Title: Influence of myocardial oxygen demand on the coronary vascular response to arterial blood gas changes in humans

Authors: Tyler D. Vermeulen[1]
Lindsey M. Boulet[1]
Mike Stembridge[2]
Alexandra M. Williams[1]
James D. Anholm[3]
Prajan Subedi[3]
Chris Gasho[3]
Philip N. Ainslie[1]
Eric O. Feigl[4]
Glen E. Foster[1]

Affiliations: [1]Centre for Heart, Lung, and Vascular Health, School of Health and Exercise Science, University of British Columbia, Kelowna, Canada.
[2]Cardiff School of Sport, Cardiff Metropolitan University, Cardiff, UK
[3]Loma Linda University, Loma Linda, CA, USA
[4]Department of Physiology and Biophysics, University of Washington, Seattle, WA, USA

Correspondence: Glen E. Foster, PhD.
School of Health and Exercise Science
Faculty of Health and Social Development
University of British Columbia.
3333 University Way,
Kelowna, BC, V1V 1V7

Telephone: 250-807-8224
Fax: 250-807-9665
Email: glen.foster@ubc.ca

Running Head: Effect of cardiac work on the coronary response to O_2 & CO_2

Abstract

It remains unclear if the human coronary vasculature is inherently sensitive to changes in arterial PO_2 and PCO_2 or if coronary vascular responses are the result of concomitant increases in myocardial O_2 consumption/demand (MVO_2). We hypothesized that the coronary vascular response to PO_2 and PCO_2 would be attenuated in healthy men when MVO_2 was attenuated with β_1-adrenergic receptor blockade. Healthy men (n=11; age: 25 ± 1 years) received intravenous esmolol (β_1-adrenergic receptor antagonist) or volume-matched saline in a double-blind, randomized, crossover study, and were exposed to poikilocapnic hypoxia, isocapnic hypoxia, and hypercapnic hypoxia. Measurements made at baseline and following 5-min of steady state at each gas manipulation included left anterior descending coronary blood velocity (LAD_V; Doppler echocardiography), heart rate and arterial blood pressure. LAD_V values at the end of each hypoxic condition were compared between esmolol and placebo. Rate pressure product (RPP) and left-ventricular mechanical energy (ME_{LV}) were calculated as indices of MVO_2. All gas manipulations augmented RPP, ME_{LV}, and LAD_V but only RPP and ME_{LV} were attenuated (4 – 18%) following β_1-adrenergic receptor blockade ($P<0.05$). Despite attenuated RPP and ME_{LV} responses, β_1-adrenergic receptor blockade did not attenuate the mean LAD_V vasodilatory response when compared to placebo during poikilocapnic hypoxia (29.4 ± 2.2 vs. 27.3 ± 1.6 cm/s) and isocapnic hypoxia (29.5 ± 1.5 vs. 30.3 ± 2.2 cm/s). Hypercapnic hypoxia elicited a feed-forward coronary dilation that was blocked by β_1-adrenergic receptor blockade. These results indicate a direct influence of arterial PO_2 on coronary vascular regulation that is independent of MVO_2.

New & Noteworthy

In humans, arterial hypoxemia led to an increase in epicardial coronary artery blood velocity. β_1-adrenergic receptor blockade did not diminish the hypoxemic coronary response despite reduced myocardial O_2 demand. These data indicate hypoxemia can regulate coronary blood flow independent of myocardial O_2 consumption. A plateau in the LAD_{Vmean}-RPP relationship suggested a β_1-adrenergic receptor mediated, feed-forward epicardial coronary artery dilation. In addition, we observed a synergistic effect of PO_2 and PCO_2 during hypercapnic hypoxia.

Keywords

Hypoxia, hypercapnia, coronary blood flow, β_1-adrenergic blockade, myocardial oxygen demand

Introduction

The limited anaerobic capacity of the myocardium and near maximal O_2 extraction from the coronary circulation at rest requires a close match of myocardial O_2 demand and coronary blood flow (14). Myocardial O_2 consumption/demand is closely related to myocardial contractile force and heart rate. Physiological increases in myocardial O_2 demand require coronary vasodilation to increase coronary blood flow thereby maintaining O_2 delivery and cardiac function. Mechanisms responsible for matching coronary blood flow to myocardial O_2 demand have been extensively reviewed and include vascular smooth muscle responses, endothelial release of vasoactive substances, adrenergic stimulation and metabolic feedback control to changes in arterial O_2 and CO_2 tensions (41). However, in healthy humans, separating the direct vascular effects of vasoactive stimuli from the indirect effects on myocardial O_2 demand is challenging due to the redundant and highly integrated mechanisms involved.

Exposure to acute systemic hypoxia or hypercapnia leads to increased sympathetic nerve activity (SNA) and associated catecholamine release from sympathetic nerve terminals and adrenal medulla (47). Increased catecholamines stimulate β-adrenergic receptors within the myocardium leading to positive chronotropic and inotropic responses that elevate heart rate (HR) and myocardial contractility (7). In addition, increases in total systemic vascular resistance elevates cardiac afterload and in combination with positive chronotropy and inotropy increases myocardial O_2 demand and must therefore be accompanied by an increase in O_2 delivery via coronary blood flow to maintain cardiac function. This coronary blood flow response is thought to be regulated by feed-forward coronary vascular β-adrenergic receptor activation and local metabolic feedback control of the coronary vasculature (29). These mechanisms facilitate an

indirect influence of increased SNA on coronary blood flow regulation making it difficult to establish any direct influences of independent physiological stimuli.

The arterial partial pressure of O_2 (PaO_2) and CO_2 ($PaCO_2$) are postulated to have both direct and indirect effects on coronary vascular tone in both animals (1, 8, 9, 19, 21, 29, 38) and humans (6, 16, 31, 44, 46, 48). Cardiomyocyte hypoxia exposure causes the release of vasoactive metabolites, including adenosine and nitric oxide, that relax vascular smooth muscle and dilate the coronary vasculature (11, 35). Studies in humans (4, 6, 31) and animals (29) consistently show a dose-response relationship between coronary blood flow and reductions in arterial PO_2. When the concomitant increases in myocardial workload are statistically controlled, coronary blood flow remains increased in response to hypoxemia (4). Similar to the coronary hypoxic response, hypercapnia leads to increases in coronary blood flow in both animals (8) and humans (44, 48). Boulet *et al.* (6) recently reported that the coronary blood flow response to manipulations in arterial PO_2 and PCO_2 are equally attributable to direct vascular effects and indirect effects associated with increases in myocardial O_2 demand. Interestingly, when hypoxia and hypercapnia are combined, a synergistic influence on coronary blood flow is present in a closed-chest animal model (9). In humans, it remains to be determined if manipulation of myocardial O_2 demand influences the coronary vascular response to hypoxemia with combined manipulations in arterial PCO_2.

The purpose of this investigation was to determine the influence of myocardial O_2 demand on the coronary vascular response to acute alterations in combined arterial PO_2 and PCO_2 stimuli in healthy humans. Using esmolol, a fast acting β_1-adrenergic receptor antagonist, to minimize myocardial O_2 requirements by reducing HR and myocardial contractility (7), we hypothesized that the coronary blood velocity response to end-tidal gas manipulations would be

attenuated when myocardial O_2 demand was reduced. In contrast to our hypothesis, the coronary vasodilator response to hypoxemia was conserved during blockade of β_1-adrenergic receptors.

Methods

Ethical Approval

The protocol for this study was approved by the Clinical Research Ethics Board at the University of British Columbia and conformed to Canada's Tri-Council Policy Statement for the ethical conduct for research involving humans as well as the Declaration of Helsinki. All participants provided written, informed consent prior to experimentation.

Participants

Eleven healthy males with no history of cardiovascular or pulmonary disease participated in this study. Participants completed a questionnaire to screen for previous cardiovascular or pulmonary disease and to ensure they met inclusion criteria. Participants were excluded if they were hypertensive (systolic blood pressure > 140 mmHg; diastolic blood pressure > 90 mmHg), obese (BMI > 30 kg/m^2), or if the blood velocity from the left anterior descending coronary artery could not be sufficiently measured by transthoracic Doppler ultrasound. Pulmonary function was assessed by spirometry according to recommended guidelines (28) and included measurement of the forced expiratory volume in 1s (FEV_1) to forced vital capacity (FVC) ratio (FEV_1/FVC). Participants who did not achieve a FEV_1/FVC ratio >75% of predicted were excluded from the experiment. Participants refrained from exercise, alcohol and caffeine for 24 hours prior to experimentation. All participants provided written, informed consent prior to experimentation. All experiments were conducted in Kelowna, BC, Canada at an elevation of 344m.

Experimental Design

Experimental Protocol

In a double blind, placebo controlled, randomized crossover design, participants received an intravenous infusion of (1) a cardiac specific β_1-adrenergic receptor antagonist, esmolol (Brevibloc, Baxter Healthcare Corporation), or (2) volume matched 0.9% saline. A minimum of 45 minutes (5-biological half-lives of esmolol) separated drug and placebo experiments to ensure no carry-over effects (39). Esmolol was initially infused as a 500 μg/kg bolus over 1 minute followed by a 150 μg/kg/min continuous maintenance infusion for the remainder of the experimental protocol. Previously, a similar esmolol infusion protocol has been found to have a comparable effect as propranolol, a non-specific β-adrenergic receptor antagonist, in reducing heart rate (HR), mean arterial pressure (MAP) (32) and rate pressure product (RPP) (27) responses to exercise. Following instrumentation, participants were instructed to lay supine in a left lateral decubitus position to allow for optimal echocardiographic windows. The initial bolus was infused and following 5 minutes of maintenance infusion participants breathed room air through the mouthpiece for a minimum of 5 minutes. Baseline echocardiographic measurements were acquired following 5 minutes of room air breathing. A dynamic end-tidal forcing system was utilized to manipulate the partial pressure of end-tidal oxygen ($P_{ET}O_2$) and carbon dioxide ($P_{ET}CO_2$) to desired levels as previously described (42, 43). Participants were not blinded to the desired end-tidal gas manipulations. Following baseline measurements, participants were exposed to poikilocapnic ($P_{ET}CO_2$ = uncontrolled), isocapnic ($P_{ET}CO_2$ = baseline) and hypercapnic ($P_{ET}CO_2$ = +5 mmHg from baseline) hypoxia ($P_{ET}O_2$ = 45 mmHg) consecutively in the described order (figure 1A and B). We have previously reported that the $P_{ET}O_2$-to-PaO_2 gradient ranges between 5.9 ± 0.4 and 6.7 ± 0.7 mmHg while the $PaCO_2$-to-$P_{ET}CO_2$ gradient ranges between 0.2 ± 0.2 and -0.9 ± 0.3 mmHg during similar end-tidal gas manipulations (43).

Cardiovascular and respiratory measurements were collected continuously while echocardiographic measurements were collected following 5 minutes of steady state in each end-tidal gas manipulation.

Instrumentation and Cardiorespiratory Measurements

Initially, a 25-gauge intravenous catheter was placed into the antecubital vein and connected to an infusion pump (ALARIS^{TM} PC Pump 8100, CareFusion, San Diego, CA, USA). Participants were then instrumented with a lead II electrocardiogram connected to a bio amp (FE132, ADinstruments, Colorado Springs, CO, USA) to measure HR, a finger probe and pulse oximeter (7500FO, Nonin Medical Inc., Plymouth, MN, USA) to measure oxyhemoglobin saturation (SpO_2), and a finger cuff to measure beat-by-beat blood pressure by finger pulse photoplethysmography (Finometer PRO; Finapress Medical Systems, Amsterdam, Netherlands). The blood pressure signal was calibrated to a reconstructed brachial artery waveform via return-to-flow calibration prior to initiating the infusion at the start of each experimental condition (18). In addition, an automated brachial blood pressure monitor was placed on the upper left arm and used to verify the beat-by-beat blood pressure measurement (CARESCAPE^{TM} V1000 Vital Signs Monitor, GE, Fairfield, CT, USA). Throughout the experimental protocol, participants breathed through a mouthpiece attached in series to a bacteriological filter, a pneumotachograph (HR 800L, Hans Rudolph, Shawnee, KS, USA) with a differential pressure transducer (1110 series, Hans Rudolph) to measure respiratory flow and frequency, and a two-way non-rebreathing valve (2600 series, Hans Rudolph). The pneumotachograph was calibrated prior to experiments with a 3-liter syringe. Respired gases were sampled at the mouth and analyzed by a gas analyzer (ML206, ADinstruments) to measure the $P_{ET}O_2$ and $P_{ET}CO_2$. Prior to experiments, the gas

analyzer was calibrated with gases of known concentration. Commercially available software (LabChart V7.1, ADinstruments) was used to collect respiratory and cardiovascular variables for offline analysis with a sampling frequency of 200 Hz.

Echocardiographic measurements

All echocardiographic images were collected by two experienced sonographers using a commercially available ultrasound machine (Vivid E9, GE) with a M5S 5 MHz ultrasound probe or a 3V 3D-array ultrasound transducer, and saved for offline analysis with commercially available software (EchoPAC v.13, GE). The epicardial portion of the left anterior descending (LAD) coronary artery near the left ventricular apex was visualized as previously described to obtain mean LAD blood velocity (LAD_{Vmean}) and maximum LAD blood velocity (LAD_{Vmax}) during diastole (6, 25). The measurement of LAD_{Vmean} and LAD_{Vmax} with echocardiography has previously been validated against invasive Doppler guide-wire measurements (30). Left ventricular end-systolic (ESV) and end-diastolic (EDV) volumes were measured using a modified Simpson's biplane method which allowed calculation of stroke volume (SV; EDV-ESV) and ejection fraction (EF) (26). Simpson's biplane method has previously been validated for accurate and reproducible measurements of left ventricular volumes (26, 34). Blood pressure and EF measurements were used to calculate an estimate of the left ventricular end-systolic elastance (E_{es}) and used as an index of myocardial contractility as previously described (6, 10). All echocardiographic measurements are reported as an average of 3 cardiac cycles.

Myocardial O_2 Demand Estimation

Two indices of myocardial O_2 demand were calculated. First, the minute mechanical energy of the left-ventricle (ME_{LV}) was estimated from the derived area bound by the E_{es} and a simplified pressure-volume loop as previously described (6). Briefly, the total energy (PV_A) was taken as the sum of stroke work and the elastic potential energy. The PV_A in mmHg was converted to joules (J) using a factor of 1.3×10^{-4} and multiplied by HR to give the ME_{LV} reported in J/min as previously described (6, 10). The second index of myocardial O_2 demand RPP, was calculated as the product of HR and beat-by-beat systolic blood pressure (SBP). The measurement of RPP has been shown to correlate well with direct myocardial O_2 demand measurements (22).

Statistical Analysis

Our primary outcome variable is the difference in LAD_{Vmean} between placebo and esmolol in each hypoxic condition. Our sample size was estimated based on previously published data from our laboratory (6) such that a difference in LAD_{Vmean} of 2 cm/s between placebo and esmolol could be resolved with a pooled standard deviation of 1.9 cm/s and a power >0.85. A two-by-four repeated measures analysis of variance was used to compare cardiovascular, respiratory and echocardiographic measurements between drug condition (i.e. placebo or esmolol) and each end-tidal gas manipulation (i.e. baseline, poikilocapnic, isocapnic, and hypercapnic hypoxia). When significant F-ratios were present, a Tukey's HSD post-hoc test was applied to determine where statistical differences lay. Additionally, a mixed effect linear model was constructed using LAD_{Vmean} as the dependent variable, drug as a categorical predictor, and $P_{ET}O_2$, $P_{ET}CO_2$, and RPP as continuous predictors. The model contained a random subject intercept to account for correlation between measurements. Backwards elimination of non-significant effects was performed on the linear mixed effect model. This process was repeated including ME_{LV} rather

than RPP as a continuous predictor. The Pearson's product-moment test was used to determine if a correlation existed between RPP and ME_{LV}. Reported measurements represent mean ± SE. Statistical significance was set at $P < 0.05$ for all comparisons.

Results

Participants

Participants were all male and had a mean age of 25 ± 1 years, weight of 73 ± 3 kg, height of 177 ± 2 cm and a BMI of 23.2 ± 0.5 kg/m^2. Lung function was normal in all subjects with an average FVC of 110 ± 3% of predicted, FEV_1 of 98 ± 3% of predicted and a FEV_1/FVC ratio of 89 ± 2% of predicted. Randomization resulted in 6 participants receiving esmolol as the first infusion condition, and 5 participants receiving placebo as the first infusion. Participants received a volume of 461 ± 16 ml of saline or esmolol over a period of 59 ± 2 min.

Respiratory response

Table 1 provides respiratory measurements during baseline and each end-tidal gas manipulation for placebo and β_1-adrenergic receptor blockade. Tidal volume and minute ventilation were similar to baseline during poikilocapnic hypoxia and increased during isocapnic and hypercapnic hypoxia. Breathing frequency was increased significantly during hypercapnic hypoxia. Figure 1A and B provide 15-sec group mean (n=11) $P_{ET}CO_2$ and $P_{ET}O_2$ values during end-tidal gas manipulation with and without β_1-adrenergic receptor blockade. The hypoxic stimulus was similar between placebo and β_1-adrenergic receptor blockade with $P_{ET}O_2$ and SpO_2 both being reduced from baseline and not different between poikilocapnic, isocapnic or hypercapnic hypoxia. $P_{ET}CO_2$ was also similar between placebo and β_1-adrenergic receptor blockade, and was reduced from baseline during poikilocapnic hypoxia, consistent with baseline during isocapnic hypoxia and increased from baseline during hypercapnic hypoxia.

Cardiovascular response

Table 2 outlines select cardiovascular measurements during baseline and each end-tidal gas manipulation with placebo and β_1-adrenergic receptor blockade. All end-tidal gas manipulations caused an increase in SBP from baseline during placebo, however, with β_1-adrenergic receptor blockade only hypercapnic hypoxia increased SBP. A significant interaction effect for SBP was identified; post-hoc analysis determined SBP was attenuated by β_1-adrenergic receptor blockade during poikilocapnic ($P = 0.04$) and hypercapnic hypoxia ($P < 0.01$) but not isocapnic hypoxia ($P = 0.06$). Diastolic blood pressure (DBP) and MAP were not influenced by poikilocapnic and isocapnic hypoxia but increased from baseline with hypercapnic hypoxia. Both DBP and MAP were unaffected by β_1-adrenergic receptor blockade. Heart rate increased from baseline in response to all end-tidal gas manipulations ($P < 0.01$) and tended to be reduced by β_1-adrenergic receptor blockade ($P = 0.09$). Left ventricular EDV was similar between drug conditions ($P = 0.9$) while ESV tended to be greater with β_1-adrenergic receptor blockade ($P = 0.09$). Isocapnic hypoxia reduced both EDV and ESV ($P < 0.05$), while hypercapnic hypoxia only reduced ESV ($P < 0.01$). During placebo, E_{es} increased from baseline during all end-tidal gas manipulations and was attenuated by β_1-adrenergic receptor blockade.

Coronary vascular response

The LAD_{Vmean} and LAD_{Vmax} responses to end-tidal gas manipulations with placebo infusion and β_1-adrenergic receptor blockade are outlined in figure 2A and B. During both placebo and β_1-adrenergic receptor blockade both LAD_{Vmean} and LAD_{Vmax} increased from baseline during exposure to all end-tidal gas manipulations. No differences in LAD_{Vmean} or LAD_{Vmax} were observed between poikilocapnic, isocapnic or hypercapnic hypoxic conditions. β_1-adrenergic receptor blockade had no significant influence on the LAD_{Vmean} and LAD_{Vmax} responses amongst

all end-tidal gas manipulations. The final mixed effect linear model for LAD_{Vmean} included subject as a random effect ($P < 0.001$), and $P_{ET}O_2$ ($P < 0.001$) and $P_{ET}CO_2$ ($P < 0.01$) as fixed effects. Both drug ($P = 0.71$) and RPP ($P = 0.08$) were non-significant predictors and excluded from the final model. Similarly, when RPP was replaced by ME_{LV}, subject was included as a random effect ($P < 0.001$), and $P_{ET}O_2$ ($P < 0.002$) and $P_{ET}CO_2$ ($P < 0.01$) as fixed effects. Both drug ($P = 0.92$) and ME_{LV} ($P = 0.15$) were non-significant predictors of the LAD_{Vmean} response to hypoxemia. However, if the relationship between LAD_{Vmean} and RPP or ME_{LV} are considered regardless of end-tidal gases, then mixed effect linear modeling indicates that both RPP ($P < 0.001$) and ME_{LV} ($P < 0.001$) are significant predictors of LAD_{Vmean}. Thus, the relationship between LAD_{Vmean} and our indices of myocardial O_2 demand are left-shifted by esmolol (RPP model: $P < 0.05$); ME_{LV} Model: $P = 0.06$; See Figure 3A & B) and suggests that LAD_{Vmean} is 2.4 ± 1.0 cm/s greater during β_1-adrenergic blockade compared with control ($P = 0.03$) at a standardized myocardial O_2 demand.

Myocardial O_2 Demand

Measurements of RPP and ME_{LV} were significantly correlated ($r = 0.74$, $P < 0.01$) with each other and their response to end-tidal gas manipulation during placebo and β_1-adrenergic receptor blockade are presented in figure 2C and D. All end-tidal gas manipulations caused RPP to increase from baseline during placebo and β_1-adrenergic receptor blockade. A significant interaction was identified and post-hoc analysis determined that β_1-adrenergic receptor blockade attenuated the RPP response during hypercapnic hypoxia ($P < 0.01$) but not significantly during poikilocapnic ($P = 0.11$) or isocapnic hypoxia ($P = 0.07$). All end-tidal gas manipulations caused an increase in ME_{LV} from baseline during both placebo and β_1-adrenergic receptor blockade.

Hypercapnic hypoxia caused a further increase in ME_{LV} compared to poikilocapnic ($P < 0.01$) and isocapnic hypoxia ($P < 0.01$). During β_1-adrenergic receptor blockade the ME_{LV} response was reduced across all end-tidal gas manipulation conditions, but no interaction was present. Figure 3A and B outline both indices of myocardial O_2 demand and LAD_{Vmean} responses to gas manipulations during control and β_1-adrenergic receptor blockade. Both figures indicate that during β_1-adrenergic receptor blockade the myocardial O_2 demand response was attenuated.

Discussion

To our knowledge, this is the first study in healthy humans to measure the coronary vascular response to combined arterial PO_2 and PCO_2 manipulations with and without β_1-adrenergic receptor blockade. The data show that despite reductions in RPP and ME_{LV} due to β_1-adrenergic receptor blockade, the coronary blood velocity response was conserved during poikilocapnic and isocapnic hypoxia. This indicates a direct influence of hypoxemia independent of myocardial O_2 demand. Furthermore, a synergistic effect of PO_2 and PCO_2 was observed during hypercapnic hypoxia as evidenced by a plateau in the LAD_{Vmean}-RPP relationship, suggesting a feed-forward epicardial coronary artery dilation that was absent during β_1-adrenergic receptor blockade. In contrast to our hypothesis, coronary hypoxemic vasodilation was conserved despite a significant attenuation of myocardial O_2 demand following β_1-adrenergic receptor blockade.

Sympathetic feed-forward coronary vasodilation

Our results showed a significant increase in RPP during hypercapnic hypoxia without any further increase in LAD_{Vmean} leading to an observed plateau in the LAD_{Vmean}-RPP relationship that was not present during β_1-adrenergic receptor blockade (see Figure 3A). We interpret this plateau in the LAD_{Vmean}-RPP relationship during hypercapnic hypoxia (denoted by dagger in Figure 3A) as a feed-forward β_1-adrenergic vasodilation in the epicardial LAD thereby attenuating the recorded rise in velocity despite an increase in total blood flow. The β_1-adrenergic receptor blockade with esmolol abolished this feed-forward dilation and attenuated RPP. At all other time points, feed-forward β_1-adrenergic dilation is absent, RPP and ME_{LV} tend to be reduced, yet LAD_{Vmean} are similar (see Figure 2). The suggested β_1-adrenergic mediated response within the epicardial artery is supported by the distribution of β-adrenergic receptor subtypes along the coronary

vascular tree. Larger conduit vessels (diameter > 100 μm) exhibit a 2-fold greater distribution of β_1-adrenergic receptors compared to β_2-adrenergic receptors; whereas smaller resistance vessels (diameter < 100 μm) have a greater β_2-adrenergic receptor distribution with approximately 85% of receptors being of the β_2-adrenergic receptor subtype (3, 12, 14). Furthermore, both β_1- and β_2-adrenergic receptors have been shown to contribute to coronary vasodilation in response to β-adrenergic receptor agonists in a non-beating heart model (40) and closed-chest canines under cardiac pacing (29).

The conserved coronary vascular response to physiological stimuli we observed during β_1-adrenergic receptor blockade (Figure 2A and B) is consistent with a recent investigation in healthy humans assessing the coronary vascular response to isometric handgrip exercise. Maman *et al.* (27) illustrated in humans that the coronary vascular response to exercise was impaired during non-specific β-adrenergic receptor blockade (propranolol) but not β_1-adrenergic receptor blockade (esmolol) despite similar reductions in markers of myocardial O_2 demand. This finding suggests that β_2-adrenergic receptors in the coronary microcirculation were responsible for the coronary vascular response during β_1-adrenergic receptor blockade. The present data are consistent with the results of Maman *et al.* (27), and provide the first evidence of a feed-forward β_1-adrenergic receptor mediated human epicardial vasodilation during hypercapnic hypoxia. However, neither study can rule out a change in coronary O_2 extraction nor the effect of parasympathetic withdrawal.

Influence of oxygen and carbon dioxide manipulations

In the current data, we observed a synergistic effect of hypoxia and hypercapnia on the human coronary vascular response that was attenuated by β_1-adrenergic receptor blockade. Similar

findings were reported previously in canines under cardiac pacing during which the coronary blood flow response to hypoxia was augmented when combined with hypercapnia (9) Specifically, during hypercapnic hypoxia, a significant increase in RPP and ME_{LV} did not lead to a complimentary increase in LAD_{Vmean}. As described above, this finding suggests dilation in the epicardial artery that attenuated the recorded rise in LAD_V despite increases in blood flow. In contrast, the current results show the LAD_{Vmean} response to hypoxia was not further attenuated by poikilocapnia (see Figure 2). In the present study, the reduction in PCO_2 during poikilocapnic hypoxia may not have been sufficient to attenuate LAD_{Vmean} or the change in LAD_{Vmean} may have been too small to detect. Recent investigations into the isolated effect of CO_2 using large changes in $P_{ET}CO_2$ (+7-10 mmHg) consistently show increases in coronary blood flow and velocity (6, 44, 48). However, the influences of smaller changes in $P_{ET}CO_2$ (+4-5 mmHg) have led to conflicting results with some investigations observing increases in coronary blood flow (4) and velocity (6) and others showing no effect (44, 48). The confounding influence of hyperoxia may be responsible for these discrepant findings (44, 48).

The current findings of increased LAD_V in response to hypoxia corroborate previous investigations and there is general agreement that hypoxemia leads to an increase in coronary blood flow in both animals (5, 21, 35) and humans (4, 6, 17, 31). Evidence that hypoxemia has a direct vascular effect in humans is supported by studies which have found that the coronary blood flow response remained after normalizing for changes in myocardial O_2 demand (4, 31). In addition, a study from our laboratory, Boulet *et al.* (6), found nearly equal contributions of hypoxia and cardiac O_2 demand toward the coronary vascular response using multiple regression analysis. The data from the current study are consistent with Boulet *et al.* (6) and support a direct role for hypoxemia in coronary vascular regulation that is independent from changes in

myocardial O_2 demand (see Figure 2). Specifically, we experimentally manipulated myocardial O_2 demand and observed no change in the LAD_V response to poikilocapnic and isocapnic hypoxia. Our data are consistent with the adenine nucleotide hypothesis suggesting that the coronary vascular response to hypoxemia in health is related to endothelial purinergic receptor activation from the release of ATP and its metabolites from erythrocytes rather than pathological cardiomyocyte hypoxia (14, 15, 36).

Methodological considerations

The current study utilized noninvasive transthoracic Doppler echocardiography to measure LAD_V which was used as an index for coronary blood flow. This method of non-invasively quantifying the coronary vascular response to physiological stimuli has been used in multiple investigations (6, 27, 31, 33, 37) and permits comparison between studies. The measurement of LAD_V has previously been validated against direct measurements of coronary blood flow using coronary Doppler guidewires and is highly correlated (30). Previous measurements of LAD diameter recorded with multiple imaging modalities have consistently shown that LAD diameter does not change during acute hypoxia when compared to rest (13, 20). However, our observation that LAD_V versus RPP relationship plateaued during hypercapnic hypoxia suggests that LAD dilation took place during this condition at our measurement site thereby attenuating the rise in LAD_V despite an increase in blood flow. This plateau was absent during β_1-adrenergic receptor blockade.

Our data show reduced myocardial O_2 demand in RPP and ME_{LV} measurements due to β_1-adrenergic receptor blockade with esmolol (Table 2, and Figure 2). Esmolol was chosen due to its fast mechanism of action, and quick elimination half-life, allowing a single subject to be

tested in one laboratory visit (39) thereby minimizing the larger day-to-day variability and associated extraneous factors. Previous investigations comparing esmolol to propranolol, a nonspecific β-adrenergic receptor antagonist, show comparable HR and MAP reducing effects of esmolol at an infusion dose similar to that used in the present study (32). Higher doses of esmolol have previously been used in experimental studies (2, 23), however there is a lack of strong evidence to suggest a higher dose of esmolol would have resulted in a greater reduction in HR and blood pressure in response to hypoxia (45). Interestingly, esmolol did not significantly reduce HR during end-tidal gas manipulations (Table 2). Although this is in contrast to previous experiments involving exercise interventions (27, 32), it may be the result of a greater parasympathetic to sympathetic balance during hypoxia compared with exercise interventions. The dose of esmolol used currently resulted in a clinically relevant reduction in myocardial O_2 demand as the RPP we observed with esmolol during hypercapnic hypoxia is similar to that experienced by hypertensive patients undergoing treatment with β-adrenergic receptor antagonists (24).

Conclusion

The current data confirm that LAD_V correlates with RPP and ME_{LV} but also indicate that hypoxemia directly increases coronary blood flow independent from changes in myocardial O_2 demand, potentially through feedback adenine-nucleotide release from red blood cells in response to low blood oxyhemoglobin saturation. Additionally, we found a synergistic effect of O_2 and CO_2 on the coronary vasculature that manifested as a feed-forward β_1-adrenergic dilation in the epicardial artery that was abolished by β_1-adrenergic receptor blockade. These findings

demonstrate a direct influence of arterial PO_2 on coronary vascular regulation that is independent from associated changes in myocardial O_2 consumption.

Acknowledgements

The authors would like to thank our volunteers for the willingness to participate.

Grants

This research was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC; GEF & PNA), the Canadian Foundation for Innovation (GEF) and the Canada Research Chair program (PNA). GEF is a Michael Smith Foundation for Health Research Scholar. LMB was supported by a NSERC Canada Graduate Scholarship. TDV was supported by an NSERC Undergraduate Student Research Award.

Disclosures

No disclosures to report, financial or otherwise.

Author Contributions

L.M.B., M.S., J.D.A, P.N.A., and G.E.F. conception and design of research; T.D.V., L.M.B., M.S., A.M.W., J.D.A., P.S., C.G., P.N.A., G.E.F. performed experiments; T.D.V. and G.E.F. analyzed data; T.D.V., L.M.B., M.S., A.M.W., J.D.A., P.S., C.G., P.N.A., E.O.F., G.E.F; interpreted results of experiments; T.D.V. and G.E.F. prepared figures; T.D.V. and G.E.F. drafted manuscript; T.D.V., L.M.B., M.S., A.M.W., J.D.A., P.S., C.G., P.N.A., E.O.F., G.E.F. edited and revised manuscript; T.D.V., L.M.B., M.S., A.M.W., J.D.A., P.S., C.G., P.N.A., E.O.F., G.E.F. approved final version of manuscript.

References

1. **Alexander CS**, **Liu SM**. Effect of hypercapnia and hypocapnia on myocardial blood flow and performance in anaesthetized dogs. *Cardiovasc Res* 10: 341–348, 1976.
2. **Alexander R**, **Binns J**, **Hetreed M**. A controlled trial of the effects of esmolol on cardiac function. *Br J Anaesth* 72: 594–595, 1994.
3. **Barbato E**. Role of adrenergic receptors in human coronary vasomotion. *Heart* 95: 603–608, 2009.
4. **Beaudin AE**, **Brugniaux JV**, **Vohringer M**, **Flewitt J**, **Green JD**, **Friedrich MG**, **Poulin MJ**. Cerebral and myocardial blood flow responses to hypercapnia and hypoxia in humans. *Am J Physiol Heart Circ Physiol* 301: H1678-H1686, 2011.
5. **Berne RM**. Cardiac nucleotides in hypoxia: possible role in regulation of coronary blood flow. *Am J Physiol* 204: 317–322, 1963.
6. **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.
7. **Brodde OE**, **Bruck H**, **Leineweber K**. Cardiac adrenoceptors: physiological and pathophysiological relevance. *J Pharmacol Sci* 100: 323–337, 2006.
8. **Broten TP**, **Feigl EO**. Role of myocardial oxygen and carbon dioxide in coronary autoregulation. *Am J Physiol Heart Circ Physiol* 262: H1231-H1237, 1992.
9. **Broten TP**, **Romson JL**, **Fullerton DA**, **Van Winkle DM**, **Feigl EO**. Synergistic action of myocardial oxygen and carbon dioxide in controlling coronary blood flow. *Circ Res* 68: 531–542, 1991.

10. **Chen CH**, **Fetics B**, **Nevo E**, **Rochitte CE**, **Chiou KR**, **Ding PA**, **Kawaguchi M**, **Kass DA**. Noninvasive single-beat determination of left ventricular end-systolic elastance in humans. *J Am Coll Cardiol* 38: 2028–2034, 2001.

11. **Feigl EO**. Coronary Physiology. *Physiol Rev* 63: 1–205, 1983.

12. **Feigl EO**. Neural control of coronary blood flow. *J Vasc Res* 35: 85–92, 1998.

13. **Foster GE**, **Deng Z**, **Boulet LM**, **Mehta P**, **Wei J**, **Fan Z**, **Dharma-kumar R**, **Merz C**, **Li D**, **Nelson M**. Changes in left ventricular function and coronary blood flow velocity during isocapnic hypoxia; a cardiac magnetic resonance imaging study. *J Cardiovasc Magn Reson* 18: P126, 2016.

14. **Goodwill AG**, **Dick GM**, **Kiel AM**, **Tune JD**. Regulation of Coronary Blood Flow. *Compr Physiol* 7: 321–382, 2017.

15. **Gorman MW**, **Rooke GA**, **Savage MV**, **Jayasekara MPS**, **Jacobson KA**, **Feigl EO**. Adenine nucleotide control of coronary blood flow during exercise. *Am J Physiol Heart Circ Physiol* 299: H1981-H1989, 2010.

16. **Grover RF**, **Lufschanowski R**, **Alexander JK**. Alterations in the coronary circulation of man following ascent to 3,100 m altitude. *J Appl Physiol* 41: 832–838, 1976.

17. **Grubbström J**, **Berglund B**, **Kaijser L**. Myocardial oxygen supply and lactate metabolism during marked arterial hypoxaemia. *Acta Physiol Scand* 149: 303–310, 1993.

18. **Guelen I**, **Westerhof BE**, **Van Der Sar GL**, **Van Montfrans GA**, **Kiemeneij F**, **Wesseling KH**, **Bos WJ**. Finometer, finger pressure measurements with the possibility to reconstruct brachial pressure. *Blood Press Monit* 8: 27–30, 2003.

19. **Gurevicius J**, **Salem MR**, **Metwally AA**, **Silver JM**, **Crystal GJ**. Contribution of nitric oxide to coronary vasodilation during hypercapnic acidosis. *Am J Physiol Heart Circ*

Physiol 268: H39–H47, 1995.

20. **Heinonen I**, **Luotolahti M**, **Vuolteenaho O**, **Nikinmaa M**, **Saraste A**, **Hartiala J**, **Koskenvuo J**, **Knuuti J**, **Arjamaa O**. Circulating N-terminal brain natriuretic peptide and cardiac function in response to acute systemic hypoxia in healthy humans. *J Transl Med* 12: 189, 2014.

21. **Herrmann SC**, **Feigl EO**. Adrenergic blockade blunts adenosine concentration and coronary vasodilation during hypoxia. *Circ Res* 70: 1203–1216, 1992.

22. **Hoeft A**, **Sonntag H**, **Stephan H**, **Kettler D**. Validation of myocardial oxygen demand indices in patients awake and during anesthesia. *Anesthesiology* 75: 49–56, 1991.

23. **Hoiland RL**, **Ainslie PN**, **Bain AR**, **Macleod DB**, **Stembridge M**, **Drvis I**, **Madden D**, **Barak OF**, **MacLeod DM**, **Dujic Z**. Beta 1-blockade increases maximal apnea duration in elite breath hold divers. *J Appl Physiol* 122: 899-906, 2016.

24. **Kokkinos P**, **Chrysohoou C**, **Panagiotakos D**, **Narayan P**, **Greenberg M**, **Singh S**. Beta-blockade mitigates exercise blood pressure in hypertensive male patients. *J Am Coll Cardiol* 47: 794–798, 2006.

25. **Krzanowski M**, **Bodzoń W**, **Dimitrow PP**. Imaging of all three coronary arteries by transthoracic echocardiography. An illustrated guide. *Cardiovasc Ultrasound* 1: 16, 2003.

26. **Lang RM**, **Bierig M**, **Devereux RB**, **Flachskampf FA**, **Foster E**, **Pellikka PA**, **Picard MH**, **Roman MJ**, **Seward J**, **Shanewise J**, **Solomon S**, **Spencer KT**, **St. John Sutton M**, **Stewart W**. Recommendations for chamber quantification. *Eur J Echocardiogr* 7: 79–108, 2006.

27. **Maman SR**, **Vargas AF**, **Ahmad TA**, **Miller AJ**, **Gao Z**, **Leuenberger UA**, **Proctor DN**, **Muller MD**. Beta-1 versus beta-2 adrenergic control of coronary blood flow during

isometric handgrip exercise in humans. *J Appl Physiol* 123: 337-343, 2017.

28. **Miller MR**, **Hankinson J**, **Brusasco V**, **Burgos F**, **Casaburi R**, **Coates A**, **Crapo R**, **Enright P**, **van der Grinten CP**, **Gustafsson P**, **Jensen R**, **Johnson DC**, **MacIntyre N**, **McKay R**, **Navajas D**, **Pedersen OF**, **Pellegrino R**, **Viegi G**, **Wanger J**, **Force AT**. Standardisation of spirometry. *Eur Respir J* 26: 319–338, 2005.

29. **Miyashiro JK**, **Feigl EO**. Feedforward control of coronary blood flow via coronary beta-receptor stimulation. *Circ Res* 73: 252–263, 1993.

30. **Momen A**, **Kozak M**, **Leuenberger UA**, **Ettinger S**, **Blaha C**, **Mascarenhas V**, **Lendel V**, **Herr MD**, **Sinoway LI**. Transthoracic Doppler echocardiography to noninvasively assess coronary vasoconstrictor and dilator responses in humans. *Am J Physiol Heart Circ Physiol* 298: H524-H529, 2010.

31. **Momen A**, **Mascarenhas V**, **Gahremanpour A**, **Gao Z**, **Moradkhan R**, **Kunselman A**, **Boehmer JP**, **Sinoway LI**, **Leuenberger UA**. Coronary blood flow responses to physiological stress in humans. *Am J Physiol Heart Circ Physiol* 296: H854-H861, 2009.

32. **Muller MD**, **Ahmad TA**, **Vargas Pelaez AF**, **Proctor DN**, **Bonavia AS**, **Luck JC**, **Maman SR**, **Ross AJ**, **Leuenberger UA**, **McQuillan PM**. Esmolol infusion versus propranolol infusion: effects on heart rate and blood pressure in healthy volunteers. *J Appl Physiol* 122: 511-519, 2016.

33. **Muller MD**, **Gao Z**, **McQuillan PM**, **Leuenberger UA**, **Sinoway LI**. Coronary responses to cold air inhalation following afferent and efferent blockade. *Am J Physiol Heart Circ Physiol* 307: H228–H235, 2014.

34. **Otterstad JE**, **Froeland G**, **St John Sutton M**, **Holme I**. Accuracy and reproducibility of biplane two-dimensional echocardiographic measurements of left ventricular dimensions

and function. *Eur Heart J* 18: 507–13, 1997.

35. **Park KH**, **Rubin LE**, **Gross SS**, **Levi R**. Nitric oxide is a mediator of hypoxic coronary vasodilatation. Relation to adenosine and cyclooxygenase-derived metabolites. *Circ Res* 71: 992–1001, 1992.

36. **Pradhan RK**, **Feigl EO**, **Gorman MW**, **Brengelmann GL**, **Beard DA**. Open-loop (feed-forward) and feedback control of coronary blood flow during exercise, cardiac pacing, and pressure changes. *Am J Physiol Heart Circ Physiol* 310: H1683–H1694, 2016.

37. **Ross AJ**, **Gao Z**, **Pollock JP**, **Leuenberger UA**, **Sinoway LI**, **Muller MD**. β-Adrenergic receptor blockade impairs coronary exercise hyperemia in young men but not older men. *Am J Physiol Heart Circ Physiol* 307: H1497-H1503, 2014.

38. **Rowe GG**, **Castillo CA**, **Crumpton CW**. Effects of hyperventilation on systemic and coronary hemodynamics. *Am Heart J* 63: 67–77, 1962.

39. **Sum CY**, **Yacobi A**, **Kartzinel R**, **Stampfli H**, **Davis CS**, **Lai CM**. Kinetics of esmolol, an ultra-short-acting beta blocker, and of its major metabolite. *Clin Pharmacol Ther* 34: 427–434, 1983.

40. **Trivella MG**, **Broten TP**, **Feigl EO**. β-Receptor subtypes in the canine coronary circulation. *Am J Physiol Heart Circ Physiol* 28: H1575-H1585, 1990.

41. **Tune JD**, **Gorman MW**, **Feigl EO**. Matching coronary blood flow to myocardial oxygen consumption. *J Appl Physiol* 97: 404–415, 2004.

42. **Tymko MM**, **Ainslie PN**, **MacLeod DB**, **Willie CK**, **Foster GE**. End tidal-to-arterial CO2 and O2 gas gradients at low- and high-altitude during dynamic end-tidal forcing. *Am J Physiol Integr Comp Physiol* 308: R895-906, 2015.

43. **Tymko MM**, **Hoiland RL**, **Kuca T**, **Boulet LM**, **Tremblay JC**, **Pinske BK**, **Williams**

AM, **Foster GE**. Measuring the human ventilatory and cerebral blood flow response to CO2: a technical consideration for the end-tidal-to-arterial gas gradient. *J Appl Physiol* 120: 282–296, 2016.

44. **Tzou WS**, **Korcarz CE**, **Aeschlimann SE**, **Morgan BJ**, **Skatrud JB**, **Stein JH**. Coronary flow velocity changes in response to hypercapnia: assessment by transthoracic Doppler echocardiography. *J Am Soc Echocardiogr* 20: 421–426, 2007.

45. **Volz-Zang C**, **Eckrich B**, **Jahn P**, **Schneidrowski B**, **Schulte B**, **Palm D**. Esmolol, an ultrashort-acting, selective beta 1-adrenoceptor antagonist: pharmacodynamic and pharmacokinetic properties. *Eur J Clin Pharmacol* 46: 399–404, 1994.

46. **Wyss CA**, **Koepfli P**, **Fretz G**, **Seebauer M**, **Schirlo C**, **Kaufmann PA**. Influence of altitude exposure on coronary flow reserve. *Circulation* 108: 1202–1207, 2003.

47. **Xie A**, **Skatrud JB**, **Puleo DS**, **Morgan BJ**. Exposure to hypoxia produces long-lasting sympathetic activation in humans. *J Appl Physiol* 91: 1555–1562, 2001.

48. **Yang HJ**, **Yumul R**, **Tang R**, **Cokic I**, **Klein M**, **Kali A**, **Sobczyk O**, **Sharif B**, **Tang J**, **Bi X**, **Tsaftaris SA**, **Li D**, **Conte AH**, **Fisher JA**, **Dharmakumar R**. Assessment of myocardial reactivity to controlled hypercapnia with free-breathing T2-prepared cardiac blood oxygen level-dependent MR imaging. *Radiology* 272: 397–406, 2014.

TABLES

Table 1. Effect of β_1-adrenergic receptor blockade on respiratory measurements at baseline and during poikilocapnic-, isocapnic-, and hypercapnic hypoxia.

		Baseline	Poikilocapnic Hypoxia	Isocapnic Hypoxia	Hypercapnic Hypoxia	Drug	Time	Interaction
$\dot{V}_E$ (l/min)	Placebo	12.3 ± 0.5	14.9 ± 0.8	21.8 ± 2.3*	38.0 ± 3.6*	P = 0.12	**P < 0.01**	P = 0.59
	Esmolol	11.9 ± 0.4	13.3 ± 0.6	18.7 ± 1.0*	34.1 ± 3.4*			
V_T (l)	Placebo	0.8 ± 0.0	1.0 ± 0.1	1.3 ± 0.1*	2.0 ± 0.1*	**P < 0.01**	**P < 0.01**	P = 0.34
	Esmolol	0.7 ± 0.0	0.9 ± 0.1	1.1 ± 0.1*	1.8 ± 0.1*			
f_b (/min)	Placebo	14 ± 1	14 ± 1	15 ± 1	17 ± 1*	P = 0.41	**P < 0.01**	P = 0.83
	Esmolol	15 ± 1	14 ± 1	16 ± 1	18 ± 1*			
SpO_2 (%)	Placebo	98 ± 0	82 ± 1*	80 ± 1*	80 ± 1*	**P < 0.05**	**P < 0.01**	P = 0.88
	Esmolol	98 ± 1	81 ± 1*	79 ± 1*	80 ± 1*			
$P_{ET}O_2$ (mmHg)	Placebo	94.1 ± 1.5	42.6 ± 0.5*	43.5 ± 0.2*	43.4 ± 0.2*	**P < 0.01**	**P < 0.01**	**P < 0.01**
	Esmolol	89.7 ± 1.2†	42.3 ± 0.6*	43.9 ± 0.3*	43.7 ± 0.1*			
$P_{ET}CO_2$ (mmHg)	Placebo	37.5 ± 0.8	33.1 ± 0.8*	37.0 ± 0.7	42.0 ± 0.6*	P = 0.13	**P < 0.01**	P = 0.38
	Esmolol	39.1 ± 0.6	34.3 ± 0.8*	37.9 ± 0.5	42.6 ± 0.5*			

Values represent mean ± SEM, n = 11. *indicates significant difference from baseline (P<0.05); †indicates significant different from placebo condition. V_E, minute ventilation; V_T, tidal volume; f_b, frequency of breathing; SpO_2, oxyhemoglobin saturation; $P_{ET}O_2$, partial pressure of end-tidal oxygen; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide.

Table 2. Effect of β_1-adrenergic receptor blockade on cardiovascular measurements at baseline and during poikilocapnic-, isocapnic-, and hypercapnic hypoxia

		Baseline	Poikilocapnic Hypoxia	Isocapnic Hypoxia	Hypercapnic Hypoxia	Drug	Time	Interaction
HR	Placebo	55 ± 3	68 ± 2*	67 ± 4*	73 ± 4*	P = 0.10	**P < 0.01**	P = 0.09
(/min)	Esmolol	55 ± 3	66 ± 3*	65 ± 3*	69 ± 3*			
SBP	Placebo	119 ± 2	124 ± 3*	127 ± 4*	141 ± 4*	**P < 0.05**	**P < 0.01**	**P < 0.05**
(mmHg)	Esmolol	114 ± 2	116 ± 3†	119 ± 3	124 ± 4*†			
DBP	Placebo	63 ± 2	62 ± 2	62 ± 1	68 ± 2*	P = 0.61	**P < 0.01**	P = 0.56
(mmHg)	Esmolol	62 ± 1	62 ± 2	61 ± 2	66 ± 2*			
MAP	Placebo	82 ± 1	83 ± 1	84 ± 1	92 ± 2*	P = 0.90	**P < 0.01**	P = 0.13
(mmHg)	Esmolol	79 ± 2	80 ± 2	81 ± 2	85 ± 2*			
EF	Placebo	61 ± 1	63 ± 2	63 ± 2	65 ± 1	P = 0.08	P = 0.07	P = 0.63
(%)	Esmolol	60 ± 1	59 ± 2	60 ± 2	61 ± 2			
EDV	Placebo	102 ± 5	98 ± 6	94 ± 6*	96 ± 7	P = 0.96	**P < 0.05**	P = 0.67
(ml)	Esmolol	99 ± 5	98 ± 5	95 ± 5*	96 ± 5			
ESV	Placebo	40 ± 2	36 ± 3	35 ± 3*	34 ± 3*	P = 0.09	**P < 0.01**	P = 0.45
(ml)	Esmolol	40 ± 3	40 ± 3	38 ± 4*	37 ± 3*			
E_{es}	Placebo	1.7 ± 0.1	1.9 ± 0.1*	2.2 ± 0.2*	2.3 ± 0.2*	**P < 0.01**	**P < 0.01**	P = 0.23
(mmHg/ml)	Esmolol	1.6 ± 0.1	1.7 ± 0.2	1.8 ± 0.1*	1.9 ± 0.1*			
RPP	Placebo	6597 ± 320	8510 ± 397*	8656 ± 683*	10366 ± 772*	**P = 0.03**	**P < 0.01**	**P < 0.01**
(mmHg/min)	Esmolol	6320 ± 364	7706 ± 394*	7794 ± 448*	8478 ± 463*†			
ME_{LV}	Placebo	64.4 ± 2.7	79.4 ± 5.1*	75.9 ± 6.1*	92.7 ± 5.9*	**P = 0.02**	**P < 0.01**	P = 0.17
(J/min)	Esmolol	60.9 ± 3.8	71.9 ± 3.3*	70.4 ± 3.8*	78.3 ± 3.9*			
LAD_{Vmean}	Placebo	20.4 ± 1.8	29.4 ± 2.2*	29.5 ± 1.5*	30.4 ± 2.4*	P = 0.78	**P < 0.01**	P = 0.37
(cm/s)	Esmolol	20.8 ± 1.8	27.3 ± 1.6*	30.3 ± 2.2*	31.8 ± 3.2*			
LAD_{Vmax}	Placebo	28.8 ± 2.9	40.1 ± 3.2*	42.7 ± 2.6*	43.2 ± 3.4*	P = 0.93	**P < 0.01**	P = 0.32
(cm/s)	Esmolol	32.8 ± 3.2	38.0 ± 2.8*	40.2 ± 3.0*	43.4 ± 4.2*			

Values represent mean ± SEM, n = 11. *indicates significant difference from baseline (P<0.05); †indicates significant different from placebo condition. HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; EF, ejection fraction; EDV, end diastolic volume; ESV, end systolic volume; E_{es}, end systolic elastance; RPP, rate pressure product; ME_{LV}, mechanical energy of the left ventricle; LAD_{Vmean}, mean left anterior descending coronary blood velocity; LAD_{Vmax}, maximum left anterior descending coronary blood velocity.

FIGURE LEGENDS

Figure 1. Experimental schematic of end-tidal gas measurements. (A) Partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$) and (B) partial pressure of end-tidal oxygen ($P_{ET}O_2$) during baseline, poikilocapnic, isocapnic and hypercapnic hypoxia. Values represent 15-sec mean ± SEM of all subjects both with (open squares) and without (closed squares) β_1-adrenergic receptor blockade (n = 11).

Figure 2. Coronary blood velocity and myocardial O_2 demand responses to end-tidal gas manipulations with (dashed lines) and without (solid lines) β_1-adrenergic receptor blockade. (A) Mean left anterior descending coronary blood velocity (LAD_{Vmean}) response, (B) Maximum left anterior descending coronary blood velocity (LAD_{Vmax}) response. (C) Rate pressure product response (RPP), (D) Left ventricular mechanical energy (ME_{LV}) response. Values represent mean ± SEM, n = 11. * denotes a significant change from respective baseline ($P < 0.05$), † indicates significant difference between β_1-adrenergic blockade and placebo conditions. P-values along x-axis in panel C are post-hoc analysis values comparing placebo to β_1-adrenergic receptor blockade.

Figure 3. Changes in coronary blood velocity compared to changes in myocardial O_2 demand to end-tidal gas manipulations with (dashed lines) and without (solid lines) β_1-adrenergic receptor blockade. Values are mean ± SEM, n = 11. † denotes significant difference between esmolol and placebo in the hypercapnic hypoxia condition for RPP. BL, baseline; PH, poikilocapnic hypoxia; IH, isocapnic hypoxia; HH, hypercapnic hypoxia; RPP, rate pressure

product; ME_{LV}, mechanical energy of the left ventricle; LAD_{Vmean}, mean left anterior descending coronary blood velocity.