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### Article

**Citation** (please note it is advisable to refer to the publisher's version if you intend to cite from this work)

**Peace, A, Pinna, V, Timmen, F, Speretta, G, Jones, H, Lotto, RR, Jones, IS and Thijssen, DHJ (2019) Role of blood pressure in mediating carotid artery dilation in response to sympathetic stimulation in healthy, middle-aged individuals. American Journal of Hypertension. ISSN 0895-7061**

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1 Role of blood pressure in mediating carotid artery dilation in response  
2 to sympathetic stimulation in healthy, middle-aged individuals.

3

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**WORD COUNT: 4,837**

17

**ABSTRACT WORD COUNT: 235**

18

**FIGURES: 3**

19

**TABLES: 3**

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25

**DISCLOSURES**

26

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

27 **ABSTRACT**

28 **Objectives.** Carotid artery diameter responses to sympathetic stimulation, i.e. carotid artery reactivity  
29 (CAR), represents a novel test of vascular health and relates to cardiovascular disease/risk. This study  
30 aims to understand the relationship between the increase in blood pressure and carotid artery  
31 diameter response during the CAR-test in healthy, middle-aged men.

32 **Methods.** Sample consisted of 40 normotensive men (aged 31-59) with no history of cardiovascular  
33 disease or currently taking medication. Non-invasive ultrasound was used to measure carotid artery  
34 diameter during the cold pressor test (CPT), with CAR% being calculated as the relative change from  
35 baseline (%). Mean arterial pressure (MAP) was measured with beat-to-beat blood pressure recording.

36 **Results.** CAR% was  $4.4 \pm 5.4\%$ , peaking at  $92 \pm 43$ s. MAP increased from  $88 \pm 9$  mmHg to  $110 \pm 15$  mmHg,  
37 peaked at  $112 \pm 38$  seconds, which was significantly later than the diameter peak ( $P=0.04$ ). The  
38 correlation between resting MAP and CAR% was weak ( $r=0.209$   $P=0.197$ ). Tertiles based on resting  
39 MAP or MAP-increase revealed no significant differences between groups in subject characteristics  
40 including age, BMI or CAR% (all  $P>0.05$ ). Subgroup analysis of individuals with carotid constriction  
41 ( $n=6$ ) versus dilation ( $n=34$ ), revealed no significant difference in resting MAP or increase in MAP  
42 ( $P=0.209$  and  $0.272$ , respectively).

43 **Conclusion.** Our data suggests that the characteristic increase in MAP during the CPT does not  
44 mediate carotid artery vasomotion.

45

46 **KEYWORDS:** Endothelial function, coronary arteries, carotid artery reactivity test, cold pressor test,  
47 cardiovascular risk, blood pressure

## 48 Introduction

49 Cardiovascular disease (CVD) is the leading cause of mortality, accounting for approximately 31% of  
50 all deaths worldwide (1). Coronary artery disease (CAD) is the largest subtype of CVD and a growing  
51 burden due to modern lifestyle and an ageing population, is (1). The vascular endothelium plays an  
52 important role in regulating vascular tone, thereby contributing to the health and integrity of the  
53 vasculature. Several studies have revealed the importance of a healthy endothelium in the prevention  
54 of progression of atherosclerosis and development of cardiovascular disease (2-5). The sympathetic  
55 nervous system, largely through  $\alpha$ - and  $\beta$ -adrenergic receptors on the endothelium, contribute to the  
56 regulation of vascular tone (6). Indeed, sympathetic stimulation leads to marked vasodilation in  
57 central arteries, including the coronary arteries. In the progression of atherosclerosis, function and/or  
58 presence of endothelial  $\alpha$ - and  $\beta$ -adrenergic receptors may be altered, resulting in vasoconstriction in  
59 response to sympathetic stimulation in patients with established cardiovascular disease (6, 7).  
60 Interestingly, the presence of coronary artery constriction during sympathetic stimulation has  
61 independent prognostic value for future cardiovascular events (8)

62

63 Similar to coronary arteries, carotid artery dilation occurs during sympathetic stimulation in healthy  
64 individuals using the cold pressor test (9). Furthermore, we found that this carotid artery reactivity  
65 (CAR) relates to the magnitude of coronary artery vasomotion (9), but also has independent prognostic  
66 value for future cardiovascular events in patients with peripheral artery disease (10). Whilst the role  
67 of the sympathetic nervous system in mediating coronary and carotid artery responses are well  
68 established, relatively little is known about the potential role of the increase in blood pressure *per se*.

69

70 Recently, we found that an alpha-1-receptor blocker partially abrogated the increase in carotid artery  
71 diameter during the CAR-test, but also attenuated the blood pressure increase (11). In the same study,  
72 lower body negative pressure, another stimulus for sympatho-excitation, did not cause an increase in  
73 blood pressure or carotid artery diameter. Also others have linked changes in blood pressure, directly

74 linked to increases in sympathetic activity, to conduit artery dilation(12). Furthermore, an increase in  
75 blood pressure may affect vasomotion as a hemodynamic stimulus, whilst the magnitude of blood  
76 pressure increase may reflect sympathetic drive (13, 14). Therefore, an increase in blood pressure may  
77 represent the dilator stimulus for the carotid artery diameter during the CAR-test. To better  
78 understand the link between blood pressure and carotid artery vasomotion, we investigated the  
79 relationship between the timing and magnitude of the sympathetically-induced elevation in blood  
80 pressure and carotid artery diameter responses in healthy, middle-aged men. We included this group  
81 as they demonstrate a good diversity of blood pressure and diameter responses to the cold pressor  
82 test, which will help to better answer our research question.

83

## 84 **Methods**

### 85 **Participants**

86 Forty healthy men aged 31-59 years old with no history of CVD were recruited. Exclusion criteria were:  
87 a history of CVD, history of diabetes, currently using cardiac medication for heart rate, blood pressure  
88 or cholesterol, Raynaud's syndrome. Local ethical approval from the Liverpool John Moores University  
89 was sought and gained (17/NW/0347). Informed consent was obtained and formally documented.  
90 Participants completed a health questionnaire, including medical history and CVD related lifestyle risk  
91 factors.

### 92 **Procedure**

93 Participants were asked to abstain from smoking for at least 6 hours, from vigorous exercise for at  
94 least 24 hours prior to attending the laboratory and to avoid dietary products that can influence  
95 endothelial function, such as caffeine, alcohol, chocolate, and vitamin C for at least 18 hours (9, 15).  
96 Upon arrival participants body weight and height were measured, and were instructed to lie on a bed  
97 in a quiet, light and temperature controlled room. A finometer (Finapres Medical Systems,

98 Amsterdam, The Netherlands) was used to measure beat-to-beat blood pressure and the resting blood  
99 pressure was measured with an automated sphygmomanometer (Dinamap Procare 100, GE Medical  
100 Systems Ltd., Buckinghamshire, UK). Participants laid supine for at least 5 minutes before they  
101 underwent the cold pressure test (CPT). The Cold Pressor Test is a sympathetic nervous system  
102 stimulus consisting of 1 minute baseline, 3 minutes with the left hand submerged in cold water (~4°C).  
103 During the CPT, carotid artery diameter and blood flow velocity were measured continuously using  
104 ultrasound sonography (Terason 3300, Terason Labs, Burlington, Massachusetts, USA).

## 105 Measurements

### 106 Blood pressure

107 The blood pressure was measured with an automated sphygmomanometer (16) on the left arm while  
108 the participant was laying supine. This measure was used to determine the resting blood pressure and  
109 to calibrate the beat-to-beat blood pressure values. The finometer cuff was attached on the second  
110 phalanx of the right index or middle finger. The finometer was calibrated to the height of the heart  
111 and was allowed to auto-calibrate for 2 minutes. This has previously been demonstrated to be a  
112 reliable and reproducible measure of beat-to-beat blood pressure monitoring (17).

### 113 Cold pressor test

114 During the CPT, the left hand was immersed in a bucket of cold water (~4°C). The water temperature  
115 was measured with a digital thermometer (Quartz digi-thermo, Fischer scientific, Loughborough, UK)  
116 and controlled by adding crushed ice to maintain a stable water temperature. The participant was  
117 asked to position themselves close to the left edge of the bed, to ensure the hand could easily move  
118 into the water without significant movement of the neck. This enabled assessment of the carotid  
119 artery. After a 1 minute baseline diameter recording, participants were instructed to place their hand  
120 in the ice water for 3 minutes. They were instructed not to speak, and to breathe normally during the  
121 ultrasound assessment of the carotid artery in order to prevent hyperventilation (9, 18, 19).

### 122 Carotid Artery Diameter

123 The left common carotid artery diameter was assessed using ultrasound sonography (Terason 3300,  
124 Terason Labs, Burlington, Massachusetts, USA). Using a longitudinal view of the artery, the carotid  
125 bulb was identified as an anatomical landmark to standardise approximate scanning area between  
126 individuals. The common carotid artery, proximal from the carotid bulb, was identified and image was  
127 optimised so that the artery walls were clearly defined (figure 1). Doppler velocity assessments were  
128 also recorded at the lowest possible insonation angle (always  $<60^\circ$ ). The carotid artery diameter was  
129 calculated with edge detection software (20). On-screen calibration points were selected with the  
130 calibration tool which the software calculated the pixel-to-centimetre ratio. Calibration points were  
131 used for the diameter and the pulse wave velocity. A rectangle containing the largest straight artery  
132 segment was selected as the Region of Interest (ROI), ensuring that the vessel walls were in focus. The  
133 software marked the vessel walls within the ROI with lines and calculated the number of pixels in each  
134 vertical column between the lines. From the pixel distance the software calculated the lumen  
135 diameter in centimetres.

#### 136 Carotid Artery Response (CAR%)

137 The CAR% is the relative change in carotid artery diameter above or below baseline expressed as a  
138 percentage. The average diameter during the 1 minute baseline measurement was calculated and set  
139 as the baseline value. Subsequently, the diameter of the carotid artery was measured during the CPT,  
140 and averaged over 10 second periods, resulting in 24 periods. The average, maximum and minimum  
141 percentages, were calculated. If the average percentage change was an increase in diameter (dilation),  
142 the CAR% is expressed as the maximum percentage. Conversely, if the average percentage change  
143 was a decrease in diameter (constriction), the CAR% is expressed as the minimum percentage (9, 18,  
144 19) .

#### 145 Blood pressure

146 The beat-to-beat blood pressure data was processed in the same was as the CAR% calculation. Beat-  
147 to-beat blood pressure was measured and then MAP calculated. Baseline was the average mean

148 arterial pressure (MAP) during the 1 minute. Next, the average MAP during the CPT is calculated for  
149 each 10 second period, matching the epochs for diameter as described earlier. The systolic and  
150 diastolic blood pressure measured with the sphygmomanometer is used to calculate the MAP before  
151 testing. The beat-to-beat blood pressure data was calibrated using assessment of resting blood  
152 pressure using an automated sphygmomanometer (Dynamap) placed around the left arm and  
153 performed twice (with a 5-minute rest period in between). The maximum change in blood pressure  
154 was expressed as the maximum increase ( $\Delta$ MAP) and maximum percent increase in MAP (relative  
155  $\Delta$ MAP) compared to baseline during the CPT.

## 156 Statistical analysis

157 All data were presented as mean  $\pm$  SD. Statistical analysis was performed using IBM SPSS Statistics 25  
158 (IBM SPSS; IBM Corp., Armonk, New York, USA). Pearson correlations were employed to examine the  
159 relation between baseline MAP and the change in blood pressure during the cold pressor test ( $\Delta$ MAP)  
160 *versus* the CAR% (i.e. relative change in diameter compared to baseline), whilst we also examined the  
161 relation between the timing of the peak responses in BP *versus* CAR%. Participants were divided in  
162 tertiles based on the relative change in BP during the CPT: low (<15%), medium (15-30%) and high  
163 (>30%). One-way ANOVA was used to examine difference between groups in general characteristics  
164 including age, BMI and cardiovascular risk and CAR%. Tukey post-hoc analysis was performed to  
165 examine which groups differed from each other. Statistical significance was at  $p < 0.05$ .

## 166 Results

167 In response to the CPT, diameter immediately changed and demonstrated a gradual increase with an  
168 average peak at  $92 \pm 43$  seconds, which was followed by a gradual decline (Figure 2A). The mean CAR%  
169 was  $4.4 \pm 5.4$ , with six participants demonstrating a constriction of the carotid artery during the CPT  
170 (ranging from -7.6 to -0.74%). During the CPT, MAP began to increase within 30s, followed by a  
171 gradual increase that peaked at  $112 \pm 38$  seconds (Figure 2A). The timing of the peak MAP ( $112 \pm 38$ s)  
172 was significantly later than the peak in diameter ( $92 \pm 43$ s, difference in peak  $20 \pm 5$ s, Wilcoxon-test;



173 P=0.04). There was no significant correlation between peak CAR% and peak MAP (R=0.03 P=0.29), nor  
174 between the timing of the CAR% and MAP (R=0.03 P=0.30).

175

176 After dividing the group into tertiles (based on the relative increase in blood pressure), no significant  
177 differences were found between groups in subject characteristics (e.g. age, weight, BMI, MAP and  
178 family history), baseline diameter or CAR% (Table 3). No differences were found between groups  
179 when, individuals were divided into tertiles based on absolute blood pressure responses (data not  
180 shown). There was no correlation between the relative increase in blood pressure and CAR% (r=0.27  
181 p=0.09).

182

183 Based on the distinct vasomotor responses during the CPT, we compared groups with carotid artery  
184 dilation (n=34) *versus* constriction (n=6). Nevertheless, baseline diameter and CAR% were similar  
185 (Table 4). Importantly, participants who demonstrated carotid artery constriction revealed a similar  
186 increase in BP compared to individuals with carotid dilation (Table 4).

187

## 188 Discussion

189 Our primary aim was to understand the relationship between changes in blood pressure and carotid  
190 artery diameter during the CPT. We present the following findings. First, the start of dilation and the  
191 timing of the peak carotid artery diameter response preceded blood pressure changes during the cold  
192 pressor test. Second, we found no differences in baseline characteristics including age, weight, BMI or  
193 in the magnitude of carotid artery dilation when comparing groups based on the magnitude of blood  
194 pressure increase. This finding is supported by the lack of correlation between the relative changes in  
195 carotid artery diameter and blood pressure during the CPT. Finally, individuals who demonstrated  
196 carotid artery vasoconstriction also demonstrated a comparable increase in blood pressure during  
197 sympathetic stimulation compared to those with vasodilation. Taken together, our study suggests that

198 the characteristic increase in blood pressure during sympathetic stimulation may not directly relate to  
199 carotid artery vasomotion in healthy middle-aged men.

200

201 The CPT is a frequently used procedure to activate the sympathetic nervous system in humans. As  
202 expected, and in line with several previous studies, blood pressure gradually increased after a period  
203 of 20-30 seconds. The increase in blood pressure is most likely the result of (nor)adrenaline release,  
204 mediating a vasoconstriction response in peripheral arteries that cause an increase in total peripheral  
205 resistance. (21, 22) Interestingly, we found that the timing of the start of carotid artery dilation, but  
206 also the timing of the peak diameter change, significantly preceded the blood pressure response. This  
207 suggests that, contrary to our hypothesis, carotid artery response is not directly linked or driven by  
208 the increase in blood pressure response during the cold pressor test. To further support this  
209 conclusion, we found no relation between the degree of blood pressure increase and the CAR% during  
210 the CPT. However, it should be noted that the lack of correlation may relate to the presence of  
211 confounding factors influencing vascular tone. Closely controlling for factors potentially affecting  
212 endothelial function (e.g. drugs, supplements, behavioural aspects) at least partly prevented such  
213 impacts. A final strong argument against a key role for blood pressure in mediating the carotid artery  
214 vasomotor response during the cold pressor test is the presence of vasoconstriction in some  
215 individuals. Intriguingly, a significant increase in blood pressure was found in these individuals, which  
216 did not differ from the blood pressure response found in subjects with carotid artery dilation.

217

218 Despite the absence of a relation between the diameter and blood pressure response, both responses  
219 seem strongly related to sympathetic stimulation. In fact, a previous study found that muscle  
220 sympathetic nerve activity bursts are associated with concomitant increases in blood pressure and  
221 peripheral conduit artery diameter responses(12). Furthermore, catecholamine-release during  
222 sympathetic stimulation seem directly related to carotid artery responses, whilst catecholamines may

223 also be responsible for the increased peripheral artery resistance and blood pressure changes (23).  
224 Differences in sensitivity of receptors or mechanisms contributing to vasomotion between central (i.e.  
225 carotid) and peripheral arteries may explain the difference in timing of the blood pressure and  
226 diameter responses. Nonetheless, given their dependence on catecholamines (24), we expected a  
227 relation between the magnitude of blood pressure and diameter response. One potential explanation  
228 for the lack of relation is that catecholamine-release is less strongly related to vascular responses than  
229 anticipated. Indeed, Cummings *et al.* found adrenalectomised participants do not demonstrate an  
230 increase in adrenaline, noradrenaline or dopamine during the CPT, despite the presence of an increase  
231 in blood pressure of comparable magnitude as in healthy individuals. Therefore, peripheral artery  
232 responses (and therefore blood pressure) to the CPT may be independent of catecholamine release  
233 (25), whilst catecholamines may be important for carotid artery diameter responses. At least, our data  
234 suggests no direct link between blood pressure *per se* and carotid artery diameter response to the CPT  
235 in healthy individuals, despite both parameters change markedly in response to CPT. Based on the  
236 important role of blood pressure during the CPT, and the possible link with vasomotion, we  
237 recommend performing beat-by-beat blood pressure measurements when examining the CAR.

238

239 An important factor to consider is that structural properties of the artery may influence the dilator  
240 response. A previous study demonstrated a negative correlation between baseline carotid diameter  
241 and CAR% in non-diseased average risk, high risk, and coronary artery disease patients, but not with  
242 the carotid artery intima-media wall thickness (IMT) (7). In contrast, Van Mil *et al.* reported no  
243 correlation between the baseline carotid diameter or IMT and CAR% in healthy people (9), whilst also  
244 others found no correlation between coronary artery baseline diameter and dilation response (26). In  
245 our study, we found a significant, but weak, inverse correlation between the baseline carotid diameter  
246 and the CAR%, implying that a smaller baseline diameter correlates with a larger CAR%. This  
247 observations fits with several previous studies examining peripheral arteries, where a smaller brachial  
248 or femoral artery is related to a larger dilation in response to increases in shear stress (27, 28). The

249 presence of a correlation between diameter and CAR% in our study, whilst largely absent in previous  
250 work, may relate to the inclusion of healthy individuals only. For example, previous work in peripheral  
251 arteries also found a weaker or non-existing correlation between baseline diameter and dilator  
252 responses in older and diseased populations. This may be explained by the impact of older age and/or  
253 cardiovascular risk factors in those groups that affect both baseline diameter and dilator response,  
254 consequently affecting the (weak) inverse relation between both parameters in healthy young  
255 individuals. At least, our observation suggests that structural characteristics of the artery wall should  
256 be considered when examining the CAR% responses, but unlikely affect or interfere with the blood  
257 pressure increase (and subsequent diameter response) during the CPT.

258

259 *Limitations.* One potential limitation is that the results of our study only apply to middle-aged men,  
260 making extrapolation to other (diseased) groups difficult. This is important since distinct populations  
261 have demonstrated different CAR% and/or blood pressure response (29, 30), whilst also physical  
262 activity may affect the blood pressure and/or CAR% (31). Nonetheless, it seems unlikely that these  
263 confounding factors, despite their role in changing blood pressure and/or CAR%, affects the relation  
264 between the blood pressure and diameter increase during the CPT. Another limitation is that our study  
265 did not explore the causal link between blood pressure and CAR%. Such a study would require direct  
266 manipulation of the blood pressure response during sympathetic stimulation. Monahan et al (2013)  
267 examined the impact of the CPT on the left anterior descending artery with  $\alpha$ - and  $\beta$ -adrenergic  
268 antagonists (32), and found adrenergic blockage to abolished coronary artery vasodilation. In  
269 agreement, we examined carotid and coronary artery responses during the CPT with and without  $\alpha_1$ -  
270 receptor blockade, and reported abolished carotid and coronary artery responses when combined  
271 with  $\alpha_1$ -receptor blockade(33). Whilst this provides evidence for the role of adrenergic receptors in  
272 contributing to carotid (and coronary) artery vasodilation during the cold pressor test, the presence

273 of an increase in blood pressure during blockade hampered conclusions pertaining to the role of blood  
274 pressure *per se*.

275

276 In conclusion, findings from our study suggest that carotid artery diameter changes during the CPT  
277 may not be related to the characteristic increase in blood pressure. The start and peak of the diameter  
278 precedes that of the blood pressure, whilst no correlation is present between the magnitude of the  
279 blood pressure response and CAR%. Moreover, even individuals who present carotid artery  
280 vasoconstriction demonstrate an increase in blood pressure, making it unlikely that the blood pressure  
281 rise should be regarded as the dilator stimulus. Nonetheless, the change in blood pressure during the  
282 CAR% may still be relevant, especially to understand the link to the sympathetic nervous system. This  
283 work suggests the CAR% provides relevant information, independent of the increase in blood pressure  
284 during the CPT.

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372

373 Tables

374 **Table 1.** Correlation between CAR% and the blood pressure variables during CPT for all participants  
 375 and the dilation group.  $\Delta$ BP is the absolute difference between peak and baseline blood pressure,  
 376 whereas relative  $\Delta$ BP is the percent increase from baseline to the peak blood pressure.  
 377 A: BP Change defined as low (<15%), medium (15-30%) and high (>30%)

378

	All participants		Dilation group (N=34)	
	<i>Pearson correlation</i>	<i>p-value</i>	<i>Pearson correlation</i>	<i>p-value</i>
Baseline diameter	-0.354	0.025	-0.588	<0.001
Baseline MAP	0.209	0.197	0.298	0.087
Peak MAP	0.359	0.023	0.422	0.013
$\Delta$ MAP	0.326	0.040	0.327	0.059
Relative $\Delta$ BP	0.272	0.089	0.226	0.199
MAP change <sub>A</sub>	0.331	0.037	0.271	0.121

379



380 **Table 2.** CAR and blood pressure results of the groups divided based on relative  $\Delta$ MAP (low= <15%,  
 381 medium = between 15% and 30%, high = >30%). Relative  $\Delta$ MAP is the percent increase from baseline  
 382 to the peak MAP.

	Low relative $\Delta$ BP (n=8)	Medium relative $\Delta$ BP (n=20)	High relative $\Delta$ BP (n=12)	p-value
Age	42.6 $\pm$ 9.3	40.9 $\pm$ 9.2	44.3 $\pm$ 10.6	0.613
Weight (kg)	83.5 $\pm$ 12.3	81.2 $\pm$ 11.7	83.9 $\pm$ 15.3	0.823
Height (m)	1.76 $\pm$ 0.08	1.77 $\pm$ 0.07	1.79 $\pm$ 0.07	0.599
BMI (kg/m <sup>2</sup> )	26.7 $\pm$ 2.2	26.0 $\pm$ 3.5	26.1 $\pm$ 4.6	0.889
Positive family history	1.5 $\pm$ 0.76	1.2 $\pm$ 0.8	1.3 $\pm$ 0.8	0.664
Baseline diameter (cm)	0.67 $\pm$ 0.05	0.65 $\pm$ 0.05	0.69 $\pm$ 0.08	0.219
CAR%	1.3 $\pm$ 4.6	4.5 $\pm$ 4.5	6.4 $\pm$ 6.5	0.109
Baseline MAP (mmHg)	95 $\pm$ 11	85 $\pm$ 8	90 $\pm$ 9	0.053
Peak MAP(mmHg)	102 $\pm$ 13	106 $\pm$ 10	125 $\pm$ 15 <sup>ab</sup>	<0.001
Relative $\Delta$ MAP (%)	7.5 $\pm$ 4.3	23.7 $\pm$ 3.9 <sup>a</sup>	38.0 $\pm$ 4.0 <sup>ab</sup>	<0.001

383 <sup>a</sup> Post-hoc significantly different from group 1

384 <sup>b</sup> Post-hoc significantly different from group 2.

385

386

387

388 **Table 3:** Participant characteristics and CAR% when divided into groups based on the presence of  
 389 diameter dilation or vasoconstriction. P-values refer to an unpaired t-test.

390

	<b>Dilator (34)</b>	<b>Constrictor (6)</b>	<b>p-value</b>
Age	42.08±9.3	43.17±12	0.904
Weight (kg)	81.05±11.2	90.3±18.7	0.343
Height (m)	1.779±0.072	1.750±0.050	0.648
BMI (kg/m <sup>2</sup> )	25.58±2.9	29.37±5.3	0.060
Number of risk factors	0.441±0.504	0.167±0.408	0.372
Baseline diameter (cm)	0.67±0.054	0.66±0.104	0.430
CAR%	5.7±4.7	-2.9±2.48	<0.001
Baseline MAP (mmHg)	88.7±7.4	91.5±8	0.464
Peak MAP (mmHg)	110±15	109±19	0.288
Relative ΔMAP (%)	22.3±10	20±15	0.518

391

392

393 **Figure Legends**

394 **Figure 1.** A) Carotid artery ultrasound alongside the cold pressor test (CPT). B) A healthy ultrasound  
395 image demonstrating wall tracking (yellow) used to calculate vessel diameter and C)  
396 Diameter of the carotid artery during both 1: Baseline measurement and 2: In response to  
397 the CPT. Demonstrating a healthy dilatory response. Adapted from (19).

398

399 **Figure 2.** Mean and Standard Deviation participants during the cold pressor test (CPT) **A)** Mean arterial  
400 pressure (MAP) response to the CPT and **B)** Diameter response to the CPT. One-Way ANOVA  
401 performed to compare baseline vs increase in both MAP and diameter. \* denotes  $P < 0.05$

402

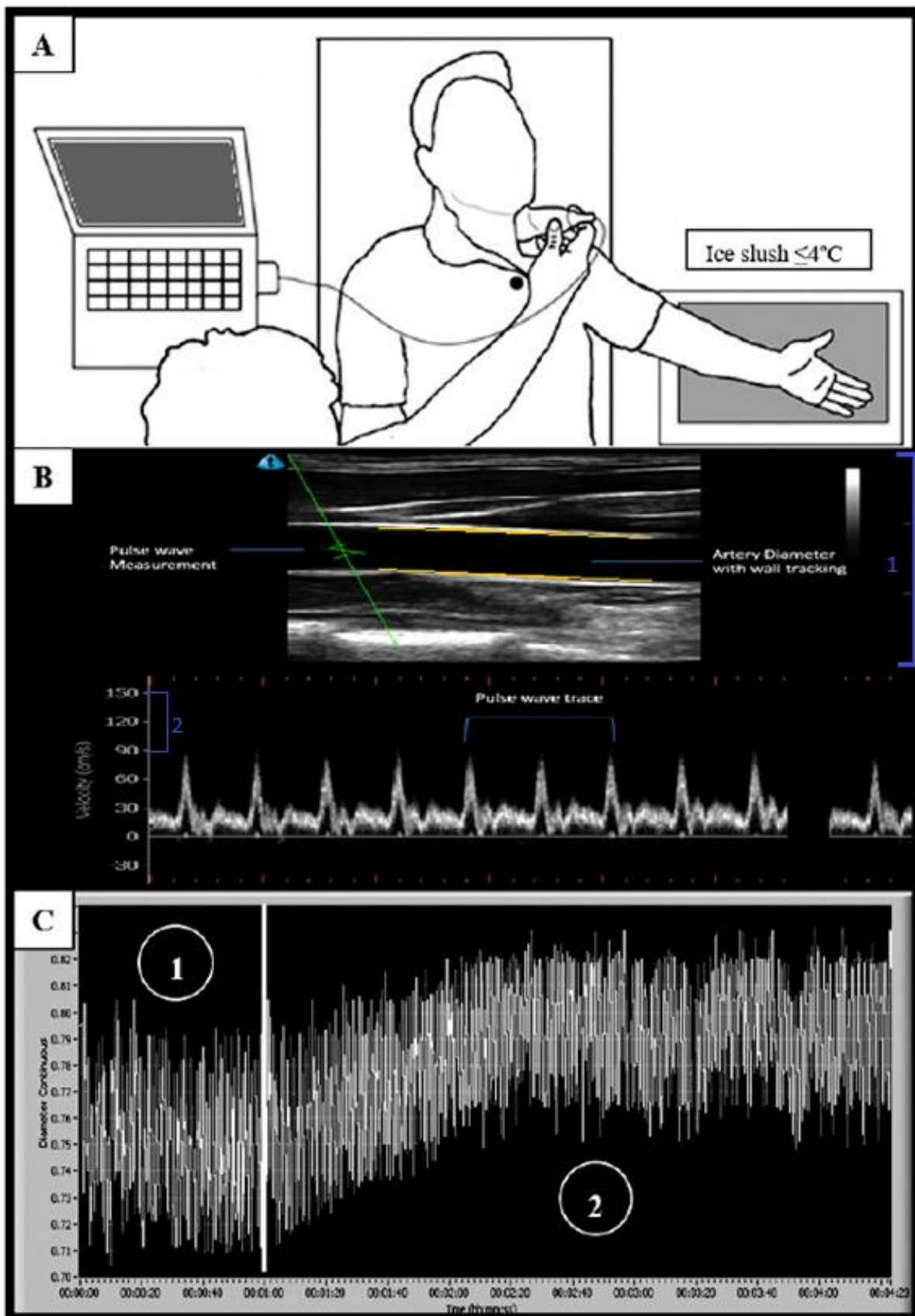
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Figure 1

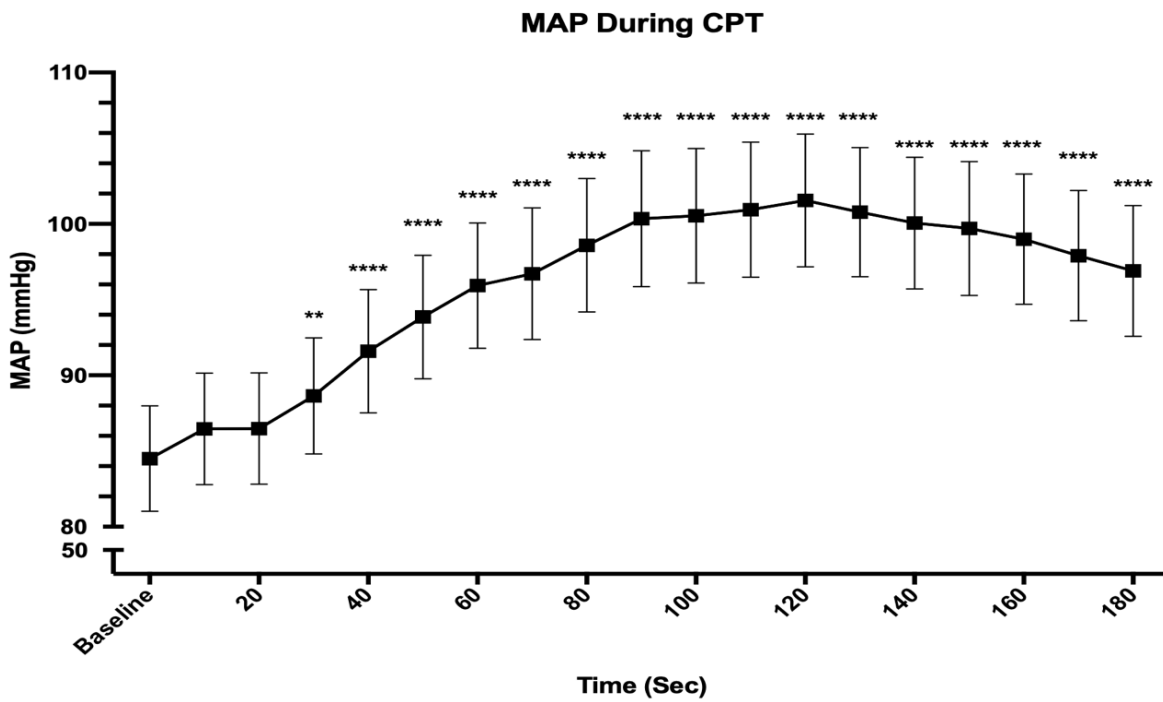
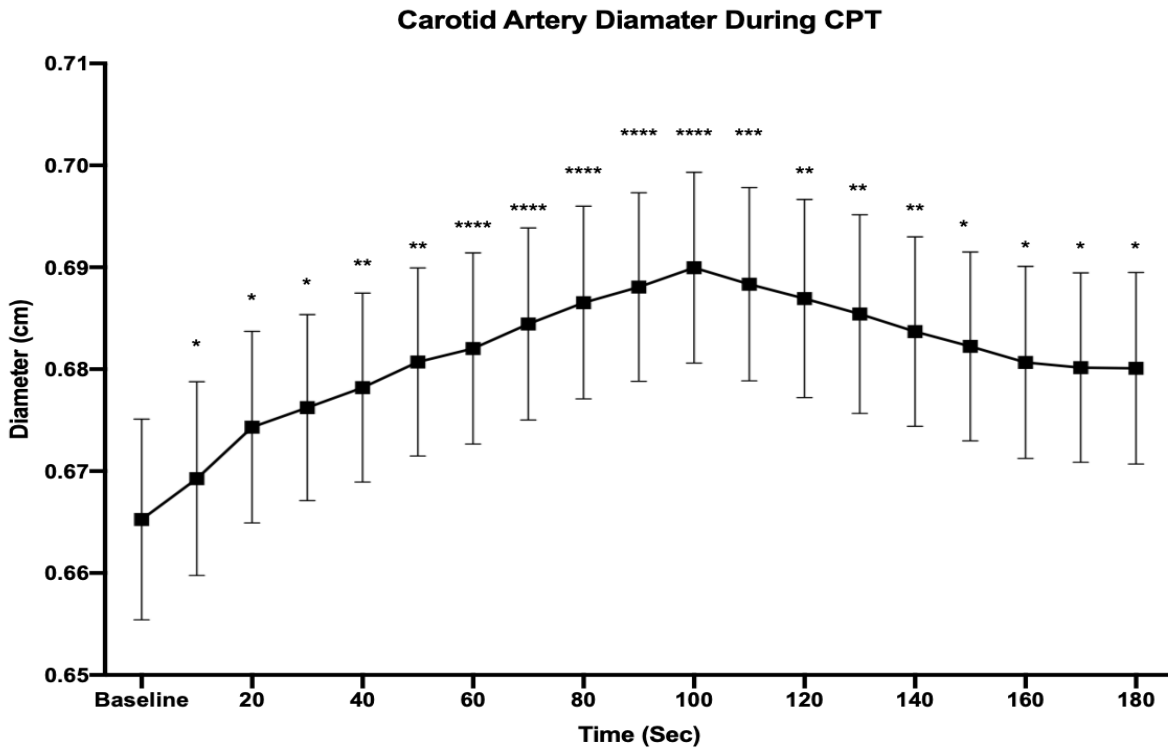


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408

409

Figure 2



410