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### Article

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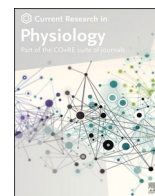
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## Carotid artery vasoreactivity correlates with abdominal aortic vasoreactivity in young healthy individuals but not in patients with abdominal aortic aneurysm

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### ABSTRACT

**Background:** Sympathetic stimulation of central arteries, such as coronary and carotid arteries, cause vasodilation in healthy subjects, but vasoconstriction in those with increased cardiovascular risk. This study compared vasoreactivity to sympathetic stimulation between abdominal aorta and carotid artery in healthy young individuals (young group,  $n = 20$ ), in patients with abdominal aortic aneurysm (AAA group,  $n = 20$ ) and in a healthy older group, age- and gender matched with AAA group (matched group,  $n = 18$ ).

**Method:** All subjects underwent cold pressor test, while performing concomitantly duplex ultrasound of abdominal aorta and carotid artery vasoreactivity. Observer-independent software was used to analyze and calculate magnitude and timing of maximum vasodilation or vasoconstriction. Pearson's correlation coefficient was calculated to investigate vasoreactivity between arteries.

**Results:** Carotid artery reactivity [Interquartile range 25%, Interquartile range 75%] did not significantly differ between the young, matched and AAA group (3.5% [1.4, 4.7], 2.6% [2.0, 4.1] and 2.2% [-1.9, 3.7], respectively,  $p = 0.301$ ). Abdominal aortic responsiveness demonstrated larger differences between young (4.9% [-0.2, 8.4]), matched (3.3% [-2.5, 4.4]) and individuals with AAA (0.5% [-3.9, 4.1],  $p = 0.059$ ). Pooled analysis showed a significant correlation between carotid and abdominal aortic vasoreactivity ( $r = 0.444$ ,  $p = 0.001$ ). Subgroup analyses demonstrated significant correlation between both arteries in young ( $r = 0.636$ ,  $p = 0.003$ ), but not matched ( $r = -0.040$ ,  $p = 0.866$ ) or AAA group ( $r = 0.410$ ,  $p = 0.129$ ).

**Conclusions:** Sympathetic stimulation induces powerful vasodilation of the carotid artery and abdominal aorta, which is significantly correlated in healthy individuals. No such correlation is present in abdominal aortic aneurysm patients. This suggests the aneurysm alters local abdominal aorta vasoreactivity, but not the carotid artery.

### 1. Introduction

An abdominal aortic aneurysm (AAA) represents a serious, local dilation of the aorta that is associated with life-threatening complications. It is widely acknowledged that AAA has a multifactorial pathogenesis and consistently exists in the context of atherosclerosis. Pathological processes known to contribute to aneurysm formation in general are inflammation and dysregulation of matrix remodeling and

repair of the aortic wall (Ailawadi et al., 2003; Abdul-Hussien et al., 2010; Sun, 2012). Although these factors are systemically present, AAA represents a local process with a characteristic local structural abnormality, e.g. widening, of the abdominal aorta (Ailawadi et al., 2003; van Mil et al., 2019). Similarly, studies have also linked the presence of generalized endothelial dysfunction by investigating corresponding pathogenic mechanisms to the development of AAA (Kaneko et al., 2011; Siasos et al., 2015). Measures of endothelial function in the carotid

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**Table 1**  
Determined parameters of the CPT.

Parameter	Definition
Baseline diameter (cm)	Mean diameter of the baseline
AUC (cm*s)	Diameter area under the curve from start CPT
Peak diameter (cm)	AUC >0, maximum value diameter AUC <0, minimum value diameter
Time to peak (s)	Time from start CPT to peak
Maximum reactivity (mm)	Peak minus the mean diameter of the baseline
Maximum reactivity (%)	Percentage change of the diameter
Slope of the curve (mm/s)	Slope from baseline diameter at start CPT to peak
Duration of response (s)	Vasodilation: Time duration of diameter change >1.5% Vasoconstriction: Time duration of diameter change <1.5%
AUC of response (cm*s)	Diameter area under the curve of the response (>1.5% or <-1.5%)
Effective reactivity (mm)	Area under the curve of the response/duration of response
Impact factor (cm*s <sup>2</sup> )	Area under the curve of the response * duration of response

artery, to determine vasoreactivity, have demonstrated to predict risk for future cardiovascular events in patients with peripheral artery disease (van Mil et al., 2019). Such prognostic information is also crucial for AAA patients since they have a 2.5 times higher risk on development of future cardiovascular events (Newman et al., 2001; Bahia et al., 2015). However, endothelial function is typically examined in peripheral arteries, with little work directly focusing on functional characteristics of the abdominal aorta.

Sympathetic stimulation elicits differential responses depending on the vascular bed. Interestingly, arteries such as the carotid and coronary arteries show strong similarity in their vasoreactivity to a sympathetic stimulus (van Mil et al., 2017). Specifically, sympathetic stimulation causes vasodilation of both arteries in healthy subjects, whilst this response is diminished or even reversed to vasoconstriction in patients with coronary heart disease (Nabel et al., 1988; Zeiher et al., 1989; Schächinger et al., 2000). Studies have related this response to the integrity and function of the endothelium (Zeiher et al., 1989). Previous work demonstrated that abdominal aorta diameter in healthy individuals also shows strong vasodilation in response to sympathetic stimulation (Chandraratna et al., 2009). This raises the question whether the abdominal aorta, similar to other elastic arteries, show such reactivity in healthy individuals, and in those with AAA. This allows a better understanding of whether functional responses of the abdominal aorta are impaired in AAA patients. Moreover, through simultaneous evaluation of both the carotid artery and abdominal aorta, this allows us to examine whether such impairment in AAA patients is a local or systemic phenomenon. This is relevant since this could help to better understand the role of local or systemic measures of endothelial function in AAA.

The purpose of this study is to evaluate the carotid and abdominal

aortic diameter reactivity to sympathetic stimulation between healthy young, older individuals with AAA and age- and gender matched healthy individuals. We hypothesize that individuals with AAA demonstrate an attenuated vasodilator response in both arteries compared to their peers, whilst this attenuated response is further exaggerated in the abdominal aorta. Adopting this novel approach of examining central artery diameter reactivity to sympathetic stimulation may improve knowledge of the pathophysiological process in AAA patients, which can help in risk assessment and thereby clinical management and surveillance of AAA patients.

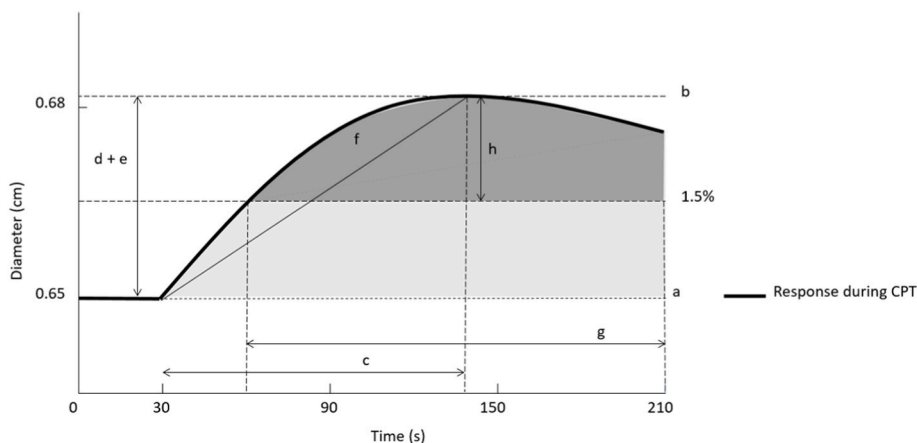
## 2. Methods

### 2.1. Ethics approval

This study was designed as a prospective observational two-center study, both located in the Netherlands. The study was approved by the regional Medical Ethical Committee (CMO, 2019–5560) and the local Institutional Review Boards. An identifier (NCT04035252) was assigned at the US National Library of Medicine (<https://ClinicalTrials.gov>). This study was conducted according to the ethical guidelines of the 1975 Declaration of Helsinki.

### 2.2. Study population

Participants were divided over three groups of twenty individuals each: (1) healthy young adults between the age of 18 and 40 years old (2) individuals with an untreated AAA (i.e. diameter between 3.0 and 5.0 cm), and (3) age- and gender-matched healthy older individuals



**Fig. 1.** Definitions of the parameters of the data analysis, where (a) is the mean baseline diameter, (b) is the peak diameter, (c) the time to peak, (d) and (e) show the reactivity, (f) is the slope of the curve, (g) is the duration of the response, (h) is the effective reactivity, the dark grey area is the AUC of the response and the dark grey and light area together is the AUC. See Table 1 for parameter definitions.

**Table 2**  
Participant characteristics.

	Young healthy group (n = 20)	Matched healthy Group (n = 20)	AAA Group (n = 18)	P-value
Male gender (n, %)	12.0, 60.0	18.0, 90.0	17.0, 94.4	0.012 <sup>a</sup>
Age (years)	23.5 [21.0, 26.0]	67.5 [64.5, 70.0]	69.5 [67.0, 79.0]	<0.001 <sup>a</sup>
BMI (kg/m <sup>2</sup> )	22.8 [21.8, 24.7]	25.1 [22.5, 25.9]	24.3 [22.8, 25.7]	0.110
Systolic BP (mmHg)	124.0 [114.25, 134.0]	133.0 [123.0, 152.8]	146.0 [135.0, 160.0]	0.001 <sup>a</sup>
Diastolic BP (mmHg)	65.5 [63.0, 72.3]	79.0 [75.3, 85.3]	83.0 [79.0, 90.0]	<0.001 <sup>a</sup>
Heart rate (beats/min)	61.0 [57.3, 67.8]	63.0 [554.3, 68.8]	62.5 [60.0, 71.0]	0.695
Current smoker (n, %)	5.0, 25.0	2.0, 10.0	6.0, 33.3	0.191
Estrogen use (n, %)	5.0, 25.0	0.0, 0.0	0.0, 0.0	0.102
<b>Comorbidities (n, %)</b>				
Diabetes Mellitus	0.0, 0.0	0.0, 0.0	2.0, 11.1	0.089
Hypertension	0.0, 0.0	0.0, 0.0	7.0, 38.9	0.005 <sup>a</sup>
Hyperlipidemia	0.0, 0.0	2.0, 10.0	6.0, 33.3	0.022 <sup>a</sup>
<b>SVS/AAVS score<sup>b</sup> (n, %)</b>				
1 Absent	20.0, 100.0	19.0, 95.0	12.0, 66.7	0.003 <sup>a</sup>
2 Mild	0.0, 0.0	1.0, 5.0	6.0, 33.3	0.004 <sup>a</sup>
3 Moderate	0.0, 0.0	0.0, 0.0	0.0, 0.0	1.000
4 Severe	0.0, 0.0	0.0, 0.0	0.0, 0.0	1.000
<b>Medication use (n, %)</b>				
Acetylsalicylic acid	0.0, 0.0	0.0, 0.0	9.0, 50.0	<0.001 <sup>a</sup>
Antiplatelet drugs	0.0, 0.0	2.0, 10.0	6.0, 33.3	0.003 <sup>a</sup>
Anticoagulants	0.0, 0.0	1.0, 5.0	3.0, 16.7	0.084
Antihypertensives	0.0, 0.0	0.0, 0.0	7.0, 38.9	<0.001 <sup>a</sup>
Statins	0.0, 0.0	3.0, 15.0	10.0, 55.6	<0.001 <sup>a</sup>
Analgesic	0.0, 0.0	1.0, 5.0	7.0, 38.9	0.001 <sup>a</sup>
Sympathomimetics	0.0, 0.0	1.0, 5.0	1.0, 5.6	0.420

<sup>a</sup> Significant difference between the three groups (P < 0.05).

<sup>b</sup> SVS/AAVS score is a comorbidity severity score of the Society of Vascular Surgery and American Association for Vascular Surgery.

(matching on AAA patients; further described as matched group throughout the manuscript). Individuals with an age over 18 years and without cardiovascular history, antihypertensive medication or high blood pressure (i.e. >140 mmHg for systolic and/or >90 mmHg for diastolic pressure) were approached for enrollment in the young healthy and in the matched group. Exclusion criteria were increased risk for coronary spasms (based on the Rose questionnaire (Rose et al., 1977)), (history of) carotid artery disease, a body mass index (BMI)  $\geq 30$  kg/m<sup>2</sup>, connective tissue diseases (i.e. Marfan's syndrome and scleroderma), Raynaud's phenomena, chronic pain syndrome, open wounds on the upper extremities and/or the presence of an arteriovenous fistula or shunt. Furthermore, patients with recent (less than three months) presence of angina pectoris, myocardial infarction, cerebral infarction, heart failure and/or treatment for peripheral artery disease were excluded. All participants provided written informed consent prior to participation.

### 2.3. Experimental design

All participants were planned for a single visit of approximately 30 min. Conform guidelines for measuring vascular function, participants were requested to fast for a minimum of 6 h before assessment to ensure standardized and qualitative measurements (Thijssen et al., 2019). Additionally, participants were asked to avoid consumption of alcohol, caffeine, chocolate and products with high vitamin C content for 18 h, and to abstain from strenuous exercise for 24 h prior to the hospital visit (Thijssen et al., 2019).

The medication use (type, dose and start date), and demographic information (age, gender, resting blood pressure and heart rate, and if applicable, date of last menstrual period and use of hormonal contraceptives) were obtained. Additionally, cardiovascular risk factors and diseases, like hypertension, hyperlipidemia, (history of) smoking, diabetes mellitus, vascular disease, cardiac disease, renal disease, pulmonary disease, and/or (history of) cancer were registered.

Participants rested in supine position in a temperature-controlled room with dimmed lighting for a minimum of 10 min before starting baseline measurements. Resting blood pressure and heart rate were obtained using an automatic sphygmomanometer. A curved probe was

placed right above the bifurcation of the abdominal aorta and ultrasound parameters were set to further improve visibility of the lumen-arterial wall interface using a longitudinal, B-mode image. Concomitantly, participants were positioned with the neck extended to allow assessment of the left common carotid artery (CCA). The CCA diameter was visualized also in longitudinal plane, 2 cm below the bifurcation using a linear probe. The measurement consisted of an ultrasound measurement where the CCA and abdominal aorta were recorded concomitantly for in total 210 s, including a 30 s baseline period and 3 min cold pressor test (CPT). The CPT entailed submersion of the participant's left hand in water of  $\leq 4$  °C. The blood pressure was measured every 60 s after emerging the hand in the ice water. All blood pressure measurements were measured at the right arm, using an automatic sphygmomanometer. All ultrasound measurements were performed by three experienced ultrasonographers.

### 2.4. Data analysis

The measured data were saved as an Audio Video Interleave (AVI) file. The AVI files were loaded into BloodFlow Software (Version 4.0; National Instruments LabVIEW, Austin, TX, USA). This software uses a special developed edge-detection and wall-tracking algorithm in order to determine the diameter of the vessel lumen. A region-of-interest (ROI) of the artery was selected where the lumen-arterial wall interface was clearly visible during the entire video. The diameter was determined multiple times per frame, where the number of detected segments of the vessel wall depended on the size of the ROI. Every frame gives a median diameter, that can be used for further analysis. These data were manually filtered on major artefacts, e.g. caused by swallowing, and probe movement. The diameter and time data were transferred to MatLab R2018b (Mathworks, Natick, MA, USA). First, outliers were removed using a threshold of 2.5 times Median Absolute Deviation (MAD) for the baseline and CPT data separately. Then, the missing data were filled using linear interpolation between available data points. However, if there was data missing at the first seconds of the baseline this interpolation started from the first known value. Moreover, if there was data missing at the end of the CPT, interpolation was performed using a constant of the mean of the last ten values. Additionally, a moving mean

**Table 3** Results of different parameters CPT test for both arteries and all the groups<sup>a</sup>, where YH=young healthy group, MH = matched healthy group, AAA = AAA group.

	Carotid artery			Abdominal aorta			P-value
	YH (n = 19)	MH (n = 20)	AAA (n = 18)	YH (n = 20)	MH (n = 20)	AAA (n = 15)	
Mean baseline diameter (cm)	0.64 [0.61, 0.68] <sup>c,f</sup>	0.71 [0.65, 0.77] <sup>d,f</sup>	0.76 [0.71, 0.80] <sup>e,f</sup>	1.45 [1.36, 1.65] <sup>f</sup>	1.70 [1.60, 1.94] <sup>f</sup>	3.40 [2.90, 3.91] <sup>f</sup>	<0.001 <sup>b,g</sup>
AUC (cm*s)	1.08 [0.08, 2.36] <sup>f</sup>	1.54 [0.61, 2.63]	1.08 [-0.63, 2.73]	6.01 [0.05, 14.17] <sup>f</sup>	4.24 [-1.19, 7.08]	0.12 [-8.90, 8.11]	0.262
Maximum reactivity (%)	3.48 [1.44, 4.66]	2.63 [1.98, 4.12]	2.19 [-1.88, 3.68]	4.85 [-0.15, 8.43]	3.27 [-2.48, 4.38]	0.53 [-3.89, 4.14]	0.059
Maximum peak diameter (cm)	0.66 [0.62, 0.71] <sup>c,f</sup>	0.72 [0.68, 0.77] <sup>d,f</sup>	0.78 [0.73, 0.81] <sup>d,f</sup>	1.49 [1.37, 1.74] <sup>f</sup>	1.79 [1.59, 1.93] <sup>f</sup>	3.45 [2.94, 3.89] <sup>f</sup>	<0.001 <sup>b,g</sup>
Maximum reactivity (mm)	0.25 [0.09, 0.28] <sup>f</sup>	0.20 [0.15, 0.28]	0.17 [-0.15, 0.27]	0.73 [-0.01, 1.32] <sup>f</sup>	0.55 [-0.38, 0.76]	-0.20 [-1.21, 1.23]	0.239
Time to peak (s)	90.16 [52.23, 116.80] <sup>e</sup>	152.33 [78.63, 178.24] <sup>d</sup>	116.07 [80.71, 168.97]	112.22 [81.41, 168.45]	108.10 [53.60, 174.58]	104.00 [59.17, 173.91]	0.978
Slope (mm/s)	3.30*10 <sup>-3</sup> [0.80*10 <sup>-3</sup> , 4.20*10 <sup>-3</sup> ] <sup>f</sup>	1.40*10 <sup>-3</sup> [0.90*10 <sup>-3</sup> , 2.50*10 <sup>-3</sup> ]	1.30*10 <sup>-3</sup> [-1.20*10 <sup>-3</sup> , 3.10*10 <sup>-3</sup> ]	6.00*10 <sup>-3</sup> [-0.50*10 <sup>-3</sup> , 12.10*10 <sup>-3</sup> ] <sup>f</sup>	4.00*10 <sup>-3</sup> [-5.30*10 <sup>-3</sup> , 8.00*10 <sup>-3</sup> ]	3.40*10 <sup>-3</sup> [-13.00*10 <sup>-3</sup> , 9.70*10 <sup>-3</sup> ]	0.266
Duration response (s)	78.24 [39.24, 130.94] <sup>f</sup>	81.22 [27.11, 153.18]	77.18 [38.86, 131.50]	137.53 [72.14, 180.01] <sup>f</sup>	106.32 [40.50, 123.57]	100.92 [62.41, 154.60]	0.187
AUC response (cm*s)	0.35 [0.06, 1.64]	0.25 [0.05, 0.90]	0.14 [-0.16, 0.85]	3.28 [0.64, 10.63]	1.01 [-0.35, 3.13]	-0.37 [-4.02, 6.21]	0.130
Effective reactivity (mm)	0.06 [0.03, 0.12]	0.03 [0.02, 0.09]	0.02 [-0.03, 0.07]	0.21 [0.09, 0.70]	0.10 [-0.08, 0.25]	-0.10 [-0.32, 0.44]	0.132
Impact factor (cm*s <sup>2</sup> )	20.67 [1.07, 198.88]	18.45 [0.87, 128.93]	8.58 [-9.16, 114.50]	442.07 [42.46, 1461.36]	107.33 [-12.45, 469.71]	-996 [-513.54, 980.61]	0.129

<sup>a</sup> Data represented as median [interquartile range: Q1, Q3].  
<sup>b</sup> Significant difference between the three groups (P < 0.05).  
<sup>c-e</sup> Median in row with different superscript letter differ (P < 0.05), as analyzed by One-way ANOVA.  
<sup>f</sup> Significant difference between carotid artery and abdominal aorta (P < 0.05).  
<sup>g</sup> No post-hoc testing is performed since the difference is inherent to group definitions.

of the data was calculated using a 10 s time window. From this moving mean, the baseline diameter, diameter area under the curve (AUC), peak diameter, time to peak, maximum reactivity (cm), maximum reactivity (%), slope of the curve, duration of response, AUC of response, effective reactivity and the impact factor were calculated. The definition and graphical explanation of these parameters can be found in respectively Table 1 and Fig. 1. The classification of the vasomotor reactivity was based on the AUC; when the AUC was positive, the maximum positive reactivity was calculated, when the AUC was negative, the maximum negative reactivity was calculated. A maximum reactivity between -1.5% and 1.5% was considered as a non-response.

2.5. Statistical analysis

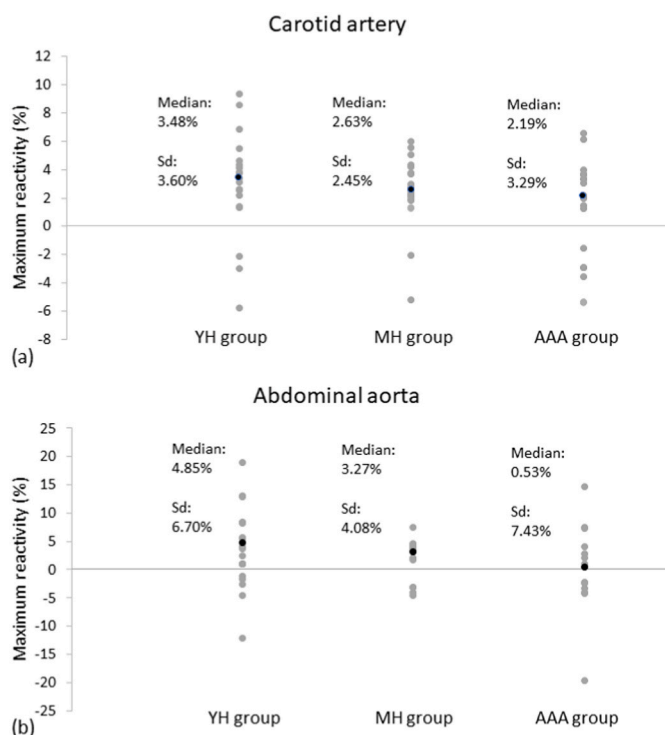
Normality of baseline characteristics and vasomotor response data of all groups separately were determined based on visual inspection and tested for normal distribution using the Shapiro-Wilk test. Baseline characteristics are presented as median and interquartile range (IQR). Categorical variables are presented as number followed by percentage. Pearson’s correlation coefficient was calculated between the reactivity parameters of the two arteries in all participants for all groups together and separately. Differences in the continuous reactivity parameters between the abdominal aorta and CCA within every group were calculated with the Wilcoxon Signed Rank test since data did not meet normality criterion. Furthermore, differences between the three groups were calculated with the Kruskal-Wallis test. Post-hoc analysis was performed on variables showing differences among all groups, to determine between which specific groups the differences existed. For correction of multiple testing, One-way ANOVA analysis was used using Bonferroni for continuous variables. P-values < 0.05 were considered as significant. Statistical analysis was performed in IBM SPSS Statistics version 25 (IBM Corporation, Armonk, NY, USA).

3. Results

A total of 60 individuals were included initially. There were two protocol deviations in the AAA group; both patients were previously diagnosed with carotid disease (i.e. 30% stenosis and occlusion, respectively) of the right carotid artery. These two subjects were excluded for analysis and not replaced according to the protocol. This resulted in 58 individuals being enrolled for analysis in this study divided over three groups: 20 healthy young adults, 20 matched healthy older individuals and 18 individuals with an untreated AAA. Data of the abdominal aorta of three participants of the AAA group could not be used, due to extensive thrombotic plaque formation disturbing the wall-tracking algorithm, and low quality ultrasound measurement. Data of the carotid artery of one participant of the young healthy group could not be used, because analysis could not be performed due to technical difficulties during the measurement. So final numbers per group were 19 young healthy, 20 matched and 18 AAA participants for the carotid artery and 20 young healthy, 20 matched and 15 AAA participants for the abdominal aorta. Baseline characteristics of all participants are depicted in Table 2. One AAA participants had a saccular AAA, while the other patients had fusiform AAA. Median maximum AAA diameter extracted from hospital records was 40 mm, while the median measured AAA diameter during this study was 34 [29, 39] mm (Table 3).

**Carotid artery.** At baseline the carotid artery diameters of the AAA and matched group were significantly larger than the young group (p < 0.001). During CPT, the young, matched and AAA group all revealed an increase in diameter (respectively 3.5% [1.4, 4.7], 2.6% [2.0, 4.1], 2.2% [-1.9, 3.7]), which did not significantly differ between groups (Figs. 2 (a) and 3(a)).

**Abdominal aorta.** For the abdominal aorta, a significant difference in the baseline diameter was found between groups (p < 0.001). The matched individuals demonstrate a significantly larger diameter than young subjects, whilst the AAA group showed the largest baseline



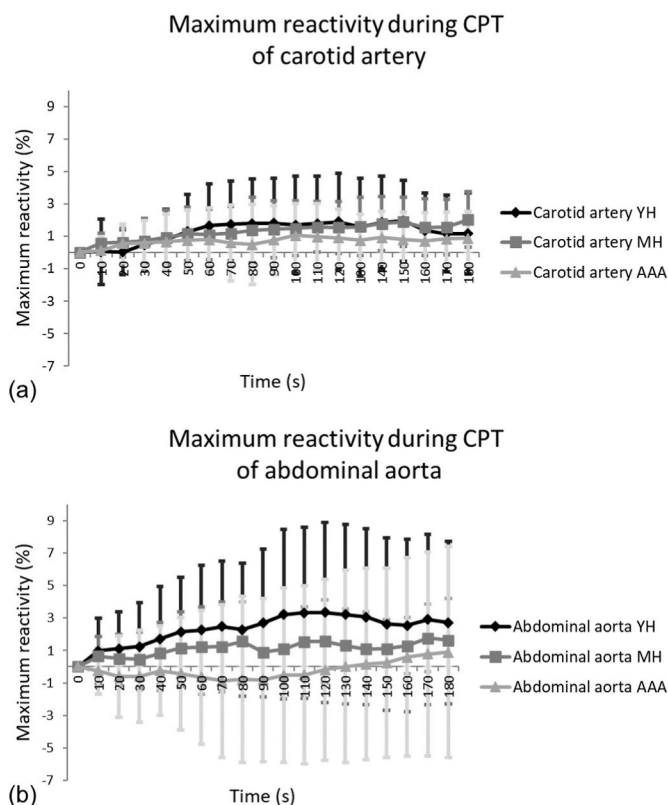
**Fig. 2.** Maximum reactivity per participant per group where YH=young healthy group (n = 20), MH = matched healthy group (n = 20), AAA = AAA group (n = 18). Every participant is visualized by a grey dot which represents their maximum vasoreactivity for the carotid artery (a) and abdominal aorta (b). The black dot visualizes the mean vasoreactivity per group per artery. There was no significant difference between groups in carotid artery (p = 0.301) and abdominal aorta (p = 0.059).

diameter. During CPT, an increase in diameter was found in healthy young (4.9% [-0.2, 8.4]), which was attenuated in the matched individuals (3.3% [-2.5, 4.4]) and reversed to no response in those with AAA (0.5% [-3.9, 4.1], p = 0.059, Figs. 2(b) and 3(b)).

**Correlation.** When data from all participants were pooled, a significant positive correlation was found between carotid and abdominal aorta for baseline diameter (r = 0.601), AUC (r = 0.313), maximum reactivity (%) (r = 0.444), maximum peak (r = 0.577), maximum reactivity (cm) (r = 0.391) and effective diameter change (r = 0.288). Fig. 4(A) illustrates the responsiveness of the carotid artery and abdominal aorta per participant. For the subgroups, a significant correlation between carotid and abdominal aortic maximum reactivity (%) was found in healthy young (r = 0.636, p = 0.003, Fig. 4(b)), but not in matched group (r = -0.040, p = 0.866, Fig. 4(c)) or in AAA group (r = 0.410, p = 0.129, Fig. 4(d)).

#### 4. Discussion

The first outcome of this study is that sympathetic stimulation leads to marked vasodilation of the carotid artery, without differences between the three groups (young, matched and AAA group). Second, marked vasodilation was also found in the abdominal aorta during sympathetic stimulation in healthy young subjects, which was attenuated in the healthy matched individuals, and even reversed to no response in patients with an AAA. Third, the significant positive correlation between the carotid artery and abdominal aorta vasoreactivity in the young healthy group, was absent in the AAA group and the matched healthy group. Whilst this work reinforces previous work, in that healthy central arteries demonstrated strong and comparable responses to sympathetic stimulation (van Mil et al., 2017), this study also revealed a locally disrupted vasoreactivity of the abdominal aorta in patients with

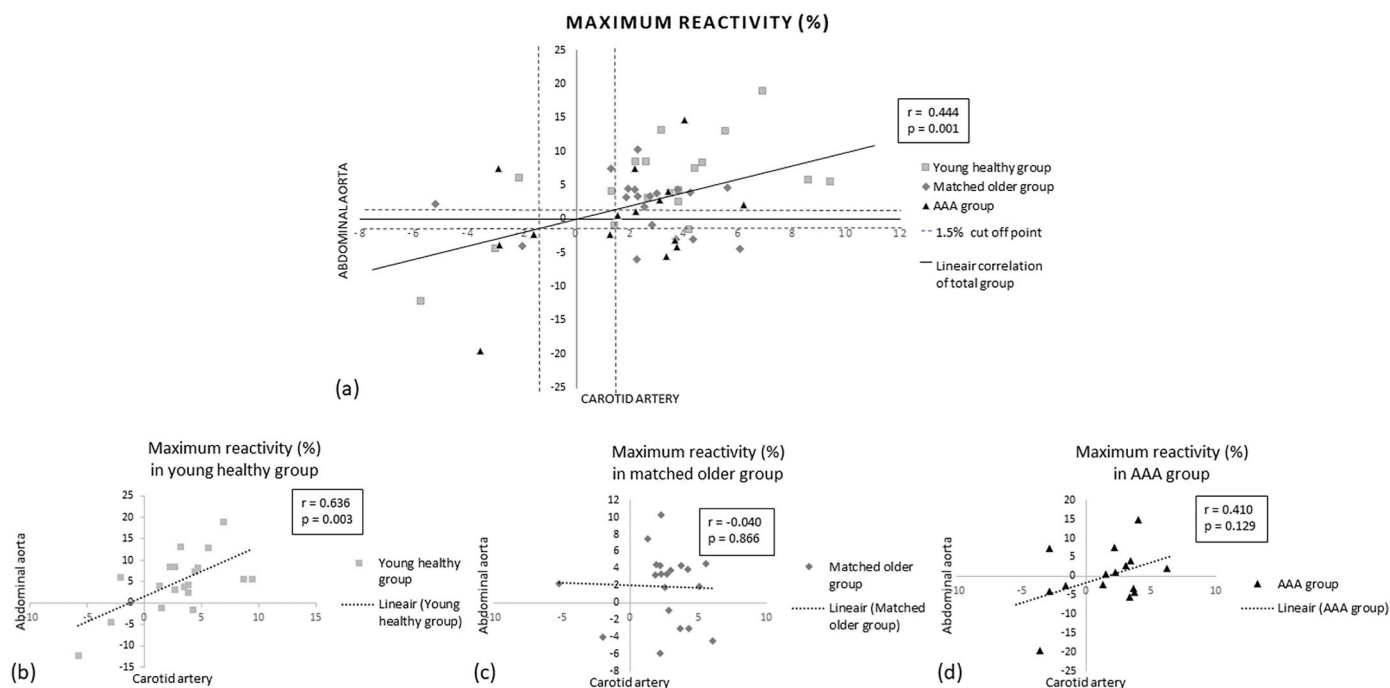


**Fig. 3.** Mean reactivity during CPT. Visualization of vasoreactivity of the carotid artery (a) and abdominal aorta (b) with standard deviation (colored line) for the young healthy group (black), older healthy group (dark grey) and AAA group (light grey) during CPT. These reactivity curves can be compared with the AUC per group per artery, where the carotid artery and abdominal aorta did not significantly differ between groups (respectively, p = 0.834 and p = 0.262).

#### AAA.

In healthy young individuals, the relative maximum reactivity of the abdominal aorta (4.9% [-0.2, 8.4]) seemed larger than the carotid artery (3.5% [1.4, 4.7]), however not significant. The larger diameter of the aorta unlikely explains relatively larger vasoreactivity, especially since previous work found that larger conduit artery diameter is associated with smaller vasodilation in response to shear stress stimuli (Herrington et al., 2001; Silber et al., 2001; Thijssen et al., 2008). A potential explanation can be the higher amount of smooth muscle cells in the abdominal aorta, which are stimulated by  $\alpha$ 1-,  $\alpha$ 2- and  $\beta$ -adrenergic receptors during sympathetic stimulation and cause vasodilation (Barbato, 2009; Chandraratna et al., 2009; van Mil et al., 2018). Despite the absolutely larger response in the aorta, a positive significant correlation was found between the abdominal aorta and carotid artery in the young healthy group.

In contrast to the strong vasodilation in the young healthy group in both arteries, the AAA group demonstrated an attenuated response of the carotid artery and even no response of the abdominal aorta. Previous research suggested a systemic response of the central arteries to sympathetic stimulation, which was present in healthy individuals as well as in those with cardiovascular risk factors and/or disease (Rubenfire et al., 2000; van Mil et al., 2017). However, our results suggest that local changes due to an aneurysm alters the response of the abdominal aorta compared to the carotid artery, which did not significantly differ with the other groups. This raises questions about the potential explanation for the absence of response in the aorta in AAA patients. One potential explanation is that older age of AAA patients contributes to these effects, supported by the observations in the matched cohort. However,



**Fig. 4.** An overview of the maximum reactivity of the carotid artery and abdominal aorta per person. (a) Every diamond, square and triangle represent a participant of the young healthy ( $n = 19$ ), matched healthy ( $n = 20$ ) or AAA group ( $n = 15$ ), respectively. The dotted line represents the cut-off point of a reactivity of 1.5% and the black line represents the correlation of the total group. (b, c, d) Illustrates the maximum reactivity per person in respectively the young healthy group (b), matched healthy group (c) and AAA group (d), where the dotted line represents the correlation of each group.

comparing the AAA group with the matched healthy group, there seemed to be a small, but not significant, difference in baseline diameter and vasoreactivity for both arteries. These results support the evidence that the vessels are not only affected by age (Sugiyama et al., 1996; Astrand et al., 2005; Tri et al., 2019; Amabili et al., 2020), but also by vascular disease (Nabel et al., 1988; Länne et al., 1992; Sonesson et al., 1997; Makita et al., 2000; Johnsen et al., 2009; Puri et al., 2013).

Another explanation for our observed local changes in the abdominal aorta in AAA patients can be the critical role of the endothelium in vasoreactivity, where endothelial dysfunction, present in atherosclerosis, affects the vasomotor response (Zeiber et al., 1989). In fact, atherosclerotic vessel parts have already shown to have a loss of normal dilator function, resulting in vasoconstrictive response (Nabel et al., 1988; Zeiber et al., 1989; Länne et al., 1992). This highlights the potential role of local processes in mediating the abnormal vasoconstrictive response of the aorta in AAA. Overall, local changes of the abdominal aorta, likely related to impairment or even (partial) denudation of the endothelium, may contribute to the vasoconstrictive response during sympathetic stimulation and explains the lack of correlation between the carotid artery and abdominal aorta.

To further test local processes of the AAA, vasoreactivity of the aortic neck was compared with the aneurysm response in one AAA patient. The aortic neck, located just above the start of the aneurysm, showed a dilation (3.5%), which was comparable to the carotid artery response (3.3%). However, a constriction (−5.5%) was found at the level of the aneurysm. This supports the assumption that AAA causes a local effect on the vasomotor response of the abdominal aorta. The aortic neck therefore could be more equivalent with the carotid artery than the AAA, maybe because it is exposed to comparable systemic factors, without being affected by the local process of AAA. Hence, no more data on the aortic neck of AAA patients were available, but are needed in order to study the possible effects of the disease on different parts of the vascular tree.

A limitation of this study is that this test only measures a 2D plane. This results in a difference between the measured baseline diameter of

the AAA group during this study (34 mm) and the median maximum diameter of the AAA group based on electronic patient files (40 mm). Literature demonstrates a non-linear behavior and distensibility of both arteries (Kamenskiy et al., 2012; Amabili et al., 2020). Therefore, the measured vasomotor response can vary in different directions. Another limitation could be the inclusion of a saccular aneurysm. Due to the geometry, flow is likely to be different from fusiform aneurysms, which could influence the diameter response and therefore also affect the comparison with the carotid artery. However, only one patient had a saccular aneurysm and analysis without the results of this patient did not change any of the results. Furthermore, differences in arterial carbon dioxide (CO<sub>2</sub>) might affect the arterial diameter response (Tymko et al., 2017). However, heavily breathing of participants was not observed during measurements and if any CO<sub>2</sub> changes occurred, this is unlikely to affect the abdominal aorta. As far as known, the abdominal aorta is less likely to react on changes in CO<sub>2</sub> concentrations due to different auto-regulation processes than the carotid artery. The carotid artery is affected by changes in cerebral perfusion which reacts on changes in CO<sub>2</sub> concentration (Tymko et al., 2017). Thereby, the primary findings of this study are less likely to be affected. Additionally, a study of Hoiland et al. (2017) demonstrated that the primary stimulus of dilation is mainly caused by shear stress rather than CO<sub>2</sub> increase. Furthermore, the small sample size is a limitation. These limitations should be kept in mind, while interpreting the results.

This study suggests that local processes of the AAA can only be evaluated in the abdominal aorta itself. This contrasts with previous work in patients with coronary artery disease where they found correlations between (affected) coronary arteries and carotid arteries. A possible explanation may relate to the pathophysiological background of both pathologies. Coronary artery disease is importantly driven by a stenotic disease process (atherosclerosis), one which is typically present throughout the entire vascular tree. In contrast, AAA refers to a strong, locally dilatory pathophysiological process, rarely causing similar abnormalities elsewhere in the vasculature. Although carotid artery vasoreactivity may not reflect the local AAA process, it seems a potential

surrogate measure for systemic processes like atherosclerosis, leading to cardiovascular disease. Therefore, results are needed on the prognostic value of this measure on cardiovascular events in AAA patients to determine the value of this measure. Especially since other studies already demonstrated differences in reactivity in patients with risk for cardiovascular events (Zeiber et al., 1989; Chandraratna et al., 2009; van Mil et al., 2017). Therefore, two studies are started where AAA patients who are under surveillance (NCT03989011) and AAA patients who are scheduled for repair (NCT04183426) undergo CPT in order to determine the prognostic value for cardiovascular events.

## 5. Conclusion

In conclusion, sympathetic stimulation induces powerful vasodilation of the carotid and abdominal aorta, which is significantly correlated in healthy young individuals. No such correlation is present in AAA patients and in matched healthy individuals. The AAA patients even demonstrated no response of the abdominal aorta. This suggests that the response of the abdominal aorta is altered compared to the carotid artery, most likely due to local vascular impairment.

## Availability of data and materials

Since the data is not anonymous, it is not online available. However, the data is available upon request.

## Competing interests

The authors declare that they have no competing interests.

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## CRedit authorship contribution statement

**Jenske J.M. Vermeulen:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – original draft, Visualization, Supervision, Project administration. **Anne-Jet S. Jansen:** Methodology, Software, Investigation, Resources, Writing – review & editing. **Sam van de Sande:** Methodology, Investigation, Resources, Writing – review & editing. **Yvonne A.W. Hartman:** Investigation, Writing – review & editing. **Suzanne Holewijn:** Writing – review & editing, Supervision, Funding acquisition. **Michel M.P.J. Reijnen:** Writing – review & editing, Supervision, Funding acquisition. **Dick H.J. Thijssen:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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