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# Role of the ACE2/Angiotensin 1–7 axis of the Renin-Angiotensin System in Heart Failure

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## Abstract

Heart failure remains the most common cause of death and disability, and a major economic burden, in industrialized nations. Physiological, pharmacological, and clinical studies have demonstrated that activation of the renin-angiotensin system is a key mediator of heart failure progression. Angiotensin converting enzyme 2 (ACE2), a homologue of ACE, is a monocarboxypeptidase that converts angiotensin II (Ang II) into angiotensin 1–7 (Ang 1–7) which, by virtue of its actions on the Mas receptor, opposes the molecular and cellular effects of Ang II. ACE2 is widely expressed in cardiomyocytes, cardiofibroblasts, and coronary endothelial cells. Recent preclinical translational studies confirmed a critical counter-regulatory role of ACE2/Ang 1–7 axis on the activated renin-angiotensin system that results in heart failure with preserved ejection fraction. While loss of ACE2 enhances susceptibility to heart failure, increasing ACE2 level prevents and reverses the heart failure phenotype. ACE2 and Ang 1–7 have emerged as a key protective pathway against heart failure with reduced and preserved ejection fraction. Recombinant human ACE2 has been tested in phase I and II clinical trials without adverse effects while lowering and increasing plasma Ang II and Ang 1–7 levels, respectively. This review discusses the transcriptional and post-transcriptional regulation of ACE2 and the role of the ACE2/Ang 1–7 axis in cardiac physiology and in the pathophysiology of heart failure. The pharmacological and therapeutic potential of enhancing ACE2/Ang 1–7 action as a novel therapy for heart failure is highlighted.

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NONE.

**Keywords**

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The renin-angiotensin system (RAS) is a peptidergic system that functions in the homeostatic control of the cardiovascular and renal systems and in regulating extracellular fluid volume. Inhibition of the RAS plays a central role in alleviating the increased morbidity and mortality of patients with heart failure (HF).[1, 2] The RAS consists of a series of enzymatic reactions that result in generation of angiotensin (Ang) II. In the first step, renin (an aspartyl proteinase secreted by kidney into the circulation) cleaves hepatic peptide angiotensinogen to produce Ang I in the blood. Ang I is then hydrolyzed by angiotensin-converting enzyme (ACE) in the second step, producing the octapeptide Ang II. This biologically active peptide acts on Ang II type 1 and type 2 receptors ($AT_1R$ and $AT_2R$) (Figure 1A). Ang II promotes vasoconstriction, inflammation, salt and water reabsorption, and oxidative stress via the activation of $AT_1R$.[3] These detrimental effects of Ang II/$AT_1R$ have encouraged the quest for a counter-regulatory axis of the activated RAS. RAS was initially thought to function as a systemic entity not localized to any specific tissue. However, this notion of systemic RAS was challenged by observations that many tissues are capable of synthesizing the key components of RAS,[4–6] including heart,[4, 7, 8] kidney,[9] vasculature,[9] pancreas,[6, 10] retina,[11, 12] brain,[6, 13] and others. The local RAS could produce peptides at the tissue level that show autocrine effects (on the cells where they are being produced), paracrine effects (on neighboring cells), or endocrine effects (on a distant organ or tissue; via systemic circulation).[6, 14]

Our conception of the RAS family has seen substantial changes with the identification of angiotensin-converting enzyme 2 (ACE2), a homologue of ACE. ACE2 is a monocarboxypeptidase that degrades Ang I into a nonapeptide, Ang 1–9 and Ang II into a heptapeptide, Ang 1–7 (Figure 1A). The discovery of ACE2, Ang 1–9, and Ang 1–7 unravels a distinct enzymatic pathway for degradation of Ang I and Ang II as endogenous negative regulation of RAS activation. Moreover, ACE2 has been identified as an important RAS regulator capable of mitigating the deleterious actions mediated by Ang II and $AT_1R$. This is of particular importance in pathological conditions where the RAS is activated. Ang 1–7 is a biologically active peptide exerting a wide array of actions, many of which are opposite to those attributed to Ang II.[15–18] In 2003, an endogenous orphan receptor, Mas (MasR), was identified as the Ang 1–7 receptor. A779, a MasR antagonist, has been shown to block the majority of Ang 1–7 effects.[17, 19–22] Ang 1–9 has also shown beneficial biological effects via $AT_2R$ that result in cardioprotection.[23–26] Thus, while ACE/Ang II/$AT_1R$ is a well-established axis of the RAS, the ACE2/Ang 1–7/MasR and ACE2/Ang 1–9/$AT_2R$ axes have emerged as physiological antagonists that counter-regulate the activate RAS.[16, 27–31] Taken together, the cardioprotective effects of ACE2 can be attributed to i) degradation of Ang I to Ang 1–9, limiting the availability of substrate for ACE action, ii) degradation of Ang II, limiting its detrimental effects, and iii) generation of Ang 1–7, exerting its cardioprotective effects. Several lines of evidence suggest that ACE2 level and/or activity balances two different arms. Decreased ACE2 activity results in activation of the

Ang II/$AT_1R$ axis, contributing to increased progression of heart disease. Increased ACE2 level/activity leads to activation of ACE2/Ang 1–9 and ACE2/Ang 1–7 axes, leading to protection against heart disease (Figure 1B). In this review, we highlight the role of ACE2/Ang 1–7 in counter-regulation of Ang II actions, different approaches to manipulating ACE2/Ang 1–7 levels, and the potential of enhancing ACE2 action as a therapy for HF.

## ACE2: Discovery, biochemistry, and regulation

### a. Discovery of ACE2 and its differences from ACE

ACE2 or ACE homologue (ACEH) was discovered as a zinc metalloproteinase by two different groups in 2000. ACE2 was initially identified from human HF and lymphoma cDNA libraries[32, 33] and was later shown to serve as a receptor for the SARS coronavirus.[34] It was found to possess an apparent signal peptide, a transmembrane domain, and a single metalloproteinase active site containing an HEXXH zinc-binding domain.[32, 33] ACE2 is a type I transmembrane protein with an extracellular N-terminal domain containing the catalytic site and an intracellular C-terminal tail. Similar to ACE, the catalytic site of ACE2 is exposed (an 'ectoenzyme') to circulating vasoactive peptides.[35] Expression of a soluble truncated form of ACE2 in CHO cells produced a glycoprotein of 120 kDa that was able to cleave Ang I and II but not bradykinin.[33] Other critical residues typical of the ACE family are conserved in ACE2. Tipnis et al. discovered that the ACE2 gene contains 18 exons, with several having considerable size similarity to the first 17 exons of human ACE.[33] The metalloproteinase catalytic domains of ACE2 and ACE are 42% identical according to the findings of Donoghue et al.[32] In spite of such similarity though, unlike ACE, ACE2 does not convert Ang I to Ang II. In fact, ACE2 activity is inhibited by EDTA but is unaffected by ACE inhibitors such as captopril and Lisinopril.[32, 33, 36] Further research revealed a major difference in enzymatic actions of ACE and ACE2. ACE acts a dipeptidyl carboxypeptidase (removing a dipeptide from the C-terminus of substrate) whereas ACE2 acts as a mono-carboxypeptidase (removing a single amino acid) that degrades Ang I to generate the nonapeptide Ang 1–9 and Ang II to generate the heptapeptide Ang 1–7.[32, 33] Later studies focused on ACE2 purification and characterization of its catalytic activity, showing a pH optimum of 6.5 and enhancement of ACE2 activity by monovalent anions, including $Cl^-$ and $F^-$, but not $Br^-$.[37] This is consistent with the activity of ACE.[38] However, ACE2 was later shown to possess one $Cl^-$ binding site compared to two $Cl^-$ sites in ACE.[39] Out of 126 biological peptides tested with ACE2 using LC-MS, ACE2 hydrolyzed three peptides with high efficiency: Ang II, apelin-13, and dynorphin A 1–13. ACE2 also showed a preference for cleaving C-terminal amino acids with peptides ending in Pro-X, where X is a hydrophobic amino acid.[38, 40] This cleavage preference of ACE2 was supported by a key experiment in which a dipeptide, Pro-Phe, completely inhibited ACE2 activity at 180 μM with Ang II as the substrate.[41] In a search for the active site residues of ACE2, site-directed mutagenesis revealed that Arg273 is critical for substrate binding and its replacement causes complete loss of enzyme activity.[39]

The difference in ACE and ACE2 enzymatic activity became more evident upon the discovery that human ACE2 catalytic efficiency is 400-fold higher with Ang II as a substrate than with Ang I.[38, 42] To further unravel the biological role and importance of ACE2, several

ACE2 inhibitors were designed and synthesized via substrate-based[43] and structure-based[44] pharmacophore design and virtual screening. MLN-4760, a potent and selective inhibitor developed with substrate-based design, has been a key tool for *in vivo* and *in vitro* studies.[43] In the last 15 years, distinct roles of ACE2 have been discovered ranging from catalytic activities with various substrates, functional SARS coronavirus receptor, and an amino acid transporter.[34, 40, 45, 46] ACE2 was initially thought to be expressed only in heart, kidney, and testes,[33] but was eventually found to be widely expressed in various organ systems including the cardiovascular system, kidneys, lungs, and brain, in which it exerts important actions to maintain cardiovascular homeostasis.[47–52] In the heart, ACE2 is localized to cardiomyocytes (contracting cardiac muscle cells), cardiac fibroblasts, and the coronary vascular endothelium.[53, 54] MasR is also present on cardiomyocytes, cardiac fibroblasts, and endothelial cells.[19, 55–57]

### b. Proteolytic processing, transcriptional, and post-transcriptional regulation of ACE2

Various molecules are shed from cell surfaces by the action of a disintegrin and metalloproteinase (ADAM) 17, also known as tumor necrosis factor-α converting enzyme (TACE).[58–60] ADAM17-mediated proteolysis of ACE2 releases an enzymatically active ectodomain from the cell surface, generating a soluble, active form of the enzyme. Lambert et al. confirmed the ectodomain shedding of heterologously expressed ACE2 in HEK293 cells and endogenously expressed ACE2 in Huh7 cells. Small interfering RNA (siRNA) against ADAM17 reduced the shedding of ACE2 and ADAM17 overexpression increased it, providing direct evidence of ADAM17-mediated ectodomain shedding of ACE2. Lambert et al. later discovered that calmodulin, a ubiquitous calcium binding protein, associates with ACE2 and prevents its shedding, an action inhibited by calmodulin inhibitors.[61] However, increased ACE2 shedding mediated by calmodulin inhibitors was only partially blocked by metalloproteinase inhibitor, suggesting the involvement of alternate proteolytic pathways not yet identified.[61] The initial observation of ACE2 shedding was further confirmed and shown to be a constitutive and regulated phenomenon in various cell types including CHO cells, fibroblasts, 3T3-L1 adipocytes, neurons, cardiomyocytes, and proximal tubular cells.[53, 62–64] In particular, we identified a positive feedback mechanism in the RAS whereby Ang II facilitates the loss of its negative regulator, ACE2.[53] Ang II action on $AT_1R$ leads to phosphorylation (mediated by p38 mitogen-activated protein kinase [MAPK]) and activation of ADAM17, resulting in increased ACE2 shedding (Figure 2).[53, 65] Shedding of membrane-bound ACE2 is likely responsible for the loss of myocardial ACE2[66, 67] and elevation in plasma ACE2 activity in HF that correlates with worsened prognosis.[68, 69] The biological and clinical significance of ACE2 ectodomain shedding is yet to be fully characterized. The inhibition of ectodomain shedding of ACE2 by manipulating the enzyme activity of ADAM17 could have therapeutic potential in HF.

A reporter system using the 3′-UTR of an ACE2 transcript was used to determine the functionality of putative microRNA (miRNA) binding sites identified *in vitro*. In a luciferase reporter assay containing ACE2 3′-UTR, miR-421 strikingly decreased ACE2 protein levels while loss of miR-421 reversed these effects, implying that miR-421 modulates ACE2 expression via post-translational repression rather than degradation of mRNA transcripts. This identified miR-421 as a potential regulator of ACE2 and was the first demonstration of

post-transcriptional regulation of ACE2.[70] ACE2 mRNA expression is also regulated by Sirtuin 1 (SIRT1). Energy stress by hypoxia and adenosine monophosphate kinase (AMPK) activation by 5-amino-4-imidazolecarboxamide riboside (AICAR) increase the cellular ratio of NAD+ to NADH and increase ACE2 expression.[71] SIRT1, in the presence of a possible but unknown cofactor, binds to the promoter region of *ACE2* and this binding is promoted by AICAR. AICAR-induced ACE2 expression is inhibited by an inhibitor of SIRT1, providing strong evidence for the SIRT1-mediated transcriptional regulation of ACE2 under conditions of energy stress (Figure 2).[71] Similarly, apelin also increases ACE2 promoter activity *in vitro* and upregulates ACE2 expression in failing hearts *in vivo* (Figure 2).[72] Therapeutically, agents that increase ACE2 expression (SIRT1 activators, apelin) or inhibitors of negative regulators of ACE2 (TACE or miR-421) could be utilized to enhance ACE2 activity and counteract cardiovascular diseases including HF.

## Role of ACE2/Ang 1–7 in HF

Heart failure is a growing epidemic with high morbidity and mortality at an international scale. Acute and chronic HF is characterized by activation of several signaling pathways associated with pathological hypertrophy and maladaptive ventricular remodeling. HF is caused by damage to or loss of cardiomyocytes and contributes to diminished systolic performance and diastolic dysfunction in the failing heart.[73, 74] HF involves changes in cardiac structure, myocardial composition, myocyte deformation, and multiple biochemical and molecular alterations, collectively referred to as adverse myocardial remodeling. Despite improvements in medical and surgical therapies, cardiac diseases remain the leading cause of death in North America, with ischemic and hypertensive heart disease as the leading cause of HF.[75–77]

Diabetes mellitus and obesity are major causes of morbidity and mortality in all parts of the world including North America.[78] Diabetes mellitus is characterized by insulin insufficiency that is frequently associated with severe cardiovascular complications and increased risk for hypertension, HF, and myocardial infarction (MI).[79–81] Obesity itself is an independent risk factor for development of HF with preserved ejection fraction (HF-pEF), independent of other comorbid conditions.[82–84] The rising global tide of obesity and diabetes will likely contribute further to the increasing prevalence of systolic and diastolic HF.[78, 80, 85–87] Although the mechanisms underlying the intertwined relationship among diabetes, obesity, hypertension, and cardiovascular events remain to be fully defined, major culprits that have been implicated are cardiovascular inflammation, oxidative stress, mitochondrial dysfunction, and insulin resistance, all closely linked with abnormalities in the RAS.[88–91]

Neurohormonal changes such as activation of the RAS and increased Ang II levels play a pivotal role in adverse myocardial remodeling and progression to HF.[2, 92, 93] Indeed, pharmacological antagonism of the RAS using ACE inhibitors (ACEi) or $AT_1R$ blockers (ARB) is a cornerstone of current medical therapy for human HF, including diabetic cardiomyopathy.[75, 94] While these pharmacotherapies for HF provide benefits, patients with HF continue to be plagued by clinical deterioration, high morbidity, and mortality.[77] Irrespective of the capacity of ACE inhibitors to inhibit ACE action, Ang II levels can remain elevated in optimally treated HF patients. About 50% of the patients using ongoing

ACEi therapy exhibit elevated levels of Ang II, the result of activation of mast cell chymase.[95–98] Therefore, there is an urgent need to identify alternative strategies to minimize the detrimental effects of Ang II and treat HF.

ACE2, by virtue of its action on Ang I and Ang II, is nature's endogenous ACE inhibitor at the cellular level (Figure 3). Ang 1–9, the product of ACE2 degradation of Ang I, has recently shown promising anti-hypertrophic, anti-fibrotic, and anti-hypertensive effects. These beneficial effects result in cardioprotection against hypertension and MI.[23–26] Adenoviral delivery of Ang 1–9 in H9c2 cardiomyocytes has shown anti-hypertrophic effects comparable to adenoviral Ang 1–7 delivery.[23] Moreover, RhoA/Rho kinase inhibition has shown potent anti-hypertensive effects that were mediated via the upregulation of vascular and plasma ACE2 and increased plasma Ang 1–9 levels, without an increase in Ang 1–7 levels.[26] This suggests a potential role for Ang 1–9 in the anti-hypertensive effects of RhoA/Rho-kinase inhibition.

Both Ang I and Ang II can function as the preferred substrate for ACE2. Studies using recombinant human ACE2 (rhACE2) and ACE2 purified from sheep tissues showed Ang II as a preferred substrate for ACE2.[33, 38, 41, 67, 99, 100] In sheep, conversion from Ang I to Ang 1–9 was not detected while the proximal tubules contained robust ACE2 activity that converted Ang II to Ang 1–7.[101] In contrast, changes in ACE2 correlated with plasma Ang 1–9 levels in rats.[102] In a recent study Ye et al. demonstrated that rhACE2 generated Ang 1–7 and Ang 1–9 while recombinant murine ACE2 generated predominantly Ang 1–7.[103] In addition, the therapeutic effects of rhACE2 is highly dependent on Ang 1–7 action in rodents[30, 67, 100] and in human studies rhACE2 clearly lowered plasma Ang II levels resulting in increased plasma Ang 1–7 levels.[104–106] However, it remains possible that the contribution of Ang 1–9 in ACE2's beneficial effects may be underestimated and requires further investigation with a clear emphasis on human studies.

Ang 1–7 activates MasR and exerts various effects, the majority of which antagonize Ang II's effects.[15, 20] These effects include i) activation of the phosphatidylinositol 3-kinase (PI3K)-Akt-endothelial nitric oxide synthase (eNOS) pathway; ii) inhibition of protein kinase C (PKC)-p38 MAPK pathways and iii) inhibition of collagen expression to limit cardiac fibrosis (Figure 3).[19, 107, 108] To understand the relative contributions of inhibiting the Ang II/$AT_1$R axis and activating the Ang 1–7/MasR axis to cardioprotective effects, we studied the effects of irbesartan and Ang 1–7 supplementation in pressure-overload-induced HF in ACE2 knockout mice.[30] We found functional redundancy in the anti-fibrotic and anti-hypertrophic effects and suppression of pathological signaling. The cardioprotective effects of irbesartan and Ang 1–7 were equivalent, suggesting similar significance of both axes.

### a. Role of ACE2/Ang 1–7 in hypertension

Activated RAS and Ang II are established key mediators of hypertension, therefore ACE2 is hypothesized to be a potent modulator of blood pressure and its deficiency leads to hypertension. In a preclinical model of hypertension, ACE2 gene maps to a defined quantitative trait locus on the X-chromosome previously identified as a quantitative locus for blood pressure.[7] Recent studies suggest an association between ACE2 activity and blood pressure levels.[109, 110] Serum ACE2 activity was higher in patients with hypertension

compared to healthy individuals. In hypertensive patients with type 1 diabetes, serum ACE2 activity was positively correlated with systolic blood pressure in both males and females.[110] These studies suggest that elevated ACE2 may be a "compensatory response" to the hypertension. Indeed, the anti-hypertensive role of ACE2 has also been established in various preclinical models of hypertension.[28, 111–113] Lentiviral overexpression of ACE2 results in increased expression of anti-hypertensive components of RAS (Ang 1–7, MasR and $AT_2R$) attenuating the elevated blood pressure.[111, 112] Similarly, rhACE2 pretreatment alleviated hypertension induced by acute Ang II infusion and was associated with decreased plasma Ang II and increased plasma Ang 1–7 levels.[99] Cyclodextrin-encapsulated Ang 1–7, AVE0091, and CGEN856S (MasR agonists) have shown blood pressure-lowering effects in hypertensive animals.[114] The anti-hypertensive effects of ACE2/Ang 1–7 generated interest in potential cardioprotective effects against hypertensive heart diseases, a group of disorders that includes HF, ischemic heart disease, hypertensive heart disease, and left ventricular hypertrophy.

### b. Role of ACE2/Ang 1–7 in HF with reduced ejection fraction (HF-rEF)

ACE2 plays a critical role in the control of cardiac physiology and altered ACE2 expression or activity is linked to the progression of heart disease (Figure 1B). In heart, ACE2 is expressed in various cells including the cardiomyocytes, cardiac fibroblasts, and coronary endothelial cells,[115] where it negates Ang II actions and also activates Ang 1–7/MasR signaling (Figure 3). ACE2 expression is highly affected by pathological disease conditions, suggesting its role in counter-regulating the development of cardiac diseases. In the human population, genetic variations in the *ACE2* gene correlate with susceptibility to cardiovascular disease.[116–118] Single nucleotide polymorphisms of *ACE2* are associated with variation in septal wall thickness, ventricular hypertrophy,[116] and coronary artery disease.[117]

The first report on the role of ACE2 as an essential regulator of cardiac function came soon after its discovery.[7] In that study, ACE2 knockout mice showed reduced systolic function. The decrease in systolic function was both sex- and time-dependent, with more severe abnormalities in male than in female mice, and a more pronounced phenotype in older animals. ACE2 knockout mice also showed increased Ang II levels, which were rescued with genetic ablation of ACE.[7] Consistently, we found age-dependent dilated cardiomyopathy in ACE2 knockout mice. This resulted in reduced systolic function along with increased cardiac inflammation and oxidative stress.[29] Myocardial ACE2 protein levels were decreased in pressure-overload-induced HF, suggesting an inverse relationship between myocardial ACE2 protein levels and disease progression.[22, 67] In addition, loss of ACE2 resulted in worsened pathological remodeling in response to pressure-overload-induced biomechanical stress. This was associated with systolic dysfunction and ventricular dilation. Both were deemed due to activation of the myocardial NAPDH oxidase system, superoxide production, and matrix metalloproteinase (MMP) activation, which was attributed to increased local Ang II levels (Figure 3; Table).[30, 119, 120] Post-MI remodeling and coronary artery disease is one of the most common causes of HF.[121] MI increased ACE2 mRNA expression in humans, mice, and rats,[122, 123] whereas loss of ACE2 or inhibition of ACE2 by C16, resulted in worsening of MI-induced cardiac dysfunction, increased infarct size, MMP

activation, cardiac extracellular matrix disruption, and inflammation (Table).[123, 124] Lentiviral[125, 126] or adenoviral[127] overexpression of ACE2 ameliorated MI-induced cardiac remodeling. In addition, lentiviral infection of cultured fibroblasts decreased the acute hypoxic exposure-induced production of collagen.[128]

Importantly, Ang 1–7 treatment has shown noticeable cardioprotective effects in preclinical models of non-ischemic and ischemic cardiomyopathy.[15, 21, 126, 129] Ang 1–7 suppressed cardiomyocyte growth *in vitro* and inhibited myocardial infarction-induced ventricular hypertrophy *in vivo*. Ang 1–7 also decreased myocardial levels of pro-inflammatory cytokines (TNFα and IL-6) leading to alleviation of cardiac inflammation.[21, 126] These results confirm the important contribution of Ang 1–7 in the cardioprotective effects of ACE2 (Figure 4).

### c. Role of ACE2/Ang 1–7 in HF with preserved ejection fraction (HF-pEF)

HF-pEF, also termed diastolic HF, is often associated with a normal or smaller heart size and diastolic filling abnormalities. It accounts for approximately 30% of all HF patients, with a similar mortality rate to patients with HF-rEF.[84, 130] Ang II-induced diastolic dysfunction is a clinically relevant, widely accepted preclinical model of HF-pEF. We and others found that loss of ACE2 resulted in worsened cardiac dysfunction, cardiac hypertrophy, and fibrosis, leading to greater diastolic dysfunction in response to Ang II (Table).[67, 131] Importantly, treatment with rhACE2 decreased plasma and myocardial Ang II levels and increased plasma Ang 1–7 levels, providing definitive evidence for a key role of ACE2 in the metabolism of Ang II.[67] Furthermore, rhACE2 attenuated pathological changes mediated by Ang II, reducing myocardial hypertrophy and fibrosis and correcting diastolic dysfunction. However, treatment with rhACE2 did not affect baseline plasma Ang II, Ang 1–7, or blood pressure in wild-type mice. This suggests that substrate availability is a limiting factor in ACE2 enzymatic activity.[132] The pursuit of molecular mechanisms for these actions identified rhACE2's capacity to inhibit the Ang II effects on TGF-β1 activation and collagen production.[57, 67, 133] Loss of ACE2 also resulted in increased production of reactive oxygen species (ROS) via NADPH oxidase 2 activation, which is also suppressible by rhACE2.[67] Lentiviral overexpression of ACE2 protects the heart against myocardial injuries induced by Ang II in rats, confirming the role of ACE2 in counteracting HF-pEF.[13430] We assessed the contribution of Ang 1–7/MasR activation to the favorable effects shown by rhACE2 in the Ang II-induced murine HF model; inhibition of Ang 1–7/MasR signaling resulted in loss of rhACE2 mediated cardioprotective effects. However, this observation does not rule out the potential contribution of Ang 1–9 to the protective effects of rhACE2. An appropriate preclinical study is required to assess the relative contributions of Ang 1–9 and Ang 1–7.[100] ACE2 is an endogenous regulator of activated RAS-induced HF-pEF and enhancing ACE2 has a marked beneficial effect.

### d. Role of ACE2/Ang 1–7 in diabetes and obesity-associated cardiomyopathy

Diabetes and obesity are major causes of morbidity and mortality in all parts of the world including Canada.[78] Studies of the ACE2/Ang 1–7 axis in diabetes and obesity-associated cardiac dysfunction have shed light on the critical role of this pathway in counter-regulation of the Ang II/$AT_1$R axis (Figure 4). In human type 1 diabetes, elevated plasma ACE2

activity correlated with microvascular and macrovascular complications, increased systolic blood pressure, and the duration of diabetes,[110] strongly supporting a key clinical role for the ACE2 system in cardiovascular disease that is secondary to diabetes. The role of ACE2 in diabetic cardiovascular complications has been studied in various preclinical models of diabetes. Tools such as ACE2 knockout mice,[123, 135] adenoviral ACE2 gene transfer,[136] rhACE2,[137] ACE2 activators and inhibitors,[138–140] Ang 1–7 supplementation,[141] Ang 1–7/MasR activator (AVE0991)[142] and Ang 1–7/MasR receptor blockade (A779)[142] have been utilized to assess the role of ACE2/Ang 1–7 in diabetic cardiovascular complications.

We studied the role of ACE2 in preventing progression of type 1 diabetic cardiovascular complications[135] using a clinically relevant animal model of diabetes, the Akita mouse. Akita type 1 diabetic hearts show diastolic dysfunction associated with reduced levels of the cardiac SERCA2a and increased myocardial lipotoxicity.[143] Loss of ACE2 in these hearts, in Akita/ACE2 knockout double mutants, resulted in systolic dysfunction.[135] Akita/ACE2 knockout hearts exhibited increased NADPH oxidase activity, ROS production, and protein kinase C and MMP activation, leading to increased degradation of the cardiac extracellular matrix. This study demonstrated a key role for ACE2 as a negative regulator of activated RAS in diabetic cardiomyopathy.[135] Further studies have validated our findings for this essential role of ACE2 in diabetic cardiomyopathy.[136, 138–140, 144] We also identified beneficial effects of Ang 1–7 in type 2 diabetic cardiomyopathy. By reducing cardiac hypertrophy, lipotoxicity, and adipose inflammation, in combination with increased adipose triglyceride lipase, Ang 1–7 completely rescued diastolic dysfunction in the db/db type 2 diabetic murine model.[141, 145]

Obesity is characterized by excessive fat accumulation in adipose tissues throughout the body and is the most common nutritional disorder in industrialized countries. Obesity is associated with increased morbidity and mortality and is a risk factor for development of HF-pEF, independent of other comorbid conditions.[82–84, 146] We studied the role of ACE2 in obesity induced by high fat diet and associated cardiac dysfunction.[147] Loss of ACE2 was associated with worsened obesity-associated HF-pEF due to increased epicardial adipose tissue inflammation, myocardial lipotoxicity, and cardiac metabolic abnormalities (Table). These findings coupled with the protective effects of ACE2/Ang 1–7 in the vasculature supports a key role of adipose tissue inflammation and microvascular dysfunction in the pathogenesis of HF-pEF.[69, 148] Importantly, Ang 1–7 prevented these changes and rescued HF-pEF in ACE2 knockout mice, validating its critical role in ACE2-mediated cardioprotection (Figure 4). As such, enhancing the ACE2/Ang 1–7 pathways represents a potential therapy for HF-pEF, which currently lacks effective therapies.

## Therapeutic approaches and potential of enhancing ACE2/Ang 1–7 in HF

Irrespective of the capacity of ACEi to inhibit ACE action, Ang II levels can remain elevated in optimally treated HF patients; about 50% of patients using ongoing ACEi therapy exhibit elevated levels of Ang II.[95–98] The generation of plasma and tissue Ang II by non-ACE related enzymes such as chymase suggests that enhancing ACE2 action may indeed have a unique therapeutic role.[67, 96] In fact, ACEi and ARB have been shown to upregulate the expression of ACE2 or prevent the loss of ACE2.[102, 149] ADAM17-mediated ACE2

shedding represents a mechanism by which Ang II induces a positive feedback mechanism in the tissue-localized RAS leading to its dysregulation. This results in the neurohumoral imbalance that is typical of HF.[150] Inhibiting TACE-mediated shedding of ACE2 from the surface of cardiac cells, leading to retention of ACE2 enzymatic activity within the cardiac microenvironment, might have therapeutic potential. ACE2 is post-transcriptionally regulated by miR-421, inhibition of which may result in increased ACE2 expression. As ACE2 is also subject to transcriptional regulation by SIRT1 and apelin, SIRT1 activators or apelin may have therapeutic benefits by enhancing the actions of ACE2.

A well-studied tool to enhance ACE2 action is rhACE2. A randomized, double-blinded, placebo-controlled study administered Intravenous rhACE2 to healthy human subjects and found that the rhACE2 was well-tolerated. Despite marked changes in angiotensin system peptide concentrations, hypotension was absent, suggesting the presence of effective compensatory mechanisms in healthy volunteers.[106] rhACE2 is primarily responsible for the conversion of Ang II into Ang 1–7 but can also convert Ang 1–10 into Ang 1–9.[151] In healthy human volunteers treated with rhACE2, Ang II levels were reduced but Ang 1–7 levels were increased or remained unchanged.[104, 106] Importantly, in a recently completed phase II trial in patients with acute lung injury, rhACE2 resulted in sustained reduction in plasma Ang II levels and elevation in Ang 1–7 levels.[105] We propose that assessment of plasma RAS peptide levels can allow the tailoring of rhACE2 therapy for human HF. rhACE2 provided beneficial effects against Ang II-induced HF-pEF and pressure-overload-induced HF-rEF in murine models of HF (Table).[67] Thus, using rhACE2 as a therapy is very much a viable option and the advancement of rhACE2 in clinical trials provides the translational impact of rhACE2 findings in murine models.[104, 105] Several ACE2 activators and Ang 1–7/MasR agonists have been developed. In addition, novel approaches, including oral ACE2 and Ang 1–7 biencapsulated in plant cells, have been designed and used in preclinical studies, showing promising cardioprotective effects.[152–156] Lastly, gene therapy approaches could be utilized to achieve the tissue-specific delivery of ACE2/Ang 1–7.

Autologous cell-based therapy using putative progenitor cells such as $CD34^+$ cells could be an attractive therapeutic approach for diabetic vascular complications. However, these cells are dysfunctional in diabetic individuals. Peripheral $CD34^+$ cells isolated from patients with diabetes exhibit reduced proliferative potential and migratory function, which could be attributed to decreased eNOS activity, increased ROS levels, and advanced glycation end-products.[157, 158] As ACE2 and Ang 1–7 are potent activators of eNOS[19] and anti-oxidants,[100, 135] the ACE2/Ang 1–7/MasR axis should improve $CD34^+$ cell function and result in increased reparative efficacy. Indeed, Ang 1–7 increased the vascular reparative function of $CD34^+$ cells isolated from patients with diabetes.[159]

## Conclusions

ACE2 has emerged as the dominant mechanism for negative regulation of the RAS, by metabolizing Ang II into the beneficial peptide Ang 1–7. This important biochemical and physiological property is being harnessed as potential therapy for HF. Since the discovery of ACE2 in 2000, tremendous progress has been made in elucidating its biochemical actions and its key role in heart disease and HF. ACE2 is widely expressed and regulates the

fundamental cellular biology of cardiomyocytes, cardiofibroblasts, and coronary endothelial cells in both HF-rEF and HF-pEF models. Ang 1–7 has also emerged in HF models as a physiologically active peptide with protective effects. Enhancing Ang 1–7 action may also provide marked therapeutic effects in HF. Clinical and experimental studies clearly support a physiological and pathophysiological role for ACE2/Ang 1–7 in HF, and studies indicate that increasing/activating ACE2/Ang 1–7 results in beneficial effects to prevent heart disease and HF. Further experimental studies are required that combine rhACE2/ACE2 activators with RAS blockers (such as ACE inhibitors or $AT_1R$ blockers) to determine if this combined approach offers additional benefits.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations

| | |
|---|---|
| **3′-UTR** | 3′ untranslated region |
| **ACE** | Angiotensin converting enzyme |
| **ACE2** | Angiotensin converting enzyme 2 |
| **ACEi** | ACE inhibitor |
| **ADAM17** | A disintegrin and metalloproteinase 17 |
| **ADH** | Antidiuretic hormone |
| **AICAR** | 5-amino-4-imidazolecarboxamide riboside |
| **AMPK** | Adenosine monophosphate kinase |
| **Ang** | Angiotensin |
| **APA** | Aminopeptidase A |
| **ARB** | $AT_1R$ blocker |
| **$AT_1R$** | Angiotensin II type 1 receptor |
| **$AT_2R$** | Angiotensin II type 2 receptor |

| | |
|---|---|
| **CPA** | Carboxypeptidase A |
| **DAG** | Diacyl glycerol |
| **eNOS** | Endothelial nitric oxide synthase |
| **ERK1/2** | Extracellular signal-regulated kinase 1/2 |
| **IP3** | Inositol triphosphate |
| **HF** | Heart failure |
| **HF-pEF** | Heart failure with preserved ejection fraction |
| **HF-rEF** | Heart failure with reduced ejection fraction |
| **MAPK** | Mitogen activated protein kinase |
| **MasR** | Mas receptor |
| **MI** | Myocardial infarction |
| **miRNA** | MicroRNA |
| **MMP** | Matrix metalloproteinases |
| **NAD+** | Nicotinamide adenine dinucleotide – oxidized form |
| **NADH** | Nicotinamide adenine dinucleotide – reduced form |
| **NEP** | Neutral endopeptidase |
| **Nox2** | NADPH oxidase 2 |
| **PCP** | Prolyl carboxypeptidase |
| **PEP** | Prolyl endopeptidase |
| **NADPH** | Nicotinamide adenine dinucleotide phosphate |
| **PKC** | Protein kinase C |
| **PI3K** | Phosphatidylinositol 3-kinase |
| **PLC** | Phospholipase C |
| **RAS** | Renin-angiotensin system |
| **ROS** | Reactive oxygen species |
| **rhACE2** | Recombinant human ACE2 |
| **siRNA** | Small interfering RNA |
| **SIRT1** | Sirtuin 1 |
| **TACE** | Tumor necrosis factor-α converting enzyme |

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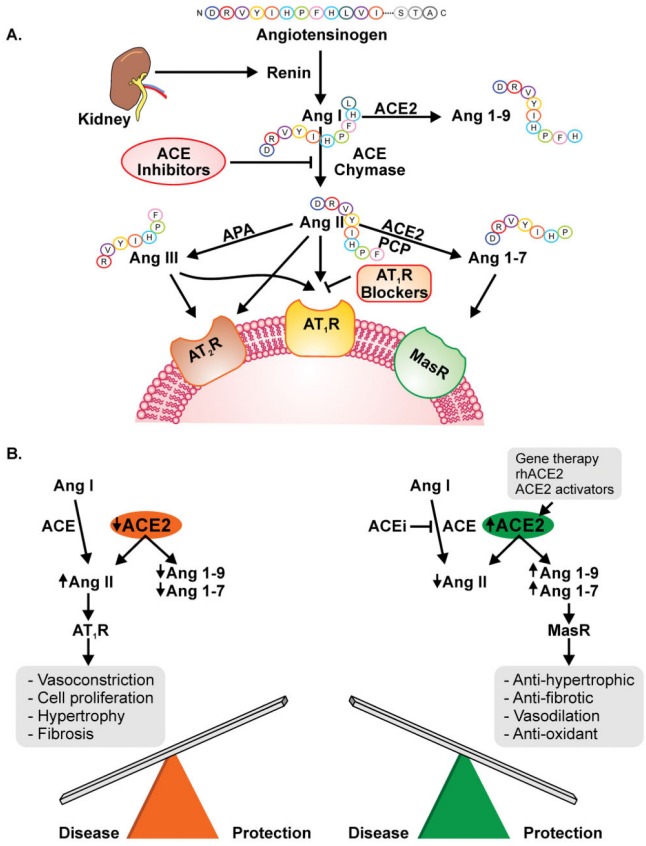


**Figure 1. The enzymatic cascade of the RAS, key receptor systems, and the biological effects mediated by Ang II and Ang 1–7**

(A) The RAS cascade showing the angiotensin peptide metabolic pathway. Angiotensinogen, as the starting substrate, is cleaved by renin to Ang I. Ang I is cleaved by ACE to Ang II, which is cleaved by ACE2 to Ang 1–7. Ang II acts on $AT_1$ and $AT_2$ receptors. Ang 1–7 acts on Mas receptors and counterbalances the Ang II/$AT_1$R actions. (B) Decreased ACE2 shifts the balance in the RAS to the Ang II/$AT_1$R axis, resulting in disease progression. Increased ACE2 (by rhACE2, gene delivery, or ACE2 activators) shifts the balance to the Ang 1–7/MasR axis, leading to protection from disease.

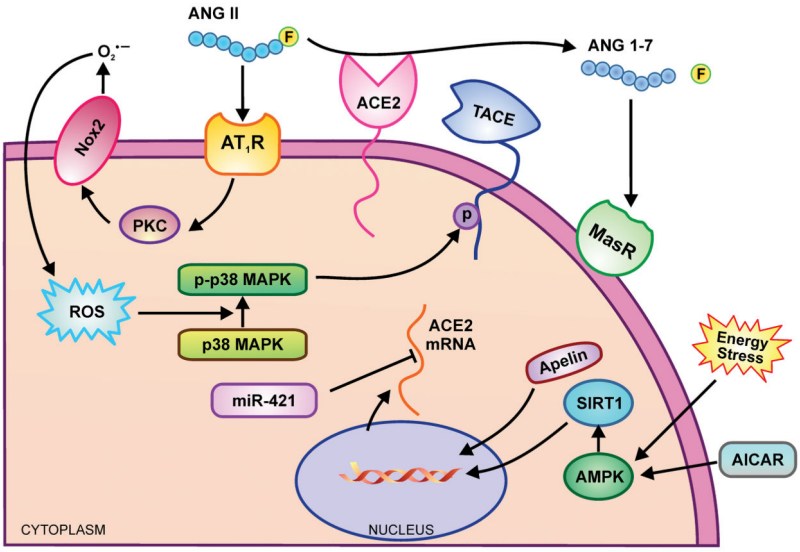


**Figure 2. Transcriptional, post-transcriptional, and post-translational regulation of ACE2**

ACE2 expression is transcriptionally regulated by energy stress and activation of AMPK via SIRT1, which binds to the promoter region and facilitates ACE2 mRNA expression. Similarly, apelin binds to the promoter region of *ACE2* and enhances its expression. ACE2 mRNA is subject to post-transcriptional regulation by miR-421, which regulates protein expression. Ang II, the main effector peptide of the RAS, is produced by ACE and chymase in the heart and other tissues. ACE2, a monocarboxypeptidase, degrades Ang II into a heptapetide, Ang 1–7. Ang II, via its action on $AT_1R$, promotes NOX2-dependent ROS formation. This leads to phosphorylation and activation of p38-MAPK and ultimately results in TACE phosphorylation (Thr735) and activation. Activated TACE proteolytically cleaves ACE2 and releases the active ACE2 ectodomain.

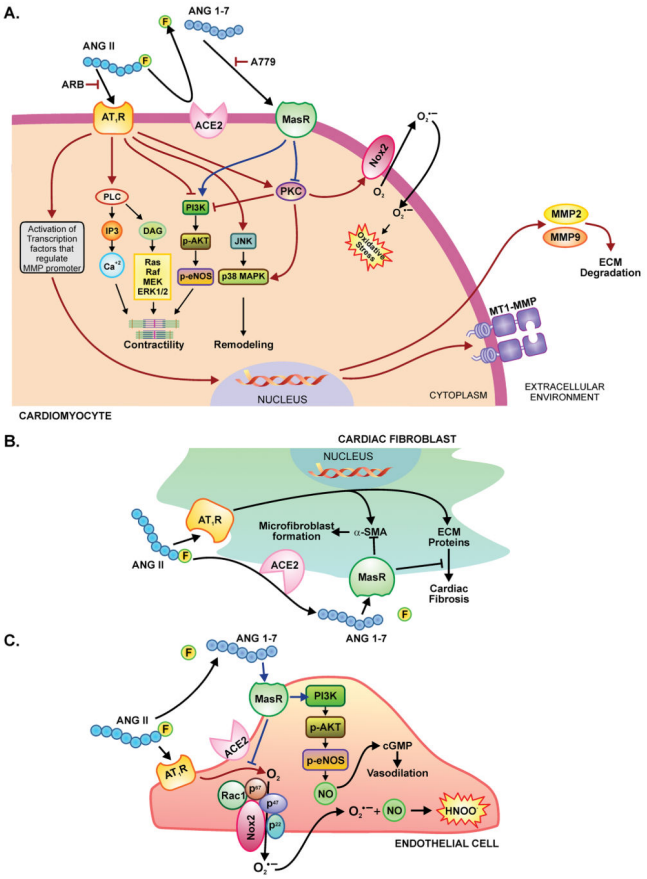


**Figure 3. Cardiac effects of the Ang II/AT$_1$R axis and counter-regulation by the ACE2/Ang 1–7/MasR axis**

ACE-mediated generation of Ang II results in activation of various signaling pathways in cardiomyocytes, cardiac fibroblasts, and endothelial cells, resulting in adverse cardiac remodeling and cardiac dysfunction. Activation of the ACE2/Ang 1–7/MasR axis counter-regulates Ang II/AT$_1$R mediated effects and also stimulates cardiac contractility mediated by the PI3K-Akt-eNOS pathway.

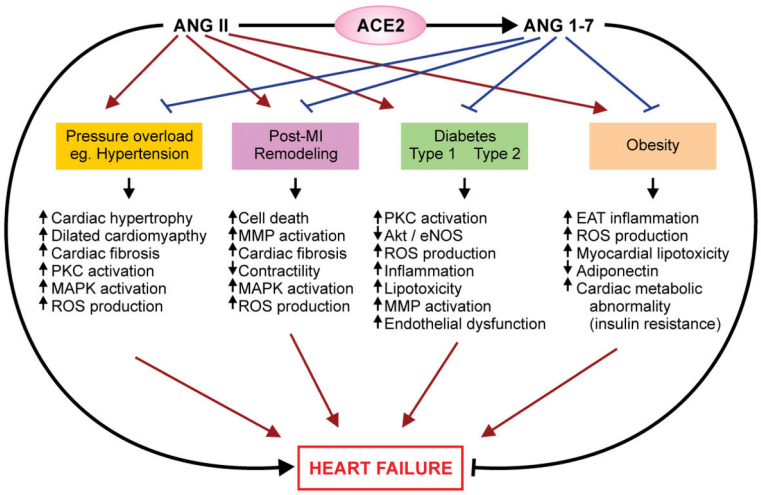


**Figure 4. Central role of the ACE2/Ang 1–7 axis in HF: non-ischemic cardiomyopathy, MI, diabetic cardiomyopathy, and obesity-associated cardiac dysfunction**

Ang II/$AT_1R$ is critically involved in the disease progression leading to non-ischemic, ischemic, and diabetic cardiomyopathy and to obesity-associated cardiac dysfunction. By converting Ang II to Ang 1–7, ACE2 shifts the balance to the cardioprotective ACE2/Ang 1–7/MasR axis. EAT: epicardial adipose tissue.

## Table

Interventions to modulate ACE2 levels or activity and their effects in experimental models of heart failure.

| Experimental intervention | Experimental model | Observation |
|---|---|---|
| **Gain of Function** | | |
| Lentiviral overexpression | LAD* coronary artery ligation | 6 weeks post-surgery: complete rescue of cardiac output, a 41% rescue of ejection fraction, a 44% rescue in contractility, and a 53% rescue in LV anterior (infracted) wall thinning compared to control rats[117] |
| Lentiviral overexpression | SHR | Attenuation of high blood pressure in the SHR, 18% reduction in left ventricular wall thickness, 12% increase in left ventricular end diastolic, and a 21% increase in end systolic diameters in lenti-ACE2-treated SHR; attenuation of peri-vascular fibrosis[103] |
| Lentiviral overexpression | Ang II infusion | Attenuation of the increased heart weight/body weight and myocardial fibrosis induced by Ang II infusion[126] |
| Lentiviral overexpression | Cardiac fibroblasts – hypoxia/re-oxygenation | Attenuation of both basal and hypoxia/re-oxygenation- induced collagen production by fibroblasts[120] |
| Adenoviral overexpression | LAD coronary artery ligation | 4 weeks after ACE2 gene transfer: reduced LV volume and extent of myocardial fibrosis, increased LV ejection fraction and levels of ACE2 activity[119] |
| rhACE2 | Ang II infusion | Blunted the hypertrophic response and expression of hypertrophy markers; decreased ROS production; inhibited pathological signaling[68]; rhACE2 administration to WKY rats reduced Ang II infusion-induced pressor response, myocardial hypertrophy, pathological signaling, and superoxide production[24] |
| rhACE2 | SHR | 14-day administration of rhACE2 partly corrected hypertension, ROS production, and pathological signaling in the heart[24] |
| rhACE2 | Transverse aortic constriction | rhACE2 partially prevented the pressure-overload- induced dilated cardiomyopathy and mRNA expression of disease markers and pro-fibrotic genes[68] |
| ACE2 activator (DIZE) | LAD coronary artery ligation | DIZE attenuated the MI-induced decrease in fractional shortening by 89%, improved dP/dtmax by 92%, and reversed ventricular hypertrophy by 18%[151] |
| **Loss of Function** | | |
| ACE2KO | Ang II infusion | Worsened cardiac fibrosis and pathological hypertrophy in ACE2KO mice[68] |
| ACE2KO | Transverse aortic constriction | Eccentric cardiac remodeling, increased pathological hypertrophy, and worsening of systolic performance; increased ROS production[67, 111, 112] |
| ACE2KO | LAD coronary artery ligation | Enhanced susceptibility to MI, with increased mortality, infarct expansion, and adverse ventricular remodeling[115] |
| ACE2KO | Type 1 diabetes; Akita | Loss of ACE2 in type 1 diabetic mice resulted in HF-rEF with background HF-pEF in Akita mice[129] |
| ACE2KO | High fat diet- induced obesity | Loss of ACE2 worsens epicardial adipose tissue inflammation, myocardial metabolic abnormalities, and lipotoxicity, resulting in HF-pEF[141] |
| ACE2 inhibitor (MLN4760) | (mRen2)27 hypertensive rats | Increased cardiac Ang II levels; increases in LV anterior, posterior, and relative wall thicknesses; increased interstitial collagen fraction area and cardiomyocyte hypertrophy[157] |
| ACE2 inhibitor (DX600) | Ang II stimulation of cultured cardiac fibroblasts | DX600 increased superoxide production and expression of CTGF, FKN, and phosphorylated ERK1/2; rhACE2 reduced these effects of Ang II[158] |
| ACE2 Inhibitor (C16) | Coronary artery ligation | Increase in MI size and reduction in LV % fractional shortening[116] |

*ACE2KO: ACE2 knockout; CTGF, connective tissue growth factor; DIZE, diminazene aceturate; ERK1/2, extracellular signal-regulated kinase 1/2; FKN, fractalkine; LAD, left anterior descending; LV, left ventricle; SHR, spontaneously hypertensive rats.