysis of main effects revealed greater NO in CD34⁺ cells of the sedentary group with data collapsed across treatment conditions (P = 0.005). The sedentary group also had higher O₂⁻ levels at baseline (P < 0.05; Fig. 1B). O₂⁻ levels increased in the endurance-trained group with acute exercise (P < 0.05) but did not change in the sedentary group (P > 0.05). Treatment with apocynin reduced O₂⁻ levels in the sedentary group both before and after exercise relative to their baseline vehicle-control condition (P < 0.05) such that baseline group differences were completely abolished (P < 0.05). However, apocynin treatment had no effect on O₂⁻ in CD34⁺ cells from the trained group.

**CD34⁺ cells.** The sedentary and endurance-trained groups had similar NO levels at baseline (Fig. 2A). In the trained group, there was no effect of acute exercise on NO levels, although apocynin (~45%, P < 0.05) increased NO levels both at baseline and after acute exercise. Acute exercise tended to increase NO in the sedentary group by 38% (P = 0.10), and this increase resulted in a significant difference between the groups after acute exercise (56% greater NO levels in the sedentary group; P < 0.05). Apocynin increased NO levels in CD34⁺ cells from the sedentary group by 46% at baseline and by 41% after acute exercise compared with their baseline vehicle-control condition (both P < 0.05). At baseline, NO levels increased with apocynin treatment to a greater extent in CD34⁺ cells of the sedentary group compared with the trained group (group difference of 40%, P < 0.05). The difference between the groups was not statistically significant in apocynin-treated CD34⁺ cells after acute exercise. There were no significant effects of training status, acute exercise, or apocynin on O₂⁻ levels in CD34⁺ cells (Fig. 2B).

**Gene Expression**

**CD34⁺ cells.** Endurance-trained subjects had ~2-fold greater eNOS mRNA levels in CD34⁺ cells compared with sedentary subjects at baseline (P < 0.05), and a similar trend was observed after acute exercise (P = 0.08; Fig. 3A). iNOS mRNA levels in the sedentary group were ~2-fold greater than those of the trained group at baseline and after acute exercise (both P < 0.05; Fig. 3B). VEGF mRNA levels were 20% higher in the trained group at baseline (P < 0.05) but the difference between the two groups was not significant after
acute exercise (Fig. 3C). SOD1 mRNA levels were ~25% and 40% higher in cells of the trained group before and after acute exercise, respectively \((P < 0.05)\), with the sedentary group having a significant acute exercise-induced decrease in SOD1 mRNA (Fig. 3D). There were no significant effects of acute exercise or training status on SOD2 or GPX1 mRNA levels (Fig. 3, E and F). p47\textsuperscript{phox} mRNA levels were higher in the trained subjects compared with the sedentary group at baseline (~40\%, \(P < 0.05\)) and after acute exercise (~50\%, \(P < 0.05\); Fig. 3G). Both groups had significant acute exercise-induced decreases in p47\textsuperscript{phox} mRNA levels \((P < 0.05)\). gp91\textsuperscript{phox} mRNA levels in CD34\textsuperscript{+} cells were similar between groups but differed after exercise by ~40\% \((P < 0.05); \text{Fig. 3H}\). Both groups had significant exercise-induced reductions in gp91\textsuperscript{phox} mRNA levels \((P < 0.05)\).

**CD34\textsuperscript{+} cells.** eNOS mRNA levels were ~25\% lower in the sedentary group at baseline and increased with acute exercise in the sedentary group \((P < 0.05, \text{Fig. 4A})\). However, there were no differences in iNOS mRNA levels between groups or
effects of acute exercise for either group (Fig. 4B). Endurance-trained subjects had ~25% higher baseline VEGF mRNA levels than the sedentary group at baseline (P < 0.05, Fig. 4C), and groups had similar VEGF mRNA levels after acute exercise. There were no differences between groups in SOD1, SOD2, or GPX1 (Fig. 4, D–F) at baseline, and acute exercise-induced increases were only evident in the sedentary group (P < 0.05 for SOD1 and SOD2, P = 0.08 for GPX1). There were no differences between groups or with acute exercise in expression of p47^phox or gp91^phox (Fig. 4, G and H).

**DISCUSSION**

The major findings in the CD34\(^+\) cell fraction are 1) intracellular NO and O\(_2^-\) levels are higher in sedentary than endurance-trained men; 2) the effect of training status on O\(_2^-\), but not NO levels, is NADPH oxidase dependent; and 3) mRNA levels of a number of angiogenic and antioxidant genes are lower in sedentary than trained men. Together, these findings point to increased nitro-oxidative stress in the sedentary state. Importantly, these data were obtained from a carefully selected and screened study sample of healthy, lean, young men with low risk for CV disease. Thus the molecular changes associated with impaired angiogenic potential of circulating CD34\(^+\) cells may occur very early in the disease-related process associated with a sedentary lifestyle.

Our finding of higher intracellular NO in CD34\(^+\) cells of the sedentary group was contrary to our original hypothesis. Data from human and animal studies indicate that maintaining optimal levels of intracellular NO in vascular endothelial cells is a key mechanism by which exercise improves CV health (14, 24). Additionally, we and others have previously shown that cultured EPCs seem to exhibit similar NO-related biology compared with fully differentiated en-
dothelial cells. For example, cultured angiogenic cells up-regulate endothelial and angiogenic genes in response to shear stress (40). EPCs from patients with coronary artery disease had undetectable eNOS and impaired migratory capacity that was reversed on treatment with a NO donor (16). We previously reported that cultured EPC colonies from endurance-trained individuals had higher NO levels compared with sedentary individuals (20). Together, these previous data provided a strong rationale for our original hypothesis that trained men would have higher NO in circulating CD34+/H11001 cells.

These previous investigations were based on the premise that eNOS is the primary source of NO in endothelial cells, and, by extension, in angiogenic cells that take on an endothelial phenotype in culture. However, the iNOS isoform also produces NO in large quantities in hematopoietic cells (32). The expression and activity of iNOS relative to eNOS can determine whether NO performs beneficial physiological functions (e.g., vasodilation in ECs, progenitor/angiogenic cell migration and homing) or harmful pathophysiological functions (e.g., inflammation, nitro-oxidative damage to cell components, and apoptosis) (25, 29, 35). These adverse consequences of high NO are amplified in the presence of excess O₂·, as the interaction of these two radicals results in the rapid and spontaneous formation of peroxynitrite (32). Peroxynitrite increases iNOS expression (5) and promotes uncoupling of the eNOS reaction (12). Thus the present findings of greater iNOS gene expression, lower eNOS gene expression, greater NO levels, and greater NADPH oxidase-derived O₂· production in the sedentary group are suggestive of a state of increased nitro-oxidative stress in CD34+ cells of sedentary individuals. However, our cross-sectional study design allows us to conclude that we have identified an association, but not necessarily a cause-effect relationship, between a sedentary lifestyle and increased nitro-oxidative stress.

Fig. 4. mRNA levels of eNOS (A), iNOS (B), VEGF (C), SOD1 (D), SOD2 (E), GPX-1 (F), p47phox (G), and gp91phox (H) in CD34+ cells of endurance-trained and sedentary men before and after acute exercise. I: RT-PCR agarose gel electrophoresis products shown are representative of data presented in A–H; separate images from the same gel are shown for each target gene. *Statistically significant difference between groups within the given experimental condition (P < 0.05). †Statistically significant within-group change relative to baseline control sample (P < 0.05).
It is also possible that our present finding of higher levels of NO in cells from the sedentary group was in contrast with our previous finding of higher NO levels in cultured EPCs from trained compared with sedentary men (20) because of the difference between cultured and freshly isolated cell characteristics. Our use of freshly isolated cells in the present study is a very different approach compared with using cultured PBMC-derived EPCs grown in a powerful endothelial growth environment (i.e., exposure of high concentrations to known endothelial growth factors for 5 days), as in our previous study. It is reasonable to suggest that cells from exercise-trained subjects may be primed for endothelial-directed differentiation under angiogenic growth conditions, owing to their higher expression of eNOS (in Ref. 20, and replicated in both cell fractions of the present study) and VEGF gene expression.

It was surprising that NO and O$_2^-$ levels increased with exercise in CD34$^+$ cells from the trained group but not the sedentary group, which also contrasted with our previous data (20). An important aspect of our study with respect to the acute exercise data is that we obtained samples at only one time point after exercise. A time course experiment is warranted to determine whether NO and/or O$_2^-$ in freshly isolated CD34$^+$ cells are unaffected by acute exercise in sedentary individuals, or whether training status determines the temporal nature of the response.

Treatment of CD34$^+$ cells with apocynin reduced intracellular O$_2^-$ in the sedentary group and normalized the baseline O$_2^-$ difference between groups, suggesting that the training-related difference in O$_2^-$ observed at baseline was a result of increased NADPH oxidase enzymatic activity in the sedentary group. Excessive NADPH oxidase-derived O$_2^-$ has been implicated in oxidative stress-related dysfunction of progenitor cell angiogenic activities (12). Our data provide the first evidence that NADPH oxidase activity is elevated in freshly isolated CD34$^+$ cells of sedentary individuals, and it is important to emphasize that because we matched groups for age and BMI, we are confident that we have isolated the effect of chronic exercise training to the extent possible given the cross-sectional study design. Nevertheless, these findings will require confirmation in a future prospective study.

The major findings in the CD34$^-$ cell fraction are 1) training status modifies the regulatory effect of NADPH oxidase enzyme activity on intracellular NO concentrations, as indicated by the effect of apocynin on NO being different between the sedentary and trained groups; 2) regular endurance exercise is associated with enhanced angiogenic gene expression (i.e., VEGF and eNOS); and 3) acute exercise increases antioxidant gene expression in CD34$^-$ cells of sedentary individuals.

The greater response of NO levels to apocynin treatment in the sedentary group than in the trained group is possibly indicative of greater basal NADPH oxidase enzymatic activity in the sedentary group. This finding is in line with our previous observations in putative EPC colonies, most of which are CD34$^-$ (20). Thus further research is required to link the role of NADPH oxidase enzymatic activity and its regulatory effects on NO levels with angiogenic actions of particular CD34$^-$ cellular subsets. In addition, our data suggest that the angiogenic properties of CD34$^-$ cells may be enhanced by acute and chronic endurance exercise, as indicated by the higher levels of basal VEGF and eNOS mRNA in trained compared with sedentary subjects, and also by the acute exercise-induced increased in antioxidant genes in CD34$^-$ cells of sedentary subjects. However, CD34$^-$ cells as a whole should probably not be thought of as angiogenic per se, as these cells are mostly white blood cells (T-cells, monocytes, etc.) with primarily immune function, and it would be interesting to know which cell populations within the CD34$^-$ fraction are driving the present results. A reasonable hypothesis for future research is that the functions of angiogenic CD34$^-$ monocyte and T-cell fractions (4, 6, 15, 22, 23) may benefit from acute (based on our finding of increased antioxidant gene expression) and chronic (based on our finding of higher eNOS and VEGF gene expression) endurance exercise.

Limitations

It would have been advantageous to have selected additional subpopulations within the CD34$^+$ fraction, e.g., CD34$^+$/VEGFR2$^+$ or CD34$^+$/CD45$^-$ EPCs; however, cell yields in pilot studies were too low to perform NO or O$_2^-$ assays. Thus we chose to examine only two subfractions based on the presence or absence of the CD34 antigen, which yielded an appropriate number of cells to work with and seemed reasonable given their accepted role as proangiogenic cells under certain conditions. We did not measure all aspects of angiogenic function (e.g., in vitro capillary formation, migration, and assessments of target genes at the protein level), and clearly data generated from such assays would have aided the interpretation of the unexpected finding of higher NO levels in CD34$^+$ cells of the sedentary group.

Conclusions

The present study provides the first evidence that physical inactivity is associated with increased nitro-oxidative stress in CD34$^+$ cells, and it appears that the notion that higher levels of NO are necessarily associated with beneficial cellular outcomes will require further investigation. In particular, the relative contribution of eNOS vs. iNOS activities to CD34$^+$ cell-mediated vascular endothelial maintenance needs to be examined. As we have investigated these outcomes in healthy young men at low risk for CV disease, we have isolated the effects of exercise training independent of other confounding factors. Thus we anticipate that the information provided by our study will be particularly useful for the development of therapeutic applications of CD34$^+$ cells in regenerative medicine. The efficacy of cell therapy trials may be improved if exercise training as a strategy to reduce nitro-oxidative stress were employed in conjunction with cell infusions. However, further investigation is required to confirm the nitro-oxidative stress at the functional level (e.g., nitration of proteins) in CD34$^+$ cells. Overall, our data provide a strong rationale for further research to clarify the mechanisms of exercise-induced improvements in the vascular repair capacity of circulating CD34$^+$ PBMCs.

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

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