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van Mil, AC, Tymko, MM, Kerstens, TP, Stembridge, M, Green, DJ, Ainslie, PN and Thijssen, DHJ (2018) Similarity between carotid and coronary artery responses to sympathetic stimulation and the role of alpha-1 receptors in humans. Journal of Applied Physiology (1985). 125 (2). pp. 409-415. ISSN

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

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16 **Short title: SNS activation & central artery responses**

17
18 **WORD COUNT: 4604**

19 **ABSTRACT WORD COUNT: 250**

20 **FIGURES: 5**

21 **TABLES: 2**

22
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27

28 **ABSTRACT**

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

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

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

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

52

53 **KEYWORDS:** carotid artery, coronary artery endothelial function, sympathetic nervous
54 system, cardiovascular disease, α 1-adrenoceptors

55 NEWS AND NOTEWORTHY

56

57 • We showed distinct carotid artery responses to cold pressor test (i.e. dilation) and
58 lower-body negative pressure (i.e. constriction).

59 • Blockade of α 1-receptors significantly attenuated dilator responses in carotid and
60 coronary arteries during CPT, whilst no changes were found during LBNP.

61 • Our findings indicate strong similarity between carotid and coronary artery responses
62 to distinct sympathetic stimuli, and for the role of α -receptors.

63 ABBREVIATION LIST

64

65 Cardiovascular disease (CVD)

66 Cardiac output (CO)

67 Cold pressor test (CPT)

68 Common carotid artery (CCA)

69 Diastolic blood pressure (DBP)

70 Heart rate (HR)

71 Left anterior descending (LAD)

72 Left anterior descending artery, mean diastolic velocity (LADV_{mean})73 Left anterior descending artery, peak diastolic velocity (LADV_{max})

74 Lower body negative pressure (LBNP)

75 Partial pressure of end-tidal carbon dioxide (P_{ET}CO₂)76 Partial pressure of end-tidal oxygen (P_{ET}O₂)

77 Rate pressure product (RPP)

78 Systolic blood pressure (SBP)

79 Sympathetic nervous system (SNS)

80 Stroke volume (SV)

81 Velocity time integral (VTI)

82 INTRODUCTION

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

97

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

107 No previous study examined the potential underlying mechanisms mediating carotid artery
108 vasomotion during SNS activation. Work in both animal and human coronary arteries
109 revealed a central role for α_1 -receptors to mediate vasomotor responses during SNS activation
110 (17, 23, 26). In line with this previous work, we expect that α_1 -receptors, at least in part,
111 contribute to the carotid artery responses to CPT and LBNP. Therefore, our first aim is to
112 examine the impact of activation of the SNS, either through the CPT (i.e. elevates SNS
113 activity and blood pressure)(18, 46) or LBNP (i.e. elevates SNS activity, with preserved blood
114 pressure)(21, 45) on carotid artery diameter. Our second aim was to assess the role of α_1 -
115 adrenoreceptors to these carotid artery responses by using an oral, selective α_1 -adrenoreceptor
116 blocker (i.e. Prazosin).

117

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

126

127 **METHODS**

128 **Ethical approval**

129 This study was approved by the Human Ethics Committee of the University of British
130 Columbia and conformed to the standards set by the Declaration of Helsinki. All volunteers
131 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/m²), 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 ($23\pm 1^\circ\text{C}$). 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 -receptor activity, 0.05mg per kg body
155 weight)(1, 22).

156

157 **Experimental measures**

158 *Common carotid artery diameter and velocity.* Left carotid artery diameter and red blood cell
159 velocity were recorded simultaneously and continuously during baseline (1-minute) and
160 sympathetic stimuli (i.e. 3-minutes CPT, and ~18-minutes LBNP). Carotid artery image
161 acquisition was performed using a 10-MHz multifrequency linear array handheld probe
162 attached to a high resolution ultrasound machine (15L4, Terason T3200, Burlington, MA,
163 USA). When an optimal image was found, 2-3 cm proximal from the bifurcation, the probe
164 was held stable and the ultrasound parameters were set to optimise the longitudinal, B-mode
165 image of the lumen-arterial wall interface. Continuous pulsed wave Doppler velocity
166 assessments were also obtained and were collected at the lowest possible insonation angle
167 (always $<60^\circ$). Assessment was performed by an experienced sonographer (ACCM), whom
168 has an hour-to-hour reproducibility (i.e. coefficient of variation) of CCA baseline diameter of
169 0.8% and reproducibility of 0.8% for the peak CCA diameter, in line with previous findings
170 (44).

171

172 *Coronary artery velocity.* Before and during both CPT and LBNP, the left anterior descending
173 (LAD, cm) coronary artery velocity was examined using transthoracic ultrasound. This
174 assessment was performed simultaneously with CCA diameter and velocity responses. All
175 echocardiographic measurements were collected by a trained sonographer (MS) on a
176 commercially available ultrasound system (Vivid E9; GE, Fairfield, CT) using a broadband
177 M5S 5 MHz or a 3V 3D-array transducer. In a previous study the Cronbach's alpha reliability
178 test revealed alpha values of 0.81 and 0.89 for both max and mean LAD velocities,
179 respectively, suggesting good consistency between LAD velocity measurements (5).
180 Participants assumed a left lateral position to allow for data collection. The LAD was imaged
181 using a modified parasternal short axis view from the fourth or fifth left intercostal space, and

182 was assessed using pulsed-wave Doppler. The transducer was positioned such that a 2- to 3-
183 mm segment of the LAD was imaged along the long axis, taking care to align the pulse-wave
184 cursor with the length of the vessel. With a sample volume (2.0 mm) positioned over the color
185 Doppler signal in the LAD, measurements of the LAD velocity were collected during the
186 sympathetic tests.

187

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

198

199 **Sympathetic stimuli**

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

207 (P_{ET}CO₂) and oxygen (P_{ET}O₂) were clamped at baseline values for the entire duration of the
208 protocol to reduce the potential impact of hyperventilation on the vascular responses, upon an
209 end-tidal forcing approach described extensively elsewhere (43). To reduce measurement
210 error, CPT procedures were repeated twice and averaged for analyses (44).

211

212 *Lower body negative pressure.* The participant was positioned in the supine position on a tilt
213 bed, and strapped into a custom-made airtight, lower-body suction chamber at the level of the
214 iliac crest (42). The LBNP chamber was then moved from supine position into a left lateral
215 position (~25-30°) to ensure adequate coronary imaging. The lower body negative pressure
216 test consisted of a 5-minute baseline, followed by progressive 2-minute stages, using
217 increments of -10 mmHg, to -80 mmHg or until pre-syncope. LBNP was terminated when *a*)
218 pre-syncope occurred, defined by a sustained drop in systolic blood pressure <80 mmHg for
219 more than 10 seconds (24), or *b*) upon participants request due to the onset of subjective
220 symptoms (e.g. feelings of dizziness, nausea, faintness). During the Prazosin condition,
221 participants were unable to last longer than -40mmHg during the LBNP test. For reliable
222 comparison between the control and drug condition, we chose to only include data until -
223 30mmHg.

224

225 **Data analysis**

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

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

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

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

256

257 Statistical analyses

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

274

275

276 RESULTS**277 Carotid artery responses: different SNS stimuli.**

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

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

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

289

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

291 *Cold pressor test.* Prazosin increased baseline CCA diameter, and decreased shear (
292 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
293 no changes were found in carotid blood flow and blood velocity (10.9 ± 1.8 to 10.8 ± 1.5 ml/sec
294 $P=0.405$, and 30.7 ± 6.6 to 27.7 ± 5.2 cm/sec, $P=0.051$). Prazosin caused an abolished CPT-
295 induced increase in diameter (Figure 3). Prazosin attenuated the increase in blood pressure
296 during CPT, and resulted in a larger increase in cardiac output, heart rate and RPP during the
297 CPT (n=9, Table 2). We found no change in stroke volume (Table 2), whilst we also found no
298 change in CCA flow, shear and velocity (data not shown).

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

306

307 ***Carotid artery responses versus coronary artery responses.***

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

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

319

320

321 **DISCUSSION**

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

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

334

335 **Carotid artery responses to sympathetic stimulation.**

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

357

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

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

372

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

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

388

389 **Carotid artery *versus* coronary artery.**

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

405

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

421

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

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

435

436 *Methodological considerations.* A strength of our study was that we controlled for end-tidal
437 gases at baseline values, during both CPT and LBNP, and the α_1 -receptor blockade condition.
438 Fluctuations and alterations in $P_{ET}CO_2$ are known to directly influence the diameter of the CCA
439 (35) and LAD VTI (5). Following our α_1 blockade, which directly affects mean arterial
440 pressure and ventilatory regulation during sympathetic activation, clamping $P_{ET}CO_2$ and
441 $P_{ET}O_2$ to baseline values reduced the possible interference with our carotid and coronary
442 artery responses.

443

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

451

452 **Acknowledgements**

453 We would like to thank Kevin Wildfong for his valuable contribution to the experiments, and
454 Glen Foster for providing the necessary equipment and laboratory space.

455

456 Author contribution statement

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

463

464 Sources of funding

465 Philip N. Ainslie, and the experimental studies which were conducted in his laboratory, was
466 supported by a Canadian Research Chair in Cerebrovascular Physiology, a Natural Sciences
467 and Engineering Research Council Discovery Grant and Canadian Foundation of Innovation
468 Grant. MT is funded by a Natural Sciences and Engineering Research Council Doctoral
469 award. DJG is supported by a National Health and Medical Research Council (NHMRC)
470 Principal Research Fellowship (APP1080914). ACCM is financially supported by a Top
471 Institute for Food and Nutrition-grant.

472

473 Disclosures

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

475

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- 627
- 628

629 **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															

630 Hemodynamic and coronary responses during Cold pressor test (averaged per 10 second intervals). P-values refer to 2-way repeated measures

631 ANOVA's, for within participant comparison (time), between trial comparison, and the interaction time*trial. *Symbols denote P<0.05

632 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±174	7923±174	7929±1831	8024±1766	8192±1741	8348±1895	8708±1916	<0.001	0.027	0.025
Rate pressure product - Prazosin	8954±261	9253±253	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	5	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±0.0		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±0.0	0.207±0.0		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.

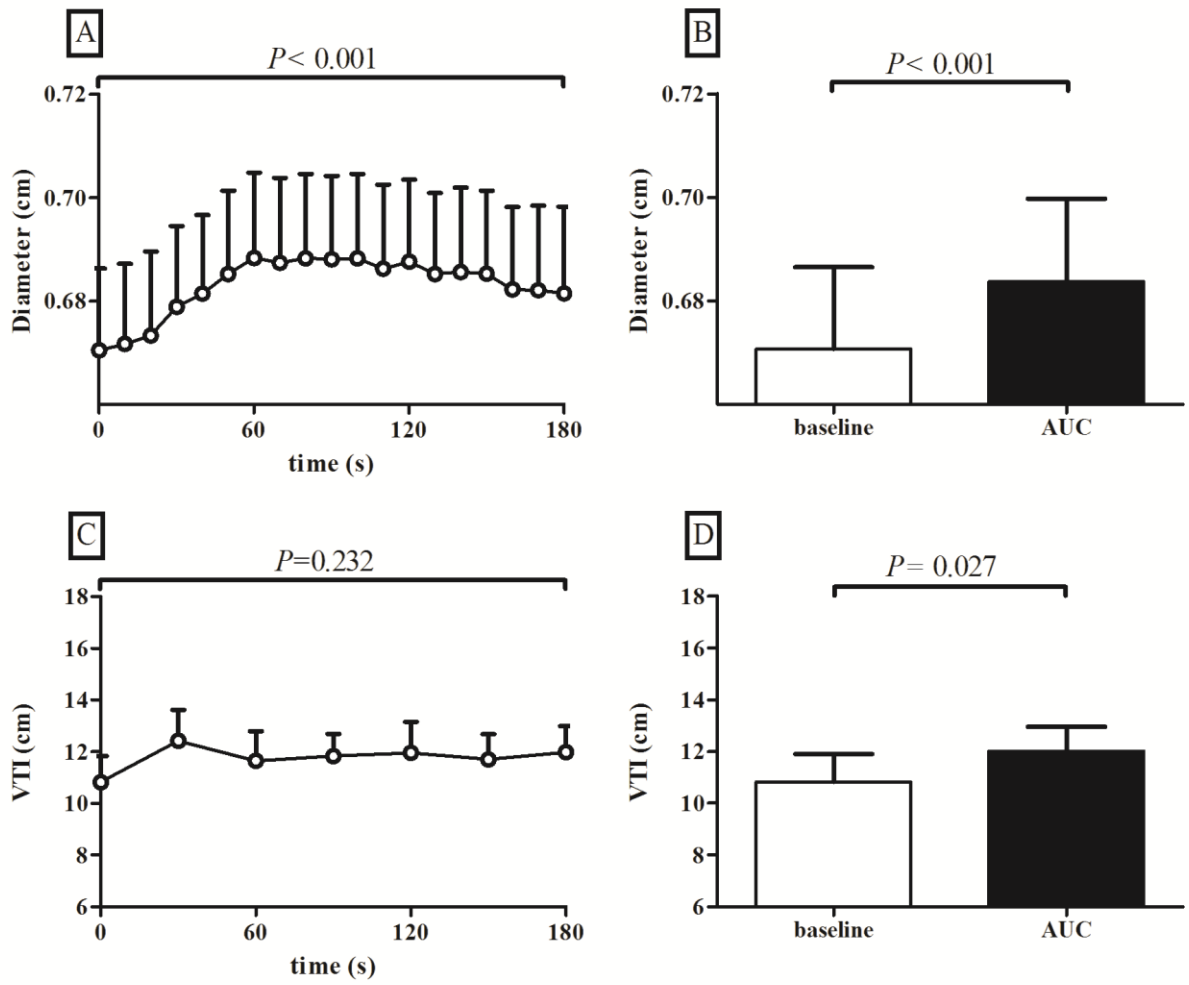
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661 **FIGURE 4.** Responses of the carotid artery (n=9) and the LAD coronary artery (n=5) to
662 LBNP, Control *versus* Prazosin condition. A. Diameter change of the carotid artery over time.
663 B. Percentage change in carotid diameter at baseline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

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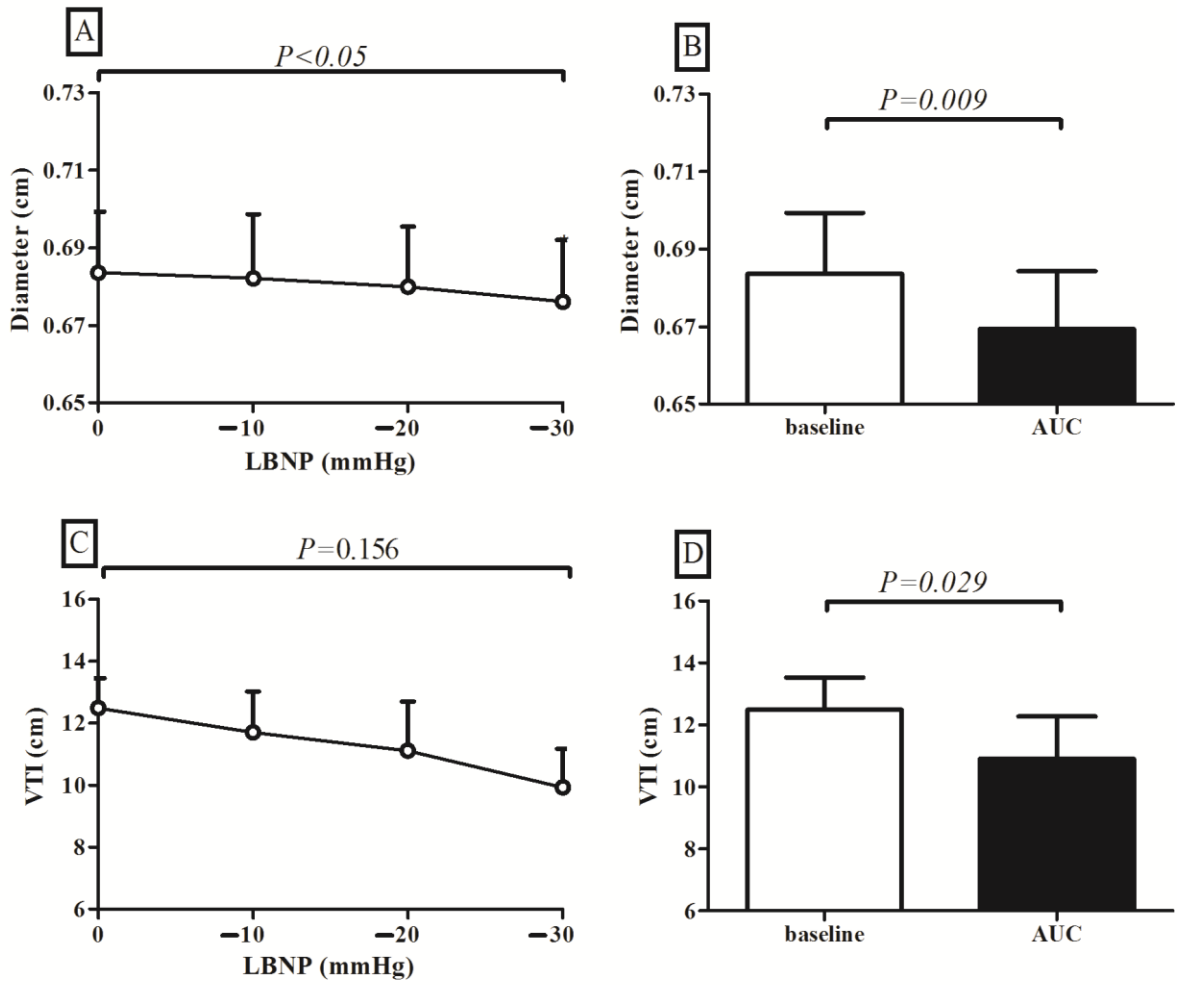
669 **FIGURE 5.** Correlation between the carotid artery diameter response (CAR%) and coronary
670 LAD response (change in the velocity time integral (VTI in cm)) pooled for the cold pressor
671 test and lower body negative pressure test (n=16).

672 **FIGURE 1.**



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675 **FIGURE 2.**

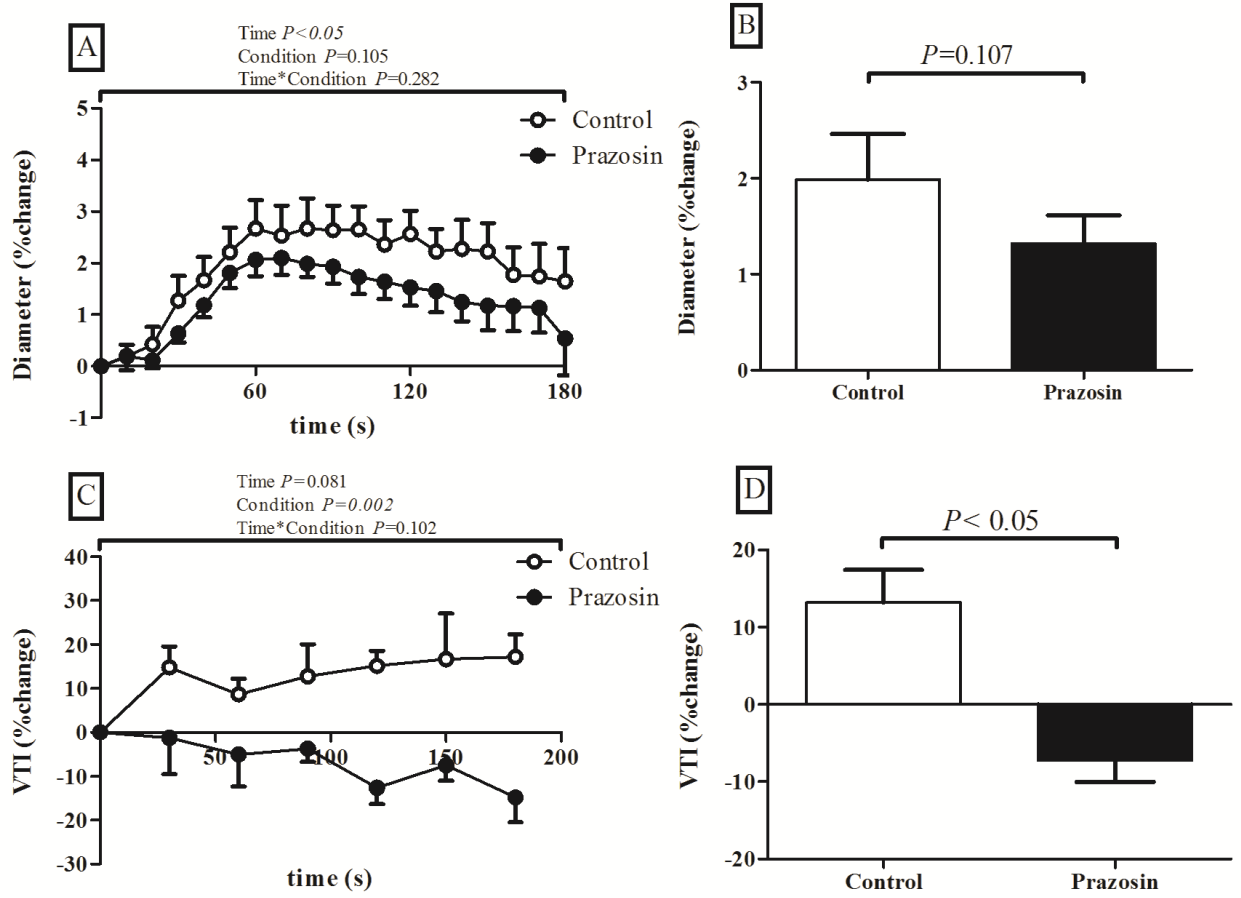


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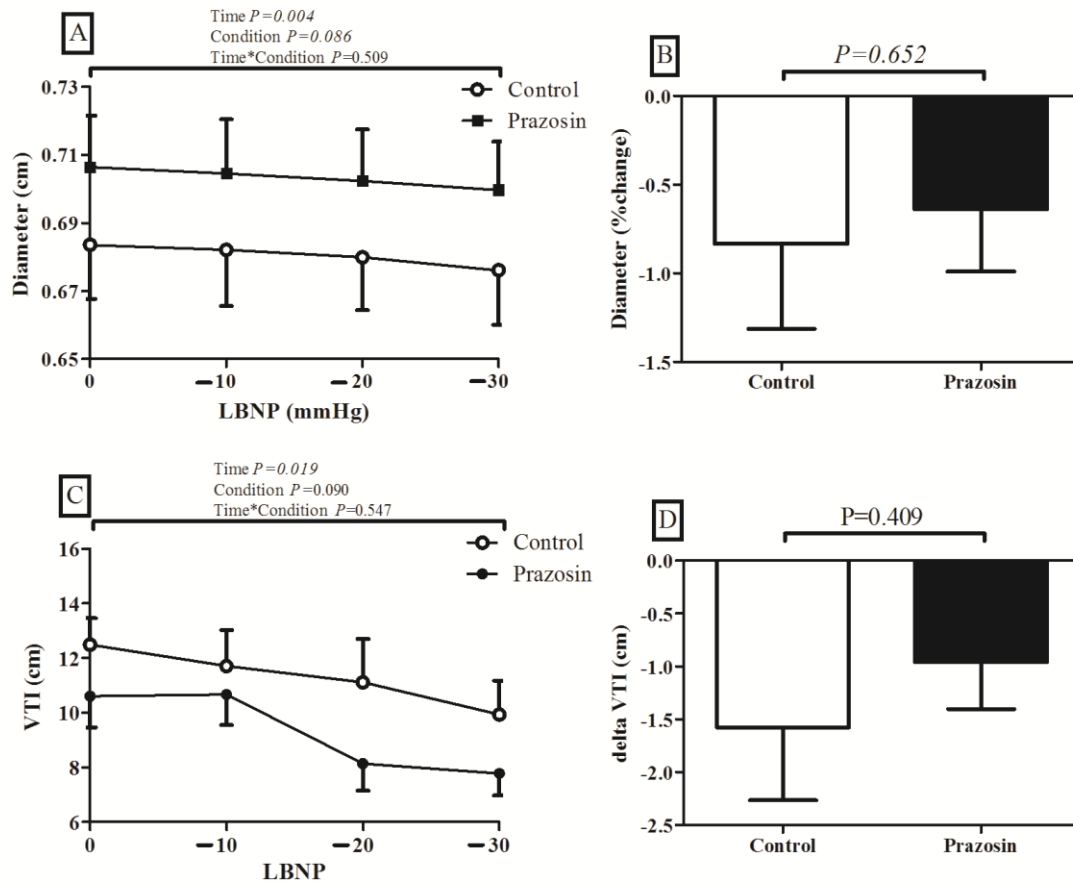
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679 **FIGURE 3.**
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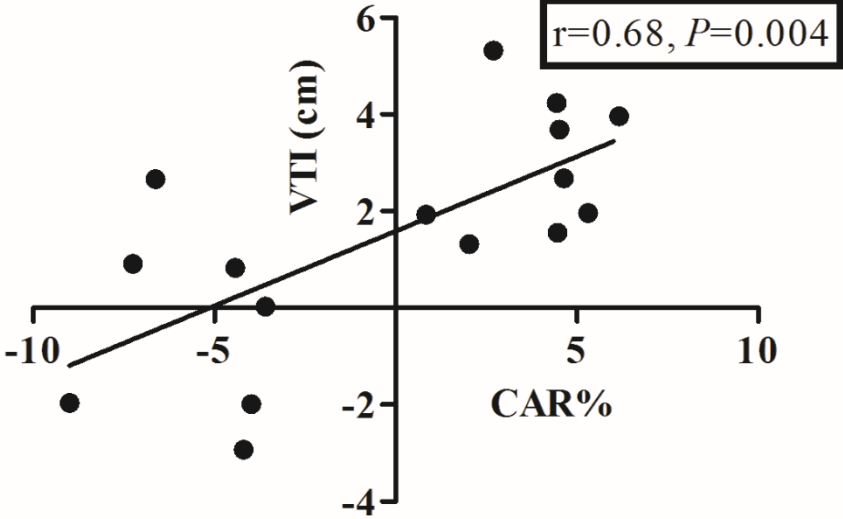
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683 **FIGURE 4.**
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687 **FIGURE 5.**
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