THE INFLUENCE OF PROLONGED SITTING WITH BRIEF HOURLY STAIR CLIMBING ON POSTPRANDIAL CIRCULATING MICROVESICLES

by

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ABSTRACT

Sedentary behaviour is a major risk factor for chronic diseases of the cardiovascular system, including impairments in vascular function. Physical inactivity such as prolonged sitting alters concentrations of circulating microvesicles. Vascular function is partially regulated by microvesicles (MVs), as the molecules they transport facilitate cell-to-cell communication leading to structural and functional changes of blood vessels and their component cells. Along with inactivity impairments, high carbohydrate diet transiently impedes postprandial vascular function. However, regular and acute aerobic exercise counteracts this dysfunction. Further, interrupting prolonged sitting with short bouts of exercise improves cardiorespiratory fitness and postprandial markers of cardiometabolic health, particularly in overweight individuals.

The purpose of this study was to examine the influence of hourly stair snack interruptions to prolonged sitting and high or low carbohydrate diets on postprandial circulating MVs in two populations. Individuals of healthy weight (n = 11; males) and elevated waist circumference (n= 3/5; males/females) completed three experimental trials in a randomized crossover design: i) sedentary with low carbohydrate meals, ii) sedentary with high carbohydrate meals, and iii) hourly stair snacks (ascending 55 steps in 15-30s) with high carbohydrate meals. MVs of leukocyte, granulocyte, platelet, activated-, and apoptotic-endothelial cell derivations were quantified using a Cytoflex flow cytometer.

Linear mixed model analysis demonstrated that neither diet (high or low carbohydrate) nor incorporation of hourly stair snacks altered concentrations of postprandial circulating MVs from pre-prandial baseline state throughout a five-hour bout of sitting. An absence of influence was observed in both healthy weight individuals and those with elevated waist circumference. Minor differences were observed in select MV populations with diet or condition; however, the transient changes are likely not physiologically significant given the natural variability of MV populations in circulation. This result is consistent with the limited volume and intensity of the stair snack intervention and complexity of factors influencing MV release that extend beyond the scope of the study. The results of this study suggest that an acute bout of prolonged

sitting does not alter concentrations of circulating MVs and this influence is not changed by carbohydrate consumption. These findings are an important step in beginning to understand how sedentary behaviour alters vascular function and for establishing practical and accessible exercise interventions.

Keywords: Microvesicles, Prolonged sitting, Flow Cytometry, Postprandial microvesicles, Stair Climbing, Brief intense exercise

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Chapter 1: Introduction

Effects of Sedentarism on Cardiovascular Health

Nearly 85% of Canadian adults 20-79 years of age do not meet the recommended 150 minutes of physical activity per week[1]. Further to this inactivity, the majority of an average individual's waking hours are spent sitting[2–4]. Sedentary behaviour is the most severe form of physical inactivity, defined as ≤1.5 metabolic equivalents while sitting or in reclined posture[5], and is a major contributor to chronic disease, particularly those affecting the cardiovascular system[6,7]. Cardiovascular health is largely determined by the function of endothelial cells forming the lining of blood vessels: endothelium[8]. The endothelium acts as a protective barrier as well as mediator of leukocyte adhesion and migration into peripheral tissues, inflammation, vascular tone, coagulation, and vessel permeability[9]. These functions are modulated by physical and chemical stimuli in the lumen of blood vessels, the most important being blood flow[7,10]. Increased blood flow causes increased shear stress forces on the endothelium, the mechanotransduction of which results in elevated production of vasoactive compounds, one of which is nitric oxide (NO)[11–13]. NO is largely produced by endothelial nitric oxide synthase (eNOS) through the oxidation of L-arginine, however, additional NO is produced in disease states by inducible NOS in inflamed endothelial cells and leukocytes[12]. Suvorava *et al*. demonstrated reductions in eNOS production and endothelium-dependent vasodilation resulting from abrupt physical inactivity in mice[14]. Although this particular study observed rodents[14], several human bed-rest and step-reduction studies have since shown significant reductions in flow-mediated vasodilation (FMD), a non-invasive surrogate measure of endothelial function, particularly that of the superficial femoral and popliteal arteries, following physical inactivity[7,15] or sedentary behaviour[6,8,16–22].

Importantly, in addition to NO, other vasoactive compounds also contribute to the regulation of endothelial function. The balance between relaxation- and constriction-inducing products of the arachidonic acid pathway is particularly important in regulating vascular tone[23,24]. Specifically, there are six major endothelial cyclooxygenase-derived factors, eicosanoids, that act in both autocrine and paracrine manners to regulate endothelium

function[24]. Those promoting vasodilation include prostaglandin I_2 (PGI_2, prostacyclin) and prostaglandin D_2 (PGD_2), while prostaglandin H_2 (PGH_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and thromboxane A_2 (TxA_2) function as vasoconstrictors[23,24]. In addition, prostaglandin E_2 (PGE_2) can function as either a vasodilator or constrictor depending on its concentration and which of the four smooth muscle cell receptors it interacts with[24]. Although TxA_2 is the major contributor to endothelium-dependent contraction induced by arachidonic acid[24], endothelin-1 (ET-1) is the predominate vasoconstrictor regulating vascular tone[25]. ET-1 interacts directly with receptors on vascular smooth muscle and endothelial cells to induce contraction[26]. Although ET-1 is a potent vasoconstrictor, its short half-life in circulation[26] makes it a well suited functional opposite to NO. The explicit effects of physical inactivity on concentrations of each of these vasoactive compounds remains under investigation, however, studies on primates have demonstrated increases in vasoconstricting eicosanoids in activity-restricted individuals compared to their physically active counter parts[27]. Further, discrepancies exist over the influence of prolonged sitting on ET-1, with some studies indicating no change[28] while others observe increases over time[29].

In addition to vasoactive compounds, the sympathetic nervous system (SNS) also contributes to the regulation of endothelial function, and thus cardiovascular health. The SNS contributes to cardiovascular homeostasis, particularly vascular tone, compliance, and blood pressure, through arterial vasoconstriction[25,30,31]. Sympathetic efferent excitation increases vessel wall stiffness, and thus blood pressure, via vascular smooth muscle contraction[30]. This direct modulation of arterial stiffness may occur alongside a passive increase in blood pressure through endothelial cell signalling; demonstrating the integrated nature of vascular smooth muscle cell regulation by the SNS and endothelium[25]. SNS activity is commonly measured as muscle sympathetic nerve activity (MSNA)[32]. The influence of physical inactivity on MSNA remains unknown and inconsistencies abound in the published literature. For example, Kamiya *et al*. demonstrated an increase in MSNA following 120 days of head-down tilt bed rest[33], whereas Shoemaker *et al*. observed a decrease in MSNA burst frequency following 14 days using the same inactivity model[32]. Further, Just *et al*. observed no change in sympathetic vasoconstrictor responsiveness between a rodent inactivity model (hindlimb-unweighting) and controls following 21 days[31]. In contrast to other findings, this study suggests short duration

inactivity does not influence sympathetic nerve regulation of skeletal muscle vasculature[31]. Beyond elucidating the influence of inactivity on MSNA, current research has not yet characterized the effect of inactivity-induced changes in SNS activity on vascular function.

In addition to declines in endothelium-dependent functions, inactivity studies have demonstrated reduced metabolic[34,35], muscular[36], and vascular function from reductions in daily step count. Krogh-Madsen *et al*. found a 2-week reduction in step count impaired peripheral insulin sensitivity, demonstrated by a 2.8% decline in lean leg mass and 17% glucose infusion rate reduction during a hyperinsulinemic-euglycemic clamp[37]. Further, Teixeria *et al*. observed reduced FMD of the popliteal artery following 5 days of step reduction[7]. Given the more severe nature of sedentary behaviour it is unsurprising sedentary studies have also shown profound systemic physiological impairments from inactivity including insulin resistance, contributing to type 2 diabetes mellitus[38,39], as well as muscle mass and strength decline[40]. Stephens *et al.* found a significant reduction in insulin sensitivity following one day of sedentary behaviour[41]. Specifically, the amount of insulin required to clear infused glucose was elevated in young, healthy individuals when sedentary (sitting an average of 16.9 hrs/day) compared to active (sitting an average of 5.8 hrs/day)[41]. Although there is an established connection between sedentary behaviour and disease risk, the mechanisms responsible for vascular maladaptation, including the role of circulating factors, remain largely unknown.

Role of Microvesicles in Cardiovascular Health

Microvesicles (MVs) are anucleate, lipid-membrane vesicles produced through cytoskeletal reorganization, particularly by disruption of phosphatidylserine asymmetry in the plasma membrane[42–44]. First referred to as ‘Platelet dust’ in 1967, MVs play an important role in coagulation of platelet-free plasma[45,46]. Electron microscopy revealed the platelet origin of the first observed MVs[47], a cell type now known to be one of many capable of MV release when activated, apoptotic, or necrotic[44]. MVs are a type of extracellular microvesicle distinct from exosomes and apoptotic bodies because of their size and outward vesiculation process of release[43,48]. The term medium extracellular vesicles is used to describe MVs when classification is based on size[49]. The surface of MVs contains transmembrane proteins[43,44]

including cell membrane-specific antigens denoting the cell type from which they are derived[42]. The relatively large diameter of MVs, 0.1-1μm, facilitates function in cell-to-cell communication through transport of cytosolic components including lipids, proteins, mRNA, and miRNA to distant recipient cells[43,44]. The particular action exerted on recipient cells depends on the cell type from which the MVs originated (e.g. endothelial, platelet, smooth muscle, leukocyte, granulocyte, or erythrocyte) and the conditions under which they are formed[42,43].

The functions of the predominant MV phenotype in plasma, platelet-derived microvesicles (PMVs), are thrombogenic in nature[42,43] harbouring transmembrane tissue factor and its associated agonists to initiate coagulation[45]. In addition to thrombotic functions, PMVs interact with endothelial cell-derived microvesicles (EMVs) to induce EMV activation and up-regulation of cell adhesion molecules thereby influencing their effect on endothelial cells[43]. The effects of circulating MVs on endothelial cells varies with some EMVs inducing oxidative stress[50] and inflammation[51], while other MVs cause endothelial dysfunction or induce pro-angiogenic pathways[42,44,52,53]. Alterations to endothelial cell function directly effects the endothelium; a major determinant of cardiovascular health. Modulations in MV concentrations are associated with several cardiovascular risk factors and disease states incurring chronic vascular damage[42,43]. For example, patients with coronary artery disease, type 2 diabetes mellitus, and renal failure exhibit elevated concentrations of circulating EMVs[8,42,54]. The role of MVs in cell-to-cell communication and their ability to robustly regulate cell function, including that of endothelial cells, makes them important mediators of cardiovascular health.

Role of Microvesicles in Vascular Adaptation to Exercise

Shear stress is integral in the regulation of NO by endothelial cells, and exercise-induced alterations in shear are a major stimulus for vascular adaptation to exercise[55]. Although blood flow forces are integral in this vascular response, occurrence of endothelial adaptation in vasculature lacking direct exercise-induced shear stress suggests a role of circulating factors such as MVs in vascular adapation[11]. A wide variety of MVs with substantial differences in concentration exist naturally in circulation[44]. As observed with function, the dynamics of MV responses to stimuli like physical activity are specific to the cell type from which the MVs are

derived[43]. Following strenuous exercise, concentrations of circulating PMVs are transiently elevated[11]. This pro-angiogenic response mirrors the increases in vascular stress induced by exercise, as SNS activation and increases in circulating metabolites such as adenosine diphosphate are suggested PMV formation agonists[11,42]. Further, application of post-exercise PMVs to endothelial cells *in vitro* increases angiogenic activity with regards to tubule formation, proliferation, and migration[11]. Alternatively, concentrations of circulating EMVs remain unchanged following strenuous exercise[11]. Notably, observations regarding exercise-induced changes to EMV circulation are inconsistent in the literature. Although the majority of research demonstrates a lack of change[11,56,57], other studies have reported both increases[58] or decreases in concentration[59]. These discrepancies may be due to the contradictory nature of stimuli during exercise, as cytokines released promote EMV formation while increased shear stress supresses MV release[42]. Additionally, inconsistencies may arise from failure to correct for blood volume when quantifying MVs following exercise[42] or insufficient exercise intensity for endothelial activation and thus EMV release[11]. The pro-angiogenic response exhibited by PMVs, in addition to the general absence of an exercise-induced increase in EMVs associated with vascular damage, suggests MVs play an integral role in vascular adaptation to exercise stress response[11,42,43].

Effect of Sedentary Behaviour on Circulating Microvesicles

In contrast to the accumulating research investigating the influence of exercise on MV populations and their role in vascular adaptation, few studies have considered the effects of physical inactivity on circulating MVs. Boyle *et al*. demonstrated that 5 days of step-reduction increased concentrations of $CD31^{+}/CD42b^{-}$ EMVs but not $CD62e^{+}$ EMVs[15]. Although this alteration in EMV concentration occurred alongside a reduction in popliteal artery FMD, the changes were not correlated[15]. These inactivity-induced changes in circulating MVs have also been observed in sedentary behaviour studies. Navasiolava *et al.* observed an increase in circulating EMVs following 3 days of enforced physical inactivity using dry immersion. In the 4 days of physical inactivity following this initial observation, EMV concentrations only slightly increased demonstrating further endothelial effects with prolonged muscle disuse[8]. Importantly, quantification was completed on EMVs lacking CD41 and expressing CD31 ($CD31^{+}/CD41^{-}$)[8], and therefore represent MVs released from apoptotic endothelial cells[15]. In

both this and Boyle *et al.'s* step reduction study, the increase in these specific EMVs, in addition to the lack of change in plasma soluble CD62E, indicates the MV response occurred without a significant endothelial stimulus for inflammation[8]. Notably, dry immersion is a severe form of sedentary behaviour, with little-to-no leg movement for long durations. Prolonged sitting is also a sedentary behaviour; however, results of such investigations are inconsistent with other physical inactivity studies. Evans *et al.* observed a decrease in activated and apoptotic EMVs in overweight individuals following 180 minutes of sitting[28]. This decrease occurred with and without inclusion of a calf-raise intervention and persisted after accounting for shifts in plasma volume[28]. This research begins to characterize the effects of physical inactivity and sedentary behaviour on circulating MVs; however, a paucity of mechanistic investigations has thus far prevented determination of the role of MVs in inactivity-induced vascular maladaptation.

Postprandial Changes in MVs and Cardiovascular Function

The association of postprandial hyperglycemia[60–66], hyperinsulemia[67], and hyperlipidemia[66,68–70], with risks of cardiovascular disease is well documented. Vascular function, as measured by FMD, is transiently impaired following a high-carbohydrate[61–63,65] or high-fat meal[64,71]. Inconsistencies have been observed in the duration of impairment due to an abundance of contributing factors including test meal composition, habitual physical activity level[64], and glucose tolerance[69]. However, published studies demonstrates peak reduction in FMD occurring 60-120 minutes following a high-carbohydrate[61–65,72] or high-fat meal[64,71]. In addition to impaired FMD, platelet aggregation is also altered postprandially. Ahuja *et al.* observed a significant reduction in maximum platelet aggregation 120 minutes postprandially following consumption of high-carbohydrate or high-fat meals with varied glycemic indices[73,74]. Consumption of a high-fat meal also significantly increases concentrations of tumor necrosis factor[75], interleukin-6, and adhesion molecules ICAM-1 and VCAM-1; cytokines that when chronically elevated are predictive of increased cardiovascular disease risk[69]. In conjunction with these functional changes, postprandial alterations in MV concentrations have also been observed. Consumption of a high-fat meal increases the plasma concentrations of apoptotic EMVs ($CD31^{+}$/ $CD42^{-}$) beginning 1 hour postprandially[68], with elevation lasting upwards of 6 hours[76]. While the majority of an average person's day is spent

sitting[2–4], it is also spent in the postprandial state[70], therefore, it is important to consider postprandial changes in cardiovascular function in sedentary behaviour studies.

Effect of Physical Activity on Postprandial Cardiovascular Function

Physical activity can greatly impact the degree of cardiovascular impairment observed postprandially. Das *et al.* demonstrated participation in regular exercise (aerobic, cross-training, or resistance) prevents reductions in FMD seen in sedentary age-matched counterparts following consumption of either high-carbohydrate or high-fat mixed meals[64]. Importantly, postprandial increases in blood insulin, glucose, and triglycerides still occur in habitually active individuals, however, to a lesser extent than in sedentary individuals[64]. In addition to habitual activity, acute exercise can also mitigate postprandial modulation of vascular function. A single bout of endurance exercise one day prior to a high-carbohydrate meal elevates vascular function to the extent that the postprandial FMD decline remains above sedentary control impairment levels[77]. Similarly, acute high intensity interval exercise one day before a high-fat meal prevents postprandial FMD impairment[78]. Notably, discrepancies related to the efficacy of acute exercise depend on the study population and marker of vascular function. For example, in adults with obesity acute aerobic or whole-body resistance exercise one day prior to a high-carbohydrate meal does not alter postprandial impairments in FMD or increases in blood insulin and glucose[79]. Further, Harrison *et al.* demonstrated acute exercise does not prevent postprandial increases in apoptotic EMVs following high-fat meal consumption[76]. The effect of physical activity on postprandial changes to other MV populations remains unknown.

Effect of Brief Exercise Interventions on Vascular Function During Prolonged Sitting

Given the profound effect of sedentary behaviour on cardiovascular function, interventions for prolonged sitting have been investigated. Interventions for pre- and post-prolonged sitting demonstrate efficacy in mitigating vascular impairment. Specifically, sitting-induced reductions in popliteal artery FMD are prevented by a 45 min bout of cycling immediately prior to three hours of prolonged sitting[19]. Conversely, reduced popliteal artery FMD from six hours of uninterrupted sitting is reversed when followed by 10 minutes of walking[20]. While pre-sitting activities provide the dysfunction prevention that is lacking from post-sitting interventions, the duration of protection afforded by preceding bouts of exercise is

unknown. As such, interrupting prolonged sitting with bouts of brief exercise has also been explored. While interventions as small as intermittent fidgeting can mitigate FMD impairment accompanying prolonged sitting[18], hourly bouts of exercise prevent impairment altogether. Specifically, McManus *et al.* prevented superficial femoral artery FMD impairment in children by interrupting sitting hourly with 10 minutes of moderate intensity exercise[17]. Similarly, interrupting sitting with hourly 5 minute walks prevented dysfunction in healthy adults[22]. Although these interventions are effective, the duration of interruption required may limit practicality. The novel concept of "sprint/stair snacks", in which brief (20-30 second) bouts of vigorous intensity exercise are separated by hours of rest[80,81], may be a time-efficient alternative to walking interventions for prolonged sitting. Sprint snacks emerged from the endurance training alternative of low-volume sprint interval training, in which 10-30 second bouts of vigorous exercise are repeated with short duration periods of recovery[82]. While sprint snacks involve much longer periods of rest between bouts, studies have demonstrated their efficacy in improving cardiorespiratory fitness. Specifically, completing three 20s bouts of cycling[80] or stair climbing (60 steps)[81] separated by 1-4 hours of rest can improve cardiorespiratory fitness in sedentary adults. Further, breaking up prolonged sitting with hourly stair snacks lowered postprandial insulin and free fatty acids in overweight individuals[83]. Notably, the stair snack intervention was insufficient in lowering hyperglycemia regardless of weight[83]. The influence of exercise snacks on endothelial function, or circulating markers of cardiovascular function such as MVs, has yet to be determined.

Objectives

The purpose of the present study was to investigate the effect of breaking up prolonged sitting with brief hourly stair sprints on concentrations of postprandial circulating microvesicles. To do so, four major objectives were addressed. Firstly, describe how prolonged sitting with consumption of high or low carbohydrate meals alters concentrations of circulating MVs from the pre-prandial baseline state. Secondly, determine the influence of carbohydrate content on postprandial changes in MV concentrations observed with prolonged sitting. Thirdly, assess the efficacy of brief hourly stair-sprint interventions in prevention of prolonged sitting and postprandial induced changes in circulating MVs. Fourthly, determine if the efficacy of the stair sprint intervention is dependent on waist circumference.

Chapter 2: Methods

Study Approval

Approval for the study was granted by the University of British Columbia Clinical Research Ethics board (ID H17-01747) and it was registered on ClinicalTrials.gov (NCT03374436). Approval for MV analysis was granted through a data and biological samples agreement by the Thompson Rivers University Research Ethics Board (ID 102457). The study conformed to the Declaration of Helsinki and written informed consent was given by all participants.

Study Design and Participants

The study design and methods have previously been reported[83]. Briefly, two randomized cross-over design studies were conducted involving three 9-hr experimental trials: 1) sedentary with low-carbohydrate meals (LC), 2) sedentary with high-carbohydrate meals (HC), and 3) hourly stair snacks with high-carbohydrate meals (ACT) (Figure 1). Sedentary trials involved participants sitting for the entire 9-hr test with minimal other movement. Stair snack trials required participants to sit for the entire trial apart from when they performed stair snacks involving ascending 55 steps in 15-30 seconds at a pace deemed challenging by the participant. Stair snacks began at 60 mins into the trial and were performed every hour and immediately prior to meal consumption at 180 and 360 mins. Experimental trials were performed 3-7 days apart to ensure no carryover effects.

The same high or low carbohydrate meal was consumed at 0 (immediately following baseline sampling), 180, and 360 mins within each experimental trial, and all meals were matched for calories (approx. 530 kcal) across conditions and diets. The low carbohydrate meal was designed to elicit a minimal postprandial glycemic response and consisted of three extra large eggs, 32 g of cheddar cheese, 15 ml of olive or canola oil (according to participant preference), and 15 ml of frozen corn (7.5 g carbohydrate, 30 g protein, and 42 g fat). Conversely, the high carbohydrate meal was designed to induce a large glycemic response and

included a peanut butter and jam sandwich with 400 ml of orange juice from concentrate (97 g carbohydrate, 11 g protein, and 11 g fat). Water was provided ad libitum across all experimental trials.

All participants were recruited through the University of British Columbia Okanagan campus (Kelowna, British Columbia). Study one consisted of young healthy weight (HW) males and served as a pilot test of the stair snack intervention without the influence of the menstrual cycle on microvesicle release. Males included in study one were 18-35 years of age and had a BMI of 18.5-24.9 $kg \cdot m^2$. Subsequently, study two was conducted in individuals with overweight characterized by an elevated waist circumference (EWC) and included both sexes. An elevated waist circumference was defined according to the World Health Organization waist circumference cut off points for overweight or obesity and association with disease risk and included women with values ≥88 cm and men with values ≥102 cm[84]. All participants in study two were 18-69 years of age and of the women studied, five were postmenopausal while three were premenopausal. Of the premenopausal women, two used an intrauterine device and one used oral contraceptive. All experimental trials for the premenopausal women were conducted in the follicular phase of the menstrual cycle (days 3-9 following menstruation). In both studies participants were excluded from the experiment for any of the following reasons: previous diagnosis of diabetes; currently taking insulin, oral hypoglycemic drugs, or any medication affecting blood glucose; diagnosed cardiovascular diseases; current smoker; allergy to eggs or peanuts; participation in serious exercise training (>5 days per week); medical or orthopedic conditions limiting physical activity; or adherence to a specialty diet including vegan or ketogenic diets.

Baseline anthropometric measurements of height, weight, waist circumference, and hip circumference were taken, and study eligibility was confirmed by a registered dietician prior to completion of experimental trials. Further interviews were conducted preceding each trial to standardize participant diet and activity. A 24-hr food recall was conducted at the first trial and participants were asked to repeat the same diet prior to all testing days; a control verified via dietician interview. In addition, participants were asked to abstain from alcohol and exercise the day before each trial. Self-report of activity standardization and hours of sleep the

night prior to each testing day were recorded. Activity trackers (Mio Slice watch, Canada) were used to confirm activity leading up to testing days in study one but not in study two due to technical issues. Finally, participants were instructed to fast for ≥ 10 hours overnight before each test day.

A Williams latin square design and online randomizer (https://statpages.info/latinsq) were employed to randomize completion of the three experimental trials. Each trial began between 7 and 8:30 am with confirmation of diet and activity standardization as previously described. Participants were equipped with an intravenous catheter (BD Nexiva; Becton Dickinson, Franklin Lakes, NJ, USA) inserted into an antecubital vein to allow for repeat blood sampling and an activity monitor (Mio Slice Watch, Canada) for step count and heart rate monitoring. In ACT trials stair climbing was supervised by a technician and rating of perceived exertion (RPE; category-ratio 0-10 scale), total time for each stair climb, and heart rate following each climb was recorded.

Blood samples were drawn via intravenous catheter prior to meal consumption at time 0 mins and again every 30 mins throughout each 9-hour trial, for a total of 19 draws per condition. Samples were collected in tubes containing ethylenediamine tetraacetic acid (EDTA-K_2) and centrifuged for 15 mins at 1550 x g and 4^{o}C before being stored at -80^{o}C until further analysis.

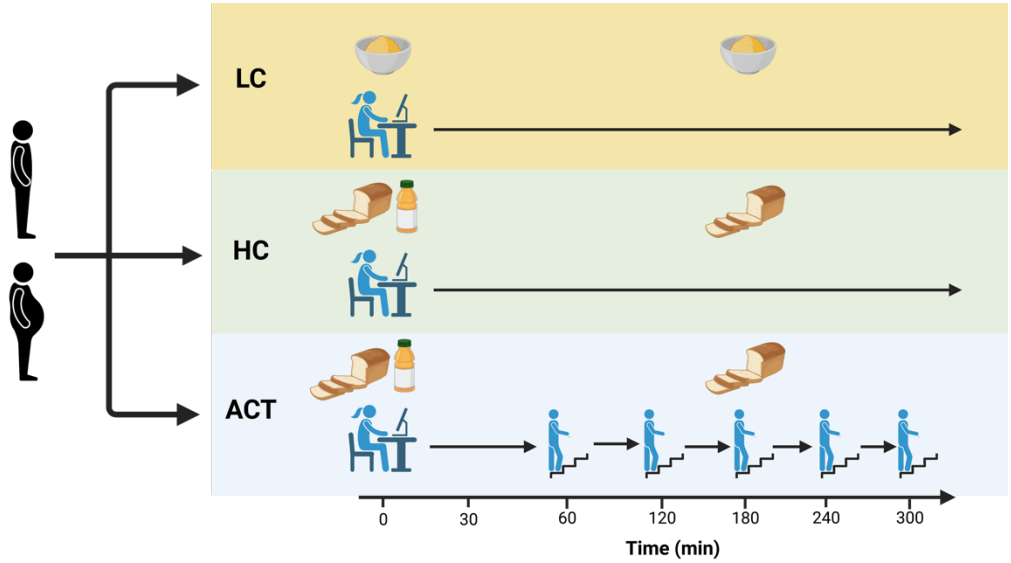

Figure 1. Overview of study design for first 5-hrs of each condition or diet. Blood samples were drawn every 30 mins.

Microvesicle Analysis

Concentrations of circulating MVs throughout the first 5-hrs of each experiment were determined using flow cytometry. Specifically, plasma samples from baseline, 60, 120, 180, 240, and 300 mins were analyzed from each experimental trial (Figure 1). Plasma samples were collected as previously described and transported from University of British Colombia Okanagan campus (Kelowna, British Columbia) to Thompson Rivers University (Kamloops, British Columbia) where they were stored at -80°C until batch analysis. Upon analysis samples were thawed and centrifuged at 13 000 x g at a fixed angle and room temperature for 2 mins to obtain platelet-free plasma[85]. Following centrifugation 50µl of platelet-free plasma was incubated with 2.5 µl of Fc receptor blocker (TruStain FcX, BioLegend, USA) for 10 mins to limit non-specific binding of antibodies.

Each sample was analyzed using three panels to quantify five different MV populations and four controls. The MV populations measured include total leukocyte-derived microvesicles (LMVs), granulocyte-derived microvesicles (GMVs), platelet derived

microvesicles (PMVs), as well as activated and apoptotic endothelial cell-derived microvesicles (EMVs). LMVs were measured as CD45-phycoerythrin (CD45-PE) positive events and GMVs were identified as CD66b-fluoresceinisothiocyanate (CD66b-FitC) positive events. Each of these were measured in individual panels using 2.5 µl of the appropriate antibody (both from BioLegend, USA). A third panel was used to measure PMVs as CD41-Brilliant Violet 421 (CD41-BV421) positive, activated EMVs as CD62e-phycoerythrin (CD62e-PE) positive, and apoptotic EMVs as CD31-allophycocyanin (CD31-APC) positive and CD41-BV421 negative events. A master mix was prepared immediately before staining panel three, such that each sample was incubated with 3 µl CD62e-PE, 3 µl CD31-APC, and 1.5 µl CD41-BV421 (all from BioLegend, USA). Samples were incubated for 25 minutes and diluted with 445 µl of 0.22 µm double-filtered phosphate buffered saline (PBS) before centrifugation at 18 407 x g and room temperature for 30 mins. In addition to an unstained control for each sample, a MV-free fluorescence control for each sample and panel was prepared by mixing 80 µl of supernatant with 30 µl of 0.22 µm double-filtered PBS. Subsequently, 340 µl of sample supernatant was removed and MV pellet was resuspended in 30 µl of 0.22 µm double-filtered PBS.

The CytoFlex flow cytometer (V2-B3-R2, C09745 Beckman Coulter) was used with the 405 nm laser triggering violet side scatter and a flow rate of 10 µl/min for 90 seconds per sample. A mixture of silica and polystyrene beads (ApogeeMix, Apogee Flow Systems) of varying sizes (80, 110, 180, 240, 300, 500, 590, 880, and 1300 nm) were used to determine particle gate boundaries (gating strategy described in Appendix B). Additionally, MV-free controls were used to determine the upper limits of the horizontal gate representing background noise. A MV was defined as anything distinct from noise and 180-1000 nm in diameter in accordance with the reported definition of a MV[43] and the lower bounds of accurate sizing capability of the current experiment. CytExpert software (Beckman Coulter) was used to analyze each sample and quantify MV concentrations (events/µl). A compensation matrix for CD31-APC and CD62e-PE in panel three was created using single-stained samples and values of 0.05 APC-PE% and 2.46 PE-APC% were applied to all panel three samples. Concentrations of MV were corrected according to unstained and MV-free controls, as well as dilution factor to account for pre-analytical steps (equation 1).

$$\text{Corrected MV Concentration (events/µl)} = \frac{\big(x - (a + b)\big) * c}{50}$$

Equation 1. Formula for corrected concentration of MVs (events/µl) in which x = concentration of MVs in stained samples as measured by flow cytometry (events/µl), a = concentration of events in MV-free fluorescence control (events/µl), b = concentration of events in unstained control (events/µl), and c = final volume of diluted plasma sample (µl).

Statistical Analysis

Given the different participant populations and timing, corrected MV concentrations for HW and EWC groups were analyzed separately. Parametric assumptions of skewness and normality were assessed by z score and Q-Q plots. All data were natural log transformed to fulfill parametric assumptions. Two linear mixed model analyses were conducted for each MV population using JASP (version 0.13) and employed type III sum of squares and Satterthwaite approximation. The main linear mixed model included only condition or diet (LC vs. HC and HC vs. ACT) as a fixed effect and participant as a random effect. Subsequent analyses including time and condition or diet as fixed effects with participant as a random effect were used to assess the impact of time, condition or diet, and their interaction on circulating MVs. Significant main effects and interactions were followed up with pre-planned contrasts to compare time points within and across conditions using Bonferroni corrections. Condition (HC vs. ACT) and diet (LC vs. HC) were analyzed separately to determine the influence of each manipulation on concentrations of microvesicles independently. Although this elevated the risk of type I error, the use Satterthwaite approximation in the model[86] and lack of significant differences in parameters despite pre-planned contrasts indicated type I error was very unlikely. In all analyses significance was set at $p < 0.05$ and values are reported as mean ± SD.

Chapter 3: Results

Study Population

A total of 11 healthy weight participants and 8 individuals with elevated waist circumference completed the study. Baseline characteristics of all participants are described in table 1. Within the healthy weight group there was no difference in the number of steps walked on the day preceding each of the 3 trials (P = 0.903). Further, neither study group demonstrated differences in the number of hours of sleep the night preceding each trial (HW P = 0.164; EWC P = 0.533). Characteristics of the stair snacks for the ACT condition and steps count during each trial are summarized for each study group in table 2. Notably, the average time taken to complete a stair snack was greater in the EWC group (29.7 ± 12.6 s) than in the HW group (15.6 ± 1.3 s).

Table 1. Baseline characteristic of study participants for both healthy weight and elevated waist circumference study groups. Values are presented as mean (SD).

Characteristic	**HW**	**EWC**
No. Participants (M/F)	11 (11/0)	8 (3/5)
Age, years	23.1 (4.4)	52.3 (12.4)
Weight, kg	74.7 (5.9)	101.6 (21.4)
Body Mass Index, $kg \cdot m^{-2}$	24.2 (2.1)	34.5 (6.6)
Waist Circumference, cm	80.2 (4.9)	107.9 (10.7)
Hip Circumference, cm	99.7 (4.3)	121.1 (13.2)
Waist-to-hip Ratio	0.80 (0.03)	0.89 (0.05)
Resting Heart Rate, bpm	62 (12)	67 (11)

Table 2. Characteristics of the stair climbing snacks for healthy weight and elevated waist circumference study groups. Values are presented as mean (SD).

Characteristic	HW			EWC		
	LC	**HC**	**ACT**	**LC**	**HC**	**ACT**
Steps day of trial	164 (173)	100 (90)	951 (359)	396 (383)	407 (537)	900 (287)
Mean RPE for Stair Snack	-	-	4.6 (2.3)	-	-	4.1 (2.3)
Mean HR for Stair Snack	-	-	102 (17)	-	-	105 (21)
Mean Stair Climbing Time (seconds)	-	-	15.6 (1.3)	-	-	29.7(12.6)

Technical Variation

Baseline samples from each condition were used to determine the day-to-day coefficient of variation (CV) accounting for both inter-assay variation between flow cytometer batch analyses and physiological day-to-day variability between testing days. CV values for each antibody were calculated using non-transformed data and are detailed in Table 3. The largest inter-assay variation was observed in concentrations of apoptotic EMVs ($CD31^+/CD41^-$). This is consistent with the greatest presence of outliers for $CD31^+/CD41^-$ events.

Table 3. Day-to-day coefficients of variation for each antibody averaged for all participants within each study cohort. Each participant CV was calculated from the baseline value of each condition.

Participant Group	CD45	CD66b	CD62e	CD41	$CD31^+/CD41^-$
EWC	21.5	21.7	24.9	22.7	50.0
HW	21.5	22.8	21.5	51.3	53.6

Study 1: Healthy Weight

Total Leukocyte-derived Microvesicles

Concentrations of circulating LMVs remained stable over time across all experimental diets and conditions in HW individuals. Liner mixed model analysis including diet or condition as the fixed effect indicated no significant difference between LC and HC diet ($F(1, 9.96) = 0.084$, $p = 0.777$) nor sedentary (HC) and stair snack (ACT) conditions ($F(1, 9.95) = 0.625$, $p = 0.448$). Inclusion of time as a fixed effect also displayed no significant main effect of diet or condition (LC vs. HC $F(1, 9.98) = 0.186$, $p = 0.675$; HC vs. ACT $F(1, 9.94) = 0.656$, $p = 0.437$, Figure 2A). A significant main effect of time was found when collapsed across LC and HC diets ($F(5, 15.99) = 3.671$, $p = 0.031$) but not when collapsed across HC and ACT conditions ($F(5, 14.39) = 2.634$, $p = 0.069$) (Figure 2A). Further, a significant time by diet or condition interaction was observed (LC vs. HC $F(5, 76.77) = 2.612$, $p = 0.031$; HC vs. ACT $F(5, 77.35) = 2.843$, $p = 0.021$). However, subsequent pairwise contrasts did not reveal any significant differences between time points within condition, nor within time points across conditions (Appendix A, Tables 52, 54, and 55).

Granulocyte-derived Microvesicles

The concentration of circulating GMVs exhibited similar patterns across all diets, conditions, and time points in HW individuals. Linear mixed model analysis with the fixed effect of diet or condition displayed no significant difference in GMVs between LC and HC diets ($F(1, 10.20) = 0.984$, $p = 0.344$) nor HC and ACT conditions ($F(1, 9.81) = 1.191$, $p = 0.301$). Inclusion of time as a fixed effect also indicated no significant main effect of diet or condition (LC vs. HC $F(1, 10.21) = 0.941$, $p = 0.354$; HC vs. ACT $F(1, 9.86) = 1.311$, $p = 0.279$) (Figure 2B). The main effect of time was significant when collapsed across HC and ACT conditions ($F(5, 17.06) = 3.219$, $p = 0.032$) but not across LC and HC diets ($F(5, 15.74) = 2.831$, $p = 0.052$) (Figure 2B). Further pairwise comparisons of time over HC and ACT conditions indicated GMVs were significantly lower at 180 mins (5757 ± 3157 events/μl) than at baseline (6684 ± 32917 events/μl) ($p = 0.038$). Finally, no significant time by diet or condition interaction was observed in circulating GMVs (LC vs. HC $F(5, 75.03) = 2.047$, $p = 0.082$; HC vs. ACT $F(5, 77.68) = 1.981$, $p = 0.091$).

Activated Endothelial Cell-derived Microvesicles

In HW participants circulating activated EMVs did not differ in concentration across time, diet, or condition. Linear mixed model analysis indicated no significant difference between HC and LC diets ($F(1, 10.09) = 2.787$, $p = 0.126$) nor HC and ACT conditions ($F(1, 9.58) = 2.001E\text{-}5$, $p = 0.997$) when collapsed over time. Further analysis including time as a fixed effect also displayed no significant main effect of diet or condition (LC vs. HC $F(1, 10.11) = 2.523$, $p = 0.143$; HC vs. ACT $F(1, 9.73) = 0.004$, $p = 0.949$, Figure 2C). Time had a significant main effect when collapsed over HC and ACT conditions ($F(5, 15.94) = 3.476$, $p = 0.026$, Figure 1C), however, pairwise comparisons indicated no significant difference at any time points when compared to baseline (Appendix A, Table 56). No main effect of time was observed when collapsed across LC and HC diets ($F(5, 16.61) = 2.589$, $p = 0.065$, Figure 2C) and no significant time by diet or condition interaction was found in circulating EMVs (LC vs. HC $F(5, 76.96) = 1.061$, $p = 0.389$; HC vs. ACT $F(5, 77.44) = 0.682$, $p = 0.638$).

Platelet-derived Microvesicles

Concentrations of circulating PMVs in HW participants were similar across all conditions, diets, and time points. Linear mixed model analysis with diet or condition or diet collapsed over time indicated no significant difference between LC and HC diets ($F(1, 10.06) = 0.323$, $p = 0.582$), nor HC and ACT conditions ($F(1, 10.20) = 0.797$, $p = 0.393$). Inclusion of time as a fixed effect also displayed no significant effect of diet or condition (LC vs. HC $F(1, 10.05) = 0.214$, $p = 0.654$; HC vs. ACT $F(1, 10.51) = 0.927$, $p = 0.357$), nor of time when collapsed across diet or condition (LC vs. HC $F(5, 13.47) = 1.746$, $p = 0.191$; HC vs. ACT $F(5, 15.41) = 2.296$, $p = 0.096$) (Figure 2D). Further, no significant time by diet or condition interaction was observed in PMVs (LC vs. HC $F(5, 67.43) = 2.015$, $p = 0.088$; HC vs. ACT $F(5, 77.19) = 1.247$, $p = 0.296$).

Apoptotic Endothelial Cell-derived Microvesicles

In HW participants concentrations of circulating apoptotic EMVs were similar over time, diets, and conditions. Linear mixed model analysis indicated no significant difference between HC and LC diets ($F(1, 9.99) = 0.356$, $p = 0.564$), nor sedentary (HC) and stair snacks (ACT) conditions ($F(1, 9.81) = 0.013$, $p = 0.912$) when collapsed over time. Inclusion of time as a fixed effect also displayed no significant difference between diet or condition (LC vs. HC $F(1, 10.33) = 0.214$, $p = 0.653$; HC vs. ACT $F(1, 9.77) = 0.005$, $p = 0.944$) nor time when collapsed across diet or condition (LC vs. HC $F(5, 26.30) = 1.358$, $p = 0.272$; HC vs. ACT $F(5, 21.28) = 0.539$, $p = 0.744$) (Figure 2E). No significant time by diet interaction was found between LC and HC diets ($F(5, 95.66) = 1.646$, $p = 0.155$). The time by condition interaction was significant when comparing HC and ACT conditions ($F(5, 87.07) = 3.545$, $p = 0.006$), however, subsequent pairwise comparison indicated no significant differences between time points within condition, nor within time points across conditions (Appendix A, Table 53).

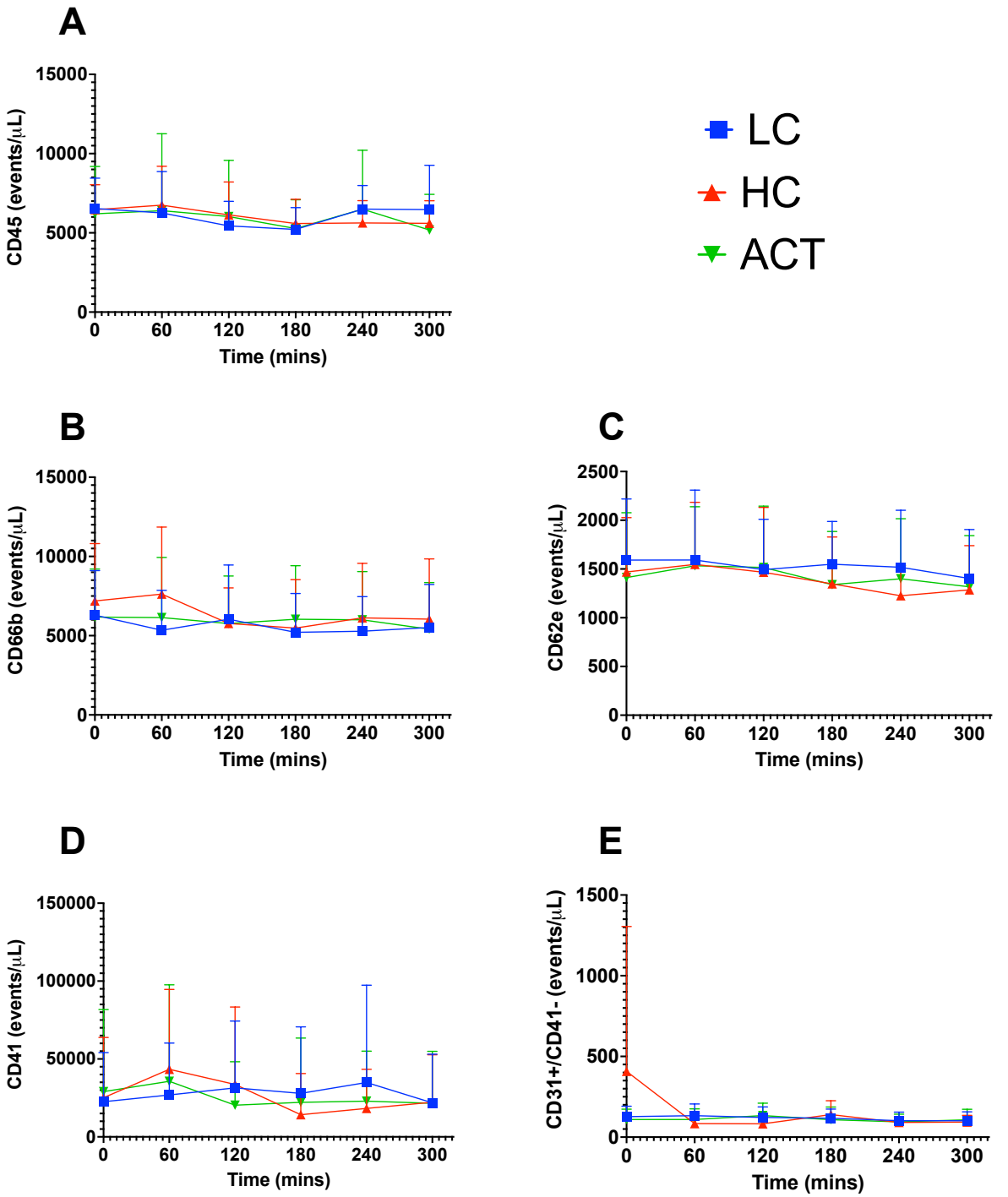

Figure 2. Concentration of circulating microvesicles over time in healthy weight individuals. Participants consumed 2 identical low-carbohydrate (LC) or high-carbohydrate (HC and ACT) meals at 0 and 180 mins and remained sedentary (LC, HC) or performed hourly 15-30s stair climbs (ACT). a) LMVs b) GMVs c) EMVs d) PMVs e) apoptotic EMVs.

Study 2: Elevated waist Circumference

Total Leukocyte-derived Microvesicles

Concentrations of circulating LMVs were relatively stable over conditions, diets, and time in EWC individuals. Linear mixed model analysis with condition collapsed over time demonstrated no significant difference between HC and LC diet ($F(1, 8.03) = 0.933$, $p = 0.362$) nor sedentary (HC) and stair snack (ACT) conditions ($F(1, 7.00) = 5.397$, $p = 0.053$). Subsequent analysis including time as a fixed effect, also indicated no main effect of diet or condition (LC vs. HC $F(1, 6.62) = 0.720$, $p = 0.426$; HC vs. ACT $F(1, 7.24) = 5.091$, $p = 0.057$, Figure 3A). There was no significant main effect of time ($F(5, 9.78) = 2.318$, $p = 0.123$, Figure 2A) or interaction between time and condition ($F(5, 56.00) = 2.017$, $p = 0.090$) when comparing stair snack (ACT) to sedentary (HC) conditions. However, a significant main effect of time ($F(5, 10.32) = 3.405$, $p = 0.045$, Figure 3A) and interaction of time and diet ($F(5, 52.12) = 5.919$, $p < 0.001$) was observed between LC and HC diets. Pairwise comparison revealed in the LC treatment LMVs were significantly lower at 240 mins (5230 ± 1477 events/μl) than at baseline (8546 ± 2688 events/μl) ($p < 0.016$). Further, LMVs were significantly lower at 240 mins (5230 ± 1477 events/μl) in the LC condition as compared to the HC condition (7627 ± 2170 events/μl) ($p < 0.016$).

Granulocyte-derived Microvesicles

Circulating GMV concentrations did not differ with change in diet, activity, or over time in individuals with EWC. Linear mixed model analysis indicated no significant difference between HC and LC diet ($F(1, 81.07) = 0.256$, $p = 0.614$) nor sedentary (HC) and stair snack conditions (ACT) ($F(1, 6.88) = 3.166$, $p = 0.119$) when collapsed over time. Further analysis including the fixed effect time, also demonstrated no significant main effect of diet or condition (LC vs. HC $F(1, 6.10) = 0.056$, $p = 0.821$; HC vs. ACT $F(1, 7.33) = 2.917$, $p = 0.130$), nor time (LC vs. HC $F(5, 8.72) = 2.321$, $p = 0.131$; HC vs. ACT $F(5, 11.86) = 0.686$, $p = 0.643$) (Figure 3B). Additionally, no significant interaction between time and diet or condition was observed for circulating GMVs (LC vs. HC $F(5, 48.89) = 0.494$, $p = 0.130$; HC vs. ACT $F(5, 54.97) = 1.656$, $p = 0.161$).

Activated Endothelial Cell-derived Microvesicles

Concentrations of circulating microvesicles derived from EMVs did not differ across condition, diet, or over time in the EWC group. Linear mixed model analysis with condition/diet collapsed over time displayed no significant difference between HC and LC diet ($F(1, 6.52) = 0.251$, $p = 0.633$) nor sedentary (HC) and stair snack conditions (ACT) ($F(1, 7.00) = 1.482$, $p = 0.263$). Subsequent analysis including time also indicated no significant main effect of diet or condition (LC vs. HC $F(1, 4.66) = 2.695$, $p = 0.988$; HC vs. ACT $F(1, 7.07) = 1.430$, $p = 0.270$) nor of time (LC vs. HC $F(5, 10.92) = 1.847$, $p = 0.185$; HC vs. ACT $F(5, 13.03) = 0.392$, $p = 0.846$) on EMV concentrations (Figure 3C). Further, no significant interaction was found between time and diet or condition (LC vs. HC $F(5, 55.32) = 0.032$, $p = 0.999$; HC vs. ACT $F(5, 63.00) = 1.596$, $p = 0.174$).

Platelet-derived Microvesicles

The concentration of circulating PMVs was similar across all conditions, diets, and time points in EWC individuals. Linear mixed model analysis indicated no significant difference between HC and LC diet ($F(1, 6.03) = 3.493$, $p = 0.111$) nor sedentary (HC) and stair snack conditions (ACT) ($F(1, 7.00) = 0.004$, $p = 0.954$) when collapsed over time. Further analysis including the fixed effect time, also indicated no significant main effect of diet or condition (LC vs. HC $F(1,8.90) = 3.778$, $p = 0.084$; HC vs. ACT $F(1,7.30) = 0.003$, $p = 0.956$), nor time (LC vs. HC $F(5, 11.59) = 1.365$, $p = 0.306$; HC vs. ACT $F(5, 14.30) = 2.705$, $p = 0.064$) on concentrations of circulating PMVs (Figure 3D). Additionally, no significant interaction between diet/condition and time was observed on PMVs (LC vs. HC $F(5, 58.51) = 0.956$, $p = 0.452$; HC vs., ACT $F(5, 70.01) = 0.915$, $p = 0.476$).

Apoptotic Endothelial Cell-derived Microvesicles

Concentrations of circulating apoptotic EMVs remained fairly stable in EWC individuals over time across all diets and conditions. Linear mixed model analysis with condition as a fixed effect indicated no significant difference in apoptotic EMVs between LC and HC diets ($F(1, 6.25) = 0.016$, $p = 0.902$) nor sedentary (HC) and stair snack (ACT) conditions ($F(1, 7.00) = 0.511$, $p = 0.498$). Inclusion of time as a fixed effect also demonstrated

no significant difference between diet or condition (LC vs. HC $F(1, 6.59) = 0.027$, p 0.873; HC vs. ACT $F(1, 7.53) = 0.445$, $p = 0.525$, Figure 3E). Further, no significant main effect of time ($F(5, 11.35) = 1.794$, $p = 0.192$) nor interaction between time and condition ($F(5, 63.00) = 1.690$, $p = 0.150$) was found when comparing HC and ACT conditions (Figure 3E). Conversely, similar comparison of the LC and HC diets displayed a significant main effect of time ($F(5, 11.84) = 4.302$, $p = 0.018$) and diet by time interaction ($F(5, 58.66) = 3.443$, $p = 0.009$) (Figure 3E). Subsequent pairwise comparisons indicated concentrations of circulating apoptotic EMVs were significantly lower at 180 mins (159 ± 116 events/μl) compared to baseline (272 ± 166 events/μl) in the LC condition ($p < 0.016$).

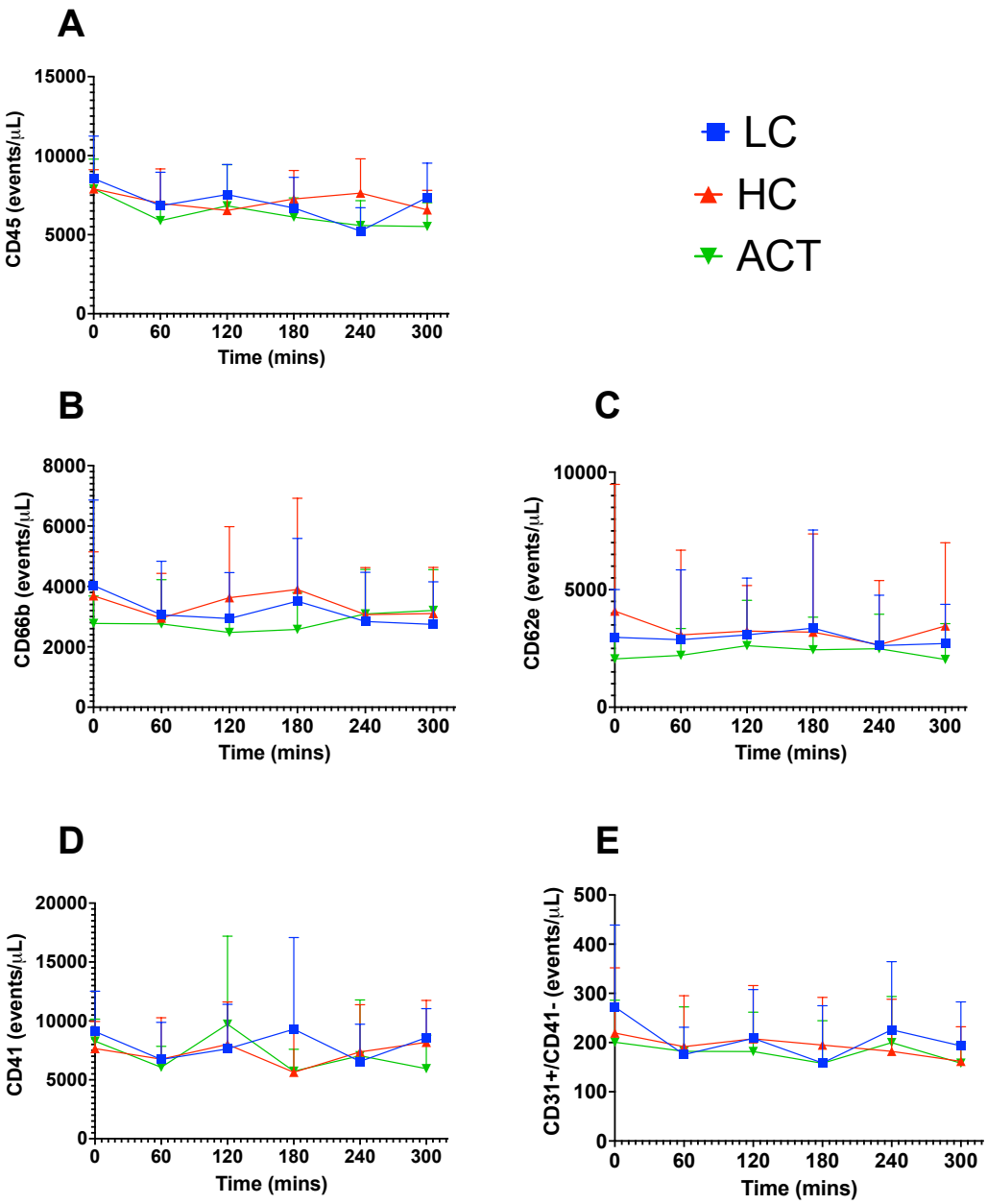

Figure 3. Concentration of circulating microvesicles over time in individuals with elevated waist circumference. Participants consumed 2 identical low-carbohydrate (LC) or high-carbohydrate (HC and ACT) meals at 0 and 180 mins and remained sedentary (LC, HC) or performed hourly 15-30s stair climbs (ACT). a) LMVs b) GMVs c) EMVs d) PMVs e) apoptotic EMVs.

Chapter 4: Discussion

The central goal of this thesis was to examine the influence of hourly stair snack interruptions to prolonged sitting and high or low carbohydrate diets on postprandial circulating MVs in two populations: individuals of healthy weight and those with elevated waist circumference. Using flow cytometry concentrations of LMVs, GMVs, PMVs, apoptotic and activated EMVs were determined throughout three experimental trials: i) sedentary with low carbohydrate meals, ii) sedentary with high carbohydrate meals, and iii) hourly stair snack with high carbohydrate meals.

The main findings of this study are that neither high or low carbohydrate diet nor incorporation of hourly stair snacks altered concentrations of postprandial circulating microvesicles throughout a five-hour bout of sitting in either study population. Minor differences were observed in select MV populations with diet or condition; however, they are likely not physiologically significant given the natural variability of MV populations in circulation.

Influence of Diet on Postprandial Circulating MVs during Prolonged Sitting

The carbohydrate content of meals consumed during a bout of prolonged sitting did not influence concentrations of circulating MVs in healthy weight individuals. A similar result was observed with elevated waist circumference, as quantities of GMVs, PMVs, and activated EMVs were consistent in both high and low carbohydrate sedentary trials. However, in EWC participants some individual time points differed between diets or over time within diets for LMVs and apoptotic EMVs, respectively. This difference may be due to the presence of outliers in $CD31^{+}/CD41^{-}$ MVs and high day-to-day variability measured for this population.

LMVs were reduced at one time point in the EWC group with consumption of low-carbohydrate compared to high carbohydrate meals, however, this small and transient decrease likely does not result in a physiologically meaningful response. Further, the marker of LMVs used and range of physiological effects from different LMVs also make it difficult to suggest physiological importance. The marker of LMVs used in the present study, CD45, is used to assess total leukocyte-derived MVs[87]. Although this marker is widely used in MV studies, it is

not 100% co-expressed with other antigens specific to sub-LMV populations[87,88]. Therefore, the change in LMVs observed may not be accurately capturing changes in all neutrophil-, monocyte-, and lymphocyte-derived MVs. Further, each of these sub-LMV populations have diverse physiological effects on endothelial cells with neutrophil-derived MVs exerting anti- or proinflammatory effects, monocyte-derived MVs inducing thrombogenicity as well as apoptosis or angiogenesis, and lymphocyte-derived MVs inducing NO production[87]. Given this and the inability of the present study to measure which LMV population contributed to the reduction observed, it is not possible to conclude how carbohydrate consumption during prolonged sitting influences circulating LMVs.

Importantly, the lack of change observed in circulating MVs with high or low carbohydrate diets during prolonged sitting supports recent findings involving arterial stiffness. Specifically, Kelsch *et al.* found that although prolonged sitting increases arterial stiffness, this increase was not exacerbated by consumption of a high-glycemic index meal[89]. Although the mechanisms of increasing arterial stiffness for prolonged sitting[90] and high-carbohydrate consumption[91,92] differ, this result suggests that those of prolonged sitting predominate. Prolonged sitting increases blood pooling in the lower limbs, causing decreased venous return and stroke volume[93]. Such a reduction in stroke volume results in decreased shear stress[89], which is an important mediator of MV release[28,94–96]. Therefore, the similar concentrations of MVs between the two sedentary trials, regardless of diet, may be due to the predominate mechanism effecting their release being the same: prolonged sitting.

Influence of Interrupting Prolonged Sitting on Postprandial Circulating MVs

Breaking up prolonged sitting with hourly 30 second stair snacks did not alter concentrations of circulating LMVs, GMVs, PMVs, apoptotic or activated EMVs in individuals with EWC. Conversely, in HW participants GMVs were reduced at one time with the sedentary and stair snack conditions. Importantly, concentrations of GMVs returned to baseline in all succeeding time points. Quantities of all other MV populations studied were unaffected by the stair snack intervention in HW individuals. Given the paucity of research into the influence of exercise snacks on MVs, it is important to consider the specifics of the stair snack intervention when assessing the significance of results in the present study.

Implications of the Stair Snack Intervention

Previous exercise snack studies have used this type of brief exercise bout to improve cardiorespiratory fitness[80,81]. As such it was hypothesized that stair snacks may impact circulating MV populations, however, this was not observed. This may relate to the specifics of the stair snack intervention. A major difference between the stair snacks employed in this study and those of previous exercise snack investigations is the absence of a warm-up and cool-down period with each exercise bout. In the studies that demonstrated cardiorespiratory fitness improvement with sprint or stair snacks, each repeated exercise bout consisted of a warm-up of either dynamic calisthenics (jumping jacks, lunges, squats) or walking, the exercise snack, and a one minute walking cool-down[80,81]. Although the intensities of these activities are likely not significant with regards to MV formation[97], they do increase the total amount of activity per bout. Therefore, this discrepancy in duration may contribute to the lack of noticeable increases in circulating MV populations and in the postprandial metabolic profile of HW individuals previously observed with this intervention[83].

Given the time-course of MV release with exercise, we are confident that if changes in MV concentration had occurred they would have been detected given the frequent sampling schedule of the experiment. The dynamics of MV release with exercise are dependent on the cell of origin. Following an acute bout of exercise circulating PMVs are elevated immediately[57,98–100], with increases remaining up to one[11,99,100] and two[56,98,100] hours, before returning to baseline. The circulating time-course for the other MV populations (i.e. GMVs, LMVs, and EMVs) are not as well defined as that of PMVs. Chaar *et al.* demonstrated an increase in neutrophil-derived MVs lasting two hours following maximal exercise tests[98]. Concentration of another LMV, monocyte-derived MVs, depend on the training level of the individual with delayed increases (45 to 120 mins-post) in exercise trained groups[100] and no changes in untrained populations[98]. Regarding EMV release time-course, increases in activated EMVs have been noted between 45[100] and 90[58] minutes post-exercise, and return to baseline levels within two hours[100]. Conversely, apoptotic EMVs may reduce in concentration 1-3 hours[59] following exercise but not immediately following a bout[101] in healthy individuals. Importantly, these time courses were observed following exercise bouts of greater intensity

and longer duration than those employed in the current study. However, even across the variety of exercise protocols used, measurable changes, if they occurred, happened no later than 90 minutes post-exercise. In relation to prolonged sitting, circulating EMV concentrations may decrease following just 180 minutes[28]. Therefore, it is reasonable to conclude that the prolonged testing duration and hourly sampling of the current study would have been sufficient to capture a change in concentration in any of the MV populations studied occurring as a result of the exercise intervention and prolonged sitting.

The lack of change observed in MV concentrations with the activity intervention may be due to insufficiency of stair snacks to elicit exercise-induced changes. Type, volume, and intensity of exercise all influence the release of MVs into circulation. Research on the effect of exercise type on MV release is ongoing, with the majority of studies using continuous or interval running or cycling. Although these exercise modalities differ in contraction type, aerobic power matched concentric and eccentric cycling produce analogous increases in PMVs and lack of change in EMVs[57]. This indicates that type of muscle contraction may not alter post-exercise MV release[42]. Further, although stair-climbing is not commonly employed in MV studies, this supports its use as an exercise intervention that may produce shifts in MV dynamics consistent with other non-resistance exercises. That being said other aspects of stair snacks, specifically volume and intensity, may limit its impact on MV release. The small volume of exercise in stair snacks may contribute to the lack of change observed in circulating MVs in the present study. Similar to exercise type, few studies have thoroughly investigated the influence of volume, particularly small-volume exercise, on MV dynamics. Wilhelm *et al.* demonstrated that doubling exercise duration did not further increase concentrations of PMVs once elevated from baseline with 30 mins of cycling[11]. Further, exercise studies employing several minute bouts of whole body or limb exercise report increases in PMVs consistent with other exercise studies[98,99,102]. These results suggest exercise volume does not greatly influence MV release, however, they only address PMV and EMV populations and all previous studies exceed the volume of exercise in stair snacks. The greatest determinant of exercise induced MV release may be intensity. Vigorous, but not moderate, intensity exercise causes increases in circulating PMVs from baseline[11]. According to the American College of Sports Medicine, moderate intensity exercise is defined as a RPE of fairly light to somewhat hard, whereas

vigorous exercise involves a RPE of somewhat hard to very hard[103]. Given the average RPE in both the EWC and HW groups were somewhat hard to hard[104,105] in the stair snack condition, it may be that the intensity of the stair snacks bordered the moderate to vigorous ranges. This in conjunction with the extremely small volume of exercise in the stair snacks may contribute to the lack of influence observed on circulating MVs. Specifically, the intensity and volume of exercise employed in the present study may not have been sufficient to elicit changes in concentrations of circulating MVs seen with other exercise interventions.

Influence of Prolonged Sitting and Stair Snacks on Apoptotic EMVs

Contrary to other inactivity studies[8,15], no increase in apoptotic EMVs was observed in the sedentary conditions in HW participants. Further, no change was observed in the EWC group which is in contrast to the findings of Evans *et al.* who observed decreases in apoptotic EMVs in overweight individuals[28]. This lack of change is surprising given the prolonged duration of sitting utilized in the present study. A significant reduction in mean shear rate occurs after three hours of prolonged sitting[20–22], therefore it is reasonable to assume that the five hour period employed in this study would elicit a reduction in shear rate as well. *In vitro* studies have demonstrated a reduction in shear of similar magnitude to those observed with sitting, increased release of apoptotic EMVs[94]. Mechanistically, this is due to activation of the RhoA-ROCK pathway, in which low shear stress increases endothelial Rho kinase and extracellular signal-regulated protein kinase 1 and 2 activity, inducing cytoskeletal reorganization and apoptotic EMV release[94]. This occurs simultaneously with a reduction in eNOS expression, which results in lowered NO-inhibition of ABCA-1 flippase; further inducing membrane remodeling and phosphatidylserine exposure and thus apoptotic EMV release[94]. Given this pathway it is reasonable to assume that if a reduction in shear rate occurred with the prolonged sitting bout in the present study an increase in apoptotic EMVs would have been observed.

Influence of Prolonged Sitting and Stair Snacks on Activated EMVs

Along with no change in apoptotic EMVs, concentrations of activated EMVs did not differ over time with prolonged sitting nor stair snack intervention. This result agrees with the lack of increase in activated EMVs with dry immersion[8] and step-reduction[15], however, it

contrasts decreases observed with prolonged sitting in overweight individuals[28]. No change in activated EMVs is reasonable when considering the lack of effect physical inactivity has on inflammatory markers observed in previous long-term studies[8,37]. The discrepancy between the results in the present study and the investigation by Evans *et al.* into prolonged sitting may be due to antagonistic processes at play. Specifically, if the assumed prolonged sitting induced reduction in shear was accompanied by altered flow patterns, differential MV release may occur. Jenkins *et al.* demonstrated increased apoptotic and activated EMVs with disturbed blood flow, characterized by low shear stress, high retrograde flow, and oscillatory shear stress[106]. Disturbed blood flow induces the pro-inflammatory effect of the NF-κB pathway[107–109]; one that is not induced with low shear stress alone[94]. Specifically, shear stress of disturbed flow stimulates the phosphorylation of NF-κB inhibitory proteins (IκBs), causing them to degrade and release NF-κB dimers[107,108]. These translocate to the nucleus of endothelial cells and activate the transcription of pro-inflammatory genes including those for E-selectin, IL-1β, ICAM-1, and VCAM-1[108–110]. IL-1β then promotes further expression of VCAM-1 in response to disturbed flow, thereby elevating the pro-inflammatory effects of NF-κB[110]. This pro-inflammatory response to disturbed flow may contribute to the elevated activated EMVs observed by Jenkins *et al.*[106] that are not observed in earlier studies of low shear stress alone. It is possible that in conjunction with a reduction in shear rate, disturbed flow is occurring in the present study. If so, then this activated EMV release-inducing stimulus would be occurring simultaneously with the release-inhibiting effect of prolonged sitting observed by Evans *et al.*[28]. The culmination of these antagonistic effects may have resulted in the lack of change in activated EMVs observed in the present study. However, without measures of the inflammatory cytokine products of the NF-κB pathway or flow patterns it is beyond the scope of the present study to make this conclusion.

In examining the differences between the results from the present study and those of Evans *et al.* it is important to consider discrepancies in methodology. Given the massive size range of MVs from 100-1000 nm[43,44], differences in flow cytometry sensitivity can greatly influence MV measurements. Evans *et al.* employed a FACS Canto II, which is incapable of detecting vesicles 600 nm or less in diameter to a level above threshold[111]. Conversely, the same assessment showed the CytoFlex used in the present study detected vesicles down to 300

nm in diameter using the testing protocol. Therefore, it is possible that the decreases in EMVs observed by Evans *et al.* are not true decreases in concentrations of EMVs but rather reduction in the size the of EMVs to the extent they could no longer be detected.

Influence of Prolonged Sitting and Stair Snacks on LMVs

While the lack of change observed in EMV populations is surprising given the presumed reduction in shear rate that would have occurred with five hours of prolonged sitting, the absence of shift in LMVs is not unreasonable. Specifically, *in vivo* studies of variable shear rate demonstrated no correlation between reduction in shear rate and concentrations of LMVs[94]. Notably, Vion *et al.* refer to brachial artery shear rate and classifies LMVs as $CD11a^{+}$ [94], which is typically used as a marker for monocyte LMVs[87]. The marker of LMVs used in the present study, CD45, encompasses monocytes as well as other sub-LMV populations to give a measure of total LMVs[87,88]. Therefore, a lack of change in monocyte LMVs may be contributing to the consistent LMV concentrations observed in the present study. However, it is possible that other sub-LMV populations are changing in equal but opposite manners. Further investigation into each sub-LMV population is needed before the consistency observed can be attributed to true absence of influence of prolonged-sitting and/or stair snacks, and not the sum of opposing changes within the LMV population.

Influence of Prolonged Sitting and Stair Snacks on GMVs

In HW participants GMVs were reduced at one point during prolonged sitting when collapsed over the sedentary and stair snack conditions. Importantly, this result was not observed in EWC individuals or at succeeding time points in the HW study. Such a reduction in GMVs is interesting given Chaar *et al.* demonstrated an increase in polymorphonuclear neutrophils, a type of granulocyte, following exercise[98]. Research regarding GMVs and physical inactivity or acute exercise is limited. Further, GMVs appear to demonstrate both pro- and anti-inflammatory effects on vascular function. Specifically, the anti-inflammatory effects of GMVs arise from down regulation of macrophage activity[112], while application of GMVs to endothelial cells increases IL-6 release, indicating a pro-inflammatory effect[113]. Therefore, it is difficult to determine the role GMVs play in vascular response to prolonged sitting and

acute exercise. This, in addition to the GMV reduction only being observed transiently, makes it difficult to elucidate the physiological importance of the decrease.

Influence of Prolonged Sitting and Stair Snacks on PMVs

No effect of prolonged sitting or stair snacks was observed in circulating PMVs in HW or EWC individuals. PMV release occurs in response to many factors including thrombin[114], IL-6[115], norepinephrine[116], and shear stress[95]. The majority of inactivity studies do not investigate PMVs, however, some have reported the influence of inactivity on these PMV formation agonists. Inactivity in the form of two weeks of step reduction[37] or bed rest[6] does not alter concentrations of IL-6 in plasma. Further, eight hours of uninterrupted prolonged sitting does not influence plasma levels of norepinephrine[117]. Given this, it is reasonable that the prolonged sitting of the present study may not have altered concentrations of these agonists and thus quantitates of PMVs in circulation were unchanged. Of note is the contribution of shear stress in the release of PMVs. Elevated shear stress induces formation of PMVs[96] through a glycoprotein Ib_{α} mediated process[95]. Given this and the aforementioned reduction in shear rate that occurs with prolonged sitting[20–22], it is reasonable to not see an increase in PMVs over time in the sedentary condition. Absence of PMV decrease with reduction in shear rate is also consistent with *in vitro* studies showing no decrease in platelet activation when shear is reduced from moderate levels to those observed with prolonged sitting[118]. Conversely, no change in the stair snack condition is in contrast to elevations of PMVs observed in exercise studies[11]. Exercise increases mean and anterograde vascular wall shear rates, measures of shear stress,[55] which in conjunction with SNS activation and increasing adenosine diphosphate and IL-6[98] results in elevated PMV release[11,42]. However, as previously discussed exercise intensity plays a critical role in PMV response, with vigorous but not moderate intensity exercise increasing PMV concentrations[11]. Therefore, the intensity of the stair snacks may have been insufficient in prompting the elevated PMV release expected. This insufficiency may be exacerbated if the lesser intensity of stair snacks prevents the release of juvenile platelets into circulation that is typically observed with high intensity exercise[56]. Juvenile platelets are more sensitive to the PMV formation agonist norepinephrine[56], and an absence of their contribution to PMV release may have contributed to the lack of change observed in the present study.

Strengths and Limitations

The experimental design of the present study, including randomized cross-over design, use of mixed meals, frequent blood sampling, prolonged duration, and flow cytometry, are strengths of this research. Many studies investigating the impact of carbohydrate consumption use specialty glucose beverages which limits their application in understanding physiological responses in real world situations. The use of mixed meals in the present study avoids this limitation. The five-hour duration and frequent sampling of this study expands on the findings of previous short-duration prolonged sitting studies and accounts for the time-course of MV release as previously described. Additionally, the flow cytometry techniques employed are major strengths in this work. With the exception of anticoagulant, the best practices for MV flow cytometry[88,119,120] were used, including fluorescence and unstained controls for each sample. Further, linear dilution series were used to ensure absence of swarming and antibody titration was completed to avoid over saturation (see Appendix B Table 1.). A mixture of polystyrene and silica beads were used for gating to account for refractive index differences between commercial beads and MVs[119]. Finally, the number of MV populations enumerated, although still limited, gives a more complete depiction of MV response than the majority of other inactivity studies.

In conjunction with these strengths, it is important to consider the limitations of this study. The composition of the two study groups has several implications. Specifically, the small sample size of both the HW and EWC groups may have reduced the power of the study. Further, the HW group consisted of only males and thus participants in the EWC group could not be age and sex-matched. This inhibited direct comparison of stair snack and diet influence between populations. In addition to these participant-based limitations, an absence of measures for more specific MV populations and MV release mediators may limit understanding of the physiological processes at play. As previously described the marker of LMVs used, CD45, is an indicator of total LMVs and is not 100% co-expressed with other markers of specific sub-populations[87,88]. This in conjunction with the range of physiological effects of different LMV populations makes it difficult to understand the influence of prolonged sitting and carbohydrate consumption on LMVs. Further, the aforementioned absence of cytokine or direct shear stress

measurements limits assessment of how each condition and diet affect these mediators of MV release. Previous work with this study demonstrated increases in femoral artery blood flow and mean shear rate following the ACT condition and no influence of prolonged sitting[121]. Importantly, hemodynamic measures are not reported for the LC diet in HW participants or any trial of the EWC group. This prevented mechanistic evidence to support the lack of changes observed in MV concentration in response to meal carbohydrate content or waist circumference. Additionally, without such measures for both study groups it is difficult to conclude whether differences between this study and Evans *et al.* are due to discrepancies in method or true physiological shifts.

While the flow cytometry protocol is a strength of the present study, it is important to consider the implications of the anticoagulant used and lack of plasma volume correction. Using EDTA as an anticoagulant has been shown to artificially increase concentrations of PMVs compared to other anticoagulants[119]. Such an increase does not limit interpretation of the overall trends in MVs observed as all samples were exposed to the anticoagulant equally. However, an artificial increase may limit direct comparison of observed MV concentrations with previously published findings utilizing other anticoagulants. Similarly, failure to account for change in plasma volume also may limit direct comparison with published studies. Plasma volume data for prolonged sitting is limited, however, Evans *et al.* demonstrated an insignificant 6% decrease with three hours of prolonged sitting[28]. Without measures of hematocrit and hemoglobin[122] it is unknown whether such a reduction in plasma volume occurred in the present study. While this may limit comparison of MV concentrations with previous results, such comparisons are already extremely limited by the large dependency of flow cytometry measurements on sample processing and technique; factors that improve as time of publication progresses. Further, it is unlikely that correcting for a shift in plasma volume similar to that observed by Evans *et al.* would alter the MV trends observed because of the high day-to-day coefficient of variation for each population.

Another limitation of the present study is failure to account for MV clearance. Using venous blood samples to quantify circulating MVs only provides an indication of half of the MV response to prolonged siting, stair snacks, and diet. Specifically, it limits the ability to

determine that observed concentration changes are caused by altered MV release and not clearance from venous circulation. This is a limitation in many MV investigations, as research into MV clearance is ongoing, with mechanisms including endothelial uptake, phagocytosis by macrophages, and localization to the spleen, liver, and lungs purported in the literature[96]. Given this limitation, it is difficult to measure MV clearance in its entirety, however, inclusion of atrial sampling to determine arterial-venous difference would have lessened this limitation in the present study.

Future Directions

The findings of this study begin to describe MV response to prolonged sitting with consumption of a high-carbohydrate diet, however, there are still several questions that need to be addressed.

How does prolonged sitting effect blood flow patterns?

Blood flow and associated shear stress are major mediators of MV release[7,10]. Such femoral artery hemodynamics increase following the ACT condition and remain unchanged after prolonged sitting (HC) in HW participants[121]. While concentrations of MVs did not change with prolonged sitting or diet in the present study, a lack of reported blood flow measures for all trials in both study groups make it difficult to examine the physiological mechanisms at play and any potential changes to the dynamics. This is especially important given the paucity of studies that combine MV response with prolonged sitting induced changes to blood flow and shear stress. Future prolonged sitting studies should include measures of blood flow and shear to elucidate how these factors may be contributing to the MV response being observed.

What minimum duration/intensity exercise alters MV concentrations?

As described, the volume and intensity of the stair snack intervention may have been insufficient to elicit a similar MV response as observed with other exercise interventions. However, RPE scores indicate the stair snacks were well tolerated in both the HW and EWC groups. Given that lack of time and access to exercise facilities are common barriers to physical

activity[123], the accessibility of exercise snacks in everyday life warrants further research into their application. Specifically, future studies should determine the minimum duration and intensity of exercise that demonstrates protective effects against vascular impairments with prolonged sitting. While the present study appears to be below this threshold, future work could expand on exercise snacks to create an accessible and practical intervention to prolonged sitting for application in the workplace and everyday life.

How do prolonged sitting and diet influence MV clearance?

MV release is only one half of the dynamic MV response to stimuli such as inactivity, exercise, and diet. As previously described, MV clearance is difficult to measure given the broad range of uptake and localization that occurs[96]. Experimental models have demonstrated uptake of MVs is highly dependent on phosphatidyl-serine and membrane protein exposure, with localization to different organs[124–126]. Further, rate of clearance may depend on environmental stimuli[96,127]. Given this complexity in uptake and clearance, as well as the altered phosphatidyl-serine exposure that occurs with MV freeze/thaw[96], it is difficult to conclude that clearance responses observed with *in vitro* models are physiologically relevant. Future animal model, and eventual human studies, could involve infusion of a known concentration of labelled MVs combined with tissue imaging and sampling to better understand the influence of different stimuli on MV clearance under physiological conditions. Until such studies are completed, inclusion of measures of MVs in excreted fluids such as urine[85] in addition to arterial and venous sampling would provide a more complete understanding of MV dynamics. Such investigations would provide insight into whether changes in MVs observed with different stimuli are a result of altered release, clearance, or both.

Conclusions

In conclusion, this study begins to describe the effect of prolonged sitting with hourly stair sprints and high carbohydrate diet on concentrations of circulating MVs. No changes were observed in any MV population studied, LMVs, GMVs, PMVs, activated or apoptotic EMVs, with five hours of prolonged sitting regardless of carbohydrate consumption or an hourly

exercise intervention. While this result was surprising for some MV populations given previous inactivity and exercise studies, it is not unreasonable due to the limited volume and intensity of the stair snack intervention and the multitude of factors influencing MV release; many of which are beyond the scope of the present study. MVs are mediators of vascular function and can give insight into the mechanisms of vascular impairment with inactivity; however, research into the effect of prolonged sitting on MVs is lacking. This study suggests that an acute bout of prolonged sitting does not alter concentrations of circulating MVs in healthy weight individuals or those with elevated waist circumference. However, further research involving measures of MV formation agonists and MV clearance, with prolonged sitting in the post prandial state are needed to better understand all aspects of the MV response. Prolonged sitting and carbohydrate consumption are everyday realities for many individuals, with potentially serious implications for cardiovascular function. This study is an important step in beginning to understand how such sedentary behaviour alters vascular function and establish practical exercise interventions that can be implemented in day-to-day life.

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Appendix A: Data and Statistics

Table A.1. Concentration of LMVs (CD45-PE$^+$) in HW participants over time during completion of three experimental trials. Participants consumed 2 identical low-carbohydrate (LC) or high-carbohydrate (HC and ACT) meals at 0 and 180 mins and remained sedentary (LC, HC) or performed hourly 15-30s stair climbs (ACT). Concentrations are reported as events/μl and corrected for dilution factor.

Condition	Time (min)	Concentration of CD45-PE$^+$ MVs (events/μl)										
		HW 3	HW 2	HW 5	HW 6	HW 9	HW 11	HW 13	HW 14	HW 4	HW 1	HW 10
LC	0	5514	7707	5903	9514	6968	5542	5698	7933	4626	9197	3327
	60	5185	7530	5897	7578	6528	5152	4010	5555		12387	2751
	120	5867	9216	6146	5171	5711	4102	4189	5498	4205	6146	3587
	180	5237	7154	3197	6453	6150	4612	5076	4847	4314	7090	3239
	240	6061	7842	7552	8084	7112	4735	5052	5350	4684	8515	
	300	8624	11782	6875	5954	9377	4399	3967	5902	2987	8098	3171
HC	0	7063	7229	5549	5653	7728	7091	4043	6965	4495	9594	5644
	60	8055	11617	7126	8089	4948		3294	5962	7098		4542
	120	6584	9556	4870	6730	6847	5818	3283	5304	6284	9236	2946
	180	7114	5097	5628	4875	5913	6162	2972	5236	6882	8168	3442
	240	6747	6543	6349	5318	6959	5084	2868	6805	5240	6556	3395
	300	7168	5285	6682	4525	5919	5527	3776	7071	5393	7287	2943
ACT	0	6194	14905	6689	4310	5499	5308	5735	5805	4750	4916	4137
	60	10525	19698	5998	5022	4016	3931	3468	4794	4967	5239	2755
	120	11144	13323	7470	5047	4393	3452	3044	5083	4195		3149
	180	6916	10005	4949	4502	5260	4270	3827	5349	5146	4214	3543
	240	10443	15968	7539	5044	5401	4214	4449	5211	5726	4590	2859
	300	8791	10005	5544	4842	4647	4196	3733	5122	3500	3962	2625

Table A.2. Concentration of GMVs (CD66b-FitC^{+}) in HW participants over time during completion of three experimental trials. Participants consumed 2 identical low-carbohydrate (LC) or high-carbohydrate (HC and ACT) meals at 0 and 180 mins and remained sedentary (LC, HC) or performed hourly 15-30s stair climbs (ACT). Concentrations are reported as events/µl and corrected for dilution factor.

Condition	Time (min)	Concentration of CD45-PE^{+} MVs (events/µl)										
		HW 3	HW 2	HW 5	HW 6	HW 9	HW 11	HW 13	HW 14	HW 4	HW 1	HW 10
LC	0	6723	4458	1696	6581	7223	7674	3037	10067	7453	3722	10602
	60	7275	2973	1840	6731	6919	6078	2229	8978		3261	7153
	120	11099	4461	1618	5472	7261	5961	2594	7192	5170	2937	12661
	180	9698	3475	1990	4957	5736	6198	2351	6107	4622	3446	8772
	240	8493	3789	4182	5182	5354	5803	2567	9411	4807	3218	
	300	9045	5035	2603	3648	9148	5959	2784	9371	4060	3502	
HC	0	10079	4331	4296	4999	8301	8480	4439	9396	4285	4842	15668
	60	11396	4732	4261	5182	4613		2183	12135	10427		13669
	120	9147	3741	3994	4117	5840	6963	2030	9066	7030	4980	6536
	180	8299	2636	4428	2624	4641	6025	2489	12059	4751	3532	8748
	240	10321	3323	3776	3933	5760	6087	2601	11339	5184	3149	11875
	300	11541	3188	4806	3407	4105	5118	1687	13976	4214	5643	8803
ACT	0	11344	5149	4182	3943	4134	7428	3062	10233	5846	2910	9692
	60	15230	6296	4179	3172	3201	6333	2604	7883	4893	3616	10180
	120	8798	5005	4400	3353	4997	6253	2013	6394	3960		12468
	180	11658	4667	4366	4353	4409	6211	2481	9133	4255	2813	12077
	240	10839	6088	4061	4014	4050	6245	1925	9977	5417	3204	10165
	300	9815	4097	3209	3193	4657	6847	2219	10746	5218	2184	7358

Table A.3. Concentration of activated EMVs (CD62e-PE$^+$) in HW participants over time during completion of three experimental trials. Participants consumed 2 identical low-carbohydrate (LC) or high-carbohydrate (HC and ACT) meals at 0 and 180 mins and remained sedentary (LC, HC) or performed hourly 15-30s stair climbs (ACT). Concentrations are reported as events/µl and corrected for dilution factor.

Condition	Time (min)	Concentration of CD45-PE$^+$ MVs (events/µl)										
		HW 3	HW 2	HW 5	HW 6	HW 9	HW 11	HW 13	HW 14	HW 4	HW 1	HW 10
LC	0	1339	1761	765	2674	2143	2470	723	1292	1448	1431	1461
	60	1138	1826	1004	2220	2877	2409	737	1086		1601	1028
	120	1508	1553	1226	2197	1722	2549	762	1044	1174	1207	1489
	180	1385	1719	1410	2117	1840	2350	739	1359	1261	1409	1445
	240	1144	1794	1213	1953	2178	2409	426	1535	1154	1375	
	300	1275	1873	925	2396	1912	1679	728	1072	1067	1335	1165
HC	0	1058	1641	976	1930	1299	2596	717	1543	960	1413	2030
	60	1019	2042	1053	2494	1499		678	1358	2417		1362
	120	893	1549	923	2595	1294	2692	628	1283	1751	1463	1063
	180	960	1451	1213	2214	1182	2235	690	1122	1068	1276	1411
	240	1405	1141	928	1613	1155	1816	729	1061	1400	1172	1056
	300	1056	1244	933	1326	1438	2350	531	1622	1253	1289	1102
ACT	0	1548	984	1199	2184	943	3094	934	1046	1262	1036	1295
	60	1705	2252	1152	2457	1815	2261	701	880	1327	970	1340
	120	1107	2158	1202	2401	1299	2448	678	1020	1748		1097
	180	1261	1709	1090	1812	1390	2568	586	1225	1070	718	1297
	240	968	1857	1292	2239	983	2660	666	1127	1545	947	1116
	300	1285	1544	1138	1783	1427	2566	599	920	1220	994	1014

Table A.4. Concentration of PMVs (CD41-BV421^{+}) in HW participants over time during completion of three experimental trials. Participants consumed 2 identical low-carbohydrate (LC) or high-carbohydrate (HC and ACT) meals at 0 and 180 mins and remained sedentary (LC, HC) or performed hourly 15-30s stair climbs (ACT). Concentrations are reported as events/μl and corrected for dilution factor.

Cond-ition	Time (min)	Concentration of CD45-PE^{+} MVs (events/μl)										
		HW 3	HW 2	HW 5	HW 6	HW 9	HW 11	HW 13	HW 14	HW 4	HW1	HW 10
LC	0	12455	91283	13847	9475	3470	2037	9197	11442	5544	80148	9162
	60	10974	89157	26512	31450	3938	5298	4837	6197		85585	4881
	120	32137	115719	26632	17127	4094	5291	5291	1065	13324	116310	8633
	180	15734	138571	9474	30601	4053	5166	4877	1190	8252	77898	11444
	240	20356	208653	21265	43763	6207	2852	6257	1468	10313	27969	
	300	10361	108721	16681	22442	9610	3445	5353	6435	2826	45171	9381
HC	0	110509	93424	3418	7552	1962	5348	7149	21667	3102	16633	5765
	60	144715	116440	26031	41295	5273		1061	15285	15873		24139
	120	62165	157408	5269	20586	4607	6035	3453	3941	11072	88178	7534
	180	93027	11358	10603	13807	2969	3489	5636	1261	5728	2620	5716
	240	89016	19712	29335	19147	13100	2106	4107	1845	14310	3399	4640
	300	83592	12466	28974	6352	6215	3014	1198	4086	5635	80250	12695
ACT	0	118439	150428	11201	7648	2504	3503	3119	1169	5501	5338	10824
	60	105055	199649	16096	15235	2248	1910	5238	3965	10714	27759	4391
	120	37336	91177	27013	24085	1934	2088	2982	3872	6029		6742
	180	57703	136680	15213	7738	2433	4550	3432	1928	3550	3371	7118
	240	62182	104210	19020	25176	2398	4647	2754	1914	13727	11231	4941
	300	57524	108755	7616	27660	2872	3457	1378	3206	4384	14494	5444

Table A.5. Concentration of apoptotic EMVs (CD31-APC^{+}/CD41-BV421^{-}) in HW participants over time during completion of three experimental trials. Participants consumed 2 identical low-carbohydrate (LC) or high-carbohydrate (HC and ACT) meals at 0 and 180 mins and remained sedentary (LC, HC) or performed hourly 15-30s stair climbs (ACT). Concentrations are reported as events/µl and corrected for dilution factor.

Condition	Time (min)	Concentration of CD45-PE^{+} MVs (events/µl)										
		HW 3	HW 2	HW 5	HW 6	HW 9	HW 11	HW 13	HW 14	HW 4	HW 1	HW 10
LC	0	214	147	46	150	218	176	141	84	98	14	108
	60	137	135	62	131	146	116	106	77		325	94
	120	148	129	53	174	267	104	137	84	92	21	135
	180	180	75	121	70	230	109	164	110	94	25	111
	240	62	9	116	136	184	127	129	140	62	57	
	300	145	15	74	170	197	97	135	91	72	40	80
HC	0	44	27	126	120	393	104	141	161	101	3098	165
	60	17	42	40	70	180		173	36	54		142
	120	46	10	105	69	137	136	113	60	72	22	136
	180	27	118	79	168	237	114	128	101	108	345	114
	240	52	129	46	111	96	119	105	93	85	30	133
	300	35	120	55	110	141	124	117	118	101	12	95
ACT	0	69	5	93	236	166	146	153	99	76	45	114
	60	90	50	88	254	195	102	121	73	67	26	135
	120	88	113	53	192	322	121	135	68	110		132
	180	61	14	64	232	269	100	132	98	69	40	111
	240	53	37	78	154	189	91	131	89	71	39	106
	300	58	4	88	182	239	142	143	78	76	65	108

Table A.6. Concentration of LMVs (CD45-PE$^+$) in EWC participants over time during completion of three experimental trials. Participants consumed 2 identical low-carbohydrate (LC) or high-carbohydrate (HC and ACT) meals at 0 and 180 mins and remained sedentary (LC, HC) or performed hourly 15-30s stair climbs (ACT). Concentrations are reported as events/μl and corrected for dilution factor.

Condition	Time (min)	Concentration of CD45-PE$^+$ MVs (events/μl)							
		EWC 3	EWC 6	EWC 7	EWC 8	EWC 9	EWC10	EWC11	EWC 1
LC	0	9680	12015	11965	5713		6205	7735	6507
	60	6421	10647	7578	3461		6854	6351	6493
	120	8496	10142	9328	4789		6188	7455	6350
	180	6221	9512	8954	4891		7241	4991	5021
	240	5283	6326	3530	4083		7791	5412	4186
	300	6307	9114	10604	6215		8867	4627	5740
HC	0	6993	7917	8770	8097	5980	9012	9554	6829
	60	7477	11749	8110	6329	5288	5953	5696	5289
	120	6774	7454	7966	5445	5446	6925	6769	5494
	180	7312	11205	6098	6010	8125	5841	6074	7405
	240	11415	7747	6016	7923	4936	5789	9900	7291
	300	5085	8714	6822	4918	7249	5966	6768	7023
ACT	0	5736	6612	8503	7519	7298	6525	11565	9424
	60	8272	5312	6575	5331	5683	4846	6312	4782
	120	5374	5075	9823	4317	6665	9619	9943	3772
	180	5756	5172	8406	5692	6007	5658	7464	4766
	240	6249	7742	7570	5327	4232	4107	5860	3559
	300	6176	6487	7874	3344	4033	5573	6324	4339

Table A.7. Concentration of GMVs (CD66b-FitC^{+}) in EWC participants over time during completion of three experimental trials. Participants consumed 2 identical low-carbohydrate (LC) or high-carbohydrate (HC and ACT) meals at 0 and 180 mins and remained sedentary (LC, HC) or performed hourly 15-30s stair climbs (ACT). Concentrations are reported as events/μl and corrected for dilution factor.

Condition	Time (min)	Concentration of CD66b-FitC^{+} MVs (events/μl)							
		EWC 3	EWC 6	EWC 7	EWC 8	EWC 9	EWC10	EWC11	EWC 1
LC	0	3482	8896	2958	2410		1883	1501	7079
	60	2287	5617	2261	2020		1982	1581	5653
	120	2842	4222	2783	2267		1738	1111	5582
	180	3434	5061	2828	2572		1735	1540	7398
	240	2079	2688	3528	2502		2012	977	6090
	300	2433	1993	3016	1881		2465	1635	5772
HC	0	2947	5126	4202	3824	3802	2285	1442	5893
	60	2148	3391	2975	2390	3432	1816	1341	6121
	120	2379	3919	2587	2811	5988	1826	1298	8213
	180	3148	4685	1847	3042	4506	1892	1341	10733
	240	3437	2317	2541	3217	2919	1888	1606	6596
	300	2841	3355	2435	2097	5472	1815	1488	5314
ACT	0	2888	2894	2524	3538	3301	1513	1496	4037
	60	3627	2941	1927	2214	3246	1219	1239	5647
	120	2407	2088	3021	1734	3083	2764	2226	
	180	2150	1632	3330	2537	4942	1370	1424	3229
	240	3171	2572	2733	2230	4987	1653	1724	5640
	300	4922	4289	2978	1769	3053	2054	1677	4894

Table A.8. Concentration of activated EMVs (CD62e-PE$^+$) in EWC participants over time during completion of three experimental trials. Participants consumed 2 identical low-carbohydrate (LC) or high-carbohydrate (HC and ACT) meals at 0 and 180 mins and remained sedentary (LC, HC) or performed hourly 15-30s stair climbs (ACT). Concentrations are reported as events/µl and corrected for dilution factor.

Condition	Time (min)	Concentration of CD62e-PE$^+$ MVs (events/µl)							
		EWC 3	EWC 6	EWC 7	EWC 8	EWC 9	EWC10	EWC11	EWC 1
LC	0	2932	7451	2623	2342		2125	1916	1419
	60	2632	9482	2447	1361		1639	1028	1521
	120	1867	8252	2987	1780		2044	1110	3502
	180	448	12219	4930	1783		2192	997	998
	240	1995	6457	4747	1925		1510	991	748
	300	2031	5411	4813	1346		1789	1587	1996
HC	0	2547	17220	4117	2362	1794	2468	1314	908
	60	2459	11888	1832	1509	2267	2368	1464	843
	120	2709	7116	3119	2070	5048	2159	2636	1069
	180	2337	13427	1709	1722	2838	1176	1077	1260
	240	2876	9216	1189	2475	927	1649	1708	1258
	300	2624	11985	2337	1596	1729	3810	2509	1057
ACT	0	1823	3597	3255	2192	1816	1390	1400	963
	60	3680	2918	3958	1530	1309	1100	1790	1369
	120	2118	4544	6528	1651	1500	2046	1882	667
	180	1750	3354	3635	2208	4858	1322	1734	693
	240	2353	4821	2767	1536	4472	1711	1541	714
	300	2039	5620	1991	1226	2081	1307	1128	863

Table A.9. Concentration of PMVs (CD41-BV421^{+}) in EWC participants over time during completion of three experimental trials. Participants consumed 2 identical low-carbohydrate (LC) or high-carbohydrate (HC and ACT) meals at 0 and 180 mins and remained sedentary (LC, HC) or performed hourly 15-30s stair climbs (ACT). Concentrations are reported as events/μl and corrected for dilution factor.

Condition	Time (min)	Concentration of CD41-BV421^{+} MVs (events/μl)							
		EWC 3	EWC 6	EWC 7	EWC 8	EWC 9	EWC10	EWC11	EWC 1
LC	0	12844	14046	7033	10498		4835	6884	7554
	60	6765	13101	5871	5966		2579	5980	6830
	120	6883	15917	5025	6049		6788	5041	7680
	180	4031	20335	21000	4816		4244	5432	5210
	240	5327	13584	5276	6402		4323	5583	5473
	300	8123	12502	6137	5966		8691	7488	11170
HC	0	8839	11280	3518	7406	8865	8080	7511	5693
	60	4923	11633	3911	5506	12614	7183	4931	3299
	120	8087	11343	8513	10251	12474	1788	7554	4091
	180	3109	11398	3579	3541	10720	2622	4489	5677
	240	4942	12679	1768	8112	8464	2520	8618	11841
	300	6669	9786	5418	5720	11947	2681	10530	12694
ACT	0	7275	10459	8814	6123	9317	6250	7097	10948
	60	8931	5951	7599	4895	6094	3641	7122	4277
	120	3868	10537	21948	4661	6443	7013	20455	2843
	180	8909	4608	4961	5452	7457	3822	6932	3699
	240	5465	16990	5031	6725	11169	3391	3622	3944
	300	4985	12085	7558	4969	6128	3740	5126	2981

Table A.10. Concentration of apoptotic EMVs (CD31-APC^{+}/CD41-BV421^{-}) in EWC participants over time during completion of three experimental trials. Participants consumed 2 identical low-carbohydrate (LC) or high-carbohydrate (HC and ACT) meals at 0 and 180 mins and remained sedentary (LC, HC) or performed hourly 15-30s stair climbs (ACT). Concentrations are reported as events/µl and corrected for dilution factor.

Condition	Time (min)	Concentration of CD31-APC^{+}/CD41-BV421^{-} MVs (events/µl)							
		EWC 3	EWC 6	EWC 7	EWC 8	EWC 9	EWC10	EWC11	EWC 1
LC	0	312	161	208	611		195	309	110
	60	212	135	162	271		171	183	97
	120	158	126	222	422		196	153	183
	180	126	103	167	412		100	137	65
	240	148	160	248	512		263	108	143
	300	164	113	244	340		258	133	101
HC	0	263	165	188	525	147	163	209	95
	60	222	200	136	388	89	260	178	61
	120	209	194	127	428	141	257	236	68
	180	206	193	130	416	104	186	191	133
	240	169	127	156	377	75	265	232	56
	300	121	179	115	312	97	199	157	115
ACT	0	160	260	123	374	188	210	185	106
	60	263	141	175	362	144	172	127	73
	120	208	128	263	319	156	130	179	71
	180	171	104	119	338	91	207	168	65
	240	198	220	191	354	149	291	152	45
	300	197	144	128	200	137	182	190	87

Table A.11. Inter-assay coefficients of variation for each antibody averaged for each participant within each study cohort. CV was calculated from the baseline value of each condition.

Participant	CD45	CD66b	CD62e	CD4	$CD31^{+}/CD41^{-}$
HW3	12.4	25.5	18.7	73.4	84.2
HW2	43.2	9.5	28.6	30.0	17.3
HW5	9.6	43.3	22.1	57.1	42.5
HW6	41.6	25.7	16.7	13.2	46.6
HW9	16.8	33.0	42.2	28.9	25.3
HW11	16.2	7.0	12.1	45.7	96.5
HW13	18.7	22.8	15.6	47.7	27.8
HW14	15.4	4.5	19.2	89.7	93.1
HW4	2.8	27.0	20.1	29.6	25.9
HW1	32.8	25.4	17.2	118.5	93.9
HW10	26.9	26.9	24.2	30.0	36.6
EWC3	27.0	10.5	23.1	29.8	65.5
EWC6	31.9	53.8	74.5	15.8	42.9
EWC7	19.8	27.0	22.5	41.7	27.9
EWC8	17.5	23.0	4.0	28.1	26.4
EWC9	14.0	10.0	0.9	3.5	5.9
EWC10	21.2	20.4	27.6	25.5	8.5
EWC11	19.9	2.2	21.1	4.5	105.1
EWC1	21.1	27.0	25.5	33.0	117.6

Table A.12. Results of linear mixed model analysis of the effect of LC and HC conditions on concentrations of circulating LMVs ($CD45\text{-}PE^{+}$) in healthy weight individuals. The model included condition as the fixed effect, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
	1, 9.96	0.084	0.777
Model Summary	Deviance		BIC
	-17.123		20.419
Fixed Effects Estimates	Estimate		SE
Intercept	8.652		0.08
Condition	0.009		0.03
Random Effect Variance	Estimate		
Intercept	0.067		
Condition	0.007		
Residual Variance	0.036		

Table A.13. Results of linear mixed model analysis of the effect of HC and ACT conditions on concentrations of circulating LMVs (CD45-PE$^+$) in healthy weight individuals. The model included condition as the fixed effect, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
	1, 9.95	0.625	0.448
Model Summary	Deviance	BIC	
	-17.853	18.726	
Fixed Effects Estimates	Estimate	SE	
Intercept	8.622	0.091	
Condition	-0.039	0.049	
Random Effect Variance	Estimate		
Intercept	0.089		
Condition	0.024		
Residual Variance	0.032		

Table A.14. Results of linear mixed model analysis of the effect of LC and HC conditions on concentrations of circulating GMVs (CD66b-FitC$^+$) in healthy weight individuals. The model included condition as the fixed effect, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
	1, 10.20	0.984	0.344
Model Summary	Deviance	BIC	
	39.201	74.893	
Fixed Effects Estimates	Estimate	SE	
Intercept	8.577	0.143	
Condition	0.041	0.041	
Random Effect Variance	Estimate		
Intercept	0.22		
Condition	0.014		
Residual Variance	0.051		

Table A.15. Results of linear mixed model analysis of the effect of HC and ACT conditions on concentrations of circulating GMVs (CD66b-FitC^{+}) in healthy weight individuals. The model included condition as the fixed effect, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
	1, 9.81	1.191	0.301
Model Summary	Deviance		BIC
	14.522		50.892
Fixed Effects Estimates	Estimate		SE
Intercept	8.584		0.147
Condition	-0.032		0.03
Random Effect Variance	Estimate		
Intercept	0.234		
Condition	0.006		
Residual Variance	0.043		

Table A.16. Results of linear mixed model analysis of the effect of LC and HC conditions on concentrations of circulating activated EMVs (CD62e-PE^{+}) in healthy weight individuals. The model included condition as the fixed effect, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
	1, 10.09	2.787	0.126
Model Summary	Deviance		BIC
	-33.018		4.438
Fixed Effects Estimates	Estimate		SE
Intercept	7.218		0.102
Condition	-0.041		0.025
Random Effect Variance	Estimate		
Intercept	0.112		
Condition	0.004		
Residual Variance	0.031		

Table A.17. Results of linear mixed model analysis of the effect of HC and ACT conditions on concentrations of circulating activated EMVs (CD62e-PE^{+}) in healthy weight individuals. The model included condition as the fixed effect, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
	1, 9.58	2.00E-05	0.997
Model Summary	Deviance		BIC
	-20.584		16.844
Fixed Effects Estimates	Estimate		SE
Intercept	7.176		0.103
Condition	-1.14E-04		0.026
Random Effect Variance	Estimate		
Intercept	0.114		
Condition	0.004		
Residual Variance	0.034		

Table A.18. Results of linear mixed model analysis of the effect of LC and HC conditions on concentrations of circulating PMVs (CD41-BV421^{+}) in healthy weight individuals. The model included condition as the fixed effect, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
	1, 10.06	0.323	0.582
Model Summary	Deviance		BIC
	329.085		361.017
Fixed Effects Estimates	Estimate		SE
Intercept	9.348		0.311
Condition	-0.072		0.127
Random Effect Variance	Estimate		
Intercept	1.02		
Condition	0.133		
Residual Variance	0.529		

Table A.19. Results of linear mixed model analysis of the effect of HC and ACT conditions on concentrations of circulating PMVs (CD41-BV421^{+}) in healthy weight individuals. The model included condition as the fixed effect, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
	1, 10.20	0.797	0.393
Model Summary	Deviance		BIC
	313.126		346.238
Fixed Effects Estimates	Estimate		SE
Intercept	9.204		0.353
Condition	-0.068		0.077
Random Effect Variance	Estimate		
Intercept	1.327		
Condition	0.022		
Residual Variance	0.5		

Table A.20. Results of linear mixed model analysis of the effect of LC and HC conditions on concentrations of circulating apoptotic EMVs (CD31-APC^{+}/CD41-BV421^{-}) in healthy weight individuals. The model included condition as the fixed effect, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
	1, 9.99	0.356	0.564
Model Summary	Deviance		BIC
	284.203		319.072
Fixed Effects Estimates	Estimate		SE
Intercept	4.542		0.115
Condition	-0.047		0.079
Random Effect Variance	Estimate		
Intercept	0.104		
Condition	0.028		
Residual Variance	0.469		

Table A.21. Results of linear mixed model analysis of the effect of HC and ACT conditions on concentrations of circulating apoptotic EMVs (CD31-APC^{+}/CD41-BV421^{-}) in healthy weight individuals. The model included condition as the fixed effect, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
	1, 9.81	0.013	0.912
Model Summary	Deviance	BIC	
	273.834	308.414	
Fixed Effects Estimates	Estimate	SE	
Intercept	4.485	0.159	
Condition	-0.009	0.077	
Random Effect Variance	Estimate		
Intercept	0.244		
Condition	0.031		
Residual Variance	0.403		

Table A.22. Results of linear mixed model analysis of the effect of LC and HC conditions on concentrations of circulating LMVs (CD45-PE^{+}) in individuals with elevated waist circumference. The model included condition as the fixed effect, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
	1, 8.03	0.933	0.362
Model Summary	Deviance	BIC	
	-4.205	32.125	
Fixed Effects Estimates	Estimate	SE	
Intercept	8.818	0.061	
Condition	0.031	0.032	
Random Effect Variance	Estimate		
Intercept	0.025		
Condition	0.003		
Residual Variance	0.048		

Table A.23. Results of linear mixed model analysis of the effect of HC and ACT conditions on concentrations of circulating LMVs (CD45-PE$^+$) in individuals with elevated waist circumference. The model included condition as the fixed effect, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
	1, 7.00	5.397	0.053
Model Summary	Deviance	BIC	
	-2.543	34.522	
Fixed Effects Estimates	Estimate	SE	
Intercept	8.779	0.046	
Condition	-0.07	0.03	
Random Effect Variance	Estimate		
Intercept	0.013		
Condition	0.003		
Residual Variance	0.05		

Table A.24. Results of linear mixed model analysis of the effect of LC and HC conditions on concentrations of circulating GMVs (CD66b-FitC$^+$) in individuals with elevated waist circumference. The model included condition as the fixed effect, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
	1, 81.07	0.256	0.614
Model Summary	Deviance	BIC	
	22.599	56.824	
Fixed Effects Estimates	Estimate	SE	
Intercept	7.985	0.17	
Condition	0.013	0.025	
Random Effect Variance	Estimate		
Intercept	0.27		
Condition	1.40E-07		
Residual Variance	0.055		

Table A.25. Results of linear mixed model analysis of the effect of HC and ACT conditions on concentrations of circulating GMVs (CD66b-FitC^{+}) in individuals with elevated waist circumference. The model included condition as the fixed effect, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
	1, 6.88	3.166	0.119
Model Summary	Deviance	BIC	
	27.142	61.867	
Fixed Effects Estimates	Estimate	SE	
Intercept	7.936	0.147	
Condition	-0.062	0.035	
Random Effect Variance	Estimate		
Intercept	0.169		
Condition	0.005		
Residual Variance	0.058		

Table A.26. Results of linear mixed model analysis of the effect of LC and HC conditions on concentrations of circulating activated EMVs (CD62e-PE^{+}) in individuals with elevated waist circumference. The model included condition as the fixed effect, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
	1, 6.52	0.251	0.633
Model Summary	Deviance	BIC	
	109.587	141.426	
Fixed Effects Estimates	Estimate	SE	
Intercept	7.739	0.223	
Condition	0.033	0.065	
Random Effect Variance	Estimate		
Intercept	0.382		
Condition	0.019		
Residual Variance	0.138		

Table A.27. Results of linear mixed model analysis of the effect of HC and ACT conditions on concentrations of circulating activated EMVs (CD62e-PE$^+$) in individuals with elevated waist circumference. The model included condition as the fixed effect, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
	1, 7.00	1.482	0.263
Model Summary	Deviance	BIC	
	99.018	131.401	
Fixed Effects Estimates	Estimate	SE	
Intercept	7.68	0.199	
Condition	-0.091	0.075	
Random Effect Variance	Estimate		
Intercept	0.307		
Condition	0.036		
Residual Variance	0.112		

Table A.28. Results of linear mixed model analysis of the effect of LC and HC conditions on concentrations of circulating PMVs (CD41-BV421$^+$) in individuals with elevated waist circumference. The model included condition as the fixed effect, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
	1, 6.03	3.493	0.111
Model Summary	Deviance	BIC	
	100.716	134.363	
Fixed Effects Estimates	Estimate	SE	
Intercept	8.845	0.131	
Condition	-0.084	0.045	
Random Effect Variance	Estimate		
Intercept	0.123		
Condition	0.002		
Residual Variance	0.147		

Table A.29. Results of linear mixed model analysis of the effect of HC and ACT conditions on concentrations of circulating PMVs (CD41-BV421^{+}) in individuals with elevated waist circumference. The model included condition as the fixed effect, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
	1, 7.00	0.004	0.954
Model Summary	Deviance	BIC	
	122.831	156.71	
Fixed Effects Estimates	Estimate	SE	
Intercept	8.757	0.108	
Condition	-0.004	0.064	
Random Effect Variance	Estimate		
Intercept	0.078		
Condition	0.018		
Residual Variance	0.175		

Table A.30. Results of linear mixed model analysis of the effect of LC and HC conditions on concentrations of circulating apoptotic EMVs (CD31-APC^{+}/CD41-BV421^{-}) in individuals with elevated waist circumference. The model included condition as the fixed effect, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
	1, 6.25	0.016	0.902
Model Summary	Deviance	BIC	
	48.9	82.121	
Fixed Effects Estimates	Estimate	SE	
Intercept	5.146	0.154	
Condition	-0.006	0.047	
Random Effect Variance	Estimate		
Intercept	0.183		
Condition	0.01		
Residual Variance	0.071		

Table A.31. Results of linear mixed model analysis of the effect of HC and ACT conditions on concentrations of circulating apoptotic EMVs (CD31-APC^{+}/CD41-BV421^{-}) in individuals with elevated waist circumference. The model included condition as the fixed effect, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
	1, 7.00	0.511	0.498
Model Summary	Deviance		BIC
	32.227		66.77
Fixed Effects Estimates	Estimate		SE
Intercept	5.118		0.155
Condition	-0.022		0.031
Random Effect Variance	Estimate		
Intercept	0.188		
Condition	0.003		
Residual Variance	0.06		

Table A.32. Results of linear mixed model analysis of the effect of LC and HC conditions on concentrations of circulating LMVs (CD45-PE^{+}) in healthy weight individuals. The model included condition and time as the fixed effects, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
Condition	1,9.98	0.186	0.675
Time	5,15.99	3.671	0.021
Condition*Time	5,76.77	2.612	0.031

Model Summary	Deviance	BIC
	-57.442	202.233

Fixed Effects Estimates	Estimate	SE
Intercept	8.658	0.081
Condition_T(1)	0.013	0.03
Time_T (1)	0.086	0.037
Time_T (2)	0.081	0.051
Time_T (3)	-0.043	0.031
Time_T (4)	-0.1	0.038
Time_T (5)	-0.006	0.034
Condition_T (1) * Time_T (1)	-0.012	0.03
Condition_T (1) * Time_T (2)	0.08	0.033
Condition_T (1) * Time_T (3)	0.035	0.03
Condition_T (1) * Time_T (4)	0.02	0.03
Condition_T (1) * Time_T (5)	-0.065	0.031

Random Effect Variance	Estimate
Intercept	0.07
Condition_T(1)	0.008
Time_T(1)	0.005
Time_T(2)	0.016
Time_T(3)	9.241e -4
Time_T(4)	0.006
Time_T(5)	0.002
Residual Variance	0.024

Table A.33. Results of linear mixed model analysis of the effect of HC and ACT conditions on concentrations of circulating LMVs (CD45-PE^{+}) in healthy weight individuals. The model included condition and time as the fixed effects, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
Condition	1,9.94	0.656	0.437
Time	5,14.39	2.634	0.069
Condition*Time	5,77.35	2.843	0.021

Model Summary	Deviance	BIC
	-97.281	166.536

Fixed Effects Estimates	Estimate	SE
Intercept	8.624	0.091
Condition_T(1)	-0.04	0.05
Time_T (1)	0.082	0.05
Time_T (2)	0.069	0.053
Time_T (3)	-0.008	0.034
Time_T (4)	-0.065	0.038
Time_T (5)	0.009	0.029
Condition_T (1) * Time_T (1)	6.304e -4	0.022
Condition_T (1) * Time_T (2)	-0.055	0.024
Condition_T (1) * Time_T (3)	-0.008	0.023
Condition_T (1) * Time_T (4)	0.008	0.022
Condition_T (1) * Time_T (5)	0.073	0.022

Random Effect Variance	Estimate
Intercept	0.091
Condition_T(1)	0.026
Time_T(1)	0.022
Time_T(2)	0.024
Time_T(3)	0.007
Time_T(4)	0.01
Time_T(5)	0.004
Residual Variance	0.013

Table A.34. Results of linear mixed model analysis of the effect of LC and HC conditions on concentrations of circulating GMVs (CD66b-FitC^{+}) in healthy weight individuals. The model included condition and time as the fixed effects, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
Condition	1,10.21	0.941	0.354
Time	5,15.74	2.831	0.052
Condition*Time	5,75.03	2.047	0.082
Model Summary	Deviance	BIC	
	-4.935	*249.484*	
Fixed Effects Estimates	Estimate	SE	
Intercept	8.582	0.143	
Condition_T(1)	0.04	0.041	
Time_T (1)	0.125	0.052	
Time_T (2)	0.042	0.052	
Time_T (3)	-0.018	0.041	
Time_T (4)	-0.117	0.038	
Time_T (5)	-0.007	0.042	
Condition_T (1) * Time_T (1)	0.036	0.036	
Condition_T (1) * Time_T (2)	0.104	0.039	
Condition_T (1) * Time_T (3)	-0.024	0.036	
Condition_T (1) * Time_T (4)	-0.027	0.036	
Condition_T (1) * Time_T (5)	-0.032	0.036	
Random Effect Variance	Estimate		
Intercept	0.223		
Condition_T(1)	0.016		
Time_T(1)	0.016		
Time_T(2)	0.014		
Time_T(3)	0.005		
Time_T(4)	0.002		
Time_T(5)	0.005		
Residual Variance	0.033		

Table A.35. Results of linear mixed model analysis of the effect of HC and ACT conditions on concentrations of circulating GMVs (CD66b-FitC^{+}) in healthy weight individuals. The model included condition and time as the fixed effects, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
Condition	1,9.86	1.311	0.279
Time	5,17.06	3.219	0.032
Condition*Time	5,77.68	1.981	0.091

Model Summary	Deviance	BIC
	-27.329	*229.958*

Fixed Effects Estimates	Estimate	SE
Intercept	8.587	0.147
Condition_T(1)	-0.034	0.03
Time_T (1)	0.115	0.045
Time_T (2)	0.084	0.048
Time_T (3)	-0.045	0.04
Time_T (4)	-0.06	0.037
Time_T (5)	-0.007	0.035
Condition_T (1) * Time_T (1)	-0.046	0.033
Condition_T (1) * Time_T (2)	-0.059	0.035
Condition_T (1) * Time_T (3)	-0.003	0.034
Condition_T (1) * Time_T (4)	0.084	0.033
Condition_T (1) * Time_T (5)	0.03	0.033

Random Effect Variance	Estimate
Intercept	0.235
Condition_T(1)	0.007
Time_T(1)	0.01
Time_T(2)	0.011
Time_T(3)	0.005
Time_T(4)	0.003
Time_T(5)	0.001
Residual Variance	0.029

Table A.36. Results of linear mixed model analysis of the effect of LC and HC conditions on concentrations of circulating activated EMVs (CD62e-PE^{+}) in healthy weight individuals. The model included condition and time as the fixed effects, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
Condition	1,10.11	2.523	0.143
Time	5,16.61	2.589	0.065
Condition*Time	5, 79.96	1.061	0.389

Model Summary	Deviance	BIC
	-60.339	199.594

Fixed Effects Estimates	Estimate	SE
Intercept	7.223	0.103
Condition_T(1)	-0.039	0.025
Time_T (1)	0.039	0.038
Time_T (2)	0.094	0.049
Time_T (3)	0.007	0.032
Time_T (4)	0.006	0.036
Time_T (5)	-0.069	0.032
Condition_T (1) * Time_T (1)	0.004	0.03
Condition_T (1) * Time_T (2)	0.061	0.033
Condition_T (1) * Time_T (3)	0.012	0.03
Condition_T (1) * Time_T (4)	-0.038	0.03
Condition_T (1) * Time_T (5)	-0.034	0.031

Random Effect Variance	Estimate
Intercept	0.115
Condition_T(1)	0.005
Time_T(1)	0.006
Time_T(2)	0.015
Time_T(3)	0.002
Time_T(4)	0.004
Time_T(5)	0.001
Residual Variance	0.023

Table A.37. Results of linear mixed model analysis of the effect of HC and ACT conditions on concentrations of circulating activated EMVs (CD62e-PE^{+}) in healthy weight individuals. The model included condition and time as the fixed effects, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
Condition	1,9.73	0.004	0.949
Time	5,15.94	3.476	0.026
Condition*Time	5,77.44	0.682	0.638

Model Summary	Deviance	BIC
	-59.069	201.174

Fixed Effects Estimates	Estimate	SE
Intercept	7.178	0.104
Condition_T(1)	-0.002	0.026
Time_T (1)	0.023	0.051
Time_T (2)	0.11	0.042
Time_T (3)	0.033	0.044
Time_T (4)	-0.04	0.034
Time_T (5)	-0.056	0.032
Condition_T (1) * Time_T (1)	-0.023	0.029
Condition_T (1) * Time_T (2)	-0.028	0.03
Condition_T (1) * Time_T (3)	0.01	0.03
Condition_T (1) * Time_T (4)	-0.011	0.029
Condition_T (1) * Time_T (5)	0.043	0.029

Random Effect Variance	Estimate
Intercept	0.117
Condition_T(1)	0.005
Time_T(1)	0.019
Time_T(2)	0.009
Time_T(3)	0.012
Time_T(4)	0.003
Time_T(5)	0.002
Residual Variance	0.022

Table A.38. Results of linear mixed model analysis of the effect of LC and HC conditions on concentrations of circulating PMVs (CD41-BV421^{+}) in healthy weight individuals. The model included condition and time as the fixed effects, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
Condition	1,10.05	0.214	0.654
Time	5,13.47	1.746	0.191
Condition*Time	5,67.43	2.015	0.088

Model Summary	Deviance	BIC
	289.32	516.56

Fixed Effects Estimates	Estimate	SE
Intercept	9.36	0.313
Condition_T(1)	-0.057	0.124
Time_T (1)	-0.07	0.202
Time_T (2)	0.324	0.147
Time_T (3)	0.156	0.149
Time_T (4)	-0.281	0.152
Time_T (5)	-0.059	0.195
Condition_T (1) * Time_T (1)	-0.001	0.108
Condition_T (1) * Time_T (2)	0.298	0.117
Condition_T (1) * Time_T (3)	0.061	0.108
Condition_T (1) * Time_T (4)	-0.232	0.108
Condition_T (1) * Time_T (5)	-0.103	0.111

Random Effect Variance	Estimate
Intercept	1.049
Condition_T(1)	0.141
Time_T(1)	0.318
Time_T(2)	0.086
Time_T(3)	0.114
Time_T(4)	0.126
Time_T(5)	0.281
Residual Variance	0.307

Table A.39. Results of linear mixed model analysis of the effect of HC and ACT conditions on concentrations of circulating PMVs (CD41-BV421^{+}) in healthy weight individuals. The model included condition and time as the fixed effects, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
Condition	1,10.51	0.927	0.357
Time	5,15.41	2.296	0.096
Condition*Time	5,77.19	1.247	0.296

Model Summary	Deviance	BIC
	273.83	503.1

Fixed Effects Estimates	Estimate	SE
Intercept	9.224	0.354
Condition_T(1)	-0.078	0.081
Time_T (1)	-0.088	0.159
Time_T (2)	0.424	0.15
Time_T (3)	0.139	0.146
Time_T (4)	-0.354	0.177
Time_T (5)	-0.034	0.167
Condition_T (1) * Time_T (1)	-0.018	0.105
Condition_T (1) * Time_T (2)	-0.196	0.11
Condition_T (1) * Time_T (3)	-0.078	0.108
Condition_T (1) * Time_T (4)	0.158	0.105
Condition_T (1) * Time_T (5)	0.128	0.105

Random Effect Variance	Estimate
Intercept	1.352
Condition_T(1)	0.048
Time_T(1)	0.159
Time_T(2)	0.115
Time_T(3)	0.108
Time_T(4)	0.224
Time_T(5)	0.186
Residual Variance	0.287

Table A.40. Results of linear mixed model analysis of the effect of LC and HC conditions on concentrations of circulating apoptotic EMVs (CD31-APC^{+}/CD41-BV421^{-}) in healthy weight individuals. The model included condition and time as the fixed effects, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
Condition	1,10.33	0.214	0.653
Time	5,26.30	1.358	0.272
Condition*Time	5,95.66	1.646	0.155
Model Summary	Deviance	BIC	
	252.427	482.995	
Fixed Effects Estimates	Estimate	SE	
Intercept	4.55	0.111	
Condition_T(1)	-0.04	0.087	
Time_T (1)	0.273	0.131	
Time_T (2)	-0.004	0.195	
Time_T (3)	-0.138	0.152	
Time_T (4)	0.152	0.121	
Time_T (5)	-0.133	0.126	
Condition_T (1) * Time_T (1)	0.225	0.116	
Condition_T (1) * Time_T (2)	-0.198	0.126	
Condition_T (1) * Time_T (3)	-0.189	0.116	
Condition_T (1) * Time_T (4)	0.111	0.116	
Condition_T (1) * Time_T (5)	0.043	0.119	
Random Effect Variance	Estimate		
Intercept	0.104		
Condition_T(1)	0.053		
Time_T(1)	0.038		
Time_T(2)	0.243		
Time_T(3)	0.104		
Time_T(4)	0.011		
Time_T(5)	0.02		
Residual Variance	0.355		

Table A.41. Results of linear mixed model analysis of the effect of HC and ACT conditions on concentrations of circulating apoptotic EMVs (CD31-APC^{+}/CD41-BV421^{-}) in healthy weight individuals. The model included condition and time as the fixed effects, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
Condition	1,9.77	0.005	0.944
Time	5,21.38	0.539	0.744
Condition*Time	5,87.07	3.545	0.006

Model Summary	Deviance	BIC
	233.013	466.034

Fixed Effects Estimates	Estimate	SE
Intercept	4.471	0.161
Condition_T(1)	-0.006	0.078
Time_T (1)	0.237	0.205
Time_T (2)	-0.152	0.122
Time_T (3)	-0.063	0.125
Time_T (4)	0.126	0.113
Time_T (5)	-0.048	0.13
Condition_T (1) * Time_T (1)	-0.294	0.099
Condition_T (1) * Time_T (2)	0.212	0.104
Condition_T (1) * Time_T (3)	0.231	0.101
Condition_T (1) * Time_T (4)	-0.169	0.099
Condition_T (1) * Time_T (5)	0.01	0.099

Random Effect Variance	Estimate
Intercept	0.264
Condition_T(1)	0.045
Time_T(1)	0.357
Time_T(2)	0.045
Time_T(3)	0.061
Time_T(4)	0.034
Time_T(5)	0.08
Residual Variance	0.255

Table A.42. Results of linear mixed model analysis of the effect of LC and HC conditions on concentrations of circulating LMVs (CD45-PE^{+}) in individuals with elevated waist circumference. The model included condition and time as the fixed effects, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
Condition	1,6.62	0.72	0.426
Time	5,10.32	3.405	0.045
Condition*Time	5,52.12	5.919	<0.001

Model Summary	Deviance	BIC
	-56.364	186.517

Fixed Effects Estimates	Estimate	SE
Intercept	8.825	0.058
Condition_T(1)	0.024	0.028
Time_T (1)	0.154	0.042
Time_T (2)	-0.034	0.05
Time_T (3)	0.003	0.039
Time_T (4)	-3.705e -4	0.048
Time_T (5)	-0.129	0.082
Condition_T (1) * Time_T (1)	-0.04	0.036
Condition_T (1) * Time_T (2)	-7.807e -4	0.036
Condition_T (1) * Time_T (3)	-0.079	0.036
Condition_T (1) * Time_T (4)	0.017	0.036
Condition_T (1) * Time_T (5)	0.183	0.037

Random Effect Variance	Estimate
Intercept	0.024
Condition_T(1)	0.004
Time_T(1)	0.003
Time_T(2)	0.01
Time_T(3)	0.002
Time_T(4)	0.008
Time_T(5)	0.043
Residual Variance	0.023

Table A.43. Results of linear mixed model analysis of the effect of HC and ACT conditions on concentrations of circulating LMVs (CD45-PE^{+}) in individuals with elevated waist circumference. The model included condition and time as the fixed effects, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
Condition	1,7.24	5.091	0.057
Time	5,9.78	2.318	0.123
Condition*Time	5,56.00	2.017	0.09
Model Summary	Deviance	BIC	
	-47.087	197.592	
Fixed Effects Estimates	Estimate	SE	
Intercept	8.779	0.046	
Condition_T(1)	-0.07	0.031	
Time_T (1)	0.179	0.058	
Time_T (2)	-0.039	0.051	
Time_T (3)	-0.012	0.065	
Time_T (4)	0.006	0.044	
Time_T (5)	-0.032	0.063	
Condition_T (1) * Time_T (1)	0.064	0.037	
Condition_T (1) * Time_T (2)	-0.004	0.037	
Condition_T (1) * Time_T (3)	0.064	0.037	
Condition_T (1) * Time_T (4)	-0.011	0.037	
Condition_T (1) * Time_T (5)	-0.086	0.037	
Random Effect Variance	Estimate		
Intercept	0.015		
Condition_T(1)	0.006		
Time_T(1)	0.016		
Time_T(2)	0.01		
Time_T(3)	0.023		
Time_T(4)	0.004		
Time_T(5)	0.021		
Residual Variance	0.026		

Table A.44. Results of linear mixed model analysis of the effect of LC and HC conditions on concentrations of circulating GMVs (CD66b-FitC^{+}) in individuals with elevated waist circumference. The model included condition and time as the fixed effects, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
Condition	1, 6.10	0.056	0.821
Time	5, 8.72	2.321	0.131
Condition*Time	5, 48.89	0.494	0.779

Model Summary	Deviance	BIC
	-19.125	218.979

Fixed Effects Estimates	Estimate	SE
Intercept	7.992	0.172
Condition_T(1)	0.006	0.026
Time_T (1)	0.144	0.071
Time_T (2)	-0.068	0.047
Time_T (3)	-0.005	0.05
Time_T (4)	0.085	0.06
Time_T (5)	-0.094	0.073
Condition_T (1) * Time_T (1)	-0.012	0.039
Condition_T (1) * Time_T (2)	-0.038	0.039
Condition_T (1) * Time_T (3)	0.036	0.039
Condition_T (1) * Time_T (4)	-0.023	0.039
Condition_T (1) * Time_T (5)	0.034	0.039

Random Effect Variance	Estimate
Intercept	0.235
Condition_T(1)	0.003
Time_T(1)	0.028
Time_T(2)	0.006
Time_T(3)	0.008
Time_T(4)	0.017
Time_T(5)	0.03
Residual Variance	0.027

Table A.45. Results of linear mixed model analysis of the effect of HC and ACT conditions on concentrations of circulating GMVs (CD66b-FitC^{+}) in individuals with elevated waist circumference. The model included condition and time as the fixed effects, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
Condition	1,7.33	2.917	0.13
Time	5,11.86	0.686	0.643
Condition*Time	5,54.97	1.656	0.161

Model Summary	Deviance	BIC
	9.222	246.314

Fixed Effects Estimates	Estimate	SE
Intercept	7.936	0.147
Condition_T(1)	-0.062	0.036
Time_T (1)	0.065	0.067
Time_T (2)	-0.089	0.054
Time_T (3)	0.018	0.06
Time_T (4)	-0.025	0.056
Time_T (5)	0.004	0.054
Condition_T (1) * Time_T (1)	-0.067	0.05
Condition_T (1) * Time_T (2)	0.016	0.05
Condition_T (1) * Time_T (3)	-0.013	0.051
Condition_T (1) * Time_T (4)	-0.087	0.05
Condition_T (1) * Time_T (5)	0.065	0.05

Random Effect Variance	Estimate
Intercept	0.17
Condition_T(1)	0.007
Time_T(1)	0.017
Time_T(2)	0.004
Time_T(3)	0.008
Time_T(4)	0.005
Time_T(5)	0.003
Residual Variance	0.047

Table A.46. Results of linear mixed model analysis of the effect of LC and HC conditions on concentrations of circulating activated EMVs (CD62e-PE^{+}) in individuals with elevated waist circumference. The model included condition and time as the fixed effects, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
Condition	1,4.66	2.70E-04	0.988
Time	5,10.92	1.847	0.185
Condition*Time	5,55.32	0.032	0.999

Model Summary	Deviance	BIC
	82.714	307.148

Fixed Effects Estimates	Estimate	SE
Intercept	7.773	0.223
Condition_T(1)	-0.001	0.073
Time_T (1)	0.108	0.085
Time_T (2)	-0.064	0.083
Time_T (3)	0.159	0.109
Time_T (4)	-0.103	0.126
Time_T (5)	-0.166	0.106
Condition_T (1) * Time_T (1)	-0.009	0.075
Condition_T (1) * Time_T (2)	-0.014	0.075
Condition_T (1) * Time_T (3)	0.009	0.075
Condition_T (1) * Time_T (4)	-0.006	0.076
Condition_T (1) * Time_T (5)	-0.006	0.075

Random Effect Variance	Estimate
Intercept	0.387
Condition_T(1)	0.032
Time_T(1)	0.013
Time_T(2)	0.011
Time_T(3)	0.05
Time_T(4)	0.082
Time_T(5)	0.044
Residual Variance	0.1

Table A.47. Results of linear mixed model analysis of the effect of HC and ACT conditions on concentrations of circulating activated EMVs (CD62e-PE^{+}) in individuals with elevated waist circumference. The model included condition and time as the fixed effects, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
Condition	1,7.07	1.43	0.27
Time	5,13.03	0.392	0.846
Condition*Time	5,63.00	1.596	0.174
Model Summary	Deviance	BIC	
	77.406	306.523	
Fixed Effects Estimates	Estimate	SE	
Intercept	7.68	0.199	
Condition_T(1)	-0.091	0.076	
Time_T (1)	0.026	0.074	
Time_T (2)	-0.04	0.074	
Time_T (3)	0.117	0.093	
Time_T (4)	-0.026	0.092	
Time_T (5)	-0.051	0.08	
Condition_T (1) * Time_T (1)	-0.074	0.068	
Condition_T (1) * Time_T (2)	0.038	0.068	
Condition_T (1) * Time_T (3)	-0.051	0.068	
Condition_T (1) * Time_T (4)	0.083	0.068	
Condition_T (1) * Time_T (5)	0.121	0.068	
Random Effect Variance	Estimate		
Intercept	0.309		
Condition_T(1)	0.039		
Time_T(1)	0.006		
Time_T(2)	0.006		
Time_T(3)	0.033		
Time_T(4)	0.03		
Time_T(5)	0.014		
Residual Variance	0.089		

Table A.48. Results of linear mixed model analysis of the effect of LC and HC conditions on concentrations of circulating PMVs (CD41-BV421^{+}) in individuals with elevated waist circumference. The model included condition and time as the fixed effects, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
Condition	1,8.90	3.778	0.084
Time	5,11.59	1.365	0.306
Condition*Time	5,58.51	0.956	0.452

Model Summary	Deviance	BIC
	71.171	297.123

Fixed Effects Estimates	Estimate	SE
Intercept	8.853	0.135
Condition_T(1)	-0.092	0.047
Time_T (1)	0.139	0.105
Time_T (2)	-0.102	0.077
Time_T (3)	0.042	0.079
Time_T (4)	-0.116	0.133
Time_T (5)	-0.098	0.102
Condition_T (1) * Time_T (1)	-0.006	0.074
Condition_T (1) * Time_T (2)	0.048	0.073
Condition_T (1) * Time_T (3)	0.047	0.073
Condition_T (1) * Time_T (4)	-0.154	0.074
Condition_T (1) * Time_T (5)	0.055	0.073

Random Effect Variance	Estimate
Intercept	0.136
Condition_T(1)	0.009
Time_T(1)	0.044
Time_T(2)	0.005
Time_T(3)	0.007
Time_T(4)	0.097
Time_T(5)	0.041
Residual Variance	0.096

Table A.49. Results of linear mixed model analysis of the effect of HC and ACT conditions on concentrations of circulating PMVs (CD41-BV421^{+}) in individuals with elevated waist circumference. The model included condition and time as the fixed effects, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
Condition	1,7.30	0.003	0.956
Time	5,14.30	2.705	0.064
Condition*Time	5,70.01	0.915	0.476
Model Summary	Deviance	BIC	
	81.654	310.24	
Fixed Effects Estimates	Estimate	SE	
Intercept	8.757	0.108	
Condition_T(1)	-0.004	0.068	
Time_T (1)	0.19	0.102	
Time_T (2)	-0.067	0.083	
Time_T (3)	0.135	0.15	
Time_T (4)	-0.207	0.077	
Time_T (5)	-0.048	0.132	
Condition_T (1) * Time_T (1)	0.056	0.075	
Condition_T (1) * Time_T (2)	-0.014	0.075	
Condition_T (1) * Time_T (3)	0.046	0.075	
Condition_T (1) * Time_T (4)	0.063	0.075	
Condition_T (1) * Time_T (5)	-0.006	0.075	
Random Effect Variance	Estimate		
Intercept	0.084		
Condition_T(1)	0.027		
Time_T(1)	0.039		
Time_T(2)	0.01		
Time_T(3)	0.134		
Time_T(4)	0.002		
Time_T(5)	0.095		
Residual Variance	0.108		

Table A.50. Results of linear mixed model analysis of the effect of LC and HC conditions on concentrations of circulating apoptotic EMVs (CD31-APC^{+}/CD41-BV421^{-}) in individuals with elevated waist circumference. The model included condition and time as the fixed effects, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
Condition	1,6.59	0.027	0.873
Time	5,11.84	4.302	0.018
Condition*Time	5,58.66	3.443	0.009
Model Summary	Deviance	BIC	
	3.598	238.578	
Fixed Effects Estimates	Estimate	SE	
Intercept	5.148	0.153	
Condition_T(1)	-0.008	0.047	
Time_T (1)	0.202	0.074	
Time_T (2)	-0.056	0.062	
Time_T (3)	0.069	0.048	
Time_T (4)	-0.124	0.054	
Time_T (5)	-0.016	0.069	
Condition_T (1) * Time_T (1)	-0.071	0.047	
Condition_T (1) * Time_T (2)	0.028	0.047	
Condition_T (1) * Time_T (3)	0.002	0.046	
Condition_T (1) * Time_T (4)	0.171	0.046	
Condition_T (1) * Time_T (5)	-0.083	0.046	
Random Effect Variance	Estimate		
Intercept	0.184		
Condition_T(1)	0.013		
Time_T(1)	0.027		
Time_T(2)	0.013		
Time_T(3)	0.002		
Time_T(4)	0.007		
Time_T(5)	0.021		
Residual Variance	0.038		

Table A.51. Results of linear mixed model analysis of the effect of HC and ACT conditions on concentrations of circulating apoptotic EMVs (CD31-APC^{+}/CD41-BV421^{-}) in individuals with elevated waist circumference. The model included condition and time as the fixed effects, participant as a random effect, and employed type III sum of squares and Satterthwaite approximation. Results are those of the maximum random effects structure of the model and estimate values are from natural log transformed data.

ANOVA Summary	df	F	p
Condition	1,7.53	0.445	0.525
Time	5,11.35	1.794	0.192
Condition*Time	5,63.00	1.69	0.15
Model Summary	Deviance	BIC	
	-8.042	231.756	
Fixed Effects Estimates	Estimate	SE	
Intercept	5.118	0.155	
Condition_T(1)	-0.022	0.033	
Time_T (1)	0.132	0.056	
Time_T (2)	-0.01	0.051	
Time_T (3)	0.043	0.053	
Time_T (4)	-0.055	0.057	
Time_T (5)	-0.016	0.079	
Condition_T (1) * Time_T (1)	0.001	0.043	
Condition_T (1) * Time_T (2)	0.017	0.043	
Condition_T (1) * Time_T (3)	-0.028	0.043	
Condition_T (1) * Time_T (4)	-0.102	0.043	
Condition_T (1) * Time_T (5)	0.083	0.043	
Random Effect Variance	Estimate		
Intercept	0.19		
Condition_T(1)	0.006		
Time_T(1)	0.01		
Time_T(2)	0.006		
Time_T(3)	0.008		
Time_T(4)	0.011		
Time_T(5)	0.035		
Residual Variance	0.036		

Table A.52. Results of post-hoc pairwise contrasts of time points within and across HC and ACT conditions on concentrations of circulating LMVs (CD45-PE^{+}) in healthy weight individuals following a significant interaction of condition and time in linear mixed model analysis. Values are from natural log transformed data and p-values reported are calculated using Bonferroni corrections. Significance was set at $p < 0.05$.

Contrast	Estimate	SE	df	t	p*	Bonferroni corrected p
HC 0, HC 60	-0.042	0.097	14.33	-0.436	0.669	10.704
HC 0, HC 120	0.082	0.083	14.799	0.99	0.338	5.408
HC 0, HC 180	0.155	0.067	18.802	2.318	0.032	0.512
HC 0, HC 240	0.145	0.075	16.22	1.929	0.071	1.136
HC 0, HC 300	0.149	0.07	17.492	2.142	0.047	0.752
ACT 0, ACT 60	0.069	0.094	13.195	0.732	0.477	7.632
ACT 0, ACT 120	0.098	0.084	15.407	1.175	0.258	4.128
ACT 0, ACT 180	0.139	0.067	18.802	2.086	0.051	0.816
ACT 0, ACT 240	7.845e -14	0.075	16.22	1.041e -12	1	16
ACT 0, ACT 300	0.19	0.07	17.492	2.73	0.014	0.224
ACT 0, HC 0	-0.079	0.109	14.264	-0.728	0.478	7.648
ACT 60, HC 60	-0.19	0.111	15.232	-1.721	0.105	1.68
ACT 120, HC 120	-0.095	0.109	14.621	-0.873	0.397	6.352
ACT 180, HC 180	-0.064	0.109	14.264	-0.586	0.567	9.072
ACT 240, HC 240	0.066	0.109	14.264	0.611	0.551	8.816
ACT 300, HC 300	-0.12	0.109	14.264	-1.104	0.288	4.608

p* are unadjusted p values

Table A.53. Results of post-hoc pairwise contrasts of time points within and across HC and ACT conditions on concentrations of circulating apoptotic EMVs (CD31-APC^{+}/CD41-BV421^{-}) in healthy weight individuals following a significant interaction of condition and time in linear mixed model analysis. Values are from natural log transformed data and p-values reported are calculated using Bonferroni corrections. Significance was set at $p < 0.05$.

Contrast	Estimate	SE	df	t	p*	Bonferroni corrected p
HC 0, HC 60	0.895	0.333	16.206	2.691	0.016	0.256
HC 0, HC 120	0.825	0.329	14.205	2.508	0.025	0.4
HC 0, HC 180	0.235	0.256	28.423	0.921	0.365	5.84
HC 0, HC 240	0.589	0.334	16.24	1.765	0.096	1.536
HC 0, HC 300	0.642	0.303	18.749	2.115	0.048	0.768
ACT 0, ACT 60	-0.117	0.323	14.616	-0.363	0.722	11.552
ACT 0, ACT 120	-0.226	0.334	14.879	-0.678	0.508	8.128
ACT 0, ACT 180	-0.015	0.256	28.423	-0.057	0.955	15.28
ACT 0, ACT 240	-0.019	0.334	16.24	-0.057	0.955	15.28
ACT 0, ACT 300	0.031	0.303	18.749	0.102	0.92	14.72
ACT 0, HC 0	-0.6	0.25	50.617	-2.399	0.02	0.32
ACT 60, HC 60	0.412	0.263	56.56	1.57	0.122	1.952
ACT 120, HC 120	0.452	0.256	53.159	1.764	0.083	1.328
ACT 180, HC 180	-0.35	0.25	50.617	-1.399	0.168	2.688
ACT 240, HC 240	0.008	0.25	50.617	0.033	0.974	15.584
ACT 300, HC 300	0.011	0.25	50.617	0.044	0.965	15.44

p* are unadjusted p values

Table A.54. Results of post-hoc pairwise contrasts of time points within and across LC and HC conditions on concentrations of circulating LMVs (CD45-PE$^+$) in healthy weight individuals following a significant interaction of condition and time in linear mixed model analysis. Values are from natural log transformed data and p-values reported are calculated using Bonferroni corrections.. Significance was set at $p < 0.05$.

Contrast	Estimate	SE	df	t	p*	Bonferroni corrected p
HC 0, HC 60	-0.087	0.088	22.554	-0.99	0.333	5.328
HC 0, HC 120	0.082	0.072	28.972	1.143	0.262	4.192
HC 0, HC 180	0.155	0.07	41.175	2.193	0.034	0.544
HC 0, HC 240	0.145	0.07	41.152	2.075	0.044	0.704
HC 0, HC 300	0.149	0.08	25.03	1.868	0.073	1.168
LC 0, LC 60	0.097	0.086	21.159	1.133	0.27	4.32
LC 0, LC 120	0.176	0.072	28.972	2.463	0.02	0.32
LC 0, LC 180	0.218	0.07	41.175	3.096	0.004	0.064
LC 0, LC 240	0.039	0.072	43.526	0.549	0.586	9.376
LC 0, LC 300	0.059	0.08	25.03	0.74	0.466	7.456
HC 0, LC 0	0.002	0.085	34.14	0.021	0.983	15.728
HC 60, LC 60	0.186	0.091	41.807	2.036	0.048	0.768
HC 120, LC 120	0.096	0.085	34.14	1.132	0.266	4.256
HC 180, LC 180	0.065	0.085	34.14	0.769	0.447	7.152
HC 240, LC 240	-0.104	0.087	35.946	-1.202	0.237	3.792
HC 300, LC 300	-0.088	0.085	34.14	-1.036	0.308	4.928

p* are unadjusted p values

Table A.55. Results of post-hoc pairwise contrasts of time points on concentrations of circulating LMVs (CD45-PE$^+$) in healthy weight individuals following a significant main effect of time in linear mixed model analysis of HC and ACT conditions. Values are from natural log transformed data and p-values reported are calculated using Bonferroni corrections. Significance was set at $p < 0.05$.

Contrast	Estimate	SE	df	t	Bonferroni corrected p
0, 60 mins	0.031	0.077	10.845	0.403	1.000
0, 120 mins	0.161	0.063	12.315	2.550	0.125
0, 180 mins	0.176	0.059	19.673	2.970	0.038
0, 240 mins	0.123	0.063	15.873	1.949	0.346
0, 300 mins	0.202	0.072	10.863	2.810	0.086

Table A.56. Results of post-hoc pairwise contrasts of time points on concentrations of circulating activated EMVs (CD62e-PE$^+$) in healthy weight individuals following a significant main effect of time in linear mixed model analysis of HC and ACT conditions. Values are from natural log transformed data and p-values reported are calculated using Bonferroni corrections. Significance was set at $p < 0.05$.

Contrast	Estimate	SE	df	t	Bonferroni corrected p
0, 60 mins	-0.087	0.084	10.314	-1.031	1.000
0, 120 mins	-0.010	0.084	10.460	-0.120	1.000
0, 180 mins	0.064	0.055	14.853	1.160	1.000
0, 240 mins	0.080	0.064	12.073	1.249	1.000
0, 300 mins	0.092	0.063	10.234	1.454	0.879

Table A.57. Results of post-hoc pairwise contrasts of time points within and across LC and HC conditions on concentrations of circulating LMVs (CD45-PE$^+$) in individuals with elevated waist circumference following a significant interaction of condition and time in linear mixed model analysis. P-value reported is calculated using Bonferroni corrections. Significance was set at $p < 0.05$.

Contrast	Estimate	SE	df	t	p*	Bonferroni corrected p
HC 0, HC 60	0.149	0.089	20.585	1.669	0.11	1.76
HC 0, HC 120	0.19	0.078	45.594	2.451	0.018	0.288
HC 0, HC 180	0.097	0.09	15.073	1.078	0.298	4.768
HC 0, HC 240	0.06	0.105	12.607	0.574	0.576	9.216
HC 0, HC 300	0.19	0.096	13.779	1.984	0.068	1.088
LC 0, LC 60	0.227	0.094	22.895	2.404	0.025	0.4
LC 0, LC 120	0.112	0.083	46.678	1.348	0.184	2.944
LC 0, LC 180	0.212	0.096	16.8	2.207	0.042	0.672
LC 0, LC 240	0.505	0.11	14.214	4.609	0.001	0.016
LC 0, LC 300	0.108	0.101	15.623	1.074	0.299	4.784
HC 0, LC 0	-0.031	0.092	34.07	-0.341	0.735	11.76
HC 60, LC 60	0.047	0.093	32.914	0.501	0.62	9.92
HC 120, LC 120	-0.11	0.092	33.507	-1.191	0.242	3.872
HC 180, LC 180	0.083	0.093	32.934	0.893	0.378	6.048
HC 240, LC 240	0.414	0.092	35.106	4.509	0.001	0.016
HC 300, LC 300	-0.113	0.093	32.83	-1.222	0.23	3.68

p* are unadjusted p values

Table A.58. Results of post-hoc pairwise contrasts of time points within and across LC and HC conditions on concentrations of circulating apoptotic EMVs (CD31-APC^{+}/CD41-BV421^{-}) in individuals with elevated waist circumference following a significant interaction of condition and time in linear mixed model analysis. Values are from natural log transformed data and p-values reported are calculated using Bonferroni corrections. Significance was set at $p < 0.05$.

Contrast	Estimate	SE	df	t	p*	Bonferroni corrected p
HC 0, HC 60	0.159	0.121	16.138	1.313	0.208	3.328
HC 0, HC 120	0.06	0.118	18.561	0.51	0.616	9.856
HC 0, HC 180	0.084	0.103	33.051	0.81	0.424	6.784
HC 0, HC 240	0.23	0.14	11.782	1.648	0.126	2.016
HC 0, HC 300	0.254	0.141	12.305	1.799	0.097	1.552
LC 0, LC 60	0.356	0.128	17.942	2.782	0.012	0.192
LC 0, LC 120	0.206	0.124	20.779	1.657	0.113	1.808
LC 0, LC 180	0.567	0.11	35.607	5.157	0.001	0.016
LC 0, LC 240	0.205	0.146	13.332	1.403	0.183	2.928
LC 0, LC 300	0.301	0.148	13.88	2.043	0.06	0.96
HC 0, LC 0	-0.157	0.132	22.932	-1.186	0.248	3.968
HC 60, LC 60	0.04	0.13	23.145	0.309	0.76	12.16
HC 120, LC 120	-0.011	0.131	22.65	-0.087	0.932	14.912
HC 180, LC 180	0.327	0.132	22.917	2.469	0.021	0.336
HC 240, LC 240	-0.182	0.133	23.115	-1.369	0.184	2.944
HC 300, LC 300	-0.109	0.132	22.976	-0.83	0.415	6.64

p* are unadjusted p values

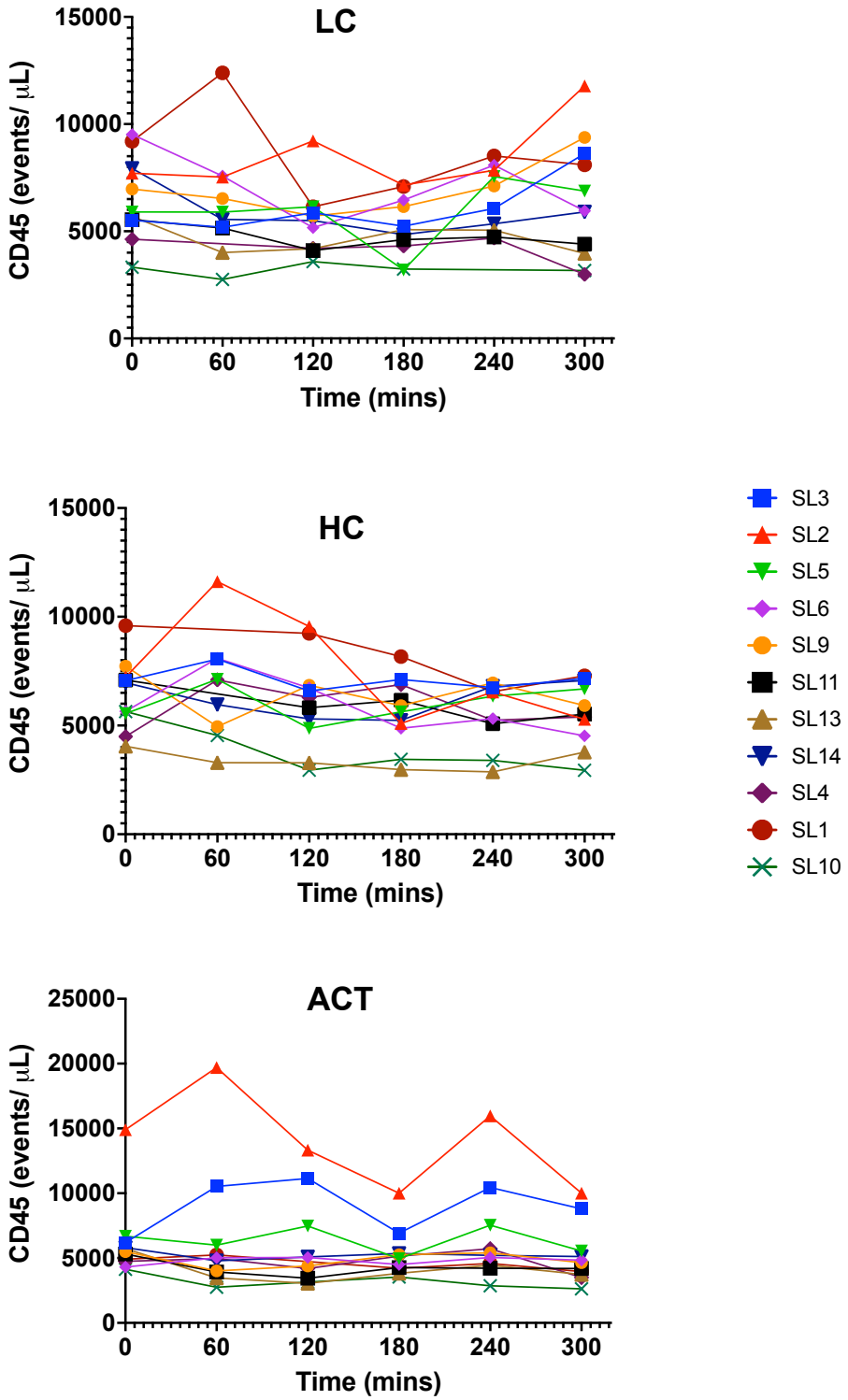

Figure A.1. Concentration of circulating LMVs (CD45-PE^{+}) in different healthy weight individuals over time under three experimental conditions. Participants consumed 2 identical low-carbohydrate (LC) or high-carbohydrate (HC and ACT) meals at 0 and 180 mins and remained sedentary (LC, HC) or performed hourly 15-30s stair climbs (ACT).

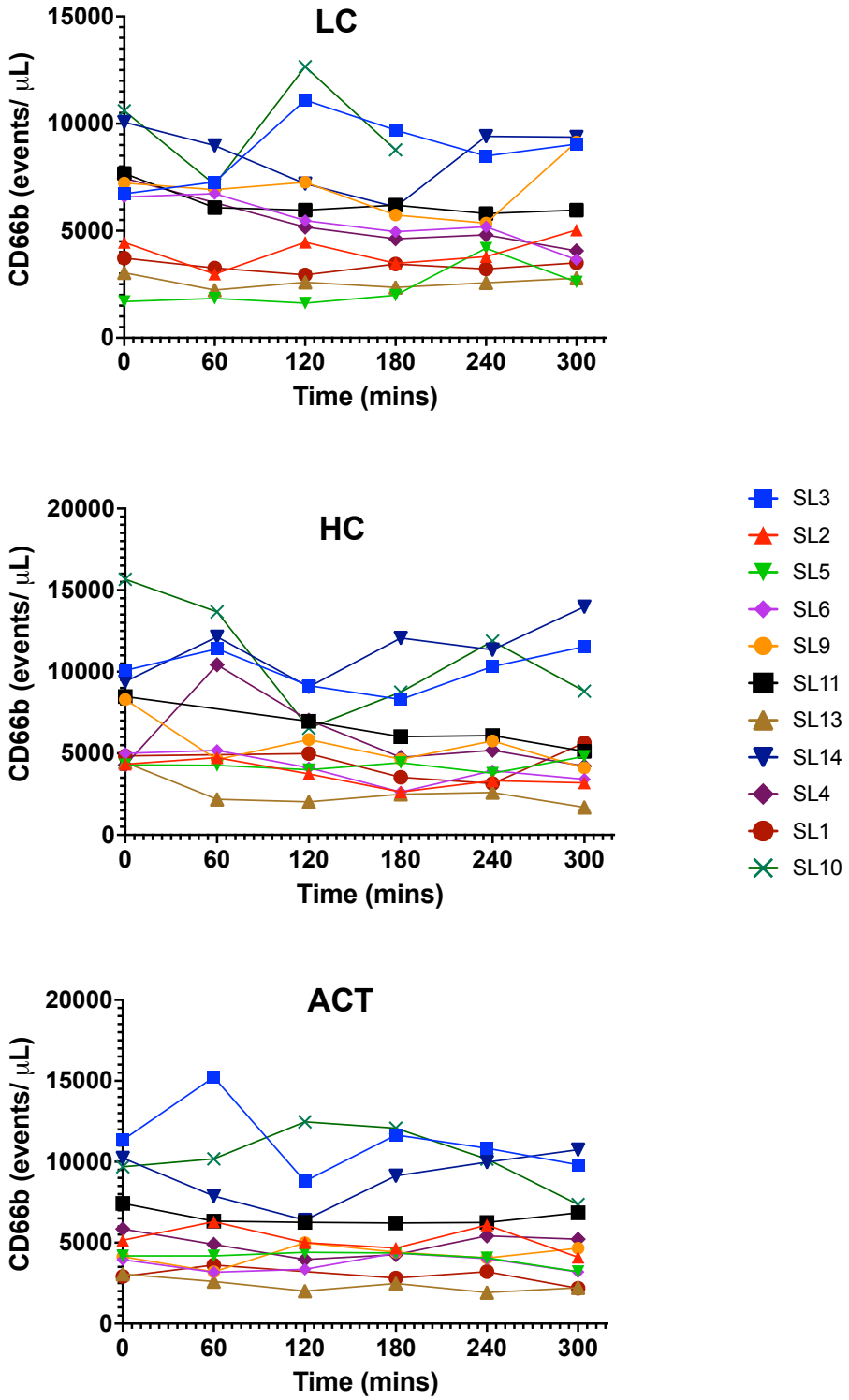

Figure A.2. Concentration of circulating GMVs (CD66b-FitC^{+}) in different healthy weight individuals over time under three experimental conditions. Participants consumed 2 identical low-carbohydrate (LC) or high-carbohydrate (HC and ACT) meals at 0 and 180 mins and remained sedentary (LC, HC) or performed hourly 15-30s stair climbs (ACT).

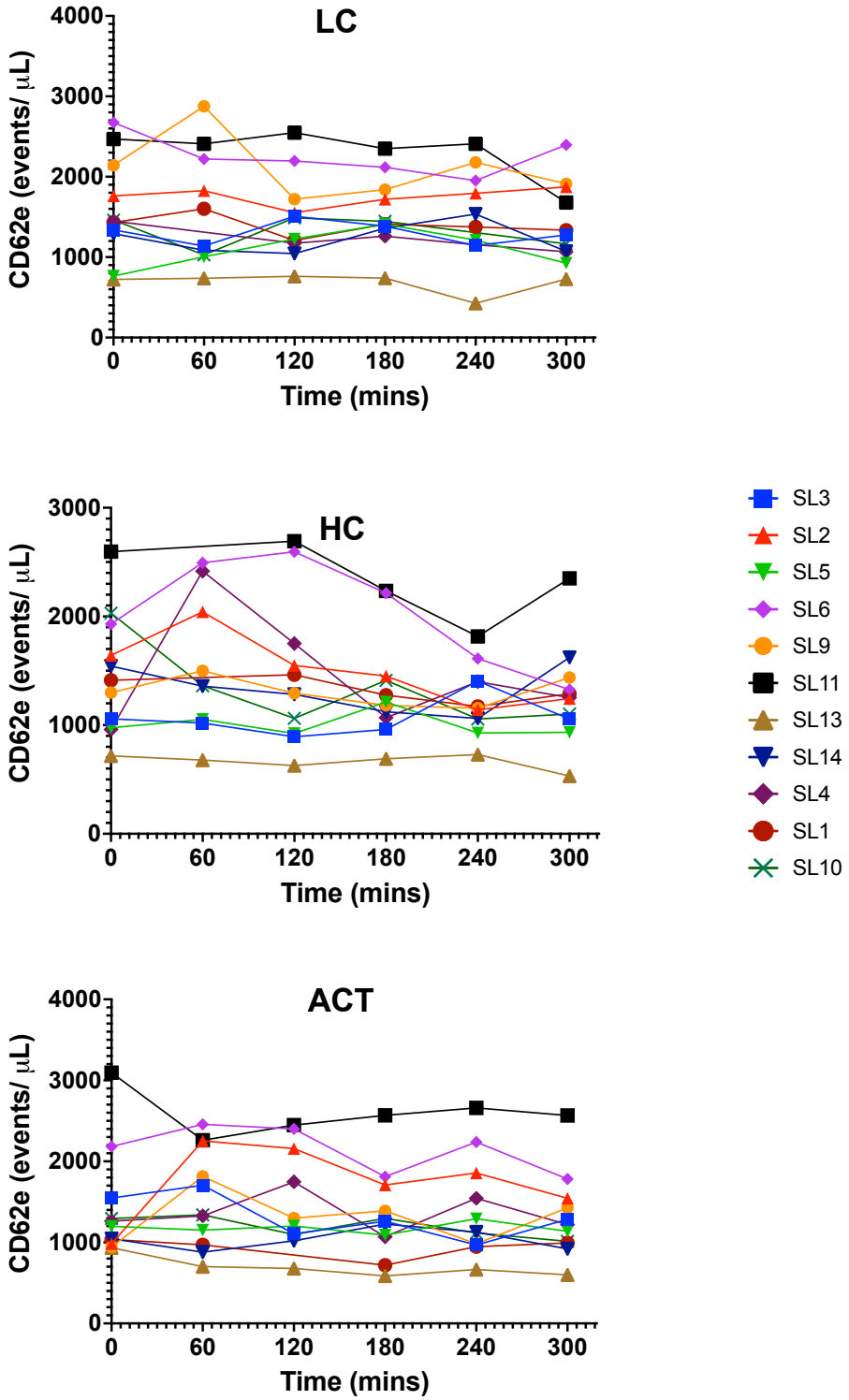

Figure A.3. Concentration of circulating activated EMVs (CD62e-PE^{+}) in different healthy weight individuals over time under three experimental conditions. Participants consumed 2 identical low-carbohydrate (LC) or high-carbohydrate (HC and ACT) meals at 0 and 180 mins and remained sedentary (LC, HC) or performed hourly 15-30s stair climbs (ACT).

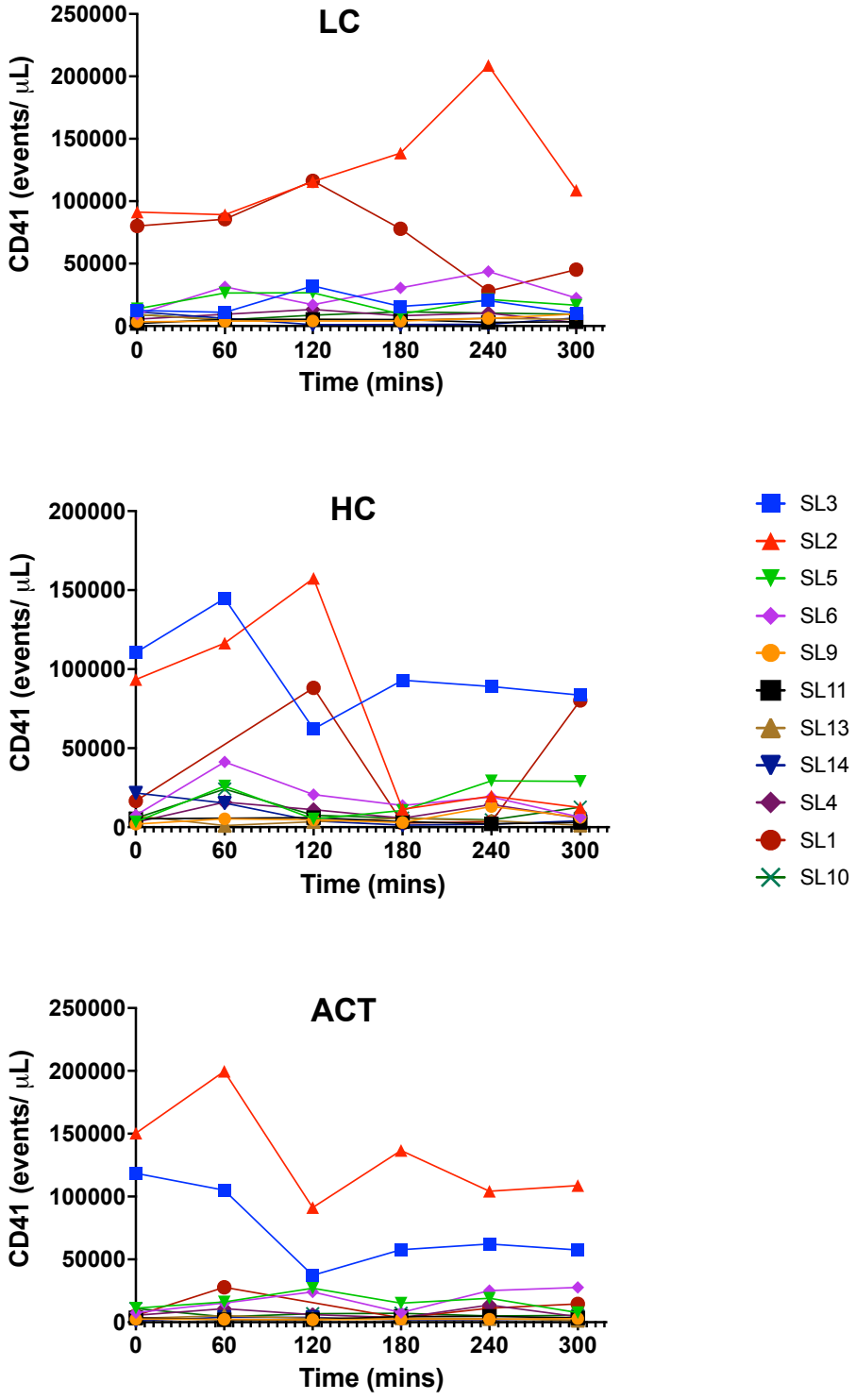

Figure A.4. Concentration of circulating PMVs (CD41-BV421^{+}) in different healthy weight individuals over time under three experimental conditions. Participants consumed 2 identical low-carbohydrate (LC) or high-carbohydrate (HC and ACT) meals at 0 and 180 mins and remained sedentary (LC, HC) or performed hourly 15-30s stair climbs (ACT).

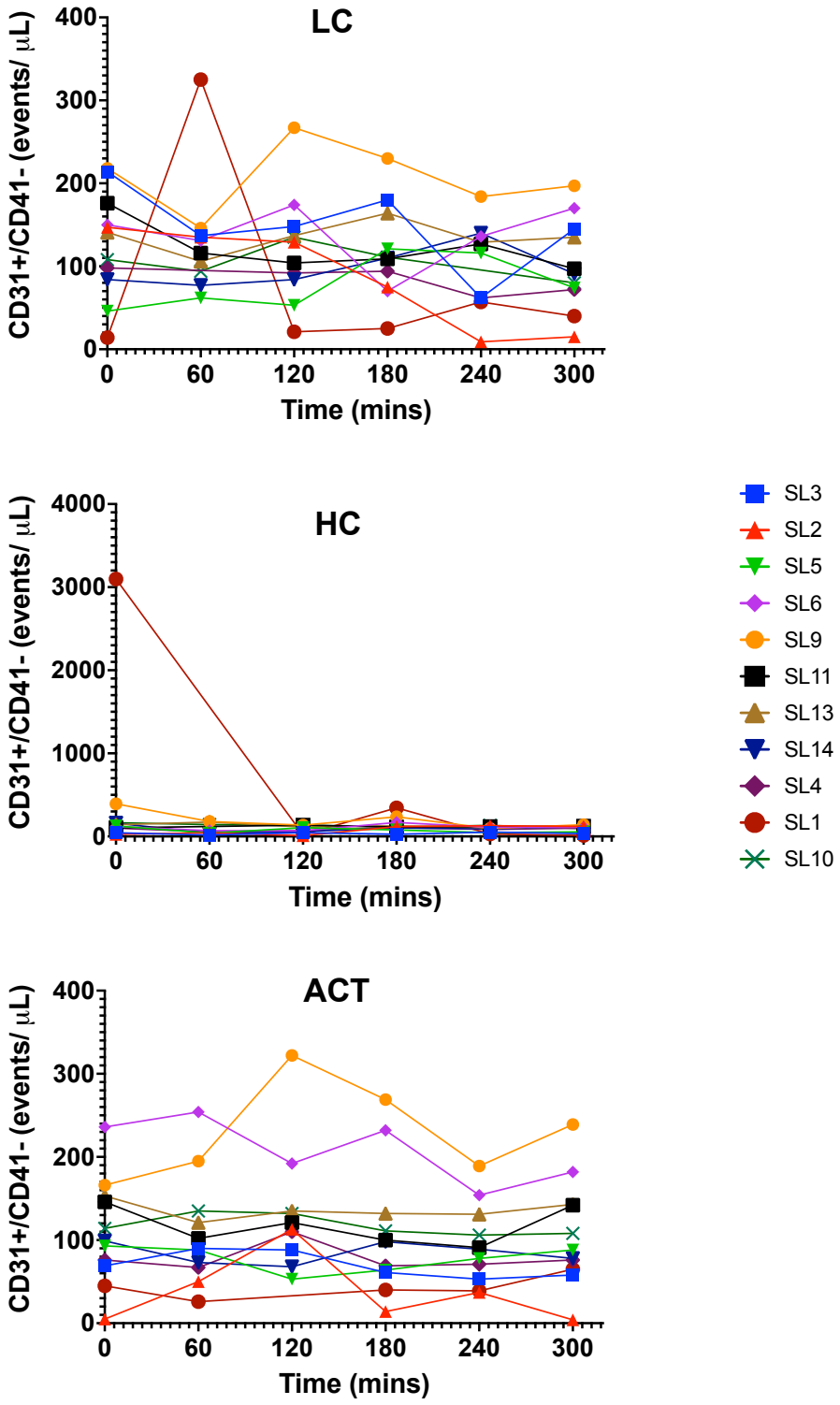

Figure A.5. Concentration of circulating apoptotic EMVs (CD31-APC^{+}/CD41-BV421^{-}) in different healthy weight individuals over time under three experimental conditions. Participants consumed 2 identical low-carbohydrate (LC) or high-carbohydrate (HC and ACT) meals at 0 and 180 mins and remained sedentary (LC, HC) or performed hourly 15-30s stair climbs (ACT).

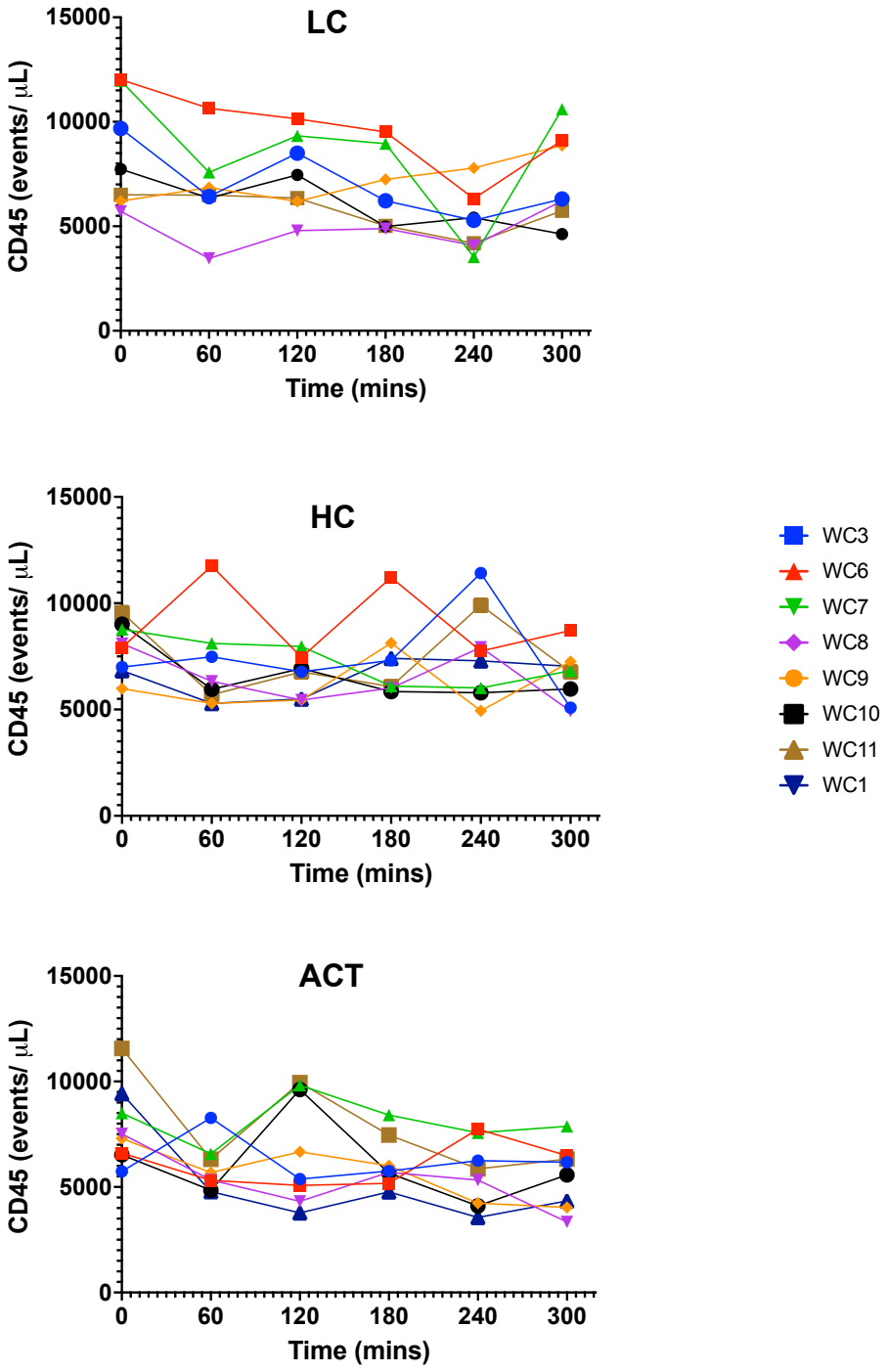

Figure A.6. Concentration of circulating LMVs (CD45-PE^{+}) in different individuals with elevated waist circumference over time under three experimental conditions. Participants consumed 2 identical low-carbohydrate (LC) or high-carbohydrate (HC and ACT) meals at 0 and 180 mins and remained sedentary (LC, HC) or performed hourly 15-30s stair climbs (ACT).

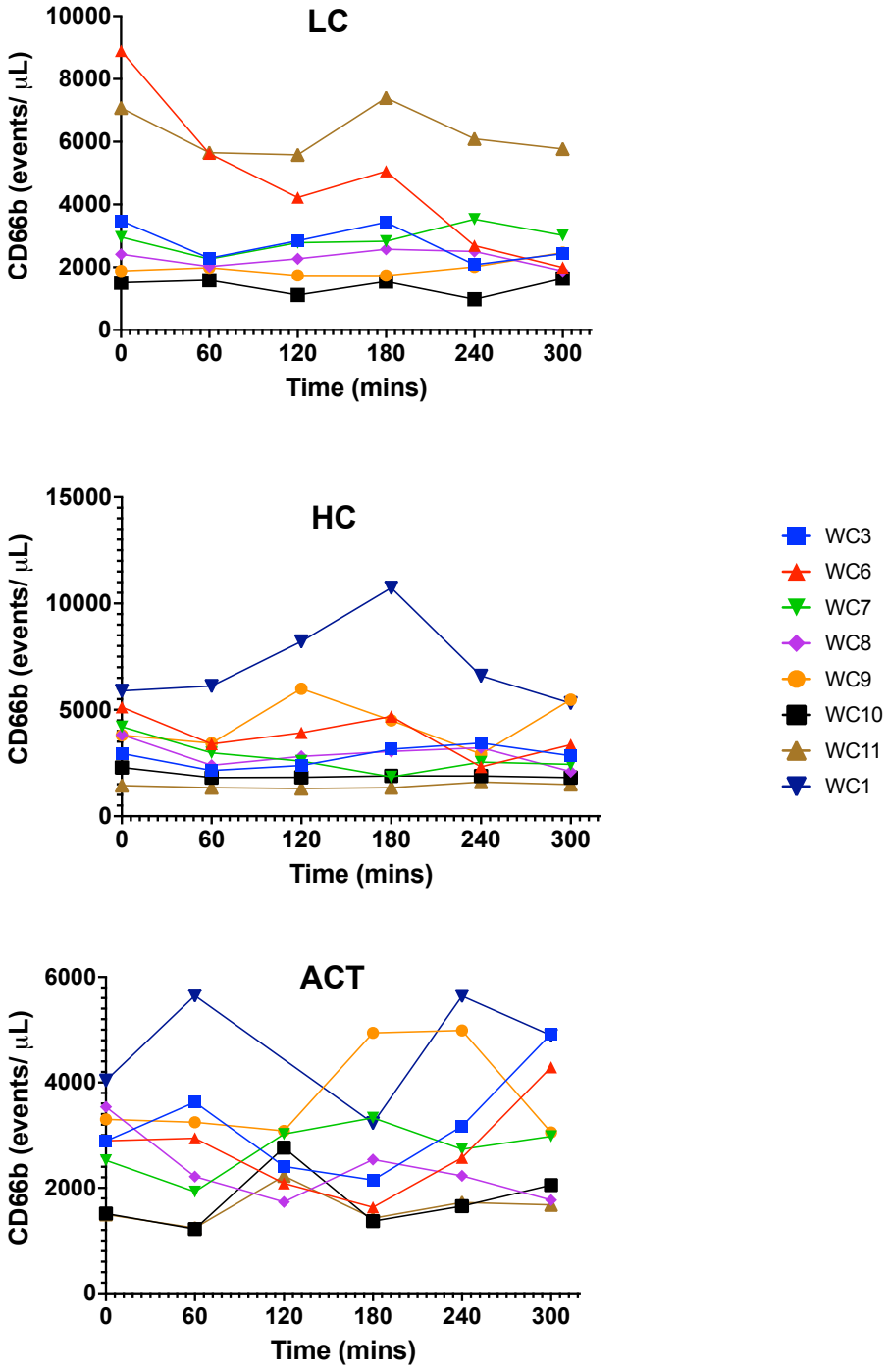

Figure A.7. Concentration of circulating GMVs (CD66b-FitC^{+}) in different individuals with elevated waist circumference over time under three experimental conditions. Participants consumed 2 identical low-carbohydrate (LC) or high-carbohydrate (HC and ACT) meals at 0 and 180 mins and remained sedentary (LC, HC) or performed hourly 15-30s stair climbs (ACT).

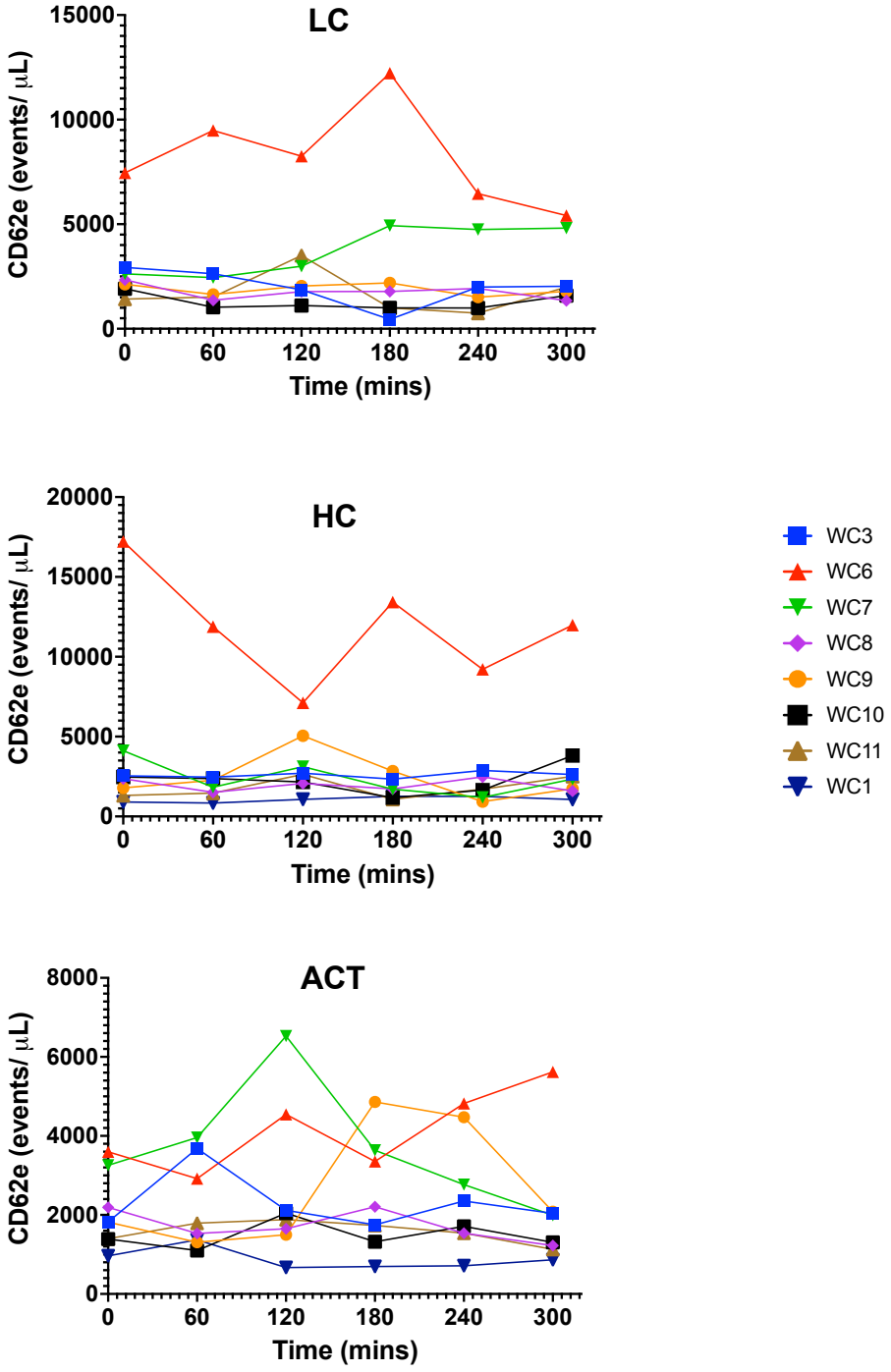

Figure A.8. Concentration of circulating activated EMVs (CD62e-PE^{+}) in different individuals with elevated waist circumference over time under three experimental conditions. Participants consumed 2 identical low-carbohydrate (LC) or high-carbohydrate (HC and ACT) meals at 0 and 180 mins and remained sedentary (LC, HC) or performed hourly 15-30s stair climbs (ACT).

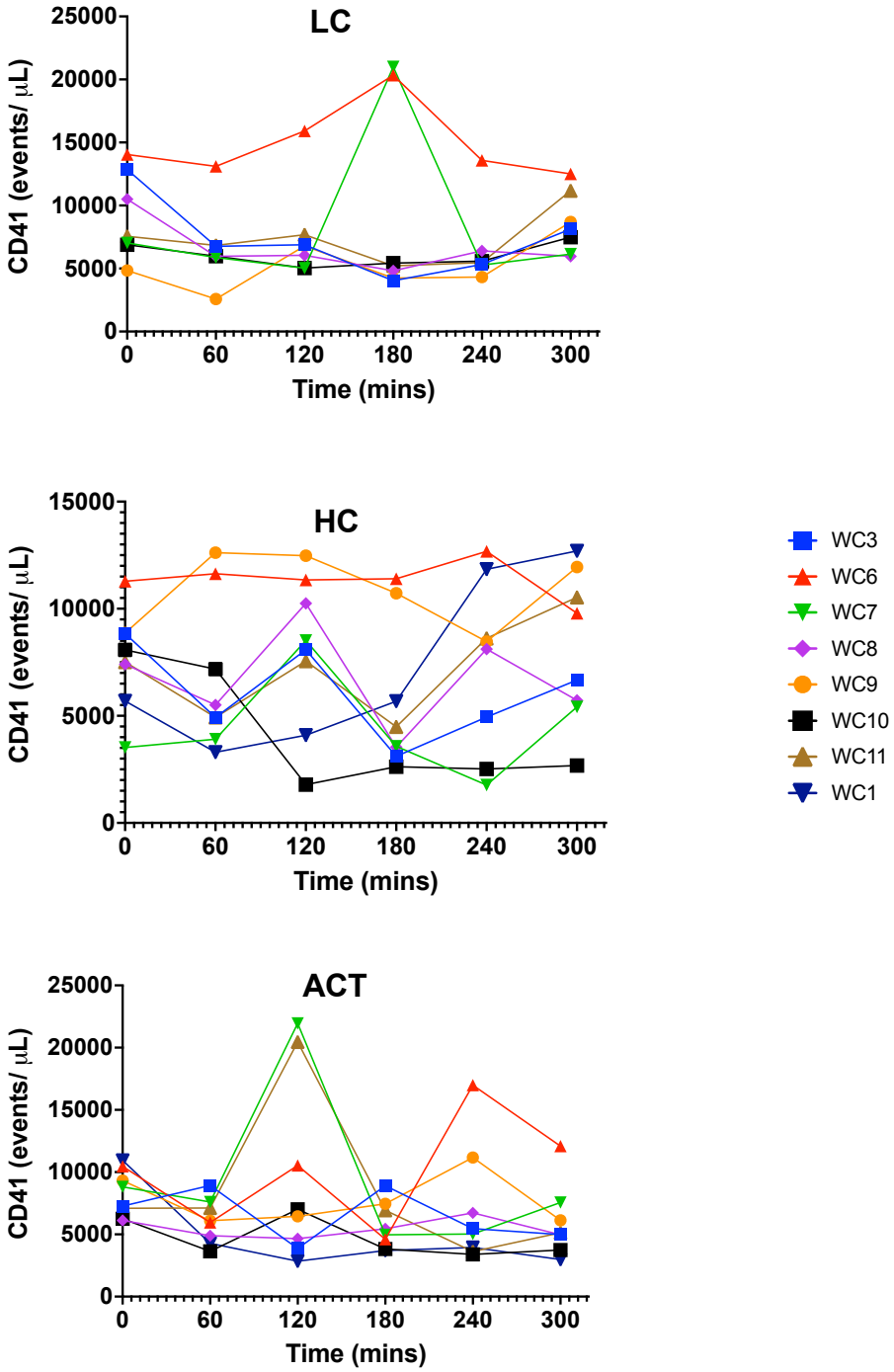

Figure A.9. Concentration of circulating PMVs (CD41-BV421^{+}) in different individuals with elevated waist circumference over time under three experimental conditions. Participants consumed 2 identical low-carbohydrate (LC) or high-carbohydrate (HC and ACT) meals at 0 and 180 mins and remained sedentary (LC, HC) or performed hourly 15-30s stair climbs (ACT).

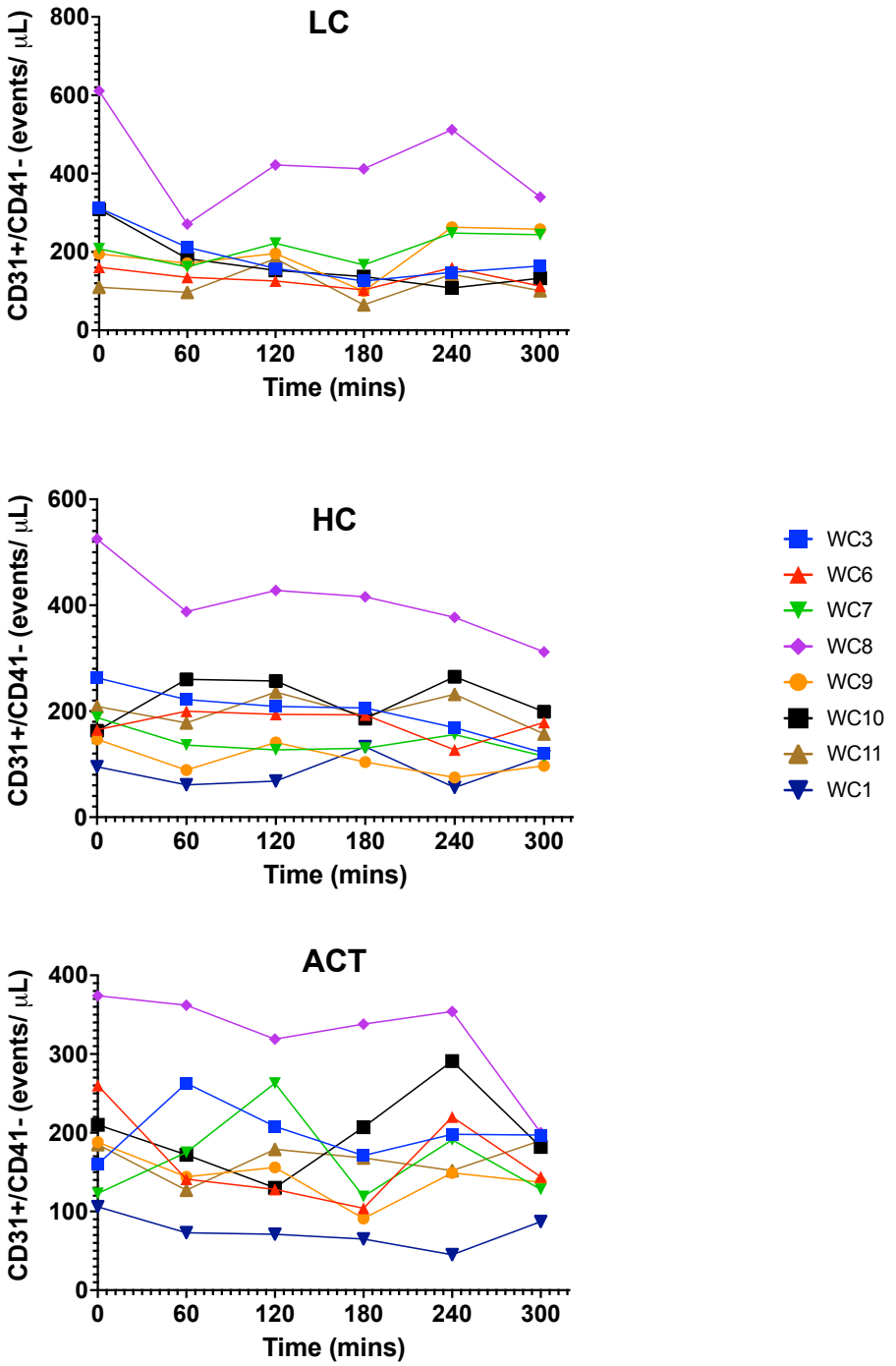

Figure A.10. Concentration of circulating apoptotic EMVs (CD31-APC^{+}/CD41-BV421^{-}) in different individuals with elevated waist circumference over time under three experimental conditions. Participants consumed 2 identical low-carbohydrate (LC) or high-carbohydrate (HC and ACT) meals at 0 and 180 mins and remained sedentary (LC, HC) or performed hourly 15-30s stair climbs (ACT).

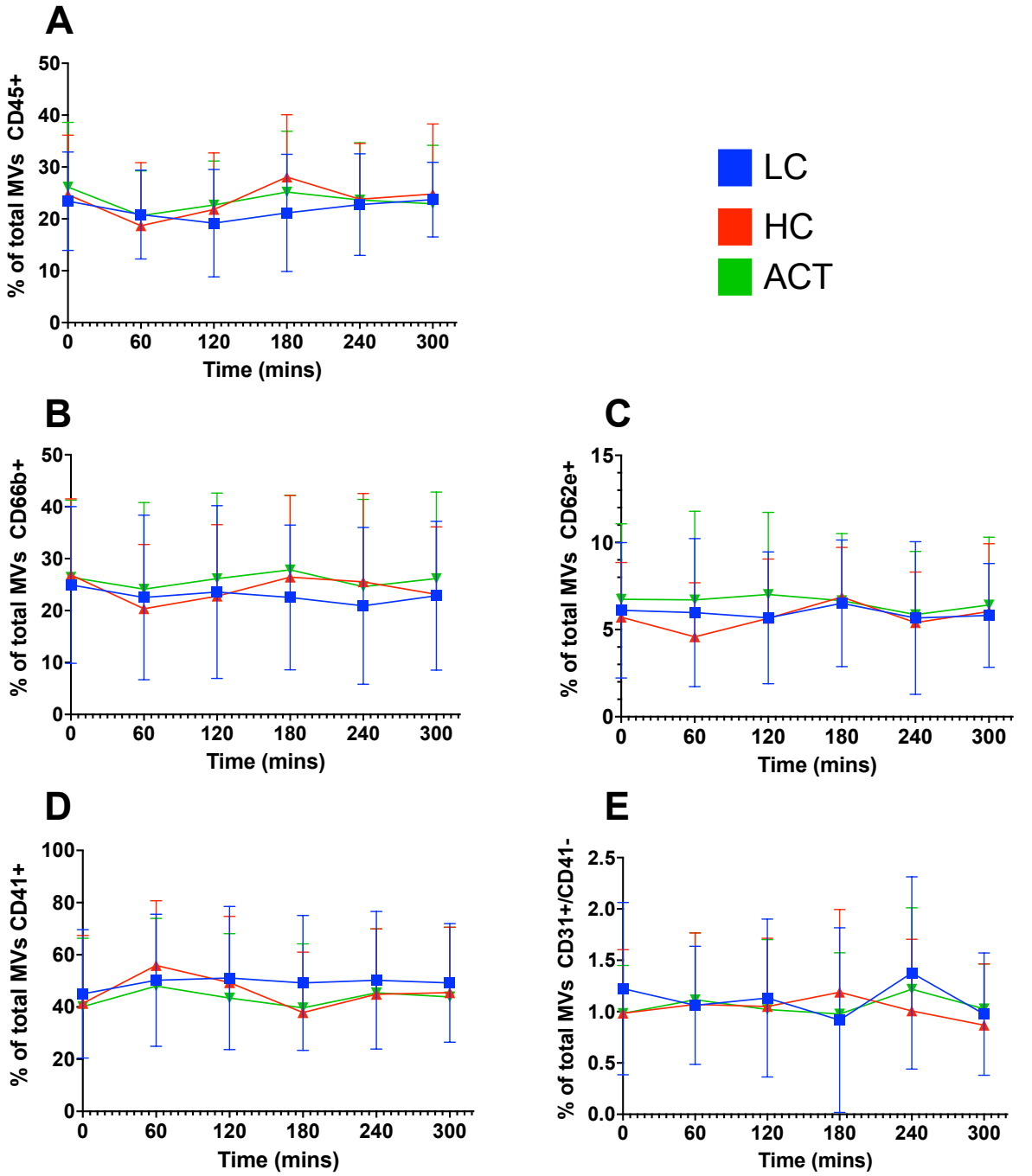

Figure A.11. Percentage of total circulating microvesicles that are a) LMVs (CD45-PE^{+}), b) GMVs (CD66b-FitC^{+}), c) activated EMVs (CD62e-PE^{+}), d) PMVs (CD41-BV421^{+}), and e) apoptotic EMVs (CD31-APC^{+}/CD41-BV421^{-}) in healthy weight individuals over time under three experimental conditions. Participants consumed 2 identical low-carbohydrate (LC) or high-carbohydrate (HC and ACT) meals at 0 and 180 mins and remained sedentary (LC, HC) or performed hourly 15-30s stair climbs (ACT).

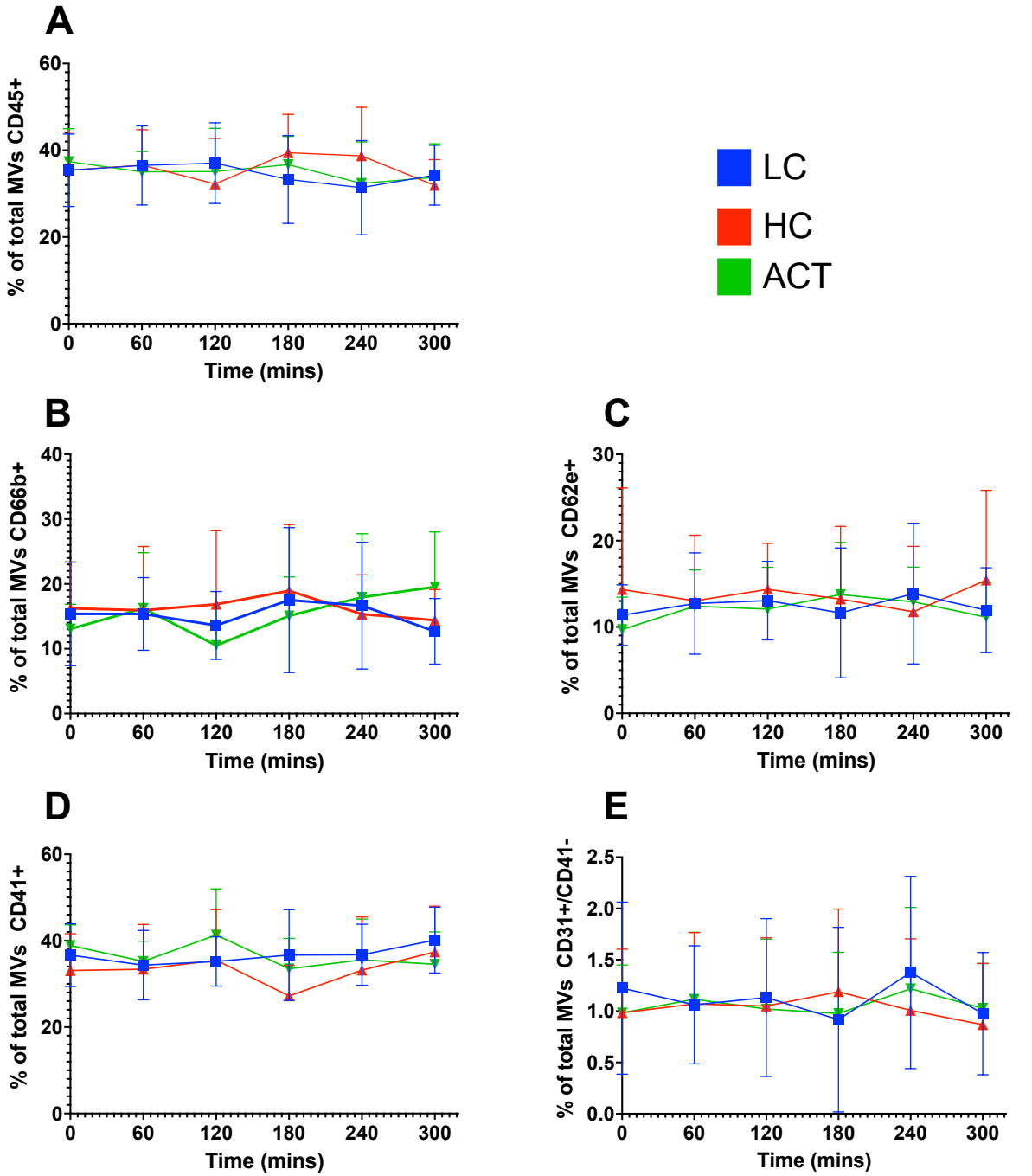

Figure A.12. Percentage of total circulating microvesicles that are a) LMVs (CD45-PE^{+}), b) GMVs (CD66b-FitC^{+}), c) activated EMVs (CD62e-PE^{+}), d) PMVs (CD41-BV421^{+}), and e) apoptotic EMVs (CD31-APC^{+}/CD41-BV421^{-}) in individuals with elevated waist circumference over time under three experimental conditions. Participants consumed 2 identical low-carbohydrate (LC) or high-carbohydrate (HC and ACT) meals at 0 and 180 mins and remained sedentary (LC, HC) or performed hourly 15-30s stair climbs (ACT).

Appendix B: Gating Strategy, Antibody Titration, and Example Dot Plots

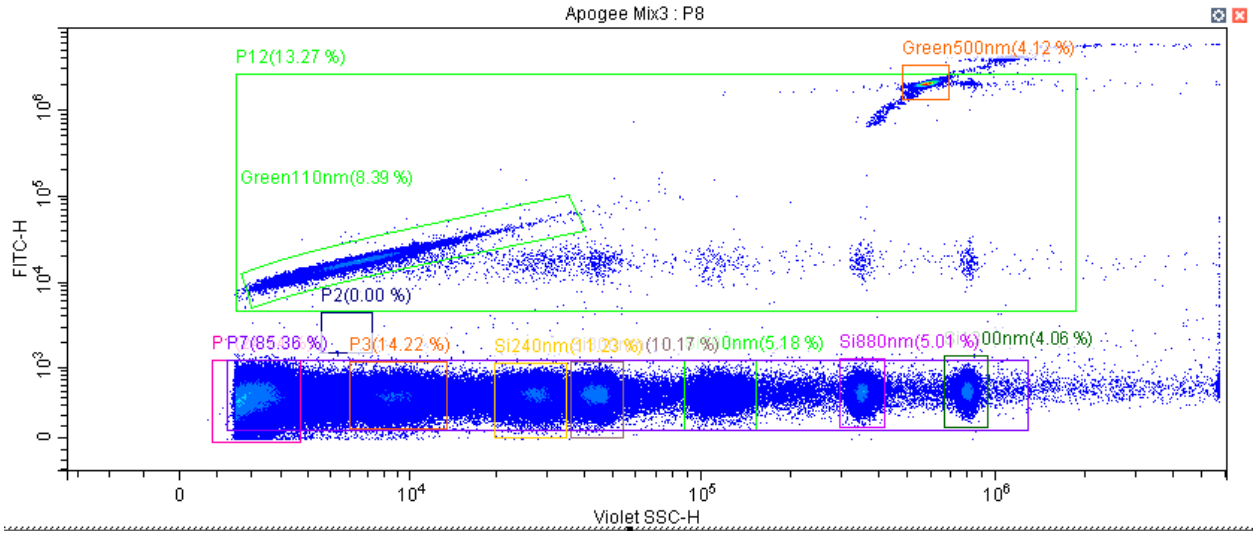

Figure B.1. Dot plot of violet side scatter-height (VSSC-H) by FITC-Height (FITC-H) with ApogeeMix of silica and polystyrene beads. Bead populations of 240-1300 nm in diameter and fluorescently labeled 110 nm beads appeared as distinct populations and were gated accordingly. The non-fluorescent 180 nm population was not distinct from noise and required further adjustments to gate as lower boundary for MV gate. The gate P7 was added around noise and all non-fluorescent bead populations.

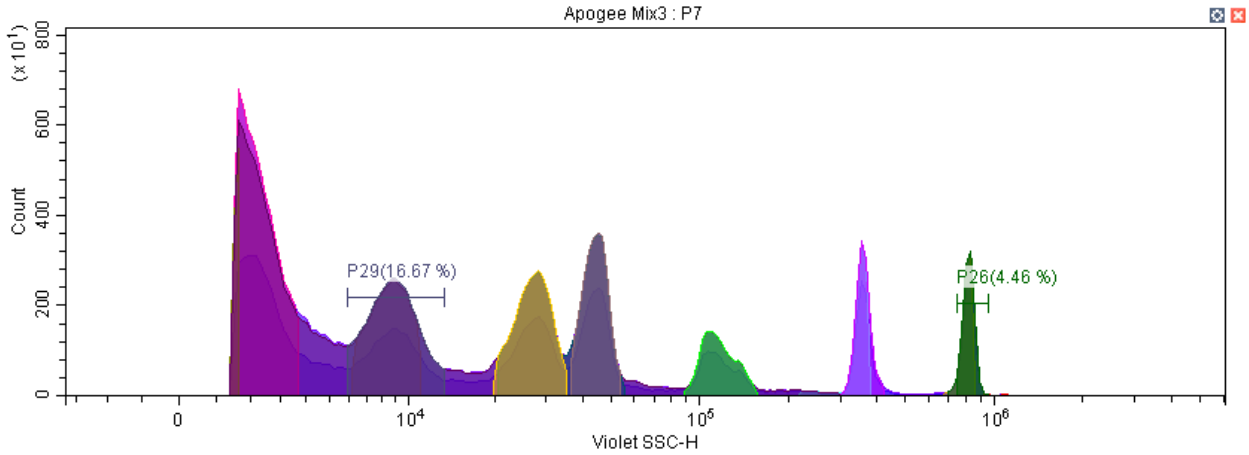

Figure B.2. Histogram of event count by violet side scatter-height of all events within the P7 gate in figure 13. The gate P29 was set around normal distribution of first population distinct from noise, representing 180 nm silica beads.

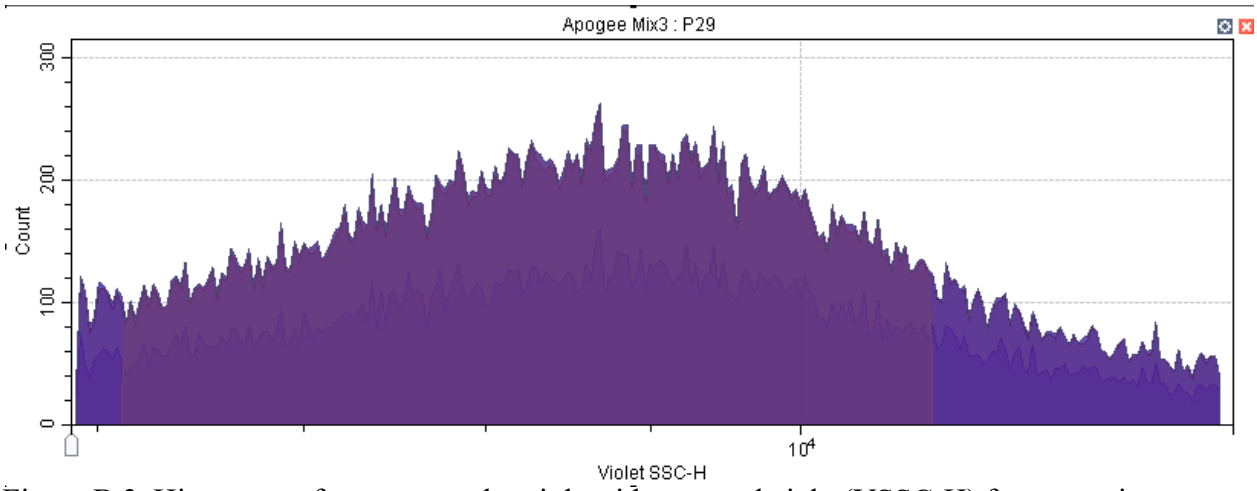

Figure B.3. Histogram of event count by violet side scatter-height (VSSC-H) for events in gate P29 of the figure 14. Maximum and minimum VSSC-H were adjusted until normal distribution occupied entire plot.

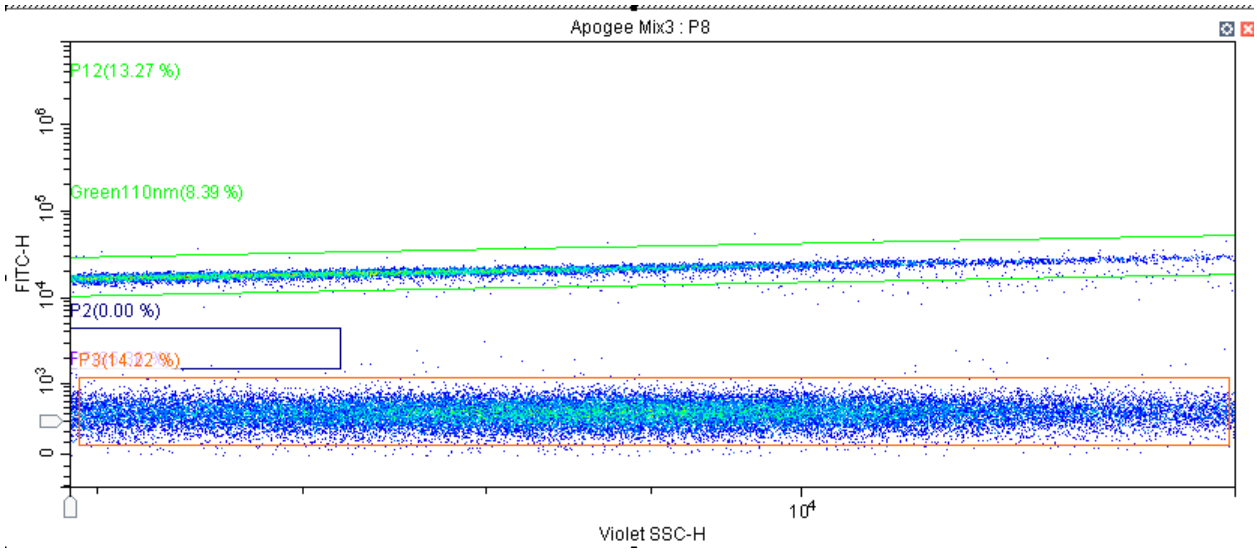

Figure B.4. Dot plot of FITC-height (FITC-H) by violet side scatter-height (VSSC-H) of ApogeeMix silica and polystyrene beads. Maximum and minimum VSSC-H values of the plot adjusted to those from figure 15. Gate P3 set around non-fluorescent population to the entire width of the plot to encompass 180 nm silica bead population.

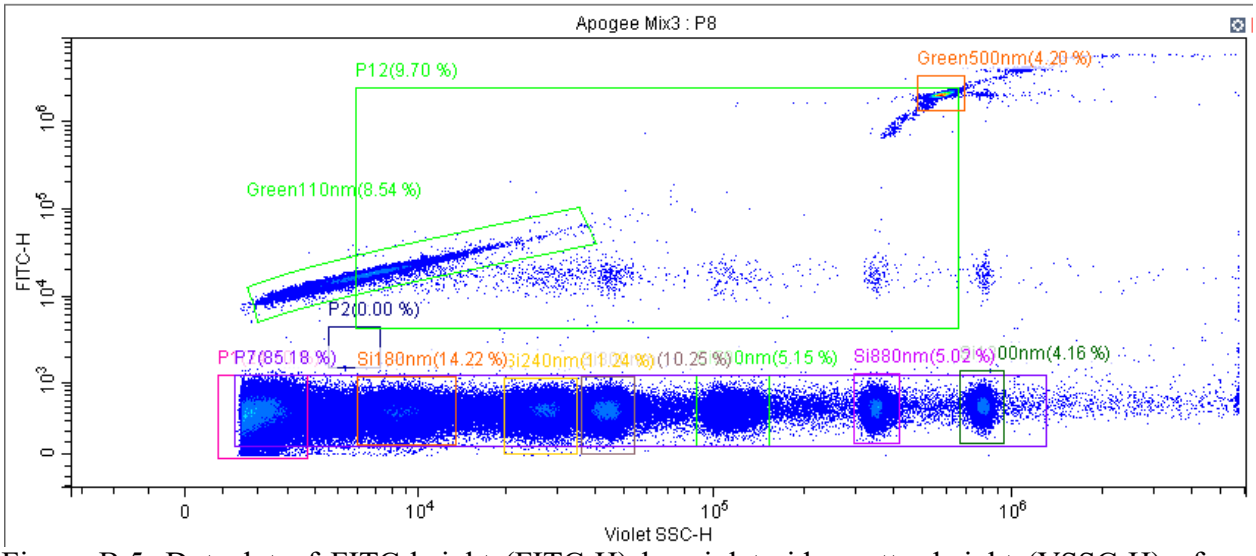

Figure B.5. Dot plot of FITC-height (FITC-H) by violet side scatter-height (VSSC-H) of ApogeeMix silica and polystyrene beads with all fluorescent and non-fluorescent bead populations (110, 180, 240, 300, 500, 590, 880, 1300 nm) gated. Gate P12 was set as the particle gate with lower VSSC-H boundary to the smallest bounds of the 180 nm silica bead gate and upper VSSC-H boundary to between the 880 and 1300 nm bead gates. Size of P12 in relation to FITC-H was set according to MV-free stained controls and platelet poor plasma unstained controls for each antibody (CD66b-FITC, CD45-PE, CD31-APC, CD41-BV421, CD62e-PE).

Table B.1. Concentration of positive events in plasma singly stained with varied volumes of antibody for antibody titration of 50 µl of plasma. Concentrations are given in events/µl and are corrected for MV free controls for each sample.

Volume of Antibody (µl)	**Concentration of events (events/µl)**				
	CD45-PE	**CD66b-FitC**	**CD62E-PE**	**CD31-APC**	**CD41-BV421**
0.5	-	-	-	-	2026
1.0	-	-	-	-	2672
1.5	-	-	-	-	3007
2.0	3753	2523	463	36	-
2.5	2939	2315	660	138	-
3.0	3189	2841	939	257	-
3.5	1978	1582	521	166	-

*dashes indicate antibody volume and type combinations that were not tested

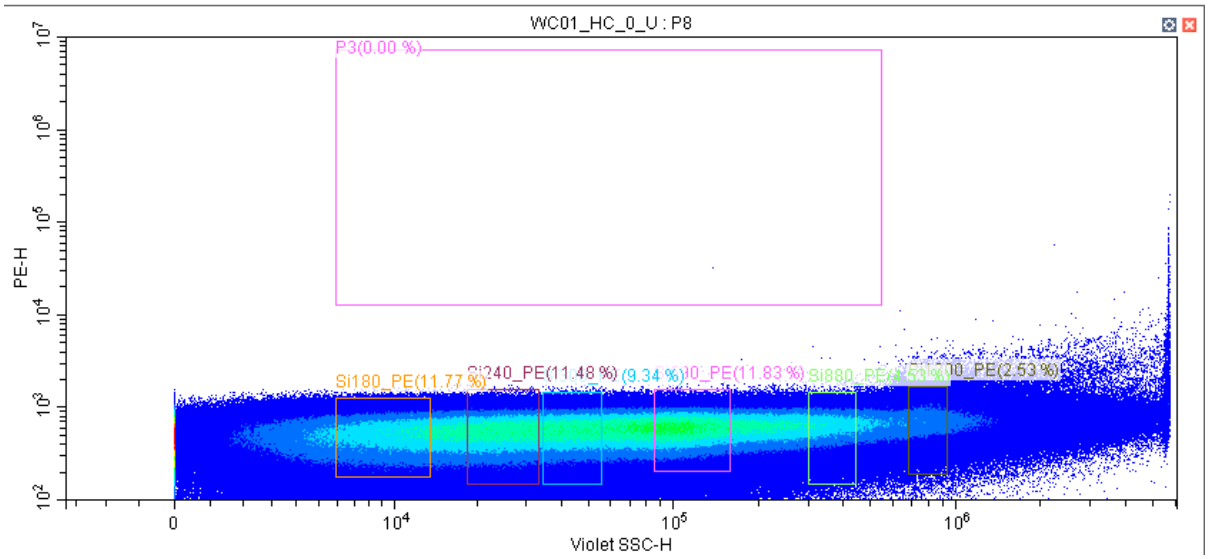

Figure B.6. Dot plot of PE-Height (PE-H) by violet side scatter-height (VSSC-H) of an unstained control for a plasma sample. Particle gate P3 denotes PE^{+} events subtracted from MV counts of corresponding stained sample.

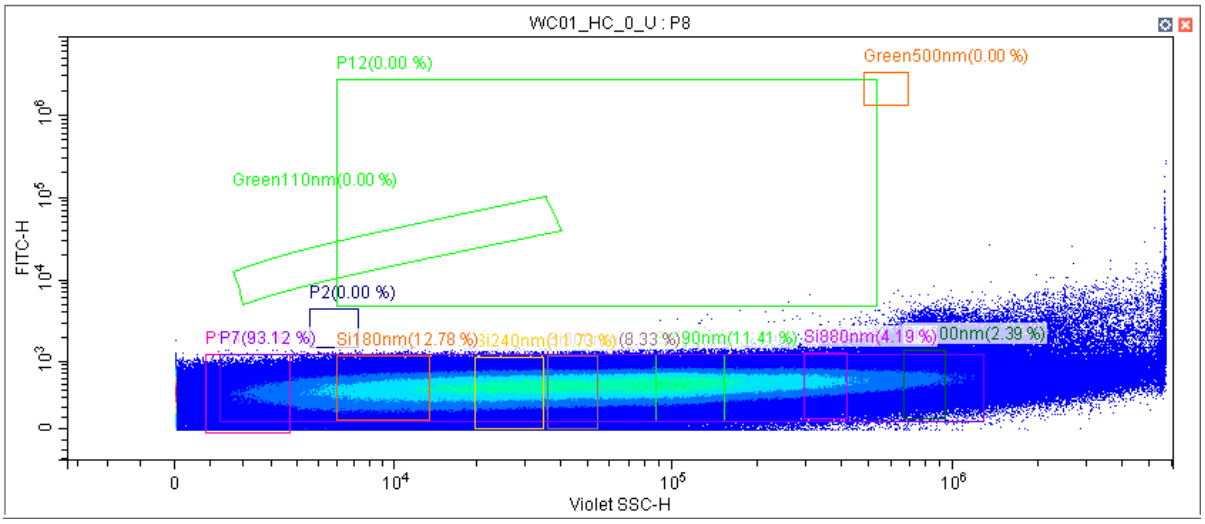

Figure B.7. Dot plot of FitC-Height (FitC-H) by violet side scatter-height (VSSC-H) of an unstained control for a plasma sample. Particle gate P12 denotes $FitC^{+}$ events subtracted from MV counts of corresponding stained sample.

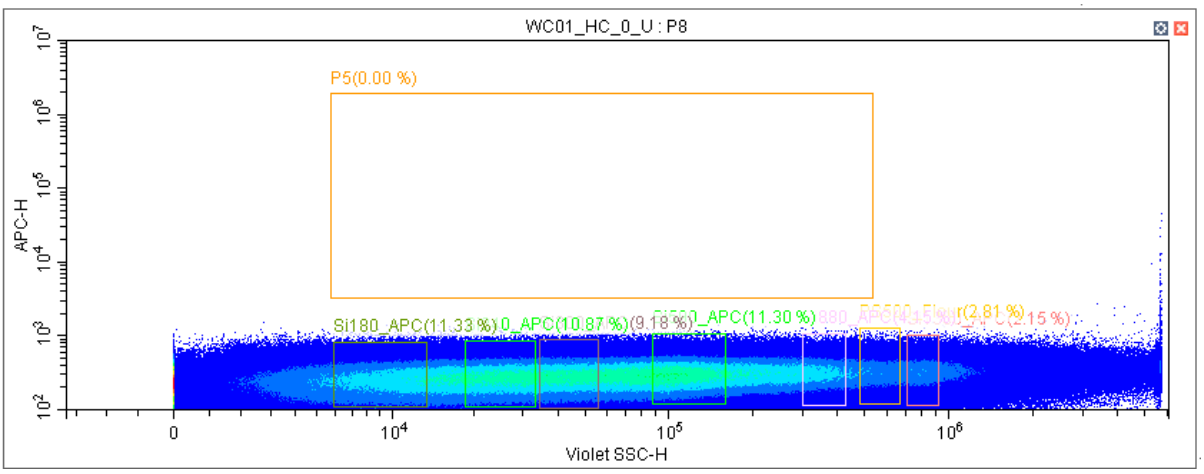

Figure B.8. Dot plot of APC-Height (APC-H) by violet side scatter-height (VSSC-H) of an unstained control for a plasma sample. Particle gate P5 denotes APC^{+} events subtracted from MV counts of corresponding stained sample.

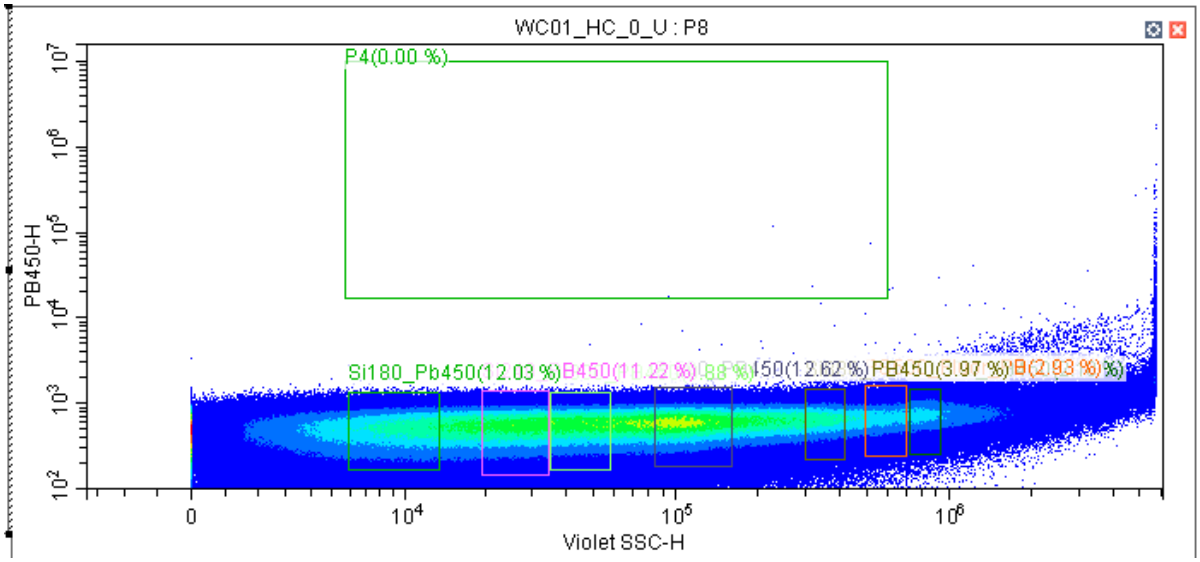

Figure B.9. Dot plot of BV421-Height (BV421-H) by violet side scatter-height (VSSC-H) of an unstained control for a plasma sample. Particle gate P4 denotes $BV421^{+}$ events subtracted from MV counts of corresponding stained sample.

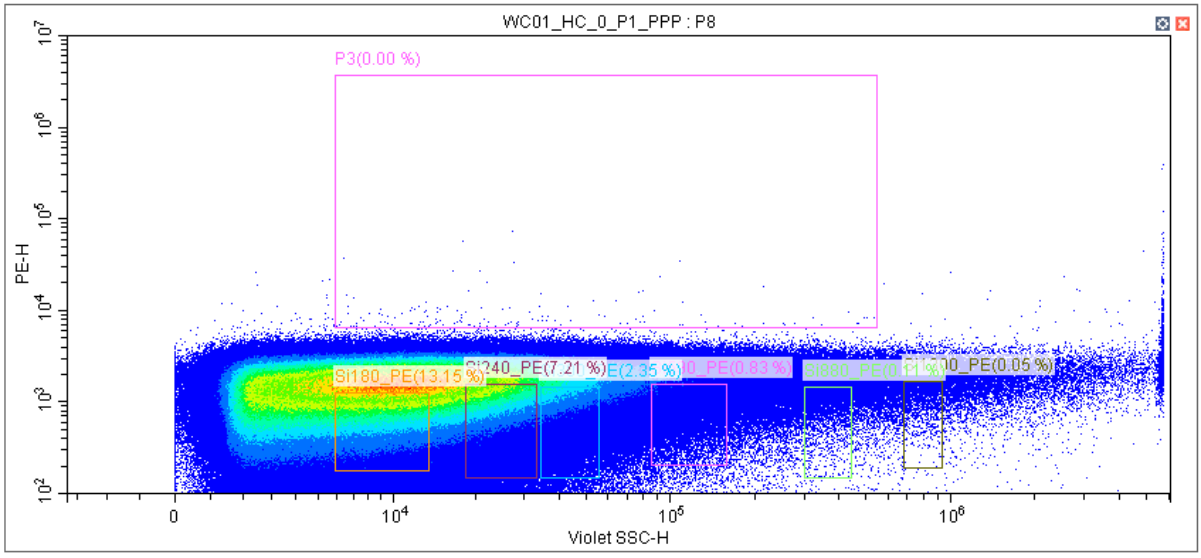

Figure B.10. Dot plot of PE-Height (PE-H) by violet side scatter-height (VSSC-H) of a CD45-PE stained MV-free plasma sample. Particle gate P3 denotes CD45-PE^{+} events subtracted from MV counts of corresponding stained sample.

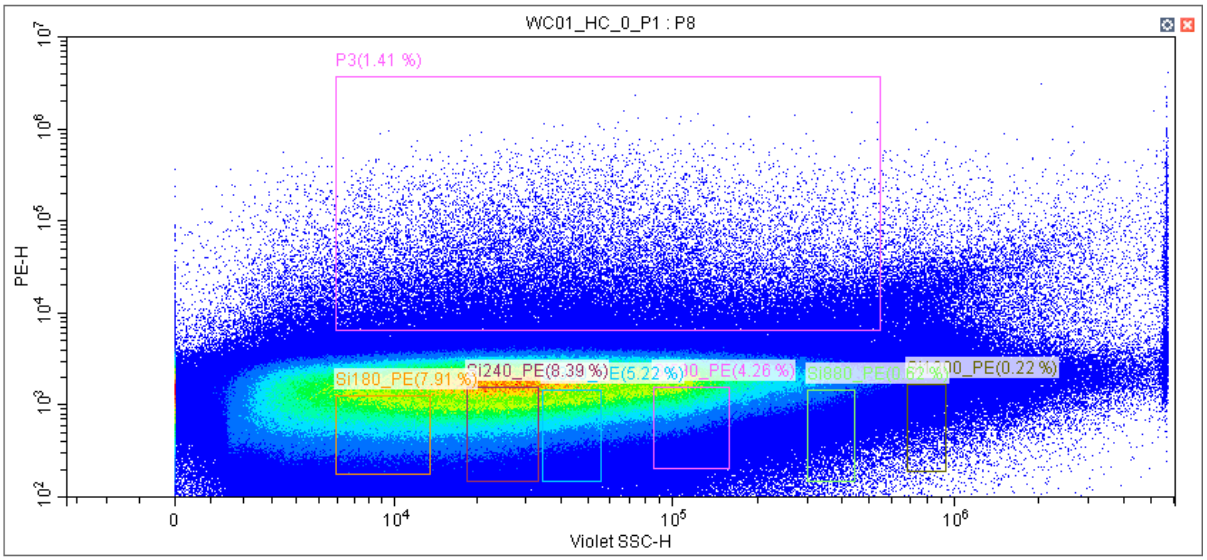

Figure B.11. Dot plot of PE-Height (PE-H) by violet side scatter-height (VSSC-H) of an CD45-PE stained plasma sample. Particle gate P3 denotes CD45-PE^{+} events and the uncorrected LMV count.

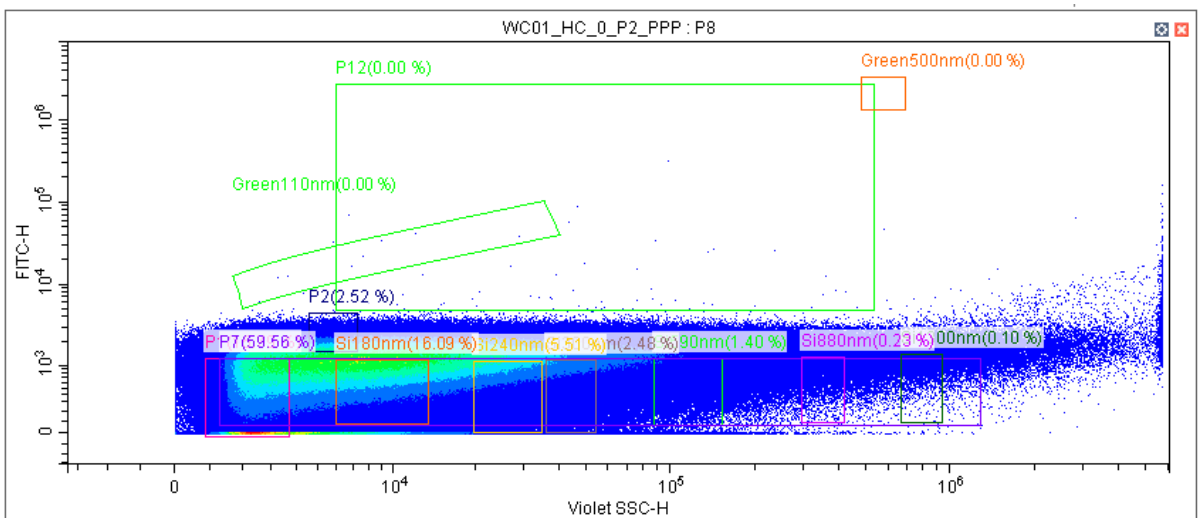

Figure B.12. Dot plot of FitC-Height (FitC-H) by violet side scatter-height (VSSC-H) of a CD66b-FitC stained MV-free plasma sample. Particle gate P12 denotes CD66b-FitC^{+} events subtracted from MV counts of corresponding stained sample.

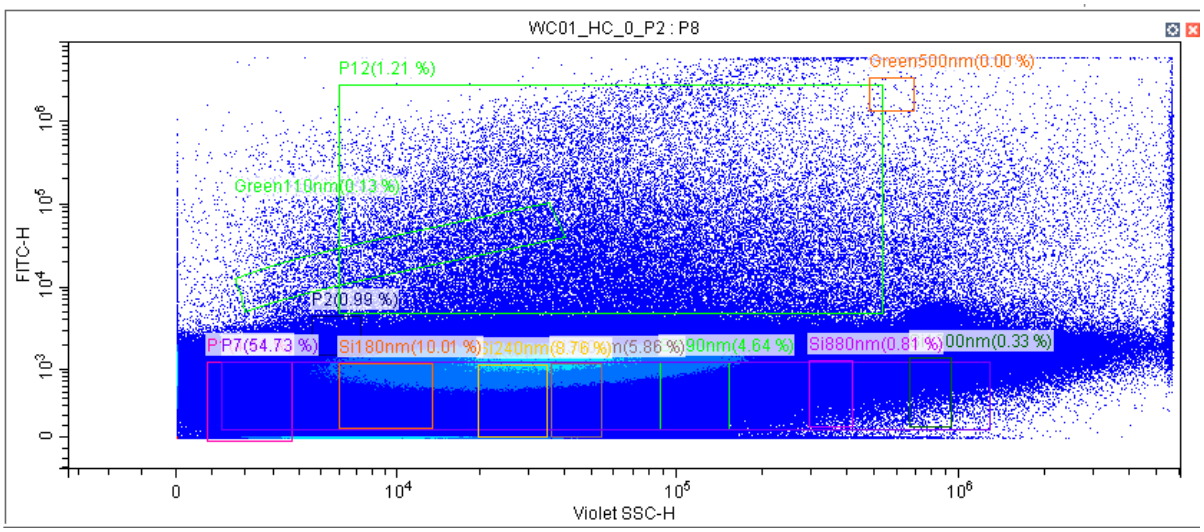

Figure B.13. Dot plot of FitC-Height (FitC-H) by violet side scatter-height (VSSC-H) of a CD66b-FitC stained plasma sample. Particle gate P12 denotes CD66b-FitC^{+} events and the uncorrected GMV count.

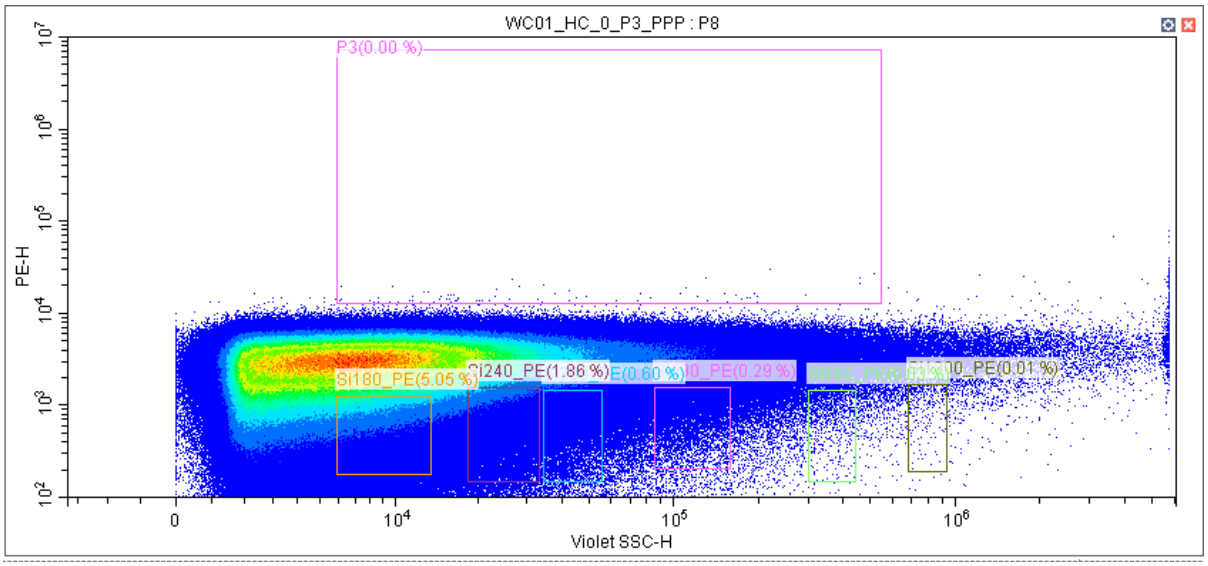

Figure B.14. Dot plot of PE-Height (PE-H) by violet side scatter-height (VSSC-H) of a CD62e-PE stained MV-free plasma sample. Particle gate P3 denotes CD62e-PE^{+} events subtracted from MV counts of corresponding stained sample.

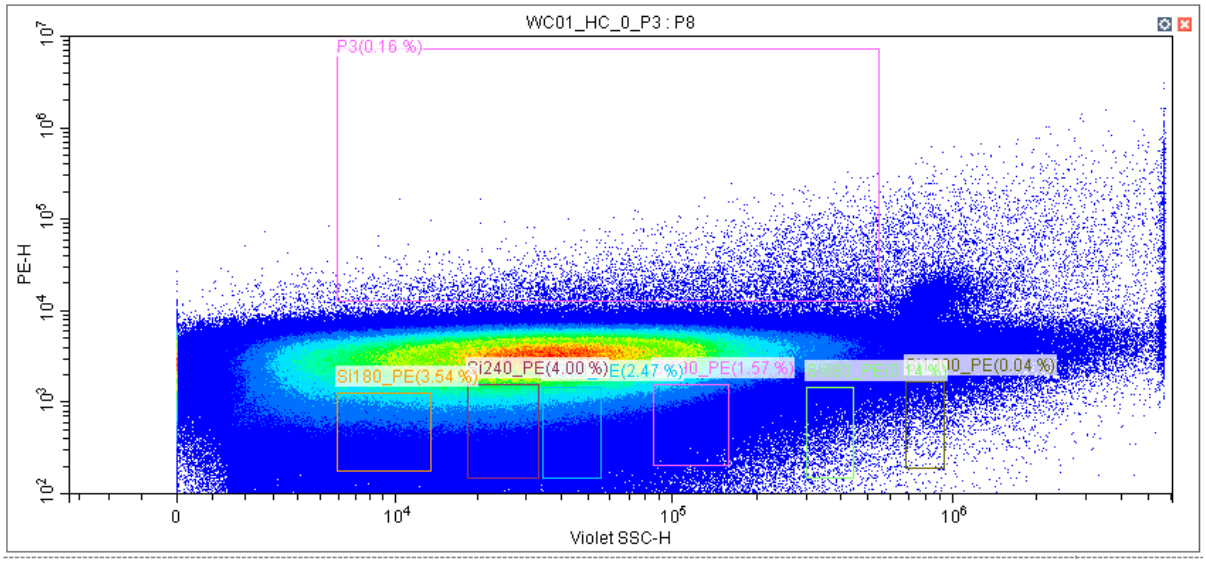

Figure B.15. Dot plot of PE-Height (PE-H) by violet side scatter-height (VSSC-H) of a CD62e-PE stained plasma sample. Particle gate P3 denotes CD62e-PE^{+} events and the uncorrected activated EMV count.

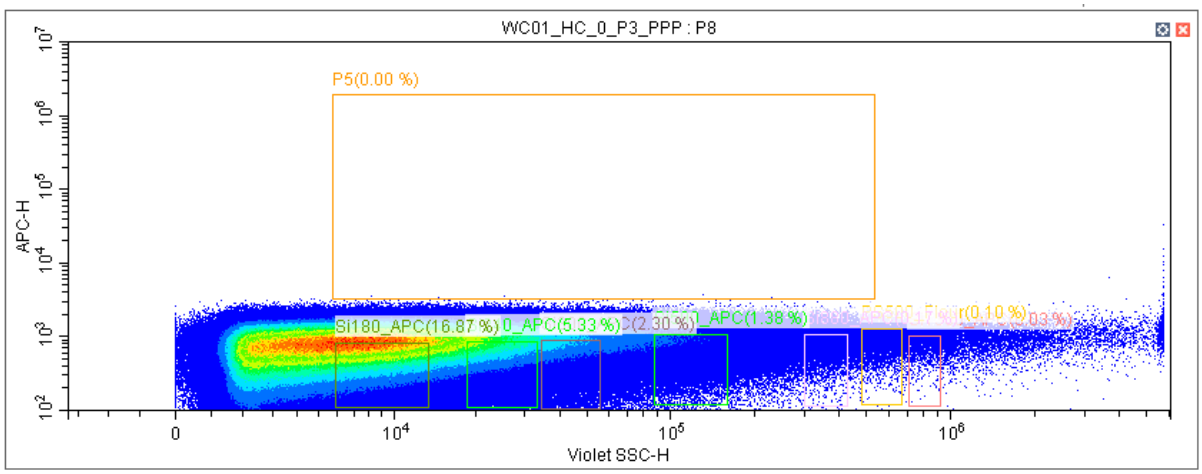

Figure B.16. Dot plot of APC-Height (APC-H) by violet side scatter-height (VSSC-H) of a CD31-APC stained MV-free plasma sample. Particle gate P5 denotes CD31-APC^{+} events subtracted from MV counts of corresponding stained sample.

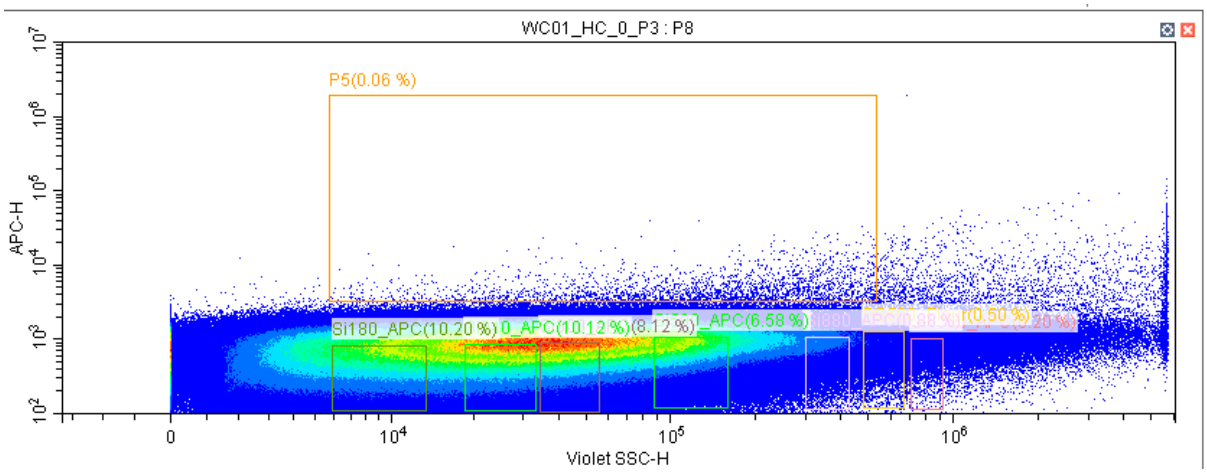

Figure B.17. Dot plot of APC-Height (APC-H) by violet side scatter-height (VSSC-H) of a CD31-APC stained plasma sample. Particle gate P5 denotes CD31-APC^{+} events. Events observed as CD31-APC^{+}/CD41-BV421^{-} form the uncorrected apoptotic EMV count.

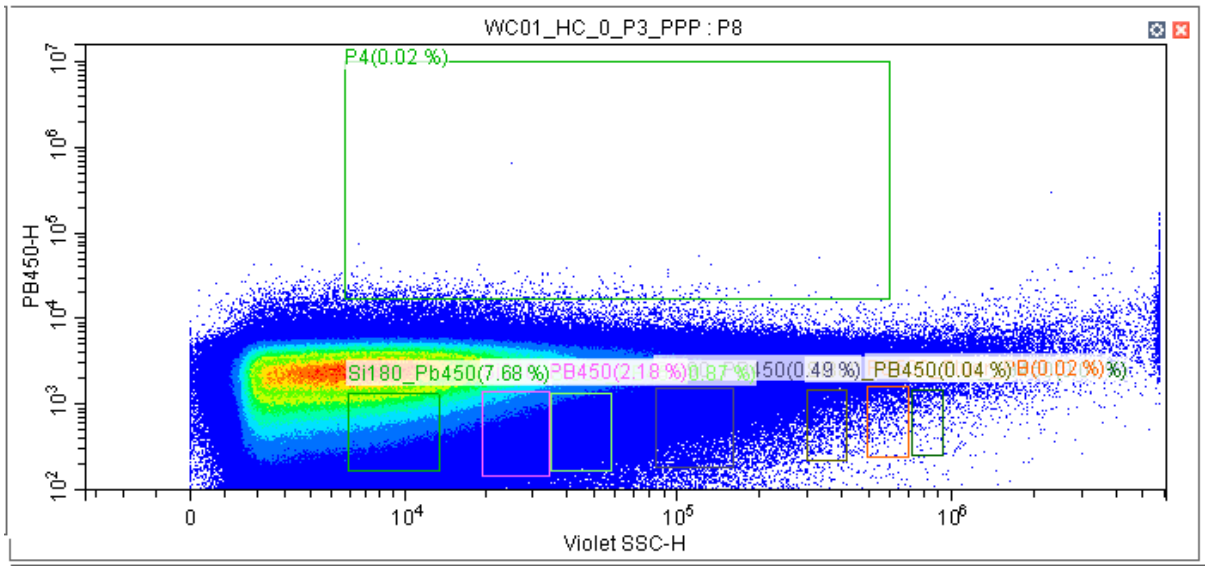

Figure B.18. Dot plot of BV421-Height (BV421-H) by violet side scatter-height (VSSC-H) of a CD41-BV421 stained MV-free plasma sample. Particle gate P4 denotes CD41-BV421^{+} events subtracted from MV counts of corresponding stained sample.

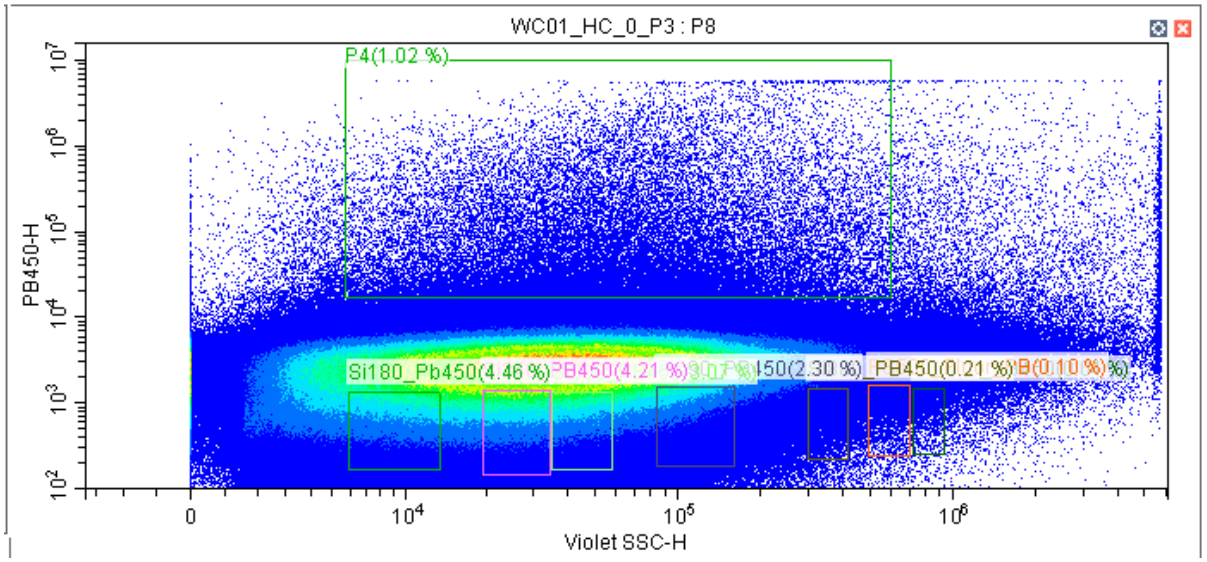

Figure B.19. Dot plot of BV421-Height (BV421-H) by violet side scatter-height (VSSC-H) of a CD41-BV421 stained plasma sample. Particle gate P4 denotes CD41-BV421^{+} events. Events observed as CD31-APC^{+}/CD41-BV421^{-} form the uncorrected apoptotic EMV count.