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# Short-term exercise training improves flow-mediated dilation and circulating angiogenic cell number in older sedentary adults.

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**Running Title:** Short-term exercise training endothelium effects

**ABBREVIATIONS**

Ab, antibody
CAC, circulating angiogenic cell
CT, cycle threshold
CVD, cardiovascular disease
DAF-M, 4-amino-5-methylamino-2′,7′-difluorofluorescein diacetate
DXA, dual x-ray absorptiometry
ECG, electrocardiogram
EDTA, ethylenediaminetetraacetic acid
FACS, fluorescent activated cell sorting
FMD, flow-mediated dilation
GRECC, Geriatric Research Education and Clinical Center
$H_2$DCFDA, 2 μM 2′,7′-dichlorodihydrofluorescein diacetate
HDL-C, high-density lipoprotein cholesterol
LDL-C, low-density lipoprotein cholesterol
NO, nitric oxide
PBMC, peripheral blood mononuclear cell
PCR, polymerase chain reaction
PEG, polyethylene glycol
ROS, reactive oxygen species
$VO_{2max}$, maximal oxygen consumption

**ABSTRACT**

Cardiovascular disease (CVD) risk increases with age due, in part, to impaired endothelial function and decreased circulating angiogenic cell (CAC) number and function. We sought to determine if 10 days of aerobic exercise training improves endothelial function, CAC number and intracellular redox balance in older sedentary adults. **METHODS**: Eleven healthy subjects (4 men, 7 women), 61 ± 2 yrs of age participated in 60 minutes of aerobic exercise at 70% $VO_2$max for 10 consecutive days while maintaining body weight. Before and after training, endothelial function was measured as flow-mediated dilation of the brachial artery and fasting blood was drawn to enumerate three CAC subtypes. Intracellular ROS and NO in CD34+ CACs were measured using fluorescent probes and reinforced via qPCR. **RESULTS**: Flow-mediated dilation improved significantly following training (10 ± 1.3% before vs.16 ± 1.4% after training; $P<0.05$). Likewise, CD34+/KDR+ number increased 104% and KDR+ number increased 151% ($P<0.05$ for both), although CD34+ number was not significantly altered ($P>0.05$). Intracellular NO and ROS levels in CD34+ CACs were not different after training ($P>0.05$ for both). mRNA expression of SOD1, eNOS and NOX-2 and p47phox in CD34+ CACs was not significantly altered with training ($P>0.05$). **CONCLUSIONS**: Ten consecutive days of aerobic exercise increased flow-mediated dilation and CAC number in older, previously sedentary adults, but did not affect intracellular redox balance in CD34+ CACs. Overall, these data indicate that even short-term aerobic exercise training can have a significant impact on CV risk factors.

**Keywords:** short-term exercise; circulating angiogenic cells; aerobic exercise training; endothelial function

## INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death in developed countries around the world and it is estimated that by 2030, over 40% of the population will have some form of CVD with direct medical costs tripling as a result (Heidenreich et al. 2011). Advancing age is a risk factor for the development of CVD due, in part, to impaired endothelial function (Seals et al. 2011). With aging, an imbalance occurs between inflammatory, oxidative stress, and vasodilatory factors that lead to vascular endothelial dysfunction and contribute to the increased risk of developing CVD (Seals et al. 2011).

Chronic aerobic exercise has been found to prevent the age-related dysfunction of the endothelium due, in part, to increased bioavailability of the vasodilator nitric oxide (NO) and improved intracellular redox balance. Our lab and others found that endurance exercise-trained older adults had greater endothelial function compared to sedentary older adults (Seals et al. 2008, Witkowski et al. 2009, DeVan and Seals 2012) and similar endothelial function compared to younger adults (Seals et al. 2008, DeVan and Seals 2012), emphasizing the importance of aerobic exercise training for the preservation of endothelial function with age. Additionally, several studies have reported significant improvements in endothelial function when patients with established coronary artery disease, type 2 diabetes, or other cardiometabolic diseases completed 6-12 weeks of aerobic exercise training, demonstrating that exercise training has the ability to improve endothelial function even in clinical populations (Kwon et al. 2011, Currie et al. 2013, Kim et al. 2014, Schreuder et al. 2014).

Several factors likely contribute to the age-related development of endothelial dysfunction. Circulating angiogenic cells (CACs) are involved in the repair and maintenance of the vascular endothelium, with the number and function of certain CACs being inversely related to CVD risk (Hill et al. 2003, Bielak et al. 2009, Bakogiannis et al. 2012). The term CAC is broad and refers to circulating cells with angiogenic potential. CD34 is the most commonly studied cell surface marker that has been studied and identifies cells with progenitor-like characteristics (Mackie and Losordo 2011). Higher CD34+ cell number is associated with lower prevalence of CVDs (Bielak et al. 2009, Fleissner and Thum 2011). Other CACs express the vascular endothelial growth factor receptor 2 (KDR) and it is believed

that CD34+/KDR+ cells have greater angiogenic properties than cells with only the CD34+ marker. Indeed, Bonello et al. found that greater mobilization of CD34+ and CD34+/KDR+ CACs after percutateous coronary intervention was predictive of target lesion revascularization and negatively predicted major cardiovascular events within 6 months of the procedure (Bonello et al. 2012). Even in the absence of traditional CVD risk factors, CAC number and function are significantly reduced in older compared to younger adults (Hoetzer et al. 2007, Koutroumpi 2012).

Aerobic exercise training in both younger and older adults is associated with increases in CAC number and function. Van Craenbroeck et al., (2010) found that CD34+/KDR+ CAC number increased significantly in a group of heart failure patients after 6 months of aerobic exercise training (Van Craenenbroeck et al. 2010a). Regular aerobic exercise also improves intracellular endothelial nitric oxide synthase (eNOS) synthesis in CACs, which is believed to contribute to their role in enhancing endothelial repair (Hoetzer et al. 2007, Van Craenenbroeck and Conraads 2010). Our lab found that CD34+ CACs from endurance-trained younger adults had a more favorable redox balance (Jenkins et al. 2011a) and enhanced paracrine function (Landers-Ramos et al. 2015) compared to their sedentary counterparts. These studies emphasize the importance of regular physical activity for optimal CAC number and function, independent of other CVD risk factors, and they serve as a precedent for studying redox balance in CD34+ CACs.

Previous studies found that 3-12 weeks of exercise training improves both CAC and endothelial cell function (Hoetzer et al. 2007, Gatta et al. 2012, Currie et al. 2013, Grace et al. 2015). However, improvements in aerobic capacity and glucose tolerance have been observed after just 6-10 days of exercise training (Rogers et al. 1988a, 1988b, Baynard et al. 2009, Liu et al. 2015). It is currently unknown if short-term exercise training results in positive endothelial and CAC responses. Significant reductions in CAC number have been observed in our lab with just 10 days of exercise cessation in older athletes (Witkowski et al. 2009) and significant reductions in Colony-forming Unit (CFU) CAC number and intracellular NO have been observed within the same timeframe in younger adults (Guhanarayan et al. 2014). We hypothesized that 10 days of endurance exercise training would improve flow-mediated

dilation (FMD) and increase CAC number and redox balance in CD34+ CACs of older previously sedentary adults.

## METHODS

### Screening and Standard Assessments

Subjects were healthy, non-smoking men and women 50-80 years of age. All subjects reported being generally sedentary for at least 5 years. Women were post-menopausal for >2 years and not prescribed hormone replacement therapy. Subjects were not taking any medications for hypertension or dyslipidemia, or those shown to affect CAC function (Everaert et al. 2010), and had no evidence of diabetes, CVD or pulmonary disease. Exclusion criteria were as follows: systolic blood pressure ≥130 mmHg, diastolic blood pressure ≥90 mmHg, serum total cholesterol ≥200 mg/dl; low-density lipoprotein-cholesterol (LDL-C) ≥130 mg/dl; high-density lipoprotein-cholesterol (HDL-C) ≤ 35 mg/dl; fasting glucose ≥100 mg/dl. The University of Maryland College Park and the University of Maryland School of Medicine Institutional Review Boards approved all study procedures and subjects provided written informed consent.



### Study Design

*Initial Testing:* Subjects reported to the laboratory at the Baltimore Veterans Affairs Medical Center Geriatric Research Education and Clinical Center (GRECC) in Baltimore the morning after an overnight (~12 hr) fast. Subjects underwent flow-mediated dilation (FMD), maximal oxygen consumption ($VO_2max$), and body composition tests were taken. Baseline blood sampling was taken on a separate day as part of the initial testing. Height, weight, seated blood pressure and BMI were measured, and body composition was assessed using a dual-energy x-ray absorptiometry (DXA) scan.

*Exercise Training:* Subjects participated in treadmill aerobic exercise training daily for 10 days at the Baltimore Veterans Affairs Medical Center GRECC exercise facility. Subjects exercised by walking or running at an intensity of ~70% $VO_{2max}$ (prescribed as 65-75% HR reserve) for 60 min. Heart rates were monitored during all sessions and subjects were supervised for at least 9 of the 10 days. Subjects were weighed daily and instructed to maintain the same body weight throughout the 10-day training period by increasing daily caloric intake slightly to balance the increased caloric expenditure. Compliance to the exercise training prescription was 100%.

*Final Testing*: After the 10-day aerobic exercise training program subjects returned to the laboratory and blood sampling and FMD tests were repeated. All tests were performed 24hrs after the last bout of exercise, and following a 12hr fast to account for any residual effects of the last exercise bout or differences in food intake prior to blood sampling.

**Flow-mediated Dilation (FMD)**

FMD of the brachial artery was assessed in subjects in the morning after a 12 hour fast; subjects refrained from ingesting any vasoactive substances on the morning of the test. Lying in a supine position, an automatic blood pressure cuff was placed on the right arm for intermittent blood pressure and heart rate monitoring throughout the study. Electrodes were placed to monitor a one lead electrocardiogram (ECG) from the ultrasound system for ECG gating of measurements. Another blood pressure cuff was placed on the subject's upper left arm well above the antecubital fossa. The brachial artery was imaged with 2D ultrasound (Philips HDI 5000) in a longitudinal orientation just above the antecubital fossa. Both 2D images of the artery and Doppler assessment of blood velocity through the artery were assessed at baseline. A blood pressure cuff was then inflated to 200 mm Hg and kept inflated for 5 minutes. The cuff was then deflated to induce a hyperemic stimulus. Longitudinal ultrasound images were recorded continuously from 30 seconds before, to 2 minutes after cuff deflation. Mid-artery Doppler assessment of the hyperemic velocity was recorded 15 seconds after cuff deflation. After release of the cuff, the maximum artery dilation was imaged and recorded, typically occurring approximately 1 minute after release. Ultrasound images of a standardized magnification were printed at baseline and maximal dilation for measurement. Images were ECG-gated to onset of the R-wave for all measurements. Lumen diameter was measured manually with calipers by a trained sonographer with >15 years of experience, and the median of five evenly-spaced diameter measurements obtained within a 5-cm segment was used for analyses. Procedures were performed on the same brachial artery location and with the same cuff position at each time point. Measurements were highly reproducible as blinded re-measurement of 10 tests revealed a CV of <3% and $r^2$=0.97. Results are presented as percent change in brachial artery diameter

(hyperemia minus baseline) at 0 and 10 days after participating in the prescribed exercise-training program.

**Dual-Energy X-ray Absorptiometry (DXA)**

Fat mass, fat-free mass, and percent body fat were measured by DXA (Prodigy, LUNAR Radiation Corp., Madison, WI).

**Framingham Coronary Heart Disease Risk Score**

Risk scores were calculated for each subject based on the Framingham study algorithms (Wilson et al. 1998) to estimate an individual's 10-year risk for coronary heart disease using the following parameters: age, gender, total cholesterol, HDL-C, systolic blood pressure, cigarette smoking and whether the individual is currently on medications to manage high blood pressure (Wilson et al. 1998).

**Maximal Oxygen Consumption ($VO_{2max}$)**

$VO_{2max}$ was measured by indirect calorimetry (Quark, Cosmed USA, Chicago, IL) during a constant-speed treadmill protocol with 2% increases in incline every 2 min until exhaustion as previously described (Prior et al. 2014). $VO_{2max}$ was defined as the highest oxygen consumption value obtained for a 30-second increment. $VO_2$ was considered maximum if the following standard criteria were met: respiratory exchange ratio >1.10 or a plateau in $VO_2$ with an increase in workload.

**Blood Sample Analyses**

Blood samples were obtained in tubes containing 15% potassium EDTA (Becton Dickinson) for measurement of glucose and lipoprotein lipid levels and enumeration and isolation of CACs. Plasma glucose levels were measured with a glucose analyzer (2300 STAT Plus, YSI, Yellow Springs, OH). Lipoprotein lipid levels were analyzed using an automated colorimetric assay as previously described (Joseph et al. 2011). Briefly, HDL-C was measured in the supernatant after precipitation with dextran sulfate, and LDL-C was calculated using the Friedewald equation: LDL-C = total cholesterol − (triglycerides/5 + HDL-C).

**Circulating Angiogenic Cell Number**

Flow cytometry was used to determine the number of CD34+, CD34+/KDR+ and KDR+ CACs.

Total peripheral blood mononuclear cells (PBMCs) were separated from whole blood using density centrifugation (Ficolle-Paque Plus; GE Healthcare). A total of $1x10^6$ PBMCs were FcR blocked (Miltenyi Biotech), immunostained with monoclonal anti-human CD34-FITC (BD Biosciences) and PE-VEGFR-2 (aka KDR; R&D Systems), and fixed in 2% paraformaldehyde. Flow cytometry analyses were performed in the University of Maryland Baltimore Flow Cytometry Core Facility with an Epics EliteESP flow cytometer (Beckman Coulter, Inc., Brea, CA). The forward-side-scatter plot was used to identify the lymphocyte gate and a total of 100,000 events per sample were acquired.

**Immunomagnetic Cell Separation**

PBMCs were isolated from venous blood samples using density gradient centrifugation (Ficoll, GE Healthcare). CD34+ was selected as the cell type to perform intracellular measures on based on the frequency of this cell type within total PBMCs, previous work in our lab supporting the use of similar intracellular measures in the same cell type (Jenkins et al. 2011a, 2011b), and literature identifying cells with the CD34 marker as progenitor cells with angiogenic properties (Schatteman et al. 2000, Harraz et al. 2001, Awad 2006, Mackie and Losordo 2011, Losordo et al. 2011). The CD34+ fraction was purified using two rounds of immunomagnetic cell separation according to the manufacturer's instructions (EasySep® Immunomagnetic Cell Separation Kits, STEMCELL Technologies) using an antibody (Ab) specific for CD34. This isolation approach has been published previously from our laboratory (Jenkins et al. 2011a, 2011b, Landers-Ramos et al. 2015) and results in ~50% purity of the cell populations from non-mobilized blood, which is equivalent to or greater than other published purity results (Asahara 1997, Schatteman et al. 2000, Awad 2006). The validity of the CD34+ PBMC immunomagnetic selection procedure has been previously confirmed in our laboratory via FACS analyses and confirmed via mRNA expression (Jenkins et al. 2011a, 2011b, Landers-Ramos et al. 2015) as positively selected CD34+ cells displayed strong expression of the target antigens with minimal presence of the target in the negative fractions (data not shown).

**Measurement of Intracellular NO and ROS**

These experiments were performed in duplicate as previously described (Jenkins et al. 2011a, 2011b, Landers-Ramos et al. 2015) with minor modifications. Briefly, $1.5 \times 10^5$ cells were stained with 10 μM 4-amino-5-methylamino-2′,7′-difluorofluorescein (DAF-FM) diacetate for determination of intracellular NO levels or 2 μM 2′,7′-dichlorodihydrofluorescein diacetate ($H_2$DCFDA) for determination of intracellular ROS levels (Molecular Probes). Cells were incubated with fluorescent dyes in a final volume of 150 μl serum-free PBS for 30 min at 37°C. After incubation, cells were washed with PBS and NO and ROS fluorescence was quantified using a fluorescent microplate reader (Biotek H1 Synergy Hybrid Reader, Winooski, VT) using excitation and emission filters of 488 and 535 nm, respectively. All fluorescent probes were validated using positive and negative controls as described previously by our lab (Jenkins et al. 2011a, 2011b, Landers-Ramos et al. 2015). Briefly, we observe several fold increases in intracellular DAF-FM and $H_2$DCF-DA signals in the presence of an NO donor, *Diethylenetriamine NONOate (*50 μM) and *3-Morpholinosydnonimine* (200 μM), respectively. Pretreatment with 50 U/mL PEG-catalase reduces $H_2$DCF-DA signal by nearly 40% after exposure to hydrogen peroxide (500 μM). Intra-assay coefficients of variation for ROS and NO were 4.0% and 3.4%, respectively (Landers-Ramos et al. 2015).



**Assessment of Gene Expression by RT-PCR**

Total RNA was extracted from freshly isolated CACs using the TriZol reagent, quantified using a spectrophotometer (BioTek H1 Synergy Hybrid Reader, Winooski, VT) and reverse transcribed to cDNA (Life Technologies, Grand Island, NY). Quantitative real-time polymerase chain reaction (PCR) was performed using Applied BioSystems 7300 Real-Time PCR System. Primer Assays were purchased from IDT (Coralville, IA) and optimal concentration for efficacy of >90% was determined. Primer sequences are listed in Table 1. Each reaction was performed in duplicate on a 96-well plate and contained iTaq Universal Probes Supermix (Biorad, Hercules, CA), respective primer probe, and the cDNA template. The PCR conditions used were as follows: 95°C for 3 min, followed by 50 cycles of 95°C for 15 sec, and 60°C for 45 sec. *GAPDH* primers were used as a control gene and *GAPDH* cycle thresholds (CTs) were

not different across time in the present study. mRNA expression values are presented as $2^{-\Delta CT}$ where $\Delta C_T$ is the $C_T$ of the target gene minus *GAPDH* control for each condition.

**Statistical Analysis**

Sample size calculations were performed *a priori* based on effect size estimates from findings published in the literature regarding our primary study outcomes- changes in CAC number (Witkowski et al. 2009, Van Craenenbroeck et al. 2010b, Gatta et al. 2012) and changes in %FMD (Franzoni et al. 2005, Witkowski et al. 2009, Grace et al. 2015) with exercise training- indicating between 80-95% power to detect differences with a sample size of n=10. Statistical analyses were completed using IBM SPSS Statistics 21 (IBM, Armonk, NY). Assumptions of homoscedasticity and normality were verified for all outcome measures. A repeated-measures MANOVA was used to test for effects of training (baseline vs. 10 days after exercise training) and sex (men vs. women). For CAC number analyses, when data were not normally distributed, data were analyzed using Wilcoxon signed-rank tests. Statistical significance was accepted at $P \leq 0.05$. Values are expressed as mean ± standard error of the mean.

## RESULTS

Subject characteristics can be found in Table 2. Four men and 7 women completed the study. All subjects were sedentary but were otherwise healthy according to BMI and cardiometabolic risk factors. There were no significant changes in body weight with the 10 days of training.

### Flow-mediated Dilation

FMD increased significantly and substantially after ten days of aerobic exercise training in these sedentary older men and women ($P<0.05$; Figure 1). Values increased from 25 ± 2 mm to 27 ± 2.3 mm in response to hyperemia at baseline and from 26 ± 2.7 mm to 30 ± 3 mm after ten days of exercise training with no changes in baseline diameter as a result of training ($P>0.05$) but significantly greater hyperemia-induced diameter after exercise training ($P<0.05$). Baseline and hyperemia-induced time-averaged mean velocity were similar before and after the exercise training period ($P>0.05$ for both). FMD responses increased for nearly every subject after training with no distinguishable differences found as a function of sex.

### Flow Cytometry Analyses

CD34+/KDR+ cell number increased by 104% and KDR+ cell number increased by 151% after the 10-day aerobic exercise-training program ($P<0.05$ for both, Figure 2A and 2B). Although not statistically significant, CD34+ cell number was numerically 48% higher after 10 days of endurance exercise training ($P=0.28$, Figure 2C). Notably, this difference appears to be driven by one individual. No differences in CAC number as a function of sex were found. There was no statistically significant correlation between ΔFMD and ΔCD34+/KDR+, ΔKDR+ or ΔCD34+ ($P>0.05$ for all).

### Intracellular Nitric Oxide and Reactive Oxygen Species

CD34+ intracellular ROS levels were not significantly different after 10 days of aerobic exercise training compared to baseline levels (20880 ± 4098 vs. 21449 ± 3139 arbitrary units (a.u.), respectively; $P=0.85$). There were also no significant differences in intracellular NO levels before and after 10 days of aerobic exercise training (24245 ± 3739 vs. 23235 ± 4374 a.u., respectively; $P=0.73$). There was large individual variability in both ROS and NO at baseline, after 10 days of exercise training but no

differences in intracellular ROS or NO were found as a function of sex.

**RT-PCR**

Expression of genes associated with the production of NO (*eNOS*) and ROS (*SOD1*, *NOX-2*, and *p47phox*) was measured in CD34+ CACs. *SOD1* mRNA expression was not significantly different after 10 days of aerobic exercise training compared to baseline (P>0.05, Figure 3A). *eNOS* mRNA was numerically 42% lower after training compared to baseline but this was not significantly different due to large individual variation (P=0.13, Figure 3B). *NOX-2* mRNA expression was not significantly different after 10 days of aerobic exercise training compared to baseline (P>0.05, Figure 3C). Although not statistically different, *p47phox* mRNA expression was 63% greater after 10 days of aerobic exercise training compared to baseline (P>0.05, Figure 3D). No differences in mRNA expression of any targets were found as a function of sex.

## DISCUSSION

Aerobic exercise training is beneficial for individuals of all ages and has been used successfully as a means of preventing and reversing age- and CVD-related endothelial dysfunction (Seals et al. 2008, Van Craenenbroeck et al. 2010b, DeVan and Seals 2012, Grace et al. 2015). We tested whether a short-term aerobic exercise-training program results in positive cardiovascular changes, specifically in terms of FMD, CAC number and CAC intracellular measures. The major findings of this study were that just 10 days of aerobic exercise training was sufficient to increase CD34+/KDR+ and KDR+ CAC number and improve FMD in previously sedentary older adults.

Aging is associated with the development of CVD and dysfunction of the vascular endothelium that, in part, can be attributed to a decrease in NO bioavailability from oxidative stress and reduced antioxidant defenses (Seals et al. 2011). However, studies comparing older athletes to non-athletes have found that lifelong exercise can attenuate this age-related development of endothelial dysfunction (Seals et al. 2008, DeVan and Seals 2012). Even 6-8 weeks of exercise training in older adults has been found to improve FMD in both healthy individuals (Grace et al. 2015) and those with CVD (Green et al. 2003, Kim et al. 2014). However, few studies have assessed the effects of short-term exercise on FMD. Our finding that 10 days of daily exercise improves FMD are concordant with one previous report that as little as 4 days of hand-grip exercise (20 minutes per day) improved brachial artery FMD (Allen et al. 2003). Conversely, Baynard et al (2009) found that 10 consecutive days of aerobic exercise training did not improve FMD in obese older adults (Baynard et al. 2009). Because the present study included leaner subjects, this suggests that obesity may mitigate the timeframe for FMD adaptations to aerobic exercise training.

Low CAC number is associated with older age (Heiss et al. 2005), physical inactivity (Heiss et al. 2005, Volaklis et al. 2012) and reduced CV health status (Koutroumpi 2012). The progression of CVD may be due, in part, to a lack of a sufficient number of CACs to aid in the preservation of endothelial integrity with age (Hill et al. 2003). An acute bout of exercise has been shown to mobilize CACs from the bone marrow and increase circulating cell number (Mobius-Winkler et al. 2009, Sandri et al. 2010); thus,

routine exercise is expected to result in regular, intermittent mobilization of CACs. The shortest exercise training period found in the previous literature that investigated CAC number found increases in CD34+/KDR+ CACs after 3 weeks in older heart failure patients exercising for 30 min twice a day at 75-85% $VO_2$max (Gatta et al. 2012). To our knowledge, the current study is the first report of increased basal numbers of CD34+/KDR+ and KDR+ CACs after just 10 days of endurance exercise training in healthy older adults.

Contrary to our hypothesis, we did not detect a significant increase in the number of CD34+ CACs after 10 days of aerobic exercise training; however, this finding is concordant with reports showing no significant changes in CD34+ number (Cesari et al. 2012, Keser et al. 2013) despite differences in CD34+/KDR+ number (Cesari et al. 2012) after exercise training. This suggests that the signals elicited by exercise affect mobilization of CACs with an endothelial lineage to a greater degree or that exercise may be causing a shift in CACs to a more endothelial phenotype [i.e., those expressing KDR, also known as the receptor for vascular endothelial growth factor-2 (VEGFR-2)]. Increases in NO bioavailability and signaling factors such as VEGF and SDF-1, among others, result in the release of CACs from the bone marrow (Fleissner and Thum 2011). It is interesting to speculate that endothelial factors may preferentially induce mobilization of CACs with a more endothelial phenotype (e.g., CD34+/KDR+ and KDR+), but not the entire population of CD34+ cells. However, direct measurements of these factors as well as evidence of preferential release of these cells with exercise are necessary in order to confirm these speculations.

Previous studies have found strong correlations between CAC number and FMD response to hyperemia (Hill et al. 2003, Witkowski et al. 2009). Indeed, some have found that the use of CAC number to predict vascular function in adults was equivalent to or greater than traditional CV risk factors (Hill et al. 2003). In the present study, change in % FMD measures with ten days of exercise training was not significantly correlated with change in any CAC subtype number. Heiss et al. (2005) found that attenuations in the maintenance of vascular integrity with older age were related more to reductions in

CAC function (i.e. CAC migration and proliferation) than quantitative differences in CAC numbers (Heiss et al. 2005). Thus, future work should aim to determine whether functional measures in CACs serve as an alternative predictor of vascular homeostasis in older adults in response to exercise training.

When markers of oxidative stress were assessed, we found that there was no effect of 10-day exercise training on eNOS, SOD1, NOX2 or p47phox mRNA expression, or on intracellular ROS or NO measures in CD34+ CACs. Our lab has previously found more favorable CD34+ CAC intracellular redox balance (lower NO and superoxide levels) in young endurance-trained adults compared to their sedentary counterparts (Jenkins et al. 2011a). Our previous and current studies differ in two major ways in that subjects were younger, college-aged men, and that the study design was cross-sectional with the endurance-trained subjects reporting being regularly physically active for the past 5 years (Jenkins et al. 2011a). As such, we can speculate that if improvements in intracellular redox balance occur with aerobic exercise training, the age of the individual and/or the length of the exercise-training period may impact these effects. Future studies should include a younger control group to better address these questions.

An acute intense bout of exercise is known to elicit increases in both ROS and NO in many tissues (especially in sedentary individuals), whereas regular, repeated exercise bouts result in a more blunted effect as redox balance improves compared to baseline levels (Nikolaidis et al. 2012). However, to our knowledge, a time course of potential improvements in CAC intracellular redox balance has not yet been established. Thus, we could speculate that if improvements in intracellular redox balance occur in CD34+ CACs from older adults with exercise, a training program longer than 10 days would be necessary to elicit these improvements.

It is also possible that intracellular ROS and NO levels may differ in CAC subpopulations other than CD34+. Indeed, Guhanarayan et al. (Guhanarayan et al. 2014) found that 10 days of reduced physical activity is sufficient to decrease CFU number and CFU intracellular NO, but this was not evident in the freshly isolated CD34+ CACs. This suggests that there is a cell-type specific response and that

some CACs could adapt or respond more quickly to changes in their intracellular environment. In the present study, our cell sampling methods did not yield adequate numbers of cells to assess ROS and NO changes in CD34+/KDR+ and KDR+ CACs. As our lab has previously found differences in both CD34+ cell number and intracellular redox balance as a function of reduced physical activity (Witkowski et al. 2009) and aerobic exercise (both chronic and acute) (Jenkins et al. 2011a), respectively, we selected this cell type as our focus for mRNA and intracellular measures of ROS and NO. Future studies should investigate intracellular ROS and NO in other CAC subtypes to determine if differences in intracellular redox state are cell-type specific.

When interpreting our findings, it is important to consider that despite being older and sedentary our subjects were otherwise healthy, not on medication, and free of traditional CVD risk factors (Table 2). In fact, FMD measures at baseline were high compared to some other studies assessing FMD in both older (Green et al. 2003, Pierce et al. 2011) and younger (Allen et al. 2003, Pierce et al. 2011, Llewellyn et al. 2012) adults. Thus, although interesting and in line with some other studies (Seals et al. 2008, DeVan and Seals 2012, Gatta et al. 2012), it is unclear whether our findings can be extrapolated to populations with CVD.

There is some disagreement in the field regarding the ideal cuff placement to determine the most accurate endothelial-dependent FMD response. In the current study, we used upper arm occlusion rather than forearm occlusion. Using this method, the buildup of metabolites as a result of tissue ischemia can contribute to the dilatory response making the response only partly mediated by NO (Doshi et al. 2001). However, Vogel et al. (2000) found better separation of subjects with and without coronary risk factors using the upper arm technique compared to the forearm occlusion (Vogel et al. 2000). Thus, although the method used in the current study is valid, interpretation of our FMD data in relation to other studies using forearm occlusion may be difficult. Indeed, this may explain the discrepancies between the present study and the Baynard et al. (2009) study that employed a similar short-term aerobic exercise program (Baynard et al. 2009).

Notably, adherence rates to the 10-day exercise program were 100%, despite relatively high

exercise intensity (70% of the subjects' $VO_2$max) and duration (60 min) of daily exercise. This exercise program falls within the higher range of the recommended physical activity for older adults prescribed by the American College of Sports Medicine (American College of Sports Medicine et al. 2009), and the program was well-tolerated by the subjects indicating that healthy older adults are capable of exercise training in this capacity and may derive some novel CV health benefits within just the first 10 days after adoption. Given the multitude of benefits of regular exercise, it is critical that clinicians continue to prescribe and educate older adults about the benefits of aerobic exercise training.

Although women are at a lower risk for CVD at a younger age, older men and women have similar rates of CV events and CVD is the leading cause of death for both men and women (Gillespie et al. 2013). As such, it is important that we include both sexes when studying CACs that may be used in cell therapies. In this study we detected no significant differences in outcome measures as a function of sex. However, future studies with a larger sample size of men and women so that the study is adequately powered to detect sex differences are necessary to determine whether differences in CAC number or function exist as a function of sex.



In the present study, body composition was assessed at baseline but not after the ten-day exercise-training period. Previous studies indicate that an exercise training program of this length would not be expected to substantially alter body composition (Cononie et al. 1994, Houmard et al. 1995, Kang et al. 1996, Cox et al. 1999, Youngren et al. 2001). We did find that although most CVD risk factors assessed were unchanged, total and LDL cholesterol were significantly reduced after the ten-day exercise-training period. Thus, it appears that in this short-term exercise training program, enhancements in novel CVD risk factors (i.e. CAC number and FMD) occur in the absence of changes in aerobic fitness and some conventional risk factors, but not total nor LDL cholesterol.

Although we examined the effects of our short-term exercise training program on enumeration of three different CAC subtypes, the present study did not comprehensively examine all pro-angiogenic PBMCs. Indeed, a number of different subtypes of PBMCs with pro-angiogenic potential have been identified including CD34-/CD31+ (Landers-Ramos et al. 2015), CD62E+ (Lansford et al. 2015),

angiogenic T-cells (Hur et al. 2007, Kushner et al. 2010), and angiogenic monocytes (Favre et al. 2012), among others. Future studies investigating both the number and function of a variety of different pro-angiogenic PBMCs would provide a more comprehensive assessment of the heterogeneous response of CACs to aerobic exercise training.

In conclusion, 10 days of aerobic exercise training in previously sedentary, but otherwise healthy older men and women improved FMD and increased CD34+/KDR+ and KDR+ CAC number; however, these beneficial adaptations appear to be independent of alterations in CD34+ cell number and intracellular redox balance. Together, these data provide the rationale for future studies investigating the effects of regular exercise on CAC populations, as well as the time course of CV adaptations with aerobic exercise training in both healthy and CVD patients who may benefit from exercise as a treatment or prevention strategy.



## COMPETING INTERESTS

No competing interests are reported.

## AUTHOUR CONTRIBUTIONS

RQL, EES, SJP and JMH conceived and designed the research experiments. RQL, KJC, LMG and CNA performed the experiments and collected the data. Analysis and interpretation of data was performed by RQL, EES, SJP and JMH. RQL drafted the manuscript; KJC, LMG, CNA EES, SJP and JMH edited and revised the manuscript and provided important intellectual content. All authors approved the final version of the manuscript.

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## REFERENCES


Allen, J.D., Geaghan, J.P., Greenway, F., and Welsch, M.A. 2003. Time Course of Improved Flow-Mediated Dilation after Short-Term Exercise Training. Med Sci Sports Exerc **35**(5): 847–853. doi: 10.1249/01.MSS.0000064931.62916.8A.

American College of Sports Medicine, Chodzko-Zajko, W.J., Proctor, D.N., Fiatarone Singh, M.A., Minson, C.T., Nigg, C.R., Salem, G.J., and Skinner, J.S. 2009, July. American College of Sports Medicine position stand. Exercise and physical activity for older adults. doi: 10.1249/MSS.0b013e3181a0c95c.

Asahara, T. 1997. Isolation of Putative Progenitor Endothelial Cells for Angiogenesis. Science **275**(5302): 964–966. doi: 10.1126/science.275.5302.964.

Awad, O. 2006. Differential Healing Activities of CD34+ and CD14+ Endothelial Cell Progenitors. Arteriosclerosis, Thrombosis, and Vascular Biology **26**(4): 758–764. doi: 10.1161/01.ATV.0000203513.29227.6f.

Bakogiannis, C., Tousoulis, D., Androulakis, E., Briasoulis, A., Papageorgiou, N., Vogiatzi, G., Kampoli, A.-M., Charakida, M., Siasos, G., Latsios, G., Antoniades, C., and Stefanadis, C. 2012. Circulating endothelial progenitor cells as biomarkers for prediction of cardiovascular outcomes. Curr. Med. Chem. **19**(16): 2597–2604.

Baynard, T., Carhart, R.L., Weinstock, R.S., Ploutz-Snyder, L.L., and Kanaley, J.A. 2009. Short-term exercise training improves aerobic capacity with no change in arterial function in obesity. Eur J Appl Physiol **107**(3): 299–308. doi: 10.1007/s00421-009-1126-2.

Bielak, L.F., Horenstein, R.B., Ryan, K.A., Sheedy, P.F., Rumberger, J.A., Tanner, K., Post, W., Mitchell, B.D., Shuldiner, A.R., and Peyser, P.A. 2009. Circulating CD34+ Cell Count is Associated with Extent of Subclinical Atherosclerosis in Asymptomatic Amish Men, Independent of 10-Year Framingham Risk. Clin Med Cardiol **3**: 53–60.

Bonello, L., Harhouri, K., Baumstarck, K., Arnaud, L., Lesavre, N., Piot, C., Paganelli, F., Dignat-George, F., and Sabatier, F. 2012. Mobilization of CD34+ KDR+ endothelial progenitor cells predicts target lesion revascularization. J. Thromb. Haemost. **10**(9): 1906–1913. doi: 10.1111/j.1538-7836.2012.04854.x.

Cesari, F., Sofi, F., Gori, A.M., Corsani, I., Capalbo, A., Caporale, R., Abbate, R., Gensini, G.F., and Casini, A. 2012. Physical activity and circulating endothelial progenitor cells: an intervention study. European Journal of Clinical Investigation **42**(9): 927–932. doi: 10.1111/j.1365-2362.2012.02670.x.

Cononie, C.C., Goldberg, A.P., Rogus, E., and Hagberg, J.M. 1994. Seven consecutive days of exercise lowers plasma insulin responses to an oral glucose challenge in sedentary elderly. J Am Geriatr Soc **42**(4): 394–398.

Cox, J.H., Cortright, R.N., Dohm, G.L., and Houmard, J.A. 1999. Effect of aging on response to exercise training in humans: skeletal muscle GLUT-4 and insulin sensitivity. J. Appl. Physiol. **86**(6): 2019–2025.

Currie, K.D., Dubberley, J.B., McKelvie, R.S., and MacDonald, M.J. 2013. Low-Volume, High-Intensity Interval Training in Patients with CAD. Med Sci Sports Exerc **45**(8): 1436–1442. doi: 10.1249/MSS.0b013e31828bbbd4.

DeVan, A.E., and Seals, D.R. 2012. Vascular health in the ageing athlete. Experimental Physiology: no–no. doi: 10.1113/expphysiol.2011.058792.

Doshi, S.N., Naka, K.K., Payne, N., Jones, C.J., Ashton, M., Lewis, M.J., and Goodfellow, J. 2001. Flow-mediated dilatation following wrist and upper arm occlusion in humans: the

contribution of nitric oxide. Clin. Sci. **101**(6): 629–635.
Everaert, B.R., Van Craenenbroeck, E.M., Hoymans, V.Y., Haine, S.E., Van Nassauw, L., Conraads, V.M., Timmermans, J.-P., and Vrints, C.J. 2010. International Journal of Cardiology. International Journal of Cardiology **144**(3): 350–366. Elsevier Ireland Ltd. doi: 10.1016/j.ijcard.2010.04.018.
Favre, J., Terborg, N., and Horrevoets, A.J.G. 2012. The diverse identity of angiogenic monocytes. European Journal of Clinical Investigation **43**(1): 100–107. doi: 10.1111/eci.12009.
Fleissner, F., and Thum, T. 2011. Critical role of the nitric oxide/reactive oxygen species balance in endothelial progenitor dysfunction. Antioxid. Redox Signal. **15**(4): 933–948. doi: 10.1089/ars.2010.3502.
Franzoni, F., Ghiadoni, L., Galetta, F., Plantinga, Y., Lubrano, V., Huang, Y., Salvetti, G., Regoli, F., Taddei, S., and Santoro, G. 2005. Physical activity, plasma antioxidant capacity, and endothelium-dependent vasodilation in young and older men. American Journal of Hypertension **18**(4): 510–516. doi: 10.1016/j.amjhyper.2004.11.006.
Gatta, L., Armani, A., Iellamo, F., Consoli, C., Molinari, F., Caminiti, G., Volterrani, M., and Rosano, G.M.C. 2012. International Journal of Cardiology. International Journal of Cardiology **155**(3): 409–413. Elsevier Ireland Ltd. doi: 10.1016/j.ijcard.2010.10.045.
Gillespie, C.D., Wigington, C., Hong, Y., Centers for Disease Control and Prevention (CDC). 2013. Coronary heart disease and stroke deaths - United States, 2009. MMWR Surveill Summ **62 Suppl 3**: 157–160.
Grace, F.M., Herbert, P., Ratcliffe, J.W., New, K.J., Baker, J.S., and Sculthorpe, N.F. 2015. Age related vascular endothelial function following lifelong sedentariness: positive impact of cardiovascular conditioning without further improvement following low frequency high intensity interval training. Physiological Reports **3**(1): e12234–e12234. doi: 10.14814/phy2.12234.
Green, D.J., Walsh, J.H., Maiorana, A., Best, M.J., Taylor, R.R., and O'Driscoll, J.G. 2003. Exercise-induced improvement in endothelial dysfunction is not mediated by changes in CV risk factors: pooled analysis of diverse patient populations. AJP: Heart and Circulatory Physiology **285**(6): H2679–H2687. doi: 10.1152/ajpheart.00519.2003.
Guhanarayan, G., Jablonski, J., and Witkowski, S. 2014. Circulating angiogenic cell population responses to 10 days of reduced physical activity. Journal of Applied Physiology **117**(5): 500–506. doi: 10.1152/japplphysiol.00087.2014.
Harraz, M., Jiao, C., Hanlon, H.D., Hartley, R.S., and Schatteman, G.C. 2001. CD34- blood-derived human endothelial cell progenitors. Stem Cells **19**(4): 304–312. doi: 10.1634/stemcells.19-4-304.
Heidenreich, P.A., Trogdon, J.G., Khavjou, O.A., Butler, J., Dracup, K., Ezekowitz, M.D., Finkelstein, E.A., Hong, Y., Johnston, S.C., Khera, A., Lloyd-Jones, D.M., Nelson, S.A., Nichol, G., Orenstein, D., Wilson, P.W.F., Woo, Y.J., on behalf of the American Heart Association Advocacy Coordinating Committee, Stroke Council, Council on Cardiovascular Radiology and Intervention, Council on Clinical Cardiology, Council on Epidemiology and Prevention, Council on Arteriosclerosis, Thrombosis and Vascular Biology, Council on Cardiopulmonary, Critical Care, Perioperative and Resuscitation, Council on Cardiovascular Nursing, Council on the Kidney in Cardiovascular Disease, Council on Cardiovascular Surgery and Anesthesia, and Interdisciplinary Council on Quality of Care and Outcomes Research. 2011. Forecasting the Future of Cardiovascular Disease in the United States: A

Policy Statement From the American Heart Association. Circulation **123**(8): 933–944. doi: 10.1161/CIR.0b013e31820a55f5.

Heiss, C., Keymel, S., Niesler, U., Ziemann, J., Kelm, M., and Kalka, C. 2005. Impaired Progenitor Cell Activity in Age-Related Endothelial Dysfunction. Journal of the American College of Cardiology **45**(9): 1441–1448. doi: 10.1016/j.jacc.2004.12.074.

Hill, J.M., Zalos, G., Halcox, J.P.J., Schenke, W.H., Waclawiw, M.A., Quyyumi, A.A., and Finkel, T. 2003. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N. Engl. J. Med. **348**(7): 593–600. doi: 10.1056/NEJMoa022287.

Hoetzer, G.L., Van Guilder, G.P., Irmiger, H.M., Keith, R.S., Stauffer, B.L., and DeSouza, C.A. 2007. Aging, exercise, and endothelial progenitor cell clonogenic and migratory capacity in men. J. Appl. Physiol. **102**(3): 847–852. doi: 10.1152/japplphysiol.01183.2006.

Houmard, J.A., Hickey, M.S., Tyndall, G.L., Gavigan, K.E., and Dohm, G.L. 1995. Seven days of exercise increase GLUT-4 protein content in human skeletal muscle. J. Appl. Physiol. **79**(6): 1936–1938.

Hur, J., Yang, H.M., Yoon, C.H., Lee, C.S., Park, K.W., Kim, J.H., Kim, T.Y., Kim, J.Y., Kang, H.J., Chae, I.H., Oh, B.H., Park, Y.B., and Kim, H.S. 2007. Identification of a Novel Role of T Cells in Postnatal Vasculogenesis: Characterization of Endothelial Progenitor Cell Colonies. Circulation **116**(15): 1671–1682. doi: 10.1161/CIRCULATIONAHA.107.694778.

Jenkins, N.T., Landers, R.Q., Prior, S.J., Soni, N., Spangenburg, E.E., and Hagberg, J.M. 2011a. Effects of acute and chronic endurance exercise on intracellular nitric oxide and superoxide in circulating CD34+ and CD34- cells. Journal of Applied Physiology **111**(3): 929–937. doi: 10.1152/japplphysiol.00541.2011.

Jenkins, N.T., Landers, R.Q., Thakkar, S.R., Fan, X., Brown, M.D., Prior, S.J., Spangenburg, E.E., and Hagberg, J.M. 2011b. Prior endurance exercise prevents postprandial lipaemia-induced increases in reactive oxygen species in circulating CD31+ cells. J. Physiol. (Lond.) **589**(Pt 22): 5539–5553. doi: 10.1113/jphysiol.2011.215277.

Joseph, L.J., Prigeon, R.L., Blumenthal, J.B., Ryan, A.S., and Goldberg, A.P. 2011. Weight loss and low-intensity exercise for the treatment of metabolic syndrome in obese postmenopausal women. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences **66**(9): 1022–1029. doi: 10.1093/gerona/glr093.

Kang, J., Robertson, R.J., Hagberg, J.M., Kelley, D.E., Goss, F.L., DaSilva, S.G., Suminski, R.R., and Utter, A.C. 1996. Effect of exercise intensity on glucose and insulin metabolism in obese individuals and obese NIDDM patients. Diabetes Care **19**(4): 341–349.

Keser, I., Suyani, E., Aki, S.Z., and Sucak, A.G.T. 2013. Transfusion and Apheresis Science. Transfusion and Apheresis Science **49**(2): 302–306. Elsevier Ltd. doi: 10.1016/j.transci.2013.06.007.

Kim, C., Choi, H.E., Jung, H., Kang, S.H., Kim, J.H., and Byun, Y.S. 2014. Impact of Aerobic Exercise Training on Endothelial Function in Acute Coronary Syndrome. Ann Rehabil Med **38**(3): 388. doi: 10.5535/arm.2014.38.3.388.

Koutroumpi, M. 2012. Circulating endothelial and progenitor cells: Evidence from acute and long-term exercise effects. WJC **4**(12): 312. doi: 10.4330/wjc.v4.i12.312.

Kushner, E.J., MacEneaney, O.J., Morgan, R.G., Van Engelenburg, A.M., Van Guilder, G.P., and DeSouza, C.A. 2010. Blood Cells, Molecules, and Diseases. Blood Cells, Molecules, and Diseases **44**(2): 74–78. Elsevier Inc. doi: 10.1016/j.bcmd.2009.10.009.

Kwon, H.R., Min, K.W., Ahn, H.J., Seok, H.G., Lee, J.H., Park, G.S., and Han, K.A. 2011. Effects of Aerobic Exercise vs. Resistance Training on Endothelial Function in Women with

Type 2 Diabetes Mellitus. Diabetes Metab J **35**(4): 364. doi: 10.4093/dmj.2011.35.4.364.
Landers-Ramos, R.Q., Sapp, R.M., Jenkins, N.T., Murphy, A.E., Cancre, L., Chin, E.R., Spangenburg, E.E., and Hagberg, J.M. 2015. Chronic endurance exercise affects paracrine action of CD31+ and CD34+ cells on endothelial tube formation. AJP: Heart and Circulatory Physiology **309**(3): ajpheart.00123.2015. doi: 10.1152/ajpheart.00123.2015.
Lansford, K.A., Shill, D.D., Dicks, A.B., Marshburn, M.P., Southern, W.M., and Jenkins, N.T. 2015. Effect of acute exercise on circulating angiogenic cell and microparticle populations. Experimental Physiology **101**(1): 155–167. doi: 10.1113/EP085505.
Liu, M., Gillis, L.J., Persadie, N.R., Atkinson, S.A., Phillips, S.M., and Timmons, B.W. 2015. Effects of Short-Term Exercise Training With and Without Milk Intake on Cardiometabolic and Inflammatory Adaptations in Obese Adolescents. Pediatr Exerc Sci. doi: 10.1123/pes.2015-0053.
Llewellyn, T.L., Chaffin, M.E., Berg, K.E., and Meendering, J.R. 2012. The relationship between shear rate and flow-mediated dilation is altered by acute exercise. Acta Physiol **205**(3): 394–402. doi: 10.1111/j.1748-1716.2012.02417.x.
Losordo, D.W., Henry, T.D., Davidson, C., Sup Lee, J., Costa, M.A., Bass, T., Mendelsohn, F., Fortuin, F.D., Pepine, C.J., Traverse, J.H., Amrani, D., Ewenstein, B.M., Riedel, N., Story, K., Barker, K., Povsic, T.J., Harrington, R.A., Schatz, R.A., the ACT34-CMI Investigators. 2011. Intramyocardial, Autologous CD34+ Cell Therapy for Refractory Angina. Circulation Research **109**(4): 428–436. doi: 10.1161/CIRCRESAHA.111.245993.
Mackie, A.R., and Losordo, D.W. 2011. CD34-positive stem cells: in the treatment of heart and vascular disease in human beings. Tex Heart Inst J **38**(5): 474–485.
Mobius-Winkler, S., Hilberg, T., Menzel, K., Golla, E., Burman, A., Schuler, G., and Adams, V. 2009. Time-dependent mobilization of circulating progenitor cells during strenuous exercise in healthy individuals. Journal of Applied Physiology **107**(6): 1943–1950. doi: 10.1152/japplphysiol.00532.2009.
Nikolaidis, M.G., Kyparos, A., Spanou, C., Paschalis, V., Theodorou, A.A., and Vrabas, I.S. 2012. Redox biology of exercise: an integrative and comparative consideration of some overlooked issues. Journal of Experimental Biology **215**(10): 1615–1625. doi: 10.1242/jeb.067470.
Pierce, G.L., Donato, A.J., LaRocca, T.J., Eskurza, I., Silver, A.E., and Seals, D.R. 2011. Habitually exercising older men do not demonstrate age-associated vascular endothelial oxidative stress. Aging Cell **10**(6): 1032–1037. doi: 10.1111/j.1474-9726.2011.00748.x.
Prior, S.J., Blumenthal, J.B., Katzel, L.I., Goldberg, A.P., and Ryan, A.S. 2014. Increased Skeletal Muscle Capillarization After Aerobic Exercise Training and Weight Loss Improves Insulin Sensitivity in Adults With IGT. Diabetes Care **37**(5): 1469–1475. doi: 10.2337/dc13-2358.
Rogers, M.A., Yamamoto, C., Hagberg, J.M., Martin, W.H., Ehsani, A.A., and Holloszy, J.O. 1988a. Effect of 6 d of exercise training on responses to maximal and sub-maximal exercise in middle-aged men. Med Sci Sports Exerc **20**(3): 260–264.
Rogers, M.A., Yamamoto, C., King, D.S., Hagberg, J.M., Ehsani, A.A., and Holloszy, J.O. 1988b. Improvement in glucose tolerance after 1 wk of exercise in patients with mild NIDDM. Diabetes Care **11**(8): 613–618.
Sandri, M., Bernhard Beck, E., Adams, V., Gielen, S., Lenk, K., Höllriegel, R., Mangner, N., Linke, A., Erbs, S., Möbius-Winkler, S., Scheinert, D., Hambrecht, R., and Schuler, G. 2010. Maximal exercise, limb ischemia, and endothelial progenitor cells. Eur J Cardiovasc Prev

Rehabil: 1. doi: 10.1097/HJR.0b013e32833ba654.
Schatteman, G.C., Hanlon, H.D., Jiao, C., Dodds, S.G., and Christy, B.A. 2000. Blood-derived angioblasts accelerate blood-flow restoration in diabetic mice. J. Clin. Invest. **106**(4): 571–578. doi: 10.1172/JCI9087.
Schreuder, T.H.A., Green, D.J., Nyakayiru, J., Hopman, M.T.E., and Thijssen, D.H.J. 2014. Time-course of vascular adaptations during 8 weeks of exercise training in subjects with type 2 diabetes and middle-aged controls. Eur J Appl Physiol **115**(1): 187–196. doi: 10.1007/s00421-014-3006-7.
Seals, D.R., DeSouza, C.A., Donato, A.J., and Tanaka, H. 2008. Habitual exercise and arterial aging. J. Appl. Physiol. **105**(4): 1323–1332. doi: 10.1152/japplphysiol.90553.2008.
Seals, D.R., Jablonski, K.L., and Donato, A.J. 2011. Aging and vascular endothelial function in humans. Clin. Sci. **120**(9): 357–375. doi: 10.1042/CS20100476.
Van Craenenbroeck, E.M., and Conraads, V.M. 2010. Endothelial progenitor cells in vascular health: Focus on lifestyle. Microvascular Research: 1–9. Elsevier Inc. doi: 10.1016/j.mvr.2009.12.009.
Van Craenenbroeck, E.M., Beckers, P.J., Possemiers, N.M., Wuyts, K., Frederix, G., Hoymans, V.Y., Wuyts, F., Paelinck, B.P., Vrints, C.J., and Conraads, V.M. 2010a. Exercise acutely reverses dysfunction of circulating angiogenic cells in chronic heart failure. European Heart Journal **31**(15): 1924–1934. doi: 10.1093/eurheartj/ehq058.
Van Craenenbroeck, E.M., Hoymans, V.Y., Beckers, P.J., Possemiers, N.M., Wuyts, K., Paelinck, B.P., Vrints, C.J., and Conraads, V.M. 2010b. Exercise training improves function of circulating angiogenic cells in patients with chronic heart failure. Basic Res. Cardiol. **105**(5): 665–676. doi: 10.1007/s00395-010-0105-4.
Vogel, R.A., Corretti, M.C., and Plotnick, G.D. 2000. A comparison of brachial artery flow-mediated vasodilation using upper and lower arm arterial occlusion in subjects with and without coronary risk factors. Clin Cardiol **23**(8): 571–575.
Volaklis, K.A., Tokmakidis, S.P., and Halle, M. 2012. Acute and chronic effects of exercise on circulating endothelial progenitor cells in healthy and diseased patients. Clin Res Cardiol **102**(4): 249–257. doi: 10.1007/s00392-012-0517-2.
Wilson, P.W., D'Agostino, R.B., Levy, D., Belanger, A.M., Silbershatz, H., and Kannel, W.B. 1998. Prediction of coronary heart disease using risk factor categories. Circulation **97**(18): 1837–1847.
Witkowski, S., Lockard, M.M., Jenkins, N.T., Obisesan, T.O., Spangenburg, E.E., and Hagberg, J.M. 2009. Relationship between circulating progenitor cells, vascular function and oxidative stress with long-term training and short-term detraining in older men. Clin. Sci. **118**(4): 303–311. doi: 10.1042/CS20090253.
Youngren, J.F., Keen, S., Kulp, J.L., Tanner, C.J., Houmard, J.A., and Goldfine, I.D. 2001. Enhanced muscle insulin receptor autophosphorylation with short-term aerobic exercise training. Am. J. Physiol. Endocrinol. Metab. **280**(3): E528–33.

**Table 1. RT-PCR genes and primer sequences**

| Symbol | Gene name | Primer Sequence |
|---|---|---|
| *eNOS* | endothelial nitric oxide synthase | F: CCCTGTAGTTTCCGTGGA |
| | | R: TTCCCTCGTGTGAAGAACTG |
| *NOX2* | NADPH oxidase 2 | F: TCAGCATGCAGTTGAAATTCAG |
| | | R: TTCCTCTTTGTCTGGTATTACCG |
| *p47phox* | neutrophil cytosolic factor 1 | F: CCTGTTCTCTGGATTGATCGC |
| | | R: GCACTATGTGTACATGTTCCTG |
| *SOD1* | Superoxide dismutase 1 | F: CCTCTCTTCATCCTTTGGC |
| | | R: AAGGTGTGGGGAAGCATT |
| *GAPDH* | | F: TGTAGTTGAGGTCAATGAAGGG |
| | | R: ACATCGCTCAGACACCATG |

**Table 2. Subject Characteristics (n=11)**

| | Baseline | After Training |
|---|---|---|
| Age (years) | 61 ± 2.1 | - |
| VO2max (L/min) | 2.2 ± 0.1 | 2.1 ± 0.2 |
| VO2max (mL/kg/min) | 29.3 ± 1.5 | 28.7 ± 1.6 |
| Framingham Risk (%) | 5.3 ± 1.5 | 5.3 ± 1.9 |
| Fat mass (kg) | 24.6 ± 1.7 | - |
| Lean Mass (kg) | 46.5 ± 3.2 | - |
| Body Fat (%) | 34 ± 2.2 | - |
| Height (cm) | 169 ± 2.2 | - |
| Weight (kg) | 74 ± 4.3 | 74 ± 4.4 |
| BMI ($kg/m^2$) | 26 ± 0.9 | 26 ± 1.1 |
| SBP (mm Hg) | 117 ± 6 | 117 ± 5 |
| DBP (mm Hg) | 71 ± 2.3 | 70 ± 3.3 |
| MAP (mm Hg) | 87 ± 3.3 | 86 ± 3.3 |
| Cholesterol (mg/dL) | 198 ± 9.6 | 185 ± 9.3* |
| HDL (mg/dL) | 56 ± 4.5 | 56 ± 5.6 |
| LDL (mg/dL) | 121 ± 7.4 | 109 ± 7.6* |
| TC/HDL (mg/dL) | 3.7 ± 0.4 | 3.6 ± 0.4 |
| LDL/HDL (mg/dL) | 2.3 ± 0.3 | 2.2 ± 0.3* |
| Triglycerides (mg/dL) | 106 ± 12.8 | 100 ± 13.9 |
| Glucose (mg/dL) | 92 ± 2.9 | 93 ± 3.9 |

*Significantly different from baseline value; Data presented as mean ± SEM

## FIGURE CAPTIONS

**Figure 1.** Effects of ten days of endurance exercise training on brachial artery flow-mediated dilation in older previously sedentary adults (n=11). Left panels represent means at baseline and after training and right panels represent individual data with black lines indicating men and gray lines indicating women. * Indicates statistically significant from baseline at P<0.05.

**Figure 2.** Basal cell number counts of CD34+/KDR+ (A), KDR+ (B), and CD34+ (C) CACs before and after 10 days of endurance-exercise training (n=10). Left panels represent means and right panels represent individual data with black lines indicating men and gray lines indicating women. * Indicates statistically significant from baseline at P<0.05.

**Figure 3.** Effects of ten days of endurance exercise training on SOD1 (A), eNOS (B), NOX2 (C), and $p47^{phox}$ (D) real-time mRNA expression for freshly isolated CD34+ circulating angiogenic cells (n=11). GAPDH was used to normalize all data. Left panels represent means and right panels represent individual data with black lines indicating men and gray lines indicating women. * Indicates statistically significant from baseline at P<0.05.

**Figure 1.**

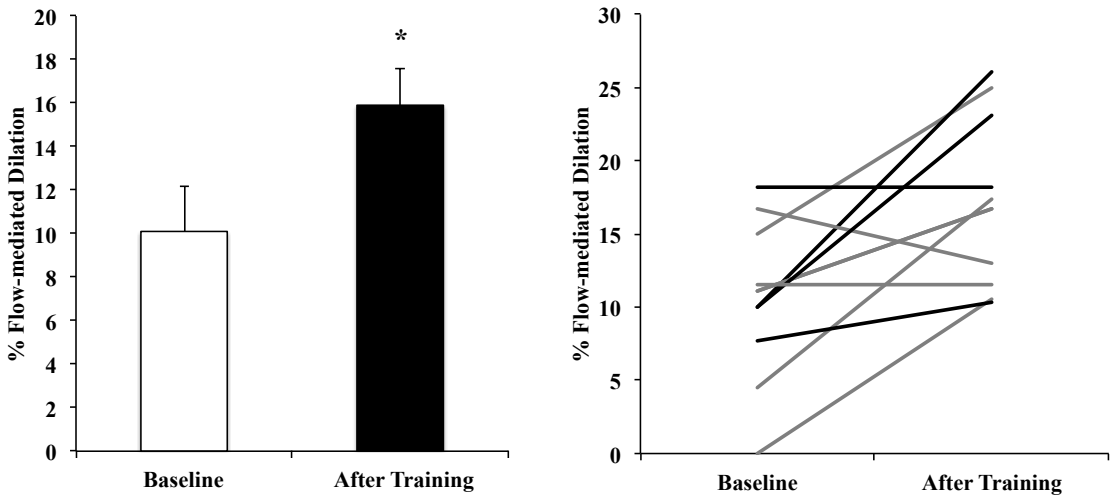

**Figure 2.**

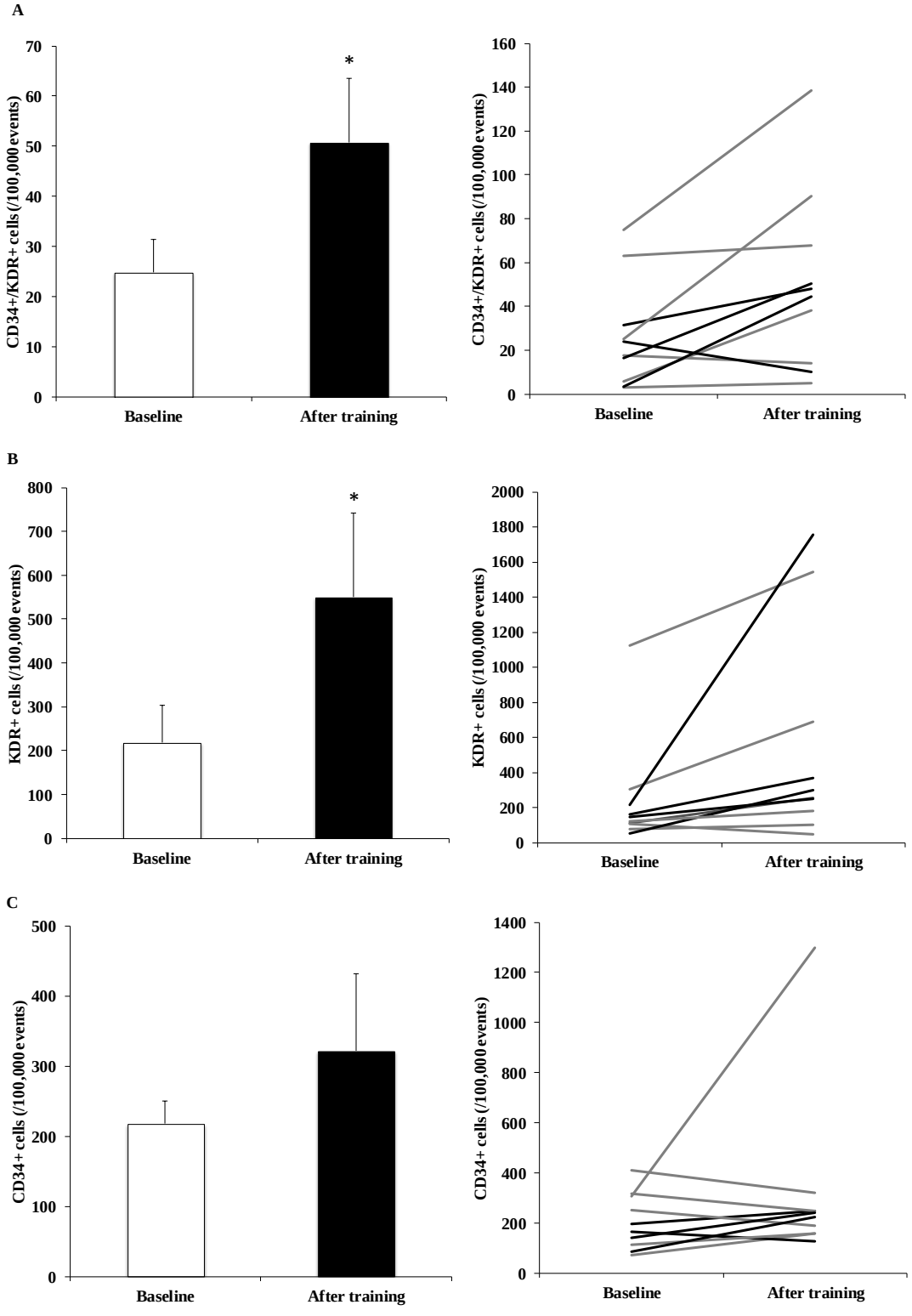
A
CD34+/KDR+ cells (/100,000 events)
70
60
50
40
30
20
10
0
*
Baseline
After training
160
140
120
100
80
60
40
20
0
Baseline
After training
B
KDR+ cells (/100,000 events)
800
700
600
500
400
300
200
100
0
*
Baseline
After training
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Baseline
After training
C
CD34+ cells (/100,000 events)
500
400
300
200
100
0
Baseline
After training
1400
1200
1000
800
600
400
200
0
Baseline
After training

Figure 3.