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Relationship Between Circulating Progenitor Cells, Vascular Function and Oxidative Stress with Long-Term Training and Short-Term Detraining in Older Men

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Relationship between circulating progenitor cells, vascular function and oxidative stress with long-term training and short-term detraining in older men

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Abstract

Exercise may contribute to the maintenance of vascular function via enhanced liberation and action of bone-marrow-derived progenitor cells. Activity related changes in oxidative stress may also influence the number and function of these cells. In the present study, we sought to determine (i) whether adaptations in reactive hyperaemic FBF (forearm blood flow) response associated with long-term endurance exercise and short-term detraining were related to resting putative progenitor cell number and function, and (ii) whether oxidative stress affected these factors. Participants included men with a history of more than 30 years of moderate-to-high-intensity exercise (HI group) and healthy low-active age- and BMI (body mass index)-matched control subjects (LO group). Vascular reactive hyperaemic FBF response, resting $CD34^+$ and $CD34^+/VEGFR2^+$ (vascular endothelial growth factor receptor 2^+] cell number, CFU-EC (colony-forming unit-endothelial cell) count and CFU-EC senescence were evaluated. Oxidative stress measures included OxLDL (oxidized low-density lipoprotein) and TAC (total antioxidant capacity). These measures were assessed following 10 days of detraining in the HI group. The HI group had greater peak reactive hyperaemic FBF responses compared with the LO group, despite no difference in resting $CD34^+$ cell number, $CD34^+/VEGFR2^+$ cell number, CFU-EC colonies or CFU-EC senescence. With detraining in the HI group, $CD34^+$ cells declined 44 %, and the percentage change in $CD34^+/VEGFR2^+$ cells was positively correlated with the change in FBF response to reactive hyperaemia. The percentage change in $CD34^+/VEGFR2^+$ cells and the percentage change in EPC (endothelial progenitor cell) senescence with detraining were related to the percentage change in TAC. These results reveal that changes in reactive hyperaemic FBF are closely related to activity dependent dynamic changes in $CD34^+/VEGFR2^+$ cell number, which may be influenced by alterations in oxidative stress.

Keywords

aging; antioxidant; endothelial progenitor cell; exercise; forearm blood flow; physical inactivity

INTRODUCTION

Disruption of vascular endothelial integrity and function is a major contributor to the development of atherosclerosis and CVD (cardiovascular disease) [1]. Circulating HPCs (haemapoietic progenitor cells) and EPCs (endothelial progenitor cells) may play an

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important role in the maintenance of vascular integrity [2,3]. Reduced EPC number and function have been associated with death from CVD [4], CVD risk factors [5], future coronary events [6] and endothelial dysfunction [7].

Physical activity is associated with improved endothelial health and vascular function [8–10]. In general, an acute bout of exercise increases the number of EPCs in the blood in healthy individuals and in patients with CVD or CVD risk factors [11,12]. Endurance exercise training has been associated with improved EPC clonogenic and migratory capacities in healthy men [13]. Short-term exercise training (4–12 weeks) has also been associated with increased circulating EPCs in patients with CAD (coronary artery disease) [14], CAD risk factors [15] and chronic heart failure [16], but not in healthy young and older men [17]. Therefore it is well documented that short-term exercise training increases resting EPC number and function in patients with CVD; however, it remains unclear what effect long-term exercise training has on EPCs in healthy individuals.

EPCs are sensitive to changes in redox potential and have a high expression of the antioxidant enzymes catalase, glutathione peroxidase and MnSOD (manganese superoxide) [18,19]. Disruptions in redox state can detrimentally affect EPC function [20]. *In vitro* evidence indicates that the highly atherogenic oxLDL [oxidized LDL (low-density lipoprotein)] decreased EPC number, proliferative capacity, migration, adhesion [21] and senescence [22]. Exercise training influences oxidative stress and antioxidant capacity [23,24]. Therefore it is possible that changes in oxidative stress status that occur with changes in physical activity status could influence circulating EPC number and/or function.

The aim of the present study was to investigate the influence of long-term endurance exercise training on the association of $CD34^+$ cells, $CD34^+/VEGFR2^+$ [VEGF (vascular endothelial growth factor) receptor 2^+] cells, CFU-EC (colony-forming unit-endothelial cell) count, and CFU-EC senescence with reactive hyperaemic FBF (forearm blood flow) response. To achieve this purpose, we studied healthy highly active men with a long-term physical activity history (HI group) and low-active sedentary but healthy men (LO group). In addition, through the use of short-term detraining, we sought to evaluate the influence of changes in physical activity state in the HI group on the reactive hyperaemic FBF response and EPCs. Finally, we investigated differences in markers of oxidative stress, antioxidant capacity and NO bioavailability as potential contributors to changes observed in EPCs.

MATERIALS AND METHODS

Participants

Written informed consent was obtained from all subjects, and the study was approved by the University of Maryland College Park Institutional Review Board. A total of 12 healthy long-term endurance-trained men (HI group) and 11 healthy age- and BMI (body mass index)-matched sedentary men (LO group) participated in the study. To verify physical activity status for inclusion, all participants completed a physical activity questionnaire. The LO group did not participate in or have a recent history of moderate-to-intense regular endurance exercise (< 2 days/week; 20 min/session). Ten of the HI group participated in 10 days of detraining following baseline testing. Physical examination, resting BP (blood pressure), blood chemistry and screening for CVD via a 12-lead ECG stress test were performed to verify study eligibility. Participants were non-smokers and were not taking medication that has been demonstrated to affect EPC number and/or function [25]. Participants were tested following an overnight fast, 24 h without alcohol and caffeine and 48 h without vitamins or medication prior to testing.

Testing procedures

Participants underwent treadmill $\dot{V}_{O2max}$ [maximal $\dot{V}_{O_2}$ (oxygen consumption)] testing, assessment of reactive hyperaemia, venipuncture for blood samples and body composition testing. Testing of men in the HI group occurred 24 h after the last exercise session to eliminate the acute effects of physical activity. The men in the HI group who volunteered for detraining (n = 10) returned following the 10 days for repeated measures of reactive hyperaemic FBF response, blood-derived parameters and body composition. Those participating in detraining were instructed to maintain a stable weight for the 10 days of training cessation and recorded morning and evening weight in a data log. FBF and NO testing occurred following 3 days of a low-nitrate diet [26].

$\dot{V}_{O2max}$—Treadmill $\dot{V}_{O2max}$ testing was performed under physician supervision. Heart rate, ECG and BP were monitored throughout the test. Expired air was sampled using indirect calorimetry (Oxycon Pro; Viasys). Treadmill testing was a modification of the Bruce protocol where treadmill speed was determined by the investigator based on heart rate such that $\dot{V}_{O2max}$ was achieved between 8 and 12 min. For all of the tests, three out of four criteria were met for a valid $\dot{V}_{O2max}$: RER (respiratory exchange reserve) ≥1.15, maximal heart rate (age-predicted) was reached, a plateau in the $\dot{V}_{O_2}$ increase with an increase in work rate (< 250 ml $\dot{V}_{O_2}$ increase) was observed, or the subject indicated exhaustion.

FBF response to reactive hyperaemia—FBF was evaluated in the non-dominant arm with strain gauge plethysmography at baseline and during reactive hyperaemia as described previously in our laboratory [27]. Briefly, basal FBF was measured in triplicate. Forearm ischaemia was achieved by a 5-min occlusion with the upper-arm pressure cuff inflated to a pressure that was 50 mmHg above the participant's resting systolic BP. Following forearm ischaemia, FBF was measured every 15 s continuously (measurement for 7 s and rest for 8 s) for 3 min. Reactive hyperaemic measures included peak FBF and minimum FVR (forearm vascular resistance; mmHg · ml^{-1} · min^{-1} · 100 ml^{-1} of tissue). As the maximal hyperaemic response has been demonstrated to occur within 1 min of occlusion release [28], the AUC (area under the curve) for the 1 min of the hyperaemic response (AUC_{1min} in ml/100 ml) was calculated according to the trapezoidal rule.

Circulating progenitor cell number—$CD34^+$ (HPC) and $CD34^+/VEGFR2^+$ (putative EPC) number were determined by flow cytometry. PBMCs (peripheral blood mononuclear cells) were separated via density centrifugation (Ficoll–Paque Plus; GE Healthcare). A total of 1×10^6 mononuclear cells were immunostained with FITC-conjugated monoclonal antibodies specific for human CD34 (BD Biosciences) and PE (phycoerytherin)-conjugated monoclonal antibodies specific for human VEGFR2 (R&D Systems). At least 5×10^5 cells were labelled with appropriate controls for isotype and positive antibodies. All cells were FcR-blocked (Miltenyi Biotech) and fixed in 4 % paraformaldehyde. Flow cytometry was performed in the Flow Cytometry/Cell Sorting CORE Laboratory with a Beckman Coulter Epics Elite ESP flow cytometer and cell sorter. The forward-side-scatter plot was used to identify the lymphocyte gate. A total of 100 000 events per sample were acquired. Reproducibility was tested with a subset of samples (r = 0.90, P < 0.001).

CFU-EC count—The CFU-EC assay was performed with the EndoCult Liquid Medium Kit (Stem Cell Technologies), according to the manufacturer's specifications. Colonies were counted after 5 days of culture by two independent observers to address reproducibility (r = 0.98, P < 0.001). A colony was defined as a central core of round cells with more elongated sprouting cells at the periphery. The endothelial lineage of these cells has been confirmed previously via immunocytochemical staining for vWF (von Willebrand factor), VEGFR2 and CD31 [7].

CFU-EC senescence—Cellular senescence is an indicator of exhaustion of the replicative potential of a cell [29], and higher EPC senescence has been related to increased CVD risk [7]. CFU-EC senescence was determined using a β-gal (β-galactosidase) Cellular Senescence assay kit (Chemicon International). After CFU-EC enumeration on day 5, the culture medium was changed and cells were incubated for an additional 2 days. On day 7, cells were fixed and SA-β-gal (senescence-associated β-gal) stain solution was added to the wells. Cells distant from central colonies from four randomly selected fields that contained 100–200 cells each were analysed. β-Gal-positive cells were counted as senescent, and senescence was quantified by the average percentage of β-gal-positive cells in each of the four wells.

NOx (nitrate/nitrite) assay—Plasma was filtered via centrifugation with 10 kDa cut-off centrifugal ultrafilters (Millipore Corporation) at 9000 ***g*** and 4°C for 50 min. NOx concentration of the filtered plasma was analysed via a modification of the Griess reaction, as described previously [30]. Absorbance was read at 541 nm (Emax; Molecular Devices). Samples were assayed in duplicate. Inter- and intra-assay CVs (coefficients of variation) were 9.9 and 3.4 % respectively.

Plasma OxLDL—Plasma OxLDL levels were measured using a commercially available competitive ELISA kit (Mercodia) as described previously [31]. Two-level control samples (Mercodia Oxidized LDL Control Kit) were assayed to confirm assay performance. All samples were analysed in a single assay to eliminate inter-assay variability. The intra-assay CV was 6.8 %.

TAC (total antioxidant capacity)—TAC (aqueous and lipid-soluble antioxidants, including vitamins, proteins, lipids, glutathione and uric acid) in plasma was analysed in duplicate using a commercial kit (Cayman Chemical) developed from the protocol described by Miller and Rice-Evans [32]. All samples were analysed in a single assay to eliminate inter-assay variability. The intra-assay CV was 4.1 %.

Body composition—Height and weight were recorded and BMI (kg/m^2) was calculated for all subjects. To quantify the percentages of lean and fat mass, body composition was assessed via dual-energy X-ray absorptiometry (Discovery A, software version 12.7.1; Hologic).

Framingham CHD (coronary heart disease) risk—To assess conventional CHD risk factors, risk scores for CHD were calculated based on equations from the Framingham study [33].

Statistical analysis

Sample size ($\beta = 0.8$, $\alpha = 0.05$) for the study was calculated *a priori* based on effect size estimates from findings published in the literature for changes in $CD34^+/VEGFR2^+$ cells [12,14], CFU-EC colony number [12,14], CFU-EC senescence [7] and FBF with exercise training [34]. Student's *t* tests were used to test for differences between the HI and LO groups. Where data were not normally distributed, the non-parametric Mann–Whitney *U* test (Wilcoxon rank-sum test) was used to compare differences between groups. Paired Student's *t* tests were used to examine differences between the HI group before and after detraining. Where paired data were not normally distributed, the non-parametric Wilcoxon signed-rank test was used to compare athletes before and after detraining. Differences between groups for the reactive hyperaemic FBF response were tested using a repeated measures ANOVA. Correlation coefficients were used to examine the relationships between variables. Multiple regression analysis was used to determine the predictors of $CD34^+$/

VEGFR2$^+$ cells, CFU-EC colony number and CFU-EC senescence. Data transformation was performed to satisfy test assumptions where appropriate. An α level of 0.05 was used to indicate statistical significance.

RESULTS

Participant characteristics

Groups were successfully matched for age and BMI (Table 1). Running was the predominant physical activity mode in the HI group, as all 12 HI participants engaged in running with an average of 5 ± 0.4 days/week. Six of the 12 HI participants also reported cycling, ranging from occasionally to 3 days/week. Swimming was reported in three of the HI group, ranging from occasionally to 3 days/week. Other modes of exercise (i.e. weights and rowing) were reported in three HI participants (range of 1 to 3 days/week). The HI group had a significantly and substantially higher $\dot{V}_{O2max}$ than the LO participants. Body weight and BMI did not change in the men in the HI group who participated in the 10-day detraining (Table 1); however the percentage body fat was significantly ($P < 0.001$) greater after detraining (Table 1). Compared with the LO participants, the HI group had lower body fat and diastolic BP, and a better blood chemistry profile, including lower plasma triacylglycerols (triglycerides), higher HDL (high-density lipoprotein)-cholesterol and lower VLDL (very-low-density lipoprotein)-cholesterol; importantly, none of these factors correlated with the main outcome variables of reactive hyperaemic FBF response, CD34$^+$ cell number, CD34$^+$/VEGFR2$^+$ cell number, CFU-EC number or CFU-EC senescence.

Resting TAC was significantly lower in the HI group compared with the LO group (2.25 ± 0.05 compared with 2.54 ± 0.06 mmol/l; $P = 0.002$). There were no differences in OxLDL and NOx between the groups.

Reactive hyperaemic FBF

Resting FBF ranged from 0.95 to 3.23 ml · min^{-1} · 100 ml^{-1} of tissue and was not different between the HI and LO groups (Figure 1). Following cuff occlusion, FBF increased significantly in the HI ($P < 0.001$) and LO ($P < 0.001$) groups (838 ± 90 and 609 ± 74 % respectively). Peak FBF was significantly greater in the HI group compared with the LO group ($P = 0.03$), which corresponded to a lower minimum FVR in this group ($P = 0.03$).

There were no significant changes in any of the FBF measurements in the HI group following the 10 days of detraining. However, peak FBF remained significantly higher and minimum FVR remained significantly lower in the HI group after detraining compared with the LO group [19 ± 2 compared with 13± 1 ml · min^{-1} · 100 ml^{-1} ($P = 0.005$) and 5 ± 1 compared with 7 ± 1 mmHg · ml^{-1} · min^{-1} · 100 ml^{-1} ($P = 0.004$) respectively].

Progenitor cells

When the HI and LO groups were combined, the CFU-EC count was negatively correlated with the Framingham risk percentage ($r = -0.49$, $P = 0.02$). In addition, regression analysis indicated that circulating OxLDL levels significantly predicted CFU-EC colonies ($P = 0.03$) when the groups were combined. However, CD34$^+$ cells (447 ± 143 compared with 341 ± 140 cells/100 000 events; $P = 0.26$), CD34$^+$/VEGFR2$^+$ cells (99 ± 39 compared with 43 ± 13 cells/100 000 events; $P = 0.23$), CFU-EC colonies (18 ± 4 compared with 14± 4; $P = 0.26$) and CFU-EC senescence (23.4 ± 3.7 compared with 22.6 ± 5.1 %; $P = 0.45$) at rest were not different between the HI and LO groups.

Responses to detraining

As shown in Figure 2, HPCs significantly declined by 44 % in the HI group following 10 days of detraining (322 ± 91 compared with 179 ± 37 cells/100 000 events; $P = 0.03$). $CD34^+/VEGFR2^+$ cell number (Figure 3), CFU-EC colonies and CFU-EC senescence tended to decrease with detraining, although the declines were not significant [89 ± 44 vs. 16 ± 7 cells/100 000 events ($P = 0.13$); 18 ± 4 compared with 12 ± 4 colonies ($P = 0.34$); and 23 ± 3 compared with 16 ± 3 % ($P = 0.11$) respectively]. Overall, the percentage change in the $CD34^+/VEGFR2^+$ cell number correlated significantly with baseline $CD34^+/VEGFR2^+$ cell number ($r = -0.76$, $P = 0.01$) in the ten HI participants who discontinued training.

In the HI participants who underwent detraining, the percentage change in $CD34^+/VEGFR2^+$ cells from before to after the 10 days was significantly correlated with the percentage change in AUC_{1min} in response to reactive hyperaemia ($r = 0.70$, $P = 0.02$). In the regression analysis, the percentage change in the AUC significantly predicted the change in $CD34^+/VEGFR2^+$ number with detraining ($P = 0.02$). In addition, the percentage change in CFU-EC senescence with detraining was significantly related to the percentage change in TAC from before to after detraining ($r = -0.66$, $P = 0.04$). There was a trend for a relationship between the percentage change in $CD34^+/VEGFR2^+$ cell number and percentage change in TAC ($r = 0.62$, $P = 0.056$).

DISCUSSION

The results of the present study provide evidence that circulating $CD34^+/VEGFR2^+$ cells at rest are associated with dynamic changes in vascular reactivity with short-term detraining, but not with vascular adaptations resulting from long-term exercise training in men. Furthermore, changes in putative EPC number and senescence with short-term detraining may be influenced by plasma antioxidant capacity.

Previous studies have indicated that an acute exercise bout increases circulating bone-marrow-derived endothelial-targeted cells in the blood [11,12]; however, results from studies evaluating resting EPC number following exercise training in humans are not consistent. Specifically, groups have reported that exercise training improves resting $CD34^+/VEGFR2^+$ cells in patients with CAD [14] and chronic heart failure [16] and $CD34^+/VEGFR2^+/CD133^+$ cells in patients with CAD and CAD risk factors [15], but not in healthy young and older men [17] trained for 8 weeks. Our present results revealed no difference in $CD34^+$ or $CD34^+/VEGFR2^+$ cells at rest between our sedentary control and athlete groups, who had dramatically different life-long exercise habits. It appears that exercise training, either short-term (8 weeks [17]) or long-term (>30 years in our present study), does not affect resting $CD34^+/VEGFR2^+$ cells in healthy individuals, whereas exercise training may increase resting circulating EPCs in individuals with CVD and, therefore, endothelial damage. Laufs et al. [14] reported a 75 % increase in $CD34^+/VEGFR2^+$ cells with 28 days of training (from 23 ± 7 to 40 ± 12 $CD34^+/VEGFR2^+$ cells/100 000 events) in patients with stable CAD. Notably, $CD34^+/VEGFR2^+$ cells before training in this population were lower than those of our healthy sedentary participants and our sedentary group had greater $CD34^+/VEGFR2^+$ cell number at rest (43 ± 14 cells/100 000 events) than the CAD patient population had following exercise training [14]. Therefore the similarity in $CD34^+/VEGFR2^+$ cell number between athletes and low-active sedentary participants may have been because our control group was disease-free.

In the HI group, we found that $CD34^+$ cells decreased following 10 days with no exercise. $CD34^+$ cells have been negatively correlated with CVD risk [35] and independently predicted cardiovascular events, total events and death [36]. Additionally, a low $CD34^+$ count was reported to significantly increase adverse outcome risk in patients with the

metabolic syndrome [36]. There is little information regarding resting $CD34^+$ cells and exercise; specifically, these bone-marrow-derived HPCs have been found to be both higher at rest in middle-aged runners compared with sedentary controls [37] and not different in older trained men compared with older untrained men [17]. To our knowledge, the present study is the first to report a decrease in these cells in long-term trained runners following a cessation of exercise.

In an important finding, the detraining-induced changes in $CD34^+/VEGFR2^+$ cells were significantly correlated with changes in forearm vascular response to reactive hyperaemia. The relationship between EPC number and vascular function has been shown in the coronary vasculature in patients with CAD [38], but not with respect to short-term dynamic changes in the peripheral vasculature in healthy individuals. The role of EPCs in the vasculature has been investigated for over 10 years; however, there are few *in vivo* results showing a relationship between the number of $CD34^+/VEGFR2^+$ cells in the circulation and a vascular functional measure. The present study is the first report of a relationship between these cells with both reactive hyperaemic FBF response and Framingham risk score, supporting further the role of these cells in the maintenance of a healthy vasculature.

Relatively short-term detraining (10 days) in the HI group revealed a large degree of individual variability in the change in $CD34^+/VEGFR2^+$ cell number. The literature to date on changes in $CD34^+/VEGFR2^+$ EPCs with acute exercise and training also has revealed a significant degree of individual variability [12,14,17]. In our present analysis, we found that the change in $CD34^+/VEGFR2^+$ cell number with detraining ranged from −99 to 142 %. In fact, the response to exercise of most health-related phenotypes is typified by individuals that have differing grades of response [39]. It is possible that individual differences in changes in EPCs with detraining could be the result of uninvestigated physiological factors or genetic influences, although further studies are necessary to confirm this speculation.

In our present study, we observed relationships between oxidative stress variables and EPCs. Oxidative stress represents the balance between the pro- and anti-oxidant forces in a system and may play a role in vascular integrity and function. Elevated plasma OxLDL levels promote atherosclerosis [40], have been shown in patients with CAD and predict future cardiovascular events in these patients [41]. OxLDL decreases EPC clonogenic capacity [42], increases EPC senescence [22] and decreases VEGF-stimulated differentiation of cultured EPCs [43]. We found that plasma OxLDL was a significant predictor of CFU-EC colony number, independent of physical activity status. To our knowledge, the present study is the first to report *in vivo* evidence that plasma OxLDL is significantly related to CFU-EC colonies in healthy individuals.

EPCs have been shown to exhibit a high expression of antioxidant enzymes such as catalase, MnSOD and glutathione peroxidase [18,19], which may be necessary for survival in oxidative-stress-rich vascular regions damaged by atherosclerosis. *In vitro* evidence has shown that incubation of EPCs with vitamins C and E had positive effects on EPC number [44]. To date, there is little *in vivo* evidence supporting this relationship. Our present analyses showed that, with detraining, increased TAC was related to increased circulating $CD34^+/VEGFR2^+$ cells and there was a trend for a relationship between decreased TAC and greater CFU-EC senescence. These findings support the relationship between dynamic changes in oxidative stress and EPCs with detraining. Intense exercise training decreases circulating antioxidants [24] and 10 days of physical inactivity may have allowed TAC to recover in some athletes. Although these results are intriguing, the measurement of TAC only gives an overview of antioxidant status. Future studies are needed to elucidate the relationship between specific antioxidant enzymes, such as glutathione peroxidase, MnSOD

and catalase, and EPCs as well as the signalling cascades involved with exercise training and inactivity.

Although HI participants predominantly performed lower-body (running) exercise training for over 30 years, we observed significant differences in the peak reactive hyperaemic FBF response and AUC_{1min} compared with that of sedentary LO participants. Furthermore, with cessation of exercise training for 10 days, we observed a decline in reactive hyperaemic FBF response in the HI group, supporting a systemic effect of exercise on vascular function.

One limitation of our present study is the large degree of individual variability in our EPC measurements. We believe that the individual variability is indicative of other factors that may have been present but not measured in the current study. Our sample size precluded an investigation of potential factors that may have been responsible for differences between responders and non-responders to detraining. Although we did not find a significant difference in resting $CD34^+/VEGFR2^+$ cells between HI and LO groups, our LO group was healthy, which may make this group unique for their age. Further studies are needed to elucidate the molecular mechanisms that underlie the relationship between dynamic changes in EPCs, vascular function and oxidative stress.

We utilized the reactive hyperaemic FBF response to measure vascular function. In addition to NO, other factors such as adenosine, mechanical and neurogenic factors may also play a role in the vasodilatory response to reactive hyperaemia. However, studies utilizing NOS (NO synthase) agonists or blockade (N^G-monomethyl-l-arginine) have shown that endothelium-derived NO plays a significant role in the reactive hyperaemic vasodilatory response [45,46], and there is a strong correlation between peak reactive hyperaemic FBF and peak vasodilation via acetylcholine ($r = 0.89$ [47]).

In conclusion, circulating $CD34^+/VEGFR2^+$ cells at rest appear to be associated with dynamic changes in reactive hyperaemic FBF response with short-term detraining in men. Furthermore, changes in circulating $CD34^+/VEGFR2^+$ cells that occur with short-term detraining may be influenced by oxidative stress. Our findings, in concert with the available literature on exercise and progenitor cells, support EPCs as a potentially important mechanism by which regular exercise maintains healthy vascular function.

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Abbreviations

AUC	area under the curve
BMI	body mass index
BP	blood pressure
CAD	coronary artery disease
CFU-EC	colony-forming unit-endothelial cell
CHD	coronary heart disease

CV	coefficient of variation
CVD	cardiovascular disease
EPC	endothelial progenitor cell
FBF	forearm blood flow
FVR	forearm vascular resistance
***β*-gal**	*β*-galactosidase
HDL	high-density lipoprotein
HI	highly active with a long-term physical activity history
HPC	haemapoietic progenitor cell
LDL	low-density lipoprotein
LO	low-active sedentary but healthy
MnSOD	manganese superoxide
NOx	nitrate/nitrite
OxLDL	oxidized LDL
TAC	total antioxidant capacity
VEGF	vascular endothelial growth factor
VEGFR	VEGF receptor
VLDL	very-low-density lipoprotein
$\dot{V}_{O_2}$	oxygen consumption
$\dot{V}_{O_{2max}}$	maximal $\dot{V}_{O_2}$

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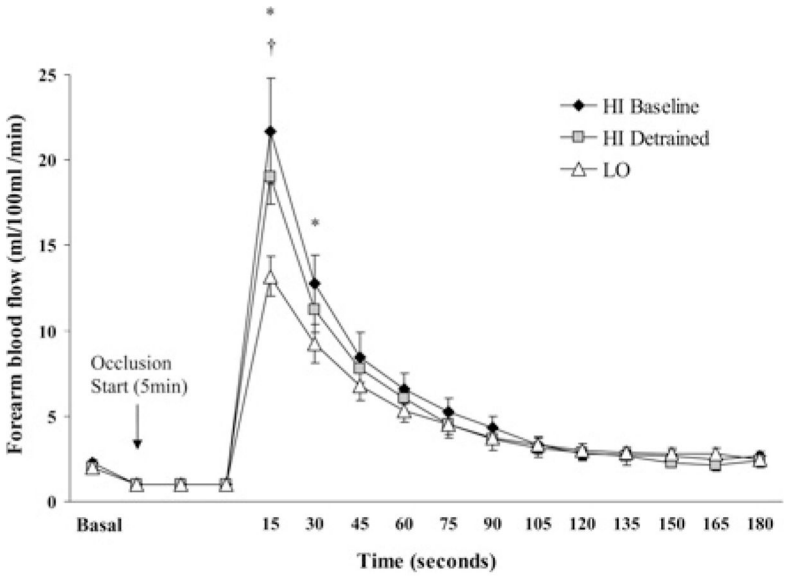

Figure 1. Reactive hyperaemic response in the HI group before and after detraining and the LO control subjects

Values are means ± S.E.M. Baseline and detrained represent the results for the ten men in the HI group who underwent 10 days of detraining. LO group, $n = 11$. *$P < 0.05$ for the HI baseline group compared with the LO group; †$P < 0.05$ for the HI detrained group compared with the LO group.

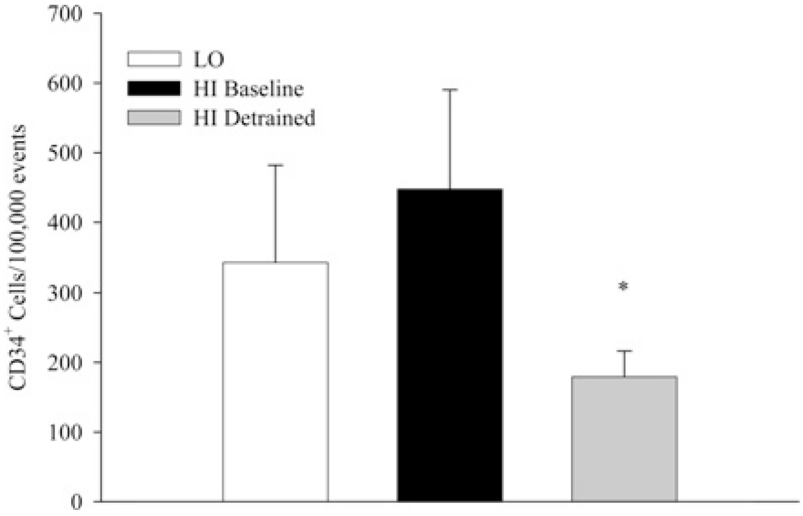

Figure 2. CD34+ cells before and after 10 days of detraining
*$P < 0.05$ compared with the HI baseline group.

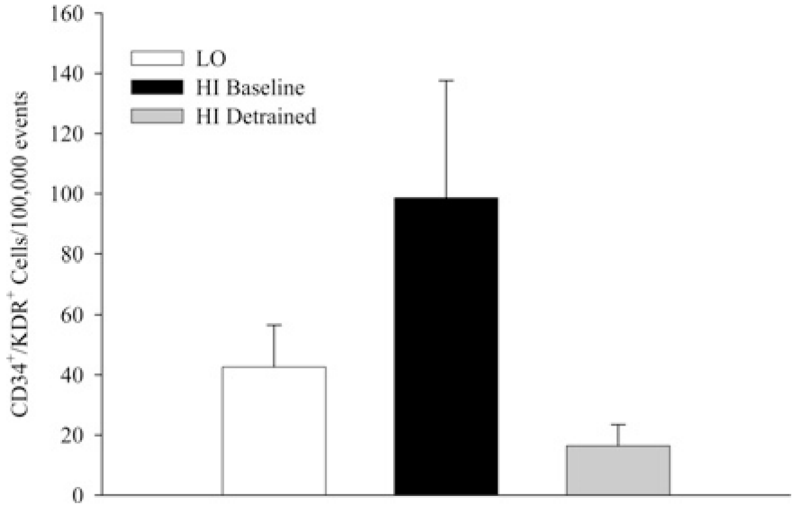

Figure 3. $CD34^+/VEGFR2^+$ cells before and after 10 days of detraining

Values are means ± S.E.M. Because data for $CD34^+/VEGFR2^+$ cell number were not normally distributed, data were analysed using the non-parametric Wilcoxon signed rank test.

Table 1

Descriptive data of the study populations

	Group		
Characteristic	**LO (*n* = 11)**	**HI baseline (*n* = 12)**	**HI detrained (*n* = 10)**
Age (years)	64 ± 2	62 ± 2	62 ± 2
Height (m)	1.76 ± 0.02	1.78 ± 0.03	1.75 ± 0.02
Weight (kg)	73.0 ± 2.4	70.1 ± 2.9	67.6 ± 2.3
BMI (kg/m^2)	23.6 ± 0.7	22.0 ± 0.8	22.1 ± 0.7
Body fat (%)	23.5 ± 1.8	18.0 ± 1.3*	18.1 ± 1.1†
SBP (mmHg)	129 ± 3	122 ± 3	114 ± 3
DBP (mmHg)	85 ± 2	79 ± 2*	77 ± 2†
Glucose (mg/dl)	99 (91–122)	94.5 (83–104)	–
Cholesterol (mg/dl)	194.2 ± 10.6	199.1 ± 8.9	–
Triacylglycerols (mg/dl)	103.0 ± 13.5	66.2 ± 8.4*	–
HDL-cholesterol (mg/dl)	51.0 ± 4.6	71.2 ± 3.3*	–
LDL-cholesterol (mg/dl)	122.5 ± 11.4	114.8 ± 8.4	–
VLDL-cholesterol (mg/dl)	20.6 ± 2.7	13.2 ± 1.7*	–
Total cholesterol/HDL-cholesterol	4.2 ± 0.5	2.9 ± 0.2*	–
Framingham 10-year CHD risk (%)	12.82 ± 2.2	7.67 ± 0.86*	–
$\dot{V}_{O_{2max}}$ (ml/kg of body weight)	28.1 ± 1.7	50.0 ± 1.9*	–
$\dot{V}_{O_{2max}}$ (litres/min)	2.07 ± 0.12	3.51 ± 0.17*	–
Days/week running	–	5 ± 0.4	
Miles/week running	–	36 ± 3	
Years exercising	–	32 ± 3	

Values are means ± S.E.M. For data that were not normally distributed, medians (lowest value-highest value) are shown, and *P* values were obtained from a-Mann–Whitney *U* test.

*$P \leq 0.05$ compared with the LO group;

†$P \leq 0.05$ compared with the HI baseline group. Owing to participant burden, not all parameters were measured following detraining.