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Working under pressure: coronary arteries and the endothelin system

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Abstract

Endogenous endothelin-1-dependent (ET-1) tone in coronary arteries depends on the balance between ET_A and ET_B receptor-mediated effects and on parameters such as receptor distribution and endothelial integrity. Numerous studies highlight the striking functional interactions that exist between nitric oxide (NO) and ET-1 in the regulation of vascular tone. Many of the cardiovascular complications associated with cardiovascular risk factors and aging are initially attributable, at least in part, to endothelial dysfunction characterized by a dysregulation between NO and ET-1. The contribution of the imbalance between smooth muscle ET_{A/B} and endothelial ET_B receptors to this process is poorly understood. An increased contribution of ET-1 that is associated with a proportional decrease in that of NO accompanies the development of coronary endothelial dysfunction, coronary vasospasm, and atherosclerosis. These data form the basis for the rationale of testing therapeutic approaches counteracting ET-1-induced cardiovascular dysfunction. It remains to be determined whether the beneficial role of endothelial ET_B receptors declines with age and risk factors for cardiovascular diseases, revealing smooth muscle ET_B receptors with procontracting and proinflammatory activities.

Keywords

vascular tone; inflammation; shear stress; coronary blood flow

Endothelin-1 (ET-1) is one of the most potent vasoconstrictors identified so far (74). This peptide induces a long-lasting contraction of isolated canine and simian coronary arteries with a half-maximal effective concentration ($-\log[EC_{50}]$) of 7.96 ± 0.23 and 8.24 ± 0.09 , respectively, which is at least one order of magnitude lower than values reported for other contracting peptides such as ANG II, with the exception of urotensin II [$-\log[EC_{50}] \pm 9.46 \pm 0.11$ and 9.56 ± 0.05 , in canine and simian left circumflex coronary artery, respectively; (30)]. In fact, ET-1 elicits its effects through two receptors: ET_A receptors, which are located in vascular smooth muscle cells (VSMC), mediate constriction, whereas ET_B receptors, located on vascular endothelial cells (EC), mediate dilation (5, 9, 15, 17, 56, 59). Additionally, ET_B receptors can also be expressed on VSMC and elicit constrictions (65). It

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is a known fact that ET-1 is released continuously, mostly from EC by a constitutive pathway, and contributes to the regulation of the vascular tone, in general (15, 17, 56). Nitric oxide (NO), however, strongly inhibits the release of ET-1 from the native endothelium (12, 50); for this reason, it has been suggested that NO and ET-1 regulate each other through an autocrine feedback loop (50). In addition to EC, ET-1 is also produced by VSMC, cardiomyocytes, leukocytes, macrophages, various neurons, and other cells (40). This peptide is also proinflammatory and promotes VSMC proliferation (4, 23, 28, 38, 39, 57). Thus, ET-1 contributes to the cardiovascular homeostasis by regulating basal vascular tone and remodeling (15, 40). We believe that coronary vessels, because of the ET-1/NO interdependence and because of their unique hemodynamic features (see below), are very susceptible to higher circulating and locally produced ET-1. Indeed, the coronary endothelium is prone to dysfunction and highly sensitive to damage which, with time, accumulates faster in the coronary than in other vascular beds. In addition, coronary endothelial dysfunction is associated with a decline in the contribution of NO in favor of a growing influence of ET-1. Is the increased influence of ET-1 with time in the coronary bed only secondary to the loss of NO, or is it due to a change in the ratio of ET_A and ET_B receptors expression (Fig. 1)? In any case, these questions support the proinflammatory and procontracting role of ET-1 via its predominant activation on smooth muscle receptors (33, 52, 63). This remains to be demonstrated, but it could become the basis for the use of ET receptor antagonists to treat cardiovascular arterial disease (CAD). In this review, data from experimental studies and clinical observations in patients are presented, implicating ET-1 in the physiological and pathophysiological control of the coronary circulation. We propose that the concomitant rise in ET-1, by counteracting the beneficial regulatory impact of NO, is deleterious to the coronary bed, further magnifying the damages associated with the normal mechanical constraints of the cardiac cycle.

What Pressure Does ET-1 Impose on the Coronary Arterial Bed?

Despite the accumulation of data demonstrating that ET-1 is a powerful vasoconstrictor, there is evidence that, in physiological conditions, the activation of endothelial ET_B receptors by ET-1 leads to dilation.

ET-1 exerts a dilatory tone in the coronaries in physiological conditions

In healthy animals, in which low levels of blood ET-1 are measured, intracoronary injections of low doses of ET-1 induce a decrease in vascular resistance: in anesthetized dogs, for example, an intracoronary bolus injection of sarafotoxin 6c (S6c), a selective ET_B receptor agonist, induces a decrease in coronary resistance for doses lower than 1 µg (65). Similarly, the injection of ET-1 in isolated rat hearts leads to a drop in coronary perfusion pressure at low concentrations of ET-1 (15). In coronary arterial rings isolated from young and healthy pigs, the activation of endothelial ET_B receptors induces a significant relaxation (19) through the release of NO and prostacyclin (17, 56). In addition, we know that in the human forearm circulation, the increase in blood flow induced by ET_A receptor blockade is blunted by ET_B receptor antagonism and NOS inhibition (70). Nonetheless, the dilatory role of ET_B receptors is not significant in isolated human coronary arterial rings (54). However, there is a limitation in these studies: most in vitro studies are performed in human coronary vessels

isolated from explanted hearts of patients undergoing cardiac transplantation for ischemic heart disease, vessels in which the expression of endothelial ET_B receptors is limited, except in the neovascularization of the atherosclerotic plaque (6), and with a pronounced endothelial dysfunction (66). Nonetheless, data obtained in coronary arteries isolated from the explanted hearts of patients with idiopathic heart disease suggest that the activation of endothelial ET_B receptors by endogenous ET-1 counteracts the contractile effect of smooth muscle ET_A receptors (67), an effect lost in coronary arteries isolated from ischemic hearts (66).

One final argument supporting the dilatory effect of ET-1 on the coronary arteries is that the basal production of ET-1 is five times greater toward the lumen than in the interstitial space (14), which would favor ET_B receptor stimulation on the endothelium, although it is quickly washed out by coronary flow. In addition, data from the latter study demonstrated that the concentration of free ET-1 in the cardiac interstitial fluid never goes higher than 1 pg/ml (0.4 pM) in healthy animals, which is below the coronary constricting tone. Similar conclusions were reached using ET-1 receptor blockade (13), supporting a vasodilatory tone associated with the stimulation of the endothelial ET_B auto-receptors in the heart, in physiological conditions.

One should also not underestimate the importance of the concentration of ET-1 in determining the dilatory vs. constricting coronary response, because of the heterogeneous distribution of ET-1 receptors. Saturation experiments using iodinated ligands, competition experiments, and reactivity studies using ET-1 receptor antagonists and autoradiography revealed that the expression of ET_A receptors is dominant in the coronaries compared with that of ET_B receptors (7, 54). The overall effects of ET-1 on vascular tone in vivo are the clear result of the balance between the contraction mediated by VSMC ET_A and ET_B receptors and the dilation mediated by endothelial ET_B receptors (17, 68). As mentioned earlier, systemic exogenous administration of ET-1 induces a biphasic response composed of an initial reduction in blood pressure (ET_B receptor-mediated) followed by a sustained hypertensive phase (ET_A receptor-mediated) (20). It should be noted as well that activation of smooth muscle ET_B receptors triggers contractions. In anesthetized dogs, S6c induces constrictions following intracoronary bolus injections greater than 1 µg (65). In isolated coronary arteries from explanted human hearts, S6c induces contractions in some but not all the donors studied (54). The heterogeneity in the contraction to ET_B receptor stimulation was related to the diameter: this response was low when present in coronary arteries of less than 400 µm of diameter, suggesting little smooth muscle ET_B receptor expression.

The Coronary Bed Is Prone to ET-1-Dependent Contraction with Age and in Pathological Conditions

The first argument in favor of a contractile response to ET-1 in the coronaries resides in the regulation of its production at the gene level. In cultured cells, it has been shown that ET-1 mRNA is upregulated by inflammatory factors such as transforming growth factor-β (TGF-β), TNF-α, interleukins, insulin, and ANG II, and downregulated by NO, PGI₂, and shear stress (12, 16, 44, 45, 51, 55). When considering these regulatory mechanisms within the

coronary circulation, shear stress is a key element and NO is the key effector. Shear stress represents the tangential force per unit area created by the flow of blood over the luminal surface of the endothelium. The vascular endothelium-dependent dilation to shear stress enables a fine-tuning of nutrient delivery to accommodate metabolic demand (48, 69). Importantly, it is the stability of shear stress, rather than its level, that contributes to the maintenance of a healthy endothelium (69) and thus to a large extent to a healthy vasculature. In contrast to other vascular beds, wall shear stress in coronary arteries is uneven. Blood flow varies throughout the cardiac cycle (37): blood flows in the coronary arteries during diastole and is at its lowest level during systole as the contracting myocardium squeezes the subendocardial coronary arteries. Mechanical stress is, therefore, greatest in the coronary circulation (69). In turn, it is not surprising that the coronary circulation is the prime site for endothelial dysfunction. It has been reported that because of these unique physiological hemodynamic features, coronary arteries display an unusual gene pattern compared with the aorta: a five-fold lower eNOS and a 2.5-fold higher ET-1 mRNA expression (22). Such a pattern predisposes coronary arteries to endothelial dysfunction and atherosclerosis. Therefore, on the basis of only its physiological characteristics, the coronary circulation should be prone to an increased influence of ET-1 with age: the accumulation of age-related damages would favor ET-1 expression in contrast to that of eNOS and exacerbate endothelial dysfunction (Fig. 1).

The second argument supporting an ET-1-dependent pro-constricting tone arises from the demonstration that patients with atherosclerosis have elevated plasma levels of ET-1, and an upregulation of ET-1 and its receptors has been described in atherosclerotic arteries and plaques (9, 21, 31, 47). Big ET-1 and ET-1 immunoreactivity has been found in atherosclerotic regions (24, 36). These observations have led to the hypothesis that ET-1 may be associated with the pathogenesis of atherosclerosis (25, 39).

In 1998, an important preclinical study (8) demonstrated that chronic ET_A receptor inhibition improved aortic endothelial dysfunction and reduced the development of atherosclerosis in ApoE knockout mice. Several studies have subsequently demonstrated the beneficial effects of acute intracoronary infusion of the ET_A receptor antagonist BQ123 on coronary diameter and coronary flow in patients with CAD. In healthy and CAD patients of less than 60 years of age, infusion of BQ123 (100 nmol/min, for 60 min) increased similarly coronary diameter and coronary flow (46). When narrowing the analysis of the dilatory effects of BQ123 to angiographically normal vessels, vessels with plaques and at stenosis, a higher dilation was observed after intracoronary infusion of BQ123 (40 nmol/min for 60 min) in patients with CAD (42); in this study, compared with the dilation to nitroglycerin, ET-1 contributed to 39% of coronary vasomotor tone in healthy and angiographically clean vessels, 74% of tone in CAD arteries, and 106% of tone at stenosis. The contribution of NO was, however, not determined: one would assume that the more severe the disease condition, the less NO would be produced, and the more ET-1 would contribute to tone. This hypothesis was tested in 44 patients with CAD in a study published that same year: Halcox et al. (34) provided the first evidence that ET-1, via ET_A receptors, contributed to the reduction of endothelial dilatory function. The intracoronary infusion of BQ123 (200 nmol/min, for 60 min) in patients with atherosclerosis resulted in coronary artery dilation and an improvement of ACh-induced endothelium-dependent dilation. The greatest improvement

associated with the intracoronary infusion of the ET_A receptor antagonist was observed in patients with the greatest endothelial dysfunction (34), suggesting that ET-1 contributes to the acute inactivation of NO. In porcine coronary arteries subjected to ischemia reperfusion injury, another model of endothelial dysfunction, the vasoreactivity to exogenous ET-1 was increased and associated with a reduction in endothelial ET_B receptor-mediated dilation and an increase in vascular smooth muscle ET_B receptor-dependent contraction (19). Böhm et al. reported that both the selective ET_A receptor (BQ123) antagonist and the combination of selective ET_A (BQ123) and ET_B receptor (BQ788) antagonists improved endothelium-dependent dilation in the coronary arteries of patients with CAD (11). Again, the dilation was higher in severely stenotic segments, demonstrating the increased importance of ET-1 in the control of vascular tone in atherosclerosis. In agreement with these data, using isolated human coronary arteries from idiopathic and atherosclerotic cardiomyopathic hearts, we demonstrated that ET-1-dependent constriction became more pronounced when the endothelial function was altered (68). Taken together, these data strongly suggest that ET-1 contributes to inactivate the acute dilatory function of the endothelium in the coronary arterial bed. Hence, the functional contribution of ET-1 appears to rise with the severity of CAD.

A third argument is the ability of ET-1, at low concentrations, to potentiate coronary contractile responses to other vasoconstrictor substances, such as norepinephrine and serotonin (32, 56). Consequently, even subthreshold concentrations of ET-1 may regulate vascular tone and reactivity. Whether this mechanism becomes predominant with age and in patients presenting with risk factors for cardiovascular diseases still needs to be demonstrated.

The potential physiological roles of ET_B receptors, in addition to acting as clearance receptors for ET-1 and stimulating NO release, remain poorly understood. This is likely due to the limited final effects of the stimulation of endothelial ET_B receptors on the in vitro vascular function, and the possible change in expression of ET-1 receptors during the development of pathologies as evidenced in pulmonary hypertension, the most studied condition associated with a dysfunction of the ET-1 system (62). A change in receptor expression is likely to change the pharmacology of the system. The consequences of receptor inhibition in young and healthy subjects or in old and diseased patients should obviously be different. Since most clinical data have been collected in an elderly population, most likely showing some degree of endothelial dysfunction, it is quite possible that our understanding of ET-1 function as a proconstrictor and proinflammatory factor is only a reflection of these data and thus may not illustrate the effects of ET-1 in young and healthy subjects. In support of this statement, the induction of an endothelial damage eliminates ET_B receptor-dependent relaxation in pig coronary arteries (19). The seminal demonstration that ACh induces a contraction of coronary arteries in patients with CAD but a dilation otherwise (49), is a good example of such a case.

Therefore, on the basis of the literature reviewed so far, one can infer that at physiological and low concentrations, ET-1 predominantly induces dilations, while at pharmacological concentrations, it induces contractions in the coronary circulation. The impact of the inevitable endothelial dysfunction when using isolated arteries from explanted human hearts

may have led researchers to underestimate the endothelial dilatory component of ET-1. The production of NO may be reduced, but an alternative explanation may be the loss of coupling between the ET_B receptor and the NO pathway without affecting the ability of NO to clear ET-1 from the circulation. For example, ACh induces a contraction of coronary vessels isolated from patients with ischemic heart disease, but substance P still produces near maximal relaxation by stimulating NO production (66). A change in the expression or coupling of the endothelial ET_B receptor cannot be excluded in an elderly population (>65 years of age) and in patients with CAD. In addition, it is possible that ET_A and ET_B receptors interact and form heterodimers in in vivo VSMC, as in rat pulmonary arteries (60, 61). We still do not know whether this pertains to the coronary circulation, what the role of the dimmers and their pharmacology is, and if the heterodimeric state varies with age and diseases (72). There are many questions with no answers.

What is the Impact of a Pathological Rise in Circulating Levels of ET-1 on Coronary Circulation?

Circulating levels of ET-1 are elevated in patients with CAD and atherosclerosis (11, 18, 34, 39, 47, 57), in patients with hypertension associated with renal failure (40) and in patients with heart failure (2, 3, 9, 17, 41). Many of the cardiovascular complications associated with aging and cardiovascular risk factors are attributable, at least in part, to endothelial dysfunction, particularly dysregulation of the vascular tone induced by an imbalance between NO and ET-1. The known reduction of NO bioavailability in association with risk factors for cardiovascular diseases and medical conditions could also be associated with a rise in ET-1. Indeed, ET-1 may subsequently feed forward by stimulating the production of damaging reactive oxygen species (29, 58, 73) and hasten the decline of endothelial function (69). In a porcine model of repetitive coronary artery endothelial injury, it has been suggested that reactive oxygen species (ROS) and ET-1 mutually stimulate the production of the other while decreasing eNOS mRNA expression (58). The inhibition of NO production by endothelial cells increases ET-1 release from the *ex vivo* porcine aorta (12), while a blockade of ET-1 receptors improves substance P-induced NO-dependent coronary dilation in patients with CAD (11). Thus, NO prevents ET-1 release, and vice versa. The rise in NO (12, 16) accounts for the decreased amount of ET-1 released by the endothelium. The mechanism underlying the inhibitory effect of ET-1 on NO release is less certain, although it has been reported that ET-1 decreases eNOS expression in human umbilical vein endothelial cells (29) and in pulmonary vascular cells (73). By increasing the production of ROS, ET-1 may inactivate NO and thus reduce its bioavailability in the coronary arteries (58). The chronic rise in ET-1 may then lead to a molecular remodeling of the endothelin system (e.g., change in the expression of receptors, enzymes, associated proteins) and could affect the cardiovascular system.

Could an acute rise in ET-1 be responsible for coronary arterial vasospasm?

There is evidence that ET-1 plasma levels are elevated in the coronary circulation of patients during angina, that ET-1 induces a long-lasting contraction in isolated coronary arteries, and that subthreshold concentrations of ET-1 potentiate the coronary constrictor effects of other vasoconstrictors, all making ET-1 an ideal candidate for the initiation and the maintenance

of coronary arterial spasm (56). It has been reported that, in acute coronary syndrome, the concentration of ET-1 in the thrombus exceeded 280 times that of ANG II, norepinephrine, and serotonin (1). Importantly, thrombus homogenates exert vasoconstrictions in isolated porcine coronary artery rings that are inhibited by the dual ET-1 receptor antagonist tezosentan (1). In a recent case report (71), a 46-year-old patient with severe coronary vasospasm and resistant to treatment was successfully cured with the dual ET_{A/B} receptor antagonist bosentan. Needless to say, the result of this case report needs confirmation; nevertheless, it does demonstrate that ET-1 is responsible, at least in part, for coronary spasms. It will be interesting to study dual vs. selective ET_A receptor antagonists in such a clinical context because of the known variability in the expression of ET-1 receptor subtypes in the coronary arteries (7, 54). The recent demonstration that ET-1 is essential for a ROS-dependent coronary spasm in pigs with coronary endothelial dysfunction (58) provides a strong rationale for testing ET receptor antagonists in spastic angina. A very recent study reports that, in a porcine model of chronic endothelial injury, the chronic administration of an ET_A receptor antagonist (TA-0201) prevented coronary artery contractions to ACh, downregulation of eNOS expression, as well as ROS generation (53), reinforcing the hypothesis of a tight relationship between ROS, ET-1, and eNOS in the regulation of coronary tone.

Clinical Inhibition of the ET-1 for Coronary Disorders

ET-1 receptor antagonists

Two classes of ET-1 receptor antagonists are currently in clinical development for the treatment of cardiovascular diseases, dual receptor antagonists, and selective ET_A receptor antagonists (10, 43). Notably, the most positive result of these developments is the licensing worldwide of ET-1 receptor antagonists for the treatment of pulmonary hypertension. The clinical studies investigating the effects of ET-1 receptor antagonists in patients with cardiovascular diseases remain, nonetheless, relatively limited. The long-term effects of ET-1 receptor antagonist treatments in CAD are not known.

Because ET-1 plays physiological roles, ET-1 receptor blockade could potentially be detrimental (25). It has been proposed that dual antagonists may have deleterious effects by preventing ET_B receptor-mediated dilation, reducing the potential benefit of ET-1 receptor inhibition in the treatment of atherosclerosis (34, 35). In a different setting, however, targeted cardiac ET-1 overexpression has been shown to be sufficient to induce the expression of inflammatory cytokines and an inflammatory cardiomyopathy, leading to heart failure and death (75). In this study, dual ET_{A/B} receptor inhibition improved cardiac function and survival in contrast to selective ET_A receptor inhibition. There is, therefore, a contribution of smooth muscle ET_B receptors in the inflammatory response of tissues to ET-1, something that should be kept in mind knowing that atherosclerosis is a chronic inflammatory disease. Furthermore, smooth muscle expression of ET_B receptors has been shown to increase in experimental hypercholesterolemia, promoting contractions of isolated porcine coronary arterial rings at low concentrations (0.1 nM) of ET-1 and S6c (36). This phenomenon contrasts with the dilatory effects of ET-1 and S6c at low concentrations in healthy coronary arteries (see above), reinforcing the hypothesis that there is indeed a

molecular remodeling of the coronary artery ET-1 system in pathological conditions. In a pathological context such as atherosclerosis, the dual inhibition of ET_{A/B} receptors should thus probably be privileged to limit smooth muscle activation of ET_A and ET_B receptors.

Endothelin-converting enzyme inhibition

ET-1 is produced by the proteolytic cleavage of big ET-1 by endothelin-converting enzymes (ECE). It has been proposed that ECE activity may modulate cardiovascular risk in patients with CAD as vascular ECE activity is inversely correlated with serum LDL levels and blood pressure (57). In addition, together with ET-1, ECE is abundantly present in human coronary arteries at different stages of atherosclerosis (38). The development of ECE inhibitors lags significantly behind the development of ET receptor antagonists. The use of ECE inhibitors has so far been limited due to their potentially severe side effects, such as angioedema (64). Furthermore, strategies have been directed toward the discovery of simultaneous inhibitors of ECE, an-giotensin-converting enzyme (ACE) and neutral endopeptidase that degrade natriuretic peptides and bradykinins, such as CGS-35601 (26), therefore increasing the vasodilatory potential of the endothelium by preventing the production of ET-1 and ANG II, as well as preserving bradykinin from degradation (27). These compounds have not entered the clinic yet, but side effects similar to those induced by ECE inhibitors may be expected.

Perspectives and Significance

Coronary vessels, due to their unique hemodynamic features, are highly sensitive to ET-1. We believe that ET-1, by counteracting the beneficial regulatory impact of NO, is deleterious to the coronary bed and magnifies the damage associated with the normal mechanical constraints of the cardiac cycle. Our understanding is that ET-1 may impose a mild dilatory tone by activating endothelial ET_B receptors in young and healthy coronary arteries. With age and the associated damages to the cardiac cycle, endothelial damage will lead to a reduction in NO and a rise in the contribution of constricting ET-1 through the activation of smooth muscle ET_A receptors, leading to a contractile tone. Whether or not this is associated with a greater involvement of smooth muscle ET_B receptors, together with an uncoupling of endothelial ET_B from the NO pathway but without affecting the ability of NO to clear ET-1, remains to be determined experimentally. This change with age would be accelerated in the presence of risk factors for CAD. Taken as a whole, the literature strongly suggests that ET-1 is associated with endothelial dysfunction and the pathogenesis of CAD. This represents the basis of the rationale for testing dual and selective ET-1 receptor antagonists.

Conclusion

The heart has the highest resting oxygen consumption rate of all organs; increased oxygen consumption must be met by an increase in coronary blood flow. Blood flow to the heart occurs during diastole, and the vascular endothelium significantly contributes to the control of the coronary vasomotor tone. ET-1 potentiates human coronary tone by activating ET_A and ET_B receptors. Coronary tone and blood flow are normally maintained by an appropriate balance between endogenous dilators and contractile stimuli. Coronary artery disease, however, is associated with increased ET-1 levels and a contribution to the coronary active tone. This augmented ET-1 pressure is likely to promote a molecular remodeling, leading to

changes in the normal expression and sensitivity of the artery to NO, among other factors. These changes may alter the normal pharmacology of the ET-1 system, as has been demonstrated in models of pulmonary hypertension (62). Intracoronary infusions of selective ET_A and dual ET_{A/B} receptor antagonists improve coronary endothelial function and increase coronary blood flow, suggesting that ET-1 blockade may be an option to improve coronary vascular function in atherosclerotic patients.

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Fig. 1. Schematization of the potential changes in the contribution of ET-1 to the regulation of the coronary arterial tone with age and clinical changes. We propose that ET-1 does not predominantly contribute to the constricting tone of the coronary circulation in young and healthy subjects, but rather to a mild dilatory tone through activation of endothelial ET_B receptors and the release of NO. With age and the associated cumulative mechanical stress-related damages induced by the cardiac cycles on the coronary circulation, endothelial damages lead to a reduction in NO and a rise in the contribution of constricting ET-1 through the activation of smooth muscle ET_A and ET_B receptors, leading, in turn, to a contractile tone. This change may be accelerated in the presence of risk factors for cardiovascular diseases.