Similarities and differences between carotid artery and coronary artery function

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ABSTRACT

Cardiovascular disease (CVD) remains one of the leading causes of morbidity and mortality. Strategies to predict development of CVD are therefore key in preventing and managing CVD. One stratergy in predicting CVD is by examining the role of traditional risk factors for CVD (e.g. age, sex, weight, blood pressure, blood lipids, blood glucose, smoking and physical activity). Although these measures are non-invasive and simple to perform, they provide limited information of CVD prediction. Directly examining functional characteristics of arteries that are involved in the pathophysiological changes that contribute to the development of CVD improve prediction of future CVD. Nevertheless, examining the function of arteries susceptable to atherosclortic changes, such as the coronary arteries, is invasive, expensive, and associated with high risk for complications. More accessible arteries can be used as a surrogate measure of coronary artery function. For example, the carotid artery may be a superior surrogate measure of coronary artery function given that, the carotid artery represents a central vessel that shows similarities in vasomotor function and anatomical structure with coronary arteries. This review summarises the similarities between the carotid and coronary arteries, describes how both arteries respond to specific vasoactive stimuli, and discusses if the easily assessible carotid artery can provide information about vascular function (e.g. vasomotor reactivity to sympathetic stimulation) which is prognostic for future cardiovascular events. Finally, the impact of older age and lifestyle interventions (e.g. exercise training) on carotid artery function will be discussed.

KEYWORDS: carotid artery; coronary artery endothelial function; atherosclerosis; cardiovascular disease

INTRODUCTION

Cardiovascular disease (CVD) is an umbrella term which describes disease of the heart and blood vessels, and remains the world's leading cause of morbidity and mortality (1), accounting for approximately 31% of all deaths (2). In addition to the significant healthcare costs, CVD affects socio-economic costs through loss of productivity. Improvements in clinical management has contributed to a reduction in CVD and stroke-related mortality by 3.7% and 4.5%, respectively. (3) Nonetheless, given the ageing population and increased prevalence of established risk factors (e.g. obesity, diabetes), the prevalence of CVD is likely to continue to increase (3) . This highlights the importance of predicting the risk of future development of cardiovascular events and/or CVD.

Traditionally, predicting future cardiovascular events and/or CVD is based on the evaluation of risk factors, such as age, sex, family history of premature CVD, blood pressure, cholesterol, body weight, and glucose homeostasis (4, 5). Despite their simplicity and non-invasive nature, these risk factors provide limited predictive capacity. Based on the central role of the endothelium in the process of atherosclerosis, direct measures of coronary artery endothelial function may improve prediction of future CVD (6, 7). Indeed, endothelial health is involved in the progression of CVD, which is supported by the finding that endothelial dysfunction precedes the development of atherosclerosis (8-11). Furthermore, measures of coronary artery endothelial function independently predict future CVD (12). Given the invasive and expensive nature of assessment of coronary artery function, studies have searched for alternative tools and largely focused on assessment of peripheral arteries. This review specifically focuses on the easily accessible carotid artery. The carotid artery is a central artery which is similar to coronary arteries in anatomical

properties and vasomotor control. In this review, we explore whether the carotid artery can be used as a surrogate measure for coronary artery vascular function. For this purpose, we will provide a brief overview of the role of endothelial function in the process of atherosclerosis, leading to CVD events. Subsequently, we will discuss similarties in carotid and coronary artery function, and also highlight approaches to assess carotid artery vascular function and examine if these approaches can predict future CVD. Finally, we will discuss the impact of older age and lifestyle interventions on carotid (and coronary) artery function.

ENDOTHELIAL FUNCTION AND ATHEROSCLEROSIS

What is the role of the endothelium in atherosclerosis?

The endothelium represents a single layer of cells on the inner side of all vessels that fulfils various important actions (13). In addition to the regulation of vascular tone, the endothelium also affects platelet aggregation, leukocyte adhesion and vascular smooth muscle cell migration and proliferation. Endothelial dysfunction seems an important contributor to the process of atherosclerosis (14). Presence of endothelial dysfunction facilitates increased lipoprotein permeability and oxidation, enhanced mononuclear leukocyte adhesion and intimal accumulation, and dysregulation of the hemostatic-thrombotic balance (15). Various stimuli contribute to the development of endothelial dysfunction, including hemodynamic factors, proinflammatory cytokines, bacterial products, hypercholesterolaemia and oxidized lipoproteins (6, 15-17). Indeed, the presence of hypercholesterolaemia, hypertension, smoking, ageing and obesity are all associated with impaired endothelial function (18). Similarly, oxidative stress (19, 20) and inflammation (21) lead to lower NO-bioavailability and, consequently, impaired endothelial

function. Moreover, studies in humans using *in vivo* techniques found that the pre-clinical stage of atherosclerosis is linked to presence of endothelial dysfunction (22, 23). These studies suggest that impaired endothelial function contributes to the process of atherosclerosis and, eventually, CVD.

Can coronary artery endothelial function predict future CVD?

Coronary artery endothelial function may be a useful predictor of CVD development. To test this hypothesis, Schächinger *et al.* examined coronary artery responses to endothelium-dependent (i.e. acetylcholine, sympathetic activation, shear stress) and –independent stimuli (i.e. glyceryl trinitrate), in 147 patients at risk for coronary artery disease across a mean follow-up of 6.7 years (12). They reported that impaired endothelium-dependent and -independent coronary artery responses were independently related to higher incidence of CVD events (12). Similar findings were reported by Suwaidi *et al*., who found that coronary artery endothelial dysfunction (using coronary artery angiography combined with acetylcholine infusion) in 157 patients with coronary artery disease were associated with an increased risk of CVD events across a 28-month follow up (24). One may question if the prognostic capacity of coronary vascular endothelial dysfunction is also found in those with angiographically normal coronary arteries. Halcox and colleagues found that coronary artery endothelial dysfunction (i.e. examined using acetylcholine infusion) holds independent predictive capacity for future CVD events in both subjects with (n=132) and without (n=176) coronary artery disease across 46 month follow-up (25). Therefore, these data support the independent prognostic value of coronary artery endothelial dysfunction for future CVD events.

Use of surrogate measures of coronary artery endothelial function?

The current gold standard for examining coronary artery endothelial function involves invasive assessment using quantitative coronary angiography combined with graded intracoronary infusion of endothelium-dependent vasodilators (e.g. acetylcholine). In addition to high patient burden and costs, this procedure is associated with significant health risks (26). These limitations have driven the development of surrogate measures of coronary endothelial function. For example, venous occlusion plethysmography combined with intra-arterial infusion of acetylcholine is often used to assess peripheral resistance artery endothelial function (27). Although this procedure resembles the procedure of intra-coronary infusion of drugs (28) and independently predicts future CVD (29), this procedure is invasive, time-consuming, expensive and has therefore predominantly been used in smaller, laboratory-based research studies (30). A more frequently used, non-invasive technique to assess peripheral artery vascular function is the flow-mediated dilation (FMD). The brachial artery is imaged using ultrasonography before and 3-minutes following a 5-minute cuff-induced occlusion of the forearm (31), which leads to a largely NO-mediated dilation (32). The FMD shows good correlation with coronary artery dilator responses (28) and independently predicts CVD events (33, 34). This work indicates that a simple (surrogate) measure of coronary artery endothelial function has potential clinical importance in predicting risk for future CVD events.

CAROTID *VERSUS* CORONARY ARTERY FUNCTION

Carotid and coronary arteries represent large vessels, often referred to as “elastic arteries” or “conducting arteries”. Both arteries transport large volumes of blood away from the left ventricle to perfuse vital organs, the brain (i.e. carotid artery) and cardiac muscle (i.e. coronary arteries). To

fulfill these tasks, the walls of both arteries are resilient against the large fluctuations in blood pressure. The tunica media of coronary and carotid arteries contain a higher density of elastic fibers and fewer smooth muscle cells compared to peripheral, muscular arteries (**Figure 1**). The relatively high amount of elastin in the arterial wall stores elastic energy during systole, which is released during diastole to contribute to a constant flow of blood towards peripheral arteries (i.e. the Windkessel-effect) (35, 36). The tunica media also contain collagen fibrils that form a slack network and provide a physical guard against over-distension. Not surpisingly, carotid artery structure (i.e. wall thickness, plaque presence, calcification) shows close correlation to coronary artery structure. In addition to these similarities in carotid and coronary artery structure, both arteries may also share similar pathways to regulate vascular health and vasomotor control. For these reasons, the carotid artery may offer an easy accessible central artery that may serve as a surrogate marker for coronary artery vascular health. In this part of the review, we will describe the regulation of vascular tone of central arteries, followed by tests of carotid artery function with specific focus on their relation to coronary arteries and ability to predict future CVD events.

How is vascular tone regulated in central arteries?

Assessment of central artery blood flow has primarily focused on coronary arteries, with little work in humans focusing on the carotid arteries. As a result, the majority of our understanding around the regulation of central artery vascular tone relates to studies examining coronary arteries. These studies adopt invasive or non-invasive techniques (MRI, CT and PET) to examine changes in (regional) myocardial blood flow in response to pharmacological (e.g. adenosine, acetylcholine) or physiological stimuli (cold pressor test, exercise). It is the response of myocardial blood flow

to these stimuli that can reveal the regulation of vascular health in humans *in vivo*. Based on this work, it is demonstrated that (coronary) arteries regulate blood flow through: *1.* (local) metabolic control, and *2.* neurally mediated vasoreactivity. Moreover, an intact endothelium seems essential to enable these stimuli to contribute to the regulation of blood flow. Previous work found that in cerebral (37) and coronary arteries (38), autoregulation contributes to acute regulation of vascular tone to ensure sufficient perfusion during fluctuations in blood pressure. This involves a complex feedback loop matching perfusion driven by metabolic requirement. However, it is currently unknown to what extend carotid artery vascular tone is regulated through such mechanisms.

What is the role of metabolic control of vascular tone in central arteries?

Changes in metabolic demand of the myocardium represents an important stimulus for changes in myocardial perfusion. An increase in myocardial work and thus metabolic demand, for example during exercise, will be accompanied by proportionate changes in coronary blood flow. Using non-invasive PET measurements it was demonstrated that 2.8-fold elevation in cardiac work (derived from the rate pressure product) is matched by a 2.2-fold increase in myocardial perfusion (39). Similarly, subsequent studies from the same research group consistently found that elevations in myocardial demand are matched by comparable elevations in myocardial perfusion (39, 40). This demonstrates a central role of myocardial oxygen consumption in mediating coronary flow (41, 42). The increase in myocardial perfusion is initiated by a metabolically-mediated decrease in microvascular resistance, most likely involving adenosine as a key metabolite causing vascular smooth muscle relaxation.

It is currently unknown to what extend cerebral metabolic control contributes to the regulation of carotid artery vasomotor function. Cerebral metabolic demand may influence upstream vascular regulation, especially since cerebral metabolic demand is strongly dependent on neural activation (43). In addition, changes in end-tidal CO_2, representing an indirect measure of metabolism, is found to be a potent stimulus for intra- and extra-cranial artery vasomotion (44, 45). More specifically, increases in end-tidal CO_2 are related to a dose-dependent increase in internal carotid artery diameter, most likely mediated through elevations in shear stress (46). Importantly, although the common carotid artery also dilated during an increase in end-tidal CO_2, distinct regulatory processes seem present contributing to the dilation (46). These differences are important to take into consideration when examining vasomotor responses of the carotid artery.

What is the role of neutrally mediated control of vascular tone in central arteries?

The sympathetic nervous system contributes to the regulation of vascular tone in central arteries. One of the first studies that provided evidence for the role of the sympathetic nervous system in the regulation of central artery vascular tone found that, in the absence of myocardial metabolism, a significant change was present in coronary sinus oxygen tension during sympathetic stimulation (47). The contribution of adrenergic receptors in mediating coronary perfusion was subsequently confirmed by Mohrman *et al.* who found that α-blockade caused a decrease in coronary oxygen uptake (48). In a study including cardiac transplant patients, both denervation and regional re-innervation of cardiac segments were studied simultaneously, demonstrating increases in coronary flow in response to sympathetic stimulation were larger in re-innervated areas. This finding suggests that coronary flow is regulated to larger extent by adrenergic mechanisms than via

metabolic regulation during sympathetic stimulation (15, 49). Since it is challenging to study these factors independently, it remains unclear to what extent the metabolic and adrenergic components interact and contribute to coronary vasomotion during sympathetic activation (50)

Coronary responses to sympathetic stimulation is highly dependent on both the integrity (51-53) and function (54) of the endothelium (see also next section). The importance of the endothelium is in part explained through the presence of adreno-receptors on both the endothelium and smooth muscle cell, which respond to regional and systemic sympathetic stimuli. For example, local release of norepinephrine from adrenergic nerve terminals in the coronary arteries (abluminal) and release of catecholamines from the adrenal glands into the circulation during sympathetic stress or physical exercise all influence coronary vasomotion through stimulation of adrenergic receptors (55). Both α- and β-adrenergic receptors are involved in coronary vasomotion in response to activation of the sympathetic nervous system (50). Whilst β-receptors induce vasodilation, activation of the α-receptors concurrently induces vasoconstriction (**Figure 2**). Nevertheless, differences in adrenoreceptor subtypes are present, which ultimately contribute to a delicate balance between α-mediated vasoconstriction and β-mediated dilation.

α_1-adrenoreceptors. On the vascular smooth muscle cells, α_1-adrenoreceptors have been identified, which are typically activated through local norepinephrine release from the adrenergic nerve terminals during sympathetic stimulation. Stimulation of α_1-receptors leads to vasoconstriction of coronary arteries (50, 56, 57). Indeed, infusion of α_1-agonists in patients with coronary stenosis demonstrated augmented coronary artery vasoconstriction (50, 52, 53). However, infusion of α_1-

agonists in healthy individuals does not alter coronary vascular tone (58). The absence of a vasoconstrictor response in healthy individuals is most likely the result of simultaneous (mild) activation of β-receptors on the endothelium that counterbalances the α_1-mediated constriction.

α_2-adrenoreceptors. In addition to stimulation of α_1-receptors, stimulation of the α_2-receptors on the vascular smooth muscle cells mediate neurally-mediated constriction. However, stimulation of α_2-adrenoreceptors located on the endothelium cause dilation through NO-release (50, 58-60). Indeed, several studies using selective stimulation found that α_2-adrenergic agonists cause endothelium-dependent relaxation in coronary arteries of healthy individuals (61), whilst concurrent stimulation of α_2-receptors located on the smooth muscle cells mediate coronary artery constriction (58, 60). Interestingly, intracoronary infusion of selective α_2-agonists induced a paradoxical vasoconstriction in patients with atherosclerotic coronary arteries. This observation is likely explained through the loss of endothelial α_2-receptors in atherosclerotic coronary arteries, which under physiological conditions mediate coronary artey vasodilation. Consequently, α_2-agonists bind to the α_2-receptors located on the smooth muscle cells, facilitating coronary artery vasoconstriction. Therefore, the ultimate effect of α2-receptor stimulation depends on the net result of both the endothelium- and smooth muscle cell-located receptors and the functional integrity of the endothelium (58, 62-64).

β-receptors. The β-receptors are predominantly present on the coronary endothelium, causing vasodilation during sympathetic activation. There are 3 subtypes (β_1, β_2, β_3), located on either the endothelium or the vascular smooth muscle cells (**Figure 2**), but the β_2-receptor being the most

abundantly present and most frequently studied. In healthy participants, infusion of a β_2-receptor agonist (salbutamol) induces coronary vasodilation, suggesting a role for β_2-receptors to lower resistance. Interestingly, administration of propranolol (i.e. a non-selective β-receptor blockader), partly inhibited the coronary artery vasodilator responses to sympathetic stimulation (51). Since β_2-receptors are more strongly antagonised by propranolol than β_1-receptors, the β_1-receptor activation contributed to the remaining dilation in healthy individuals (49). In individuals with coronary artery disease, sympathetic stimulation leads to a paradoxical vasoconstriction during sympathetic stimulation. Possibly, this vasomotor response results from impaired β-receptor activation. To support this idea, intracoronary infusion of a β_2-receptor agonists (i.e. salbutamol) resulted in impaired vasodilator responses in atherosclerotic coronary arteries (51). At least, these data support a role for β-receptors to contribute to coronary artery vasodilator responses.

The majority of the work in understanding the role of adrenoreceptors in mediating central artery responses were performed in coronary arteries. Recently, Van Mil and colleagues examined common carotid (duplex ultrasound) and left anterior descending coronary artery (Doppler ultrasound) responses to sympathetic stimulation (using the cold pressor test and lower body negative pressure), both with and without α_1-receptor blockade (65). They found carotid and coronary artery dilation during the cold pressor test, with α_1-receptor blockade leading to an attenuated dilator response in both arteries. In contrast, carotid and coronary arteries demonstrated constriction in response to lower body negative pressure, which was not affected by α_1-receptor blockade. Although further work is required, these data indicate similarity between carotid and coronary responses to sympathetic tests (with the direction of these responses being dependent on

the type of sympathetic stimulation), but also similarity in the involvement of α_1-receptors mediating these responses.

What is the role of the endothelium to regulate vascular tone in central arteries?

Changes in myocardial microvascular resistance will subsequently lead to changes in upstream blood flow. For example, an increase in local myocardial metabolism will lower resistance, leading to an increase in blood flow in the supplying coronary artery. This elevation in shear stress will further augment myocardial perfusion by endothelium-dependent factors; higher flow velocities exert greater shear-stress upon the endothelium with stimulation of the endothelial nitric oxide synthase (eNOS) and release of the smooth muscle nitric oxide (NO). In addition, the marked elevation in shear stress in coronary arteries also leads to the endothelium-dependent release of prostacyclin, bradykinin and angiotensin II. The release of these vasoactive substances contribute to a further localized dilator response (66). The ability to regulate vascular tone through the local release of vasoactive substances is highly dependent on an intact and healthy endothelium. In his Nobel-prize winning experiments, Furchgott demonstrated in isolated preparations of (central) blood vessels the importance of the endothelium (67). More specifically, he revealed that relaxation of arteries in response to acetylcholine was reversed to a constrictor response upon removal of the endothelium. Similar findings have been reported by various other laboratories, including Berdeaux et al. who found that the dose-dependent dilation of dog coronary arteries during exercise is reversed to marked vasoconstriction upon removal of the endothelium (68).

The integrity of the (coronary) endothelium also seems important in mediating the vasomotor responses in response to sympathetic stimuli. A previous study examined the response to cold pressor test (CPT) using quantitative angiography and Doppler flow velocity measurements in patients with angiographically normal coronary arteries, patients with mild coronary atherosclerosis and those with advanced coronary stenosis (51). Interestingly, a marked vasodilation was found in normal coronary arteries, whilst paradoxical vasoconstrictor responses were found in the clinical groups with visible presence of atherosclerosis. Zeiher and colleagues confirmed these findings (52), but also reported that the dilation of normal (healthy) and the constriction of atherosclerotic coronary arteries with cold pressor testing exactly mirrored the response to the endothelium-dependent dilator acetylcholine. This finding demonstrates that coronary vasomotion of large epicardial arteries in response to sympathetic stimulation by the cold pressor test in humans is intimately related to the integrity of endothelial function (52). A final study explored, more directly, the coronary artery responses to adrenergic stimulation and the role of the endothelium (53). In this study, it was found that vasomotor responses to intracoronary acetylcholine mirrored the responses to phenylephrine (i.e. adrenergic stimulation) in both coronary arteries with (i.e. constriction) and without (i.e. dilation) atherosclerosis. These results further suggest the importance of (an intact) endothelium to mediate vasomotor responses to adrenergic stimulation.

CAROTID ARTERY FUNCTION TO PREDICT CARDIOVASCULAR EVENTS

In contrast to the wealth of studies examining carotid artery structure (e.g. intima media thickness), relatively few studies have examined the functional characteristics carotid artery. Several

(pre)clinical studies have examined arterial compliance as a non-invasive test of central artery function which involves assessing the carotid artery (66, 69). Although such measures may posses independent prognostic value for future CVD (66), these 'functional' measures depend on structural characteristics of the arterial wall. Assessment of carotid artery vascular function is importantly limited by practical concerns and limitations. For example, pharmacological substances are commonly applied in coronary arteries to directly examine vascular function, but is contra-indicated for the carotid artery. Alternatively, studies have also examined coronary artery responses to physiological stimuli, such as exercise or sympathetic stimulation using the cold pressor test. The cold pressor test may be relevant in the carotid artery given its simplicity, reproducibility, frequent use, and since it represents a valid test to stimulate the sympathetic nervous system.

How do carotid and coronary arteries respond to sympathetic stimulation?

Sympathetic stimulation leads to a dilator response of the coronary arteries, whilst this is attenuated or even reversed to constriction in individuals with CVD. Rubenfire and colleagues were the first to describe similar vasomotor responses in the carotid artery (70), which was referred to as carotid artery reactivity (CAR). For this purpose, CAR was measured using non-invasive ultrasound to assess carotid artery diameter changes in response to the cold pressor test (i.e. submersion of a limb in 4°C water (71). They reported that carotid dilation was present in individuals with no previous history of cardiovascular disease or significant clinical risk factors for cardiovascular disease, whilst this response was attenuated in high-risk group and vasoconstriction occurred in the group of individuals with coronary artery disease. The carotid artery response to the

endothelium-independent vasodilator glyceryl trinitrate was comparable across groups, suggesting that the distinct vasomotor responses reflect endothelial function. Following the mechanisms of central artery vasomotor function (see above), the vasoconstrictor effects of local noradrenaline release on α-receptors on the smooth muscle cells are overruled by the dilator effects of circulating catecholamines via adrenoreceptors on the endothelium. In indiviudals with (increased) CVD risk, these opposing dilator effects are less effective or even absent, subsequently leading to an attenuated dilation or even constriction.

Based on the between-study observation that sympathetic stimulation can lead to comparable responses in carotid and coronary arteries, Van Mil *et al.* explored this concept in more detail. Ultrasound was used to measure resting and peak carotid artery diameters during the cold pressor test, whilst transthoracic Doppler was used to examine left anterior descending (LAD) artery velocity in 33 healthy individuals (71). A moderate correlation was found between changes in carotid diameter and velocity *versus* LAD velocity (r=0.486 and 0.402, respectively). This correlation between carotid and coronary artery responses to the sympathetic stimulation was recently confirmed in another study by the same authors (r=0.66), where carotid and coronary artery responses were explored during various sympathetic stimuli (i.e. cold pressor test, lower body negative pressure) (65). These data indicate similarities between carotid and coronary responses to sympathetic tests and suggest that CAR may represent a surrogate marker of coronary artery vascular function.

Is carotid artery reactivity to sympathetic stimulation related to carotid structure?

The question whether CAR-response is related to carotid artery structural characteristics is relevant because previous work has found strong correlations between peripheral artery structural and functional characteristics. For example, we previously found that a larger wall-to-lumen ratio is related to exaggerated responses to vasodilator stimuli, including shear stress and glyceryl trinitrate (72). In addition, several previous studies have found a strong relationship between peripheral artery diameter and the magnitude of the vasomotor response (73, 74). Independent of the magnitude of the shear rate response, a smaller diameter was related to a larger dilator response of the flow-mediated dilation (75-77). The presence of such a relationship is important to understand the mechanisms contributing to the dilator response, but also to statistically correct for such relations using allometric scaling (78). For this purpose, previous studies have explored the possible relationship between between carotid artery wall thickness or diameter and CAR, but found no evidence of correlation (66, 70, 79). This observation suggests that CAR, a measure of carotid artery function, possesses distinct information from measures of carotid artery structure (i.e. wall thickness and diameter) and allometric scaling is not required for the analysis of the CAR.

Is carotid artery reactivity related to older age?

As vessels age, the stiffer collagen fibrils increasingly dominate the tunica media within arteries thereby reducing rebound capacity of elastic arteries, which impairs the ability to dampen the blood pressure fluctuations (80). There is convincing evidence for the presence of an age-related increase in carotid wall thickness, whilst similar age-related increases in wall thickness are found in coronary arteries (81). Since carotid artery reactivity provides information distinct from structural measures, one may question the impact of older age on carotid artery reactivity. In a previous

study, it was reported that older age is associated with an impaired carotid artery reactivity (70), although future studies are warranted to better understand this relation and the role of (age-related) factors. These observations in the carotid artery fit with other studies who examined coronary artery function, which found a lower (endothelium-dependent) response in coronary arteries of older participants (82, 83). The age-related attenuation in artery health may be due to impaired NO-pathway (83), but could also relate to dysfunction of vasoconstrictor pathway (84, 85).

Is carotid artery reactivity to sympathetic stimulation related to CVD risk?

Some previous studies have also explored the relation between CAR and traditional CVD risk factors. Rubenfire and coworkers examined CAR in 93 men and women at average risk, high risk and with coronary artery disease (70). They found that the marked dilator response in individuals with average risk was markedly attenuated in those with high risk and even reversed to vasoconstriction in individuals with coronary artery disease. Importantly, traditional cardiovascular risk factors (e.g. systolic pressure, triglycerides and high-dendity lipoprotein) correlated well with the CAR response (70). Similarly, we recently found a relation between traditional CVD risk factors and CAR . More specifically, we found that an increasing number of CVD risk factors were related to a progressively attenuated CAR response (79). These studies suggest that CAR is strongly related to (the number of) CVD risk factors.

Can carotid artery reactivity to sympathetic stimulation predict future CVD events?

Recently, we were the first to explore the potential clinical value of CAR. In a group of 172 patients with peripheral arterial disease the CAR was assessed, with a 1-year follow-up of occurrence of

cardiac and cerebrovascular events, mortality and clinical progression to angioplasty or loss of patency. Based on the CAR, individuals were dichotomized into carotid constriction (n=82) or dilation (n=90). After correction for CVD risk factors (including carotid artery wall thickness), individuals with constriction had more cardiovascular events, with a 4.1-fold increased risk for future cardiovascular events and a 2.0-fold increased risk for clinical deterioration (**Figure 3**). Since this work represents the first study in the literature, future studies are warranted to better understand the potential clinical value of CAR, including in other patient groups as well as the ability to correctly reclassify individuals at increased risk for future events. A key consideration is that whilst there are both structural and function similarities in the carotid and coronary arteries, there are differences in the shear stress experienced by both vessels. As the heart is a dynamic beating organ, this greatly affects shear stress on the walls of the coronary arteries. Although the carotid arteries does experience shear stress, this is different than that of the coronary arteries.

Can exercise training affect carotid artery reactivity?

Few studies have examined the impact of exercise training, as an established and effective lifestyle intervention, on central artery function. Hambrecht *et al.* were one of the first to directly assess the impact of exercise training on coronary arteries, and found 4-week exercise training to improve coronary endothelial function in 19 participants with asymptomatic coronary artery atherosclerosis (86). Specifically, they found that coronary artery vasoconstriction in response ~~to~~ to acetylcholine was attenuated after exercise training (86). Similarly, in a recent study, our group found improved carotid artery reactivity after 12-weeks exercise training in a group of participants with increased CVD risk. More specifically, exercise training reversed carotid artery constriction during the

reactivity test. These observations may, at least partly, contribute to the (long-term) cardioprotective effects of regular exercise training in patients with symptomatic coronary artery disease (87-89).

CONCLUSION AND FUTURE DIRECTIONS

Predicting future development and occurrence of CVD events remains a central topic in the area of Cardiology, especially if such procedures impact (personalised) treatment and decision-making in individuals with (risk for) CVD. This review specifically focused on examining carotid artery vasomotor function, based on the assumption that the central carotid artery shares several functional and structural characteristics with coronary arteries. Indeed, both arteries show some similarity in anatomy, in that both arteries show a relatively high content of elastic fibres and are prone to develop atherosclerotic plaques. Similarities were also observed between coronary and carotid artery vasomotor function, with both arteries being highly responsive to sympathetic stimulation, leading to marked vasodilation in healthy individuals and paradoxical vasoconstriction in those with disease.

Using this latter observation, studies have explored the potency of carotid artery reactivity (CAR) to sympathetic stimulation. This measure shows good relation with coronary responses to sympathetic stimulation in healthy individuals and also to CVD risk factors. Moreover, recent work revealed the potential predictive capacity of the CAR for future CVD events in individuals with peripheral arterial disease. At least, the current work on this novel technique suggests that

CAR has potential in pre-clinical and clinical work to better understand the development of CVD. Future studies are warranted to further explore these observations, especially related to the potential predictive capacity of this reactivity test for future CVD events in symptomatic and asymptomatic populations. In addition, this review highlights that distinct arteries (central *versus* peripheral) may respond differently to CVD risk factors (including older age) and/or lifestyle interventions such as exercise training. This can be taken into consideration by examining both peripheral and central arteries in (clinical) studies, especially when trying to better understand the development and prevention of CVD in humans *in vivo*.

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FIGURE LEGENDS

Figure 1. Comparison of anatomical characteristics between eleastic (e.g. coronary and carotid) and muscular (e.g. peripheral) arteries. Elastic arteries have a thin tunica adventitia and a large tunica media, containing a large amount of eleastic and collagen fibers. Under physiological conditions, there are relatively few vascular smooth muscle cells within the tunica media of the elastic arteries. Muscular arteries have a larger tunica adventitia with a smaller tunica media with few elastic and collagen fibers, but a large vascular smooth muscle cell layer.

Figure 2. Balanse in adrenergic receeptors on the endothelium and vascular smooth muscle cells of central arteries. During sympathetic stimulation, healthy endothelium will mediate vasodilation through the dilator effects of the endothelium-bound β_1-, β_2-, β_3- and α_2-receptors (green boxes), but also the β_1- and β_2-receptors on the smooth muscle cells (yellow boxes). These dilator effects oppose the constrictor effects of the α_1- and α_2-receptors on the smooth muscle cells. With progression of atherosclerosis, loss of endothelium-bound adrenoreceptors contribute to a reversal towards vasoconstriction during sympathetic stimulation (Derived from (49)).

Figure 3. Kaplan-Meier **s**urvival curves for adverse events (A), CV events (B), clinical progression (C) and all-cause mortality (D) in PAD patients (n=172) across a 1-year follow-up. We have dichotomised PAD patients in those who demonstrate coronary

constriction (CAR constriction, dotted line) or dilation during the CPT (CAR dilation, solid line). P-values relates to a Log-rank test (Derived from Van Mil *et al*. (90)).

Figure 1.

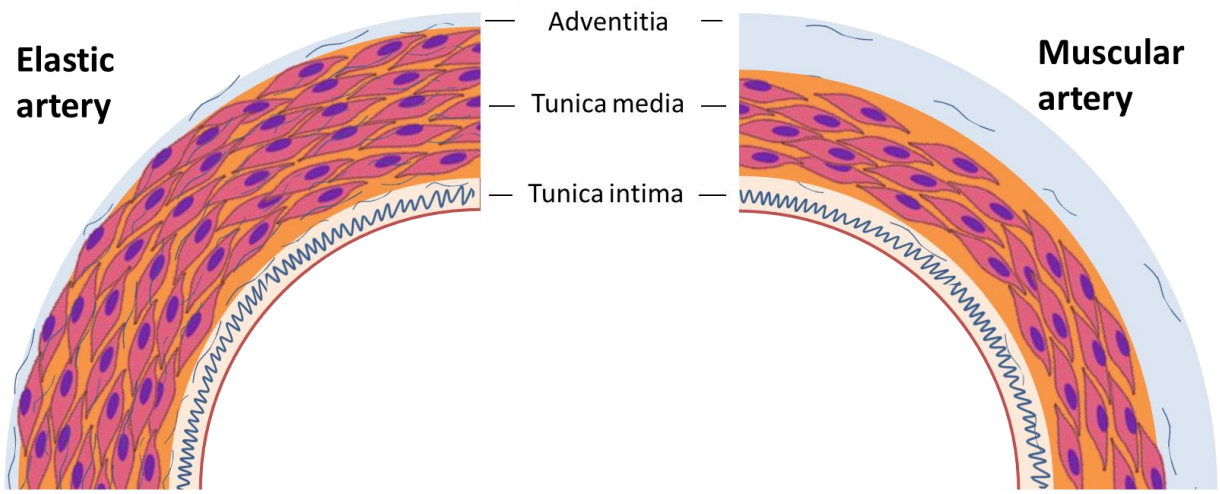

Elastic artery
Adventitia
Tunica media
Tunica intima
Muscular artery

Figure 2.

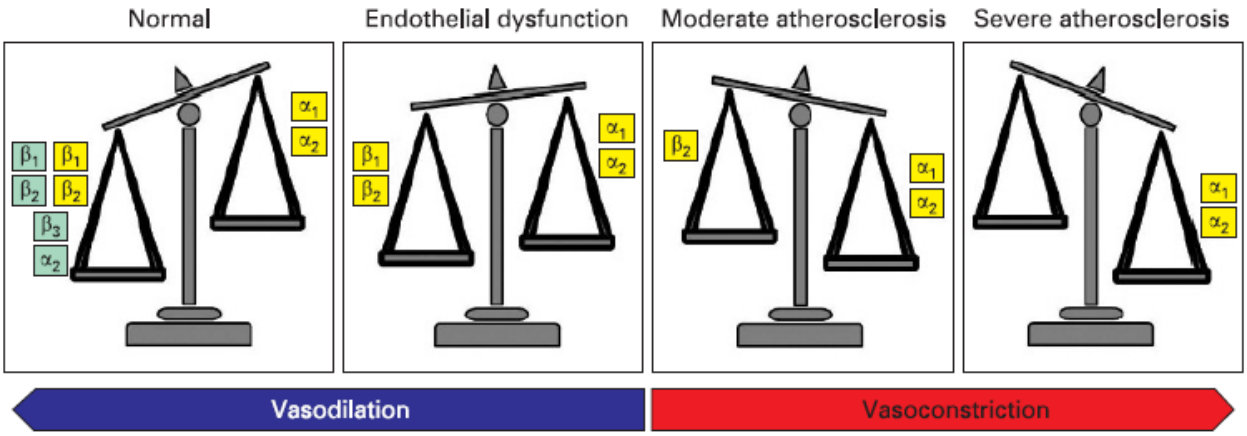
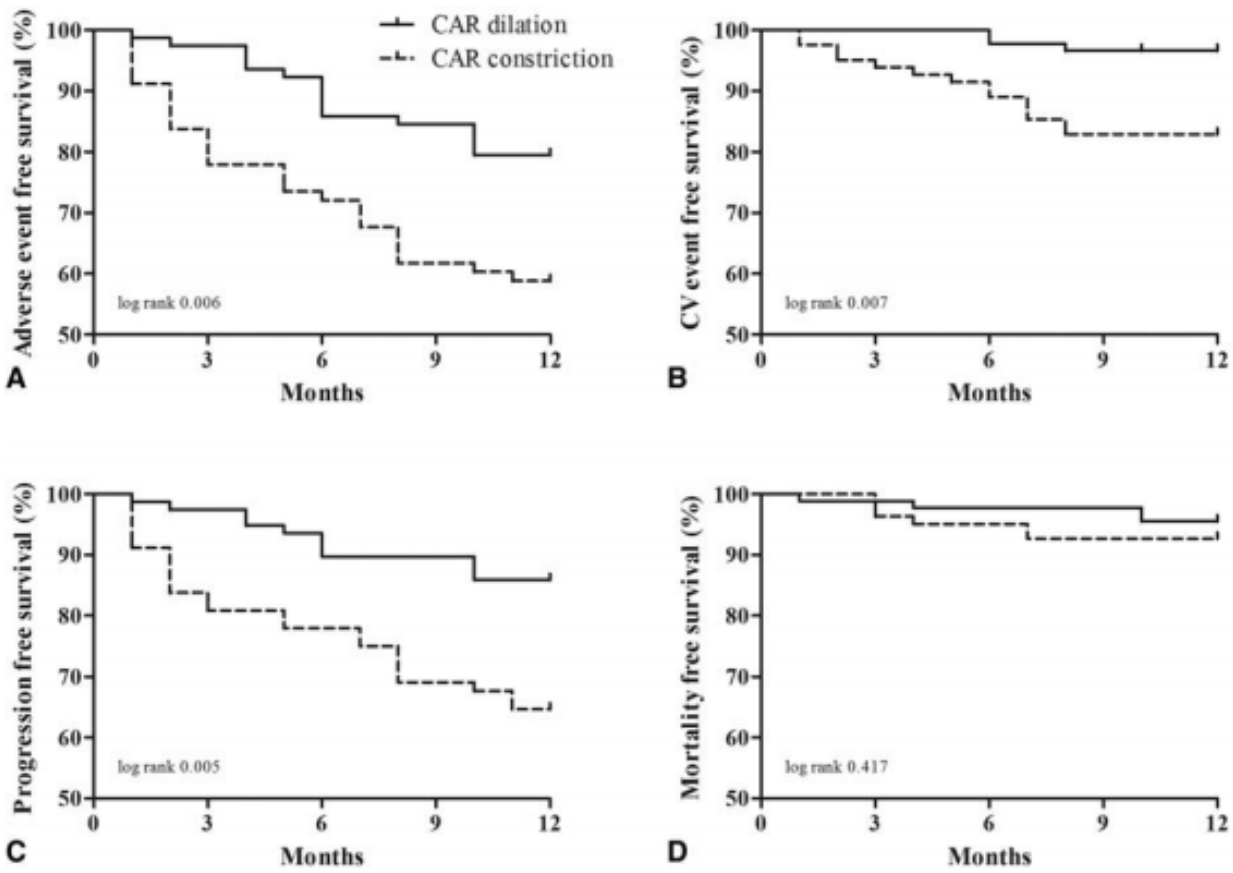

Normal
Endothelial dysfunction
Moderate atherosclerosis
Severe atherosclerosis
β1 β1 β2 β2 β3 α2
α1 α2
β1 β2
α1 α2
β2
α1 α2
α1 α2
Vasodilation
Vasoconstriction

Figure 3.


CAR dilation
CAR constriction
Adverse event free survival (%)
log rank 0.006
Months
A
CV event free survival (%)
log rank 0.007
Months
B
Progression free survival (%)
log rank 0.005
Months
C
Mortality free survival (%)
log rank 0.417
Months
D