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

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

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DISCLOSURES

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ABSTRACT

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

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

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

Conclusion. Our data suggests that the characteristic increase in MAP during the CPT does not mediates carotid artery vasomotion.

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

Introduction

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

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

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

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

Methods

Participants

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

Procedure

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

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

Measurements

Blood pressure

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

Cold pressor test

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

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°). 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

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

Statistical analysis

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

Results

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

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

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

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

Discussion

Our primary aim was to understand the relationship between changes in blood pressure and carotid artery diameter during the CPT. We present the following findings. First, the start of dilation and the timing of the peak carotid artery diameter response preceded blood pressure changes during the cold pressor test. Second, we found no differences in baseline characteristics including age, weight, BMI or in the magnitude of carotid artery dilation when comparing groups based on the magnitude of blood pressure increase. This finding is supported by the lack of correlation between the relative changes in carotid artery diameter and blood pressure during the CPT. Finally, individuals who demonstrated carotid artery vasoconstriction also demonstrated a comparable increase in blood pressure during 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

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

Limitations. One potential limitation is that the results of our study only apply to middle-aged men, making extrapolation to other (diseased) groups difficult. This is important since distinct populations have demonstrated different CAR% and/or blood pressure response (29, 30), whilst also physical activity may affect the blood pressure and/or CAR% (31). Nonetheless, it seems unlikely that these confounding factors, despite their role in changing blood pressure and/or CAR%, affects the relation between the blood pressure and diameter increase during the CPT. Another limitation is that our study did not explore the causal link between blood pressure and CAR%. Such a study would require direct manipulation of the blood pressure response during sympathetic stimulation. Monahan et al (2013) examined the impact of the CPT on the left anterior descending artery with α- and β-adrenergic antagonists (32), and found adrenergic blockage to abolished coronary artery vasodilation. In agreement, we examined carotid and coronary artery responses during the CPT with and without α_1-receptor blockade, and reported abolished carotid and coronary artery responses when combined with α_1-receptor blockade(33). Whilst this provides evidence for the role of adrenergic receptors in contributing to carotid (and coronary) artery vasodilation during the cold pressor test, the presence

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

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

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Tables

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

	All participants		**Dilation group (N=34)**	
	Pearson correlation	*p-value*	*Pearson correlation*	*p-value*
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
ΔMAP	0.326	0.040	0.327	0.059
Relative ΔBP	0.272	0.089	0.226	0.199
MAP change$_A$	0.331	0.037	0.271	0.121

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

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

383 [a] *Post-hoc significantly different from group 1*
384 [b] *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

	Dilator (34)	Constrictor (6)	p-value
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^2)	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

Figure Legends

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

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

406 **Figure 1**

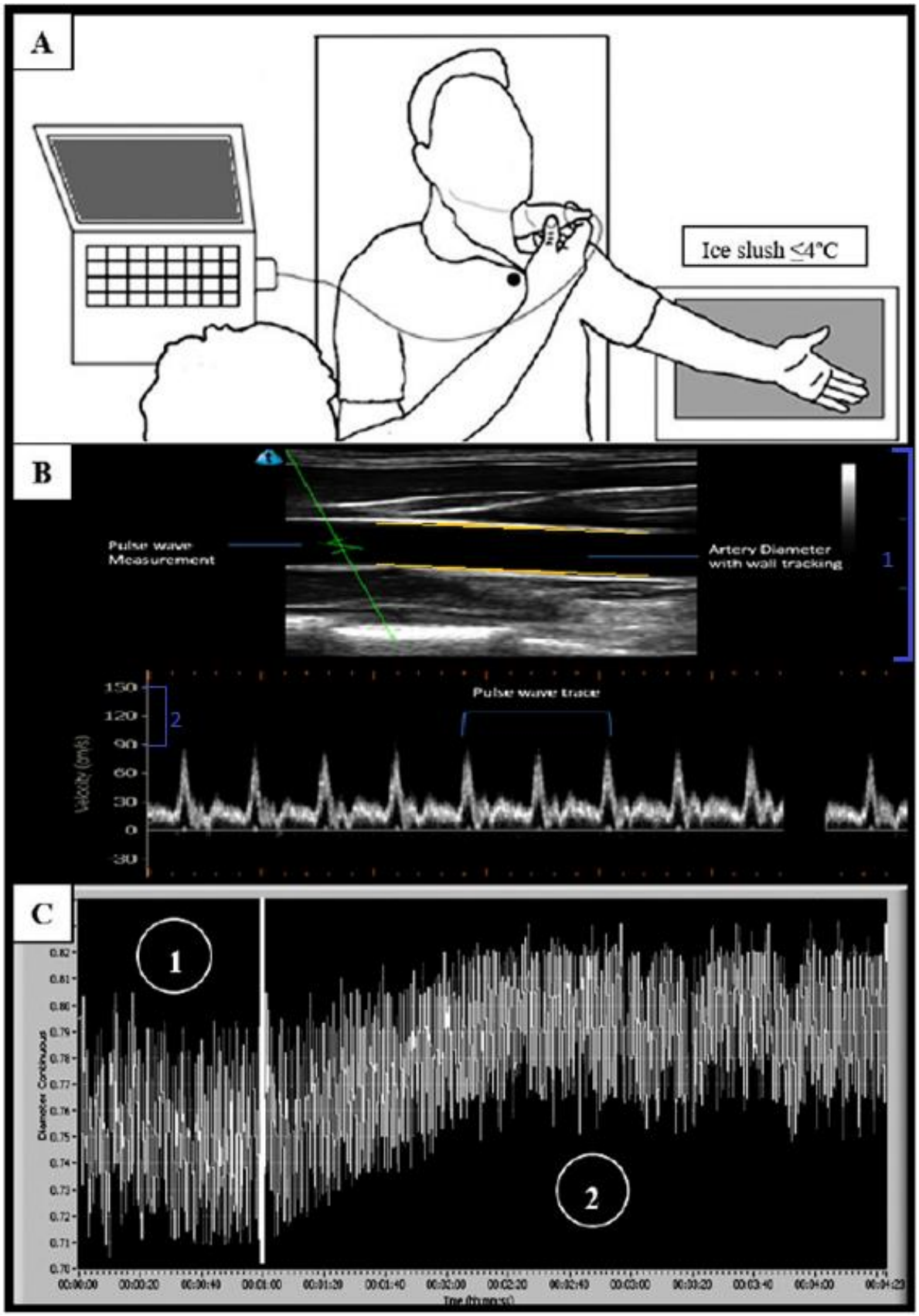

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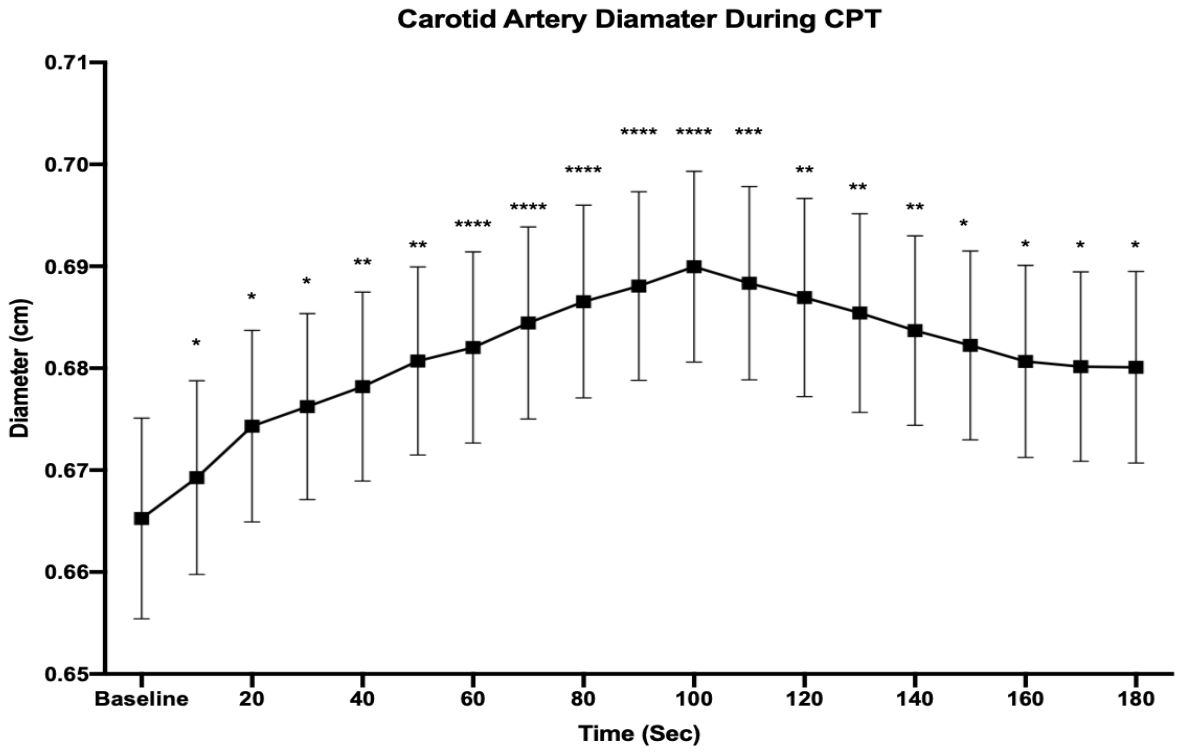
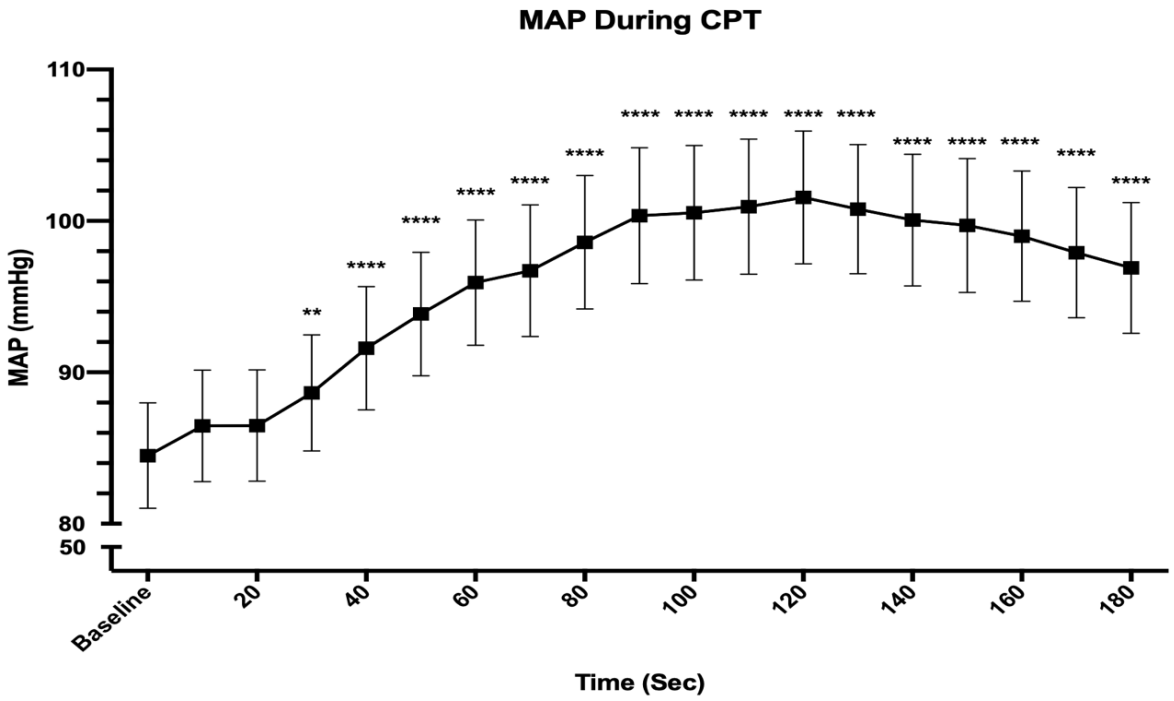
Figure 2





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