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Similarity between carotid and coronary artery responses to sympathetic stimulation and the role of alpha-1 receptors in humans.

Similarity between carotid and coronary artery responses to sympathetic stimulation and the role of alpha-1 receptors in humans

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ABSTRACT

Background. Carotid artery (CCA) dilation occurs in healthy subjects during cold pressor test (CPT), whilst the magnitude of dilation relates to cardiovascular risk. To further explore this phenomena and mechanism, we examined carotid artery responses to different sympathetic tests, with and without α_1-receptor blockade, and assessed similarity to these responses between carotid and coronary arteries.

Methods. In randomised order, 10 healthy participants (25±3 yrs) underwent sympathetic stimulation using the CPT (3-minutes left hand immersion in ice-slush) and lower-body negative pressure (LBNP). Before and during sympathetic tests, CCA diameter and velocity (Doppler ultrasound) and left anterior descending (LAD) coronary artery velocity (echocardiography) were recorded across 3-min. Measures were repeated 90-min following selective α_1-receptor blockade via oral Prazosin (0.05mg per kg bodyweight).

Results. CPT significantly increased CCA diameter, LAD maximal velocity and velocity-time integral area-under-the-curve (all $P<0.05$). In contrast, LBNP resulted in a decrease in CCA diameter, LAD maximal velocity and velocity time integral (VTI, all $P<0.05$). Following α_1-receptor blockade, CCA and LAD velocity responses to CPT were diminished. In contrast, during LBNP (-30 mmHg), α_1-receptor blockade did not alter CCA or LAD responses. Finally, changes in CCA diameter and LAD VTI-responses to sympathetic stimulation were positively correlated ($r=0.66$, $P<0.01$).

Conclusion. We found distinct carotid artery responses to different tests of sympathetic stimulation, where α_1-receptors partly contribute to CPT-induced responses. Finally, we found agreement between carotid and coronary artery responses. These data indicate similarity between carotid and coronary responses to sympathetic tests and the role of α_1-receptors that is dependent on the nature of the sympathetic challenge.

KEYWORDS: carotid artery, coronary artery endothelial function, sympathetic nervous system, cardiovascular disease, α1-adrenoceptors

NEWS AND NOTEWORTHY

- We showed distinct carotid artery responses to cold pressor test (i.e. dilation) and lower-body negative pressure (i.e. constriction).
- Blockade of α1-receptors significantly attenuated dilator responses in carotid and coronary arteries during CPT, whilst no changes were found during LBNP.
- Our findings indicate strong similarity between carotid and coronary artery responses to distinct sympathetic stimuli, and for the role of α-receptors.

ABBREVIATION LIST

Cardiovascular disease (CVD)

Cardiac output (CO)

Cold pressor test (CPT)

Common carotid artery (CCA)

Diastolic blood pressure (DBP)

Heart rate (HR)

Left anterior descending (LAD)

Left anterior descending artery, mean diastolic velocity (LADVmean)

Left anterior descending artery, peak diastolic velocity (LADVmax)

Lower body negative pressure (LBNP)

Partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$)

Partial pressure of end-tidal oxygen ($P_{ET}O_2$)

Rate pressure product (RPP)

Systolic blood pressure (SBP)

Sympathetic nervous system (SNS)

Stroke volume (SV)

Velocity time integral (VTI)

INTRODUCTION

Activation of the sympathetic nervous system (SNS) is an important and clinically-relevant prognostic stimulus to examine artery function (12, 36). During the cold pressor test (CPT), a potent sympathetic stimulus, the coronary arteries can result in a vasoconstrictor (via α_1-receptors) or vasodilatory response (via the α_2-, and β-receptors)(2). Vasodilator pathways prevail in healthy volunteers (10, 27), whereas experimental studies in patients with coronary artery disease demonstrate vasoconstriction during SNS activation (30, 47, 49). Coronary artery responses to CPT independently predict future cardiovascular events in patients at risk for cardiovascular disease (31, 32, 36), which highlights the clinical relevance of this response. However, the invasive nature of angiography make these tests impractical for large scale clinical use. Interestingly, the carotid artery shows vasodilation during SNS activation in healthy subjects, similar to coronary artery responses. This carotid dilation is abolished or even reversed to vasoconstriction in those with (increased risk for) cardiovascular disease (34, 44). To date, relatively little is known about the underlying mechanisms for the carotid artery reactivity to SNS activation.

Previous studies in peripheral conduit arteries have reported divergent responses to different tests of SNS-activation (11, 15, 25, 27, 41). To date, no previous study compared vasomotor responses of the carotid artery to distinct SNS stimuli. In line with peripheral arteries (i.e., the brachial and superficial femoral artery), we expect that distinct SNS stimuli (i.e. CPT and lower body negative pressure (LBNP)) lead to distinct carotid and coronary artery responses, as these tests mediate sympathetic activation through different pathways. More specifically, CPT evokes sympathetic activation via cold stress. The LBNP test gradually decreases central blood volume which results in progressive increases in muscle sympathetic nerve activity (8, 9), which can directly lead to constriction of the carotid diameter.

No previous study examined the potential underlying mechanisms mediating carotid artery vasomotion during SNS activation. Work in both animal and human coronary arteries revealed a central role for α_1-receptors to mediate vasomotor responses during SNS activation (17, 23, 26). In line with this previous work, we expect that α_1-receptors, at least in part, contribute to the carotid artery responses to CPT and LBNP. Therefore, our first aim is to examine the impact of activation of the SNS, either through the CPT (i.e. elevates SNS activity and blood pressure)(18, 46) or LBNP (i.e. elevates SNS activity, with preserved blood pressure)(21, 45) on carotid artery diameter. Our second aim was to assess the role of α_1-adrenoreceptors to these carotid artery responses by using an oral, selective α_1-adrenoreceptor blocker (i.e. Prazosin).

A recent study found good agreement between carotid and coronary responses to the CPT in healthy young and older subjects (44). To further explore this relationship, we aimed to compare the responses between the carotid artery diameter and left anterior descending coronary artery velocity (LAD velocity) during different SNS stimuli, with and without α_1-receptor blockade. Based on previous work (34, 44), we anticipated that there would be similarity in the magnitude and direction of the vascular responses between both the carotid artery diameter and LAD velocity, and that these responses would be partly mediated via α_1-receptors.

METHODS

Ethical approval

This study was approved by the Human Ethics Committee of the University of British Columbia and conformed to the standards set by the Declaration of Helsinki. All volunteers provided written informed consent.

132

133 **Participants**

134 We recruited 10 healthy male participants (mean age 25±3 years, height 1.78±0.1 m, and
135 weight 76±9 kg). Exclusion criteria were a history of cardiovascular disease (i.e. angina
136 pectoris, myocardial infarction, heart failure), lung disease (i.e. COPD, lung cancer), brain
137 disease (i.e. stroke, dementia), presence of Raynaud's phenomenon, scleroderma, chronic pain
138 and/or open wounds on the upper extremities, obesity (body mass index >30 kg/m^2), diabetes
139 mellitus type 1 or 2, history of smoking, or elevated blood pressure (systolic >130 mmHg;
140 diastolic >85 mmHg).

141

142 **Experimental design**

143 All participants reported to our laboratory for a single visit. They were asked to abstain from
144 strenuous exercise for 24 hours and abstain from dietary products known to affect endothelial
145 function for ≥18 hours prior to the testing session (i.e. vitamin C, caffeine and alcohol).
146 Moreover, participants were asked to fast for ≥2 hours, adapted from existing guidelines to
147 assess peripheral vascular function (38). Participants rested in the supine position for >15
148 minutes on a bed in a temperature-controlled room (23±1°C). Subsequently, participants
149 underwent LBNP and two CPT, in a randomly assigned order, with 45-minutes rest between
150 tests. All tests involved simultaneous assessment of common carotid artery (CCA) diameter
151 and velocity (ultrasound) and left anterior descending (LAD) coronary artery velocity
152 (echocardiography) before (across a 1-minute baseline) and during sympathetic stimulation.
153 The protocol was repeated 90-minutes after oral administration of Prazosin (i.e. α_1- adrenergic
154 receptor antagonist that effectively blocks 80% of α_1-recepter activity, 0.05mg per kg body
155 weight)(1, 22).

156

Experimental measures

Common carotid artery diameter and velocity. Left carotid artery diameter and red blood cell velocity were recorded simultaneously and continuously during baseline (1-minute) and sympathetic stimuli (i.e. 3-minutes CPT, and ~18-minutes LBNP). Carotid artery image acquisition was performed using a 10-MHz multifrenquency linear array handheld probe attached to a high resolution ultrasound machine (15L4, Terason T3200, Burlington, MA, USA). When an optimal image was found, 2-3 cm proximal from the bifurcation, the probe was held stable and the ultrasound parameters were set to optimise the longitudinal, B-mode image of the lumen-arterial wall interface. Continuous pulsed wave Doppler velocity assessments were also obtained and were collected at the lowest possible insonation angle (always <60º). Assessment was performed by an experienced sonographer (ACCM), whom has an hour-to-hour reproducibility (i.e. coefficient of variation) of CCA baseline diameter of 0.8% and reproducibility of 0.8% for the peak CCA diameter, in line with previous findings (44).

Coronary artery velocity. Before and during both CPT and LBNP, the left anterior descending (LAD, cm) coronary artery velocity was examined using transthoracic ultrasound. This assessment was performed simultaneously with CCA diameter and velocity responses. All echocardiographic measurements were collected by a trained sonographer (MS) on a commercially available ultrasound system (Vivid E9; GE, Fairfield, CT) using a broadband M5S 5 MHz or a 3V 3D-array transducer. In a previous study the Cronbach's alpha reliability test revealed alpha values of 0.81 and 0.89 for both max and mean LAD velocities, respectively, suggesting good consistency between LAD velocity measurements (5). Participants assumed a left lateral position to allow for data collection. 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 LAD velocity were collected during the sympathetic tests.

Blood pressure and heart rate. All continuously recorded cardiovascular measurements were acquired at 200 Hz using an analog-to-digital converter (Powerlab/16SP ML 880; ADInstruments, Colorado Springs, CO, USA) interfaced with a personal computer. Before and during CPT and LBNP, systolic and diastolic blood pressure (SBP and DBP, in mmHg, respectively), stroke volume (SV, ml), rate-pressure product (RPP, HR x SBP, a reliable indicator for myocardial oxygen demand)(14), and cardiac output (CO, L/min) were continuously measured using non-invasive finger photoplethysmography (Finometer Pro, Finapres medical systems, Amsterdam, Netherlands). Heart rate (HR, beats per minute) was recorded using three-lead electrocardiography, placed in lead II configuration (Bioamp, ML132, ADInstruments, Colorado Springs, CO, USA).

Sympathetic stimuli

Cold pressor test. The cold pressor tests (CPT) consisted of a 3-minute immersion of the left hand in a bucket of ice slush (~4.0°C)(44). The participant was positioned in supine position on a tilt bed, tilted slightly to the left lateral position (~25-30°), to facilitate arm movement in the bucket of slush without significant movement of the body, and provide adequate coronary assessment. After a 1-minute baseline period, the participants hand was immersed up to the wrist in the ice-slush for 3 minutes. The participant was instructed to remain quiet during the CPT to provide for valid CCA assessment. The partial pressures of end-tidal carbon dioxide

($P_{ET}CO_2$) and oxygen ($P_{ET}O_2$) were clamped at baseline values for the entire duration of the protocol to reduce the potential impact of hyperventilation on the vascular responses, upon an end-tidal forcing approach described extensively elsewhere (43). To reduce measurement error, CPT procedures were repeated twice and averaged for analyses (44).

Lower body negative pressure. The participant was positioned in the supine position on a tilt bed, and strapped into a custom-made airtight, lower-body suction chamber at the level of the iliac crest (42). The LBNP chamber was then moved from supine position into a left lateral position (~25-30°) to ensure adequate coronary imaging. The lower body negative pressure test consisted of a 5-minute baseline, followed by progressive 2-minute stages, using increments of -10 mmHg, to -80 mmHg or until pre-syncope. LBNP was terminated when a) pre-syncope occurred, defined by a sustained drop in systolic blood pressure <80 mmHg for more than 10 seconds (24), or b) upon participants request due to the onset of subjective symptoms (e.g. feelings of dizziness, nausea, faintness). During the Prazosin condition, participants were unable to last longer than -40mmHg during the LBNP test. For reliable comparison between the control and drug condition, we chose to only include data until -30mmHg.

Data analysis

Carotid artery responses. Analyses of diameter (cm), blood flow (ml/sec), blood velocity (cm/sec) and shear (s^{-1}) were performed using custom-designed edge-detection and wall-tracking software, which is largely independent of investigator bias, as was extensively described elsewhere (4). Baseline diameter, blood flow, blood velocity and shear were calculated as the mean of data acquired across a 1-minute baseline period (4). For the CPT, data were calculated for 10-second intervals. LBNP data was calculated per 1-minute

intervals. Subsequently, offline image analysis involves the identification of the region of interest (ROI), to allow for automated calibration on the B-mode image and velocities on the Doppler assessment (40). A ROI is drawn around the optimal B-mode image, in which a pixel-density algorithm automatically identifies the near- and far wall. Another ROI is drawn around the Doppler waveform, which is synchronized with the B-mode diameter ROI. Ultimately, this allows for blood flow and shear rate calculations (40). Peak diameter change was calculated relative to baseline diameter.

Coronary artery responses. All images were exported for offline analysis using commercially available software (EchoPAC Version 13.0; GE Medical, Horten, Norway). All echocardiographic values represent an average value of three cardiac cycles representing the clearest of five collected images for each experimental stage. The collected waveforms were analyzed to determine mean diastolic velocity (LADVmean, cm/sec), peak diastolic velocity (LADVmax, cm/sec), and the velocity time integral (VTI, cm) (19). Coronary flow velocity reliably reflects changes in absolute coronary blood flow (10, 13, 27), suggesting that an increase in flow velocity reflects coronary artery dilatation. Using observer-independent software, VTI is calculated as the integral of individual velocities across the cardiac cycle. Participants in whom at least 1 image was suboptimal, were excluded prior to analyses (5).

Blood pressure and heart rate. Analyses of systolic and diastolic pressure, heart rate, cardiac output, stroke volume, and the rate-pressure product (RPP) were performed in commercially available software (LabChart V7.1, ADInstruments, Colorado Springs, CO, USA). Measurements were averaged per 10-second bins for analyses for the CPT, and 1-minute bins for the LBNP analyses. Baseline CPT was averaged over a 3-minute period. Continuous blood pressure measurements were calibrated to automated brachial blood pressure readings during baseline (HEM-775CAN, Omron Healthcare, Bannockburn, IL, USA).

Statistical analyses

All data were presented as mean±SD unless stated otherwise. Parameters were tested for normality using a Shapiro-Wilk test. Responses of the CCA (i.e. diameter (cm), blood velocity (cm/sec), flow (ml/sec) and shear (s^{-1})) and LAD (i.e. mean velocity (cm/sec), max velocity (cm/sec) and VTI (cm) were assessed during the sympathetic stimulus with paired Students' t-tests (in case of non-parametric variables, a Wilcoxon signed-rank test was performed). Changes over time were assessed with 2-way repeated measurement ANOVA's (missing values were only imputed based on previous and consecutive measurements when available). We assessed whether CCA and LAD changes in diameter, velocity, flow and shear occurred over time (i.e. within factor 'time'), and whether this differed between conditions (i.e. between factor control vs Prazosin) were examined. In addition to the main effects, the 'time'*'condition'-interaction revealed whether the CCA and LAD changes across time differed between the control condition and Prazosin. This was done to assess the potential role of α-receptors in mediating CCA and LAD responses. The 2-way repeated measurement ANOVA's were performed with Sidak correction to account for multiple comparisons. Data were analysed using SPSS 20.0 software (IBM SPSS, IBM Corp., Armonk, NY, USA). Values for $p<0.05$ were assumed to be statistical significant.

RESULTS

Carotid artery responses: different SNS stimuli.

Cold pressor test. The CPT caused a significant increase in systolic and diastolic blood pressure and the rate-pressure product (RPP), whilst no change was found in stroke volume, heart rate and cardiac output (n=9, Table 1). Although the diameter (cm) of the CCA increased significantly during CPT ($P<0.001$, Figure 1), CCA velocity (cm/sec), flow (ml/sec)

and shear rate (s^{-1}) did not change significantly across time during CPT (P>0.05, data not shown).

Lower body negative pressure. LBNP caused a gradual, but significant increase in heart rate, a decrease in stroke volume and diastolic blood pressure, whilst systolic blood pressure and cardiac output were preserved (n=9, Table 2). LBNP caused a significant decrease in CCA diameter (cm, Figure 2), whereas no changes were found in CCA velocity (cm/sec), flow (ml/sec) and shear (s^{-1}) (data not shown).

Carotid artery response to sympathetic activation: role of α_1-receptors.

Cold pressor test. Prazosin increased baseline CCA diameter, and decreased shear (0.671±0.05 to 0.703±0.05 cm, and 185.9±50 to 159.1±40 s^{-1} respectively, all P<0.05), whilst no changes were found in carotid blood flow and blood velocity (10.9±1.8 to 10.8±1.5 ml/sec P=0.405, and 30.7±6.6 to 27.7±5.2 cm/sec, P=0.051). Prazosin caused an abolished CPT-induced increase in diameter (Figure 3). Prazosin attenuated the increase in blood pressure during CPT, and resulted in a larger increase in cardiac output, heart rate and RPP during the CPT (n=9, Table 2). We found no change in stroke volume (Table 2), whilst we also found no change in CCA flow, shear and velocity (data not shown).

Lower-body negative pressure. Baseline CCA diameter, flow and velocity were significantly larger following Prazosin adminstration (0.684±0.05 to 0.706±0.05 cm, 10.2±1.6 to 12.0±2.3 ml/sec, and 27.6±5.0 to 30.0±5.5 cm/sec, respectively, all P<0.05). All subjects reached pre-syncope at -30 or -40 mmHg. Therefore, we compared data between both sessions up to -30 mmHg. Prazosin did not alter CCA diameter responses during LBNP (Table 2, Figure 4). Prazosin exaggerated the increase in heart rate and RPP during LBNP, whilst blood pressure decreased during the Prazosin trial (n=9, Table 2).

Carotid artery responses versus coronary artery responses.

SNS stimulation. Similar to CCA responses, CPT caused a significant increase in LAD maximum velocity (n=6, baseline 0.25±0.03 to peak 0.34±0.02 cm/sec, $P<0.05$) and VTI ($P<0.05$, Figure 1). Due to the suction of the LBNP box, movement of the participants prevented assessment in 5 participants. Again in agreement with CCA responses, LBNP caused a reduction in LAD maximum velocity (n=5, Table 2) and peak VTI (cm, Figure 2). When pooled, a significant correlation was found between changes in CCA diameter and LAD peak VTI (n=20, r=0.65, $P<0.01$).

α_1-receptor blockade. Following Prazosin administration, LAD VTI were elevated and Prazosin abolished the increases in CCA diameter and LAD peak VTI (Figure 3, Table 2). During LBNP, Prazosin did not alter CCA diameter (cm), LAD peak VTI or LAD peak velocity responses (cm/sec, up to -30 mmHg; Table 2, Figure 4).

DISCUSSION

We present the following findings. First, activation of the SNS using the CPT significantly increased CCA diameter, whilst SNS activation using LBNP mediated a decrease in CCA diameter. Second, systemic blockade of the α_1-receptors significantly attenuated the dilator response of the carotid during the CPT, whilst these changes were unaltered during LBNP. This latter finding suggests the presence of distinct carotid artery responses to different types of SNS activation, with a distinct contribution of α_1-receptors mediating these responses. Furthermore, we found good agreement between the direction and magnitude of the coronary and carotid artery responses when comparing the different tests of sympathetic stimulation, but also regarding the contribution of α_1-receptors. Taken together, we found divergent responses to distinct tests of SNS activation and the role of α_1-receptors mediating these

responses, whilst similarity is found between carotid and coronary arteries in the magnitude and direction of vascular responses to sympathetic stimulation and blockade of α_1-receptors.

Carotid artery responses to sympathetic stimulation.

The CPT resulted in a characteristic dilation in the CCA of our healthy subjects, a finding observed previously in our laboratory (44) and others (34). Interestingly, these dilator responses of the carotid artery contrasts with peripheral artery responses, since brachial or superficial femoral arteries demonstrate negligible diameter changes during CPT (11, 25). Central, elastic arteries (such as the carotid artery) may thus respond differently to SNS activation using the CPT compared to muscular, peripheral arteries. This notion is further supported by observations of abdominal aorta dilation during the CPT (6). In contrast, LBNP mediated a decrease in CCA diameter. The presence of distinct artery responses to different tests of sympathetic activation has also been reported in peripheral conduit arteries (11). Both CPT and LBNP mediate sympathetic activation through different pathways, leading to distinct vascular responses in peripheral and central arteries. The CPT causes an immediate stressor response (11, 27), leading to rapid catecholamine release and blood pressure elevation. This induces β-receptor mediated vasodilation, and sympathetic blood pressure mediated constriction, respectively. The resultant of this response is an increase in CCA diameter, due to the outweighting effect of β-receptor mediated vasodilation. In contrast, the LBNP mediates a gradual, arterial baroreflex-mediated activation of the sympathetic nervous system and thus can directly decrease carotid diameter. Both sympathetic tests demonstrate distinct time-dependent changes in circulating catecholamines, with an immediate elevation after CPT, and a slower (time- and intensity-dependent) elevation during LBNP (11, 21, 27, 33). This data indicates that distinct tests of stimulation of the sympathetic nervous system lead to different carotid artery responses.

Role of α_1-receptors in carotid artery responses to sympathetic stimulation.

Under physiological conditions, α_1-receptors mediate vasoconstriction in coronary arteries during a sympathetic stimulus (23, 29). Indeed, blockade of α_1-receptors resulted in an increase in baseline CCA diameter and velocity, but also LAD velocity. However, in contrast to our hypothesis, α_1-blockade attenuated the carotid artery dilator responses during the CPT, whilst no impact of α_1-blockade was found during LBNP. One potential explanation is that the increase in baseline diameter and/or velocity (induced by α_1-receptor blockade) prevented a further increase in diameter upon additional SNS stimulation. This explanation is supported by previous work in peripheral arteries, which found that an increase in baseline diameter is associated with a smaller endothelium-(in)dependent vasodilation (37, 39). However, our data does not reveal such a relation between resting carotid diameter and peak responses (CPT control r=-0.280, Prazosin r=-0.275, LBNP control r=-0.401, Prazosin r=-0.219, all P>0.05). Therefore, CCA dilation during α_1-blockade may not explain the attenuated vasomotor responses to CPT or preserved response to LBNP.

An alternative explanation for the attenuated dilator response may relate to the pharmacological actions of α_1-receptor blockers. In healthy coronary arteries, vasoconstriction upon sympathetic stimulation is largely mediated via α_1-receptors, with only a minor role for α_2-receptors (3, 48). Previous studies in both animals and humans found that during α_1-receptor blockade, SNS activation still mediates coronary constriction through activation of α_2-receptors (7, 16, 20). Possibly, α_1-receptor blockade in our study yielded stimulation of α_2-receptors during activation of the SNS using the CPT. Consequently, the vasodilator responses may be attenuated by the constrictive actions of α_2-receptors. This hypothesis needs further exploration. A final reason for the dimished CCA dilation during CPT could reside in

the attenuated blood pressure responses. However, it is unclear whether blood pressure represents the principal contributor to the carotid dilation, especially since peak diameter responses precede peak blood pressure values. Moreover, blood pressure rises similarity between individuals who demonstrate carotid artery vasodilation versus vasoconstriction (44). Nonetheless, we cannot exclude a potential role for the blood pressure response to contribute to the carotid dilation.

Carotid artery versus coronary artery.

Our findings provide strong evidence for similarity between the carotid and coronary arteries regarding the direction and magnitude of the vasomotor response. Indeed, both carotid and coronary arteries demonstrated dilation in response to CPT, but constriction was present in both arteries during LBNP. The presence of coronary dilation to CPT (30, 49), but also coronary constriction to LBNP (27), has been reported in previous studies. This further confirms the presence of distinct artery responses to distinct stimuli to activate the sympathetic nervous system.Furthermore, α_1-receptor blockade mediated similar effects between carotid and coronary arteries for the LBNP test. During **the** CPT we observed that α_1-receptor blockade attenuated the carotid responses, whilst the coronary responses were reversed. Agreement between arteries was further supported by the presence of a significant and strong correlation between both arteries **(Figure 5)**, a finding that is in line with previous work (34, 44). A potential limitation of the echocardiographic measurement is the inability to examine blood flow. However, strong agreement is present between changes in coronary artery blood velocity and blood flow in response to sympathetic stimulation (10, 27, 28), suggesting that the increase in LAD velocity can be interpreted as true coronary vasodilation.

Despite these similarities in magnitude and direction of vascular responsiveness, it is important to emphasise that the mechanisms contributing to vascular control may differ between arteries. For example, coronary artery flow and velocity during sympathetic stimulation are dependent on both local metabolic and vasodilatory mechanisms sensitive to the rate of myocardial oxygen consumption (MVO_2).(27) For this purpose, we have calculated the rate-pressure product, a common used index for myocardial oxygen consumption (RPP, Supplemental data). The increase in RPP during the CPT suggests that the dilation of the coronary artery is, at least partly, related to the increase in myocardial oxygen uptake. Whether similar mechanisms are present in the brain to contribute to carotid artery dilation during the CPT is currently unknown. For the LBNP, we found no important role for RPP to contribute to the vascular responses in our study. When correcting our responses for potential differences for the RPP, correlation between LAD VTI and CAR(%) remained present ($r=0.66$, $P<0.05$). Future studies are required to better understand the mechanisms contributing to the vascular responses during sympathetic stimulation in both carotid and coronary arteries.

Clinical relevance. Coronary artery responsiveness to SNS stimulation, including the CPT, has shown a strong predictive ability for future cardiovascular disease and/or events (31, 32, 36). Similarity in vasomotor responsiveness between coronary and carotid arteries suggests that the carotid artery may serve as a alternative measure for coronary vascular responses to SNS stimulation. An important advantage of measuring the carotid artery is its easy accessibility, high reproducibility and the accuracy of the test. This warrants future studies to further explore the potential clinical use of examining carotid responses to SNS stimulation. To further explore the similarity between the carotid and coronaries, future studies could be performed in a catheterisation laboratory, to simultaneously measure both carotid and

coronary artery responses during sympathetic stimulation. These studies can be extended by the addition of selective α- and /or β-adrenergic agonist/antagonists, to further resolve the contribution of adrenergic receptors to sympathetically-mediated carotid and coronary artery responses.

Methodological considerations. A strength of our study was that we controlled for end-tidal gases at baseline values, during both CPT and LBNP, and the α_1-receptor blockade condition. Fluctations and alterations in $P_{ET}CO_2$ are known to directly influence the diameter of the CCA (35) and LAD VTI (5). Following our α1 blockade, which directly affects mean arterial pressure and ventilatory regulation during sympathetic activation, clamping $P_{ET}CO_2$ and $P_{ET}O_2$ to baseline values reduced the possible interference with our carotid and coronary artery responses.

To summarize, our data demonstrates that the carotid artery demonstrates distinct vascular responses to different stimuli to activate the sympathetic nerve system. Additionally, blockade of the α_1-receptors significantly attenuated the dilator responses in the carotid artery during the CPT, whilst no changes were found during LBNP, suggesting a potential role for α-receptors to contribute to vasomotor responses in carotid arteries. Finally, even though α_1-blockade resulted in disparate responses during CPT, our findings indicate strong similarity between carotid and coronary artery reactivity in response to distinct sympathetic stimuli.

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Author contribution statement

DHJT, ACCMM, MMT and PA designed the study. ACCMM, MS, MMT and TPK were involved in data collection and analyses. ACCMM and DHJT performed the statistical analyses. All authors contributed to the interpretation of the data, and writing of the manuscript. All authors provided approval of the final version and agreed to be accountable for all aspects of the work. All persons designated as authors qualify for authorship and all those who qualify for authorship are listed.

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Disclosures

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

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TABLE 1 – Cold pressor test responses

Cold pressor test		1 minute CPT								2 minutes CPT						3 minutes CPT				2-way ANOVA		
	Baseline	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	Time	Trial	Time*Trial
Stroke volume (ml) Control	105±15	106±17	106±16	105±17	105±17	105±19	105±20	105±21	104±20	104±20	103±20	105±21	106±21	106±21	106±20	106±20	106±20	107±19	107±19	0.450	0.296	0.450
Stroke volume (ml) Prazosin	112±14	113±15	114±14	114±15	113±16	112±17	111±16	110±15	111±17	111±16	109±17	110±17	110±17	111±17	112±17	111±17	111±18	112±18	112±17			
Cardiac Output (L/min) Control	6.2±1.3	6.6±1.2	6.8±1.3	6.6±1.4	6.7±1.6	6.8±1.8	7.0±1.6	6.9±1.6	6.7±1.7	6.6±1.5	6.3±1.4	6.3±1.5	6.3±1.4	6.3±1.5	6.1±1.6	6.3±1.5	6.3±1.4	6.3±1.5	6.3±1.4	0.200	0.049	0.021
Cardiac Output (L/min) Prazosin	6.7±0.9	7.4±1.0	7.5±1.2	7.5±1.3	7.7±1.5	7.7±1.6	7.5±1.6	7.6±1.6	7.5±1.4	7.5±1.4	7.5±1.4	7.6±1.3	7.6±1.2	7.4±1.1	7.6±1.1	7.6±1.1	7.6±1.0	7.7±0.9	7.6±1.0			
Heart Rate (bpm) Control	59±12	63±10	65±11	64±11	64±13	64±12	67±12	66±12	64±13	63±13	62±12	62±13	61±12	61±13	59±15	61±13	61±12	60±14	59±12	0.107	0.024	0.001
Heart Rate (bpm) Prazosin	62±12	69±13	68±11	68±11	71±13	71±14	70±13	71±14	70±15	70±14	71±14	72±14	72±14*	70±13	71±13*	71±12	71±13*	71±12*	70±13*			
Diastolic BP (mmHg) Control	79±8	83±6	81±7	84±9	86±8	88±8*	91±8*	92±9*	92±8	92±8	93±9*	92±9*	92±9*	92±9*	91±8*	90±8*	90±8*	90±8	89±8*	0.000	0.045	0.031
Diastolic BP (mmHg) Prazosin	74±9	76±9	74±9	76±9	78±9	80±9	81±10	81±9	81±9	82±8*	82±9*	82±8	83±9	80±9	81±9*	82±9*	81±9*	81±9*	80±8*			
Systolic BP (mmHg) Control	133±7	139±8	137±10	140±11	142±12	146±13	148±11*	150±12*	150±12*	150±13	151±13	150±13	151±12	151±11*	150±12*	149±11*	148±10*	148±11	148±11*	0.000	0.169	0.023
Systolic BP (mmHg) Prazosin	131±7	136±6*	135±7	136±7	138±6	140±7	141±9	140±7	140±7	140±6	141±8	141±8	141±9	139±9	140±8	141±9*	141±9*	140±9*	140±7*			
Rate pressure product - Control	7927±1754	8787±1449	8963±1654	9028±1804	9160±2048	9483±2096	9901±2047	9978±2093	9619±2114	9549±2097	9458±1931	9253±2024	9212±1982	9166±2045*	8890±2087	9077±2011	8984±1819	8883±2042	8834±1927	0.008	0.307	0.014
Rate pressure product - Prazosin	8141±1534	9380±1761	9169±1724	9284±1750	9771±1778	9953±2062	9833±2023	9960±2072	9887±2196	9858±2024	10088±2018	10158±2138	10217±2204	9705±1855	9892±1870*	9941±1667*	9981±1580*	9928±1482*	9826±1709*			
LAD velocity max Control	0.252±0.03			0.325±0.07			0.304±0.03			0.280±0.05			0.261±0.04			0.266±0.05			0.265±0.05	0.007	0.769	0.594
LAD velocity max Prazosin	0.261±0.04			0.312±0.04			0.302±0.07			0.281±0.06			0.278±0.06			0.284±0.06			0.279±0.05			
LAD velocity mean Control	0.201±0.03			0.256±0.07			0.232±0.03			0.224±0.04			0.209±0.03			0.215±0.03			0.207±0.03	0.041	0.603	0.400
LAD velocity mean Prazosin	0.199±0.03			0.25±0.02			0.226±0.03			0.228±0.05			0.231±0.04			0.231±0.04			0.228±0.04			

Hemodynamic and coronary responses during Cold pressor test (averaged per 10 second intervals). P-values refer to 2-way repeated measures ANOVA's, for within participant comparison (time), between trial comparison, and the interaction time*trial. *Symbols denote P<0.05 difference to baseline values.

633 **TABLE 2 – Lower body negative pressure responses**

Lower body negative pressure								2-way ANOVA		
	Baseline	-10		-20		-30		Time	Trial	Time*Trial
Stroke volume (ml) Control	106±13	102±12	100±12	97±12	97±13	93±14	90±14	0.000	0.687	0.086
Stroke volume (ml) Prazosin	106±12	103±14	99±16	96±15	93±16	91±16	85±18*			
Cardiac Output (L/min) Control	6.4±1.2	6.1±1.1	6.1±1.1	5.9±1.1	6.0±1.0	5.8±1.0	5.9±1.0	0.642	0.002	0.162
Cardiac Output (L/min) Prazosin	7.1±1.4	7.1±1.2	7.2±1.4	7.2±1.0	7.4±0.9	7.5±0.8	7.3±0.9			
Heart Rate (bpm) Control	61±12	60±12	61±13	62±13	63±13	64±14	67±14	0.000	0.002	0.009
Heart Rate (bpm) Prazosin	68±16	70±16	75±20	77±17*	82±17*	84±17*	89±17*			
Diastolic BP (mmHg) Control	77±9	77±9	77±9	78±9	78±9	79±9	79±10	0.177	0.160	0.060
Diastolic BP (mmHg) Prazosin	73±8	74±8	72±9	74±8	73±8	74±9	73±9			
Systolic BP (mmHg) Control	132±7	131±6	129±7	130±7	131±6	130±7	131±7	0.152	0.388	0.005
Systolic BP (mmHg) Prazosin	131±9	131±9	128±9	129±9	127±10	127±10	124±11			
Rate pressure product - Control	8048±1746	7923±1745	7929±1831	8024±1766	8192±1741	8348±1895	8708±1916	<0.001	0.027	0.025
Rate pressure product - Prazosin	8954±2618	9253±2537	9646±2963	9969±2491*	10479±2585*	10751±2568*	10960±2319*			
LAD VTI Control	12.1±2.7	11.1±3.6		9.9±3.4		8.7±1.3		0.019	0.09	0.547
LAD VTI Prazosin	9.5±0.9	9.8±1.9		8.1±2.2		7.8±1.8				
LAD velocity max Control	0.277±0.05	0.26±0.06		0.243±0.05		0.226±0.03		0.029	0.52	0.621
LAD velocity max Prazosin	0.25±0.06	0.249±0.03		0.226±0.04		0.225±0.04				
LAD velocity mean Control	0.21±0.03	0.19±0.03		0.203±0.04		0.187±0.03		0.496	0.973	0.177
LAD velocity mean Prazosin	0.197±0.03	0.207±0.02		0.188±0.03		0.20±0.03				

634 Hemodynamic and coronary responses during Lower body negative pressure test (averaged per 2 minute stages). P-values refer to 2-way
635 repeated measures ANOVA's, for within participant comparison (time), between trial comparison, and the interaction time*trial. *Symbols
636 denote P<0.05 difference to baseline values.

637 **FIGURES**

638

639 **FIGURE 1.** Responses of the carotid artery (n=9) and the LAD coronary artery (n=6) to CPT.
640 A. Diameter change of the carotid artery over time. B. Percentage change in carotid diameter
641 at baseline and during CPT (area under the curve, AUC). C. VTI change of the LAD coronary
642 artery over time. D. Percentage change in LAD coronary artery VTI at baseline and during
643 CPT (area under the curve, AUC). Data are presented as mean, error bars represent standard
644 error of the mean (SEM). White bars represent baseline measurements, black bars represent
645 peak values.

646

647 **FIGURE 2.** Responses of the carotid artery (n=9) and the LAD coronary artery (n=5) to
648 LBNP. A. The diameter change of the carotid artery over time. B. The percentage change in
649 carotid diameter at baseline and during LBNP. C. the VTI change of the LAD coronary artery
650 over time. D. The percentage change in LAD coronary artery VTI at baseline and during
651 LBNP. Data are presented as mean, error bars represent standard error of the mean (SEM).
652 White bars represent baseline measurements, black bars represent peak values.

653

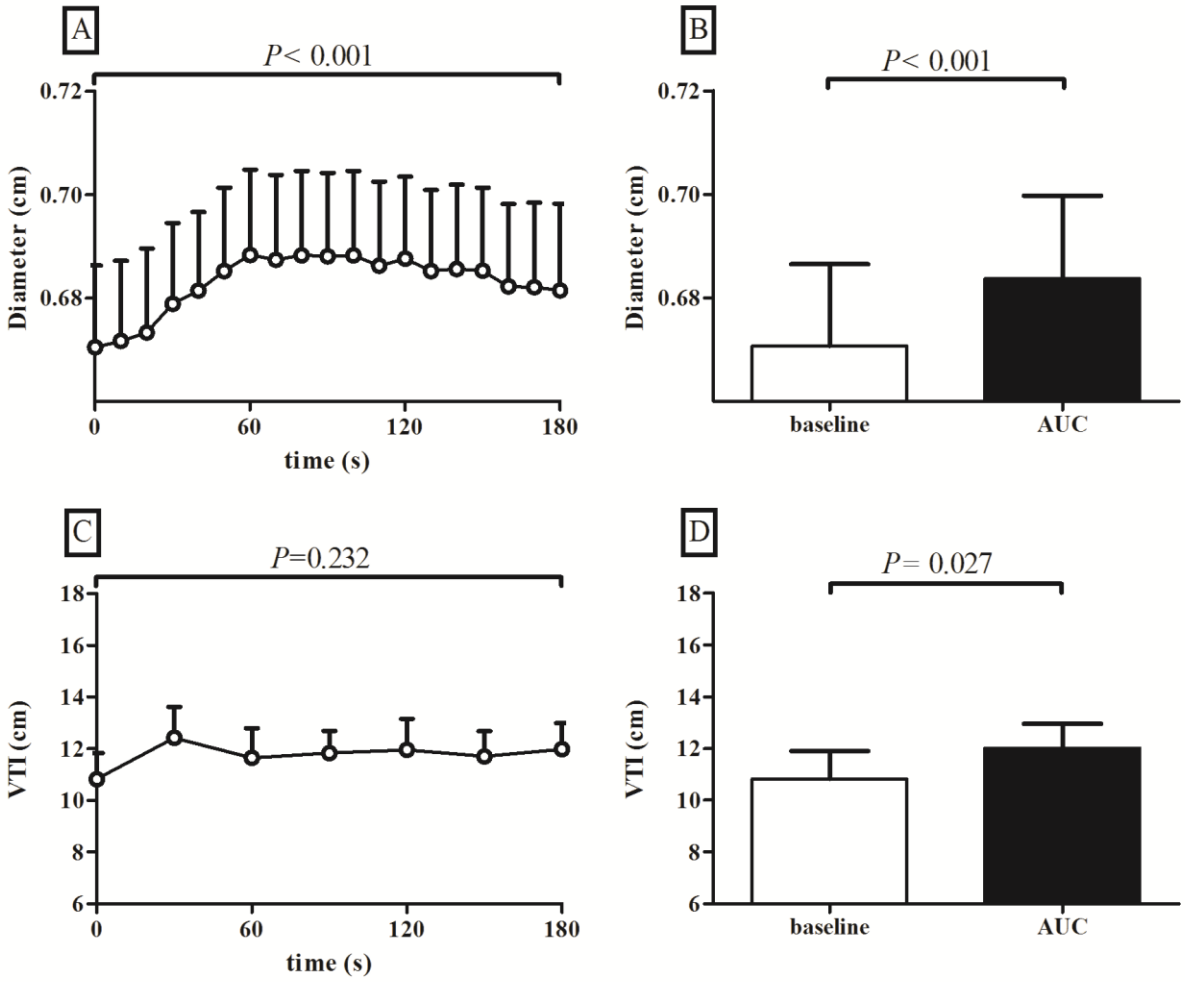
654 **FIGURE 3.** Responses of the carotid artery (n=9) and the LAD coronary artery (n=6) to CPT,
655 Control versus Prazosin condition. A. Diameter change of the carotid artery over time. B.
656 Percentage change in diameter. C. VTI change of the LAD coronary artery over time. D.
657 Percentage change in LAD coronary artery VTI at baseline and during the CPT. Data are
658 presented as mean, error bars represent standard error of the mean (SEM). White bars
659 represent the Control condition, black bars represent the Prazosin condition.

660

FIGURE 4. Responses of the carotid artery (n=9) and the LAD coronary artery (n=5) to LBNP, Control versus Prazosin condition. A. Diameter change of the carotid artery over time. B. Percentage change in carotid diameter at baseline and during the LBNP. C. VTI change of the LAD coronary artery over time. D. Percentage change in LAD coronary artery VTI at baseline and during the LBNP. Data are presented as mean, error bars represent standard error of the mean (SEM). White bars represent the Control condition, black bars represent the Prazosin condition.

FIGURE 5. Correlation between the carotid artery diameter response (CAR%) and coronary LAD response (change in the velocity time integral (VTI in cm)) pooled for the cold pressor test and lower body negative pressure test (n=16).

672 **FIGURE 1.**



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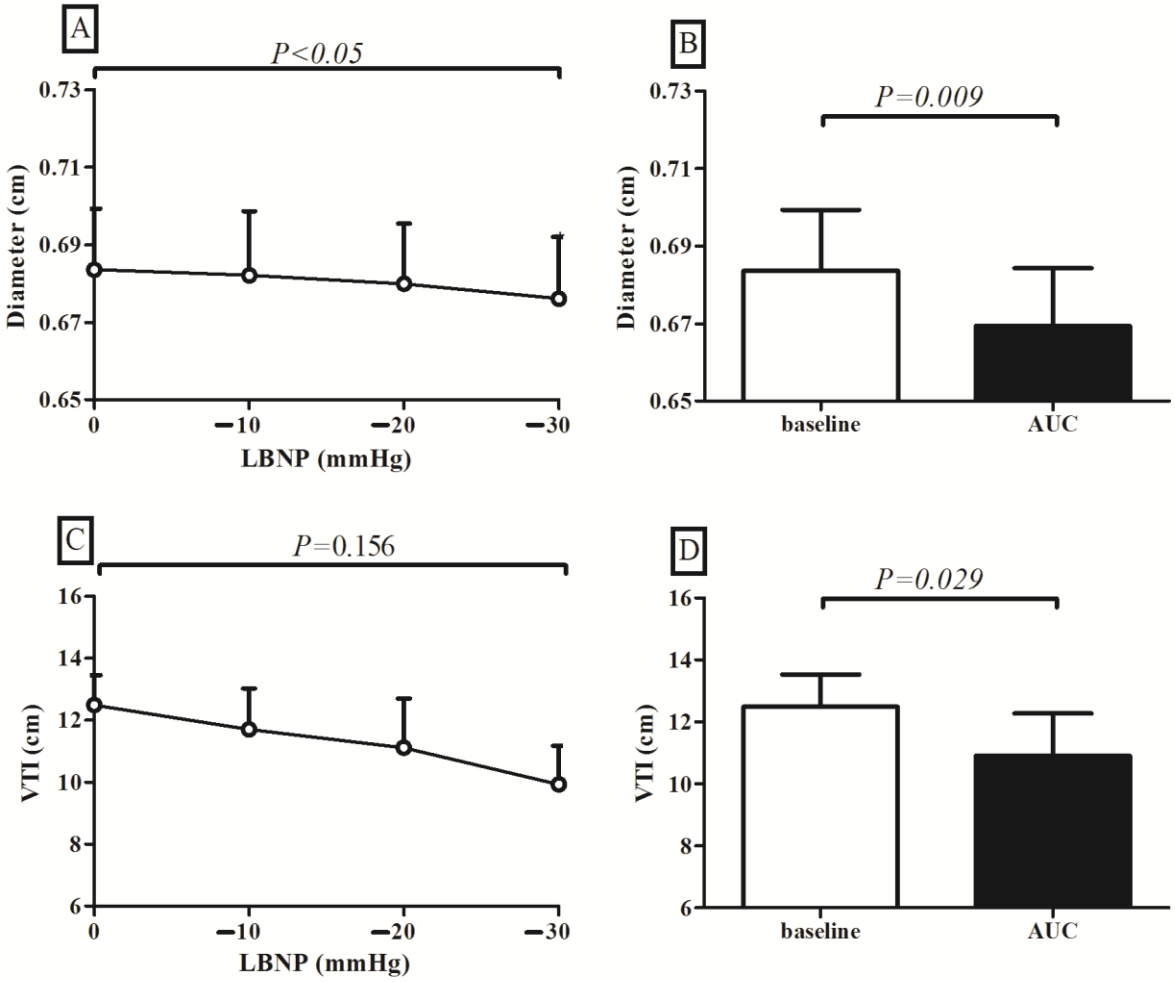
675 **FIGURE 2.**



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679 **FIGURE 3.**
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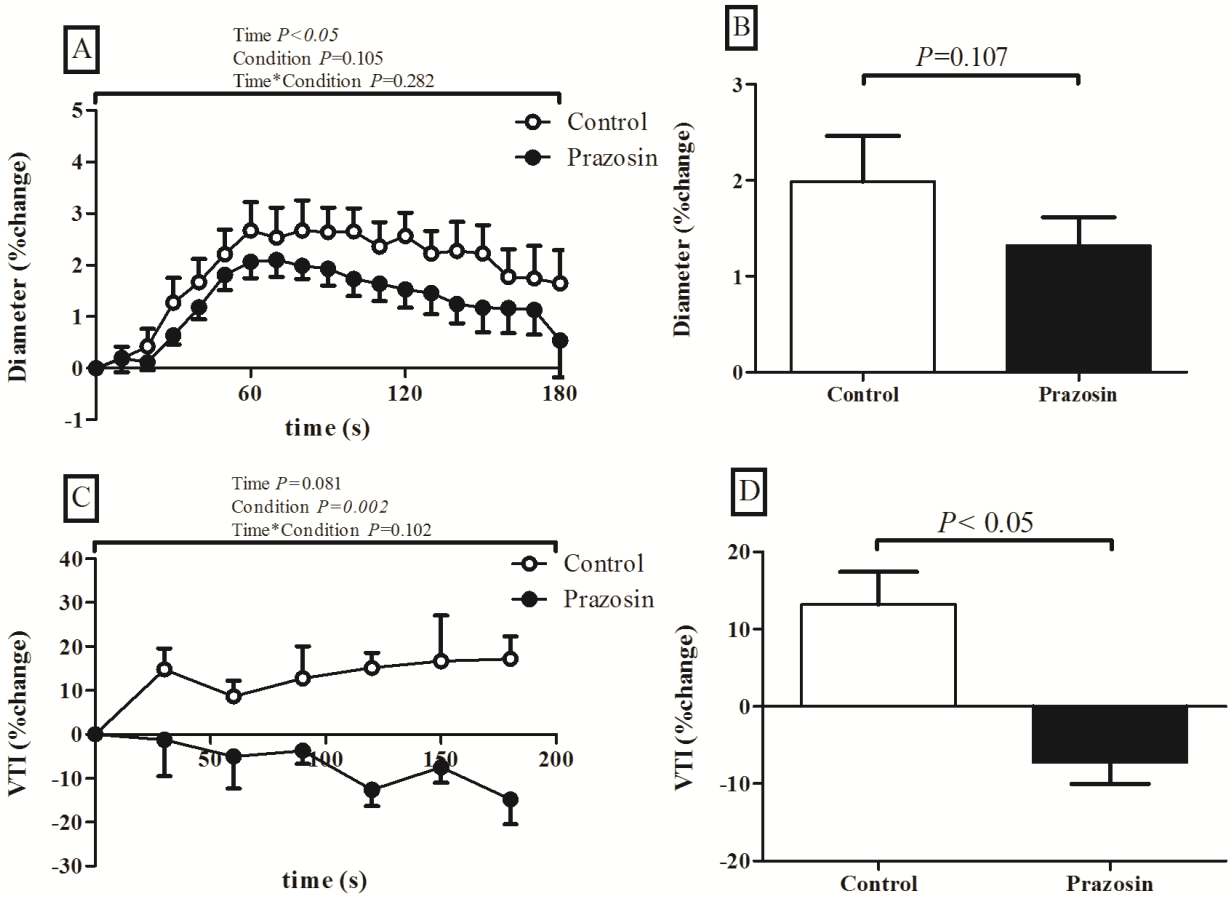

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683 **FIGURE 4.**
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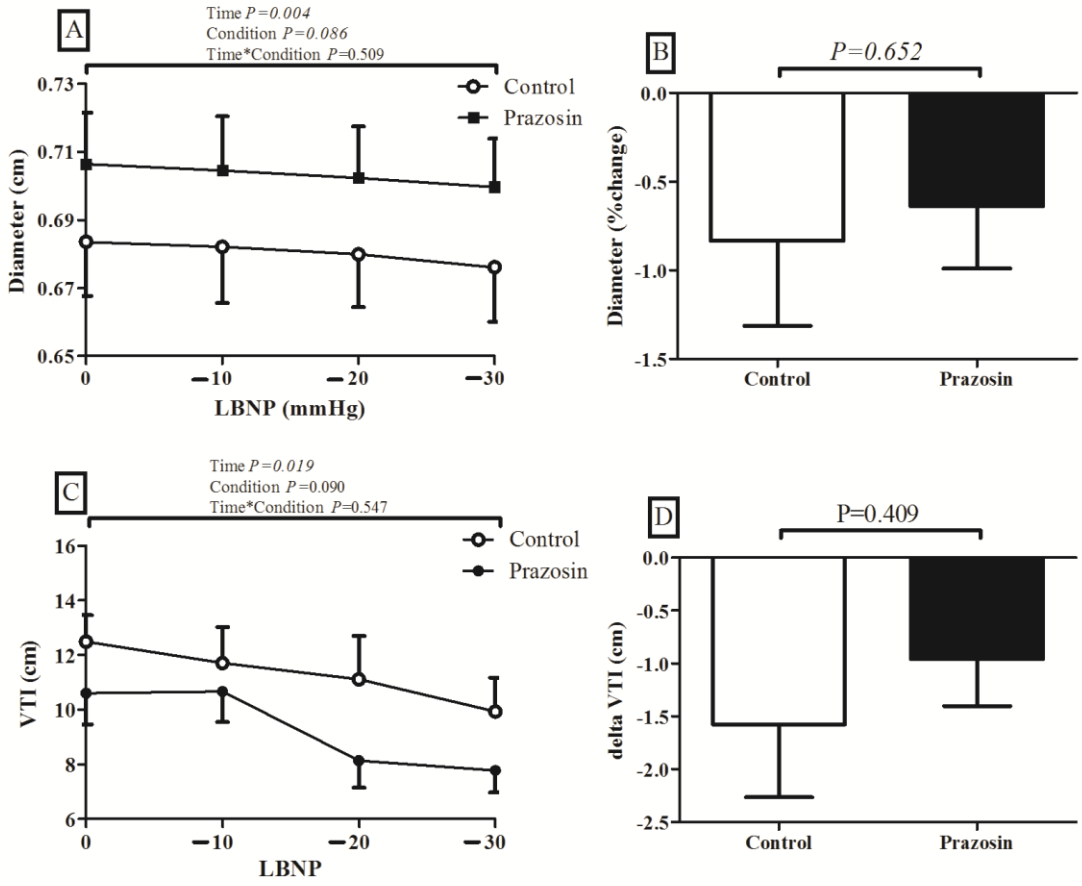

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687 **FIGURE 5.**
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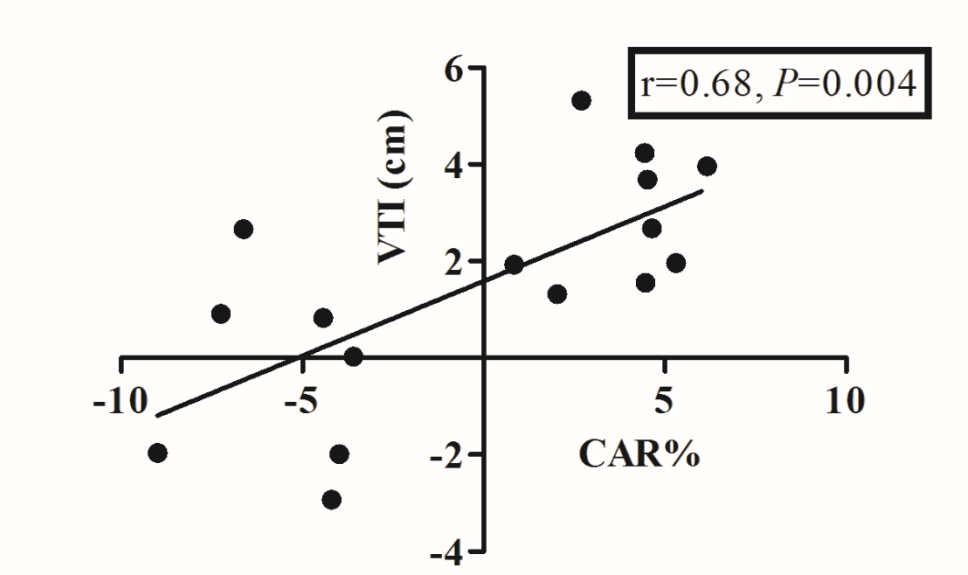

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