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

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Submitted 1 February 2016; accepted in final form 19 May 2016

Boulet LM, Stembridge M, Tymko MM, Tremblay JC, Foster GE. The effects of graded changes in oxygen and carbon dioxide tension on coronary blood velocity independent of myocardial energy demand. Am J Physiol Heart Circ Physiol 311: H326–H336, 2016. First published May 23, 2016; doi:10.1152/ajpheart.00107.2016.—In humans, coronary blood flow is tightly regulated by microvessels within the myocardium 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 because of 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 (PETCO₂) and O₂ (PETO₂). It was hypothesized that an increase in PETCO₂ (hypercapnia) or decrease in PETO₂ (hypoxia) would result in a significant increase in mean blood velocity in the left anterior descending artery (LADVmean) due to an increase in both blood gases and energy demand associated with the concomitant cardiovascular response. Cardiac energy demand was assessed through noninvasive measurement of the total left ventricular mechanical energy. Healthy subjects (n = 13) underwent a euoxic CO₂ test (PETCO₂ = −8, −4, 0, +4, and +8 mmHg from baseline) and an isocapnic hypoxia test (PETO₂ = 64, 52, and 45 mmHg). LADVmean was assessed using transthoracic Doppler echocardiography. Hypercapnia evoked a 34.6 ± 8.5% (mean ± SE; P < 0.01) increase in mean LADVmean, whereas hypoxia increased LADVmean 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 LADVmean (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 vessels; hypoxia; carbon dioxide; echocardiography; dynamic end-tidal forcing

NEW & NOTEWORTHY

Coronary blood flow in humans is responsive to both changes in cardiac effort and arterial blood gases. Using echocardiographic assessment and a noninvasively derived index of cardiac work, we present an estimation of the relative contributions to coronary reactivity during CO₂ and O₂ challenges.

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 the effect of specific stimuli on changes in coronary blood flow is investigated, it is vital to consider the effect of stimuli on cardiac energy demand. Increases in cardiac effort manifest 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 (O₂) and carbon dioxide (CO₂).

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 noninvasive and pharmacological-free assessment of CFR using echocardiography and blood gas control techniques.

Carbon dioxide has long been implicated as a regulator of myocardial blood flow with increasing partial pressure of CO₂ (PCO₂) in the coronary sinus (3, 26). 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 in-
volving 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 indexes in an effort to accurately evaluate coronary reactivity noninvasively (18). Rate pressure product (RPP) is a product of heart rate (HR) and systolic pressure, it was the first index developed, and it was adopted in clinical settings because of the simplicity of the measurements involved (18). RPP is still commonly used in coronary vascular studies, although it neglects important factors that contribute to MVO2 such as myocardial elastic potential energy and external work (41). Recently, an index of left ventricular (LV) function has been developed using a noninvasive 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 noninvasive LV pressure and volume estimations, it can be used to generate noninvasive 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 noninvasively 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 hypcapnia, 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. Second, 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.

MATERIALS AND 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 (TCP52). All participants provided written informed consent before participation in this study.

Participants

All experiments were conducted in the cardiopulmonary laboratory for experimental and applied physiology (Kelowna, BC, Canada). Twenty-two male participants were recruited for the current study; eight of these participants were excluded because of 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 (BMI ≥ 30 kg/m2), 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 1 s 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 were recorded and spirometry was performed in accordance with the standards set forth 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) to measure instantaneous 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 blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) were measured from a cuff placed on the midphalanx of the right middle finger using finger pulse photoplethysmography (Finometer PRO; Finapress Medical Systems, Amsterdam, Netherlands). Return-to-flow calibration was performed before every trial 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 nonrebreathing valve (2600 series; Hans Rudolph, Shawnee, KS). Electrocardiogram, blood pressure, respiratory flow, and expired 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 V7.0; 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-l syringe before experimentation. Minute ventilation (Vt) was calculated as a product of 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 PO2 (PETO2) and PCO2 (PETCO2) (ML206; ADInstruments). Gas analyzers were calibrated before and verified after each protocol using a calibration gas with known concentrations of CO2 and O2. Time corrections were applied to the PETO2 and PETCO2 signal to account for the gas analyzer delay, and values corresponding to the moment of end expiration were identified as the PETO2 and PETCO2. A dynamic end-tidal forcing system was used to clamp and manipulate both PETO2 and PETCO2 throughout the protocol as previously described (44, 47, 48). After instrumentation, a normoxic CO2 reactivity test was conducted first, followed by a 30-min rest and an isocapnic hypoxia reactivity test in succession; the trials were not randomized to avoid any carryover effects of hypoxia on sympathetic nervous system activation (53).

Normoxic CO2 reactivity. After instrumentation, participants breathed room air while baseline PETCO2, PETO2, and echocardiographic measurements were recorded. Immediately after baseline, PETCO2 was reduced through active hyperventilation to −8 and −4 mmHg from baseline; after the hypocapnic steps, PETCO2 was allowed
to return to baseline values. The hypercapnic protocol was then initiated, where the participants’ PETCO2 was increased to +4 and +8 mmHg using the end-tidal forcing system that continually adjusts CO2 delivery on a breath-by-breath basis to target the desired end-tidal values. Each stage lasted for ~8 min. Throughout the protocol, PETO2 was maintained at normoxic levels. The collection of echocardiographic images began following 2 min of stable end-tidal gases. This protocol was selected because it permitted the assessment of coronary blood velocity throughout the hypo- and hypercapnia range.

Isocapnic hypoxia reactivity. During an initial 10-min baseline period, resting PETO2, PETCO2, and echocardiography measurements were collected. Next, PETO2 was reduced using the end-tidal forcing system that delivers air with a low fraction of O2 on a breath-by-breath basis to target desired end-tidal values. The system held PETO2 at three stages of acute hypoxia (64, 52, 45 mmHg) that lasted 8 min while maintaining isocapnia. Echocardiographic images were collected at each stage following 2 min 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 Spo2 (37).

Measurements

Echocardiography. All echocardiographic measurements were collected on a commercially available ultrasound system (Vivid E9; GE, Fairfield, CT) using a broadband MSS 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 LADV was measured in this study and was 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% is 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 invasive 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- to 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.

Fig. 1. Representative pulsed wave Doppler recording from the left anterior descending coronary artery. A: baseline Doppler waveform. B: Doppler waveform recorded during mild hypercapnia (PETCO2 = baseline + 4 mmHg).

Pulmonary and cardiac hemodynamics. Three-dimensional triplane assessment of the left ventricle was used to obtain volume measurements; with use of 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. Because the endocardial border of each image was manually tracked with the analysis software, it was possible to estimate the longitudinal geometry of the left ventricle at three adjacent axes from one heartbeat. With use of the three measurements from the two-dimensional images, the analysis software is able to estimate the end-systolic and end-diastolic volumes by assuming a relatively circular cross-sectional area of the ventricle, similar to the Simpson’s biplane method (42). Data were collected from three heartbeats during each condition and averaged to produce one value.

LV energy demand. Two indexes 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 (MELV) was estimated using a noninvasive method (7). Second, the RPP was calculated. MEV was calculated using a noninvasive pressure-volume loop and a validated estimate of LV elastance (Esv) (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). Esv was estimated using a model developed from a group-averaged normalized elastance curve value derived from the data of 23 separate studies that used invasive measurement techniques to obtain LV and aortic pressure-volume relationships (7). Individual elastance values for each subject were estimated and corrected for ejection fraction, 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 $E_{iso}$ 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 (PVA) in milliliters per millimeters of mercury, which is then converted to Joules by applying a conversion factor of $1.3 \times 10^{-4}$. Multiplying the calculated energy by HR produces the rate of total LV mechanical energy generated ($ME_{LV}$; in 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/). LAD$_V$ 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 ANOVA with repeated measures. To determine the contribution of cardiac work and perfusion pressure on changes in LAD$_V$mean, an analysis of covariance (ANCOVA) was used to examine changes in LAD$_V$mean between the experimental stages while using ME$_{LV}$ and MAP as covariates. Tukey’s HSD test was applied to all analyses with significance criteria of an independent variable in the regression model was set at $P < 0.05$ for all comparisons.

Multiple stepwise regressions were used to determine which variables contribute to the observed changes in LAD$_V$mean in both CO$_2$ and O$_2$ reactivity protocols. The following independent variables were included in the regression equation: SpO$_2$, PETCO$_2$, 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$_V$mean to both ME$_{LV}$ and RPP as well as to correlate RPP to ME$_{LV}$. Individual coronary blood velocity reactivity was calculated as the slope of the linear regression for all three interventions (i.e., hypocapnia, hypercapnia, and hypoxia) by regressing LAD$_V$mean with either SpO$_2$ or PETCO$_2$. All data was presented as means ± SE, 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 ± SE 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 (SBP = 117 ± 5 mmHg and DBP = 64 ± 3 mmHg) and had a resting heart rate of 55 ± 2 beats/min. 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: Eucoxic Hypocapnia and Hypercapnia**

Table 1 demonstrates the cardiovascular and hemodynamic variables during each stage of hypoxic and hypercapnic. PETCO$_2$ 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 ($P < 0.01$), although it 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 hypercapnic stages ($P = 0.64$). PVA displayed an increasing trend through the hypercapnic trials, although it 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., PETCO$_2$ = +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$^{-1}$·mmHg$^{-1}$ during hypocapnia and 0.95 ± 0.11 cm$^{-1}$·mmHg$^{-1}$ during hypercapnia.

Evaluating the change in LAD$_{Vmean}$ in response to changes in PETCO$_2$ 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 $r$ values 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 PETCO$_2$ ($P < 0.01$) were identified as the major contributors to changes in LAD$_{Vmean}$ through multiple stepwise regression

Fig. 2. Noninvasive 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 ($E_{iso}$), and the area beneath it represents potential energy (PE). Data are represented as means ± SE.

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analysis. Multiple linear regression analysis indicates that 36% of the LADVmean response is related to changes in PETCO2, whereas 44% of the LADVmean response is related to changes in MELV (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. PETO2 and consequently SpO2 were well controlled during the three stages of hypoxia, whereas PETCO2 was held constant throughout the protocol (P = 0.9). LADVmax was elevated above baseline during both the 52 mmHg and 45 mmHg stages of hypoxia (P < 0.01). SBP increased significantly during the 45 mmHg PETO2 stage, whereas DBP tended to increase, but was not statistically significant (P = 0.07). Both the 52 and 45 mmHg PETO2 stages caused elevations in HR from baseline by 19.7% (P < 0.01) and 29.8% (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% (P = 0.01) and 48.3% (P = 0.01) during the 52 and 45 mmHg PETO2 stages, respectively. PVA was not significantly elevated during any of the hypoxic trials (P = 0.4). Figure 5 shows the LADVmean, MAP, CVR, ME_LV, and LADVmean as a function of ME_LV (i.e., LADVmean/ME_LV) during baseline and across the three stages of hypoxia. ME_LV was elevated from baseline during the 52 and 45 mmHg PETO2 stages of hypoxia.

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>+8 mmHg</th>
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</thead>
<tbody>
<tr>
<td>LADVmax, 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>
</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>
</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>
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<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>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>110 ± 4</td>
<td>118 ± 3</td>
<td>119 ± 3</td>
<td>125 ± 3*</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>59 ± 2</td>
<td>62 ± 2</td>
<td>64 ± 2</td>
<td>68 ± 2*</td>
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<tr>
<td>HR/min</td>
<td>58 ± 3</td>
<td>61 ± 3</td>
<td>57 ± 2</td>
<td>65 ± 3</td>
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<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>
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<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>
</tr>
<tr>
<td>RPP, mmHg·beats/min</td>
<td>6,352 ± 391</td>
<td>7,226 ± 467</td>
<td>6,758 ± 345</td>
<td>8,056 ± 457*</td>
</tr>
<tr>
<td>PVA, 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>
</tr>
</tbody>
</table>

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 1 cardiac cycle. *P < 0.05, compared with baseline.

Fig. 3. Cardiovascular and hemodynamic responses to hypo- and hypercapnia. The relationships between LADVmean (A), mean arterial pressure (MAP; B), coronary vascular resistance (CVR; C), total left ventricular (LV) mechanical energy (ME_LV; D), and LADVmean per ME_LV (E) during the hypoxic and hypercapnic trials are shown. All values are means ± SE. P values show post hoc comparison to baseline (i.e., middle data point). NSD, not significantly different.
(P < 0.01). MAP was elevated from baseline during the 45 mmHg PETO2 stage (P = 0.02). CVR decreased significantly from baseline during the 45 mmHg PETO2 stage (P = 0.02). Both the 52 and 45 mmHg PETO2 stages resulted in a significant increase in LAD Vmean (P < 0.01). When indexed against MELV, LADVmean 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 SpO2 shows no change when using ME L V or RPP as a covariate (P = 0.67; 0.26). Pearson’s product-moment correlation determined r values of 0.66 and 0.55 when correlating LAD Vmean to ME L V and RPP, respectively (P < 0.01). Correlating RPP to ME L V produced an r value of 0.71 (P < 0.01). With the use of multiple stepwise regressions analysis, it was determined that changes in ME L V (P < 0.01) and SpO2 (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 SpO2, whereas 45% of the LAD Vmean response is related to changes in ME L V (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, although a similar result is found using LAD Vmax (see Tables 1 and 2).

**DISCUSSION**

This is the first study to quantify the changes in LADv in young, healthy humans exposed to euoxic hypo- and hypercapnia and isocapnic hypoxia while noninvasively estimating total left ventricular mechanical energy. The main findings of

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**Table 2. Cardiovascular and hemodynamic responses to hypoxia**  

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>64 mmHg</th>
<th>52 mmHg</th>
<th>45 mmHg</th>
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</thead>
<tbody>
<tr>
<td>LAD Vmax, 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>
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<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>86.2 ± 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>6.505 ± 447</td>
<td>7.801 ± 374</td>
<td>8.469 ± 326*</td>
<td>9.648 ± 308*</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>

*P < 0.05, compared with baseline.

AJP-Heart Circ Physiol • doi:10.1152/ajpheart.00107.2016 • www.ajpheart.org
This study suggests that 1) exposure to hypercapnia (+4, +8 mmHg) significantly elevates both LADVmean and ME_LV, whereas hypocapnia did not; 2) hypoxia (52, 45 mmHg PETO2 stages) elicits an increase in both LADVmean and ME_LV; and 3) the sensitivity of the LAD to hypercapnia and hypoxia in humans is the combination of the inherent vascular sensitivity to CO2 and O2, 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 CO2 and the associated increase in MVO2. The current study found increases in absolute LADV 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 CO2 is not the sole stimulus for the observed increase in LADVmean. 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 LADVmean 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 MVO2, suggesting that noninvasive methods used to correct for the change in cardiac energy demand may not be valid (3, 21). Indeed, the correlations between LADVmean 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 PETCO2. 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., MVO2). Multiple stepwise regression analysis determined that of all the implicated variables (SpO2, PETCO2, MAP, SBP, DBP, RPP, and ME_LV), ME_LV and PETCO2 are the only significant predictors of changes in LADVmean. With the use of standardized beta weights, it also determined that the contributions of PETCO2 (β = 0.36) are relatively comparable with those of ME_LV (β = 0.44), contrary to previous reports. When compared with the cerebrovascular bed, absolute coronary reactivity was found to be of a similar magnitude (~4% to 5%/mmHg) (1, 4). Although the fact that 44% of the hypercapnic reactivity is due to increased cardiac effort is considered, it can be surmised that the cerebrovasculature is more sensitive to hypercapnia than the coronary circulation. The brachial artery has been found to be less sensitive to hypercapnia than cerebral vessels. However, when relative reactivity is compared, our results as well as other published

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**Fig. 5. Cardiovascular and hemodynamic responses to hypoxia.** The relationships between LADVmean (A), MAP (B), CVR (C), total ME_LV (D), and LADVmean per ME_LV (E) during the hypoxic and hypercapnic trials are shown. All values are means ± SE. P values show post hoc comparison to baseline (i.e., middle data point). NSD, not significantly different.
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 LADV$_{mean}$ 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 LADV$_{mean}$ (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) activates endothelial adenosine, 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). When compared with 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. With the consideration that 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 LADV$_{mean}$ results in no significant change with increasing hypoxia. Similar to the hypercapnic trials, a weak correlation is found between LADV$_{mean}$ and PETCO$_2$ (r = 0.55; 0.66), suggesting that using a ratio of LADV$_{mean}$ to either measure of cardiac effort might be an oversimplified solution. Stepwise multiple regressions of all possible contributors (SpO$_2$, PETCO$_2$, MAP, SBP, DBP, RPP, and ME$_{LV}$) to changes in LADV$_{mean}$ determined
that SpO2 and MELV were the only significant predictors. Through calculation of standardized units, it was determined that SpO2 and MELV were relatively equal predictors for changes in LADVmean at ~38% and 45%, respectively. This information implies that MELV 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 alone. Based on previous reports and the data from this study, the cerebral and brachial circulation have a larger reactivity to hypoxia (~1.3%/%SpO2 and ~1.5%/% SpO2, respectively) than the coronary vessels (~0.7%/% SpO2) (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 (PETCO2 = 45 mmHg) were made in healthy participants and cross-sectional area of the LAD was acquired in three subjects using cardiac MRI (13). LADVmean was similar between imaging modalities, and the cross-sectional area at baseline (22.3 ± 4.5 mm²) did not differ from hypoxia (22.4 ± 5.3 mm²). These data, albeit in a small subset of healthy humans, suggest that our measures of LADV are reflective of cardiac workload are associated with assumptions, although it is undoubtedly an important consideration for evaluating LV function in both clinical and research settings. Simple indexes 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). MELV uses certain of cardiac effort using easily obtained measurements (HR, SpO2, RPP), and the associated changes in cardiac effort during 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 MELV 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.

ACKNOWLEDGMENTS

We thank our subjects for volunteering time to complete this study.

GRANTS

This study was supported by the Natural Sciences and Engineering Research Council of Canada and Canada Foundation for Innovation.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).