

1 **Correlation of carotid artery reactivity with cardiovascular risk**  
2 **factors and coronary artery vasodilator responses in**  
3 **asymptomatic, healthy volunteers**

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5 **ANKE C.C.M VAN MIL, MSc.,<sup>A,B</sup>, YVONNE HARTMAN, MSc.,<sup>A</sup>, FREDERIEKE VAN**  
6 **OORSCHOT, BSc.,<sup>A</sup>, ANNEMIEKE HEEMELS, BSc.,<sup>A</sup>, NIKKI BAX, MSc.,<sup>A</sup>, ELLEN A.**  
7 **DAWSON, PhD,<sup>B</sup>, NICOLA HOPKINS, PhD,<sup>B</sup>, MARIA T.E. HOPMAN, PROF.,<sup>A,C</sup>, DANIEL**  
8 **J. GREEN, PROF.,<sup>B,D</sup>, DAVID L. OXBOROUGH, PhD,<sup>B</sup>, DICK H.J THIJSSSEN, PROF.,<sup>A,B</sup>**

9  
10 <sup>A)</sup> Department of Physiology, Radboudumc, Nijmegen, The Netherlands

11 <sup>B)</sup> Research institute for Sport and Exercise Sciences, Liverpool John Moores University,  
12 Liverpool, United Kingdom

13 <sup>C)</sup> Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands.TI

14 <sup>D)</sup> School of Sports Science, Exercise and Health, The University of Western Australia,  
15 Nedlands, Australia

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17 **Short title:** Relation CAR with CV risk & coronaries

18  
19 **WORD COUNT:** 5868

20 **ABSTRACT WORD COUNT:** 249

21 **FIGURES:** 4

22 **TABLES:** 4

23  
24 **Author for correspondence:**

25 Prof. Dr. Dick Thijssen, Research Institute for Sport and Exercise Sciences, Liverpool John  
26 Moores University, Tom Reilly Building, Byrom Street L3 3AF, Liverpool, United Kingdom

27 Email: D.Thijssen@ljmu.ac.uk, Tel: +441519046264

28

29 **SOURCES OF FUNDING**

30 Dr. Dick Thijssen is financially supported by the Netherlands Heart Foundation (E Dekker-  
31 stipend, 2009T064). DJG is supported by a National Health and Medical Research Council  
32 (NHMRC) Principal Research Fellowship (APP1080914).

33

34 **DISCLOSURES**

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

36

37 **ABSTRACT**

38 **Objectives.** Carotid artery reactivity (CAR%), involving carotid artery diameter responses to  
39 a cold pressor test, is a non-invasive measure of conduit artery function in humans. This study  
40 examined: 1. the impact of age and cardiovascular risk factors on the CAR% and 2. The  
41 relationship between CAR% and coronary artery vasodilator responses to the cold pressor  
42 test.

43 **Methods.** Ultrasound was used to measure resting and peak carotid artery diameters during  
44 the cold pressor test, with CAR% being calculated as the relative change from baseline (%).  
45 We compared CAR% between young (n=50, 24±3 years) and older participants (n=44, 61±8  
46 years), and subsequently assessed relationships between CAR% *and* traditional cardiovascular  
47 risk factors in 50 participants (44±21 years). Subsequently, we compared left anterior  
48 descending (LAD) artery velocity (using transthoracic Doppler) *with* carotid artery diameter  
49 (i.e. CAR%) during the cold pressor test (CPT, n=33, 37±17 years).

50 **Results.** A significantly larger CAR% was found in young *versus* older healthy participants  
51 (4.1±3.7 *versus* 1.8±2.6,  $P<0.001$ ). Participants without cardiovascular risk factors  
52 demonstrated a higher CAR% compared to those with  $\geq 2$  risk factors (2.9±2.9 *versus* 0.5±2.9,  
53  $P=0.019$ ). Carotid artery diameter and LAD velocity increased during CPT ( $P<0.001$ ).  
54 Carotid diameter and change in velocity correlated with LAD velocity ( $r=0.486$  and  $0.402$ ,  
55  $P<0.004$  and  $0.02$ , respectively).

56 **Conclusion.** Older age and cardiovascular risk factors are related to lower CAR%, whilst  
57 CAR% shows good correlation with coronary artery responses to the CPT. Therefore, CAR%  
58 may represent a valuable technique to assess cardiovascular risk, whilst CAR% seems to  
59 reflect coronary artery vasodilator function.

60

61 **KEYWORDS:** Endothelial function, coronary arteries, carotid artery reactivity test, cold  
62 pressor test, cardiovascular risk

**63 INTRODUCTION**

64 Previous studies have explored the impact of stimulation of the sympathetic nervous system,  
65 using the cold pressor test (CPT), on coronary artery responses.<sup>1-3</sup> Coronary artery responses  
66 to CPT are suggested to be endothelium-dependent.<sup>4</sup> Whilst coronary dilation is observed in  
67 healthy volunteers, participants with CV risk or disease demonstrate attenuated dilation or  
68 even constriction during the CPT.<sup>2, 4-8</sup> Moreover, CPT-induced constriction of coronary  
69 arteries independently predicts future cardiovascular (CV) events.<sup>9</sup> Non-invasive assessment  
70 of coronary artery diameter, however, is currently technically challenging, expensive and  
71 lacks sufficient temporal resolution to assess rapid changes in diameter.

72

73 Similar to coronary arteries, CPT may dilate carotid artery in asymptomatic older participants,  
74 whereas significant constriction is present in those with coronary heart disease.<sup>10</sup> No previous  
75 study examined whether the magnitude of response (i.e. dilation or constriction) of the carotid  
76 artery reactivity (CAR%) to the CPT is altered by older age and/or presence of cardiovascular  
77 risk factors. Furthermore, given similarity in vascular responsiveness between coronary and  
78 carotid arteries to CPT, with opposite responses between healthy participants (i.e. dilation)  
79 *versus* patients with coronary heart disease (i.e. constriction),<sup>9, 10</sup> one may question whether a  
80 correlation exists between coronary and carotid artery responses to the CPT, such as described  
81 previously for other measures of peripheral vascular function.<sup>11-16</sup> This would provide the first  
82 study to assess whether CAR% directly relates to coronary artery vascular function.

83

84 This study aims to better understand the potential clinical relevance of CAR% as a putative  
85 marker of cardiovascular risk and surrogate for coronary artery function. First, we examined  
86 the hypothesis that older age and increasing number of traditional cardiovascular risk factors  
87 (e.g. blood pressure, cholesterol, hypertension, diabetes, and smoking) are associated with a

88 smaller CAR% in healthy, asymptomatic participants. Secondly, we explored the relation  
89 between coronary artery and carotid artery responses to the CPT in healthy, asymptomatic  
90 participants. This work will provide important information to determine if the carotid and  
91 coronary arteries exhibit similar functional responses in the presence of cardiovascular risk  
92 factors and disease.

93

94

## 95 **METHODS**

### 96 **Participants**

97 We recruited 94 healthy participants without clinical presentation of atherosclerosis.  
98 Exclusion criteria were a history of cardiovascular disease (i.e. angina, myocardial infarction,  
99 and heart failure), presence of Raynaud's phenomenon, scleroderma, chronic pain and/or open  
100 wounds on the upper extremities. Written informed consent was obtained from all participants  
101 prior to participation. Ethical approval was obtained from local Ethics committee (Aim 1:  
102 Radboud university medical centre, Aim 2: Liverpool John Moores University), in accordance  
103 with the latest revision of the Declaration of Helsinki.

104

### 105 **Experimental design**

106 All participants (n=94) reported to our laboratory for a single visit. Participants were asked to  
107 abstain from strenuous exercise for 24 hours, fast for  $\geq 6$  hours, and to abstain from dietary  
108 products known to alter endothelial function for  $\geq 18$  hours prior to the testing sessions (i.e.  
109 caffeine, vitamin C) according to guidelines to assess peripheral vascular function.<sup>17</sup> Upon  
110 arrival, weight (kg) and height (cm) were measured and participants rested in the supine  
111 position for at least 15 minutes on a comfortable bed in a temperature-controlled room. All  
112 subjects underwent the CPT, involving continuous ultrasonography measurements of the

113 carotid artery diameter and velocity as well as haemodynamics at baseline (1-min) and during  
114 (3-min) CPT. Peak changes in diameter during CPT, presented as the relative change from  
115 baseline, represents the CAR%. To reduce measurement error, procedures were repeated after  
116 1 h and averaged for analyses. For Aim 1 (i.e. relationship CAR% & risk factors), we divided  
117 the entire study population (n=94) into young (n=50, age range 19-30 years) and older adults  
118 (n=44, age range 50-82 years). Cardiovascular risk profile was assessed in 50 participants  
119 (Radboud university medical centre, 44±21 years), who were divided in subjects with 0, 1 or  
120  $\geq 2$  cardiovascular risk factors. These different subgroups are presented in Figure 1.

121

122 For Aim 2 (i.e. CAR% vs coronary artery velocity), we studied a subgroup of 44 participants  
123 (Liverpool John Moores University), and simultaneously examined carotid artery diameter  
124 and left anterior descending coronary artery velocity responses using Doppler ultrasound  
125 during the CPT. Due to technical constraints 11 participants were excluded from analysis.  
126 This left us with 33 participants to assess the relation between CAR% and coronary artery  
127 velocity responses to the CPT (37±17 years).

128

## 129 **Experimental measures**

130 *Cold pressor test.* The CPT consisted of a 3-minute immersion of the left hand in a bucket of  
131 ice slush (~4.0°C). The participant was positioned supine on a comfortable bed, facilitating  
132 arm movement of the left hand into the bucket of ice slush without significant movement of  
133 the neck to enable assessment of the carotid and coronary arteries. After a 1-minute baseline  
134 period, the participant immersed the hand up to the wrist in the ice slush for 3 minutes. The  
135 participant was instructed not to speak and breathe normally (to prevent hyperventilation)  
136 when the hand was submerged into the ice slush.

137

138 *Carotid artery diameter, blood flow and shear rate.* Participants were positioned with the  
139 neck extended to allow assessment of the carotid artery. Left carotid artery diameter and red  
140 blood cell velocity were recorded continuously during baseline (1-minute) and CPT (3-  
141 minutes) with a 10-MHz linear array handheld probe attached to a high resolution ultrasound  
142 machine (Terason T3000, Aloka, United Kingdom). When an optimal image was found, the  
143 probe was held stable and the ultrasound parameters were set to optimise the longitudinal, B-  
144 mode image of the lumen-arterial wall interface. Continuous pulsed wave Doppler velocity  
145 assessments were also obtained and were collected at the lowest possible insonation angle  
146 (always  $<60^\circ$ ). Following a 1-minute baseline assessment of carotid artery diameter and  
147 velocity, the hand was immersed for 3-minutes with simultaneous and continuous assessment  
148 of carotid artery diameter and velocity.

149

150 *Intima-media thickness.* Previous studies found carotid artery intima-media thickness (IMT)  
151 to relate to cardiovascular risk and predict future cardiovascular disease.<sup>18</sup> To explore the  
152 relevance of studying CAR% *and* IMT, we included measurements of the IMT (mm) of the  
153 left common carotid artery. According to widely adopted recommendations, we measured the  
154 IMT approximately 2cm proximal to the bulbous. We recorded the IMT continuously for 10  
155 seconds, in 2 different perpendicular planes (differing  $90^\circ$ ). From the 2 measurements wall  
156 thickness was calculated. Analyses were performed with edge-detection and wall-tracking  
157 software, as described elsewhere.<sup>19</sup>

158

159 *Blood pressure and heart rate.* Before and during CPT, we continuously measured blood  
160 pressure using non-invasive photoplethysmography (Aim 1: Nexfin, BMEYE, Amsterdam,  
161 The Netherlands, Aim 2: Portapress, Finapres Medical Systems, Amsterdam, Netherlands).

162

163 *Cardiovascular risk factors (Aim 1; CAR% vs Risk factors)*. For the subgroups of 50  
164 participants, we performed additional assessment of cardiovascular risk factors. To examine  
165 systolic and diastolic blood pressure, we performed two assessments of blood pressure using  
166 the manual approach (sphygmomanometer, on the left arm). Hypertension was defined as  
167 systolic pressure  $\geq 140$  mmHg and/or diastolic pressure  $\geq 90$  mmHg.<sup>20</sup> We reported diagnosis of  
168 type 1 or 2 diabetes mellitus and recorded (past and current) smoking habits. We used  
169 capillary blood to assess total cholesterol, high density lipoprotein cholesterol, low density  
170 lipoprotein cholesterol and triglycerides (35  $\mu$ L blood, Mission, ACON Laboratories, Inc., San  
171 Diego, USA). Elevated cholesterol levels were defined as total cholesterol  $> 5.0$  mmol/L.<sup>21-23</sup>  
172 Based on the presence of risk factors, these participants were subdivided in; *i.* 0 risk factors,  
173 *ii.* 1 risk factor, and *iii.*  $\geq 2$  risk factors.

174

175 *Coronary artery responses (Aim 2; CAR% vs coronary artery)*. In a subgroup of 33  
176 participants ( $37 \pm 17$  years), left anterior descending (LAD) coronary artery velocity responses  
177 to the CPT were examined using transthoracic ultrasound, during simultaneous assessment of  
178 the CAR. Transthoracic assessment was performed by a highly experienced sonographer  
179 using a Vivid Q (GE Medical, Horten, Norway), with a 4 MHz phased array transducer. To  
180 this end, participants assumed a slightly left lateral position to allow access and measurement  
181 of the proximal end of the LAD from a modified parasternal window. When the vessel was  
182 detected (using color flow mapping), the Doppler sample volume was positioned in the  
183 vessel, to allow for real-time velocity assessment during the cardiac cycle. Acquisition of the  
184 coronary velocity was obtained at baseline and during CPT.<sup>24</sup>

185



**186 Data analysis**

187 *Carotid artery diameter, velocity, blood flow and shear rate.* CAR% responses were assessed  
188 for both diameter and blood flow. Analysis of the carotid artery diameter was performed  
189 using custom-designed edge-detection and wall-tracking software, which is largely  
190 independent of investigator bias, by a single blinded investigator.<sup>25</sup> Details of this technique  
191 can be found elsewhere.<sup>26</sup> Baseline diameter, velocity, shear rate,<sup>25</sup> and blood flow were  
192 calculated as the mean of data acquired across the 1 minute preceding the CPT test. After  
193 submersion of the hand in ice slush, data were calculated as the mean value for 10-second  
194 intervals, involving 8-10 full cardiac cycles. Based on this data we calculated the peak  
195 diameter change (i.e. the 10-second bin with the highest value, CAR%) and area-under-the-  
196 curve for the diameter change during CPT (CAR<sub>AUC</sub>). The peak diameter change can refer to  
197 a maximum constriction *or* dilation. The direction of this change was determined by a  
198 positive (i.e. dilation) or negative (i.e. constriction) CAR<sub>AUC</sub>. In keeping with previous work,  
199 we also calculated the diameter change at 90 seconds (CAR<sub>90</sub>).<sup>17</sup> Reproducibility (coefficient  
200 of variation, CV) of diameter responses to CPT was assessed with a 1- and 24-hour interval.  
201 Within-day CV for baseline and peak diameters was 2.2 and 2.6%, whilst day-to-day CV  
202 were 2.3% and 2.7%. Furthermore, the CAR% (i.e. maximum change in diameter) showed a  
203 within-day reproducibility of 2.6% and between-day reproducibility of 2.8%.

204

205 *Blood pressure and heart rate.* Analyses included baseline and peak mean arterial pressure  
206 (MAP, mmHg), and baseline and peak heart rate (HR, beats per minute). Analyses were  
207 performed in labchart (LabChart 7, ADInstruments, Colorado Springs, USA) and/or excel.  
208 Both MAP and HR were averaged per 30 second bins for analyses. All values were averaged  
209 over the 2 CPTs.

210

211 *Coronary artery responses.* All images were exported to DVD in raw format, for offline  
212 analyses. The coronary blood velocity was analysed using commercially available software  
213 (EchoPAC Version 7.0; GE Medical, Horten, Norway). Measurements were performed at  
214 both baseline and during CPT and included peak systolic (S), peak diastolic (D) velocity and  
215 the velocity time integral (VTI).

216

### 217 **Statistical analysis**

218 All data were presented as mean  $\pm$  SD unless stated otherwise. Statistical analysis was done  
219 using IBM SPSS Statistics 20.0 (IBM SPSS, IBM Corp., Armonk, NY, USA). For Aim 1, we  
220 examined differences between young and older groups using an independent Students' *t*-tests  
221 (when data were normally distributed, following Kolmogorov-Smirnov tests of normality) or  
222 Mann-Whitney U tests (when data was not normally distributed). Effects of CPT differences  
223 between the groups (young *vs* older, and 0 *vs* 1 *vs*  $\geq 2$  risk factors) and time (baseline *vs* CPT)  
224 was assessed by 2-way repeated measures ANOVAs. Subsequently, 50 individuals with  
225 assessment of traditional cardiovascular risk factors were categorised into presence of 0, 1 or  
226  $\geq 2$  cardiovascular risk factors. A one-way ANOVA (data normally distributed) or Kruskal-  
227 Wallis (data not normally distributed) was adopted to examine differences in our primary  
228 outcome parameters between groups. A Pearson's correlation was adopted to assess the  
229 relation between CAR% (i.e. carotid artery function) and carotid artery intima-media  
230 thickness and diameter (i.e. carotid artery structure). For Aim 2, we first examined the change  
231 in carotid artery diameter and LAD velocity in response to CPT using a paired Student's *t*-  
232 tests. Pearson's correlation coefficient was used to explore the relation between the change in  
233 carotid artery diameter (i.e. CAR%) and change in coronary artery velocity (i.e. VTI).

234

235

236 **RESULTS**

237 In healthy young subjects, CPT caused a gradual increase in carotid artery diameter that  
238 peaked around 90 seconds and, subsequently, returned towards baseline (Figure 2A). Carotid  
239 artery velocity and blood flow showed a gradual (~15%), but significant increase across the 3-  
240 minutes of the CPT-response (Figure 2B-C). Interestingly, shear rate remained around  
241 baseline levels until 90/100 seconds, after which it showed a marginal (~10%) increase  
242 (Figure 2D).

243

244 **Aim 1: CAR% versus cardiovascular risk factors**

245 *Young and older participants.* Older participants demonstrated higher weight and BMI, but no  
246 differences in height (Table 1). Systolic and diastolic blood pressure were higher in older  
247 compared to young participants (Table 1). Mean arterial pressure was lower in young  
248 compared to the older group, whilst heart rate was not different between groups (Table 2).  
249 Carotid artery diameter was larger in the older group than in young participants, whilst carotid  
250 artery shear rate was higher in the young group (Table 2). CPT induced a significant increase  
251 in heart rate and mean arterial pressure in both groups, with older participants demonstrating a  
252 larger increase in heart rate and a larger increase in mean arterial pressure (Table 2). Both  
253 groups demonstrated a significant increase in carotid artery diameter in response to the CPT  
254 (Table 2). The diameter response during the CPT was significantly larger in young compared  
255 to older humans when data were presented as the peak diameter change (i.e. CAR%), area-  
256 under-the-curve across the 3-minute CPT (i.e. CAR<sub>AUC</sub>) and diameter change at 90-seconds  
257 (i.e. CAR<sub>90</sub>) (Table 2, Figure 3A).

258

259 *Cardiovascular risk factors.* Cholesterol and LDL levels were highest in those with 1 RF  
260 compared to 0 or  $\geq 2$  RF, whilst no differences between groups were found for any of the other

261 parameters (Table 3). We found a significantly different CAR%, CAR<sub>AUC</sub> and CAR<sub>90</sub> across  
262 the 3 groups (Figure 3B, Table 3), with a smaller carotid artery dilation observed in the  
263 presence of more cardiovascular risk factors. Specifically, we found that participants with  $\geq 2$   
264 risk factors showed a smaller dilation compared to those without risk factors (Table 3). In line  
265 with the CAR%, carotid artery diameter, IMT, and IMT ratio (i.e. intima-media  
266 thickness/baseline diameter) were higher in participants with more risk factors (Table 3).  
267 However, no significant correlation was found between CAR% and carotid artery baseline  
268 diameter ( $r = -0.16$ ,  $P = 0.274$ ), IMT ( $r = -0.09$ ,  $P = 0.524$ ), or IMT ratio ( $r = -0.06$ ,  $P = 0.678$ ).

269

## 270 **Aim 2: CAR% versus coronary artery**

271 The CPT caused a significant increase in heart rate, mean arterial pressure, and carotid artery  
272 flow, velocity and shear rate (Table 4). A significant increase in carotid artery diameter was  
273 found when presented as CAR%, CAR<sub>AUC</sub> and CAR<sub>90</sub> (Table 4). Furthermore, a significant  
274 increase in LAD velocity was found during the CPT (Table 4). We found a significant,  
275 positive correlation between the CAR% and the change in LAD velocity time integral  
276 ( $r = 0.486$ ,  $P < 0.004$ , Figure 4). A significant, positive correlation was also found between  
277 changes in carotid artery velocity and flow, and the change in LAD velocity time integral  
278 ( $r = 0.402$ ,  $P = 0.021$ , and  $r = 0.368$ ,  $P = 0.035$ , respectively). This relation between carotid and  
279 coronary artery responses was reinforced when data were presented as CAR<sub>90</sub>, but not for  
280 CAR<sub>AUC</sub> ( $r = 0.361$  and  $0.258$ ,  $P = 0.039$  and  $0.146$ , respectively).

281

282

## 283 **DISCUSSION**

284 In this study we explored the relationship between age, cardiovascular risk factors and CAR%  
285 and whether carotid artery responses to CPT reflect coronary artery vascular function. We  
286 found that the CPT induces carotid artery dilation in healthy, asymptomatic young

287 participants, with no changes in shear rate. This highlights the ability of the carotid artery to  
288 dilate in response to the CPT, a functional change that is unlikely to be related to shear-  
289 mediated responses, as the dilation response of the carotid artery preceded any change in  
290 shear. Secondly, the CAR% was significantly attenuated in healthy, asymptomatic older  
291 participants, whilst presence of traditional cardiovascular risk factors was also associated with  
292 a smaller CAR%. These findings cannot be ascribed to structural characteristics of the carotid  
293 artery diameter (i.e. diameter or intima-media thickness), given the absence of a significant  
294 correlation between CAR% and these factors. Finally, a moderate-to-strong correlation was  
295 apparent between carotid artery dilation (i.e. diameter and velocity) and coronary artery  
296 dilator (i.e. velocity) responses to the CPT. These observations provide evidence that the  
297 CAR%, most likely independent of carotid artery structural characteristics, may represent a  
298 valuable test to assess arterial function and health and that it reflects coronary artery  
299 vasomotor function.

300

301 Our study reveals the novel observation that, in a healthy, asymptomatic population, who  
302 generally demonstrate carotid artery dilation in response to the CPT, the CAR% successfully  
303 distinguishes between subjects with incremental number of risk factors. Also carotid artery  
304 IMT and diameter, both predictors for CV risk,<sup>18</sup> were different between groups, with a higher  
305 value for those with  $\geq 2$  traditional cardiovascular risk factors. Since we found no correlation  
306 between CAR% and carotid IMT or diameter, it is possible that CAR% provides information  
307 that is independent from that of measures of carotid artery structure (i.e. diameter and IMT).  
308 This observation provides further support that CAR% may represent relevant information on  
309 CV risk.

310

311 Ideally, a test of (peripheral) vascular function related to CV risk should also reflect vascular  
312 health of coronary vessels, since coronary arteries are prone to the development of  
313 atherosclerosis and cardiovascular events. Previous studies have explored the relationship  
314 between measures of coronary and peripheral artery vascular function.<sup>14, 16</sup> In line with these  
315 studies, carotid artery and coronary artery responses to the CPT show a moderate-to-strong  
316 correlation, a finding that is reinforced by earlier cross-study observations of comparable  
317 coronary and carotid artery responses to the CPT; dilation in healthy subjects or constriction  
318 in those with coronary artery disease.<sup>2, 4, 10</sup> The ability for marked vasomotion of the carotid  
319 artery during the CPT is different to peripheral conduit arteries that typically show negligible  
320 change in diameter.<sup>27, 28</sup> This further highlights the potential relevance for studying the carotid  
321 artery as a surrogate for coronary artery vascular function, since both of these conduit vessels  
322 demonstrate similar responses to the CPT. The agreement between the coronary and carotid  
323 artery responses to the CPT somewhat contrasts with the lack of correlation between  
324 measures of carotid artery atherosclerosis (i.e. intima-media thickness) and coronary artery  
325 atherosclerosis (i.e. plaque burden).<sup>18</sup> Our data, nonetheless, suggest that functional, rather  
326 than structural, measures in the two vascular beds may be related.

327

328 The ability of the carotid artery to dilate (or constrict) during the CPT raises questions  
329 regarding the potential underlying mechanisms. Whilst no extant study has examined the  
330 carotid artery, several studies explored pathways contributing to coronary artery vasomotion  
331 to the CPT.<sup>1, 2, 4, 6-9, 29</sup> First, the diameter change to the CPT may be endothelium-dependent,  
332 since coronary artery responses to the CPT and acetylcholine (i.e. an endothelium-dependent  
333 stimulus) show similarity in vasomotion.<sup>4, 29</sup> To explain diameter response to the CPT, an  
334 increase in shear stress during CPT may contribute to an endothelium-dependent  
335 vasodilation.<sup>30</sup> However, the increase in shear rate during CPT occurred *after* occurrence of

336 the peak diameter (Figure 2), making changes in shear an unlikely explanation for carotid  
337 artery dilation. Another possibility is that the increase in blood pressure accounts for the  
338 diameter response to CPT. Indeed, we found a relation between increase in MAP and CAR%.  
339 However, the magnitude of increase in MAP did not differ between groups, whilst an increase  
340 in MAP was also observed in those who demonstrate a decrease in CAR%. This suggests that  
341 the increase in MAP is unlikely causally linked to carotid diameter changes. This notion is  
342 further supported when examining the timing of the peak responses, since peak diameter  
343 precede peak blood pressure responses by ~30 seconds. Nonetheless, we cannot exclude the  
344 possibility that increases in blood pressure contribute (partly) to the CAR%. Alternatively, the  
345 release of catecholamines during the CPT may contribute to vasomotion of the carotid artery  
346 during CPT,<sup>31, 32</sup> with some work linking catecholamines (e.g. norepinephrine [NE]) to  
347 coronary artery dilation in healthy vessels or constriction in diseased arteries.<sup>2, 29</sup> More  
348 specifically, NE may contribute to vasodilation via endothelium-dependent release of  
349 vasodilators,<sup>1, 33</sup> whilst a direct impact of NE on smooth muscle cells causes  
350 vasoconstriction.<sup>34, 35</sup> The balance between both effects may ultimately determines the  
351 vasomotor response, which could be influenced by endothelium dysfunction. Although these  
352 mechanisms were explored in coronary arteries, comparable mechanisms may be present in  
353 the carotid artery during the CPT. Further research is required to characterize the physiology  
354 of the carotid artery responses to sympathetic stimulation using the CPT.

355

356 *Clinical relevance.* Previous studies adopting invasive intracoronary Doppler catheters<sup>2, 4, 29</sup>  
357 and quantitative angiography,<sup>2, 4, 9, 29</sup> have shown strong predictive capacity of coronary artery  
358 responses to sympathetic stimuli for future CV disease and/or events.<sup>6, 7, 9</sup> Our observation of  
359 agreement between coronary and carotid artery responses to the CPT, combined with the  
360 relation of the CAR% with age and cardiovascular risk factors, suggest the potential utility of

361 the CAR% test. This is further supported by the observation that the CAR% provides  
362 information that seems independent from that of structural measures of the carotid artery, i.e.  
363 diameter and intima-media thickness. The potential use is further emphasised since it is easy  
364 applicable, simple, cheap, non-invasive, and requiring a minimum of training.

365

366 *Limitations.* We choose to group the number of cardiovascular risk factors, rather than  
367 explore the impact of individual risk factors, on the CAR%. Examining all individual risk  
368 factors would require a markedly larger sample size to properly perform statistical analyses,  
369 whilst our aim was to explore the relation between cardiovascular risk factors and the newly  
370 introduced CAR% in asymptomatic subjects. We strongly recommend future studies to  
371 explore the impact of individual risk factors to better understand how traditional risk factors  
372 affect CAR%. Secondly, due to technical restrictions, we were unable to collect LAD  
373 diameter to correlate diameter changes between both arteries. Since changes in diameter will  
374 affect measures of velocity, we may have underestimated the true correlation between both  
375 arteries in response to the CPT. Nonetheless, the significant correlation between both vascular  
376 beds, including the significant correlation between carotid artery and coronary artery  
377 velocities, emphasises the agreement between coronary and carotid responses to the CPT.

378

379 In conclusion, in the present study we found that older age and the presence of cardiovascular  
380 risk factors is related to a lower CAR%. Therefore, CAR% may represent a valuable  
381 technique to assess cardiovascular risk, which may be used in addition to structural measures  
382 of the carotid artery (i.e. diameter and intima-media thickness). In addition, the CAR% shows  
383 a good correlation with coronary artery responses to the CPT, which suggests that the CAR%  
384 represents a surrogate for coronary artery vasomotor function.

385



386

387 **AUTHOR CONTRIBUTIONS**

388 DHJT and DLO designed the study. DHJT, DJG and MTEH ensured funding of the project  
389 and discussed the feasibility and study design. ACCMM, YH, FO, AH, NB, EAD, NH and  
390 DLO were involved in data collection and analysis. ACCMM, DHJT performed the statistical  
391 analysis. All authors contributed to the interpretation of the data, writing of the manuscript  
392 and provided approval of the final version.

393

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498 **FIGURE LEGENDS**

499 **FIGURE 1.** Flow diagram to provide insight into the different subgroups to answer the 3  
500 aims.

501 **FIGURE 2.** The time course presented during the cold pressor test in a young healthy  
502 subpopulation (n=25). A; diameter over time (cm), B; flow velocity over time  
503 (m/sec), and C; blood flow (ml/min) and D; shear over time ( $s^{-1}$ ). Error bars  
504 represent SEM.

505 **FIGURE 3.** Carotid artery reactivity (CAR%, presented as maximal change from baseline) in  
506 a cohort of healthy, asymptomatic subjects that were divided based on age (A: 50  
507 young (black bar) *versus* 44 older humans (white bar)) and presence of  
508 cardiovascular risk factors (B: 0 risk factors (black bar, n=27), 1 risk factor (grey  
509 bar, n=11), and  $\geq 2$  risk factors (white bar, n=12)). Error bars represent SE.  
510 Statistical analysis (unpaired Students' *t*-test (A) and ANOVA (B)) revealed  
511 significant differences in CAR% between groups.

512 **FIGURE 4.** Correlation between the carotid artery diameter response (% maximum change  
513 from baseline; i.e. CAR%) and coronary left descending artery velocity response  
514 (change in the velocity time integral (VTI in cm)) during a cold pressor test in a  
515 population of healthy, asymptomatic participants (n=33). A significant, positive  
516 correlation was observed between both measurements.

517

518 **Table 1.** Subject characteristics for the comparison between young (19-30 years, n=50) and  
 519 older (>50 years, n=44) participants. P-value refers to an unpaired Student's *t*-test or \*Mann-  
 520 Whitney U test for the comparison between young and older participants.

	<b>Young</b>	<b>Older</b>	<b>P-value</b>
Sex (% male)	56%	64%	0.452
Age (years)	24±3	61±8	<0.001
Weight (kg)	69±12	77±13	0.003
Height (m)	174±8	172±8	0.100*
Body Mass Index (kg/m <sup>2</sup> )	23±3	26±4	<0.001*
Systolic blood pressure (mmHg)	118±9	134±19	<0.001*
Diastolic blood pressure (mmHg)	68±8	78±7	<0.001

521  
 522



523 **Table 2.** Carotid artery and hemodynamic baseline characteristics (averaged across a 1-minute period) and change during the cold pressor test  
 524 (averaged across the 3-minute cold pressor test) in young (19-30 years, n=50) and older (>50 years, n=44) participants. P-values refer to 2-way  
 525 repeated measures ANOVA's, for within participant comparison (CPT), between group comparison (group), and the interaction Group\*CPT.  
 526 \*Refers to Mann-Whitney U test.

	Young		Older		2-way ANOVA		
	<i>Rest</i>	<i>CPT</i>	<i>Rest</i>	<i>CPT</i>	<i>group</i>	<i>CPT</i>	<i>Group*CPT</i>
MAP (mmHg)	85±13	95±14	102±15	114±18	<0.001	<0.001	0.063
HR (bpm)	64±12	65±11	59±9	64±10	0.073	<0.001	0.006
Diameter (mm)	6.3±0.5	6.5±0.5	7.1±0.7	7.2±0.8	<0.001	<0.001	<0.001
Shear rate (1/s)	184±43	186±43	143±42	141±47	<0.001	0.905	0.318
Flow (ml/min)	9.2±2.3	10.1±2.6	10.2±2.8	10.3±3.5	0.286	0.001	0.019
<b>Carotid artery reactivity (CAR)</b>							
Diameter change (CAR%)	4.1±3.7		1.8±2.6		<0.001*		
Diameter area-under-the-curve (CAR <sub>AUC</sub> )	2.7±2.3		1.0±1.3		<0.001		
Diameter change at 90 sec (CAR <sub>90</sub> )	3.5±2.8		1.4±1.6		<0.001		

527

528 **Table 3.** Carotid artery reactivity (CAR%, presented as maximal change from baseline) in a  
 529 cohort of healthy, asymptomatic subjects categorised by the presence of cardiovascular risk:  
 530 1. 0 risk factors (n=27), 2. 1 risk factor (n=11), and 3.  $\geq 2$  risk factors (n=12). \*Post-hoc  
 531 significantly different from group 1. †Refers to Kruskal-Wallis test.

	0 risk factors (N=27)	1 risk factor (N=11)	$\geq 2$ risk factors (N=12)	P-value
Sex (% male)	52%	55%	42%	0.794
Hypertension (%)	-	9%	17%	0.115
Diabetes (%)	-	-	8%	0.312
Smoking (%)	Current	9%	17%	0.139
	No	89%	58%	
	History	11%	25%	
Cholesterol (mmol/L)	4.25 $\pm$ 0.7	6.17 $\pm$ 1.4	5.5 $\pm$ 1.3	>0.001†
HDL (mmol/L)	1.39 $\pm$ 0.3	1.30 $\pm$ 0.4	1.24 $\pm$ 0.2	0.408
LDL (mmol/L)	2.59 $\pm$ 0.7	4.0 $\pm$ 1.5	3.4 $\pm$ 1.4	0.025
Triglycerides (mmol/L)	1.3 $\pm$ 1.0	2.1 $\pm$ 1.3	1.9 $\pm$ 1.1	0.196
Baseline diameter (cm)	0.64 $\pm$ 0.06	0.70 $\pm$ 0.04*	0.74 $\pm$ 0.08*	>0.001
Intima-media thickness (mm)	0.60 $\pm$ 0.2	0.75 $\pm$ 0.1*	0.82 $\pm$ 0.1*	0.001
IMT ratio	0.09 $\pm$ 0.02	0.11 $\pm$ 0.02	0.11 $\pm$ 0.02*	0.036
<b>Carotid artery reactivity (CAR)</b>				
CAR%	2.9 $\pm$ 2.9	2.3 $\pm$ 2.2	0.5 $\pm$ 2.9*	0.060
CAR <sub>AUC</sub>	1.9 $\pm$ 1.6	1.1 $\pm$ 1.2	0.5 $\pm$ 1.5*	0.034
CAR <sub>90</sub>	2.5 $\pm$ 2.2	1.4 $\pm$ 1.4	0.9 $\pm$ 1.7*	0.037

532

533 HDL; High density lipoprotein, LDL; Low density lipoprotein.

534 **Table 4.** Coronary artery responses in all participants included for Aim 2 (n=33). P-value  
 535 refers to a paired Student's *t*-test. \*Refers to Wilcoxon Signed rank test.

	Rest	CPT	P-value
Mean arterial pressure (mmHg)	87±14	99±16	<0.001
Heart rate (bpm)	60±10	62±10	0.048
CA diameter (cm)	0.66±0.08	0.68±0.08	<0.001
CA shear rate (1/s)	158±46	174±43	<0.001
CA flow (ml/min)	9.1±2.7	10.9±3.4	<0.001
CA velocity (cm/s)	25.8±6.7	29.3±7.1	<0.001
LAD systolic velocity (cm/s)	15±3.5	18±3.4	<0.001*
LAD diastolic velocity (cm/s)	31±7	39±9	<0.001
LAD velocity time integral (cm/s)	17±4	20±4	<0.001
Diameter change (CAR%)	4.5±3.8		
Diameter area-under-the-curve (CARAUC)	2.8±2.5		
Diameter change at 90 sec (CAR90)	3.6±2.9		
Delta VTI (cm)	2.7±2.3		

536