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Effect of exercise intensity on circulating microparticles in men and women

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Abstract

Circulating microparticles (MPs) are biological vectors of information within the cardiovascular system that elicit both deleterious and beneficial effects on the vasculature. Acute exercise has been shown to alter MP concentrations, probably through a shear stress-dependent mechanism, but evidence is limited. Therefore, we investigated the effect of exercise intensity on plasma levels of $CD34^+$ and $CD62E^+$ MPs in young, healthy men and women. Blood samples were collected before, during and after two energy-matched bouts of acute treadmill exercise: interval exercise (10 × 1 min intervals at ∼95% of maximal oxygen uptake $\dot{V}_{O_2\,max}$) and continuous exercise (65% $\dot{V}_{O_2\,max}$). Continuous exercise, but not interval exercise, reduced $CD62E^+$ MP concentrations in men and women by 18% immediately after exercise (from 914.5 ± 589.6 to 754.4 ± 390.5 MPs μl^{-1}; $P < 0.05$), suggesting that mechanisms underlying exercise-induced $CD62E^+$ MP dynamics are intensity dependent. Furthermore, continuous exercise reduced $CD62E^+$ MPs in women by 19% (from 1030.6 ± 688.1 to 829.9 ± 435.4 MPs μl^{-1}; $P < 0.05$), but not in men. Although interval exercise did not alter $CD62E^+$ MPs *per se*, the concentrations after interval exercise were higher than those observed after continuous exercise ($P < 0.05$). Conversely, $CD34^+$ MPs did not fluctuate in response to short-duration acute continuous or interval exercise in men or women. Our results suggest that exercise-induced MP alterations are intensity dependent and sex specific and impact MP populations differentially.

KEYWORDS
cardiovascular health, physical activity, sex differences

1 | INTRODUCTION

Microparticles (MPs) are small, cell-derived bilayer membranes containing various concentrations of lipids, mRNAs and microRNAs, which facilitate intercellular communication within the cardiovascular system (Diehl et al., 2012; Hugel, Martínez, Kunzelmann, & Freyssinet, 2005; Morel, Jesel, Freyssinet, & Toti, 2011). Literature indicates that endothelial cells shed MPs in response to apoptosis ($CD31^+/CD42b^-$) or activation ($CD62E^+$), and MPs have been regarded as biomarkers for cardiovascular disease (Amabile et al., 2014; Boulanger, Amabile, & Tedgui, 2006; Chironi et al., 2009; Dignat-George & Boulanger, 2011; Jimenez et al., 2003; Lee et al., 2012; Schiro et al., 2014). Circulating endothelium-derived MPs are not only elevated in cardiovascular disease patients, but can predict adverse cardiovascular events and mortality, suggesting that systemic endothelial perturbations augment the risk for cardiovascular disease (Boulanger et al., 2006; Chironi et al., 2009; Dignat-George & Boulanger, 2011; Lee et al., 2012; Rautou *et al.*, 2011; Sinning et al., 2011; Werner, Wassmann, Ahlers, Kosiol, & Nickenig, 2006).

The number of circulating MPs reflects a tightly controlled relationship between MP release and uptake (Ayers et al., 2015; Curtis et al., 2013; Hoyer, Nickenig, & Werner, 2010). Recent investigations suggest that shear stress (i.e. frictional force of the blood) is a crucial regulator of MP shedding and that endocytosis via endothelial cells is the predominant MP clearance mechanism (Alexy, Rooney, Weber, Gray, & Searles, 2014; Ayers et al., 2015; Jenkins et al., 2013; Vion *et al.*, 2013). Although evidence is limited, the available studies indicate that acute exercise can alter MP concentrations, probably through a shear stress-dependent mechanism. Other mechanisms (e.g. inflammation, reactive oxygen species, hypoxia) have been shown to alter circulating

New Findings

- **What is the central question of this study?**
  What is the effect of exercise intensity on circulating microparticle populations in young, healthy men and women?
- **What is the main finding and its importance?**
  Acute, moderate-intensity continuous exercise and high-intensity interval exercise altered distinct microparticle populations during and after exercise in addition to a sex-specific response in $CD62E^+$ microparticles. The microparticles studied contribute to cardiovascular disease progression, regulate vascular function and facilitate new blood vessel formation. Thus, characterizing the impact of intensity on exercise-induced microparticle responses advances our understanding of potential mechanisms underlying the beneficial vascular adaptations to exercise.

MPs, but the extent to which these factors contribute to the rate of MP shedding or clearance is not completely understood (Ayers et al., 2015; Curtis et al., 2013; Dignat-George & Boulanger, 2011; Hoyer et al., 2010). Generally, results have varied with regard to the magnitude and direction of exercise-induced changes in MP concentrations, probably owing to the diversity among exercise protocols (Durrer et al., 2015; Guiraud et al., 2013; Lansford et al., 2016; Mobius-Winkler et al., 2009; Serviente et al., 2016; Sossdorf, Otto, Claus, Gabriel, & Lösche, 2010, 2011; Wilhelm, Gonzalez-Alonso, Parris, & Rakobowchuk, 2016). As previous investigations have used a variety of exercise protocols, it seems plausible that exercise mode, intensity and duration all influence the degree of variation in MP concentrations (Durrer et al., 2015; Guiraud et al., 2013; Lansford et al., 2016; Mobius-Winkler et al., 2009; Serviente et al., 2016; Sossdorf et al., 2010, 2011; Wilhelm et al., 2016). Moreover, we recently reported preliminary evidence that the effects of acute exercise are not uniform among endothelial MP subtypes and that they are different between men and women (Lansford et al., 2016). Finally, studies to date have not comprehensively examined the effect of exercise intensity on the time course of concentrations of MPs, representing an incomplete understanding of the role played by exercise intensity in the beneficial vascular adaptations to exercise.

Therefore, the present study aimed to characterize the effects of acute continuous and interval exercise on plasma levels of $CD34^+$ and $CD62E^+$ MPs in young, healthy men and women. Moreover, endothelial MPs are elevated in cardiovascular disease patients, but remain unaltered after acute exercise at high and moderate intensities (Guiraud et al., 2013), which indicates the that systemic endothelial impairments in cardiovascular disease are unaffected by varying intensities of acute exercise (Boulanger et al., 2006; Chironi et al., 2009; Dignat-George & Boulanger, 2011; Lee et al., 2012; Rautou et al., 2011; Sinning et al., 2011; Werner et al., 2006). In light of evidence indicating a crucial role of shear stress contributing to MP release (Ayers et al., 2015; Jenkins et al., 2013; Vion et al., 2013), in conjunction with sex differences among exercise-induced MPs (Durrer et al., 2015; Lansford et al., 2016), we hypothesized that: (i) high-intensity interval exercise (HIIE) would induce greater changes in MP levels compared with moderate-intensity continuous exercise (MICE); (ii) haematopoietic stem cell-derived $CD34^+$ MPs would be higher in women after exercise; and (iii) men would exhibit greater $CD62E^+$ MP concentrations after exercise.

2 | METHODS

2.1 | Ethical approval

The Western Institutional Review Board (Puyallup, WA, USA) approved all study procedures (approval no. 1154603), and subjects provided written informed consent before data collection. All study procedures were carried out in accordance with the *Declaration of Helsinki*, and the study was not registered in any research database.

2.2 | Screening

Healthy men and women between the ages of 18 and 40 years were recruited to perform three bouts of acute exercise at different intensities. Subjects were excluded if they smoked, participated in <30 min of vigorous activity 2 days per week or <30 min of moderate activity 3 days per week, were taking any cardiovascular or metabolic pharmacological therapies, or were taking more than three prescription or non-prescription drugs in any class.

2.3 | Experimental protocol

All subjects completed three visits to the laboratory between 05.15 and 07.30 h after a 12 h overnight fast, having abstained from alcohol and exercise for the preceding 24 h and caffeine for the previous 12 h. Subjects recorded food/beverage consumption for 24 h before the initial visit and were instructed to repeat the food/beverage intake for the 24 h before the initial visit for each subsequent visit as closely as possible to mitigate any dietary influence. Fluid intake for the 24 h preceding laboratory visits was not different between exercise bouts or groups (data not shown). Subjects were not allowed to consume any fluid during exercise, but water intake after exercise was monitored and recorded. Water consumption after exercise was not different between visits or groups (data not shown).

All experiments were carried out in an environmentally controlled (air-conditioned) room, where a normal room temperature (20–22°C) and humidity (45–55%) were maintained. Body composition was measured using dual-energy X-ray absorptiometry (iDXA; GE Healthcare, Pittsburg, PA, USA). Body composition data are available for only 19 participants (nine men and 10 women) owing to loss of data for one subject during a database back-up. Maximal oxygen uptake ($\dot{V}_{O_2\,max}$) was measured using a self-selected, constant-speed treadmill protocol. Incline increased every 2 min by 2.5% until volitional fatigue. Heart rate (Polar; Polar Electro Inc., Lake Success, NY, USA) and expired gases, via indirect calorimetry (Parvo Medics TrueOne 2400; Parvo Medics, Salt Lake City, UT, USA), were continuously measured throughout the test. Oxygen consumption was considered maximal

if a plateau in oxygen consumption was attained with increasing work, or by meeting two of the following secondary criteria: blood lactate concentration >8.0 mmol l^{-1}, rating of perceived exertion >18, respiratory exchange ratio >1.15, and peak heart rate within 10 beats min^{-1} of the age-predicated maximum. Blood lactate was measured from venous blood using a hand-held lactate analyser (Arkray Inc., Kyoto, Japan) within 5 min after completing the $\dot{V}_{O_2\,max}$ protocol.

The second and third visits consisted of a randomly assigned HIIE or MICE bout. A venous catheter was inserted into an antecubital vein for blood sampling. Blood was collected in tubes containing acid citrate dextrose (Vacutainer®; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) from subjects at seven time points: baseline, halfway through exercise, immediately after exercise, and 30, 60, 90 and 120 min after exercise. Subjects remained supine for 10 min before the baseline blood sampling. The blood sample halfway through and immediately after exercise was obtained while subjects were exercising and standing upright on the treadmill, respectively. All postexercise blood samples were obtained while subjects were in the seated position. A 3 min warm-up at 50% $\dot{V}_{O_2\,max}$ preceded each prescribed exercise bout. The HIIE consisted of 10 × 1 min intervals, with the first five intervals at 100% $\dot{V}_{O_2\,max}$ and the second five at 90% $\dot{V}_{O_2\,max}$, with 75 s of active recovery at 20–35% $\dot{V}_{O_2\,max}$ between each interval. The MICE prescription was equivalent to 65% $\dot{V}_{O_2\,max}$ and energy matched to the interval bout. A subset of nine subjects (five men and four women) completed a fourth visit to control for any circadian variations. Subjects remained seated for the entire duration of the visit. Blood was obtained at the same time points as the preceding two visits.

2.4 | Microparticle analysis

Plasma was centrifuged for 20 min at 2000*g*, and stored in 500 μl aliquots at −80°C until analysis. Microparticle concentrations were determined in batch assays as previously described (Serviente et al., 2016). Briefly, cell-free plasma was labelled with 20 μl of FITC-CD34 or PE-CD62E (Becton, Dickinson and Company) antibodies to determine MP fractions. Flow cytometric fluorescence (CyAn ADP; Beckman Coulter, Hialeah, FL, USA) was acquired for 180 s from events smaller than 1.0 μm. Microparticles were classified using 900 nm calibration beads (Polysciences, Inc., Warrington, PA, USA), and plasma concentrations were calculated using CountBright™ Absolute Counting Beads (ThermoFisher Scientific, Waltham, MA, USA). Flow cytometry data were analysed via FlowJo V10.1r5 (FlowJo, LLC, Ashland, OR, USA). $CD34^+$ MPs were assessed based on data indicating a role in paracrine signalling from haematopoietic progenitor cells, which promote and contribute to the maintenance of vascular integrity (Landers-Ramos et al., 2015; Sahoo et al., 2011). Additionally, investigations determining the effect of exercise on $CD34^+$ MPs are limited to one study (Lansford et al., 2016). We examined $CD62E^+$ MPs because increased concentrations indicate a pro-inflammatory endothelium (Jimenez et al., 2003), and in light of our previous findings demonstrating sex differences in $CD62E^+$ MP concentrations after exercise (Lansford et al., 2016).

2.5 | Statistics

Power analysis indicated that 10 participants were needed to detect exercise-induced effects on MP subpopulations of magnitudes reported previously (Lansford et al., 2016). Subject characteristics were analysed using a one-way ANOVA. Exercise duration and caloric expenditure were analysed with a two-way repeated-measures ANOVA (condition × sex). Microparticle data were analysed using a three-factor, condition × time × sex, repeated-measures ANOVA with Fisher's least significant difference tests for *post hoc* comparisons. Data are presented as means ± SD. Statistical significance was accepted at $P \leq 0.05$. P values of ≤0.10 but >0.05 were considered to be approaching statistical significance. Analysis was performed in SPSS, Version 24.0 (SPSS, IBM Corp., Armonk, NY, USA).

3 | RESULTS

3.1 | Subject characteristics

Subject characteristics are reported in Table 1. The results of a two-way ANOVA (condition × sex) show a significant interaction for

TABLE 1 Subject and exercise characteristics

Characteristic	Combined ($n = 20$)	Men ($n = 10$)	Women ($n = 10$)
Age (years)	23.6 ± 4.45	23.6 ± 3.27	23.6 ± 5.58
Height (m)	1.74 ± 0.11	1.81 ± 0.09	1.66 ± 0.06*
Mass (kg)	69.8 ± 12.3	79.5 ± 9.87	60.0 ± 3.24*
BMI (kg m^{-2})	23.0 ± 2.11	24.2 ± 1.88	21.8 ± 1.61*
FFM (kg)‡	54.6 ± 12.8	66.5 ± 7.36	44.0 ± 3.36*
Body fat (%)‡	23.3 ± 7.01	17.3 ± 4.18	28.7 ± 3.70*
$\dot{V}_{O_2\,max}$ (l min^{-1})	3.27 ± 0.86	3.98 ± 0.56	2.56 ± 0.35*
$\dot{V}_{O_2\,max}$ (ml kg^{-1} min^{-1})	46.4 ± 6.28	50.2 ± 5.08	42.6 ± 5.00*
$\dot{V}_{O_2\,max}$ (ml FFM min^{-1})‡	59.2 ± 4.72	60.4 ± 3.88	58.1 ± 5.34
Contraceptive use (yes/no)	—	—	8/2
Time between visit 1 and 2 (days)	6.65 ± 3.86	7.40 ± 4.62	5.90 ± 2.96
Time between visit 2 and 3 (days)	7.40 ± 4.95	6.10 ± 1.79	8.70 ± 6.68
Exercise duration (min)			
MICE	19.3 ± 0.76	18.9 ± 0.50*	19.8 ± 0.67*,†
HIIE	22.5 ± 0.00	22.5 ± 0.00	22.5 ± 0.00
Calories (l min^{-1})			
MICE	203.6 ± 49.0	243.3 ± 34.7	163.8 ± 18.3*
HIIE	203.6 ± 49.0	243.3 ± 34.7	163.8 ± 18.3*

Values are means ± SD. Abbreviations: BMI, body mass index; FFM, fat-free mass; HIIE, high-intensity interval exercise; MICE, moderate-intensity continuous exercise; and $\dot{V}_{O_2\,max}$, maximal oxygen uptake.

*Statistically different between men and women ($P < 0.01$).

†Statistically different between conditions ($P < 0.01$).

‡Body composition data were available on nine male subjects.

exercise duration and calories expended during exercise between men and women ($P < 0.05$). Specifically, men exercised for a shorter duration during MICE compared with women ($P < 0.05$; Table 1). Additionally, men expended more calories during MICE and HIIE compared with women ($P < 0.05$; Table 1). Menstrual cycle phase did not have any effect on subject characteristics ($P > 0.05$). Among women, the use of contraceptive therapy and the initial date of their most recent menses were self-reported. Eight women reported using contraceptive therapies with varying formulations and dosages. Menstrual cycle phase was determined by placebo pill ingestion on the morning of each exercise visit. The absence of regular menstruation owing to an intrauterine device excluded one woman from the menstrual cycle phase analysis. Among women using contraceptives during their continuous exercise visit, one woman was in the follicular phase and six in the luteal phase. Within women using contraceptives during their interval exercise visit, three were in the follicular phase and four in the luteal phase. Within non-contraceptive users, follicular and luteal phases were based on ≤16 and >16 days, respectively, from the self-reported date of previous menses onset to the morning of exercise (Fehring, Schneider, & Raviele, 2006). During MICE, both non-contraceptive users were in the luteal phase. For the HIIE visit, one woman was in the follicular phase and one in the luteal phase.

3.2 | Microparticles

Analysis of $CD34^+$ and $CD62E^+$ MP concentrations at all seven times revealed no significant interaction ($P > 0.05$; Table 2 and Figure 1a,b). Further investigation revealed a three-way interaction with $CD62E^+$ MPs that approached statistical significance during exercise; however, $CD34^+$ and $CD62E^+$ MP values were not altered 30, 60, 90 or 120 min after exercise ($P > 0.05$; Table 2 and (Figure 1a,b)). $CD34^+$ and $CD62E^+$ MP concentrations during MICE and HIIE visits were not different from MPs obtained during the time control visit ($P > 0.05$; Table 2 and Figure 2). No interactions or main effects were observed for $CD34^+$ MP counts during exercise ($P > 0.05$; Table 2). During exercise, a condition × time × sex interaction approached statistical significance for $CD62E^+$ MP concentrations ($P = 0.083$; Figure 1). Moderate-intensity continuous exercise induced an 18% decrease in $CD62E^+$ MPs from baseline (914.5 ± 589.6 MPs μl^{-1}) to postexercise (754.4 ± 390.5 MPs μl^{-1}; $P < 0.05$; Figure 1a,c). High-intensity interval exercise did not alter $CD62E^+$ MPs ($P > 0.05$; Figure 1b,d); however, postexercise the concentrations were significantly higher after HIIE (985.5 ± 596.7 MPs μl^{-1}) compared with MICE (754.4 ± 390.5 MPs μl^{-1}; $P < 0.05$; Figure 1c,d). In men only, MICE induced a 22% decrease in $CD62E^+$ MPs from halfway through exercise (875.5 ± 681.9 MPs μl^{-1}) to postexercise (678.9 ± 345.8 MPs μl^{-1}), which approached statistical significance ($P = 0.073$; Figure 1e). $CD62E^+$ MPs in men were not changed during HIIE ($P > 0.05$; Figure 1f). In men, after HIIE the $CD62E^+$ MPs were significantly higher (982.3 ± 638.9 MPs μl^{-1}) than after MICE (678.9 ± 345.8 MPs μl^{-1}; $P < 0.05$; Figures 1e,f). In women, MICE significantly reduced $CD62E^+$ MPs by 19% from baseline (1030.6 ± 688.1 MPs μl^{-1}) to postexercise (829.9 ± 435.4 MPs μl^{-1}; $P < 0.05$), with a 24% decrease approaching statistically significance halfway through exercise (785.0 ± 357.5 MPs μl^{-1}; $P = 0.059$; Figure 1e). In women, $CD62E^+$ MPs remained unchanged during HIIE ($P > 0.05$; Figure 1f). Menstrual cycle phase did not have any effect on MP concentrations ($P > 0.05$).

4 | DISCUSSION

We examined the effects of acute MICE and HIIE on plasma concentrations of $CD34^+$ and $CD62E^+$ MPs in young, healthy men and women. Overall, our results indicate that exercise-induced changes in $CD62E^+$ MPs are both sex specific and intensity dependent. Our results revealed three primary findings. First, MICE but not HIIE reduces $CD62E^+$ MP concentrations. This effect was driven by women, as our statistical analysis indicated that the reduction was significant in both the entire study cohort and in the secondary analysis of women only. Second, HIIE appears to maintain $CD62E^+$ MP concentrations, particularly in men, as evidenced by our finding that $CD62E^+$ MP levels were significantly higher after HIIE than after MICE. Finally, we also demonstrated that $CD34^+$ MPs did not fluctuate in response to short-duration acute MICE or HIIE. Overall, our study demonstrates that changes in circulating $CD62E^+$ and $CD34^+$ MP concentrations

TABLE 2 $CD34^+$ microparticles per microlitre of plasma

	Baseline	Exercise	Immediately postexercise	30 min postexercise	60 min postexercise	90 min postexercise	120 min postexercise
Combined ($n = 20$)							
Continuous	16.0 ± 18.2	15.0 ± 19.8	16.6 ± 21.5	13.1 ± 13.1	13.7 ± 14.7	12.3 ± 11.5	12.0 ± 11.6
Interval	12.5 ± 10.9	12.5 ± 11.9	12.5 ± 11.6	12.4 ± 10.6	13.4 ± 14.2	14.1 ± 13.0	12.4 ± 12.3
Men ($n = 10$)							
Continuous	18.9 ± 22.9	16.5 ± 22.9	18.0 ± 25.3	9.6 ± 7.4	11.7 ± 10.1	11.3 ± 9.4	10.0 ± 7.5
Interval	14.0 ± 8.5	12.3 ± 8.0	12.7 ± 9.6	14.6 ± 10.9	12.1 ± 8.9	14.0 ± 9.4	11.0 ± 8.6
Women ($n = 10$)							
Continuous	13.1 ± 12.6	13.5 ± 17.2	15.2 ± 18.2	16.6 ± 16.8	15.6 ± 18.6	13.3 ± 13.8	13.9 ± 14.8
Interval	11.1 ± 13.2	12.7 ± 15.3	12.3 ± 13.9	10.2 ± 10.4	14.8 ± 18.4	14.1 ± 16.4	13.8 ± 15.6
Time control ($n = 9$)	5.6 ± 5.3	7.3 ± 6.4	7.2 ± 7.1	6.1 ± 5.8	5.9 ± 4.3	5.8 ± 5.9	7.6 ± 5.5

Values are presented as means ± SD.

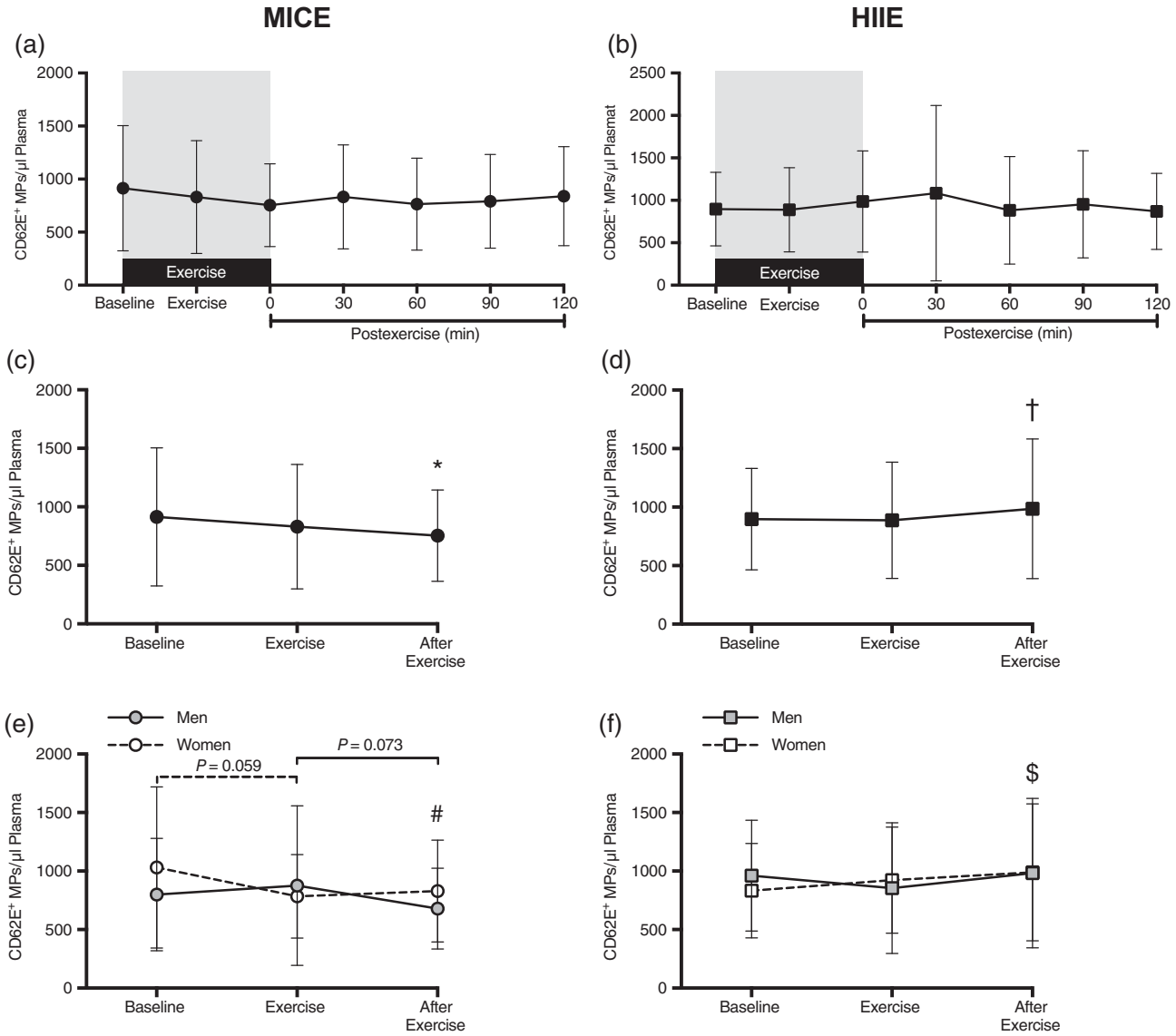

FIGURE 1 (a,b) CD62E$^+$ microparticle (MP) values in men and women combined before, during, immediately after and 2 h after moderate-intensity continuous exercise (MICE; circles) and high-intensity interval exercise (HIIE; squares), with no significant three-way interaction ($P > 0.05$; $n = 20$). (c,d) CD62E$^+$ MPs in men and women combined before, during and immediately after MICE (circles) and HIIE (squares), with a three-way interaction approaching statistical significance ($P = 0.083$; $n = 20$). (e,f) CD62E$^+$ MPs in men (grey) and women (white) before, during and immediately after MICE (circles) and HIIE (squares; $n = 10$). Data are presented as means ± SD. *Statistically significant difference from baseline within exercise condition ($P < 0.05$). †Statistically significant difference between exercise conditions at the corresponding time point ($P < 0.05$). #Statistically significant difference from baseline in women only within exercise condition ($P < 0.05$). $Statistically significant difference between exercise conditions in men only at the corresponding time point ($P < 0.05$). Brackets with *P*-values indicate data approaching statistical significance ($P \leq 0.10$)

with exercise are intensity dependent, sex specific, and not uniform between MP types.

Despite multiple studies, only a few of which have included both men and women, the effect of acute exercise on circulating CD62E$^+$ MP concentrations remains inconclusive (Durrer et al., 2015; Guiraud et al., 2013; Lansford et al., 2016; Mobius-Winkler et al., 2009; Serviente et al., 2016; Sossdorf et al., 2010, 2011; Wilhelm et al., 2016). We demonstrate that MICE lowers CD62E$^+$ MPs during and after exercise in women, but not men, whereas HIIE had no effect on CD62E$^+$ MP levels. Consistent with these results, previous investigations have found that continuous exercise does not alter CD62E$^+$ MP concentrations during or after exercise in men (Guiraud et al., 2013; Mobius-Winkler et al., 2009; Sossdorf et al., 2010; Wilhelm et al., 2016). However, in contrast to the present findings, other studies examining CD62E$^+$ MPs after exercise have produced mixed results (Durrer et al., 2015; Lansford et al., 2016; Serviente et al., 2016; Sossdorf et al., 2011). Specifically, researchers in our laboratory previously reported no change among women in CD62E$^+$ MPs after ~60 min of continuous exercise, whereas men demonstrated an increase in CD62E$^+$ MPs after ~45 min of continuous exercise at 60–70% $\dot{V}_{O_2\,max}$ (Lansford et al., 2016). We can speculate that the discrepant results between the present study and previous

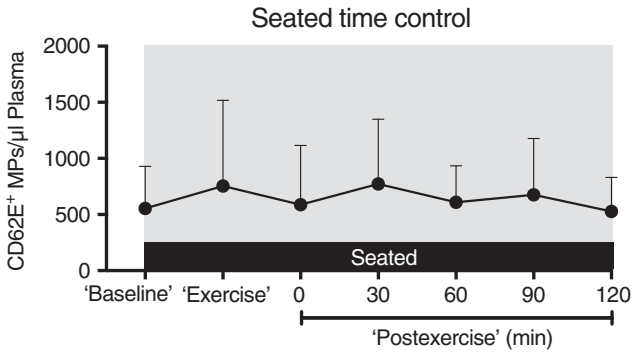

FIGURE 2 $CD62E^+$ microparticles during the seated time control visit ($n = 9$). 'Baseline', 'Exercise' and 'Postexercise' represent time points corresponding to those during the MICE and HIIE visits. Data are presented as means ± SD

investigations might be explained, in part, by the different modes and durations of exercise used across studies. In particular, cycling is the predominant mode of exercise, with durations ranging from 20 to 240 min (Durrer et al., 2015; Guiraud et al., 2013; Lansford et al., 2016; Mobius-Winkler et al., 2009; Sossdorf et al., 2010, 2011; Wilhelm et al., 2016). Further investigations are needed to clarify the kinetics of changes in endothelial MPs during and after exercise.

Acute exercise-induced shear stress partly signals the beneficial vascular adaptations to chronic exercise and is a mechanism that contributes to endothelial MP shedding (Ayers et al., 2015; Curtis et al., 2013; Padilla et al., 2011; Tinken et al., 2010). Despite a positive relationship between exercise intensity and shear rates during and after exercise (Johnson & Wallace, 2012; Padilla, Harris, Rink, & Wallace, 2008; Tanaka et al., 2006), we observed no effect of HIIE on $CD62E^+$ MP concentrations, contrary to our hypothesis. However, $CD62E^+$ MP concentrations were higher after HIIE than MICE, indicating more endothelial activation and a pro-inflammatory vascular environment after interval exercise (Jimenez et al., 2003). Interestingly, disturbed blood flow increases $CD62E^+$ MP concentrations (Jenkins et al., 2013), suggesting that the repeated increases and decreases in exercise intensity associated with HIIE might be responsible, at least in part, for the lack of change observed in $CD62E^+$ MP after interval exercise, provided MP clearance was maintained. Furthermore, laminar shear stress was recently shown to suppress $CD62E^+$ MP release (Kim et al., 2015), supporting our findings of reduced concentrations after MICE and no change after HIIE. Moreover, continuous exercise may be superior to interval exercise in acutely facilitating intercellular communication and/or vascular adaptations as endothelial cells preferentially take up endothelium-derived MPs rich in microRNAs compared with microRNA-poor MPs (Alexy et al., 2014; Diehl et al., 2012). Nevertheless, it is important to mention that HIIE training appears to have a greater potential for improving cardiovascular health compared with chronic MICE (Weston, Wisloff, & Coombes, 2014). With respect to cardiovascular disease, a recent study demonstrated that $CD62E^+$ MPs in men with stable coronary heart disease did not change after HIIE or MICE cycling (Guiraud et al., 2013). Regarding exercise mode, weight-bearing exercise and the associated impacts could differentially alter plasma MPs; however, this has not been evaluated systematically.

Haematopoietic progenitor cells, denoted by CD34, promote and contribute to the maintenance of vascular integrity through several mechanisms that augment endothelial cell function and facilitate angiogenesis (Asahara et al., 1997, 1999). Evidence suggests that paracrine signalling mediates the proangiogenic activity of haematopoietic progenitor cells via the secretion of $CD34^+$ exosomes or non-specific extracellular vesicles (Landers-Ramos et al., 2015; Sahoo et al., 2011). Contrary to our hypothesis and prior investigation, we observed no effect of continuous or interval exercise on $CD34^+$ MP levels in men or women; however, the exercise intensities, durations and mode used in the present study were different (Lansford et al., 2016). The conflicting results between studies suggest that exercise-induced changes in $CD34^+$ MPs do not rely exclusively on exercise intensity, but highlight the role of exercise duration and, possibly, surpassing a minimal duration threshold. However, given that only two studies have examined the effect of exercise on $CD34^+$ MPs, additional studies are necessary to characterize further the response to exercise and determine the functional implications of these findings.

4.1 | Limitations

Our study has limitations that warrant mention. First and foremost, plasma volume shifts were not evaluated. Changes in plasma volume during and after exercise might have impacted our results, as previous investigations indicate a reduction in plasma volume during and immediately after acute exercise (Dill & Costill, 1974; Kargotich, Goodman, Keast, & Morton, 1998). Specifically, it is possible that exercise-induced plasma volume reductions influence the observed decrease in $CD62E^+$ MPs immediately after MICE, because the magnitude of plasma volume shifts was first documented with long-duration, moderate-intensity continuous running exercise (Dill & Costill, 1974). Moreover, the postural orientation of the subjects during blood sampling might have altered plasma volumes, which could also have contributed to our observed effects on MPs. In particular, 2 h of sitting has been shown to reduce plasma volume (Hitosugi, Niwa, & Takatsu, 2000). Additionally, the location of the venepuncture might have impacted our results. Given that we were sampling blood from the vasculature of non-working muscle, it is possible that we did not fully capture the extent to which MPs are altered within the well-perfused vasculature of the working skeletal muscle during or immediately after exercise. Although this limitation is acknowledged, our rationale for obtaining blood samples from the vasculature of the upper limbs was that the non-working muscles of the arms would be representative of the systemic circulation. Finally, although exercise has been shown to modify homeostatic physiology for >24 h, we chose to limit exercise in our subjects for only 24 h preceding each visit based on the following: (a) we have previously shown that prior exercise, within ~15 h, alters basal endothelial MPs (Jenkins et al., 2011); (b) reducing physical activity to <5000 steps per day for 1, 3 or 5 days does not impact basal $CD62E^+$ MPs (Boyle et al., 2013); and (c) we used the same 24 h exercise restriction as our most recent investigation (Lansford et al., 2016).

4.2 | Conclusion

In conclusion, we demonstrate that MICE reduces $CD62E^+$ MPs immediately after exercise. Specifically, MICE lowered circulating $CD62E^+$ MP levels in women after exercise, but not men. Furthermore, we show that interval exercise alone did not elicit any changes in MPs; however, $CD62E^+$ MPs after interval exercise were higher than the value observed after continuous exercise. In addition to there being no effect of exercise on $CD34^+$ MPs, both MP populations examined in the present study remained at baseline levels for 2 h after exercise. Collectively, these results suggest that exercise-induced circulating MP concentrations are exercise intensity dependent, sex specific, and non-uniform among MP types. The present study complements and extends current knowledge on the beneficial vascular effects of exercise by examining the effects of continuous and interval exercise on the time course of concentrations of MPs populations in young, healthy men and women.

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COMPETING INTERESTS

None declared.

AUTHOR CONTRIBUTIONS

K.A.L., J.A.C., J.R.M. and N.T.J. conceived and designed the research. D.D.S., K.A.L. and H.K.H. performed the experiments. D.D.S. analysed the data and prepared the figures. All authors interpreted the results. D.D.S. and N.T.J. drafted the manuscript. All authors edited and revised the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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