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

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Short title: Relation CAR with CV risk & coronaries

WORD COUNT: 5868
ABSTRACT WORD COUNT: 249
FIGURES: 4
TABLES: 4

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SOURCES OF FUNDING

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

DISCLOSURES

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

ABSTRACT

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

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

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

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

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

INTRODUCTION

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

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

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

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

METHODS

Participants

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

Experimental design

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

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

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

Experimental measures

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

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

Intima-media thickness. Previous studies found carotid artery intima-media thickness (IMT) to relate to cardiovascular risk and predict future cardiovascular disease.[18] To explore the relevance of studying CAR% *and* IMT, we included measurements of the IMT (mm) of the left common carotid artery. According to widely adopted recommendations, we measured the IMT approximately 2cm proximal to the bulbus. We recorded the IMT continuously for 10 seconds, in 2 different perpendicular planes (differing 90º). From the 2 measurements wall thickness was calculated. Analyses were performed with edge-detection and wall-tracking software, as described elsewhere.[19]

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

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

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

Data analysis

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

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

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

Statistical analysis

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

RESULTS

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

Aim 1: CAR% *versus* cardiovascular risk factors

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

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

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

Aim 2: CAR% *versus* coronary artery

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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

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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.

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FIGURE LEGENDS

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

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

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

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

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.

	Young	**Older**	**P-value**
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^2)	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*

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Table 2. Carotid artery and hemodynamic baseline characteristics (averaged across a 1-minute period) and change during the cold pressor test (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 repeated measures ANOVA's, for within participant comparison (CPT), between group comparison (group), and the interaction Group*CPT. *Refers to Mann-Whitney U test.

	Young		**Older**		**2-way ANOVA**		
	Rest	*CPT*	*Rest*	*CPT*	*group*	*CPT*	*Group*CPT*
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*
Carotid artery reactivity (CAR)							
Diameter change (CAR%)	4.1±3.7		1.8±2.6		*<0.001**		
Diameter area-under-the-curve (CAR_{AUC})	2.7±2.3		1.0±1.3		*<0.001*		
Diameter change at 90 sec (CAR_{90})	3.5±2.8		1.4±1.6		*<0.001*		

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

		0 risk factors (N=27)	**1 risk factor (N=11)**	**≥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%	64%	58%	
	History	11%	27%	25%	
Cholesterol (mmol/L)		4.25±0.7	6.17±1.4	5.5±1.3	*>0.001†*
HDL (mmol/L)		1.39±0.3	1.30±0.4	1.24±0.2	0.408
LDL (mmol/L)		2.59±0.7	4.0±1.5	3.4±1.4	*0.025*
Triglycerides (mmol/L)		1.3±1.0	2.1±1.3	1.9±1.1	0.196
Baseline diameter (cm)		0.64±0.06	0.70±0.04*	0.74±0.08*	*>0.001*
Intima-media thickness (mm)		0.60±0.2	0.75±0.1*	0.82±0.1*	*0.001*
IMT ratio		0.09±0.02	0.11±0.02	0.11±0.02*	*0.036*
Carotid artery reactivity (CAR)					
CAR%		2.9±2.9	2.3±2.2	0.5±2.9*	0.060
CAR_{AUC}		1.9±1.6	1.1±1.2	0.5±1.5*	*0.034*
CAR_{90}		2.5±2.2	1.4±1.4	0.9±1.7*	*0.037*

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		

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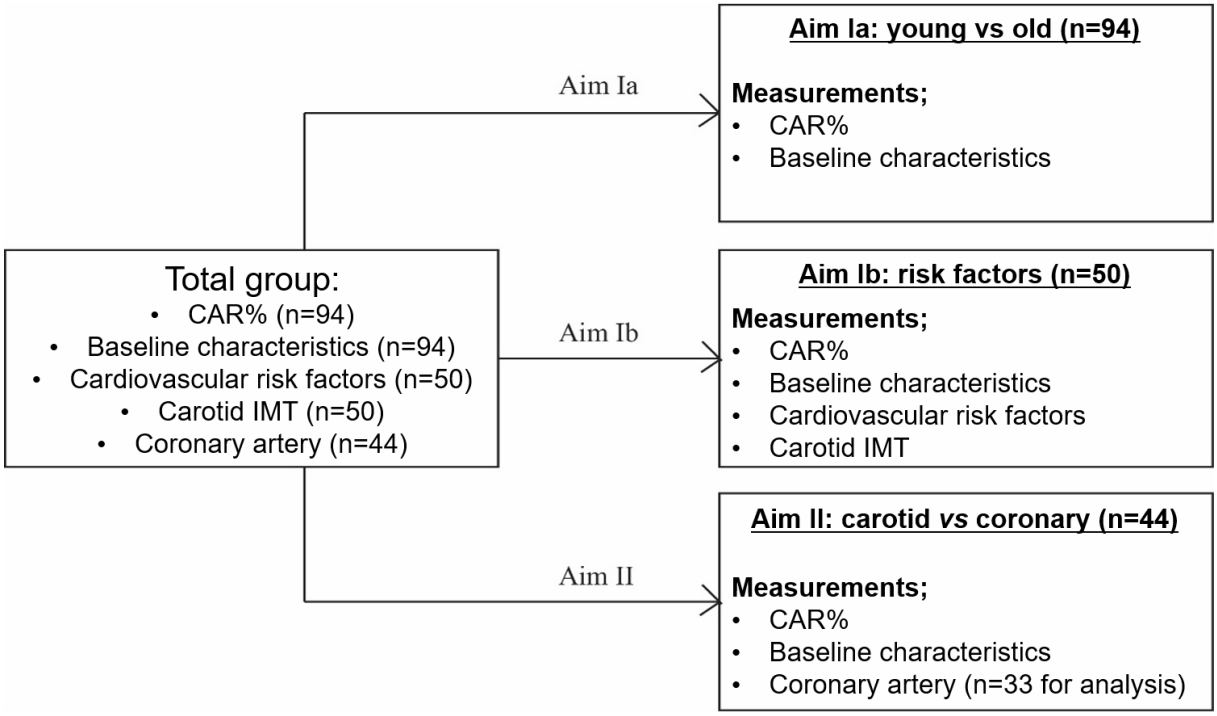
Total group:
• CAR% (n=94)
• Baseline characteristics (n=94)
• Cardiovascular risk factors (n=50)
• Carotid IMT (n=50)
• Coronary artery (n=44)
Aim Ia
Aim Ib
Aim II
Aim Ia: young vs old (n=94)
Measurements;
• CAR%
• Baseline characteristics
Aim Ib: risk factors (n=50)
Measurements;
• CAR%
• Baseline characteristics
• Cardiovascular risk factors
• Carotid IMT
Aim II: carotid vs coronary (n=44)
Measurements;
• CAR%
• Baseline characteristics
• Coronary artery (n=33 for analysis)

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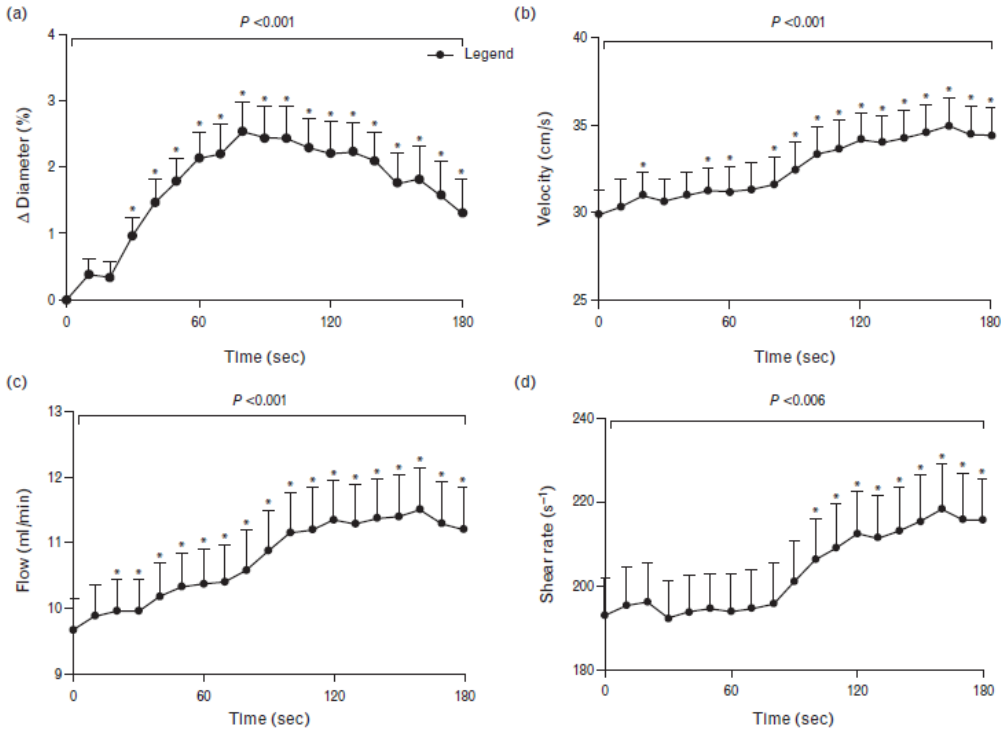

FIGURE 2 The time course presented during the cold pressor test in a young healthy subpopulation ($n = 25$). (a) Diameter over time (cm); (b) flow velocity over time (m/s); (c) blood flow (ml/min); (d) shear over time (s^{-1}). Error bars represent SEM.

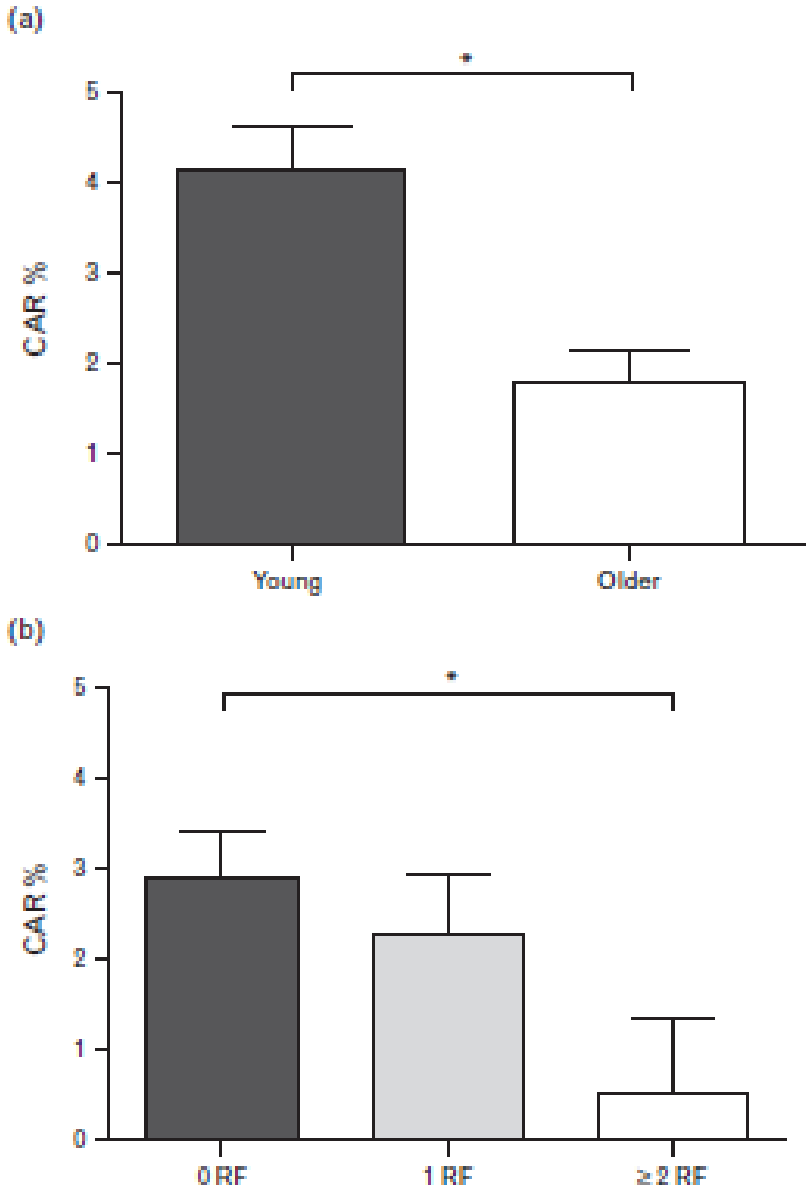

FIGURE 3 Carotid artery reactivity (CAR%, presented as maximal change from baseline) in a cohort of healthy, asymptomatic individuals who were divided on the basis of age [a: 50 young (black bar) versus 44 older humans (white bar)] and presence of cardiovascular risk factors (b: 0 risk factors (black bar, $n = 27$), one risk factor (grey bar, $n = 11$) and at least two risk factors (white bar, $n = 12$). Error bars represent SE. Statistical analysis [unpaired Students' *t*-test (a) and ANOVA (b)] revealed significant differences in CAR% between groups.

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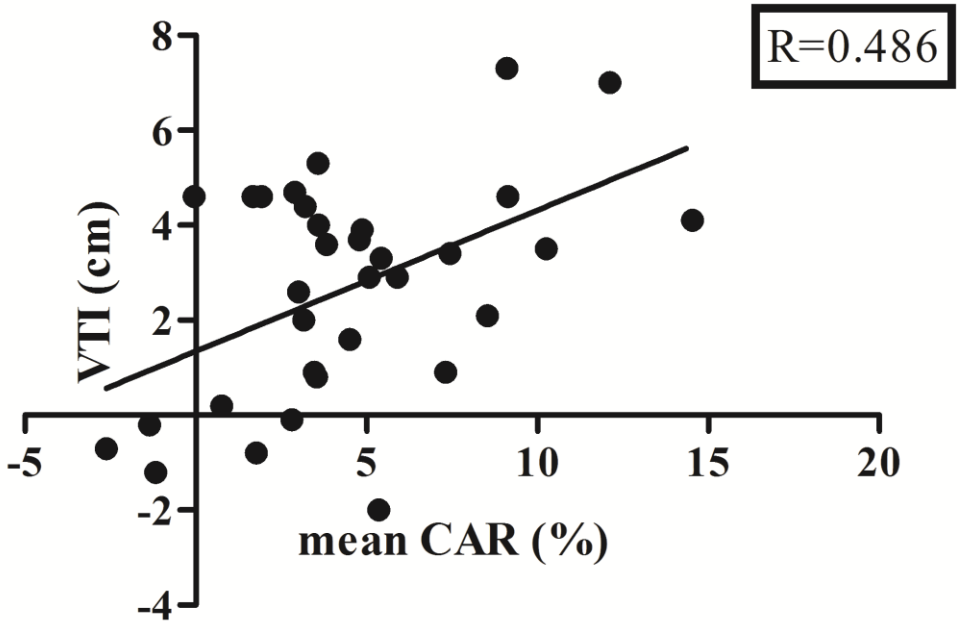

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