Red Blood Cell ATP Release and Vascular Function in Humans

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# Fasudil Study Protocol

Using a double-blind, placebo-controlled, crossover design, subjects were randomized to receive an infusion of either saline (placebo control) or fasudil for their first experimental visit. Subjects then received the opposite treatment for their second experimental visit, with at least five days and no more than two months between the first and second visit. All experimental measures were performed in the same order for each visit within a subject, with the order of hypoxia and graded-intensity rhythmic handgrip exercise trials randomized and counterbalanced between subjects. Arterial stiffness measures (augmentation index and carotid-femoral pulse wave velocity) were performed before and after placement of the venous catheter and 60 min treatment administration. For both the hypoxia and exercise trials, resting hemodynamics were measured for 2-3 min until a steady-state was observed, after which the physiological stimulus was initiated. The hypoxia trial consisted of 3 min of steady-state hypoxia at an oxygen saturation of ~80% as assessed via pulse oximetry on the earlobe ($SpO_2$; plus ~2 min for the normoxia to hypoxia transition). The exercise trial consisted of 4 min at each workload to ensure that steady-state hemodynamics were achieved. Blood samples for plasma [ATP] were taken under steady-state conditions at rest, the end of hypoxia, and the end of each exercise workload.