

# Relation Between Endothelial Microparticles and Endothelial Function Following Acute Exercise in Men with Coronary Artery Disease

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## ABSTRACT

**Purpose:** Microparticles (MP) are small membranous vesicles shed from formed elements such as circulating platelets and vascular endothelial cells. They express surface antigens characteristic of their cell-of-origin and contain DNA, RNA, miRNA and intracellular proteins. Depending on the dynamic morphological state of the parent cell, phenotypically varied MP may be released into the circulation. MP serve not only as markers, but also potential mediators of inflammation, coagulation and endothelial dysfunction. This study examined the relation between total MP, endothelial derived MP (EMP), platelet derived MP (PMP) and endothelial function (a validated surrogate measure of early atherosclerosis) in response to acute exercise in men with coronary artery disease.

**Methods:** Eight men with coronary artery disease (CAD) (65.7±4.5 yr, VO<sub>2peak</sub> 28.0±2.6 ml·kg<sup>-1</sup>·min<sup>-1</sup>, BMI 29.7±3.3 kg·m<sup>-2</sup>) underwent 20 min of treadmill walking at 66% VO<sub>2peak</sub>. Endothelial dependent dilation (EDD) of the brachial artery was assessed before and 1 h following the acute bout of exercise. Vessel diameter was determined using high resolution vascular ultrasonography (SonoSite, MicroMaxx) from flow mediated dilation (FMD) following 5 min of forearm occlusion. Endothelial independent vasodilation (EID) was measured following the administration of glyceryl trinitrate (0.4 mg). MP counts were enumerated by flow cytometry from platelet poor plasma (PPP) stored at -80°C. MP were characterized based on size (0.5 - 1.0 µm sizing gate) and their ability to bind annexin V. MP had also to bind to CD62E or CD41 to be of endothelial or platelet origin respectively.

**Results:** Compared to baseline, FMD was significantly increased at 60 min following the acute bout of exercise. There was no change in EDD or EID. There was significant relation between the percent change in total MP (annexin V+ events) and the percent changes in flow mediated dilation 60 min after the exercise bout, and between the percent change in EMP (annexin V+/CD62E+ events) and the percent changes in flow mediated dilation 60 min after the exercise bout.

**Conclusion:** Changes in endothelial function in response to acute exercise may be mediated by alterations in circulating levels of MP

## INTRODUCTION

Microparticles are tiny fragments of cells that can be found in the blood. Under normal physiological conditions, low levels of MP are continually being shed into the blood from numerous cells types including platelets and endothelial cells. There is evidence that the circulating levels of MP are increased in individuals with cardiovascular disease (1). Endothelial dysfunction is one the earliest events in the development of atherosclerosis and occurs prior to angiographic detection. Endothelial function, assessed using high-resolution ultrasonography, is used as a surrogate measure for coronary artery function. Physical activity has been shown to reduce the risk for cardiovascular disease. The purpose of this study is to determine the effects of a single bout of exercise on circulating MP and endothelial function in men with CAD.

## METHODS

**Subjects:** Eight men with CAD (Table 1.) volunteered. Ethical approval was granted by the Dublin City University Research Ethics Committee.

**Experimental Design:** Subjects visited the Vascular Research Unit (VRU) in the School of Health and Human Performance on 2 separate occasions.

**Visit 1:** Subjects performed a maximal aerobic capacity test. A 12 lead ECG, blood pressure, rating of perceived exertion, and expired gases were monitored throughout the test.

**Visit 2:** Subjects arrived after an overnight fast. Height, weight, and blood pressure were measured and a blood sample taken. Following 10 min of supine rest, baseline EID and EDD were assessed using high frequency ultrasonography. Subjects then walked on a treadmill for 20 min. Treadmill velocity and gradient were self regulated by each subject. Subjects were given the opportunity to adjust the treadmill velocity and/or grade every 5 min. Heart rate (HR) and rate of perceived exertion (RPE) were recorded at 5 min intervals. Breath by breath expiratory gases were continuously measured using open circuit spirometry. One hour post exercise a blood sample was taken and EID and EDD were measured.

**Biochemical analysis:** Blood for MP analysis was collected, prepared and stored according to a double centrifugation method (2). MP counts were quantified by flow cytometry using a BD FC500, and CXP software. MPs were identified based on size and fluorescence, using 1.0 µm sizing beads (Molecular Probes Eugene, Oregon), annexin V+ and anti-CD62+E (BD Pharmingen, San Jose, CA) monoclonal antibodies. Annexin V+ was used to identify total MP. As CD62E+ (E selectin) protein is present on endothelial microparticles, EMP were defined by events annexin V+/62E+. Samples were analysed in duplicate and microparticle numbers were expressed as counts per µL of plasma.

## RESULTS

Table 1: Subject characteristics

Variable	Result
Age	65.7 ± 1.6
Height (m)	169.9 ± 3.2
Weight (kg)	85.9 ± 4.8
BMI (kg·m <sup>-2</sup> )	29.7 ± 1.2
VO <sub>2peak</sub> (l·min <sup>-1</sup> )	2.4 ± 0.2
VO <sub>2peak</sub> (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	28.0 ± 2.6

Values are means ± SE

Table 2: MP Results

Time	MP	EMP
Baseline	499 ± 70.2	148 ± 27.4
Post exercise +1h	439 ± 66.4	133 ± 18.7

Values are means ± SE

Figure 1

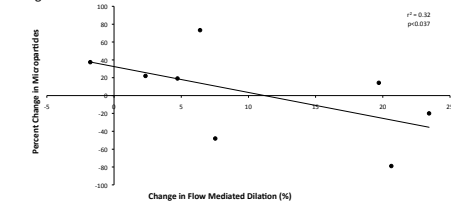


Figure 1: Significant relation between the percent change in FMD 60min post exercise and the percent change in total MP 60min post exercise

Figure 2

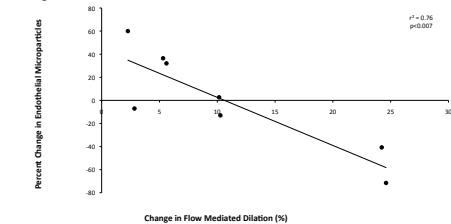


Figure 2: Significant relation between the percent change in FMD 60min post exercise and the percent change in EMP 60min post exercise

## CONCLUSION

Following an acute bout of self regulated exercise in men with CAD, there is a significant inverse relation between the percent change in total MP and endothelial function and between the percent change in EMP and endothelial function as measured by FMD. Changes in endothelial function in response to acute exercise may be mediated by alterations in circulating levels of MP.

Future research will explore this relation between MP and endothelial function.

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