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Oscillatory shear stress, flow-mediated dilatation, and circulating microparticles at sea level and high altitude

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ABSTRACT

Background and aims: Exposing the endothelium to acute periods of imposed oscillatory shear stress reduces endothelial function and elevates circulating microparticles (MPs). Oscillatory shear stress may be especially pathogenic when superimposed on hypoxia, an environmental stimulus that disrupts the endothelial milieu. We examined the effects of acute manipulation of oscillatory shear stress on endothelial function and circulating MPs at sea level (SL) and high altitude (HA).

Methods: Healthy adults (n = 12) participated, once at SL and once on the second or third day at HA (3800 m). Oscillatory shear stress was provoked using a 30-min distal cuff occlusion intervention (75 mmHg). Endothelial function was assessed before and immediately after the intervention in the brachial artery by reactive hyperaemia flow-mediated dilatation (FMD). Venous blood samples of MPs (flow cytometry) were obtained before and during the last five minutes of the shear intervention.

Results: At baseline, circulating MPs were two-fold higher at HA ($p = 0.011$) and brachial artery diameter was constricted ($p = 0.015$). Although the intervention at SL increased endothelial-derived MPs by 83 ± 39% (mean ± SEM; $p = 0.021$), FMD was unaltered. Conversely, at HA, the intervention elicited a 26 ± 11% reduction in FMD ($p = 0.020$); this reduction was inversely correlated with the change in total circulating MPs ($r = -0.737$, $p = 0.006$) and the change in endothelial-derived MPs ($r = -0.614$, $p = 0.034$).

Conclusions: The vascular endothelium appears to be susceptible to periods of oscillatory shear stress at HA, where impairments in endothelium-dependent vasodilatation may be amplified by endothelial injury. These findings have important implications for understanding the early impact of clinical situations of hypoxaemia on the vascular endothelium.

1. Introduction

The endothelium lines the vasculature and is responsive to its mechanical and chemical environment. Oscillatory, low time-averaged mean haemodynamic shear stress with a high retrograde component is considered atherogenic. For example, arterial regions chronically exposed to low shear stress have an approximately three-fold increased distribution of atherosclerotic lesions [1]. Endothelial dysfunction occurs early in the progression of atherosclerosis [2], and impaired endothelial function has been observed following acute periods of oscillatory shear stress in the conduit arteries of healthy humans. It has been established that 20–30 min of experimentally-induced oscillatory shear stress appears to blunt reactive hyperaemia flow-mediated dilatation (FMD) [3–5] and increase circulating microparticles (MPs) of endothelial origin; this release of MPs can be triggered by a disruption of endothelial cell quiescence, namely cellular activation and apoptosis [6].

Hypoxia has been shown to impair endothelial function and homeostasis. Measures of FMD during acute, passive hypoxic exposure have reported both impairments [7–10], and no change [10,11] compared to pre-hypoxic levels. Multi-day treks to high altitude (HA) have previously been reported to reduce FMD [7,8]. Measures of circulating MPs, as indices of endothelial cell homeostasis, have yielded mixed findings depending on the severity and

Abbreviations: AMS, acute mountain sickness; ANOVA, analysis of variance; FMD, flow-mediated dilatation; HA, high altitude; MPs, microparticles; OSI, oscillatory shear index; SL, sea level; SRAUC, shear rate area under the curve.

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duration of hypoxia. Acute hypoxic breathing (<80 min, simulated 3000 m and 5500 m) increases circulating MPs [12,13]. Circulating phosphatidylserine-positive and platelet-derived MPs were decreased, and circulating endothelial-derived MPs unchanged 24- and 48-h after ascent to a mild hypoxic stimulus of 2590 m [14]. Conversely, a recent study reported significant increases in circulating MPs indicative of cellular activation, but not apoptosis, with increasing altitude over 15 days up to 6200 m [15].

Alterations in shear stress profiles and hypoxic exposure individually disrupt endothelial function, however the interaction between these factors has never been explored. The purpose of this investigation was to assess whether the vascular endothelium is more susceptible to periods of oscillatory shear stress following rapid, inactive ascent to 3800 m. We examined the hypothesis that oscillatory shear stress would elicit a greater reduction in FMD and further increase in circulating MPs at HA compared to sea level (SL).

2. Materials and methods

This study was part of a larger research expedition conducted in October 2015. As such, participants took part in a number of studies (four) conducted at the University of British Columbia – Okanagan (Kelowna, British Columbia) and during two weeks at the Barcroft Station (White Mountain, California, USA). However, the *a priori*, primary research questions addressed in the current paper are novel and are exclusively dealt within this study alone – there is no overlap between this investigation and others completed on the research expedition.

2.1. Participants

Twelve normotensive (systolic blood pressure <140 mmHg, diastolic blood pressure <90 mmHg), nonsmoking participants (three female; 25 ± 1 years) free from known cardiovascular disease and risk factors and not taking medications [besides oral contraceptives (n = 1)]. completed the investigation at SL and HA. All participants were Caucasian lowlanders who had not been exposed to an altitude >3000 m for at least three months prior to the expedition. All participants provided written, informed consent, and protocols were approved by the University of British Columbia Clinical Ethics Review Board in adherence with the principles of the Declaration of Helsinki.

2.2. Study design

The testing protocol was completed on two occasions separated by two-to-five weeks. The first intervention occurred at the University of British Columbia – Okanagan in Kelowna, Canada (344 m, SL) and the second intervention the second (n = 6) or third (n = 6) morning after rapid, inactive ascent to the Barcroft Station (3800 m, HA). Participants were randomized to be tested on the second or third day and were tested at the same time of day at SL and HA to control for diurnal variation. Ten participants drove to the Barcroft Station after spending one night in Palm Springs, California (146 m), and two drove after spending one night in Bishop, California (1260 m). Brachial artery blood pressure, heart rate (HEM-775CAN, Omron Healthcare, USA), and oxyhaemoglobin saturation were measured prior to the intervention. Acute mountain sickness (AMS) was assessed using the Lake Louise AMS scoring system [16]. Diet and was matched and participants refrained from exercise at HA leading up to testing.

All participants refrained from alcohol, exercise and caffeine twelve hours prior to testing and were fasted for a minimum of six hours. The study outline is illustrated in Fig. 1. Participants arrived at the laboratory and were instructed to lay supine for 20 min. A venous blood sample was acquired from the left antecubital vein to measure circulating MPs. Endothelial function was subsequently assessed by reactive hyperaemia FMD, according to internationally recognized guidelines (please see online supplement) [17]. All measurements were performed by the same experienced sonographer with a 10 MHz multifrequency linear array probe (15L4, Terason t3200, Teratech, USA) attached to a high-resolution ultrasound machine (Terason t3200, Teratech, USA). From a previous investigation performed by the same sonographer, the median between-day coefficient of variation was 6.0% and 2.1% for brachial artery FMD and baseline diameter, respectively [18]. Standardized software approaches to acquire and analyze the Doppler ultrasound recordings were employed, as used extensively elsewhere [3,7,19,20]. The angle of insonation for the acquisition of velocity was 60°. Screen capture of the ultrasound was saved as an audio video interleave file (Camtasia Studio, Techsmith Co, Ltd, USA) for future analysis using edge-detection software [21]. A region of interest was placed around the highest quality portion of the B-mode longitudinal image of the artery. A second region of interest surrounded the Doppler strip to record blood velocity. The software automatically and continuously tracks the walls of the vessel and velocity trace within the regions of interest at a frequency 30 Hz and has a mean intraobserver coefficient of variation of 6.7% for brachial artery FMD [21]. Peak diameter was automatically detected using a moving window-smoothing function (smoothed median across time). Following the FMD, a dual-cuffing protocol was adapted from previous investigations [3,6]. One pneumatic cuff (SC5, Hokanson, USA) was placed distal to the epicondyles and inflated to 75 mmHg to provoke oscillatory shear stress and a second pneumatic cuff (SC5, Hokanson) positioned near the axilla inflated to 40 mmHg to facilitate trapping of MPs. The cuffs were inflated for 30 min. A second venous blood sample was drawn at 25 min as described above. Following the 30 min of oscillatory shear stress, the cuffs were deflated and a second reactive hyperaemia FMD performed. One-minute recordings of mean, antegrade

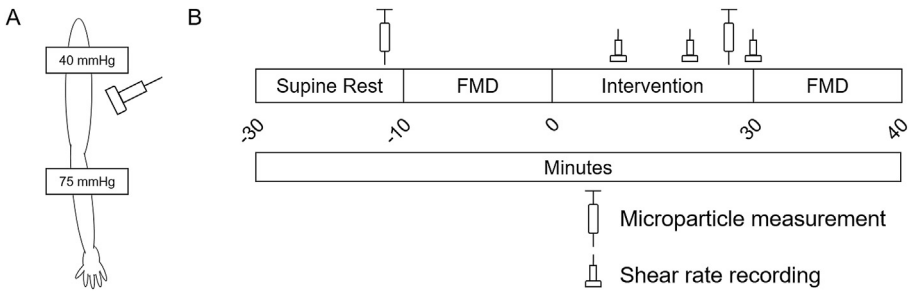

Fig. 1. Schematic of the protocol. (A) Placement of pneumatic cuffs employed to evoke oscillatory shear stress (distal cuff; 75 mmHg) and facilitating trapping of microparticles (proximal cuff; 40 mmHg). (B) Study outline, which was performed identically at sea level and, on the second or third day, at high altitude (3800 m).