Role of elastin peptides and elastin receptor complex in metabolic and cardiovascular diseases

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Abbreviations : AACE, American Association of Clinical Endocrinologists ; AMPK, Adenosine Monophosphate-Activated Protein Kinase ; CC, Cardiovascular Continuum ; CK, Creatin Kinase ; CVA, Cerebrovascular Accident ; EBP, Elastin Binding Protein ; ECM, Extracellular Matrix ; EDP, Elastin-Derived Peptides ; EGIR, European Group for Study of Insulin Resistance ; ELN, Elastin ; ERC, Elastin Receptor Complex ; HGFR, Hepatocyte Growth Factor Receptor ; IR, Insulin Receptor ; IRS, Insulin Receptor Substrate ; LKB-1, Liver Kinase-B1 ; LVEDP, Left Ventricular Diastolic Pressure ; MI, Myocardal Ischemia ; MMP, Matrix MetalloProteinase ; NAFLD, Non Alcoholic Fatty Liver Disease ; NASH, Non Alcoholic Steato Hepatitis ; NCEP, National Cholesterol Education Program ; Neu-1, Neuraminidase-1 ; NO, Nitric Oxyde ; oxLDL, oxidized Low Density Lipoprotein ; PTM, Post-Translational Modifications ; PPCA, Protective Protein/Cathepsin A ; RISK, Reperfusion Injury Salvage Kinase ; ROS, Reactive Oxygen Species ; RPP, Rate Pressure Product ; SVAS, SupraValvular Aortic Stenosis ; T2D, Type 2 Diabetes ; WBS, Willliams-Beuren Syndrome ; WHO, World Health Organisation

Keywords : Elastin, Elastin-Derived Peptides, Elastin Receptor Complex, Cardiovascular Continuum, Metabolic Syndrome, ECM-based pharmacology

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Abstract

The Cardiovascular Continuum describes a sequence of events from cardiovascular risk factors to end-stage heart disease. It includes conventional pathologies affecting cardiovascular functions such as hypertension, atherosclerosis or thrombosis and has initially be considered from the "classical" metabolic point of view. This Cardiovascular Continuum, originally described by Dzau and Braunwald, has been extended by O'Rourke, taking into account the crucial role played by aging through elastic fibers degradation in appearance of vascular stiffness, another deleterious risk factor of the continuum. However, the participation of the elastin degradation products, named elastin-derived peptides, to the Cardiovascular Continuum progression has not be considered before. Data from our lab and others, clearly showed that these bioactive peptides are central regulator of this continuum, thereby amplifying appearance and evolution of cardiovascular risk factors such diabetes or hypertension, vascular alterations such as atherothrombosis and calcification, but also non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. The Elastin Receptor Complex has been shown to be a crucial actor in these processes. We propose here to summarize the participation of these elastin-derived peptides and the Elastin Receptor Complex in such events, and introduce a revisited Cardiovascular Continuum based on their involvement, and for which elastin-based pharmacological strategies could have a strong future.

Introduction: the cardiovascular continuum and metabolic disorders

Nowadays, heart failure is no longer considered as a simple cardiovascular disease but as the consequence of complications involving diverse risk factors influencing a series of deleterious events. These later lead to the impairment of cardiovascular functions leading to end-stage heart disease. This concept, named Cardiovascular Continuum (CC), was proposed by two scientists, V. Dzau and E. Braunwald who have emphasized the importance of early identification and prevention of main risk factors leading to heart failure [1]. This model describes the progressive molecular and cellular damages of the cardiovascular system. They manifest as clinical diseases, where the risk factors are characterized by different metabolic disorders (dyslipidemia, obesity, hypertension, smoking, diabetes) and identified as the first events of this model. They initiate a cascade of mechanisms involving alteration of vasoactive mediators, inflammatory responses and remodelling of vascular extracellular matrix that affect different organs. All these pathological changes appear earlier in life and lead to tissue dysfunction causing the progressive emergence of cardiovascular diseases. Without treatment, these detrimental modifications trigger tissue injuries such as renal impairment, peripheral arterial insufficiency or stroke, and are associated with pathological remodelling. In the long run, an important degradation of vascular extracellular matrix is observed and participate in organs failure such as heart or kidney.

Some of these CC-associated metabolic disorders can be grouped into the term of metabolic syndrome which is associated with the appearance of cardiovascular diseases and type 2 diabetes (T2D). This syndrome combines the major health issues of this century like abdominal obesity, insulin resistance, dyslipidemia and hypertension [2]. Abdominal obesity is characterized by adipocytes hyperplasia and hypertrophy mostly due to increased consumption of high-calorie food and reduced physical activity. A link between obesity and T2Dis clearly recognized since few decades where obesity and its chronic low-grade inflammation state promotes insulin resistance, an altered response to insulin by its target cells creating a chronic hyperglycemia [3]. These consumption pattern changes are also responsible for the imbalance between proatherogenic and antiatherogenic lipoprotein levels called dyslipidemia that promote lipid accumulation and atherogenesis. Hypertension is frequently associated with the three metabolic disorders described above [4]. Interestingly, majority of these symptoms have been

associated in the past decades with pathological extracellular matrix remodelling or during aging, and especially with elastolysis [5].

Consequently, the classical model of CC has been extended considering a crucial factor, the aging of the vascular system. This has inspired another model, the Cardiovascular Continuum Extended, taking into account the Vascular Aging Continuum, proposed by M.O. O'Rourke [6]. In this model, fatigue and fracture of elastic lamellae in the proximal aorta are seen as a crucial events, and fundamentally participate to aortic stiffening, influencing deleterious fate of cerebral, kidney or heart tissues (Fig. 1).

However, this extension of the CC only considered the elastic role of elastin in large arteries and does not take into account the ones played by the elastin degradation products, named elastin-derived peptides (EDP), able as we'll see later in the manuscript, to directly modulate this continuum.

We propose here to summarize and discuss the roles played by elastin, elastolysis, EDP production and engagement of their cognate receptor, the Elastin Receptor Complex (ERC), in the regulation of cardiovascular risk factors and CC flow. Finally, based on recent results from our lab and others, we propose a re-revisited CC through EDP and ERC participation, in which ECM-based pharmacology could have a strong application.

Elastin remodelling, elastin-derived peptides and the Elastin Receptor Complex

Elastin biosynthesis

Tissue-specific cell behaviour is influenced by the biochemical and biophysical properties of the extracellular matrix (ECM). The molecules that composed the ECM, including collagens, elastin, proteoglycans, laminins and fibronectin, and the manner they are assembled establish the structure and the organization of the resultant ECM. Elastin is an essential component of numerous human tissues and plays a critical role in elasticity of skin, lungs and arteries. Several cell types synthesize and secrete elastin such as endothelial cells and fibroblasts. These cells produce elastin as a precursor, tropoelastin, which is transported outside the cells [7]. The elastin biosynthesis starts during the foetal stage and reaches a peak just before birth. The process of elastogenesis, which consists in formation of elastic fibers, decreases during early life to disappear at puberty [8]. Elastic fibers are composed of fibrillin-rich

microfibrils surrounding an insoluble and amorphous core of elastin [5]. Elastin has a half-life of about 70 years [9]and its production is low or inexistent during adulthood [8]. Furthermore, elastogenesis begins by the translation of the elastin gene (ELN gene) and after splicing, mature tropoelastin mRNA is exported out of the nucleus to be translated into tropoelastin in the rough endoplasmic reticulum (RER)[8]. The polypeptides formed consist in different tropoelastin isoforms with a molecular weight of about 70 kDa containing a N-terminal signal sequence of 26 amino acids which is cleaved when the protein is transferred in the RER lumen [10].Then, tropoelastin is associated with a chaperone, the Elastin Binding Protein (EBP) to prevent coacervation and premature degradation [11, 12]. The EBP-tropoelastin complex is secreted by the Golgi apparatus into the extracellular space and interacts with the rest of the Elastin Receptor Complex (ERC) composed of the Protective Protein/cathepsin A (PPCA) associated with the transmembrane sialidase, Neuraminidase-1 (Neu-1). The association of EBP with the rest of the complex, triggers an increase in sialidase activity of Neu-1, leading to the desialylation of microfibrils. This process unmasks galactosyl moiety of galacto-sugars that binds EBP on its galactolectin site which induces the release of tropoelastin. This tropoelastin monomer can be then aligned and incorporated into the growing elastic fiber [12] whereas EBP is recycled to interact with another tropoelastin molecule [12, 13].

Elastin remodelling and elastin-derived peptides

Matrix ageing is characterized by an increase in non-enzymatic protein post-translational modifications such as glycation or carbamylation and by an increase in proteolytic activities. Proteolysis of ECM macromolecules leads to the production of ECM-derived peptides that present new biological activities. These peptides, called matrikines, represent promising pharmaceutical targets. Elastin is a good example of such ECM macromolecules subject to proteolysis. Indeed, during ageing, elastin is submitted to remodelling and fragmentation by proteolysis and by several factors contributing to elastin fragility as mechanical fracture, calcification, glycation, carbamylation and peroxidation [5]. Elastin degradation is achieved by enzymes called elastases, which include serine-, cysteine-, and metalloproteinases. In the serine protease family, neutrophil elastase (NE1), proteinase 3 (PR3), and cathepsin G (CG), which are stored in neutrophil azurophilic granules, have been greatly documented [14], as well as four members of the cysteine cathepsin family (L,S, K and V) [13]. Among metalloproteinases, matrix metalloproteinases such as MMP-2, MMP-7, MMP-9 or MMP-12 clearly present elastolytic activity and are involved in elastic fibers remodelling [15, 16]. Interestingly, elastin degradation generates bioactive EDP named elastokines which harbor

the GxxPG motif. This motif adopts a type VIII β-turn conformation necessary for the bioactivity of these peptides. EDP have been described to possess different effects on various cell types like endothelial cells, monocytes and smooth muscle cells [15, 17]. For example, the most described elastokine is the VGVAPG peptide (encoded by exon 24 of human tropoelastin) but other bioactive motifs have been identified by Heinz et al. such as GVYPG, GFGPG and GVLPG [18]. Moreover, MMP-7, -9, and -12 are able to generate longer bioactive EDP as YTTGKLPYGYGPGG, YGARPGVGVGGIP, and PGFGAVPGA peptides [16]. Finally, a recent study characterized NE1 action on aortic elastin and identified other sequences such as GAGGFPGYGV [19].

The Elastin Receptor Complex (ERC)

After elastin fragmentation, produced EDP exert several biological effects by binding to their cell surface receptors. Several putative receptors for EDP have been identified as galectin-3 [20] and $\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrins [21, 22]. However, in the context of the present review, we will focus on the most prominent receptor of these elastokines, the ERC, known to be the most signalling mediator of EDP.

ERC is a heterotrimeric receptor composed of a peripheral 67kDa subunit called elastin-binding protein (EBP), which binds elastin-derived peptides, a 55 kDa protective protein/cathepsin A (PPCA) and a 61 kDa membrane-bound neuraminidase, Neu-1 [23]. The EBP subunit consists in an enzymatically spliced variant of lysosomal β-galactosidase [12] and presents two functional binding sites encompassing the elastin site on which EDP binding induces signalling pathways, and the galactolectin site whom occupation by galactosugars is associated with EDP delivery and dissociation of the complex [24]. Brassart et al. showed that among many EDP, only those with the GxxPG consensus sequence possess a conformation that allows binding to the EBP subunit [17, 25]. Binding of EDP to EBP induces Neu-1 activation which catalyzes the local conversion of GM3 [N-acetylneuraminic-α-(2-3)-galactosyl-β-(1-4)-glucosyl-(1-1')-ceramide] ganglioside I nto lactosylceramide (LacCer), a second messenger of ERC signalling pathways [26]. Indeed, EDP binding is able to activate several intracellular signalling pathways depending on the cell type: for instance, in fibroblasts, EDP activates the MEK1/2/ERK1/2 pathway through a signal dependent on protein kinase A and phosphoinositide 3-kinase γ (PI3Kγ) [27]. Another example concerns endothelial cells: a PI3Kγ/Akt/endothelial nitric oxide synthase/nitric oxide/protein kinase G module involved in ERK1/2 activation has been described to be activated by EDP [28]. Last, in smooth muscle

cells, cell proliferation mediated by EBP involves activation of Gi proteins in a concomitant manner with opening of calcium L-Type channels [29].

In these signalling processes, Neu-1 plays a central role and a lot of effort has been made to further elucidate its participation in ERC signalling. Beside its role in the generation of the second messenger LacCer described above, its catalytic activity has been shown to be dependent on an original dimerization process. In this way, two potential dimerization sequences, corresponding to two transmembrane domains (148-168 and 316-333 residues), have been identified within Neu-1 and point mutations in the 316-333 transmembrane domain inhibit significantly dimerization and sialidase activity of Neu-1 [30]. Moreover, a growing literature also shows that Neu-1 plays a major role in the regulation of many membrane receptors by desialylation such as the insulin receptor, c-Met, IGFR or PDGFR [31-33].

Taking together, all these data suggest that Neu-1 has to be seen as a crucial regulator of the membrane signallosome from the lipids but also from the protein point of view.

It is however important to note that even if the signalling processes triggered by EDP through the ERC could be responsible for the majority of EDP-related biological effects, other biological processes could involve receptor-independent phenomema. These points will be discussed in the following parts of the manuscript and are summarized in Fig. 2.

Elastin and elastin-derived peptides involvement in metabolic syndrome

Since the first formal definition by Reaven in 1988 [34] under the term “syndrome X”, the metabolic syndrome has not stopped evolving. Thereby, the definitions proposed by international organizations such as the World Health Organization (WHO), European Group for Study of Insulin Resistance (EGIR), National Cholesterol Education Program (NCEP) or the American Association of Clinical Endocrinologists (AACE) include a set of criteria comprising hypertrophy of abdominal adipose tissue, visceral adiposity first but also, insulin resistance, glucose tolerance disorders, dyslipidemia and high blood pressure. The combination of these different factors, on a same patient, increases T2D risk and/or cardiovascular diseases. According to the pandemic evolutions of obesity but also of Non Alcoholic Steato Hepatitis (NASH), T2D, the metabolic syndrome might be at the center of cardiovascular diseases [35].

The main factors predisposing to the onset of metabolic syndrome are obesity and insulin resistance. Insulin has a major anabolic function leading to storage of lipidic and

glucidic substrates such as glucose uptake in the muscle, glycogen synthesis in the liver and the muscle, lipid synthesized and stored in adipose tissue, inhibition of hepatic glucose production. All these effects result from the insulin binding to a specific membrane receptor, the insulin receptor, highly expressed on the surface of hepatocytes, myocytes and adipocytes. Insulin resistance requires multiple mechanisms and one of the most studied involves phosphorylation of serine/threonine residues on the receptor, and especially Insulin Receptor Substrate (IRS) proteins. This phosphorylation ends the receptor physiological activity by inhibiting the insulin signal transmission, and in particular towards PI3 kinase metabolic pathway. Several metabolic molecules or signalling ones are able to induce insulin resistance. Thus, free fatty acids and glucose, proinflammatory cytokines as TNF (Tumor Necrosis Factor)-α or IL (interleukin)-1β, secreted for example by adipose tissue, are involved in insulin resistance.

Interestingly, the glucose intolerance and hyperinsulinism, gradually developing, induce an important remodelling of extracellular matrix [36]. During adipocytes hypertrophy, the matrix is degraded by proteases to allow the increase in lipid droplet volume [37-39]. If matrix degradation is blocked by some genetic and pharmacological inhibitors of MMPs [40, 41] , the fat tissue cannot expand. Interestingly, as the MMPs, cathepsins are also highly expressed in adipose tissue of obese patients and contribute to the remodelling process through their proteolytic activity towards elastin and collagen. Alike MMPs invalidation, cathepsins K and L invalidations in mice, two described elastases, induce fat content loss [42, 43]. In obese patients, adipocyte expansion is fast and significant, and fibrosis onset is observed. Therefore, collagen and elastin overexpression, constitute a poorly controlled scar structure with inflammatory factors liberation and these fibrotic processes are also noticeable on the liver during NASH onset.

In consequence, an increasing literature show that the ECM may be a major actor for the emergence of metabolic syndrome. Moreover, modifications of proteases expressions such as neutrophil elastase (NE), MMPs or Cathepsin S are involved in deregulations of glucidic and lipidic homeostasis, leading to the appearance of insulin resistance, obesity or still to hepatic steatosis. Similarly, expression deficit of elastin or microfibrils could also contribute to metabolic syndrome progression. Accordingly, it has been demonstrated in humans, a high correlation between neutrophil elastase activity / circulating EDP and the metabolic syndrome markers, such as HOMA, HbA1c, BMI, and the histological grading of fibrosis and hepatic steatosis [33]. Moreover, the absence of elastases expression as NE, MMP12, and Cathepsin S, seems to protect mice from obesity, NASH and insulin resistance and as previously mentioned,

these elastases are responsible for elastin fragmentation into bioactive EDP on human and mice [33, 44-47]. We have shown that the resulting EDP are able to lead to a transient glycemia increase, by modulating glucose uptake in target tissues, namely the muscle, the liver, and adipose tissue. The chronic treatments with EDP, mimicking the chronic accumulation of EDP, leads to a glucose intolerance, explained by an insulin resistance, and to an obesity and NASH [31, 33]. This induced metabolic syndrome, might be explained by the ERC inhibitory effect on tyrosine kinase receptors such as insulin receptor and HGFR, through the Neu-1 dependent desialylation of their extracellular domains modulating their signalling properties such as inhibition of Akt and Foxo1 pathways as well as inhibition of the LBK-1/AMPK signalling. Interestingly, the ERC inhibitors used such as DANA (sialidase inhibitor) or Chondroitin Sulfate (EBP antagonist) can limit the exogenous and endogenous EDP effects in the model. Thereby, we have been able to show that the emerged Non Alcoholic Fatty Liver Disease (NAFLD) on db/db mice (mouse model to study metabolic syndrome) might be highly reduced by ERC inhibitors, but also by elastases inhibitors. NE activity blockade, limits the elastic fibers fragmentation and EDP production, but also glucose intolerance or lipids accumulation on the liver [48].

Elastin and elastin-derived peptides involvement in hypertension

Hypertension is a disease that is characterized by an abnormal and persistent increase in arterial pressure. It affects mostly the heart and blood vessels and constitutes a major cause of heart failure, kidney diseases and Cerebrovascular accident (CVA). 90 to 95% of hypertensive patients suffer from primary hypertension, meaning that their arterial pressure is constantly higher than normal rates, but without clear cause. The 5 to 10% remaining suffer from secondary hypertension that has an identifiable cause. Many factors provoke secondary hypertension such as renal blood flow obstruction, hypersecretion of aldosterone or adrenaline and noradrenaline. However, hypertension is a diagnostic that is frequently associated to obesity or/and T2D (in more than 60% of the cases). This T2D- associated hypertension, constitutes a risk factor for cardiovascular diseases. The macrovascular complications in arteries are twice more frequent on diabetic patients than in the non-diabetic one. The physiopathology of T2D vascular complications is complex and multifactorial, and still not very well-known. Moreover, T2D is also considered as a vascular accelerated aging model. It has been demonstrated that hyperglycemia and hyperinsulinemia [49, 50] play a key role on endothelium function, ROS production and arterial pressure. However, other factors might contribute to the arterial pressure

regulation. As mentioned, ECM remodelling has been described as an important element in the diabetes progression [31, 51, 52], so it could be a key factor in vascular complications [53, 54]. Indeed, the elastin fragmentation and function loss has a mechanical impact on the vessel, decreasing its compliance making it stiff [55]. Patients affected with supravalvular aortic stenosis (SVAS) or Willliams-Beuren syndrome (WBS) with a genetic deficiency of elastin show an arterial hypertension. This observation was also made using elastin haplo-insufficient murine models (Eln+/-), where the animals show a significant increase of arterial pressure with a mean arterial pressure 25-30 mmHg higher than their wild-type counterparts [55, 56]. On the contrary, elastogenesis induction in mice with elastic fibers alteration (aged mice, Eln $^{+/-}$, diabetic mice (non-published results)), allows vascular function restoration and limits arterial hypertension [57, 58]. As mentioned previously, elastin fragmentation and EDP production can be accelerated in case of hyperglycemia, leading consequently to arterial pressure modification [59]. Nevertheless, the literature data show that EDP tested *ex vivo* to an isolated organ are in favor of a vasodilation, suggesting a protective effect for arterial pressure [60]. Indeed, these peptides lead to an endothelial NOS activation and NO production, in favor of smooth muscle cells relaxation in rat. However this NO-dependent vasodilation induced by EDP is lost during aging, but could be restored in aged animal by a high glucose level [53]. This loss could be partially explained by a parallel free radical production. Indeed, the NO is quickly deactivated by the presence of superoxide ion, rapidly combined to form peroxynitrite, a highly oxidant molecule. Furthermore, occurring also with aging, the net NO production decreases, linked to a loss of calcium homeostasis and a NOS inhibitor increases, the dimethyl arginine. Consequently, the resulting endothelial dysfunction could also contribute to vascular stiffness due to the loss of NO-triggered participation in arterial compliance as well as structural remodelling [61]. In case of insulin resistance and associated hyperglycemia, a similar role could be attached to EDP, namely vasodilating effect that could compensate the hypertension induced by the elastic fibers loss. However, according to EDP and ERC inhibitory effect on the insulin receptor present in tissues as adipocyte or skeletal muscle, a different role could be proposed. Indeed, the endothelial cell, main vascular target for insulin resistance, possesses at its surface insulin receptors described as activators of NO pathway [62]. This leads to a vasodilation on a smaller scale than acetylcholine and muscarinic receptors. Therefore, these observations assume that activated ERC by EDP, could amplify the elastin fragmentation effect.

Elastin and elastin-derived peptides involvement in atherothrombosis

The dynamic balance between synthesis and proteolysis of extracellular matrix proteins contributes to the development of atherosclerosis. Altered amounts of elastin and improper assembly, modification of elastic fibers, and elastin fragmentation are associated with the disease. Damaged or degraded elastic fibers are generally not repaired and replaced by collagens and proteoglycans that stiffen the arterial wall. During atherosclerosis, vascular smooth muscle cells and macrophages can produce tropoelastin that however fails to cross-link into mature elastic fibers due to dysregulation of lysyl oxidase or any of the components of the microfibrillar scaffold required for correct fiber assembly [56, 63-67]. Due to its mineral scaffolding function, elastin is prone to calcification during atherosclerosis. Although elastin calcification without visible structural alterations can be observed in human carotid arteries obtained by endarterectomy [68], a correlation between elastin degradation and vascular calcification has been demonstrated in human and in several animal models and structural alteration of elastin precedes calcification of elastic fibers [68-72]. In addition, elastokines, by themselves, are capable of increasing expression of typical bone proteins in rat aortic smooth muscle cells which may contribute to vascular calcification [73]. Elastin is also subject to glycation and carbamylation [74, 75]. These non-enzymatic post-translational modifications that are exacerbated during chronic kidney diseases, may modulate elastin stiffness. Whether glycation and/or carbamylation of elastin affect its susceptibility to proteolysis and have direct impact on atherosclerosis progression remains to be further demonstrated.

Using $Eln^{+/-}$ mice bred with atherosclerosis-prone mice that have 60% of wild-type elastin levels, stable hypertension, and decreased aortic compliance, the Wagenseil's group has recently evaluated if elastin insufficiency and associated increase in arterial stiffness could be involved in atherosclerosis plaque deposition. From their studies, it was concluded that increased blood pressure and reduced aortic compliance are not direct causes of increased aortic plaque accumulation [76, 77]. Rather, and even if elastic fibers and artery stiffen during atherosclerosis, additional insults such as elastin fragmentation and presence of circulating elastokines are required to alter plaque accumulation [78]. Dysregulation of extracellular proteases by inflammatory cytokines, growth factors, oxidative stress and hypoxia produced during atherosclerosis, contributes to elastin fragmentation and production of elastokines [79]. MMP (MMP-2, -7, -9, -12) and cathepsin (K, L, S) -deficient mice bred with atherosclerosis-

prone mice show reduced elastin fragmentation and decreased atherosclerosis [80, 81]. Moreover, ApoE$^{-/-}$ mice harboring heterozygous mutation in the fibrillin-1 gene (C1039G$^{+/-}$) and exhibiting improper elastin structure and elastin fragmentation display unstable plaques and plaque rupture [14, 82]. Overall, these animal models demonstrate that preservation of elastic fibers integrity is correlated with reduced atherosclerosis progression.

Direct evidence for a role of elastokines in atherosclerosis was provided by the study of Gayral et al in which chronic administration of EDP or the VGVAPG peptide in atherosclerosis-prone mice increases atherosclerotic plaque deposition. Moreover, the use of chimeric LDLR$^{-/-}$ mice, a model with catalytically defective Neu-1 exclusively in haematopoietic cells obtained by bone marrow transplant from CathA/Neu-1-deficient mice to irradiated LDLR$^{-/-}$-recipient mice, showed interesting data. Indeed, this model, combining severe endogenous elastin fragmentation and marked reduction of Neu-1 activity, show reduced atherosclerosis deposition and decreased monocyte and lymphocyte infiltrates into the plaques. This has demonstrated, for the first time, the key role played by these elastokines, the ERC and its Neu-1 subunit in atherosclerosis progression [54]. Another evidence for a role of the elastokines and the ERC in atherosclerosis comes from Dale et al reporting that EDP and the VGVAPG peptide are capable of inducing macrophage polarization towards a pro-inflammatory M1 phenotype [83]. More recent data have also revealed that these elastokines enhance oxidized LDL uptake into macrophages through the ERC and its Neu-1 subunit (Kawecki et al, CMLS, revision pending). High expression of Neu-1 has been reported in peripheral blood mononuclear cells of patients with acute myocardial infarction (MI) and in macrophages present on the intima layer, within calcified regions and within the adventitia of the plaque region in human carotid arteries obtained by endarterectomy [34]. Enhanced Neu-1 expression induces a pro-inflammatory phenotype, triggers production and release of cytokines and chemokines in monocytes and promotes phagocytosis and cytokine expression in macrophages [34]. Whether the expression of the two other subunits of the ERC (EBP, PPCA) is also increased during atherosclerosis remains to be shown. However, this increased expression of Neu-1 in atherosclerotic lesions and in circulating monocytes of MI patients suggests that inhibition of Neu-1 might represent a promising strategy for managing atherosclerosis. Interestingly, ApoE-deficient mice that express hypomorphic levels of Neu-1 have reduced serum levels of VLDL and LDL cholesterol, decreased infiltration of inflammatory cells into lesions and reduced atherosclerosis [84].

Asymptomatic atherosclerosis may lead to acute events, mostly due to plaque rupture and secondary thrombosis. In addition to their role in elastogenesis and vascular architecture, many of the components of elastic fibers-associated microfibrils, such as Fibulin-1, MAGP1

and Emilin-2, have been shown to regulate hemostasis and thrombosis [85-87]. Direct evidence for a role of the elastokines in thrombosis came from the study of Kawecki et al. [88]. In their study, EDP and the VGVAPG peptide decrease platelet aggregation induced by several agonists, reduce platelet interactions with collagen under arterial shear conditions, and increase time for occlusive arterial thrombosis and tail bleeding in wild-type mice. These effects involve a direct action of these elastokines on circulating platelets and the ability of EDP to disrupt plasma von Willebrand factor interaction with collagen. Further works are now needed to address the role of these elastokines in thrombotic events occurring after plaque rupture and/or erosion.

Elastin and elastin-derived peptides involvement and myocardial ischemia/reperfusion injury

Myocardial ischemia (MI) originates from progressive narrowing of the atherosclerotic coronary arteries and compromises blood and oxygen supply to the heart, leading to ischemia of downstream tissues. In the context of acute occlusion following atherosclerotic plaque rupture, total interruption of blood supply to the myocardium leads to acute MI. Restoring blood flow by reperfusion strategies is therefore required to minimize the size of myocardial injury and to preserve cardiomyocyte contractility. Paradoxically, reperfusion triggers an oxidative burst, calcium overload, and mitochondrial damage that collectively induce cardiomyocyte apoptosis and necrosis, resulting in irreversible damage described as myocardial ischemia-reperfusion (I/R) injury [89].

The myocardium contains few elastic fibers and elasticity of the myocardium is mainly due to cardiomyocyte muscle bundles. Although elasticity of infarcted regions mostly depends on the ratio of muscle fibers to fibrotic tissue and the density of collagen cross-links, elastin is believed to play an important role in infarcted regions to prevent scar expansion, left ventricle enlargement and improve ejection fraction [90]. Indeed, after infarction, fibrosis occurs and the scar may expand if the ventricular wall lacks elasticity. The expression of recombinant elastin within the myocardial infarction has been shown to lead to the above mentioned improvements through the beneficial maintenance of tissue elasticity. Development of magnetic resonance imaging (MRI) approaches and MRI contrast agents such as the gadolinium-based elastin/tropoelastin-specific contrast agent (Gd-ESMA) have helped in evaluating tropoelastin/elastin remodeling in MI and post-MI scar remodeling in mouse models of MI [91, 92]. Both tropoelastin and elastin are present within the infarct scar and higher contrast-to-noise

ratio (CNR) values for Gd-ESMA, showing a clear tropoeastin/elastin presence, are correlated with improved ejection fraction of mice after MI. These points demonstrated that higher the elastin amount is observed in the scar, better the heart function is preserved.

Myocardial I/R injury is a strong inducer of ECM remodeling [93] and several MMP are temporally overexpressed during myocardial I/R injury [94]. As suggested by several studies using MMP inhibitors or MMP-deficient mice [95, 96], MMP play a central role in disease progression after I/R injury. Increased expression of elastases was reported in animal models of MI, such as MMP-2, MMP-9 and Cathepsin S [64, 65, 97, 98], and elastolysis was observed in early ischemia of porcine myocardium [99]. Whether EDP might exert cardioprotective effects during myocardial I/R injury was evaluated by A. Robinet et al. [100]. Using the Langendorff *ex vivo* model, A. Robinet et al elegantly demonstrated that EDP protects the myocardium against I/R injury in rats. Presence of EDP during reperfusion maintains left ventricular diastolic pressure to the normal levels observed in this *ex vivo* assay (< 50 mm Hg) and improves recovery of rate-pressure product and mean coronary flow during the reperfusion period. Myocardial infarct size is also significantly decreased in the presence of EDP. These cardioprotective effects of EDP were attributed to the ERC and involve EDP-mediated NO release and activation of the Reperfusion Injury Salvage Kinase (RISK) pathway. This pathway includes a group of pro-survival protein kinases and is a combination of two parallel cascades, PI3K-Akt and MEK1-ERK1/2. The RISK pathway confers powerful cardioprotection when activated specifically at the time of myocardial reperfusion and is shared by most cardioprotective therapies [66]. Cardioprotective effects of EDP were also observed in pre-conditioned hearts, when EDP were administrated prior to ischemia insult. Indeed, in these conditions, EDP still improve rate-pressure product to nearly total recovery, and such beneficial influence is also observed on left ventricular diastolic pressure, myocardial infarct size and creatine kinase release [100]. Overall, these data argue in favor of a cardioprotective effect of elastin and EDP during myocardial I/R injury. However, it is important to note that this cardioprotective effect has only been demonstrated in an e*x vivo* model that does not take into account other *in vivo* associated processes such as inflammation. Indeed, it has been clearly demonstrated for years that reperfusion of ischemic cells results in an oxygen radical burst actively participating to the injury [101]. Considering that EDP are highly active towards inflammatory cells and ROS production [5], the *in vivo* beneficial effects of elastin peptides in the context of ischemia/reperfusion remain to be demonstrated.

Conclusion

During aging and related processes, the elastin network suffers several alteration leading, among others processes, to the production of elastin-derived peptides (Fig. 2). EDP and ERC participation to the regulation of the CC is now clear and their involvements have to be further studied. Among their different biological effects, even if they could present beneficial effects, these later appeared very scarce compared to their deleterious role. They clearly affect the evolution of the CC and a revisited CC through their participation has to be considered (Fig. 3). They could be highly important as pharmacological targets but EDP role as predictive or prognostic factors has also to be definitively considered.

In conclusion, the different works summarized here strongly suggest that ECM and more precisely elastin-based pharmacological strategies could have a strong future to fight against cardiovascular and metabolic diseases.

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Author contributions

A.W., T.H., A.H., C.B., T.S., H.E.B., P.M., A.B., B.R-C and S.B. performed bibliographic search, summarized and analysed the content of the selected manuscripts. They prepared the figures and revised the edited version. L.M. revised the edited version. L.D. designed the sections and organization of the original outline. He performed bibliographic search and analyzed the content of the selected manuscripts. He edited the original draft and reviewed the final version of the manuscript.

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Figure Legends

Figure 1 : The Vascular Aging Continuum (adapted from O'Rourke [6]). This continuum presents interconnected events from cardiovascular risk factors such as diabetes or hypertension, to end-stage heart disease. It has been extended through the role played by aging that is mostly seen through the scope of elastic fibers degradation, leading arterial stiffness and its participation to the deleterious continuum.

Figure 2 : Elastin remodelling and functional consequences in metabolic and cardiovascular diseases. Elastin remodelling during aging and related processes leads to diverse elastin modifications and to EDP release. These peptides affect metabolic and cardiovascular diseases through ERC-dependent and independent effects. In green are indicated the ERC-engagement dependent effects. AMPK, Adenosine Monophopshate-activated Protein Kinase; CK, Creatin Kinase; EDP, Elastin-Derived Peptides; ERC, Elastin Receptor Complex; HGFR, Hepatocyte Growth Factor Receptor; IR, Insulin Receptor; LKB1, Liver Kinase B1; LVEDP, Left Ventricular Diastolic Pressure; PTM, Post-Translational Modifications; NO, Nitric Oxyde; oxLDL, oxidized Low Density Lipoprotein; RISK, Reperfusion Injury Salvage Kinase; ROS, Reactive Oxygen Species; RPP, Rate Pressure Product.

Figure 3 : The Vascular Aging Continuum revisited through EDP and ERC participation. Following elastolysis, EDP are produced and directly affects several processes involved in the cardiovascular continuum form risk factors to vascular pathologies. Most of their effects through the engagement of the ERC are deleterious and are highlighted in red, although some scarce beneficial roles have been documented (in green).

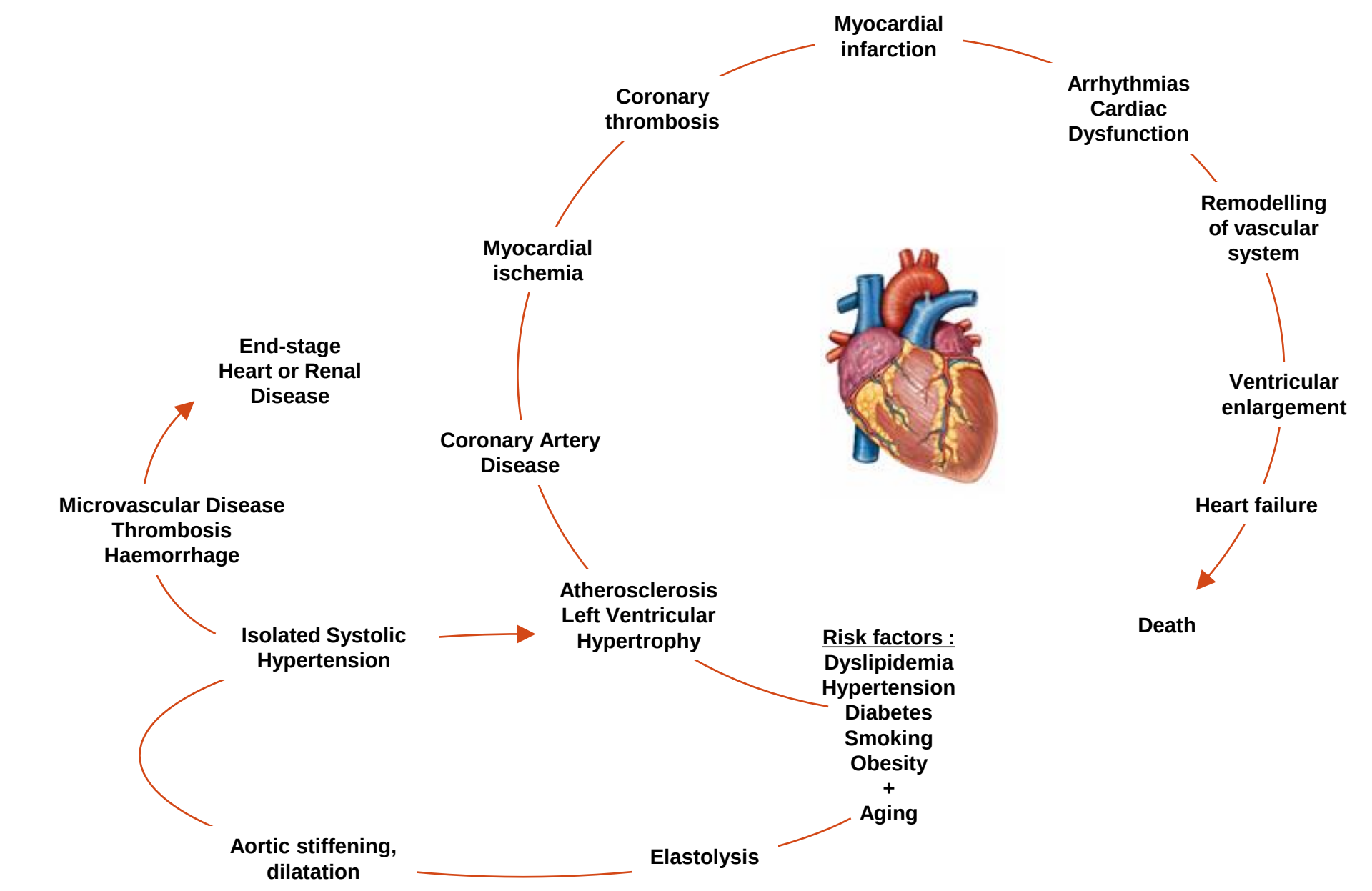
Myocardial infarction
Arrhythmias Cardiac Dysfunction
Remodelling of vascular system
Ventricular enlargement
Heart failure
Death
Coronary thrombosis
Myocardial ischemia
Coronary Artery Disease
Atherosclerosis Left Ventricular Hypertrophy
Isolated Systolic Hypertension
Microvascular Disease Thrombosis Haemorrhage
End-stage Heart or Renal Disease
Risk factors :
Dyslipidemia
Hypertension
Diabetes
Smoking
Obesity
+
Aging
Elastolysis
Aortic stiffening, dilatation

Figure 1

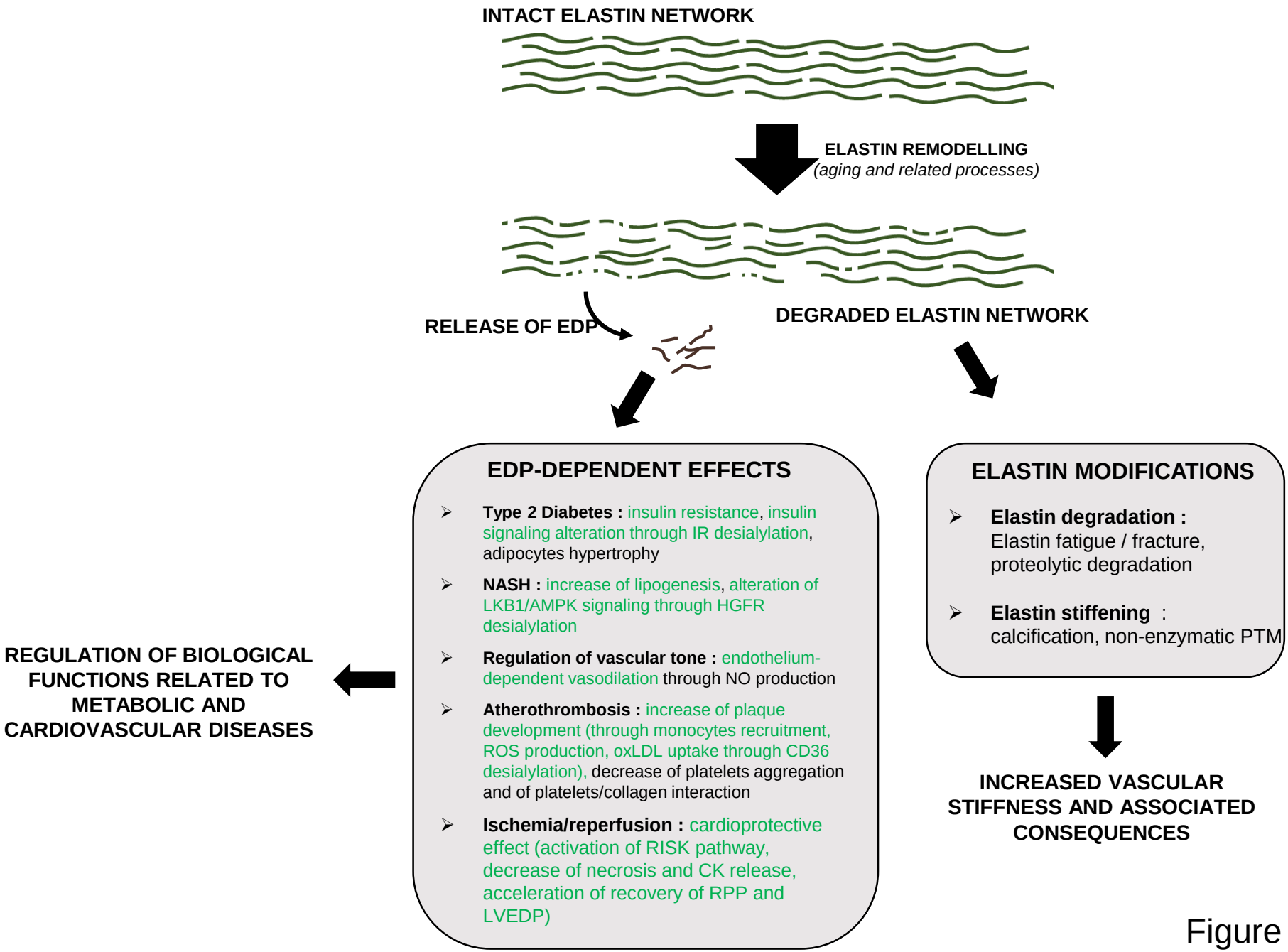
INTACT ELASTIN NETWORK
ELASTIN REMODELLING
(aging and related processes)
RELEASE OF EDP
DEGRADED ELASTIN NETWORK
EDP-DEPENDENT EFFECTS
Type 2 Diabetes : insulin resistance, insulin signaling alteration through IR desialylation, adipocytes hypertrophy
NASH : increase of lipogenesis, alteration of LKB1/AMPK signaling through HGFR desialylation
Regulation of vascular tone : endothelium-dependent vasodilation through NO production
Atherothrombosis : increase of plaque development (through monocytes recruitment, ROS production, oxLDL uptake through CD36 desialylation), decrease of platelets aggregation and of platelets/collagen interaction
Ischemia/reperfusion : cardioprotective effect (activation of RISK pathway, decrease of necrosis and CK release, acceleration of recovery of RPP and LVEDP)
ELASTIN MODIFICATIONS
Elastin degradation : Elastin fatigue / fracture, proteolytic degradation
Elastin stiffening : calcification, non-enzymatic PTM
INCREASED VASCULAR STIFFNESS AND ASSOCIATED CONSEQUENCES
REGULATION OF BIOLOGICAL FUNCTIONS RELATED TO METABOLIC AND CARDIOVASCULAR DISEASES

Figure 2

Myocardial infarction

Arrhythmias
Cardiac
Dysfunction

Remodelling of vascular system

Ventricular enlargement

Heart failure

Death

EDP and ERC participation

Coronary thrombosis

Myocardial ischemia

Coronary Artery Disease

Atherosclerosis
Calcification
Left Ventricular Hypertrophy

Risk factors :
Dyslipidemia
Hypertension
Diabetes
Smoking
Obesity
+
Aging

Elastolysis,

Aortic stiffening, dilatation

Isolated Systolic Hypertension

Microvascular Disease
Thrombosis
Haemorrhage

End-stage Heart or Renal Disease

EDP production

Figure 3