EFFECT OF EXERCISE INTENSITY ON CIRCULATING MICROPARTICLES IN MEN

AND WOMEN

by

KASEY ALEXANDRA LANSFORD

(Under the Direction of Nathan T. Jenkins)

ABSTRACT

Circulating microparticles influence vascular homeostasis by participating in angiogenesis, fibrinolysis, coagulation, and endothelial function. Microparticles can be used to characterize and understand the effect of exercise on vascular health. We investigated the effect of an acute bout of continuous and interval exercise on $CD62E^+$ endothelial microparticles (EMPs) and $CD34^+$ microparticles (MPs) in men and women. Blood samples were collected at baseline, halfway through exercise, immediately after exercise, and at 30, 60, 90, and 120 minutes after exercise. Interval exercise consisted of ten, 1-minute intervals at 100% and 90% VO_{2max} on the treadmill. The continuous bout was energy matched to the interval bout, and lasted ~22 minutes at 65% VO_{2max}. Overall, exercise produced a decrease from baseline in $CD62E^+$ EMPs, however there was no effect of exercise on $CD34^+$ MPs. This reduction in $CD62E^+$ EMPs with exercise could be contributing to the overall positive effect of exercise on cardiovascular health.

INDEX WORDS: Exercise, Endothelial, Microparticles, Cardiovascular Health

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KASEY ALEXANDRA LANSFORD

B.S., Marquette University, 2013

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment

of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2016

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KASEY ALEXANDRA LANSFORD

Major Professor:	Nathan T. Jenkins
Committee:	Ellen Evans
	Jarrod Call
	Jonathan Murrow

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
May 2016

DEDICATION

First and foremost, to God. To my husband Jeff, whose unwavering love and support has made this all possible. And to my son, James, who has brought new meaning and love into my life and has turned my world upside down in the most wonderful way possible. Finally to my mother, who has always supported and encouraged me to be the absolute best I can be.

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CHAPTER 1

INTRODUCTION

Physical activity positively influences cardiovascular health and reduces rates of cardiovascular disease, however the exact mechanisms for these beneficial adaptions are relatively unknown (Blair et al., 1989; Mora, Cook, Buring, Ridker, & Lee, 2007). Only ~60% of the physical activity-associated reduction in cardiovascular events is accounted for by established risk factors (Mora et al., 2007). To more completely understand the mechanisms of exercise-induced cardiovascular protection, further examination of events occurring in the vasculature as a result of exercise is needed.

Circulating endothelial cell-derived microparticles (EMPs) are small (≤ 1 µm) membrane fragments shed from the endothelium due to activation or apoptosis (Chironi et al., 2009). EMP levels are elevated in states of vascular disease (Dignat-George & Boulanger, 2011), however they also play a role in angiogenesis (Deregibus et al., 2007; Ullal, Pisetsky, & Reich, 2010) and fibrinolysis (Perez-Casal et al., 2009). EMPs can be used, in conjunction with other measurements, to characterize and understand the effect of exercise on vascular health. Therefore, understanding the effect of exercise on circulating MPs is crucial in furthering our knowledge of vascular homeostasis.

CD62E$^+$ EMPs are released from the endothelium due to activation (Chironi et al., 2009) and elevated levels of these EMPs are found in states of vascular disease (Amabile et al., 2009; Chironi et al., 2009). CD34 is an established marker of proangiogenic hematopoietic progenitor cells (Asahara et al., 1999; Asahara et al., 1997); however research on CD34$^+$ MPs is in its

infancy, with our lab conducting the first study examining the effect of exercise on this MP population (Lansford et al., 2015). Our goal for the current investigation of exercise-induced alterations in these MP fractions was to further our understanding of the effects of exercise on the vasculature and the balance between endothelial activation and hematopoietic potential.

Specific Aims

Aim 1: Determine the time course of changes in $CD62E^+$ and $CD34^+$ MPs before, during, and after an acute bout of interval and continuous exercise.

Aim 2: Determine sex differences in levels of $CD62E^+$ and $CD34^+$ MPs before, during, and after an acute bout of interval and continuous exercise.

Aim 3: Determine whether high intensity interval and moderate intensity continuous exercise differentially effect levels of $CD62E^+$ and $CD34^+$ MPs.

Hypotheses

Hypothesis 1: $CD62E^+$ and $CD34^+$ MPs will both increase with exercise. Specifically, $CD62E^+$ EMPs will immediately increase, and then fall below baseline values by 2 hours after exercise, while $CD34^+$ MPs will remain elevated for all time points after exercise.

Hypothesis 2: Males will experience a greater increase in $CD62E^+$ MPs immediately following both exercise prescriptions with levels dropping below baseline by 2 hours after exercise, while females will exhibit an increase in $CD34^+$ MPs after exercise.

Hypothesis 3: $CD62E^+$ and $CD34^+$ MPs will be altered to a greater extent during and after interval exercise compared to continuous exercise.

CHAPTER 2

LITERATURE REVIEW

Circulating endothelial-derived microparticles (EMPs) are shed from endothelial cells due to activation or apoptosis (Chironi et al., 2009). Patients with vascular diseases exhibit elevated plasma levels of EMPs (Dignat-George & Boulanger, 2011), and EMPs are found in atherosclerotic plaques and ischemic tissues (Leroyer et al., 2009). EMPs carry various proteins that help characterize their biological effects including coagulation, fibrinolysis, angiogenesis, endothelial function, and adhesion (Dignat-George & Boulanger, 2011). The protein composition of EMPs seems to be dependent on the stimulus triggering their release (Dignat-George & Boulanger, 2011). EMPs contain DNA and RNA that they can transfer to their target cells thereby contributing to angiogenesis (Deregibus et al., 2007; Ullal et al., 2010).

While EMPs are shown to be elevated in diseased states, they also appear to exert a positive influence over vascular health. In vivo, EMPs have both procoagulant (Mallat et al., 1999) and fibrinolytic (Perez-Casal et al., 2009) properties, contributing to vascular homeostasis. Upon injection into ischemic tissue, EMPs enhance the proangiogenic effect of bone marrow mononuclear cells injected concurrently (Leroyer et al., 2009). EMPs have also been reported to help control cell death mechanisms (Abid Hussein, Boing, Sturk, Hau, & Nieuwland, 2007) and stimulate vascular repair (Lacroix et al., 2007).

E-selectin (CD62E) is one protein marker present on MPs from activated endothelial cells (Chironi et al., 2009). In patients with right ventricular heart failure, high baseline levels of CD62E$^+$ EMPs predicted death, rehospitalization, or worsening of symptoms, whereas no

correlation was observed with other EMPs (Amabile et al., 2009). In a study of secondhand smoke exposure, endothelial function was impaired as measured by flow-mediated dilation (FMD) and CD62E$^+$ EMPs were elevated following exposure, indicating a connection between endothelial function and EMP status (Heiss et al., 2008). Studies have shown higher levels of EMPs to be predictive of major cardiovascular events in patients with acute coronary syndrome (Fan et al., 2014), stroke (Lee et al., 2012), stable coronary artery disease (Sinning et al., 2011) and those at risk for CAD (Nozaki et al., 2009). It has been suggested that EMPs could be used as a surrogate marker of endothelial health and function (Dignat-George & Boulanger, 2011). However, it is important to consider that measurement of MPs at a specific time point provides a very limited indication of the status of MPs in vivo. The level of circulating MPs is dependent upon a dynamic relationship of events leading to the release and uptake of these particles (Ayers et al., 2015).

One such stimulus for release of MPs is exercise. Few studies examine the effect of physical activity on MPs in healthy populations (Boyle et al., 2013; Durrer et al., 2015; Harrison et al., 2009; Jenkins et al., 2011; Lansford et al., 2015; Mobius-Winkler et al., 2009; Sossdorf, Otto, Claus, Gabriel, & Losche, 2011; Wahl et al., 2014), and even fewer include both males and females (Durrer et al., 2015; Lansford et al., 2015). A study examining the effect of a reduction in physical activity levels (> 10,000 steps/day reduced to < 5,000 steps/day) resulted in a significant increase in CD31$^+$/CD42b$^-$ EMPs in conjunction with a limb-specific reduction in FMD, while CD62E$^+$ EMPs remained unchanged (Boyle et al., 2013). A single bout of cycling exercise resulted in reduced values of CD62E$^+$ EMPs, 15 (Jenkins et al., 2011) and 18 (Durrer et al., 2015) hours after exercise in men, while women experienced an increase above baseline levels of CD62E$^+$ EMPs 18 hours after a single bout of high intensity interval exercise (Durrer et

al., 2015). The exercise-induced changes in CD62E$^+$ EMPs observed in the previous study (Durrer et al., 2015), is in contrast to what our lab has reported following an acute bout of exercise (Lansford et al., 2015). The contradictory results are likely due to differences in the time of the blood sample acquisition and exercise intensity.

We recently completed the first study examining the effect of exercise on CD34$^+$ MPs, in which we observed an acute exercise-induced increase in CD34$^+$ MPs in women but not men (Lansford et al., 2015). In addition, we noted higher baseline values of CD34$^+$ MPs in men compared to women (Lansford et al., 2015). CD34 is a hematopoietic progenitor cell marker shown to have potent angiogenic effects (Asahara et al., 1999; Asahara et al., 1997). These CD34$^+$ MPs observed in healthy males and females may have a proangiogenic influence on the surrounding endothelium.

The current study is a follow-up to our previous work examining the effects of acute cycling exercise on circulating angiogenic cell and MP populations (Lansford et al., 2015). Specifically, we explored the findings that immediately after acute exercise men experienced an increase in CD62E$^+$ MPs, while women experienced an increase in CD34$^+$ MPs. This illustrates sex-specific exercise-induced effects of exercise on MPs, suggesting different vascular signaling as a result of exercise in men and women (Lansford et al., 2015). A limitation of our previous study (Lansford et al., 2015) was that we only analyzed MPs at two time points (baseline and immediately after exercise), providing a limited characterization of the effects of acute exercise on MPs. In other studies examining more than two time points, CD62E$^+$ EMPs, co-expressing annexin V as a marker of apoptosis, were elevated in trained males 45 minutes after a single bout of moderate intensity cycling exercise (80% of each individual's anaerobic threshold) and had returned to baseline values two hours after exercise (Sossdorf et al., 2011). The design for the

current study was such that we examined a total of seven time points encompassing before, during, and up to two hours after exercise in men and women, thereby providing a more comprehensive characterization of the time course of exercise-induced effects on these markers than previous studies.

As supported by the sex differences in MP results from our previous study (Lansford et al., 2015), there are marked differences between the cardiovascular health of males and females. Males experience higher rates of cardiovascular disease and cardiovascular risk factors (Sader & Celermajer, 2002). An explanation for these sex-specific differences in vascular health is the vital role of sex hormones in endothelial health and function. Estrogen replacement therapy improves FMD in post-menopausal women (Lieberman et al., 1994), as well as in genetic males taking high-dose estrogen (McCrohon et al., 1997). In regards to MPs, women exhibit elevated baseline values of $CD62E^+$ EMPs compared to men (Gustafson, Shepherd, Miller, & Jayachandran, 2015; Toth et al., 2007).

Regardless of sex, there is no consensus as to the best exercise protocol for influencing cardiovascular health (Ramos, Dalleck, Tjonna, Beetham, & Coombes, 2015) and the ideal protocol is largely dependent on the study population and outcome measures. A recent meta-analysis of the impact of high-intensity interval training (HIIT) versus moderate-intensity continuous training (MICT) on vascular function summarized the results of seven randomized trials including 182 patients (Ramos et al., 2015). These studies included males and females and patients with a range of cardiovascular diseases including heart failure (Wisloff et al., 2007), hypertension (Molmen-Hansen et al., 2012), type 2 diabetes mellitus (Mitranun, Deerochanawong, Tanaka, & Suksom, 2014), and metabolic syndrome (Tjonna et al., 2008), as well as obese adults (Schjerve et al., 2008). Change in FMD, as a marker of vascular function,

was the primary outcome measure. Vascular function indicates the ability of endothelial and smooth muscle cells to release and respond to molecules responsible for vascular homeostasis (Vane, Anggard, & Botting, 1990). It is commonly assessed using FMD as a non-invasive measure to determine the ability of vessels to dilate in response to a stimulus (Celermajer et al., 1992). Each study matched caloric expenditure between exercise groups and all but one utilized a 4×4 minute interval (Molmen-Hansen et al., 2012; Schjerve et al., 2008; Tjonna et al., 2008; Wisloff et al., 2007), with the other using 4 to 6, 1-minute intervals (Mitranun et al., 2014). Overall, there was a 2.26% improvement in FMD with HIIT compared to MICT. In addition, each study that reported improved FMD with HIIT over MICT, also reported an increase in VO_{2max} for HIIT above that observed with MICT (Ramos et al., 2015). Clinically, this reduction in FMD equates to approximately a 30% reduction in cardiovascular events (Inaba, Chen, & Bergmann, 2010), providing convincing evidence that in regard to improving vascular function in patient populations, HIIT may be superior. A goal of the present study was to enhance our understanding of intensity-specific exercise-induced alterations in the vasculature in healthy males and females after a single bout of exercise. Using MPs as the measure of vascular health is complementary to previous research that utilized FMD as both measures have been established as indicators of vascular health and disease (Dignat-George & Boulanger, 2011; Ramos et al., 2015).

CHAPTER 3

EFFECT OF EXERCISE INTENSITY ON CIRCULATING MICROPARTICLES IN MEN

AND WOMEN[1]

ABSTRACT

Circulating microparticles influence vascular homeostasis by participating in angiogenesis, fibrinolysis, coagulation, and endothelial function. Microparticles can be used to characterize and understand the effect of exercise on vascular health, contributing to our knowledge of vascular homeostasis. We investigated the effect of an acute bout of continuous and interval exercise on CD62E$^+$ endothelial microparticles (EMPs) and CD34$^+$ microparticles (MPs) at seven time points, in men and women. Blood samples were collected at baseline, halfway through exercise, immediately after exercise, and at 30, 60, 90, and 120 minutes after exercise. The interval exercise consisted of ten, 1-minute intervals at 100% and 90% VO$_{2max}$ on the treadmill. The continuous bout of exercise was energy matched to the interval bout, and lasted ~22 minutes at 65% VO$_{2max}$. Overall, exercise produced a decrease from baseline in CD62E$^+$ EMPs, however there was no effect of exercise on CD34$^+$ MPs. When examining the acute effect of exercise using only the baseline and immediately after exercise time points, there was a significant sex*time interaction for CD62E$^+$ EMPs. This study illustrates a clear effect of exercise to reduce CD62E$^+$ EMPs that is most evident in males after a short bout of continuous or interval exercise, with no effect of exercise on CD34$^+$ MPs in either sex.

INTRODUCTION

Circulating endothelial cell-derived microparticles (EMPs) are small (≤1 μm) membrane fragments shed from the endothelium due to activation or apoptosis (Chironi et al., 2009). EMP levels are elevated in states of vascular disease (Dignat-George & Boulanger, 2011), however they also play a role in angiogenesis (Deregibus et al., 2007; Ullal et al., 2010) and fibrinolysis (Perez-Casal et al., 2009). EMPs can be used, in conjunction with other measurements, to characterize and understand the effect of exercise on vascular health. Therefore, understanding the effect of exercise on circulating MPs is crucial in furthering our knowledge of vascular homeostasis.

CD62E^{+} EMPs are released from the endothelium due to activation (Chironi et al., 2009) and elevated levels of these EMPs are found in states of vascular disease (Amabile et al., 2009; Chironi et al., 2009). CD34 is an established marker of proangiogenic hematopoietic progenitor cells (Asahara et al., 1999; Asahara et al., 1997), however research on CD34^{+} MPs is in its infancy, with our lab recently reporting that an acute bout of endurance exercise increases CD34^{+} MPs in women only (Lansford et al., 2015).

In the present study, we tested the time course of the effects of acute continuous and interval exercise on CD62E^{+} and CD34^{+} MPs in young, healthy adults. We also investigated whether these two exercise protocols differentially altered MP populations in a sex-specific manner. We hypothesized that exercise intensity and sex would differentially impact each MP population over the time course of the study. Specifically, we hypothesized that males would experience a greater increase in CD62E^{+} EMPs after exercise, while females would exhibit greater CD34^{+} MP concentrations after exercise. In addition, we hypothesized that interval exercise would induce a greater change in MP levels compared to continuous exercise.

METHODS

Study Participants

Healthy, young, male (n = 10) and female (n = 10) subjects between the ages of 18-40 were recruited to perform three bouts of acute exercise: a maximal graded treadmill test, a high intensity interval exercise bout, and a moderate intensity continuous exercise bout. Approval for the study was received from the Western Institutional Review Board and all subjects provided written informed consent prior to data collection. Subjects were excluded if they smoked, participated in less than 30 minutes of vigorous activity two days per week or less than 30 minutes of moderate activity three days per week, were taking any cardiovascular, metabolic, or pharmacologic therapies, or were taking more than three prescription or non-prescription drugs in any class.

Experimental Protocol

Study subjects completed at least 3 visits to the laboratory, with 5 males and 4 females completing a fourth time control visit. Subjects reported to the lab between 0515-0730 after completing a 12-hour, overnight fast, and having abstained from alcohol and exercise for the preceding 24 hours, and caffeine for the previous 12 hours for all testing sessions. Subjects completed food logs for the 24 hours prior to the initial visit, and followed that log for each subsequent session to minimize any effects of changes in diet on study outcomes.

Body composition and cardiorespiratory fitness were assessed during the baseline visit. Body composition was measured using dual-energy X-ray absorptiometry (iDXA; GE Healthcare, Fairfield, CT, USA). Maximal oxygen uptake (VO_{2max}) was measured using a graded treadmill test with the incline increasing by 2.5% every 2 minutes while maintaining a self-selected, constant speed. Indirect calorimetry was used to continuously measure oxygen

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uptake, carbon dioxide production, respiratory exchange ratio and pulmonary ventilation (Parvo Medics TrueOne 2400, Parvo Medics, Salt Lake City, UT, USA), and heart rate was measured by telemetry (Polar, Lake Success, NY, USA). The test was designed to last 6-12 minutes, and effort was considered maximal if a plateau in oxygen consumption was reached even with increasing work, or by meeting two of the following secondary criteria: blood lactate levels above 8.0 mmol/L, rating of perceived exertion greater than 18, respiratory exchange ratio above 1.15, and peak heart rate within 10 beats/min of the age-predicated maximum.

The second and third visits, involving the interval and continuous exercise bouts, were completed in random order. The subjects reported to the lab at the same time for these visits to minimize any effect of time on variations in study outcomes. A venous catheter was inserted into the antecubital vein of each subject prior to the commencement of exercise. Subjects remained in a supine position for ten minutes prior to the baseline blood draw. Subjects completed a 3-minute warm-up at 50% VO_{2max} for each session, followed by the prescribed bout of exercise. The interval bout consisted of 10 one-minute intervals with the first five intervals completed at 100% VO_{2max} and the second five at 90% VO_{2max} with 75 seconds of active recovery at 20-35% VO_{2max} between each interval. The continuous exercise bout was completed at 65% VO_{2max} and was energy matched to the interval bout. After completion of exercise, subjects remained seated and sedentary for two hours while blood samples were obtained every 30 minutes.

A subset of 9 subjects (five men, four women) returned to the lab to complete a time control visit to control for any circadian variations in MPs. These subjects arrived at the same time as their previous visits, had a venous catheter placed in their antecubital vein, and remained

sedentary for the duration of the visit. Blood was drawn at the same time points as the preceding two visits.

During the exercise and control visits, eight milliliters of blood was collected in tubes containing acid citrate dextrose (Vacutainer Tubes; Becton Dickinson, Franklin Lakes, NJ, USA) from subjects at seven time points: baseline, halfway through exercise (EX), immediately following exercise (0), and 30, 60, 90, and 120 minutes after exercise. Samples were centrifuged for 20 minutes at 22°C and 2000g. After centrifugation, 500 μL of plasma was pipetted into microcentrifuge tubes and stored at -80°.

Microparticle Analysis

MP analysis was performed on the plasma samples as previously described (Lansford et al., 2015; van Ierssel et al., 2010), to identify changes in the number of $CD62E^+$ and $CD34^+$ MPs as a result of the exercise interventions. Briefly, MP assays were completed in batches with one batch containing all of one subject's samples for both exercise visits, stained for one MP population. Cell-free plasma was obtained and stained with fluorochrome-labelled antibodies marking the MP fraction of interest, $CD34^+$ (FITC-CD34) and $CD62E^+$ (PE-CD62E) MPs (Becton Dickinson, Franklin Lakes, NY, USA), and identified using fluorescence-activated cell sorting (CyAn ADP, Beckman Coulter, Hialeah, FL, USA). Nanobead NIST 900 nm calibration beads (Polysciences, Inc., Warrington, PA, USA) were used to ensure identification of MPs as events smaller than 1.0 μm. The concentration of MPs in the original blood sample was calculated using Countbright^{TM} Absolute Counting Beads (ThermoFisher Scientific, Waltham, MA, USA). Flow cytometry data was analyzed using FlowJo version 10.1r5 (FlowJo Software, Treestar, Inc., Ashland, OR, USA).

<u>Statistical Analysis</u>

Analysis was performed in IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY, USA). Baseline differences for physical characteristics and exercise prescriptions were analyzed using Student's paired t tests. MP data were analyzed using three-factor (sex $\times$ exercise $\times$ time) repeated-measures ANOVA and Fisher's least significant difference test for follow-up tests for simple effects. Data are presented as means $\pm$ SEM with statistical significance accepted at $P \leq 0.05$.

<div align="center">

RESULTS

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<u>Subject Characteristics and Exercise Prescriptions</u>

Physical characteristics of the study subjects (10 men, 10 women) are presented in Table 3.1 and exercise intensities and durations are presented in Table 3.2.

<u>Microparticles</u>

Continuous and interval exercise differentially altered plasma CD62E$^+$ EMP concentrations. In men and women combined, continuous exercise induced a 13%, 19%, and 19% decrease from baseline in CD62E$^+$ EMPs 60, 90, and 120 minutes, respectively, after exercise, while interval exercise resulted in no significant changes in MP concentrations (Fig. 3.1*A* and *B*). Sex-specific analysis revealed a 26% decrease from baseline values, 120 minutes after continuous exercise in CD62E$^+$ EMPs in males (Fig. 3.1*C*). Interval exercise decreased CD62E$^+$ EMPs from baseline by 27%, 35%, 31%, and 40%, immediately, 30, 90, and 120 minutes after exercise, respectively in males (Fig. 3.1*D*). Females experienced a 28% decrease in CD62E$^+$ EMPs 90 minutes after continuous exercise (Fig. 3.1*E*), while no significant changes were observed with interval exercise (Fig. 3.1*F*). The time course of changes in MP levels was not significantly different between males and females or between exercise conditions. A

significant main effect of time ($P = 0.004$) was observed in response to exercise in CD62E^{+} EMPs (Fig. 3.2). Time control data were collected on a subset of subjects (5 men, 4 women), and no significant differences were observed in CD62E^{+} EMPs across the 7 time points (Fig. 3.3). In a secondary analysis of MPs with two time points, baseline and immediately after exercise (T0), there was a significant sex*time interaction for CD62E^{+} EMPs ($P = 0.027$, Fig. 3.4). After interval exercise, there was a significant decrease in CD62E^{+} EMPs from baseline values in males (Fig. 3.4*B*). CD34^{+} MPs were not altered with continuous or interval exercise in either sex (Table 3.3).

DISCUSSION

In the present study, we made the following observations: a short bout of continuous or interval exercise resulted in a decrease in CD62E^{+} EMPs from baseline levels up to 120 minutes after exercise; when examining only baseline and immediately after exercise (T0) values, the ability of exercise to reduce CD62E^{+} EMPs was most pronounced in men; and the prescribed exercise bouts had no effect on CD34^{+} MPs in either sex at any time point. To our knowledge, this is the first study to examine the time course of continuous and interval exercise-induced alterations in plasma levels of MPs and to include a non-exercising control to determine the effect of time on these MP populations. These findings indicate that exercise acutely decreases CD62E^{+} EMPs but not CD34^{+} MPs up to two hours after exercise.

Previous studies examining the effect of exercise on CD62E^{+} EMPs have produced varying results (Babbitt et al., 2013; Durrer et al., 2015; Jenkins et al., 2011; Kretzschmar et al., 2014; Lansford et al., 2015; Sossdorf et al., 2011). The decline in CD62E^{+} EMPs in the current study is in contrast to the findings from our previous study in which we reported an increase in CD62E^{+} EMPs immediately after ~45 minutes of cycling at 60-70% VO$_{2peak}$ (Lansford et al.,

2015). As the intensity employed in the previous study and the intensity for the continuous bout in the current study were similar, the differing findings are likely to reflect differences in duration rather than intensity. The results from the previous study (Lansford et al., 2015) are supported by another study examining the effect of an acute bout of endurance exercise on CD62E$^+$ EMPs in young, trained males, (Sossdorf et al., 2011) which reported an increase in Annexin V$^+$/ CD62E$^+$ EMPs from baseline 45 minutes after cycling for 90 minutes, with levels returning to baseline 2-hours post-exercise. In agreement with the current study, two additional studies examining time points 15- (Jenkins et al., 2011) and 18- (Durrer et al., 2015) hours after exercise, also report decreases in CD62E$^+$ EMPs from baseline. It would seem that the mechanisms governing the kinetics of CD62E$^+$ EMP uptake and release into the circulation can be altered with exercise. This decrease in CD62E$^+$ EMPs following exercise could be a contributing factor to the anti-inflammatory effect of exercise (Mathur & Pedersen, 2008; Tyldum et al., 2009), as well as its positive impact on endothelial health and function (Haram et al., 2006) as CD62E$^+$ EMPs are markers of activated endothelial cells (Chironi et al., 2009) and a decrease in these MPs after exercise could indicate a less activated and inflamed endothelium.

A sub-analysis of the acute effect of exercise using only the baseline and immediately after exercise (T0) values for CD62E$^+$ EMPs, revealed a significant sex*time interaction, with only males exhibiting a significant decrease in CD62E$^+$ EMPs after interval exercise. CD62E$^+$ EMPs are established markers of elevated cardiovascular disease risk (Dignat-George & Boulanger, 2011) and can be increased by disturbing blood flow indicating a dysfunctional endothelial environment (Jenkins et al., 2013). The ability of a relatively short bout of either continuous moderate intensity or high intensity interval exercise to decrease CD62E$^+$ EMPs, as illustrated in this study, could contribute to a healthier endothelium and improve overall vascular

health. This could be particularly beneficial for males as they exhibit greater prevalence and severity of cardiovascular diseases (Sader & Celermajer, 2002).

The lack of an exercise effect on CD34$^+$ MPs is contrary to our previous study identifying exercise-induced, sex-specific differences in this MP population (Lansford et al., 2015). Specifically, in our previous study, women experienced a significant increase in CD34$^+$ MPs after an acute bout of endurance cycling exercise at 60-70% VO$_{2peak}$ for ~45 minutes (Lansford et al., 2015). The absence of an exercise effect in the current study is likely due to the shorter duration or lower volume of exercise compared to the previous study. These differences between studies could indicate an influential role of a minimal exercise volume needed to induce changes in CD34$^+$ MPs. Taken together, these results suggest that CD34$^+$ MPs are released in a duration or volume-specific manner and could also have a sex-specific component, however within the scope of the current study, sex was not a factor. Determining specific exercise parameters that enhance the number of circulating CD34$^+$ MP would be beneficial in increasing our understanding of the effect of exercise on the vasculature and could have clinical ramifications, as CD34 is a hematopoietic progenitor cell marker with potent angiogenic effects (Asahara et al., 1999; Asahara et al., 1997). While cells expressing CD34 have been relatively well researched, the actions of CD34$^+$ MPs have been largely unexplored. Further investigation of the actions of CD34$^+$ MPs as defined in this study (less than 1.0 μm) is warranted. Research has shown CD34$^+$ exosomes, that are much smaller than MPs (40-90 nm), help mediate the proangiogenic activity of CD34$^+$ cells (Sahoo et al., 2011). Alternatively, CD34$^+$/KDR$^+$ MPs (defined as < 1.5 μm) derived from endothelial progenitor cells in culture, have been shown to predict aortic stiffness and contribute to a pro-apoptotic milieu in individuals with varying degrees of cardiovascular risk (Pirro et al., 2008). Research clarifying the effect of CD34$^+$ MPs,

and whether exercise alters the actions of these MPs would greatly contribute to our understanding of this MP population.

CONCLUSION

In conclusion, we identified a short bout of exercise can reduce CD62E$^+$ EMPs up to two hours after exercise, potentially identifying a contributor to the positive effect of exercise on cardiovascular health. The immediate effect of exercise is sex-specific, further contributing to differences in vascular health between men and women. Future research is needed to further our understanding of the actions of CD62E$^+$ EMPs and their impact after exercise on cardiovascular health. In addition, future study of the effect of exercise volume on CD34$^+$ MPs is needed to advance our understanding of their role in vascular adaptations to exercise. Overall, our findings indicate the ability of exercise to improve the vascular environment by a reduction in CD62E$^+$ EMPs.

Table 3.1 Subject characteristics. Values are mean ± SE. BMI, body mass index; FFM, fat-free mass; VO_{2max}, maximal oxygen uptake. * Statistically significant difference between groups ($P \leq 0.05$).

|	Men ($n = 10$)	Women ($n = 10$)
Age, years	23.6 ± 1.0	23.6 ± 1.8
Height, m	1.8 ± 0.03	1.7 ± 0.02*
Weight, kg	79.5 ± 3.1	60.0 ± 1.0*
BMI, kg/m^2	24.2 ± 0.6	21.8 ± 0.5*
Body fat, %	17.9 ± 1.4	28.7 ± 1.2*
FFM, kg	65.1 ± 2.2	42.8 ± 1.1*
VO_{2max} , l • min^{-1}	4.0 ± 0.2	2.6 ± 0.1*
VO_{2max} , ml • kg^{-1} • min^{-1}	50.1 ± 1.6	42.5 ± 1.6*
VO_{2max} , ml • kg FFM^{-1} • min^{-1}	60.9 ± 1.3	59.6 ± 1.7

Table 3.2 Exercise Intensity and Duration. Values are mean ± SE. * Statistically significant difference between groups ($P \leq 0.05$).

	Men ($n = 10$)	Women ($n = 10$)
100% VO_{2max}, ml • kg^{-1} • min^{-1}	50.1 ± 1.6	42.5 ± 1.6*
90% VO_{2max}, ml • kg^{-1} • min^{-1}	45.1 ± 1.4	38.3 ± 1.4*
65% VO_{2max}, ml • kg^{-1} • min^{-1}	32.6 ± 1.0	27.6 ± 1.0*
50% VO_{2max}, ml • kg^{-1} • min^{-1}	25.0 ± 0.8	21.3 ± 0.8*
Interval Exercise Duration, min	25.5	
Continuous Exercise Duration, min	21.8 ± 0.2	22.8 ± 0.2*

Table 3.3 Time Course of CD34$^+$ MPs. Values are presented as concentrations of CD34$^+$ MPs/µL plasma. Data are presented as mean ± SE. No significant effects of sex, time, or exercise were observed.

	Baseline	Exercise	Post-exercise Time (min)				
			0	30	60	90	120
Males (*n* = 10)							
Continuous	15.81 ± 4.19	14.03 ± 5.41	15.13 ± 5.82	8.59 ± 4.13	9.99 ± 4.38	10.87 ± 3.38	9.92 ± 3.80
Interval	11.95 ± 3.60	11.12 ± 4.01	11.91 ± 4.24	13.12 ± 3.59	12.03 ± 4.74	12.12 ± 4.20	10.89 ± 3.99
Females (*n* = 10)							
Continuous	13.11 ± 4.19	13.00 ± 5.41	14.69 ± 5.83	16.04 ± 4.13	13.95 ± 4.38	11.92 ± 3.38	12.35 ± 3.8
Interval	10.61 ± 3.60	12.21 ± 4.01	11.77 ± 4.24	10.09 ± 3.59	13.12 ± 4.74	13.17 ± 4.20	12.67 ± 3.99
Combined (*n* = 20)							
Continuous	14.46 ± 2.9	13.52 ± 3.72	14.91 ± 4.01	12.32 ± 2.97	11.97 ± 3.05	11.39 ± 2.33	11.14 ± 2.63
Interval	11.28 ± 2.48	11.66 ± 2.76	11.84 ± 2.92	11.61 ± 2.50	12.58 ± 3.27	12.65 ± 2.89	11.78 ± 2.75
Combined (*n* = 9)							
Time Control	5.83 ± 2.04	7.54 ± 2.42	7.75 ± 2.72	6.89 ± 2.72	6.57 ± 1.82	6.57 ± 2.69	8.39 ± 2.15

Figure 3.1: CD62E$^+$ EMPs with Exercise
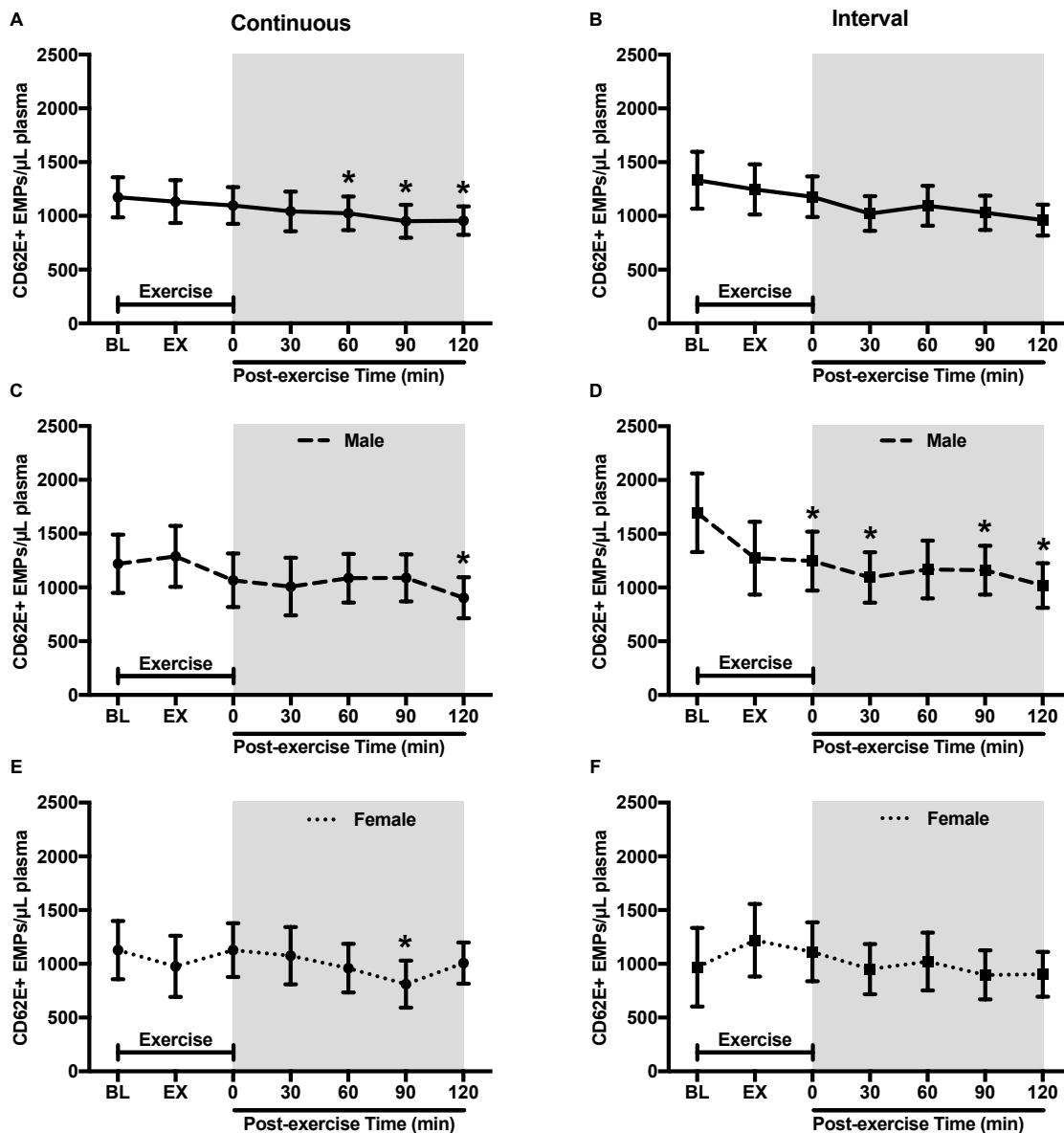
**Figure 3.1** CD62E$^+$ microparticles before, during, and after acute continuous (*A*, *C*, and *E*, •) and interval (*B*, *D*, and *F*, ■) exercise. Data for males and females combined (*A* and *B*,−), males only (*C* and *D*, ---), and females only (*E* and *F*, •••). Grey shading indicates post-exercise, sedentary recovery time. *Statistically significant change relative to baseline (*P* < 0.05).

Figure 3.2: Effect of Time on CD62E$^+$ EMPs
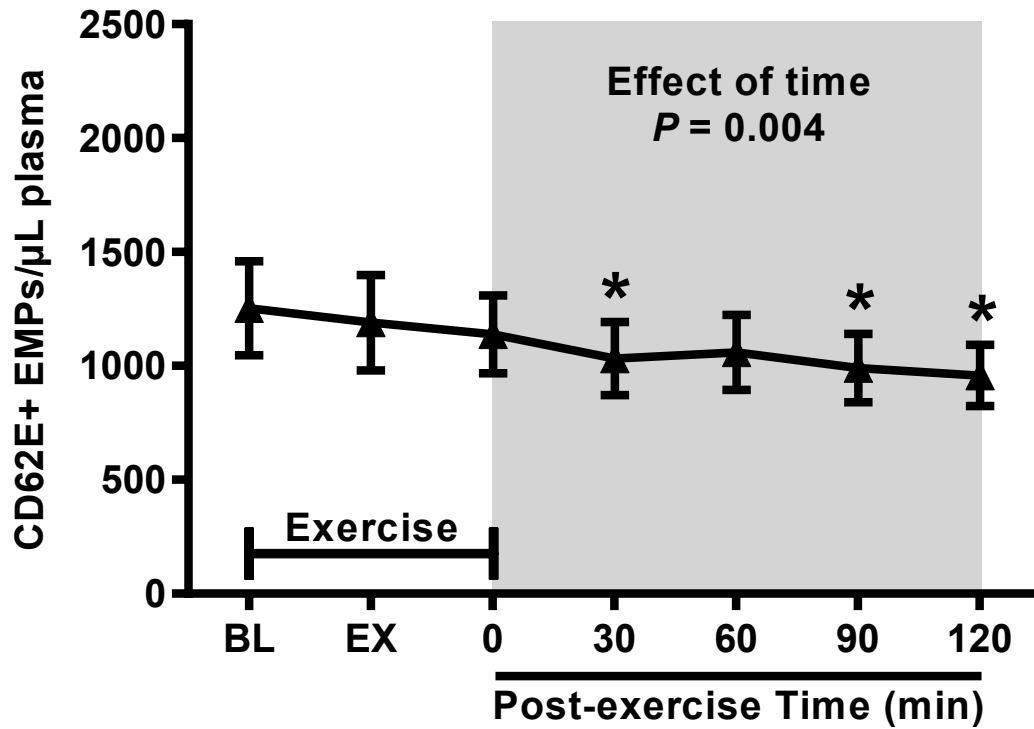
Figure 3.2 CD62E$^+$ microparticles before, during, and after acute exercise. Graph represents data from both sexes and exercise conditions collapsed together. Grey shading indicates post-exercise, sedentary recovery time. *Statistically significant change relative to baseline ($P < 0.05$).

Figure 3.3: CD62E$^+$ EMPs Time Control
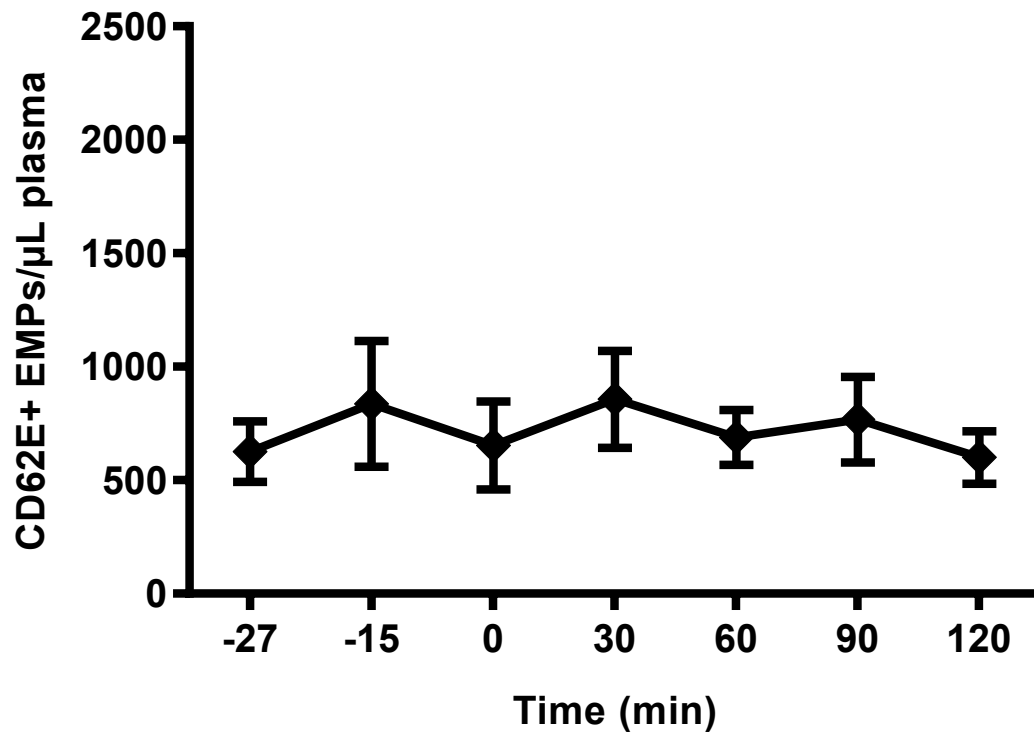
Figure 3.3 Time control for CD62E$^+$ EMPs in all nine time control subjects with data from the

males and females combined. No significant differences between time points.

Figure 3.4: CD62E$^+$ EMPs Baseline vs. After Exercise
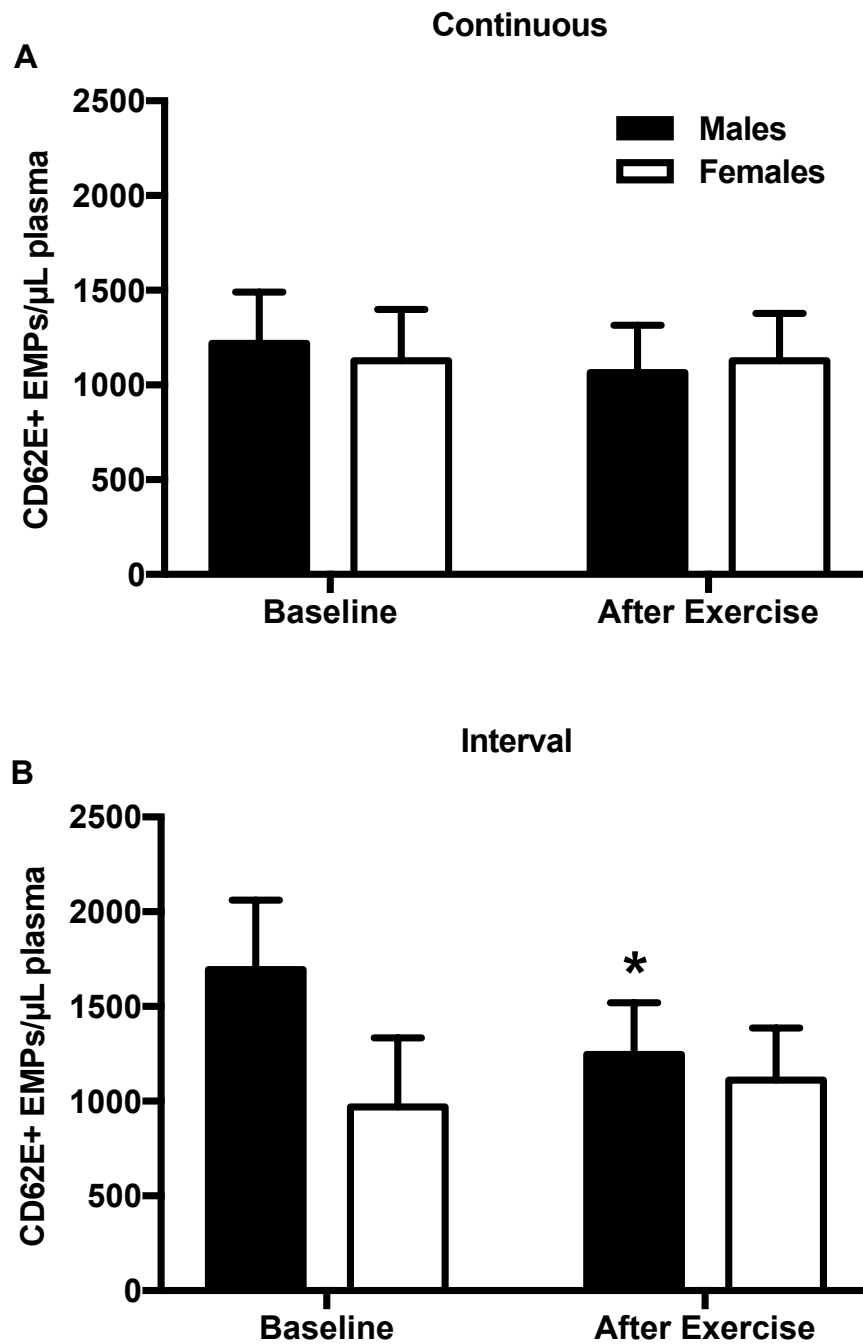
Figure 3.4 CD62E$^+$ EMPs at baseline and after exercise (T0), in males (black) and females (white). Significant time*sex interaction ($P = 0.027$). *Statistically significant change relative to baseline within the same sex ($P < 0.05$).

CHAPTER FOUR

SUMMARY AND CONCLUSION

In the present study, we made the following observations: a short bout of continuous or interval exercise resulted in a decrease in $CD62E^+$ EMPs from baseline levels up to 120 minutes after exercise; when examining only baseline and immediately after exercise (T0) values, the ability of exercise to reduce $CD62E^+$ EMPs was most pronounced in men; and the prescribed exercise bouts had no effect on $CD34^+$ MPs in either sex at any time point. To our knowledge, this is the first study to examine the time course of continuous and interval exercise-induced alterations in plasma levels of MPs and to include a non-exercising control to determine the effect of time on these MP populations. These findings indicate that exercise acutely decreases $CD62E^+$ EMPs but not $CD34^+$ MPs up to two hours after exercise.

Future studies of MPs and exercise should include characterizations of these MP populations to develop a better understanding of the significance of fluctuations in their numbers as a result of exercise. While it has been established that $CD62E^+$ EMPs are elevated in states of cardiovascular disease, their influence on the cardiovascular system after exercise remains to be determined. Once the exercise effect of $CD62E^+$ EMPs on cardiovascular health is determined, it will be possible to identify the exercise protocol that produces the optimal response in this MP population. As such, it is possible that the question of high intensity interval versus continuous moderate exercise, in relation to these MP populations, remains to be completely answered.

To our knowledge, we are the only lab studying the effect of exercise on $CD34^+$ MPs. These potentially proangiogenic MPs have yet to be completely characterized. While some

research has been conducted on smaller exosomes and larger MPs, the study of these particles is in its infancy. When both studies conducted by this lab are taken together, it would seem that changes in this population are only induced after an exercise bout of greater duration than was employed in the current study. However, it is likely that the answer is not simply one of greater duration, rather of a specific exercise volume that requires a minimal intensity and duration to be reached in order to induce alterations in the levels of $CD34^+$ MPs. Future studies should address whether there is a minimum exercise volume threshold that must be achieved in order to impact levels of $CD34^+$ MPs.

Additionally, the two studies differ in the baseline values of $CD34^+$ MPs observed in men and women. Our previous study revealed an elevated baseline level of $CD34^+$ MPs in men compared to women. Given the known differences in cardiovascular health between men and women, it would be interesting to determine what effect this MP population is having on the vascular health of each sex.

In conclusion, exercise can reduce $CD62E^+$ EMPs up to two hours after exercise, potentially identifying a contributor to the positive effect of exercise on cardiovascular health. The immediate effect of exercise is sex-specific, further contributing to differences in vascular health between men and women. Future research is needed to further our understanding of the actions of $CD62E^+$ EMPs and $CD34^+$ MPs and their impact after exercise on cardiovascular health. Overall, our findings indicate the ability of exercise to improve the vascular environment by a reduction in $CD62E^+$ EMPs.

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