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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Running head: Coronary response to O₂ & CO₂ in humans
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 CO₂ (P_{ETCO₂}) and O₂ (P_{ETO₂}). It was hypothesized that an increase in P_{ETCO₂} (hypercapnia) or decrease in P_{ETO₂} (hypoxia) would result in a significant increase in mean blood velocity in the left anterior descending artery (LADV_{mean}) 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 CO₂ test (P_{ETCO₂} = -8, -4, 0, +4, and +8 mmHg from baseline) and an isocapnic hypoxia test (P_{ETO₂} = 64, 52 and 45 mmHg). LADV_{mean} was assessed using transthoracic Doppler echocardiography. Hypercapnia evoked a 34.6 ± 8.5% (mean ± SEM; P<0.01) increase in mean LADV_{mean}, whereas hypoxia increased LADV_{mean} 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 LADV_{mean} (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.
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 CO₂ and O₂ 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,
A complementary effect was observed in follow-up studies suggesting that a decrease of PCO₂ 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 (MVO₂) 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 MVO₂ (54) or no change in coronary blood flow after normalizing for the increase in MVO₂ (55), suggesting the inherent sensitivity of the coronary vasculature to changes in PCO₂ 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 MVO₂ (56). MVO₂ 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 MVO₂ 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 MVO₂ to determine whether the sensitivity of the coronary vasculature to PO₂ and PCO₂ 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 ≥ 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
by the American Thoracic Society and the European Respiratory Society joint guidelines (28). The participants were also screened using echocardiography to ensure that coronary artery velocity was measureable. Finally, participants were asked to fill out a questionnaire to ensure they met the inclusion criteria (above).

Upon arrival for the second visit, participants were instrumented with an electrocardiogram in lead-II configuration, connected to a bio amp (FE132, ADI Instruments, Colorado Springs, CO, USA) to measure instantaneous heart rate (HR). A pulse oximeter (ML320/F, ADI Instruments) was placed on the right index finger to measure arterial oxyhemoglobin saturation (SpO₂). Beat-by-beat systolic (SBP), diastolic (DBP), and mean arterial pressure (MAP) was measured from a cuff placed on the mid-phalanx of the right middle finger using finger pulse photoplethysmography (Finometer PRO; Finapress Medical Systems, Amsterdam, the Netherlands). Return-to-flow calibration was performed prior to every trial in order to calibrate blood pressure to a reconstructed brachial artery waveform (15). The participants wore a nose clamp and breathed through a mouthpiece, bacteriological filter, and a two-way non-rebreathing valve (2600 series, Hans Rudolph, Shawnee, KS, USA). Electrocardiogram, blood pressure, respiratory flow and respired gases (O₂ and CO₂) were acquired at 200 Hz using an analog-to-digital converter (Powerlab/16SP ML 880; ADInstruments) interfaced with a personal computer and analyzed with commercially available software (LabChart V7.1, ADInstruments). Respiratory flow was measured near the mouth using a pneumotachograph (HR 800L, HansRudolph) and a differential pressure transducer (1110 series, HansRudolph), which was zeroed and calibrated using a 3-liter syringe before experimentation. Minute ventilation (V̇E) was calculated as a product of i) tidal volume (VT), which was determined using an integral of the respiratory flow signal, and ii) breathing
frequency (F<sub>B</sub>), 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<sub>2</sub> (P<sub>ET</sub>O<sub>2</sub>) and PCO<sub>2</sub> (P<sub>ET</sub>CO<sub>2</sub>) (ML206, ADinstruments). Gas analyzers were calibrated before and verified following each protocol using a calibration gas with known concentrations of CO<sub>2</sub> and O<sub>2</sub>. Time corrections were applied to the PO<sub>2</sub> and PCO<sub>2</sub> signal to account for the gas analyzer delay and values corresponding to the moment of end expiration were identified as the P<sub>ET</sub>O<sub>2</sub> and P<sub>ET</sub>CO<sub>2</sub>. A dynamic end-tidal forcing system was used to clamp and manipulate both P<sub>ET</sub>O<sub>2</sub> and P<sub>ET</sub>CO<sub>2</sub> throughout the protocol as previously described (44, 47, 48). After instrumentation, a normoxic CO<sub>2</sub> 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<sub>2</sub> Reactivity.** Following instrumentation, participants breathed room air while baseline P<sub>ET</sub>CO<sub>2</sub>, P<sub>ET</sub>O<sub>2</sub> and echocardiographic measurements were recorded. Immediately following baseline, P<sub>ET</sub>CO<sub>2</sub> was reduced through active hyperventilation to -8 and -4 mmHg from baseline, after the hypocapnic steps, P<sub>ET</sub>CO<sub>2</sub> was allowed to return to baseline values. The hypercapnic protocol was then initiated, where the participants’ P<sub>ET</sub>CO<sub>2</sub> was increased to +4 and +8 mmHg using the end tidal forcing system that continually adjusts CO<sub>2</sub> 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<sub>ET</sub>O<sub>2</sub> 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
described (36) and used to estimate right atrial pressure. An IVC, with an initial diameter ≤ 2.1 cm, that collapses more than 50% are assumed to have a normal right atrial pressure of 3 mmHg. Participants were then moved to a left lateral decubitus position for the collection of the remaining measurements.

**Coronary Blood Velocity.** Left anterior descending (LAD) coronary artery blood velocity (LADv) was measured from the distal section of the LAD using previously described echocardiographic techniques (19, 23, 49). LADv measurements made by transthoracic echocardiography have been previously shown to closely correlate with intracoronary Doppler guide wire measurements (24). LADv measurements were obtained during the last minute of baseline and each stage of the normoxic CO₂ reactivity test and the isocapnic hypoxia reactivity test. The LAD was imaged using a modified parasternal short axis view from the fourth or fifth left intercostal space and was assessed using pulsed-wave Doppler. The transducer was positioned such that a 2-3 mm segment of the LAD was imaged along the long-axis taking care to align the pulse-wave cursor with the length of the vessel. With a sample volume (2.0 mm) positioned over the color Doppler signal in the LAD, measurements of the LADv were collected during a short end-expiratory apnea. The collected waveforms were analyzed to determine mean diastolic velocity (LADvmean) and peak diastolic velocity (LADvmax) (Fig. 1). For each stage, the average value for three cardiac cycles is reported. Coronary vascular resistance (CVR) was estimated as MAP/LADvmean.

**Pulmonary and Cardiac Hemodynamics.** 3D triplane assessment of the left ventricle was used to obtain volume measurements; using a 3D-array transducer three two dimensional apical images were simultaneously obtained, 60° adjacent to each other, representing a standard four, 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$_{ETCO_2}$, 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 \( LAD_{\text{mean}} \) with either \( \text{SpO}_2 \) or \( P_{\text{ETCO}_2} \). 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/m². 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 FEV₁ of 4.65 ± 0.72 L (99 ± 2.7% of predicted values) and an FEV₁/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 hypoxic and hypercapnia. \( P_{ET}CO_2 \) was well controlled during both hypercapnic and hypocapnic stages and \( \text{SpO}_2 \) was constant throughout the protocol (P > 0.9). \( \text{LAD}_{v,\text{max}} \) 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). \( PVA \) displayed an increasing trend through the hypercapnic trials, though did not reach significance (P = 0.07). Figure 3 shows the \( \text{LAD}_{v,\text{mean}} \), MAP, CVR, ME_LV, and \( \text{LAD}_{v,\text{mean}} \) as a function of ME_LV (i.e. \( \text{LAD}_{v,\text{mean}}/\text{ME}_L \)) 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 CO₂, 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< sub>ETCO₂ = +4, +8 mmHg from baseline) induced significant increases in LAD<sub>mean</sub> from baseline. During hypocapnia, there was no significant change in LAD<sub>mean</sub> (P > 0.99). When indexed against total ME<sub>LV</sub>, LAD<sub>mean</sub> 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<sub>mean</sub> in response to changes in P<sub>ETCO₂</sub> shows no significant effect when using ME<sub>LV</sub> or RPP as a covariate (P = 0.24; P = 0.34). Correlating LAD<sub>mean</sub> to ME<sub>LV</sub> and RPP using the Pearson's product-moment correlation revealed an r-value of 0.57 and 0.55 respectively (P < 0.01). ME<sub>LV</sub> and RPP were also found to be correlated with an r-value of 0.64 (P < 0.01). Changes in ME<sub>LV</sub> (P < 0.01) and P<sub>ETCO₂</sub> (P < 0.01) were identified as the major contributors to changes in LAD<sub>mean</sub> through multiple stepwise regression analysis. Multiple linear regression analysis indicates that 36% of the LAD<sub>mean</sub> response is related to changes in P<sub>ETCO₂</sub> while 44% of the LAD<sub>mean</sub> response is related to changes in ME<sub>LV</sub> (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. \( P_{ETO2} \) and consequently \( SpO2 \) were well controlled during the three stages of hypoxia, whereas \( P_{ETCO2} \) was held constant throughout the protocol (\( P > 0.9 \)). \( LAD_{\text{Vmax}} \) 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 \( P_{ETO2} \) 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 \( P_{ETO2} \) 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 \( P_{ETO2} \) stages, respectively. \( PVA \) was not significantly elevated during any of the hypoxic trials (\( P = 0.4 \)). Figure 5 shows the \( LAD_{\text{Vmean}} \), MAP, CVR, \( ME_{LV} \), and \( LAD_{\text{Vmean}} \) as a function of \( ME_{LV} \) (i.e. \( LAD_{\text{Vmean}}/ME_{LV} \)) during baseline and across the three stages of hypoxia. \( ME_{LV} \) was elevated from baseline during the 52 and 45 mmHg \( P_{ETO2} \) stages of hypoxia (\( P < 0.01 \)). MAP was elevated from baseline during the 45 mmHg \( P_{ETO2} \) stage (\( P = 0.02 \)). CVR decreased significantly from baseline during the 45 mmHg \( P_{ETO2} \) stage (\( P = 0.02 \)). Both the 52 and 45 mmHg \( P_{ETO2} \) stages resulted in a significant increase in \( LAD_{\text{Vmean}} \) (\( P < 0.01 \)). When indexed against \( ME_{LV} \), \( LAD_{\text{Vmean}} \) 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 \( LAD_{\text{Vmean}} \) due to decreasing \( SpO2 \) 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 \( LAD_{\text{Vmean}} \) 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 LADV_{mean}. Multiple linear regression analysis implies that 38% of the LADV_{mean} response is related to changes in SpO_2 while 45% of the LADV_{mean} 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 LADV_{mean} as the outcome variable, though a similar result is found using LADV_{max} (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 \( \text{LAD}_{\text{mean}} \) and ME$_{\text{LV}}$ while hypocapnia did not, ii) hypoxia (52, 45 mmHg \( \text{PETO}_2 \) stages) elicits an increase in both \( \text{LAD}_{\text{mean}} \) and ME$_{\text{LV}}$, 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$_{\text{LV}}$ and RPP, suggests that vessel sensitivity to CO$_2$ is not the sole stimulus for the observed increase in LAD$_{\text{Vmean}}$. 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$_{\text{LV}}$ or RPP as index of work and calculating the change in the ratio of work to changes in LAD$_{\text{Vmean}}$ 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$_{V_{\text{mean}}}$ 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$_{ETCO_2}$. 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$_{ETCO_2}$, MAP, SBP, DBP, RPP and ME$_{LV}$), ME$_{LV}$ and
P$_{ETCO_2}$ are the only significant predictors of changes in LAD$_{V_{\text{mean}}}$. Using standardized beta
weights, it also determined that the contributions of P$_{ETCO_2}$ ($\beta = 0.36$) 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). MVO₂ 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 LADVmean 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 LADVmean (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 O₂ 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 $MELV_{LV}$ and RPP, occurs during hypoxia and that indexing either RPP or $MELV_{LV}$ 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 $MELV_{LV}$ ($r = 0.55; 0.66$) 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 $MELV_{LV}$) to changes in $LAD_{Vmean}$ determined that $SpO_2$ and $MELV_{LV}$ were the only significant predictors. Calculating standardized units, it was determined that $SpO_2$ and $MELV_{LV}$ were relatively equal predictors for changes in $LAD_{Vmean}$ at approximately $38\%$ and $45\%$ respectively. This information implies that $MELV_{LV}$ 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 $MELV_{LV}$ alone. Based on previous reports and the data from this study, the cerebral and brachial circulation have a larger reactivity to hypoxia ($\sim 1.3\%/\%SpO_2; \sim 1.5\%/\%SpO_2$ respectively) than the coronary vessels ($\sim 0.7\%/\%SpO_2$) (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...
approach. However, it has been demonstrated that the majority of vessel dilation occurs in the
downstream microvessels and Doppler investigation of coronary blood velocity has been shown
to be highly correlated to actual coronary flow (17, 56). Recently, measurements of coronary
blood velocity at baseline and during isocapnic hypoxia ($P_{ET}O_2 = 45$ mmHg) were made in
healthy participants and cross-sectional area of the LAD was acquired in three subjects using
cardiac MRI (13). LAD$_{\text{vmean}}$ was similar between imaging modalities and the cross-sectional
area at baseline ($22.3 \pm 4.5$ mm$^2$) did not differ from hypoxia ($22.4 \pm 5.3$ mm$^2$). These data,
albeit, in a small subset of healthy humans suggests that our measures of LAD$_{\text{v}}$ are reflective of
blood flow due to downstream microvessel dilation. To ensure the Doppler measure was
accurately estimating blood velocity, effort was made to align the long axis of the vessel with the
pulse wave cursor.

The non-invasive pressure-volume loop used to calculate ME$_{LV}$ requires the assumption
that the end diastolic pressure volume relationship (EDPVR) is equal to zero. Though untrue, it is a reasonable assumption for two reasons: (1) the study was designed for
within subject comparisons which effectively nullifies any differences in actual EDPVR between
subjects and (2) it is unlikely that, during either protocol, the compliance of the myocardium was
altered significantly, therefore there should not be any variability in EDPVR within subject. A
further limitation of the non-invasively pressure-volume loop regards the use of a reconstructed
blood pressure waveform from the finger as an estimate of systolic and diastolic left ventricular
pressures. All non-invasive estimations of cardiac workload are associated with assumptions,
though it is undoubtedly an important consideration for evaluating LV function in both clinical
and research settings. Simple indices such as RPP or the pressure work index offer quick
estimations of cardiac effort using easily obtained measurements (HR, SBP, DBP), but their
derivations lack certain variables that contribute to total cardiac effort (18). \( ME_{LV} \) employs certain important components of cardiac effort such as left ventricular end-systolic elastance and measured ventricular volumes. As demonstrated here RPP follows a similar trend in response to stimuli as \( ME_{LV} \) though they are weakly correlated during hypercapnia and hypoxia (\( r = 0.64; 0.71 \)). Further stepwise regression analysis demonstrated the increased sensitivity of \( ME_{LV} \) in predicting the observed changes in coronary blood flow, which suggests that using a comprehensive non-invasive index of cardiac effort that includes multiple parameters can offer more insight into cardiac work.

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


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

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>-8 mmHg</th>
<th>-4 mmHg</th>
<th>+4 mmHg</th>
<th>+8 mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>LADV_{max} (cm/s)</td>
<td>30.6 ± 1.1</td>
<td>30.4 ± 1.5</td>
<td>31.1 ± 1.1</td>
<td>36.0 ± 1.9</td>
<td>40.7 ± 1.8 *</td>
</tr>
<tr>
<td>PETCO₂ (mmHg)</td>
<td>40.9 ± 1.0</td>
<td>33.5 ± 0.9 *</td>
<td>37.5 ± 0.6 *</td>
<td>45.3 ± 0.8 *</td>
<td>48.8 ± 0.8 *</td>
</tr>
<tr>
<td>PETO₂ (mmHg)</td>
<td>93.7 ± 1.7</td>
<td>92.5 ± 1.5</td>
<td>92.8 ± 1.9</td>
<td>93.9 ± 1.9</td>
<td>94.5 ± 2.2</td>
</tr>
<tr>
<td>SpO₂ (%)</td>
<td>97.2 ± 0.3</td>
<td>97.7 ± 0.2</td>
<td>97.4 ± 0.3</td>
<td>97.5 ± 0.3</td>
<td>97.2 ± 0.4</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>110 ± 4</td>
<td>118 ± 3</td>
<td>119 ± 3</td>
<td>125 ± 3 *</td>
<td>134 ± 4 *</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>59 ± 2</td>
<td>62 ± 2</td>
<td>64 ± 2</td>
<td>68 ± 2 *</td>
<td>73 ± 2 *</td>
</tr>
<tr>
<td>HR (/min)</td>
<td>58 ± 3</td>
<td>61 ± 3</td>
<td>57 ± 2</td>
<td>65 ± 3</td>
<td>67 ± 2</td>
</tr>
<tr>
<td>ESV (ml)</td>
<td>48.1 ± 3.2</td>
<td>48.9 ± 2.8</td>
<td>49.8 ± 2.9</td>
<td>49.5 ± 2.5</td>
<td>46.6 ± 2.3</td>
</tr>
<tr>
<td>EDV (ml)</td>
<td>109.3 ± 5.6</td>
<td>109.6 ± 5.1</td>
<td>108.9 ± 5.4</td>
<td>108.1 ± 5.5</td>
<td>108.3 ± 5.6</td>
</tr>
<tr>
<td>RPP (mmHg*beats/min)</td>
<td>6352 ± 391</td>
<td>7226 ± 467</td>
<td>6758 ± 345</td>
<td>8056 ± 457 *</td>
<td>8975 ± 411 *</td>
</tr>
<tr>
<td>PV_{A} (J)</td>
<td>0.63 ± 0.04</td>
<td>0.67 ± 0.03</td>
<td>0.69 ± 0.03</td>
<td>0.72 ± 0.04</td>
<td>0.77 ± 0.04</td>
</tr>
</tbody>
</table>

Abbreviations: LADV_{max}, peak diastolic velocity of the left anterior descending coronary artery; PETCO₂, end-tidal partial pressure of CO₂; PETO₂, end-tidal partial pressure of O₂; SpO₂, 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.
Table 2. Cardiovascular and hemodynamic responses to hypoxia

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>64 mmHg</th>
<th>52 mmHg</th>
<th>45 mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>LADVmax (cm/s)</td>
<td>29.3 ± 1.5</td>
<td>34.1 ± 1.2</td>
<td>40.6 ± 3.2*</td>
<td>44.9 ± 1.6*</td>
</tr>
<tr>
<td>PETCO2 (mmHg)</td>
<td>40.4 ± 0.6</td>
<td>40.9 ± 0.7</td>
<td>40.5 ± 0.7</td>
<td>40.2 ± 0.7</td>
</tr>
<tr>
<td>PETO2 (mmHg)</td>
<td>93.4 ± 1.4</td>
<td>64.5 ± 0.4*</td>
<td>53.0 ± 0.30 *</td>
<td>46.3 ± 0.3 *</td>
</tr>
<tr>
<td>SpO2 (%)</td>
<td>97.7 ± 0.3</td>
<td>92.7 ± 0.4 *</td>
<td>86.3 ± 0.5 *</td>
<td>79.9 ± 0.6 *</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>117 ± 5</td>
<td>127 ± 3</td>
<td>129 ± 3</td>
<td>135 ± 3 *</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>64 ± 3</td>
<td>70 ± 3</td>
<td>71 ± 3</td>
<td>74 ± 3</td>
</tr>
<tr>
<td>HR (/min)</td>
<td>55 ± 2</td>
<td>61 ± 2</td>
<td>66 ± 2 *</td>
<td>71 ± 3 *</td>
</tr>
<tr>
<td>ESV (ml)</td>
<td>47.1 ± 2.4</td>
<td>47.7 ± 2.7</td>
<td>47.2 ± 3.0</td>
<td>46.7 ± 2.6</td>
</tr>
<tr>
<td>EDV (ml)</td>
<td>106.7 ± 5.6</td>
<td>107.0 ± 5.9</td>
<td>109.3 ± 6.0</td>
<td>105.4 ± 5.6</td>
</tr>
<tr>
<td>RPP (mmHg•beats/min)</td>
<td>6505 ± 447</td>
<td>7801 ± 374</td>
<td>8469 ± 326 *</td>
<td>9648 ± 508 *</td>
</tr>
<tr>
<td>PVA (J)</td>
<td>0.67 ± 0.04</td>
<td>0.74 ± 0.04</td>
<td>0.75 ± 0.04</td>
<td>0.77 ± 0.04</td>
</tr>
</tbody>
</table>

Abbreviations:  LADVmax, peak diastolic velocity of the left anterior descending coronary artery; PETCO2, end-tidal partial pressure of CO2; PETO2, end-tidal partial pressure of O2; SpO2, 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; PVA, energy expended by the heart in one cardiac cycle.  *P<0.05, compared to baseline
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 ($P_{ET}CO_2 = \text{baseline} + 4 \text{ mmHg}$)

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) $LADV_{\text{mean}}$, (B) mean arterial pressure (MAP), (C) coronary vascular resistance (CVR), (D) total LV mechanical energy ($MELV$) 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 $LADV_{\text{mean}}$ 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) $LADV_{\text{mean}}$, (B) mean arterial pressure (MAP), (C) coronary vascular resistance (CVR), (D) total LV mechanical energy ($MELV$) 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 $LADV_{\text{mean}}$ and $P_{ET}O_2$. Dotted lines represent individual sensitivities and solid line represents mean sensitivity.
Figure 1

A.

B.
Figure 3

A

B

C

D

E

LAD/cm^2 (cm/s)

MAP (mmHg)

CVR (mmHg/cm/s)

MEV (J/min)

LAD/MEV (cm^2/J/min)

P < 0.05

PETCO2 (mmHg)

PETCO2 (mmHg)

PETCO2 (mmHg)

PETCO2 (mmHg)
Figure 4

Hypocapnia

Hypocapnic sensitivity =
0.13 ± 0.13 cm/s/mmHg
r = -0.10; p = 0.53

Hypercapnia

Hypercapnic sensitivity =
0.95 ± 0.11 cm/s/mmHg
r = 0.37; p < 0.05
Figure 5

A

LAD (cm/s)

20

30

40

100 95 90 85 80

P < 0.05  P < 0.05

B

MAP (mmHg)

100 95 90 85 80

P < 0.05

C

CVR (mmHg/cm/s)

4.5

4.0

3.5

3.0

100 95 90 85 80

P < 0.05  P < 0.05

D

ME (J/min)

60

50

40

30

100 95 90 85 80

P < 0.05  P < 0.05

E

LAD / ME (cm/s/J/min)

0.8

0.7

0.6

0.5

100 95 90 85 80

NSD
Figure 6

Hypoxic sensitivity = 0.74 ± 0.09 cm/s/%
r = 0.63; p < 0.05