Title: The effects of graded changes in oxygen and carbon dioxide tension on coronary blood velocity independent of myocardial energy demand

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ABSTRACT

In humans, coronary blood flow is tightly regulated by microvessels within the myocardium in order to match myocardial energy demand. However, evidence regarding inherent sensitivity of the microvessels to changes in arterial partial pressure of carbon dioxide and oxygen is conflicting due to the accompanied changes in myocardial energy requirements. This study aimed to investigate the changes in coronary blood velocity while manipulating partial pressures of end-tidal CO_2 ($P_{ET}CO_2$) and O_2 ($P_{ET}O_2$). It was hypothesized that an increase in $P_{ET}CO_2$ (hypercapnia) or decrease in $P_{ET}O_2$ (hypoxia) would result in a significant increase in mean blood velocity in the left anterior descending artery (LAD_{Vmean}) due to an increase in both blood gases and energy demand associated with the concomitant cardiovascular response. Cardiac energy demand was assessed through non-invasive measurement of the total left ventricular mechanical energy. Healthy subjects (n=13) underwent a euoxic CO_2 test ($P_{ET}CO_2$ = -8, -4, 0, +4, and +8 mmHg from baseline) and an isocapnic hypoxia test ($P_{ET}O_2$ = 64, 52 and 45 mmHg). LAD_{Vmean} was assessed using transthoracic Doppler echocardiography. Hypercapnia evoked a 34.6 ± 8.5% (mean ± SEM; $P<0.01$) increase in mean $LAD_{Vmean,}$ whereas hypoxia increased LAD_{Vmean} by 51.4 ± 8.8% ($P<0.05$). Multiple stepwise regressions revealed that both mechanical energy and changes in arterial blood gases are important contributors to the observed changes in LAD_{Vmean} ($P<0.01$). In summary, regulation of the coronary vasculature in humans is mediated by metabolic changes within the heart and an inherent sensitivity to arterial blood gases.

NEW & NOTEWORTHY

Coronary blood flow in humans is responsive to both changes in cardiac effort and arterial blood gases. Using echocardiographic assessment and a non-invasively derived index of cardiac work, we present an estimation of the relative contributions to coronary reactivity during CO_2 and O_2 challenges.

Key words: coronary vessels, hypoxia, carbon dioxide, echocardiography, dynamic end-tidal forcing

INTRODUCTION

The coronary vasculature is capable of regulating myocardial perfusion to maintain oxygen delivery in the face of changing oxygen demands. The coronary resistance vessels can rapidly dilate in response to local tissue hypoxia thereby increasing coronary blood velocity in the major vessel branches (30, 56). Similarly, in response to increased cardiac energy demand, dilatation of coronary vessels will result in an increase in coronary blood flow to maintain oxygen delivery (59). When investigating the effect of specific stimuli on changes in coronary blood flow, it is vital to consider the stimuli's effect on cardiac energy demand. Increases in cardiac effort manifests as an increase in the rate of muscle contraction and the prevailing afterload, both increasing the overall energy expenditure and therefore coronary blood flow (2, 20, 56). The capacity of the coronary vessels to increase blood flow is often referred to as the coronary flow reserve (CFR) and can be assessed using a number of known stimuli, including alterations in arterial blood gases, such as oxygen (O_2) and carbon dioxide (CO_2). The magnitude of CFR can be used clinically as a predictor of disease states such as impaired left ventricular (LV) function, myocardial ischemia, the severity of stenosis during coronary artery disease and cardiac mortality (14, 31, 33). In addition, CFR can guide clinical decisions regarding patient revascularization (8). Assessing CFR in a clinical population typically involves invasive catheterization and the administration of a pharmacological agent; this study aims to demonstrate the utility of a non-invasive and pharmacological-free assessment of CFR using echocardiography and blood gas control techniques.

Carbon dioxide has long been implicated as a regulator of the coronary arteries in both animal studies and isolated preparations (9). Such studies have correlated an increase in myocardial blood flow with increasing partial pressure of CO_2 (PCO_2) in the coronary sinus (3,

26). A complementary effect was observed in follow-up studies suggesting that a decrease of PCO_2 in the coronary circulation results in an observable decrease in blood flow (3, 6, 50), which has been demonstrated using coronary sinus catheterization in both healthy subjects (36) and those with a stable cardiac disorder (21, 32, 35). Myocardial oxygen consumption (MVO_2) was measured in two of these studies and found that hypocapnia had no significant effect on overall cardiac energy demand (21, 32). These hypercapnic and hypocapnic effects have been repeated in human studies using MRI (4, 54) PET imaging (55) and echocardiography (49); all of which reported the same results as the previous invasive studies. However, some studies involving hypercapnia show either no change in MVO_2 (54) or no change in coronary blood flow after normalizing for the increase in MVO_2 (55), suggesting the inherent sensitivity of the coronary vasculature to changes in PCO_2 remains to be determined.

The limited oxygen extraction reserve of the myocardial tissue paired with an equally limited ability to perform anaerobic respiration causes the heart to rely heavily on changes in coronary blood flow to match MVO_2 (56). MVO_2 is tightly linked to cardiac work and is the main stimulus for changes in coronary blood flow; this recognition led to the development of cardiac work indices in an effort to accurately evaluate coronary reactivity non-invasively (18). RPP is a product of HR and systolic pressure, it was the first index developed and it was adopted in clinical settings due to the simplicity of the measurements involved (18). RPP is still commonly used in coronary vascular studies though it neglects important factors that contribute to MVO_2 such as myocardial elastic potential energy and external work (41). Recently, an index of LV function has been developed using a non-invasive estimation of myocardial elastance that allows an investigator to assess the elastic potential energy of the heart (7). This index is well correlated with invasive measures of myocardial contractility and has been adopted for clinical

assessments of LV function (38, 40, 52). Along with non-invasive left ventricular pressure and volume estimations, it can be used to generate non-invasive pressure volume relationships, which might provide a less variable index of total mechanical energy than RPP. This was the first coronary blood flow study to use this non-invasively derived pressure volume loop as an estimate of cardiac effort.

The purpose of this study was to observe the direct effects of acute isocapnic hypoxia, euoxic hypocapnia, and euoxic hypercapnia, on coronary blood velocity in humans. As well as to quantify the changes in cardiac energy demand as a surrogate for MVO_2 to determine whether the sensitivity of the coronary vasculature to PO_2 and PCO_2 is a product of increased cardiac effort associated with the stimulus itself. It was hypothesized that coronary blood flow would increase from baseline during hypoxia and hypercapnia and that it would decrease during hypocapnia. Secondly, it was hypothesized that cardiac effort would increase during the hypoxic and hypercapnic interventions, and cardiac effort would be the primary contributor to increases in coronary blood velocity.

METHODS

Ethical Approval

All experimental procedures and protocols were submitted to and approved by the Clinical Research Ethics Board at the University of British Columbia and conformed to the Canadian Government Tri-Council Policy Statement on research ethics (TCPS2). All participants provided written informed consent prior to participation in this study.

Participants

All experiments were conducted in the cardiopulmonary laboratory for experimental and applied physiology (Kelowna, BC). Twenty-two male participants were recruited for the current study; eight of these participants were excluded due to an inability to adequately visualize the LAD using color Doppler imaging, through the participant's intercostal acoustic windows. To avoid the known potential sex differences when assessing coronary reactivity, this study included only male participants (22). One participant was excluded for having a resting coronary velocity that exceeded two standard deviations above the group mean. Participants were excluded if they were obese (body mass index $\geq$ 30 kg m^{-2}), had a history of smoking, were hypertensive (systolic blood pressure > 140 mmHg; diastolic blood pressure > 90 mmHg), were on medication or had poor pulmonary function as determined by spirometry (ratio of forced expiratory volume in 1s to forced vital capacity < 75% of predicted). Participants included in the mean analysis were healthy males (n = 13) with no history of cardiovascular, pulmonary or neurological disease.

Experimental Protocol

Participants visited the lab on two separate occasions. On the first visit the participants' height and weight was recorded and spirometry was performed in accordance with the standards set

138 forth by the American Thoracic Society and the European Respiratory Society joint guidelines
139 (28). The participants were also screened using echocardiography to ensure that coronary artery
140 velocity was measureable. Finally, participants were asked to fill out a questionnaire to ensure
141 they met the inclusion criteria (above).

142 Upon arrival for the second visit, participants were instrumented with an
143 electrocardiogram in lead-II configuration, connected to a bio amp (FE132, ADI Instruments,
144 Colorado Springs, CO, USA) to measure instantaneous heart rate (HR). A pulse oximeter
145 (ML320/F, ADI Instruments) was placed on the right index finger to measure arterial
146 oxyhemoglobin saturation (SpO_2). Beat-by-beat systolic (SBP), diastolic (DBP), and mean
147 arterial pressure (MAP) was measured from a cuff placed on the mid-phalanx of the right middle
148 finger using finger pulse photoplethysmography (Finometer PRO; Finapress Medical Systems,
149 Amsterdam, the Netherlands). Return-to-flow calibration was performed prior to every trial in
150 order to calibrate blood pressure to a reconstructed brachial artery waveform (15). The
151 participants wore a nose clamp and breathed through a mouthpiece, bacteriological filter, and a
152 two-way non-rebreathing valve (2600 series, Hans Rudolph, Shawnee, KS, USA).
153 Electrocardiogram, blood pressure, respiratory flow and respired gases (O_2 and CO_2) were
154 acquired at 200 Hz using an analog-to-digital converter (Powerlab/16SP ML 880;
155 ADInstruments) interfaced with a personal computer and analyzed with commercially available
156 software (LabChart V7.1, ADInstruments). Respiratory flow was measured near the mouth
157 using a pneumotachograph (HR 800L, HansRudolph) and a differential pressure transducer
158 (1110 series, HansRudolph), which was zeroed and calibrated using a 3-liter syringe before
159 experimentation. Minute ventilation ($\dot{V}_E$) was calculated as a product of i) tidal volume (V_T),
160 which was determined using an integral of the respiratory flow signal, and ii) breathing

frequency (F_B), defined as the number of breaths per minute. Respired gas partial pressures were sampled near the mouth, dried with nafion tubing in desiccant, and analyzed for end-tidal PO_2 ($P_{ET}O_2$) and PCO_2 ($P_{ET}CO_2$) (ML206, ADinstruments). Gas analyzers were calibrated before and verified following each protocol using a calibration gas with known concentrations of CO_2 and O_2. Time corrections were applied to the PO_2 and PCO_2 signal to account for the gas analyzer delay and values corresponding to the moment of end expiration were identified as the $P_{ET}O_2$ and $P_{ET}CO_2$. A dynamic end-tidal forcing system was used to clamp and manipulate both $P_{ET}O_2$ and $P_{ET}CO_2$ throughout the protocol as previously described (44, 47, 48). After instrumentation, a normoxic CO_2 reactivity test was conducted first, followed by a 30-minute rest and an isocapnic hypoxia reactivity test in succession; the trials were not randomized in order to avoid any carryover effects of hypoxia on sympathetic nervous system activation (53).

Normoxic CO_2 Reactivity. Following instrumentation, participants breathed room air while baseline $P_{ET}CO_2$, $P_{ET}O_2$ and echocardiographic measurements were recorded. Immediately following baseline, $P_{ET}CO_2$ was reduced through active hyperventilation to -8 and -4 mmHg from baseline, after the hypocapnic steps, $P_{ET}CO_2$ was allowed to return to baseline values. The hypercapnic protocol was then initiated, where the participants' $P_{ET}CO_2$ was increased to +4 and +8 mmHg using the end tidal forcing system that continually adjusts CO_2 delivery on a breath-by-breath basis in response to target the desired end tidal values. Each stage lasted for approximately 8 minutes. Throughout the protocol $P_{ET}O_2$ was maintained at normoxic levels. The collection of echocardiographic images began following two minutes of stable end-tidal gases. This protocol was selected because it permitted the assessment of coronary blood velocity throughout the hypo- and hyper-capnia range.

Isocapnic Hypoxia Reactivity. During an initial ten-minute baseline period, resting $P_{ET}O_2$, $P_{ET}CO_2$ and echocardiography measurements were collected. Next, $P_{ET}O_2$ was reduced using the end tidal forcing system that delivers air with a low fraction of O_2 on a breath-by-breath basis in response to target desired end tidal values. The system held $P_{ET}O_2$ at three stages of acute hypoxia (64, 52, 45 mmHg) that lasted 8 minutes, while maintaining isocapnia. Echocardiographic images were collected at each stage following two minutes of stable end-tidal gases. This protocol was selected because it permitted the assessment of coronary blood velocity through the hypoxic range and across a linear change in SpO_2 (37).

Measurements

Echocardiography. All echocardiographic measurements were collected on a commercially available ultrasound system (Vivid E9, GE, Fairfield, CT, USA) using a broadband M5S 5 MHz or a 3V 3D-array transducer. The same trained sonographer collected all the images for the study; the sonographer has previously published test-retest reliability data for structural cardiac measurements (39). The sonographer's reliability in measuring LAD_V were measured in this study and were statistically analyzed using the Cronbach's alpha reliability test intended to determine the correlation of two separate interrogations of the same construct. Based on a sample size of 14, the alpha values were found to be 0.81 and 0.89 for the max and mean velocities respectively, suggesting good consistency between measurements. Images were captured and saved for offline analysis using commercially available software (EchoPAC v.13, GE). All echocardiographic values represent an average value of three cardiac cycles representing the clearest of five collected images for each experimental stage. Echocardiographic measurements are described in detail below. Following instrumentation, the collapsibility index of the inferior vena cava (IVC) was assessed during inspiration as previously

206 described (36) and used to estimate right atrial pressure. An IVC, with an initial diameter ≤
207 2.1cm, that collapses more than 50% are assumed to have a normal right atrial pressure of 3
208 mmHg. Participants were then moved to a left lateral decubitus position for the collection of the
209 remaining measurements.

210 ***Coronary Blood Velocity.*** Left anterior descending (LAD) coronary artery blood velocity
211 (LADv) was measured from the distal section of the LAD using previously described
212 echocardiographic techniques (19, 23, 49). LADv measurements made by transthoracic
213 echocardiography have been previously shown to closely correlate with intracoronary Doppler
214 guide wire measurements (24). LAD_V measurements were obtained during the last minute of
215 baseline and each stage of the normoxic CO_2 reactivity test and the isocapnic hypoxia reactivity
216 test. The LAD was imaged using a modified parasternal short axis view from the fourth or fifth
217 left intercostal space and was assessed using pulsed-wave Doppler. The transducer was
218 positioned such that a 2-3 mm segment of the LAD was imaged along the long-axis taking care
219 to align the pulse-wave cursor with the length of the vessel. With a sample volume (2.0 mm)
220 positioned over the color Doppler signal in the LAD, measurements of the LAD_V were collected
221 during a short end-expiratory apnea. The collected waveforms were analyzed to determine mean
222 diastolic velocity (LAD_{Vmean}) and peak diastolic velocity (LAD_{Vmax}) (Fig. 1). For each stage, the
223 average value for three cardiac cycles is reported. Coronary vascular resistance (CVR) was
224 estimated as MAP/LAD_{Vmean}.

225 ***Pulmonary and Cardiac Hemodynamics.*** 3D triplane assessment of the left ventricle was used
226 to obtain volume measurements; using a 3D-array transducer three two dimensional apical
227 images were simultaneously obtained, 60° adjacent to each other, representing a standard four,
228 three, and two chamber view. The images are then transferred to an offline workstation and

analyzed by a trained investigator. By manually tracking the endocardial border of each image with the analysis software it was possible to estimate the longitudinal geometry of the left ventricle at three adjacent axes from one heart beat. Using the three measurements from the 2D images, the analysis software is able to estimate the end systolic and diastolic volumes by assuming a relatively circular cross-sectional area of the ventricle, similar to the Simpson's biplane method (42). Data was collected from three heartbeats during each condition and averaged to produce one value.

Left Ventricular Energy Demand. Two indices were used in this study in an effort to account for the change in myocardial energy demand and its influence on coronary blood flow. First, the minute mechanical energy of the left ventricle (ME_{LV}) was estimated using a non-invasive method (7) and second, the rate-pressure product (RPP) was calculated. ME_{LV} was calculated using a non-invasive pressure-volume loop and a validated estimate of LV elastance (E_{Nd}) (7). Stroke work is defined as the area within a pressure-volume loop and, assuming that the end-diastolic pressure volume relationship is negligible and constant across experimental conditions, it can be estimated noninvasively by plotting end systolic volume (ESV), end diastolic volume (EDV), SBP and DBP for each condition (7, 44). E_{Nd} was estimated using a model developed from a group-averaged normalized elastance curve value derived from the data of 23 separate studies that employed invasive measurement techniques to obtain LV and aortic pressure-volume relationships (7). Individual elastance values for each subject were estimated and corrected for EF, the ratio of DBP to SBP and the time ratio of the isovolumic contraction period to the total systolic period (7). The isovolumic contraction period and total systolic period can be identified through a Doppler investigation of the aortic outflow in a five-chamber view time aligned to an ECG signal. Potential energy was estimated by plotting E_{Nd} tangentially to the end systolic point

with the area under the slope representing elastic potential energy (Fig. 2). The area under the pressure volume curve represents the energy expended by the heart in one cardiac cycle (PV_A) in ml•mmHg which is then converted to Joules by applying a conversion factor of 1.3 x 10^{-4}. Multiplying the calculated energy by HR produces the rate of total LV mechanical energy generated (ME_{LV}; J/min) (7). RPP was calculated as the product of the average HR and SBP for each experimental stage (18).

Statistical Analysis

Statistical comparisons and calculations were conducted in R (http://cran.r-project.org/). LADv metrics, pulmonary and cardiac hemodynamics and LV work parameters were all compared between each experimental PCO_2 and PO_2 stage using a one-way analysis of variance (ANOVA) with repeated measures. In order to determine the contribution of cardiac work and perfusion pressure on changes in LAD_{Vmean}, an analysis of covariance (ANCOVA) was used to examine changes in LAD_{Vmean} between the experimental stages while using ME_{LV} and MAP as covariates. Tukey's HSD test was applied to all analyses with significant F-ratios to determine which conditions were significantly different. Multiple stepwise regressions were used to determine which variables contribute to the observed changes in LAD_{Vmean} in both CO_2 and O_2 reactivity protocols. The following independent variables were included in the regression equation: SpO_2, $P_{ET}CO_2$, MAP, SBP, DBP, RPP and ME_{LV}. The tolerance to determine the inclusion criteria of an independent variable in the regression model was set at $P < 0.05$. Standardized beta weights were then applied to determine the predictive value of the selected independent variables. The Pearson's product-moment was used to correlate LAD_{Vmean} to both ME_{LV} and RPP as well as to correlate RPP to ME_{LV}. Individual coronary blood velocity reactivity's were calculated as the slope of the linear regression for all three interventions (i.e. hypocapnia, hypercapnia and

hypoxia) by regressing LAD_{Vmean} with either SpO_2 or $P_{ET}CO_2$. All data was presented as mean ± SEM and statistical significance was set at $P < 0.05$ for all comparisons.

RESULTS

Participants.

Participants included (n=13) in the hypercapnic and hypoxic trials had a mean ± SEM age of 25.5 ± 1.4 years, weight of 78.3 ± 2.4 kg, height of 179.8 ± 1.6 cm, and a BMI of 24.2 ± 0.5 kg/m^2. Participants were normotensive (systolic blood pressure = 117 ± 5 mmHg, diastolic blood pressure = 64 ± 3 mmHg) and had a resting heart rate of 55 ± 2 bpm. Subjects had healthy lung function with an average FEV_1 of 4.65 ± 0.72 L (99 ± 2.7% of predicted values) and an FEV_1/FVC ratio that was 99.8 ± 1.5% of predicted values.

Protocol 1: Euoxic Hypocapnia and Hypercapnia.

Table 1 demonstrates the cardiovascular and hemodynamic variables during each stage of hypo- and hyper-capnia. $P_{ET}CO_2$ was well controlled during both hypercapnic and hypocapnic stages and SpO_2 was constant throughout the protocol ($P > 0.9$). LAD_{Vmax} increased significantly above baseline during the +8 mmHg hypercapnic stage ($P < 0.01$), though did not change during hypocapnia ($P > 0.99$). Figure 1 demonstrates representative traces of coronary velocity at baseline and during hypercapnia. Both SBP and DBP were significantly elevated during both hypercapnic stages ($P < 0.02$). There was a tendency for HR to increase during hypercapnia but it did not reach significance ($P > 0.06$). EDV and ESV did not change from baseline during either the hypercapnic and hypocapnic exposure ($P = 0.99$). RPP increased 26.8 ± 7.2% and 41.3 ± 6.5% above baseline with increasing hypercapnia ($P = 0.05$) and did not change during the hypocapnic stages ($P = 0.64$). PV_A displayed an increasing trend through the hypercapnic trials, though did not reach significance ($P = 0.07$). Figure 3 shows the LAD_{Vmean}, MAP, CVR, ME_{LV}, and LAD_{Vmean} as a function of ME_{LV} (i.e. LAD_{Vmean}/ME_{LV}) across the hypocapnic and hypercapnic range. ME_{LV} showed a significant increase ($P = 0.02$) above baseline by 27.9 ± 6.1

% and 42.8 ± 6.5%, corresponding to the increasing levels of CO_2, with no change during hypocapnia (P = 0.61) (Fig. 3). MAP increased above baseline values during mild and moderate hypercapnic exposure (P < 0.01) and was unaltered by hypocapnia (P = 0.3). CVR did not significantly change from baseline (P = 0.57). Mild and moderate hypercapnia (i.e. $P_{ET}CO_2$ = +4, +8 mmHg from baseline) induced significant increases in LAD_{Vmean} from baseline. During hypocapnia, there was no significant change in LAD_{Vmean} (P > 0.99). When indexed against total ME_{LV}, LAD_{Vmean} remained unchanged from baseline. Figure 4 displays the individual and mean coronary reactivity in the hypocapnic and hypercapnic range. Mean coronary reactivity was found to be 0.13 ± 0.13 cm/s/mmHg during hypocapnia and 0.95 ± 0.11 cm/s/mmHg during hypercapnia.

Evaluating the change in LAD_{Vmean} in response to changes in $P_{ET}CO_2$ shows no significant effect when using ME_{LV} or RPP as a covariate (P = 0.24; P = 0.34). Correlating LAD_{Vmean} to ME_{LV} and RPP using the Pearson's product-moment correlation revealed an r-value of 0.57 and 0.55 respectively (P < 0.01). ME_{LV} and RPP were also found to be correlated with an r-value of 0.64 (P < 0.01). Changes in ME_{LV} (P < 0.01) and $P_{ET}CO_2$ (P < 0.01) were identified as the major contributors to changes in LAD_{Vmean} through multiple stepwise regression analysis. Multiple linear regression analysis indicates that 36% of the LAD_{Vmean} response is related to changes in $P_{ET}CO_2$ while 44% of the LAD_{Vmean} response is related to changes in ME_{LV} (R= 0.66; P<0.01).

Protocol 2: Isocapnic Hypoxia

Table 2 demonstrates the cardiovascular and hemodynamic variables at baseline and during each stage of hypoxia. $P_{ET}O_2$ and consequently SpO_2 were well controlled during the three stages of hypoxia, whereas $P_{ET}CO_2$ was held constant throughout the protocol ($P > 0.9$). LAD_{Vmax} was elevated above baseline during both the 52 mmHg and 45 mmHg stages of hypoxia ($P < 0.01$). Systolic blood pressure increased significantly during the 45 mmHg $P_{ET}O_2$ stage while diastolic blood pressure tended to increase, but was not statistically significant ($P < 0.01$; $P = 0.07$). Both the 52 and 45 mmHg $P_{ET}O_2$ stages caused elevations in HR from baseline by 19.7 ± 4.2% ($P = 0.02$) and 29.8 ±5.7% ($P <0.01$) respectively. EDV and ESV did not change from baseline during the hypoxic exposure ($P = 0.99$). RPP was elevated by 30.2 ± 5.0% and 48.3 ± 7.8% ($P < 0.01$) during the 52 and 45 mmHg $P_{ET}O_2$ stages, respectively. PV_A was not significantly elevated during any of the hypoxic trials ($P = 0.4$). Figure 5 shows the LAD_{Vmean}, MAP, CVR, ME_{LV}, and LAD_{Vmean} as a function of ME_{LV} (i.e. LAD_{Vmean}/ME_{LV}) during baseline and across the three stages of hypoxia. ME_{LV} was elevated from baseline during the 52 and 45 mmHg $P_{ET}O_2$ stages of hypoxia ($P < 0.01$). MAP was elevated from baseline during the 45 mmHg $P_{ET}O_2$ stage ($P = 0.02$). CVR decreased significantly from baseline during the 45 mmHg $P_{ET}O_2$ stage ($P = 0.02$). Both the 52 and 45 mmHg $P_{ET}O_2$ stages resulted in a significant increase in LAD_{Vmean} ($P < 0.01$). When indexed against ME_{LV}, LAD_{Vmean} did not significantly change from baseline. Mean coronary reactivity was found to be 0.74 ± 0.09 cm/s/%desaturation during hypoxia (Fig. 6).

Evaluating the changes in LAD_{Vmean} due to decreasing SpO_2 shows no change when using ME_{LV} or RPP as a covariate ($P = 0.67$; 0.26). Pearson's product-moment correlation determined an r value of 0.66 and 0.55 when correlating LAD_{Vmean} to ME_{LV} and RPP respectively ($P < 0.01$). Correlating RPP to ME_{LV} produced an r-value of 0.71 ($P < 0.01$). Using multiple stepwise

regressions analysis, it was determined that changes in ME_{LV} ($P < 0.01$) and SpO_2 ($P < 0.01$) were the major contributors to increases in LAD_{Vmean}. Multiple linear regression analysis implies that 38% of the LAD_{Vmean} response is related to changes in SpO_2 while 45% of the LAD_{Vmean} response is related to changes in ME_{LV} ($R = 0.73$; $P < 0.01$). It is important to note that all of the analyses were completed using LAD_{Vmean} as the outcome variable, though a similar result is found using LAD_{Vmax} (see tables 1 & 2).

DISCUSSION

This is the first study to quantify the changes in LADv in young, healthy humans exposed to euoxic hypo- and hyper-capnia, and isocapnic hypoxia while non-invasively estimating total left ventricular mechanical energy. The main findings of this study suggest that i) exposure to hypercapnia (+4, +8 mmHg) significantly elevates both LAD_{Vmean} and ME_{LV} while hypocapnia did not, ii) hypoxia (52, 45 mmHg $P_{ET}O_2$ stages) elicits an increase in both LAD_{Vmean} and ME_{LV}, and iii) the sensitivity of the LAD to hypercapnia and hypoxia in humans is the combination of the inherent vascular sensitivity to CO_2 and O_2, and the increase in total mechanical energy due to the cardiovascular responses associated with each stressor.

Response to Carbon Dioxide

A small number of studies have assessed the effects of hypercapnia on the coronary vasculature in humans and although it is generally accepted that an increase in blood flow occurs, there is not yet agreement on the specific contributions of vessel sensitivity to CO_2 and the associated increase in MVO_2. The current study found increases in absolute LAD_V in response to hypercapnia, which is consistent with previous studies in both healthy and clinical populations (4, 21, 49, 55). An observed increase in cardiac effort, represented by ME_{LV} and RPP, suggests that vessel sensitivity to CO_2 is not the sole stimulus for the observed increase in LAD_{Vmean}. Previous reports have suggested that there is no change in coronary blood flow in response to hypercapnia when controlling for cardiac effort (55). The accepted conclusion drawn by most animal and human studies is that the sensitivity of the coronary vasculature to hypercapnia is orders of magnitudes less than its sensitivity to hypercapnic-induced increases in cardiac work (10, 34, 55). Using ME_{LV} or RPP as index of work and calculating the change in the ratio of work to changes in LAD_{Vmean} yielded no significant change in velocity, similar to previous work.

However, there is previous evidence of a coronary response to hypercapnia with no change in cardiac effort while using direct measurements of MVO_2, suggesting that non-invasive methods used to correct for the change in cardiac energy demand may not be valid (3, 21). Indeed, the correlations between LAD_{Vmean} and RPP or ME_{LV} resulted in relatively weak relationships (r = 0.55; 0.66), inferring the need for a more detailed analysis to determine the exact individual contributions of cardiac effort and changes in $P_{ET}CO_2$. Likewise, the relationship between RPP and ME_{LV} can only account for some of the variability between two variables meant to serve as a surrogate for the same parameter (i.e. MVO_2). Multiple stepwise regression analysis determined that of all the implicated variables (SpO_2, $P_{ET}CO_2$, MAP, SBP, DBP, RPP and ME_{LV}), ME_{LV} and $P_{ET}CO_2$ are the only significant predictors of changes in LAD_{Vmean}. Using standardized beta weights, it also determined that the contributions of $P_{ET}CO_2$ ($\beta = 0.36$) are relatively comparable to those of ME_{LV} ($\beta = 0.44$), contrary to previous reports. When compared to the cerebrovascular bed, absolute coronary reactivity was found to be of a similar magnitude (~4-5%/mmHg) (1, 4). Though considering the fact that 44% of the hypercapnic reactivity is due to increased cardiac effort, it can be surmised that the cerebrovasculature is more sensitive to hypercapnia than the coronary circuit. The brachial artery has been found to be less sensitive to hypercapnia than cerebral vessels. However, when comparing relative reactivity, our results as well as other published results suggest that the coronary vessels are the least sensitive of the three (51).

Hypocapnia did not elicit any significant changes in any hemodynamic or cardiovascular variables. Most hypocapnic human studies have determined that a decrease in arterial partial pressure of CO_2 through voluntary or mechanical hyperventilation results in a corresponding decrease in coronary blood flow in both healthy subjects and those with coronary artery disease

or a cardiac disorder (21, 33, 35). MVO_2 remains unchanged during the hypocapnic exposure in these investigations, implicating the involvement of another mechanism in the control of coronary blood flow outside of myocardial energy expenditure (21, 32). The leftward shift of the oxyhemoglobin dissociation curve during alkalosis leading to an increase in oxygen extraction could offer an explanation for the observed decrease in coronary flow during hypocapnia previously observed (21, 35). Similar to previous work we found no significant change in cardiac effort; however, in contrast we observed no change in LAD_{Vmean} during hypocapnia. The duration of exposure to hypocapnia (21) or the magnitude of hypocapnia (32) may explain this discrepancy between studies.

Response to Hypoxia

Few studies have characterized the vasodilatory effects of hypoxia on the human coronary circulation; those that have are in agreement with animal studies. Similar to previous work, this study found a significant increase in LAD_{Vmean} (4, 29). Hypoxia causes a dissociation reaction in oxyhemoglobin that ultimately results in the release of ATP into the vessel lumen; it, along with its metabolites (ADP, AMP, Adenosine) activate endothelial adenosine receptors which result in smooth muscle relaxation and ultimately vessel dilation (11). This hypoxic mechanism can occur via systemic hypoxia or cardiac workload induced hypoxemia; for this reason, during an experimental hypoxic challenge, it is particularly difficult to demarcate the specific contributions of cardiac effort and the applied hypoxia (45, 46). Regardless, using an index of cardiac effort, human investigations have determined that hypoxia causes a significant increase in coronary flow above and beyond the changes in cardiac effort (4, 29). Investigations aimed at correlating coronary reactivity to cerebrovascular reactivity during hypoxia have found a similar response to decreased arterial partial pressure of O_2 in both circulations when coronary reactivity is

normalized to cardiac work (4). Compared to the previous human studies that did not report any changes in the cardiovascular or hemodynamic variables, our study found increases in SBP, MAP and HR during hypoxia. Considering these studies report a significant increase in RPP, it can be surmised that an increasing trend in HR, MAP and SBP was likely observed during hypoxia. The results presented here determine that an increase in cardiac effort, estimated by both ME_{LV} and RPP, occurs during hypoxia and that indexing either RPP or ME_{LV} to LAD_{Vmean} results in no significant change with increasing hypoxia. Similar to the hypercapnic trials, a weak correlation is found between LAD_{Vmean} and RPP or ME_{LV} ($r = 0.55$; 0.66) suggesting that using a ratio of LAD_{Vmean} to either measure of cardiac effort might be an over simplified solution. Stepwise multiple regressions of all possible contributors (SpO_2, $P_{ET}CO_2$, MAP, SBP, DBP, RPP and ME_{LV}) to changes in LAD_{Vmean} determined that SpO_2 and ME_{LV} were the only significant predictors. Calculating standardized units, it was determined that SpO_2 and ME_{LV} were relatively equal predictors for changes in LAD_{Vmean} at approximately 38% and 45% respectively. This information implies that ME_{LV} is likely a more sensitive estimate of cardiac effort than RPP and that blood pressure which contributes by both influencing cardiac afterload and coronary perfusion pressure does not appear to improve the model more than ME_{LV} alone. Based on previous reports and the data from this study, the cerebral and brachial circulation have a larger reactivity to hypoxia (~1.3%/%SpO_2; ~1.5%/%SpO_2 respectively) than the coronary vessels (~0.7%/%SpO_2) (25, 27).

Limitations

Doppler investigation of the coronary artery acts as a useful index of actual blood flow. However, B-mode images of the vessel itself lack the spatial resolution to measure the diameter. It is therefore not possible to directly quantify flow through our transthoracic ultrasound

442 approach. However, it has been demonstrated that the majority of vessel dilation occurs in the
443 downstream microvessels and Doppler investigation of coronary blood velocity has been shown
444 to be highly correlated to actual coronary flow (17, 56). Recently, measurements of coronary
445 blood velocity at baseline and during isocapnic hypoxia ($P_{ET}O_2$ = 45 mmHg) were made in
446 healthy participants and cross-sectional area of the LAD was acquired in three subjects using
447 cardiac MRI (13). LAD_{Vmean} was similar between imaging modalities and the cross-sectional
448 area at baseline (22.3 ± 4.5 mm^2) did not differ from hypoxia (22.4 ± 5.3 mm^2). These data,
449 albeit, in a small subset of healthy humans suggests that our measures of LADv are reflective of
450 blood flow due to downstream microvessel dilation. To ensure the Doppler measure was
451 accurately estimating blood velocity, effort was made to align the long axis of the vessel with the
452 pulse wave cursor.

453 The non-invasive pressure-volume loop used to calculate ME_{LV} requires the assumption
454 that the end diastolic pressure volume relationship (EDPVR) is equal to zero.
455 Though untrue, it is a reasonable assumption for two reasons: (1) the study was designed for
456 within subject comparisons which effectively nullifies any differences in actual EDPVR between
457 subjects and (2) it is unlikely that, during either protocol, the compliance of the myocardium was
458 altered significantly, therefore there should not be any variability in EDPVR within subject. A
459 further limitation of the non-invasively pressure-volume loop regards the use of a reconstructed
460 blood pressure waveform from the finger as an estimate of systolic and diastolic left ventricular
461 pressures. All non-invasive estimations of cardiac workload are associated with assumptions,
462 though it is undoubtedly an important consideration for evaluating LV function in both clinical
463 and research settings. Simple indices such as RPP or the pressure work index offer quick
464 estimations of cardiac effort using easily obtained measurements (HR, SBP, DBP), but their

465 derivations lack certain variables that contribute to total cardiac effort (18). ME_{LV} employs
466 certain important components of cardiac effort such as left ventricular end-systolic elastance and
467 measured ventricular volumes. As demonstrated here RPP follows a similar trend in response to
468 stimuli as ME_{LV} though they are weakly correlated during hypercapnia and hypoxia ($r = 0.64$;
469 0.71). Further stepwise regression analysis demonstrated the increased sensitivity of ME_{LV} in
470 predicting the observed changes in coronary blood flow, which suggests that using a
471 comprehensive non-invasive index of cardiac effort that includes multiple parameters can offer
472 more insight into cardiac work.

473 In conclusion this study demonstrated that coronary blood flow is influenced nearly
474 equally by both arterial blood gases and the associated changes in cardiac effort during
475 hypercapnia and hypoxia. This is a novel finding considering that to date, the majority of
476 investigations of human coronary vascular response to hypercapnia and hypoxia have concluded
477 that the changes in coronary flow are solely due to the increases in cardiac work and not the
478 manipulations of arterial blood gases. Furthermore, it was demonstrated that ME_{LV} is a more
479 accurate estimate of cardiac effort than RPP, which is far more commonly used. Considering the
480 profound effect cardiac workload has on coronary blood flow, using an index that includes more
481 information regarding the mechanical parameters of the heart could improve precision when
482 measuring coronary vessel sensitivity. Given the previously observed sex differences in
483 coronary reactivity, future directions would aim to extend this work to quantify and compare the
484 response differences between males and females.

ACKNOWLEDGEMENTS

We are grateful to our subjects volunteering their time to complete this study.

GRANTS

Natural Sciences and Engineering Research Council of Canada and Canada Foundation for Innovation

DISCLOSURES

None

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655 **TABLES & FIGURES**

Table 1. Cardiovascular and hemodynamic responses to hypo- and hypercapnia

	Baseline	-8 mmHg	-4 mmHg	+4 mmHg	+8 mmHg
LAD_{Vmax} (cm/s)	30.6 ± 1.1	30.4 ± 1.5	31.1 ± 1.1	36.0 ± 1.9	40.7 ± 1.8 *
$P_{ET}CO_2$ (mmHg)	40.9 ± 1.0	33.5 ± 0.9 *	37.5 ± 0.6 *	45.3 ± 0.8 *	48.8 ± 0.8 *
$P_{ET}O_2$ (mmHg)	93.7 ± 1.7	92.5 ± 1.5	92.8 ± 1.9	93.9 ± 1.9	94.5 ± 2.2
SpO_2 (%)	97.2 ± 0.3	97.7 ± 0.2	97.4 ± 0.3	97.5 ± 0.3	97.2 ± 0.4
SBP (mmHg)	110 ± 4	118 ± 3	119 ± 3	125 ± 3 *	134 ± 4 *
DBP (mmHg)	59 ± 2	62 ± 2	64 ± 2	68 ± 2 *	73 ± 2 *
HR (/min)	58 ± 3	61 ± 3	57 ± 2	65 ± 3	67 ± 2
ESV (ml)	48.1 ± 3.2	48.9 ± 2.8	49.8 ± 2.9	49.5 ± 2.5	46.6 ± 2.3
EDV (ml)	109.3 ± 5.6	109.6 ± 5.1	108.9 ± 5.4	108.1 ± 5.5	108.3 ± 5.6
RPP (mmHg•beats/min)	6352 ± 391	7226 ± 467	6758 ± 345	8056 ± 457 *	8975 ± 411 *
PV_A (J)	0.63 ± 0.04	0.67 ± 0.03	0.69 ± 0.03	0.72 ± 0.04	0.77 ± 0.04

Abbreviations: LAD_{Vmax}, peak diastolic velocity of the left anterior descending coronary artery; $P_{ET}CO_2$, end-tidal partial pressure of CO_2; $P_{ET}O_2$, end-tidal partial pressure of O_2; SpO_2, oxygen saturation of hemoglobin; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; ESV, end systolic volume; EDV, end diastolic volume; RPP, rate pressure product; PV_A, energy expended by the heart in one cardiac cycle. *P<0.05, compared to baseline

656

Table 2. Cardiovascular and hemodynamic responses to hypoxia

	Baseline	64 mmHg	52 mmHg	45 mmHg
LAD_{Vmax} (cm/s)	29.3 ± 1.5	34.1 ± 1.2	40.6 ± 3.2*	44.9 ± 1.6*
$P_{ET}CO_2$ (mmHg)	40.4 ± 0.6	40.9 ± 0.7	40.5 ± 0.7	40.2 ± 0.7
$P_{ET}O_2$ (mmHg)	93.4 ± 1.4	64.5 ± 0.4 *	53.0 ± 0.30 *	46.3 ± 0.3 *
SpO_2 (%)	97.7 ± 0.3	92.7 ± 0.4 *	86.3 ± 0.5 *	79.9 ± 0.6 *
SBP (mmHg)	117 ± 5	127 ± 3	129 ± 3	135 ± 3 *
DBP (mmHg)	64 ± 3	70 ± 3	71 ± 3	74 ± 3
HR (/min)	55 ± 2	61 ± 2	66 ± 2 *	71 ± 3 *
ESV (ml)	47.1 ± 2.4	47.7 ± 2.7	47.2 ± 3.0	46.7 ± 2.6
EDV (ml)	106.7 ± 5.6	107.0 ± 5.9	109.3 ± 6.0	105.4 ± 5.6
RPP (mmHg•beats/min)	6505 ± 447	7801 ± 374	8469 ± 326 *	9648 ± 508 *
PV_A (J)	0.67 ± 0.04	0.74 ± 0.04	0.75 ± 0.04	0.77 ± 0.04

Abbreviations: LAD_{Vmax}, peak diastolic velocity of the left anterior descending coronary artery; $P_{ET}CO_2$, end-tidal partial pressure of CO_2; $P_{ET}O_2$, end-tidal partial pressure of O_2; SpO_2, oxygen saturation of hemoglobin; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; ESV, end systolic volume; EDV, end diastolic volume; RPP, rate pressure product; PV_A, energy expended by the heart in one cardiac cycle. *P<0.05, compared to baseline

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Figure Legends

Figure 1. Representative pulsed wave Doppler recording from the left anterior descending coronary artery. (A) Baseline Doppler waveform. (B) Doppler waveform recorded during mild hypercapnia ($P_{ET}CO_2$ = baseline + 4 mmHg)

Figure 2. Non-invasive pressure volume loop during resting conditions. Derived from Echocardiographic volume measurements and photoplethysmography blood pressure measurements. The area of the pressure volume loop represents stroke work (SW), the slope plotted tangentially to the systolic mean represents LV elastance (E_{nd}) and the area beneath it represents potential energy (PE). Data is represented as mean ± SEM.

Figure 3. Cardiovascular and hemodynamic responses to hypo- and hypercapnia. The relationships between (A) LAD_{Vmean}, (B) mean arterial pressure (MAP), (C) coronary vascular resistance (CVR), (D) total LV mechanical energy (ME_{LV}) during the hypo- and hypercapnic trials. All values are mean ± SEM. *P < 0.05, compared to baseline.

Figure 4. Coronary reactivity to hypo- and hypercapnia for individual subjects. Correlation of LAD_{Vmean} and $P_{ET}CO_2$. Dotted lines represent individual sensitivities and solid line represents mean sensitivity.

Figure 5. Cardiovascular and hemodynamic responses to hypoxia. The relationships between (A) LAD_{Vmean}, (B) mean arterial pressure (MAP), (C) coronary vascular resistance (CVR), (D) total LV mechanical energy (ME_{LV}) during the hypo- and hypercapnic trials. All values are mean ± SEM. *P < 0.05, compared to baseline.

Figure 6. Coronary reactivity to hypoxia for individual subjects. Correlation of LAD_{Vmean} and $P_{ET}O_2$. Dotted lines represent individual sensitivities and solid line represents mean sensitivity.

Figure 1

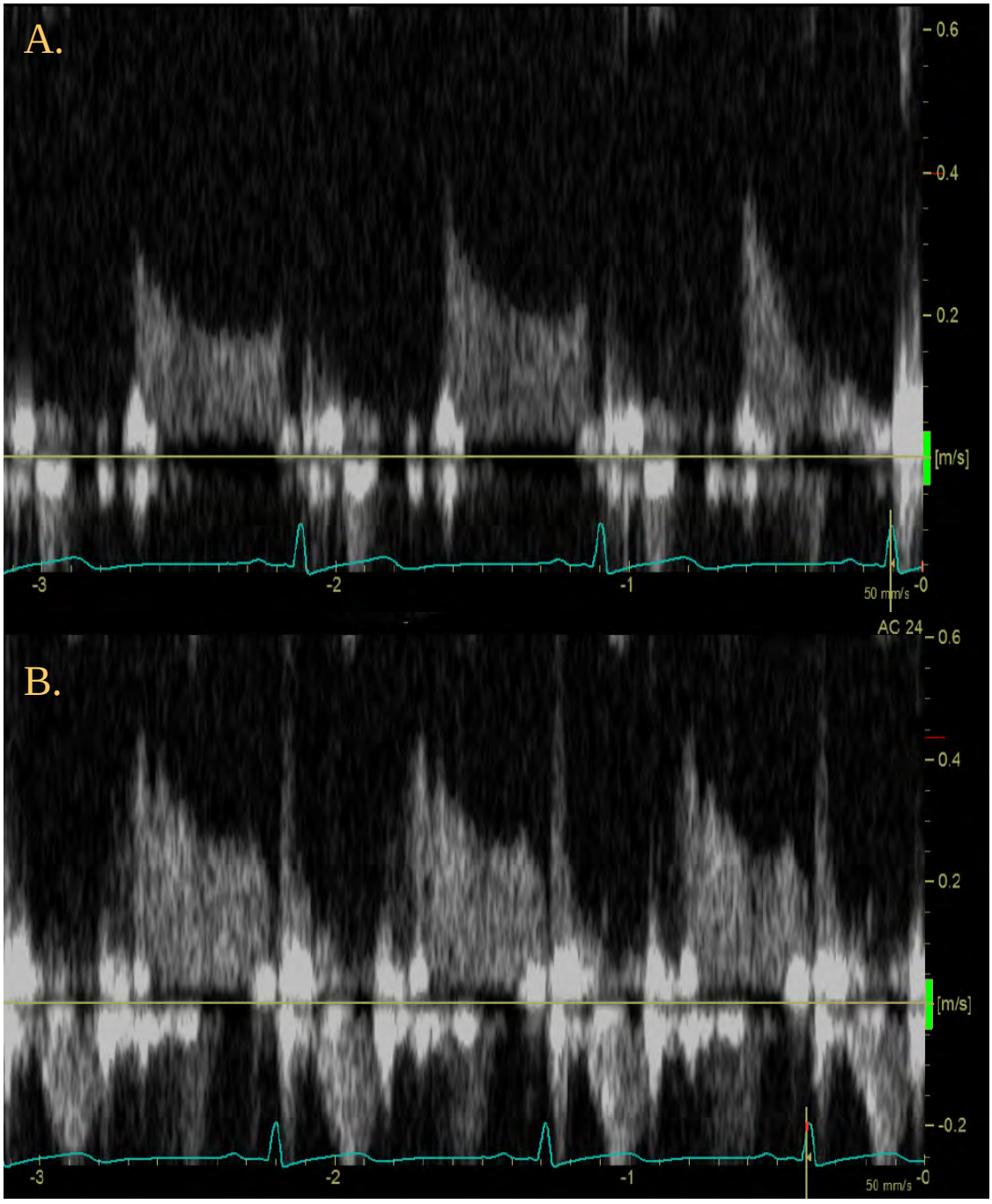


Figure 2

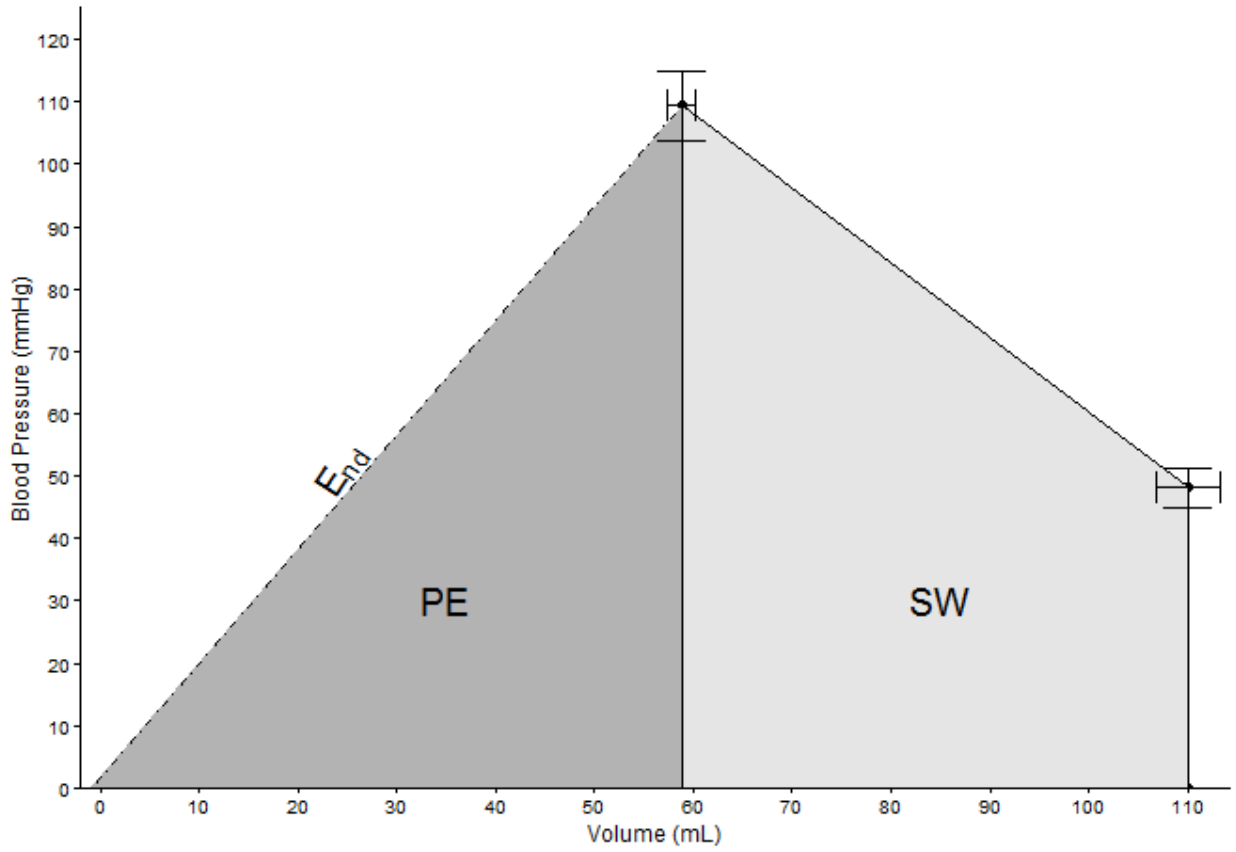

Blood Pressure (mmHg)
120
110
100
90
80
70
60
50
40
30
20
10
0
E_{end}
PE
SW
0
10
20
30
40
50
60
70
80
90
100
110
Volume (mL)

Figure 3

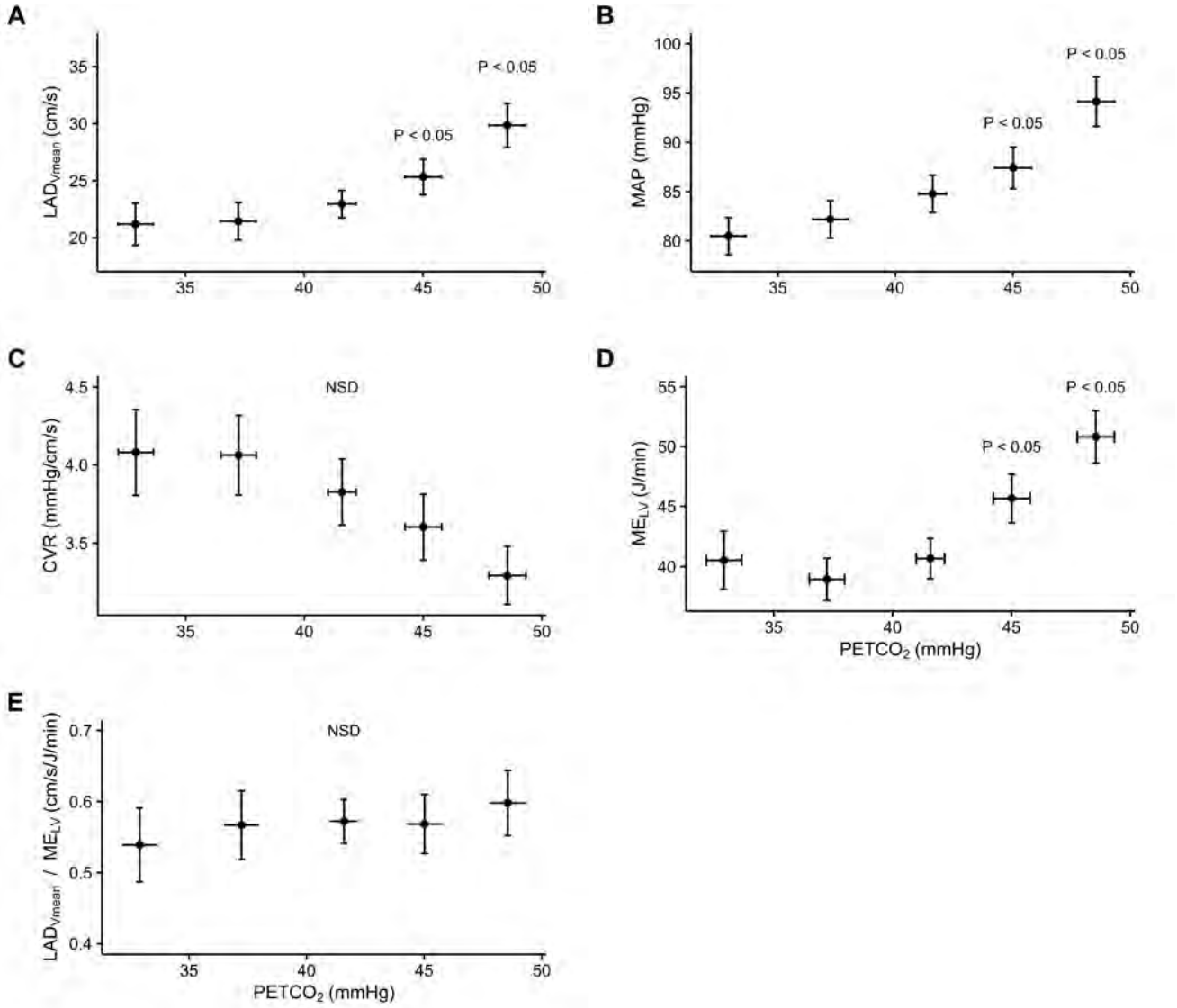

A
LADVmean (cm/s)
P < 0.05
P < 0.05
B
MAP (mmHg)
P < 0.05
P < 0.05
C
CVR (mmHg/cm/s)
NSD
D
MELV (J/min)
P < 0.05
P < 0.05
PETCO2 (mmHg)
E
LADVmean / MELV (cm/s/J/min)
NSD
PETCO2 (mmHg)

Figure 4

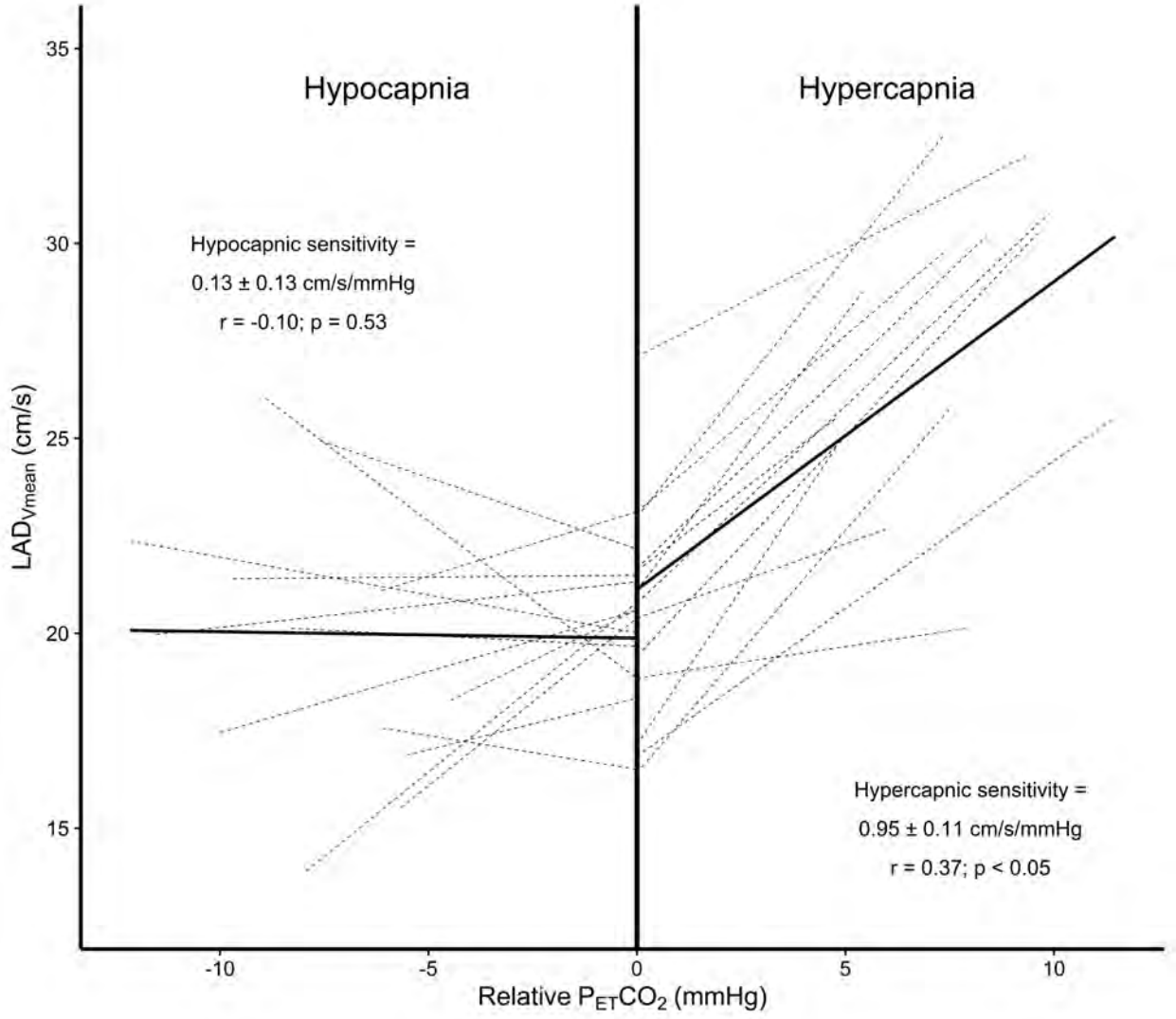

Hypocapnia
Hypercapnia
Hypocapnic sensitivity =
0.13 ± 0.13 cm/s/mmHg
r = -0.10; p = 0.53
Hypercapnic sensitivity =
0.95 ± 0.11 cm/s/mmHg
r = 0.37; p < 0.05
LADVmean (cm/s)
Relative PETCO2 (mmHg)
35
30
25
20
15
-10
-5
0
5
10

Figure 5

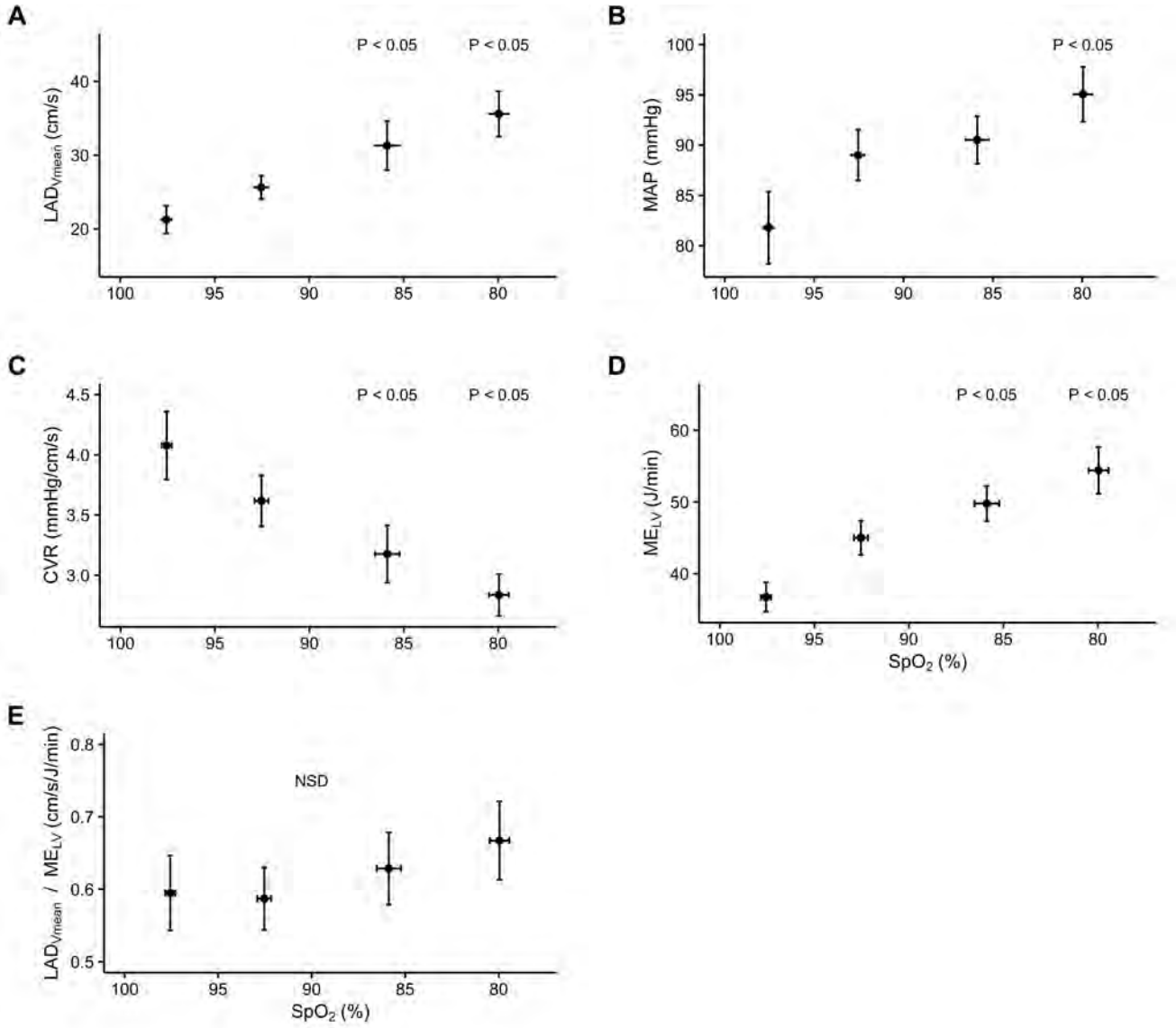
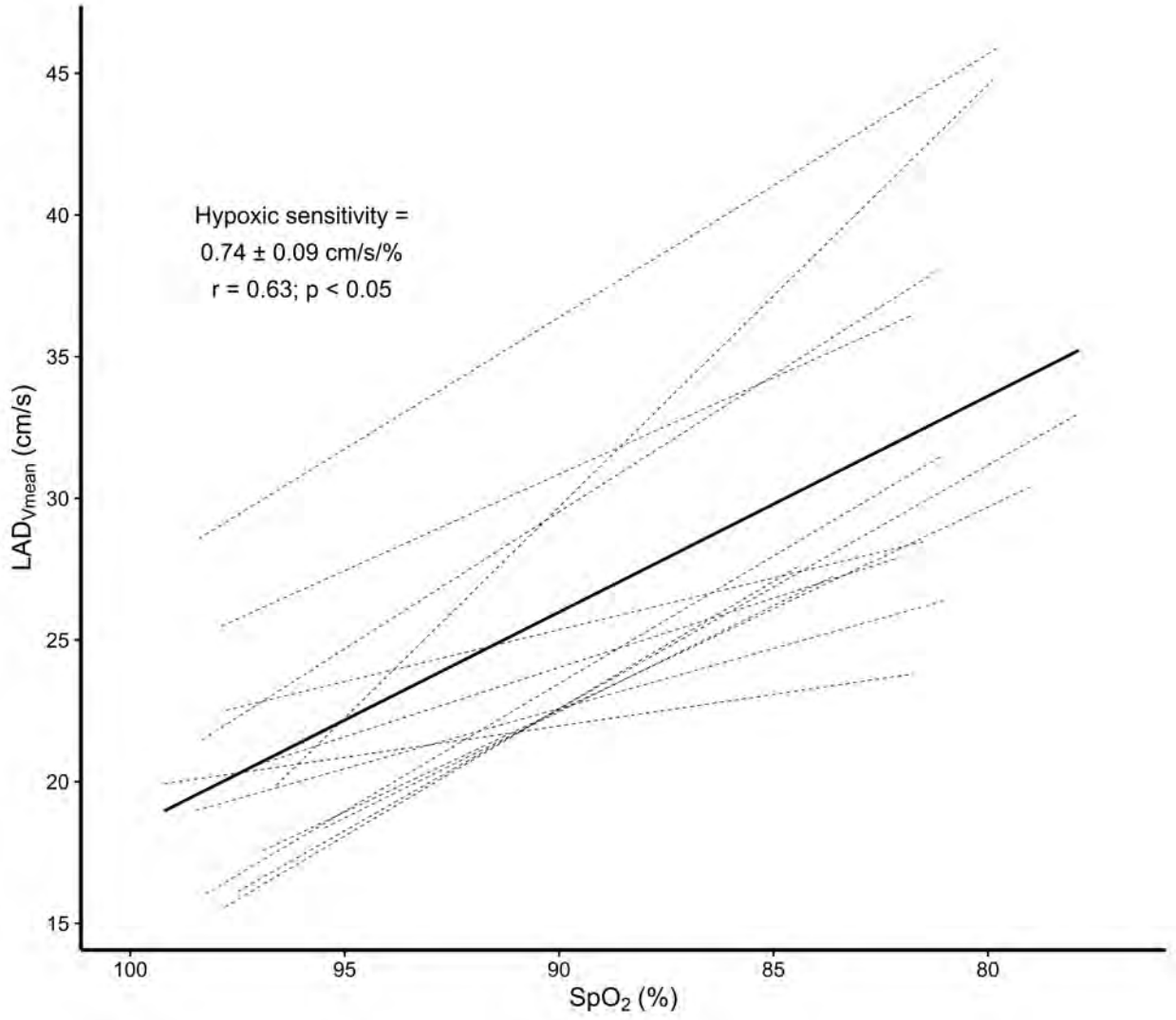

A
P < 0.05
P < 0.05
LADVmean (cm/s)
40
30
20
100
95
90
85
80
B
P < 0.05
MAP (mmHg)
100
95
90
85
80
C
P < 0.05
P < 0.05
CVR (mmHg/cm/s)
4.5
4.0
3.5
3.0
D
P < 0.05
P < 0.05
MELV (J/min)
60
50
40
SpO2 (%)
E
NSD
LADVmean / MELV (cm/s/J/min)
0.8
0.7
0.6
0.5
SpO2 (%)

Figure 6


Hypoxic sensitivity =
0.74 ± 0.09 cm/s/%
r = 0.63; p < 0.05
LADVmean (cm/s)
SpO2 (%)
45
40
35
30
25
20
15
100
95
90
85
80