Angiotensin Converting Enzyme 2: SARS-CoV-2 Receptor and Regulator of the Renin-Angiotensin System

Celebrating the 20th Anniversary of the Discovery of ACE2

Mahmoud Gheblawi[a,c#], Kaiming Wang[b,c#], Anissa Viveiros[a,c], Quynh Nguyen[b,c], JiuChang Zhong[d], Anthony J. Turner[e], Mohan K. Raizada[f], Maria B. Grant[g], Gavin Y. Oudit[a,b,c*]

[a]Department of Physiology; [b]Division of Cardiology, Department of Medicine, University of Alberta, Edmonton, Canada; [c]Mazankowski Alberta Heart Institute, University of Alberta, Edmonton, Canada; [d]Heart Center and Beijing Key Laboratory of Hypertension, Beijing Chaoyang Hospital, Capital Medical University, China; [e]School of Biomedical Sciences, University of Leeds, United Kingdom; [f]Department of Physiology and Functional Genomics, College of Medicine, University of Florida, Gainesville, Florida, USA, and; [g]Department of Ophthalmology and Visual Sciences, University of Alabama at Birmingham, USA.

[#]these authors made equal contributions.

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Address correspondence to:
Dr. Gavin Y. Oudit
Division of Cardiology
Department of Medicine
Mazankowski Alberta Heart Institute
University of Alberta
Edmonton, Alberta, T6G 2S2
Canada
Tel: 780-407-8569
gavin.oudit@ualberta.ca

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ABSTRACT

Angiotensin-converting enzyme (ACE2) has a multiplicity of physiological roles that revolve around its trivalent function: a negative regulator of the renin-angiotensin system (RAS), facilitator of amino acid transport, and the SARS-CoV and SARS-CoV-2 receptor. *ACE2* is widely expressed, including, in the lungs, cardiovascular system, gut, kidneys, central nervous system, and adipose tissue. ACE2 has recently been identified as the SARS-CoV-2 receptor, the infective agent responsible for COVID-19, providing a critical link between immunity, inflammation, ACE2, and cardiovascular disease. Although sharing a close evolutionary relationship with SARS-CoV, the receptor-binding domain of SARS-CoV-2 differs in several key amino acid residues, allowing for stronger binding affinity with the human ACE2 receptor, which may account for the greater pathogenicity of SARS-CoV-2. The loss of ACE2 function following binding by SARS-CoV-2 is driven by endocytosis and activation of proteolytic cleavage and processing. The ACE2 system is a critical protective pathway against heart failure with reduced and preserved ejection fraction including, myocardial infarction and hypertension, and against lung disease and diabetes. The control of gut dysbiosis and vascular permeability by ACE2 has emerged as an essential mechanism of pulmonary hypertension and diabetic cardiovascular complications. Recombinant ACE2, gene-delivery of *Ace2*, Ang 1–7 analogs, and Mas receptor agonists enhance ACE2 action and serve as potential therapies for disease conditions associated with an activated RAS. Recombinant human ACE2 has completed clinical trials and efficiently lowered or increased plasma angiotensin II and angiotensin 1-7 levels, respectively. Our review summarizes the progress over the past 20 years, highlighting the critical role of ACE2 as the novel SARS-CoV-2 receptor and as the negative regulator of the RAS, together with implications for the COVID-19 pandemic and associated cardiovascular diseases.

Keywords:
Angiotensin II receptor blocker, Angiotensin 1–7, Angiotensin converting enzyme 2, heart failure, recombinant human ACE2, coronavirus, renin angiotensin system, cardiovascular disease, COVID-19.

Nonstandard Abbreviations and Acronyms:

ACE	- Angiotensin converting enzyme
ACE2	- Angiotensin converting enzyme 2
ACEi	- ACE inhibitor
ADAM-17	- A disintegrin and metalloproteinase 17
AICAR - 5	-amino-4-imidazolecarboxamide riboside
AMPK	- Adenosine monophosphate kinase
Ang	- Angiotensin
ARB	- AT_1R blocker
AT_1R	- Angiotensin II type 1 receptor
CVD	- Cardiovascular disease
eNOS	- Endothelial nitric oxide synthase
ERK1/2	- Extracellular signal-regulated kinase 1/2
HF	- Heart failure
HFpEF	- Heart failure with preserved ejection fraction
HFrEF	- Heart failure with reduced ejection fraction
MAPK	- Mitogen activated protein kinase
MasR	- Mas receptor
MI	- Myocardial infarction
MMP	- Matrix metalloproteinases
NEP	- Neprilysin
Nox2	- NADPH oxidase 2
PCP	- Prolyl carboxypeptidase
NADPH	- Nicotinamide adenine dinucleotide phosphate
PH	- Pulmonary hypertension

RAS	- Renin-angiotensin system
ROS	- Reactive oxygen species
rhACE2	- Recombinant human ACE2
TACE	- Tumor necrosis factor-α converting enzyme
SARS	- Severe Acute Respiratory Syndrome
COVID-19	- Coronavirus disease 2019
SARS-CoV	- SARS Coronavirus

INTRODUCTION

Knowledge of the underlying biology and physiology of angiotensin converting enzyme 2 (ACE2) has accumulated over the last 20 years since its discovery and has provided a major stimulus to further our understanding of the renin-angiotensin system (RAS).[1-4] ACE2 has distinct roles ranging from catalytic activities with various substrates, as functional receptors for severe acute respiratory syndrome (SARS) coronaviruses (SARS-CoV), and as an amino acid transporter.[5-8] ACE2 functions as a master regulator of the RAS mainly by converting angiotensin (Ang) I and Ang II into Ang 1–9 and Ang 1–7, respectively.[1, 4] Both loss-of-function and gain-of-function approaches in experimental models of human diseases have defined a critical role for ACE2 in heart failure (HF), systemic and pulmonary hypertension, myocardial infarction (MI), and diabetic cardiovascular (CV) complications.[1] Gut dysbiosis and altered gut permeability have emerged as an important mechanism of disease controlled by the ACE2 axis in both vascular and lung diseases,[9, 10] as well as in diabetes.[11] Clinical and experimental studies support a physiological and pathophysiological role for ACE2 in cardiovascular disease (CVD), and increasing/activating ACE2 may elicit protective effects against hypertension and CVD, although this has yet to be proven clinically.[1, 12-14]

More recently, ACE2 has garnered widespread interest as the cellular receptor of SARS-CoV-2, the causative virus of the COVID-19 pandemic, which emerged from Wuhan, China, in late 2019.[4, 15, 16] ACE2 offers protection in acute lung injury, suggesting that, although it facilitates viral entry at the epithelial surface, the ACE2/Ang 1–7 axis can be carefully manipulated to mitigate SARS induced tissue injuries, which represents a potential target for therapeutic intervention.[17, 18] In experimental models of lung disease, catalytically active ACE2 alleviates pulmonary injury and vascular damage[17, 19], and prevent pulmonary hypertension, decreased lung fibrosis, arterial remodeling, and improved right ventricular performance[12] due to a combination of direct action in the lungs and via the ACE2-dependent gut-lung axis.[19, 20] In two phase II clinical trials, administration of ACE2 was shown to reduce systemic inflammation and shifted the RAS peptide balance away from Ang II towards Ang 1–7.[21, 22] Ongoing global efforts are focused on manipulating the ACE2/Ang 1–7 axis to curtail SARS-CoV-2 infection while affording maximal protective effects against lung and CV damage in patients with COVID-19. In this review, we summarize the diverse roles of ACE2, highlighting its role as the SARS-CoV-2 receptor and negative regulator of the RAS, and the implications for the COVID-19 pandemic. We also provide a framework for developing novel therapeutic strategies exploring the ACE2 pathway as it relates to CVD and COVID-19.

I. Basic Biochemistry

A. Discovery of ACE2.

Following the initial and seminal discovery of renin in 1898 by Tigerstedt and Bergman, the RAS now encompasses a complex network of enzymes, peptides, and receptors (**Figure 1**).[2, 3, 15, 16, 23-27] While many metallopeptidases cluster in small inter-related gene families (e.g., the neprilysin family), unusually, no human homolog of the vasoactive zinc-peptidase angiotensin converting enzyme (ACE) had been identified at the turn of the century. Almost simultaneously, in 2000, two independent approaches searching for such ACE homologs revealed the existence of a close relative of the *ACE* gene designated *ACEH*[2] or *ACE2*[3]. *ACEH* was cloned from a human lymphoma cDNA library and the identical *ACE2* from a human HF ventricular cDNA library, the latter emphasizing a potential role for ACE2 in CV pathologies. Expression of the *ACE2* gene was initially established in the heart, kidney and, testis, but subsequent studies have shown a much broader distribution, including the upper airways, lungs, gut, and liver (**Figure 2A**). Sequence comparison of ACE and ACE2 strongly suggested that ACE2, like ACE, was an integral transmembrane protein (and ectoenzyme) with a transmembrane anchor close to the C-terminus (type I membrane protein). A close evolutionary relationship existed between the *ACE* and *ACE2*, genes and it was presumed that the two proteins would have similar substrate specificities and involvement in the RAS.

As it turned out, important differences occur, particularly in the active site regions of the enzymes, such that the two enzymes counterbalance rather than reinforce each other's actions. Many subsequent studies over the next twenty years have revealed their inter-relationship, respective roles in the RAS, and multiple physiological and pathological actions from vasoactive peptide metabolism, importantly including not only Ang II but also apelin, to intestinal amino acid transport affecting innate immunity, to lung function and brain amyloid metabolism (converting Aβ43 to Aβ42, a substrate for ACE).[1, 4, 28] Another unexpected twist in ACE2 biology was its identification in 2003 as the cell-surface receptor for the then newly identified SARS-CoV that led to more than 8000 cases of SARS and almost 800 deaths,[5] and as the receptor for SARS-CoV-2 that is currently devastating many countries worldwide.[29, 30]

B. The ACE2 gene and basic biochemistry.

Unlike the *ACE* gene, which is located on human chromosome 17, the 40kb *ACE2* gene is located on chromosome Xp22 and contains 18 exons, most of which resemble exons in the *ACE* gene. Whereas somatic ACE contains two active sites, ACE2 possesses only a single catalytic domain. Both ACE and ACE2 act as zinc metallopeptidases but of differing substrate specificities defining their distinct and counterbalancing roles in the RAS. Whereas ACE cleaves C-terminal dipeptide residues from susceptible substrates (a peptidyl dipeptidase), ACE2 acts as a simple carboxypeptidase able to hydrolyze Ang I, forming Ang 1–9 and Ang II to Ang 1–7 (**Figure 2B**). ACE2 does not cleave bradykinin, further distinguishing its specificity from that of ACE while it is also insensitive to conventional ACE inhibitors.[2, 28] The C-terminal domain of ACE2 which has no similarity with ACE, is a homolog of a renal protein, collectrin, which regulates the trafficking of amino acid transporters to the cell surface, endowing ACE2 with multiple and distinctive physiological functions. It is the multiplicity of physiological roles that ACE2 plays that has allowed it to be hijacked by SARS-CoV-2 as a receptor, resulting in the COVID-19 pandemic.[15, 16] Structural studies have revealed the structures of both the SARS-CoV and much more recently, the SARS-CoV-2 in complex with ACE2 (**Figure 2B**).[31, 32] In the case of SARS-CoV-2, the major spike glycoprotein (S1) binds to the N-terminal region of ACE2. The knowledge of the biology and physiology of ACE2 accumulated over the last 20 years since its discovery should provide a major stimulus to understanding some of the key steps in SARS-CoV-2 infection and its ultimate prevention.

II. Role of ACE2 in COVID-19

A. COVID-19 Pandemic.

On March 11, 2020, the World Health Organization declared the outbreak of SARS-CoV-2 a global pandemic, reporting community scale transmissions occurring in every continent outside Antarctica. Since then, the outbreak has escalated to well over one million cases and caused over 60,000 deaths worldwide by the start of April 2020. However, before the emergence of SARS-CoV in 2002, coronaviruses were conventionally viewed as inconsequential pathogens circulating in nature throughout various host and intermediate species that occasionally infected humans causing only mild upper respiratory tract infections and symptoms of the common cold.[33-35] As such, to better understand the severity of global health risks posed by SARS-CoV-2 and optimize treatment for infected patients, we must recognize the role of ACE2 in SARS-CoV-2 pathogenesis. In addition to respiratory involvement, multi-organ dysfunction occurs in response to SARS-CoV-2 infections.[36-38] While respiratory symptoms are predominant, acute cardiac and kidney injuries, arrhythmias, gut, and liver function abnormalities have all been documented in infected patients, suggesting myocardial, renal, enteric and hepatic damage in COVID-19. Similarly, SARS-CoV also resulted in systemic manifestations with damages to the heart, gastrointestinal, liver, kidney, and other tissues.[39, 40]

B. ACE2 as the Receptor for SARS-CoV-2.

SARS-CoV-2 differs from the original SARS-CoV by 380 amino acid substitutions, which translates to differences in five of the six vital amino acids in the receptor-binding domain between the viral spike (S) protein with surface expressed human ACE2.[41] Viral S-proteins are well established as a significant determinant of host tropism and represents a key target for therapeutic and vaccine development. Additionally, host cell proteases are important for SARS-CoV-2 entry and infection of cells as both S-proteins and ACE2 are proteolytically modified during the process. The binding affinity of SARS-CoV-2 with ACE2 appears stronger than SARS-CoV, with alterations in several amino acid residues allowing for enhanced hydrophobic interactions and salt bridge formations, which may explain the considerably larger global influence of COVID-19 than the initial SARS.[16, 42] Moreover, SARS-CoV-2 has evolved to utilize a wide array of host proteases including cathepsin L, cathepsin B, trypsin, factor X, elastase, furin, and transmembrane protease serine 2 (TMPRSS2) for S-protein priming and facilitating cell entry following receptor binding.[43] So far, TMPRSS2 and cathepsin L/B mediates S-protein priming of SARS-CoV-2, and camostat mesylate, a serine protease inhibitor combined with cathepsin L/B inhibitor, E-64d blocked SARS-CoV-2 entry.[44] The entry of both SARS-CoV and SARS-CoV-2 into cells is facilitated by the interaction between viral S-protein with extracellular domains of the transmembrane ACE2 proteins, followed by subsequent downregulation of surface ACE2 expression (**Figure 3**).[5, 15, 29] In a cohort of 12 COVID-19 patients, circulating Ang II levels were markedly elevated compared to healthy controls (linearly correlated with viral load), providing a direct link between tissue ACE2 downregulation with systemic RAS imbalance, and facilitating the development of multi-organ damage from SARS-CoV-2 infections.[4, 45] Potential therapeutic strategies may include preventing the binding of human ACE2 and SARS-CoV-2 by blocking the receptor-binding domain (RBD) of the viral S-protein. In addition to this RBD blocking strategy, other possible treatment options may include localized use of ACE2-derived peptides, small molecule inhibitors, ACE2 antibody or single chain antibody fragment against ACE2.

C. Cardiovascular Disease in Patients with COVID-19.

In post-mortem autopsy heart tissues from twenty patients who succumbed to SARS-CoV, seven heart samples had detectable viral SARS-CoV genome, which was characterized by increased myocardial fibrosis, inflammation, and reduced myocardial ACE2 expression.[46] These patients also had a much more aggressive illness associated with earlier mortality. Additionally, bilateral pleural effusions were frequently observed during autopsy of SARS-CoV patients, further supporting the evidence of cardiac involvement.

Individuals with pre-existing diabetes, hypertension, and lung disease are at particular risk of COVID-19 infection[37, 47] and this is likely due to dysregulated RAS that occurs in these conditions.[4, 48] Significance of the SARS-CoV-2 infection in the CV system is reflected through incidences of acute myocardial injury (elevated high sensitivity troponin I levels and/or new electrocardiogram /echocardiogram abnormalities), arrhythmias, cardiac arrest, sepsis, septic shock, viral myocarditis and HF (elevated NT-proBNP levels, systolic dysfunction on cardiac magnetic resonance imaging).[49-52] Further abnormalities from laboratory tests, including elevation in D-dimers reflective of increased thrombosis risk, may lead to acute coronary syndrome, and sustained increased inflammatory cytokines levels throughout the clinical course suggest ongoing systemic and tissue inflammation in COVID-19 patients.[36, 37, 47]

D. Gut Dysbiosis and a possible link to Disease Progression in COVID-19 patients.

Ubiquitous expression of ACE2 throughout the luminal surface of the gastrointestinal (GI) tract, and most prevalently in enterocytes, may serve as a secondary site for enteric SARS-CoV-2 infection (**Figure 2A**). Leaky GI conditions in experimental models of human disease can be ameliorated and worsened with either the gain or loss of ACE2, respectively.[8, 11] COVID-19 patients also suffer from GI discomfort and diarrhea, which may arise earlier than respiratory conditions concurrent with the detection of viral RNA in feces, as seen with previous coronavirus outbreaks.[38, 47, 53-56] Moreover, common comorbidities of CVD, including diabetes and obesity, are known to affect the integrity of the GI-blood barrier and result in gut dysbiosis, bacteremia, and systemic inflammation (**Figure 4**). Development of GI leakage and gut dysbiosis have correspondingly been linked to the onset of pulmonary hypertension through the gut-lung axis and is closely related to hyperactivation of the ACE/Ang II/AT_1R axis from ACE2 loss.[20, 57] Continued viral production by host enterocytes perpetuates this situation and deteriorates conditions in the gut-lung axis.[54, 55] Evidence supports that SARS-CoV-2 infection potentially leads to degeneration of the gut-blood barrier leading to systemic spread of bacteria, endotoxins, and microbial metabolites likely affecting the host's response to COVID-19 infection and cumulating in multisystem dysfunction and septic shock.[37, 38, 47] Enteric involvement and associated worsening in patient outcomes were documented from the initial SARS-CoV outbreak in the early 2000s. Fecal viral RNA was detected in up to 70% of patients with viral shedding from the GI tract associated with a more aggressive clinical course.[54, 55] In a separate study, SARS-CoV particles were detected within the cytoplasm and surface microvilli of apical enterocytes in the ileum and colon[55] while in COVID-19 patients, SARS-CoV-2 was detected in feces suggesting fecal-oral transmission.[58] As such, the GI tract of SARS-CoV, and possibly SARS-CoV-2 patients, acts as a staging ground for sustained viral replication concurrent with disruption of the enteric ACE2 axis and adverse outcomes.[39, 47, 54, 55, 59, 60]

In addition to the direct impact of the virus on the microbiome, the predisposing disease states such as diabetes[61] and pulmonary disease have their own adverse effects on the gut microbiome,[62, 63] which may be worsened by SARS-CoV-2 infection. Ang II-dependent hypertension in animal models[64] and humans is associated with gut dysbiosis, increased gut leakiness, and gut wall pathology.[10, 63, 65, 66] There is broad support for these observations in pulmonary diseases including pulmonary hypertension, COPD, and asthma[67, 68] and in type 2 diabetes where dysbiosis characterized by decreased microbial richness and diversity, altered representation of bacterial metabolic pathways and modifications in the composition of Firmicutes (F) and Bacteroidetes (B).[69-71] ACE2 disruption in biomedical models has shown us that gut dysbiosis is quite prevalent and that this change in microbial profiles can alter systemic pathways exacerbating diabetes and hypertension. We recently showed that ACE2 deficiency magnifies diabetes-induced dysbiosis[11] characterized by an increase in peptidoglycan (PGN)-producing bacteria and loss of gut barrier integrity in *Ace2*$^{-/y}$-Akita mice. We also identified a new role of bone marrow cells in the gut. In the *Ace2*$^{-/y}$-Akita or Akita mice, the disrupted gut barrier was associated with reduced levels of circulating angiogenic cells (CACs), hematopoietic cells with reparative function. Giving exogenous CACs from wild type mice corrected gut barrier dysfunction in *Ace2*$^{-/y}$-Akita or Akita mice. Thus decreased enteric ACE2 expression from SARS-CoV-2 infection may similarly reduce CACs and compromise the integrity of the endothelium and gut epithelium leading to dysbiosis. Further examination is required to validate this link and whether it is a direct or indirect effect of viral infection.[11]

III. Link between ACE2, ADAM17, and Inflammation

A. Proteolytic Cleavage of ACE2 by ADAM-17.

Tumor necrosis factor-α (TNF-α) is a cytokine implicated in chronic inflammation, and its extracellular domain shedding and activation is driven by the membrane-bound protease coined TNF-α-converting enzyme (TACE), also known as ADAM-17.[72, 73] ADAM-17 is a type I transmembrane protein belonging to the adamalysin subfamily of Zn-dependent metalloproteases.[74] Following the discovery that ADAM-17 cleaves TNF- α, the substrate specificity of the enzyme has expanded to include various cytokines and receptors, many of which contribute to initiating and exacerbating inflammation.[75, 76] Importantly, ADAM-17 was also found to mediate proteolysis and ectodomain shedding of ACE2.[77] Enhanced ACE2 shedding resulting from RAS overactivation, and subsequent ADAM-17 upregulation drives pathogenesis in HF, atrial fibrillation, coronary artery disease, and thoracic aortic aneurysm.[1, 13, 78, 79] Ang II-mediated activation of AT_1R triggers a signaling cascade, which culminates in the activation of p38 mitogen-activated protein kinase (MAPK) and ADAM-17 phosphorylation by NAPDH oxidase 2-induced ROS formation.[80, 81] Phosphorylation enhances the catalytic activity of ADAM-17, thus increasing ACE2 shedding, resulting in loss of ACE2 at the membrane and impaired conversion of Ang II (into Ang 1–7), leading to RAS-mediated detrimental effects in a positive feedback cycle.[77, 82]

Importantly, depletion of ACE2 at the cell surface is a critical pathological outcome of SARS-CoV-2 infection. SARS-CoV-2 is endocytosed by cells in complex with ACE2; thus, the initial detrimental effects of viral infection begins with a loss of ACE2-mediated tissue protection.[83] ADAM-17 activity is upregulated upon binding of SARS-CoV to ACE2 and facilitates viral entry, while knockdown of ADAM-17 by siRNA severely attenuated SARS-CoV cellular entry.[84] The molecular mechanisms of SARS-CoV and another human coronavirus that only causes mild respiratory symptoms, HNL63-CoV, were compared. Interestingly, although HNL63-CoV also utilizes ACE2 as a receptor for cellular entry, it does not induce ADAM-17 activation and ACE2 ectodomain shedding.[84, 85] Therefore, this study elucidates the unique role of ADAM-17 mediated shedding of ACE2 in SARS-CoV infectivity and may inform the disparity in severity between coronavirus subtypes. Furthermore, loss of membrane ACE2 promotes Ang II accumulation, which also activates ADAM-17 activity, thus perpetuating membrane shedding of ACE2, RAS overactivation, and inflammation.[77]

B. ACE2, SARS-CoV-2, and Inflammation.

The modulatory effects on the Ang II/AT_1R and Ang 1–7/MasR axes make ACE2 a plausible target in preventing and treating chronic inflammation and inflammatory diseases, as highlighted by the recent COVID-19 pandemic.[29] COVID-19 patients develop pneumonia with acceleration of injury in susceptible patients to multiple organ failure[30, 86] driven in part by an inflammatory cytokine storm, and is a notable cause of death in critically ill patients.[30, 86] When the immune system is activated due to factors such as SARS-CoV-2 infection, there is an imbalance of Th17/Treg cell function and overactivation of immune cells, which secrete a large number of proinflammatory cytokines.[17, 87, 88] Imbalance in the RAS system and the loss of ACE2 in COVID-19 patients are further contributing factors to tissue and systemic inflammation.[30, 86] Lipopolysaccharide (LPS)-induced acute lung injury decreased expression of ACE2, precipitated inflammatory injury, and upregulated expression of renin, Ang II, ACE, and AT1 receptors.[89] After injection of rhACE2, lung function and pathological injury improved with attenuation of inflammation.[89] In addition, rhACE2 is beneficial and improves acute lung injury caused by SARS-CoV, acid inhalation and sepsis.[17, 18, 88]

Ace2 knockout (KO) mice showed very severe ARDS/ acute lung injury pathology, increased vascular permeability, increased pulmonary edema, neutrophil accumulation, and deterioration of lung function compared with normal WT control mice.[18, 87] ACE deficiency partially rescued the severe

phenotype of mice with a single mutation of *Ace2* in acute lung injury by further deletion of the *Ace* gene[17], suggesting that the balance of ACE2/ACE levels is the key to lung injury/lung protection during an inflammatory storm. ARBs induce ACE2, Ang 1–7, and Mas expression in line with the reduction of proinflammatory cytokines and induction of IL-10, an anti-inflammatory cytokine.[88] We showed that *Ace2* KO hypertensive mice exhibited enhancement of proinflammatory cytokines, IL-1β, IL6, TNF-α, and chemokine (C-C motif) ligand 5 while administration of rhACE2 rescued Ang II-induced T-lymphocyte-mediated inflammation.[90, 91] Blockade of Mas receptor by D-Ala7-Ang 1–7 (A-779) completely inhibited the Ang 1–7 mediated anti-inflammatory effects while AVE 0991, the agonist of Ang 1–7 receptors, mimicked the actions of Ang1-7.[88]

IV. Physiological Role of ACE2.

A. Negative Regulator of the RAS.

Discovery of ACE2 resulted in a paradigm-changing concept in all aspects of the RAS. ACE2 is a monocarboxypeptidase that converts Ang I into a nonapeptide, Ang 1–9, and Ang II into a heptapeptide, Ang 1–7 (**Figure 5A**). This distinct enzymatic pathway for degradation of Ang I and Ang II negatively regulates RAS activation and mitigates the deleterious actions mediated by Ang II and AT_1R.[1] This is of particular significance in pathological conditions where the RAS is overstimulated. Ang 1–7 is a biologically active peptide whose vast array of effects are opposite to those attributed to Ang II.[92-98] Furthermore, ACE2 can antagonize ACE independent formation of Ang II, such as from mast cell chymase.[13, 99] In 2003, an endogenous orphan receptor, Mas (MasR), was identified as the Ang 1–7 receptor. A779, a MasR antagonist, blocks the majority of Ang 1–7 effects.[94, 100-103] Ang 1–9 has also shown beneficial biological effects via the AT_2R that result in cardioprotection.[104-107] Thus, the ACE2/Ang 1–7/MasR axis has emerged as a physiological antagonist that counter-regulates the activated RAS.[93, 108-112] The cardioprotective effects of ACE2 taken together can be attributed to i) degradation of Ang I to Ang 1–9, whereby limiting action of ACE on its substrate, ii) reducing Ang II detrimental effects through degradation of the peptide, and iii) formation of Ang 1–7 which exercises cardioprotective effects. Formation of Ang 1–7 is an important mechanism of ACE2 mediated protection, as antagonism of Ang 1–7 using A779 prevented beneficial effects of rhACE2 in murine model of systolic dysfunction.[113] Diminished ACE2 activity results in activation of the Ang II/AT1R axis, contributing to the increased progression of CVD. Elevated ACE2 level and activity result in the formation of Ang 1–9 and Ang 1–7, leading to protection against CVD. (**Figure 5A**).

B. Interaction with Apelin Peptides.

The apelin family of peptides act through the apelin receptors mediating protection against CVD.[114, 115] The X-linked *APLN* gene encodes a 77 amino acids pre-pro-apelin that is subsequently cleaved by endopeptidases to various bioactive peptides from 13 to 36 amino acids in length. CVD, including HF and hypertension, is characterized by an apelin deficient state in both human myocardium and plasma.[116-118] Apelin KO mice exhibit increased infarct size and systolic dysfunction following coronary ligation and reduced myocardial contractility concomitant with increased susceptibility to HF in pressure-overload models.[119, 120] Reduced myocardial *Ace2* mRNA and ACE2 protein levels in apelin KO mice, which were rescued by infusion of apelin-13, suggest a crucial regulatory role of apelin in *Ace2* gene expression.[121] Apelin signaling through the apelin receptors specifically increased *Ace2* promoter activity leading to an increase in *Ace2* mRNA and protein.[121-123] These effects are consistent with the ability of the pyr-apelin-13 peptide to negatively regulate Ang II-mediated superoxide production, myocardial hypertrophy, dysfunction, and fibrosis[123] and analogs of apelin-17 preventing abdominal aortic rupture in low-density lipoprotein receptor KO models induced by Ang II infusion.[124] However, ACE2 through its monocarboxypeptidase activity cleaves and inactivates bioactive apelin peptides apelin-13 and apelin-36 through a negative feedback mechanism in the heart and vasculature (**Figure 5B**).[28, 125] Due to the short half-life of endogenous apelin peptides in the plasma, synthetic apelin peptide analogs resistant to ACE2

degradation and retaining their binding capability to endogenous apelin receptors elicit protection in the CV system are being explored as potential new therapies.[114, 124]

C. ACE2 as a chaperone protein for the amino acid transporter, B^0AT1 (SLC6A19).

B^0AT1 is highly expressed in the intestines and kidneys with function in the absorption of neutral amino acids.[126] The ACE2-B(0)AT1 complex is assembled as a dimer of heterodimers, with the collectrin-like domain of ACE2 mediating homo-dimerization.[16] ACE2 has a RAS-independent function, regulating intestinal amino acid homeostasis, expression of antimicrobial peptides, and the gut microbiome.[8] ACE2 is necessary for the expression of the Hartnup transporter in the intestine, and the differential functional association of mutant B(0)AT1 transporters with ACE2 in the intestine regulates the phenotypic heterogeneity of human Hartnup disorder.[126]

V. Role of ACE2 in CV and Lung Diseases

A. ACE2 and Heart Disease.

Cardiovascular disease is the leading cause of death worldwide and a major public health concern. Heart disease is characterized by the activation of several signaling pathways associated with pathological hypertrophy and maladaptive ventricular remodeling. In the heart, ACE2 is localized to cardiomyocytes, cardiac fibroblasts, epicardial adipose tissue, and the coronary vascular endothelium[77, 127, 128]; Ang 1–7/MasR is also present on cardiomyocytes, cardiac fibroblasts, and endothelial and vascular smooth muscle cells.[100, 129-131] Genetic *Ace2* deletion resulted in exacerbation of Ang II-mediated cardiorenal fibrosis and oxidative stress in the heart and kidney of hypertensive mice while administration of recombinant human ACE2 (rhACE2) remarkably rescued the Ang II-induced hypertension, pathological hypertrophy, oxidant injury and cardiac dysfunction.[90, 91]

Various ACE2 polymorphisms are linked to CVD.[132] Post-MI remodeling, and coronary artery disease are common causes of HF.[1, 133] Notably, MI increases *ACE2* mRNA expression in human, mice and rat hearts[134, 135], whereas genetic ACE2 deletion results in worsening of MI-induced cardiac dysfunction, infarct size, matrix metalloproteinase (MMP)2/MMP9 activation and extracellular matrix disruption.[134, 135] Loss of ACE2 leads to increased neutrophilic infiltration in the infarct and peri-infarct regions, resulting in upregulation of inflammatory cytokines, interferon-γ, interleukin (IL)-6, and the chemokine, monocyte chemoattractant protein-1 (MCP-1), as well as increased phosphorylation of ERK1/2 and JNK1/2 signaling pathways, changes that were blocked with an ARB ultimately resulting in improvement in myocardial function.[135] In contrast, overexpression of ACE2 and the action of Ang 1–7 ameliorates MI-induced cardiac remodeling.[136, 137] Importantly, heterozygote loss of ACE2, as seen in explanted human hearts from patients with dilated cardiomyopathy, was sufficient to increase susceptibility to heart disease.[138]

HF with preserved ejection fraction (HFpEF) is a proinflammatory state closely linked to obesity-related cardiac and microvascular dysfunction for which there are no approved therapies.[128, 139, 140] Epicardial adipose tissue (EAT) is a primary source of inflammatory cytokines that could have detrimental effects on the heart.[139] Loss of ACE2 increases macrophage polarization to proinflammatory M1-phenotype (alternatively activated, $CD11c^+$) in EAT from patients with HFpEF, with decreases in polarization to anti-inflammatory, M2-phenotype macrophages and worsening of HFpEF in response to diet-induced obesity.[139] Importantly, Ang 1–7, decreased macrophage polarization in EAT and preserved the cardiac function of obese *Ace2* KO mice.[128, 141] Ang 1–7 has potent anti-inflammatory effects in adipose tissue of obese type 2 diabetic mice and protects against diabetic cardiomyopathy and nephropathy.[141-143] The ACE2/Ang 1–7 axis also promotes browning of adipose tissue leading to improved metabolic effects and weight loss, which can confer further benefits to the CV system.[144, 145]

B. ACE2 and Vascular Disease.

Blockade of the deleterious arm of the RAS has been the mainstay of the therapeutic management of hypertensive individuals. An increase in ACE2 and the vasoprotective axis of the RAS by angiotensin-converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARBs) clearly reinforces this view (see **section VI.A below**). Furthermore, increased ACE2 expression protects against hypertension, while ACE2 deficiency exacerbates hypertension. Renal *Ace2* expression is inversely related to blood pressure in experimental models of hypertension.[25] In the spontaneously hypertensive rat (SHR) and stroke-prone SHR, renal *Ace2* mRNA levels are reduced compared to normotensive Wistar-Kyoto rats.[25] These studies support the essential role of ACE2 in maintaining healthy blood pressure. Lentiviral overexpression of ACE2 results in increased expression of anti-hypertensive components of the RAS and attenuates elevated blood pressure.[146, 147] Pretreatment with rhACE2 prevented hypertension induced by Ang II and decreased plasma Ang II while increasing plasma Ang 1–7 levels.[148] ACE2 and ADAM17 were selectively knocked down from all neurons (AC-N), which revealed a reduction of inhibitory inputs to AC-N presympathetic neurons relevant for blood pressure regulation. Mice with ACE2 selectively knocked down from Sim1 neurons in mice exhibited a blunted blood pressure elevation and preserved ACE2 activity during the development of salt-sensitive hypertension.[14] The metalloproteinase ADAM17 is responsible for mediating ACE2 shedding from the cell membrane-bound domain, which can be promoted by Ang II, and release of ACE2 as a soluble form in plasma[14, 77, 149] impairing brain ACE2 compensatory activity and thus contributing to the development of neurogenic hypertension.[150] Genetic *Ace2* deficiency is associated with the upregulation of putative mediators of atherogenesis and enhances responsiveness to proinflammatory stimuli suggestive of a key role of ACE2 in suppressing vascular inflammation and atherosclerotic disease.[151] In addition, ACE2 inhibition blocks neuropeptide catestatin-mediated protective effects in the development of atherosclerosis in $ApoE^{-/-}$ mice fed a high-fat diet.[152]

C. ACE2 in Diabetic Cardiovascular Complications.

The counter-regulatory role of the ACE2/Ang 1–7/MasR axis of the RAS has been well-characterized in the progression of diabetic complications, including CV and kidney disease.[1, 153, 154] Support for the importance of ACE2 in diabetes comes from its impact on diabetic complications wherein diabetes-induced vascular dysfunction is strongly associated with a shift in the RAS Axis towards the profibrotic, proinflammatory arm of RAS with a reduction in the protective arm (**Figure 6.**). Loss of the protective effects of the RAS is related to the regulation of tissue and circulating levels of Ang II and their sequelae in the context of diabetes.[155, 156] Alterations within the RAS are considered pivotal for the development of both diabetic micro and macrovascular complications.[1, 157]

The blockade of the proinflammatory and profibrotic arms of the RAS provides significant renoprotection in both experimental models of diabetes and in patients. While the loss of ACE2 worsens diabetic kidney injury[158], rhACE2 is therapeutic in an animal model of diabetic nephropathy[153] and experimental Alport syndrome.[159] ACEi in T1D and angiotensin receptor blockade with losartan and irbesartan in T2D retard the progression of nephropathy.[160] In diabetic renal tubules, ACE2 gene expression is decreased by ~50%, which would reduce Ang 1–7 formation and allow Ang II accumulation hence directly increasing the expression of TGF-β and connective tissue growth factor (CTGF), leading to tubulointerstitial fibrosis.[161] RAS blockade retards renal damage and ACE inhibitor therapy, as mentioned above, resulting in a compensatory increase in ACE2, leading to renoprotection.[91] Therefore support for the loss of ACE2 contributing to vascular complications in diabetes comes from strong clinical and experimental evidence.[1]

Retinopathy, the most common complication of diabetes and one of the leading causes of blindness in working-age adults, is linked to activation of oxidative stress, profibrotic, and proinflammatory arm of the RAS which can be effectively curtailed by the ACE2/Ang 1–7 axis in experimental models.[162, 163] Increased secretion of proinflammatory cytokines by bone marrow mesenchymal stem cells (MSCs) skews hematopoiesis towards the generation of an increased number of myelo-monocytic cells.[164] Target tissues

of diabetic complications secrete CCL2 in response to high glucose-induced stress[165] facilitating the homing of CCR2$^+$cells to these regions and promoting the development of vascular complications.[166-171] In addition to an increase in myeloidosis, diabetics with complications have reduced bone marrow-derived vascular reparative cells and circulating angiogenic cells (CD34$^+$cells).[11] Levels of *ACE2* mRNA were also a significant predictor of the presence of microvascular disease in diabetic patients.[172] Diabetic individuals that remained free of retinopathy despite >40 years of poor glycemic control had higher mRNA levels for genes of the vasoprotective axis (ACE2/Mas) compared to age, sex, and glycemia-matched diabetics with retinopathy.[172] In dysfunctional CD34$^+$ cells from diabetic individuals, activation of the protective arm of RAS, by exposing the cells to Ang1–7 corrected their dysfunction by restoring bioavailable NO and reducing ROS. Ang1–7 gene modification of CD34$^+$ cells restored the in vivo vasoreparative function of these cells in a mouse retinal ischemia-reperfusion injury model.[172] Moreover, intraocular administration of AAV-ACE2 or Ang1–7 reduced diabetes-induced retinal vascular leakage and inflammation, thus preventing retinopathy.[163]

Patients with diabetes have a dysregulated RAS, which may influence their vulnerability to SARS-CoV-2. Guan *et al.* examined 1099 individuals with confirmed COVID -19. Of these individuals, 173 had severe disease, and of this, 16.2% were diabetics.[47] Zhang *et al.* studied 140 patients that were hospitalized due to the severity of their COVID-19 infection, of these individuals, 12% had diabetes. It is interesting to speculate why diabetics may be more at risk for SARS-CoV-2 infection than the general population, and this may be due to the reduced ACE2 levels that are typically observed in the vasculature of diabetic individuals and diabetic animal models.[173] Indeed, loss of ACE2 was associated with marked gut dysbiosis, which was further worsened in a model with type 1 diabetes.[11]

D. ACE2 and Lung Disease.

Lung epithelial cells express high levels of ACE2, which positively correlates with airway epithelial differentiation.[17, 19, 174] Involvement of ACE2 in acute respiratory distress syndrome (ARDS), which is triggered by multiple diseases including SARS-CoV and SARS-CoV-2, has been established in multiple animal models.[18, 175] *Ace2* KO mice exhibit severe pathology of ARDS.[17, 19] Additional *Ace* deficiency, or treatment with AR1R blockers of *Ace2* KO mice rescues them from ARDS implicating the benefit of ACE2 and the critical balance of the protective vs. proinflammatory and fibrotic axes of the RAS.[18] These findings are consistent with evidence of a beneficial effect of rhACE2 on pulmonary blood flow and oxygenation in a pig model of LPS induced ARDS.[176] Age-related loss of ACE2 in the lungs correlates with the increased mortality and worsened phenotype in elderly patients with COVID-19.[174]

ACE2 has been implicated in acute lung injury (ALI) by inducing an imbalance in the RAS. Evidence includes that in ALI (i) a decrease in pulmonary ACE2 and an increase in Ang II levels occurs; (ii) supplementation with ACE2 or inhibition of Ang II improves outcomes; and (iii) a lack or decrease of pulmonary ACE2 aggravates viral-induced ALI. ACE2 is also involved in pulmonary hypertension (PH) and fibrosis.[19] Increasing ACE2 activity using rhACE2 reduced bleomycin-induced inflammation and fibrosis, resulting in improved lung function and exercise capacity[19], and the ACE2 activator, DIZE, protects animals from PH and fibrosis.[177] Moreover, oral feeding of a bioencapsulated form of ACE2 protects and arrests the progression of PH.[12] Validation of this protective effect comes from a small human study that showed that PH is characterized by reduced ACE2 activity and supplementation of these individuals with rhACE2 improved pulmonary hemodynamics and reduced oxidative and inflammatory markers.[21] Collectively, these studies unequivocally establish the conceptual framework that ACE2 is a central player in normal pulmonary function, and its imbalance leads to pulmonary diseases.

VI. Targeting ACE2 for Therapeutics

A. Pharmacological Antagonists of the RAS and ACE2 Expression.

Pathological neurohormonal activation of the RAS drives the development and progression of CVD. Current pharmacotherapies aim to achieve multilevel RAS inhibition through distinct modes of action. Although ACE2 is not the direct cellular target of these therapies, *Ace2* gene transcription, translation, and ultimately catalytic activities are modified due to the intricate nature of the RAS. Blocking the ACE/Ang II/AT_1R axis through limiting the formation and actions of Ang II potentiates the effects of ACE2 as the endogenous RAS counter-regulator. The ARBS consistently increased *Ace2* mRNA expression, protein levels, and catalytic activities in the heart, kidneys, and thoracic aorta, but the translation to protein levels and activity differs between experimental models and tissues for ACEi (**Table**).[77, 178-185] Combination of lisinopril and losartan treatment in normotensive Lewis rats abolished the increase in *Ace2* mRNA levels observed individually but retained losartan induced rise in ACE2 activity in the heart.[179] Moreover, lisinopril in normotensive Lewis rats increased *Ace2* mRNA without affecting ACE2 activity in the heart, but the opposite was observed in the kidneys.[179, 180] These findings could be attributed to tissue-specific regulation of ACE2, as higher ACE2 protein levels were reported in the heart, but ACE2 activity was higher in the kidneys of Sprague-Dawley rats, adding to the complexity of the tissue RAS.[186] In type 1 diabetic Akita angiotensinogen-transgenic mice, dual RAS blockade with perindopril and losartan normalized disease-mediated reduction in kidney *Ace2* mRNA expression and protein levels.[187] These findings suggest that the accumulation of Ang II in pathological conditions contributes to the modulatory effects of RAS blockade on ACE2.

Ang II can regulate *ACE2* expression through the AT_1R. Healthy hearts and kidneys are characterized by high levels of *ACE2* mRNA and protein expression, with moderate expression of *ACE*.[188] RAS overactivation in CVD increases AT1R stimulation by Ang II, promoting ERK1/2 and p38 MAPK signaling pathways to downregulate *ACE2* while upregulating *ACE* expression.[188] Activation of p38 MAPK upregulates ADAM17 activity though post-translational phosphorylation of the cytoplasmic domain results in shedding of surface ACE2 in a positive feedback loop and could explain the observed effects of ARBs in increasing ACE2 protein levels and activity.[77, 80, 81] Mechanisms behind the augmentation of *ACE2* mRNA levels by ACEi and ARBs require further characterization. Moreover, mineralocorticoid receptor antagonists (MRA) increased *ACE2* mRNA expression and activity in samples from chronic HF patients, wildtype mice and rats to varying degrees among different tissues, but not in the heart of a rat hypertensive disease model (**Table**).[189-191] Spironolactone, a non-selective MRA, prevented the increase in both *ACE* and *AT_1R* mRNA levels, and the associated increase in AT_1R density from aldosterone signaling in cardiomyocytes.[192, 193] Activation of mineralocorticoid receptors also stimulates overlapping downstream signaling pathways with AT_1R, including the ERK1/2 and p38 MAPK pathways mentioned before.[194, 195] Blocking these signaling pathways contributes to the observed effect of MRA on *ACE2* gene expression, surface protein levels, and activity.

B. Enhancing ACE2 action.

Promoting the ACE2/Ang 1–7/Mas signaling by rhACE2 or the Ang 1–7 receptors agonist AVE 0991 can have salutary therapeutic effects in CVD and lung disease from diverse etiologies.[1] The Ang 1–7 receptors agonist AVE 0991 has been shown to exert cardiorenal and pulmonary protective effects,[88] and treatment with rhACE2 improved the symptoms of acute lung injury, CVD, and kidney injury in various preclinical models.[17, 87, 88, 90] Maintaining ACE2 levels in patients with or predisposed to common CVD states such as diabetes, hypertension, and obesity wards off the advancement of these comorbidities in instances where the patient contracts SARS-CoV-2 by maintaining a level of ACE2/Ang1–7/MasR negative counter-regulation.

rhACE2 functionally sequesters circulating viral particles to prevent S-protein interactions with endogenous ACE2, while simultaneously regulating the systemic RAS may provide therapeutic benefits in

COVID-19 and is moving into phase II clinical trials in Europe.[196] A potential limitation of rhACE2 is the restricted penetrance and activity against tissue RAS owning to its large molecular size. Pharmacological RAS blockade agents, ARBs, in particular, are capable of modulating both systemic and tissue RAS, and simultaneously increasing ACE2 expression and activity in experimental models. The direct implications of RAS inhibition in COVID-19 patients with hypertension remain elusive, and clinical evidence is desperately needed to determine the relative benefits and risks associated with usage of these medications.[197] Nonetheless, introducing ARBs to patients already infected by SARS-CoV-2 may be an effective therapeutic option in addressing the viral-mediated RAS imbalance, and is currently under investigation in several clinical trials (ClinicalTrial.gov number NCT04312009, NCT04311177, and NCT04318418).[198-200]

Potential for ACE2 as a therapy is also facilitated by using the probiotic species *Lactobacillus paracasei* (LP), which can be engineered to express recombinant proteins. Mice treated with the recombinant LP expressing the secreted ACE2 in fusion with the non-toxic subunit B of cholera toxin (acts as a carrier to facilitate transmucosal transport) showed increased ACE2 activities in serum and tissues and reduced diabetic retinopathy.[201] These results provide proof of concept for using bioengineered probiotic species as live vectors for delivery of human ACE2 with enhanced tissue bioavailability for treating diabetic complications but could potentially be repurposed for treating CVD and COVID-19 infection.

Conclusions.

Since the discovery of ACE2 in 2000, tremendous progress has been made in elucidating its biochemical actions and fundamental role in CVD and, more recently, as the SARS-CoV-2 receptor. ACE2 is a dominant mechanism for negative regulation of the RAS by metabolizing Ang II into the beneficial peptide Ang 1–7, and this important biochemical and physiological property is being harnessed as a potential therapy in patients with HF. The activation of the RAS axis due to binding of SARS-CoV-2 to ACE2, leading to direct loss of ACE2 and indirectly via proteolytic processing and shedding, partly drives the systemic manifestations of COVID-19. Careful targeting of the RAS axes is needed in these patients to optimize their clinical outcomes, including the use of AT1 receptor blockers (ARB).

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DISCLOSURES
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REFERENCES

1. Patel VB, Zhong JC, Grant MB, Oudit GY. Role of the ace2/angiotensin 1-7 axis of the renin-angiotensin system in heart failure. *Circulation research*. 2016;118:1313-1326.
2. Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *The Journal of biological chemistry*. 2000;275:33238-33243.
3. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, Donovan M, Woolf B, Robison K, Jeyaseelan R, Breitbart RE, Acton S. A novel angiotensin-converting enzyme-related carboxypeptidase (ace2) converts angiotensin i to angiotensin 1-9. *Circulation research*. 2000;87:E1-9.
4. Wang K, Gheblawi M, Oudit GY. Angiotensin converting enzyme 2: A double-edged sword. *Circulation*. Mar 26, 2020. doi:10.1161/CIRCULATIONAHA.120.047049. [epub ahead of print]
5. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC, Choe H, Farzan M. Angiotensin-converting enzyme 2 is a functional receptor for the sars coronavirus. *Nature*. 2003;426:450-454.
6. Turner AJ, Hiscox JA, Hooper NM. Ace2: From vasopeptidase to sars virus receptor. *Trends Pharmacol. Sci.* 2004;25:291-294.
7. Clarke NE, Turner AJ. Angiotensin-converting enzyme 2: The first decade. *International journal of hypertension*. 2012;2012:307315.
8. Hashimoto T, Perlot T, Rehman A, Trichereau J, Ishiguro H, Paolino M, Sigl V, Hanada T, Hanada R, Lipinski S, Wild B, Camargo SM, Singer D, Richter A, Kuba K, Fukamizu A, Schreiber S, Clevers H, Verrey F, Rosenstiel P, Penninger JM. Ace2 links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature*. 2012;487:477-481.
9. Qi Y, Kim S, Richards EM, Raizada MK, Pepine CJ. Gut microbiota: Potential for a unifying hypothesis for prevention and treatment of hypertension. *Circulation research*. 2017;120:1724-1726.
10. Santisteban MM, Qi Y, Zubcevic J, Kim S, Yang T, Shenoy V, Cole-Jeffrey CT, Lobaton GO, Stewart DC, Rubiano A, Simmons CS, Garcia-Pereira F, Johnson RD, Pepine CJ, Raizada MK. Hypertension-linked pathophysiological alterations in the gut. *Circulation research*. 2017;120:312-323.
11. Duan Y, Prasad R, Feng D, Beli E, Li Calzi S, Longhini ALF, Lamendella R, Floyd JL, Dupont M, Noothi SK, Sreejit G, Athmanathan B, Wright J, Jensen AR, Oudit GY, Markel TA, Nagareddy PR, Obukhov AG, Grant MB. Bone marrow-derived cells restore functional integrity of the gut epithelial and vascular barriers in a model of diabetes and ace2 deficiency. *Circulation research*. 2019;125:969-988.
12. Shenoy V, Kwon KC, Rathinasabapathy A, Lin S, Jin G, Song C, Shil P, Nair A, Qi Y, Li Q, Francis J, Katovich MJ, Daniell H, Raizada MK. Oral delivery of angiotensin-converting enzyme 2 and angiotensin-(1-7) bioencapsulated in plant cells attenuates pulmonary hypertension. *Hypertension*. 2014;64:1248-1259.
13. Basu R, Poglitsch M, Yogasundaram H, Thomas J, Rowe BH, Oudit GY. Roles of angiotensin peptides and recombinant human ace2 in heart failure. *J Am Coll Cardiol*. 2017;69:805-819.
14. Mukerjee S, Gao H, Xu J, Sato R, Zsombok A, Lazartigues E. Ace2 and adam17 interaction regulates the activity of presympathetic neurons. *Hypertension*. 2019;74:1181-1191.
15. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the sars-cov-2 spike glycoprotein. *Cell*. Mar 6, 2020. doi:10.1016/j.cell.2020.02.058. [epub ahead of print]
16. Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of sars-cov-2 by full-length human ace2. *Science*. 2020;367:1444-1448.
17. Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, Yang P, Sarao R, Wada T, Leong-Poi H, Crackower MA, Fukamizu A, Hui CC, Hein L, Uhlig S, Slutsky AS, Jiang C, Penninger JM. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature*. 2005;436:112-116.

18. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, Huan Y, Yang P, Zhang Y, Deng W, Bao L, Zhang B, Liu G, Wang Z, Chappell M, Liu Y, Zheng D, Leibbrandt A, Wada T, Slutsky AS, Liu D, Qin C, Jiang C, Penninger JM. A crucial role of angiotensin converting enzyme 2 (ace2) in sars coronavirus-induced lung injury. *Nat Med*. 2005;11:875-879.
19. Rey-Parra GJ, Vadivel A, Coltan L, Hall A, Eaton F, Schuster M, Loibner H, Penninger JM, Kassiri Z, Oudit GY, Thebaud B. Angiotensin converting enzyme 2 abrogates bleomycin-induced lung injury. *J Mol Med (Berl)*. 2012;90:637-647.
20. Kim S, Rigatto K, Gazzana MB, Knorst MM, Richards EM, Pepine CJ, Raizada MK. Altered gut microbiome profile in patients with pulmonary arterial hypertension. *Hypertension*. 2020;75:1063-1071.
21. Hemnes AR, Rathinasabapathy A, Austin EA, Brittain EL, Carrier EJ, Chen X, Fessel JP, Fike CD, Fong P, Fortune N, Gerszten RE, Johnson JA, Kaplowitz M, Newman JH, Piana R, Pugh ME, Rice TW, Robbins IM, Wheeler L, Yu C, Loyd JE, West J. A potential therapeutic role for angiotensin-converting enzyme 2 in human pulmonary arterial hypertension. *Eur Respir J*. 2018;51.
22. Khan A, Benthin C, Zeno B, Albertson TE, Boyd J, Christie JD, Hall R, Poirier G, Ronco JJ, Tidswell M, Hardes K, Powley WM, Wright TJ, Siederer SK, Fairman DA, Lipson DA, Bayliffe AI, Lazaar AL. A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. *Critical Care*. 2017;21:234.
23. Bernstein KE, Xiao HD, Frenzel K, Li P, Shen XZ, Adams JW, Fuchs S. Six truisms concerning ace and the renin-angiotensin system educed from the genetic analysis of mice. *Circulation research*. 2005;96:1135-1144.
24. Page IH, Helmer OM. Angiotonin-activator, renin- and angiotonin-inhibitor, and the mechanism of angiotonin tachyphylaxis in normal, hypertensive, and nephrectomized animals. *J Exp Med*. 1940;71:495-519.
25. Crackower MA, Sarao R, Oudit GY, Yagil C, Kozieradzki I, Scanga SE, Oliveira-dos-Santos AJ, da Costa J, Zhang L, Pei Y, Scholey J, Ferrario CM, Manoukian AS, Chappell MC, Backx PH, Yagil Y, Penninger JM. Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature*. 2002;417:822-828.
26. Skeggs LT, Jr., Kahn JR, Shumway NP. The preparation and function of the hypertensin-converting enzyme. *J Exp Med*. 1956;103:295-299.
27. Goldblatt H, Lynch J, Hanzal RF, Summerville WW. Studies on experimental hypertension : I. The production of persistent elevation of systolic blood pressure by means of renal ischemia. *J Exp Med*. 1934;59:347-379.
28. Rice GI, Thomas DA, Grant PJ, Turner AJ, Hooper NM. Evaluation of angiotensin-converting enzyme (ace), its homologue ace2 and neprilysin in angiotensin peptide metabolism. *The Biochemical journal*. 2004;383:45-51.
29. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020;579:270-273.
30. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L, Wei Y, Li H, Wu X, Xu J, Tu S, Zhang Y, Chen H, Cao B. Clinical course and risk factors for mortality of adult inpatients with covid-19 in wuhan, china: A retrospective cohort study. *Lancet*. 2020;395:1054-1062.
31. Li F, Li W, Farzan M, Harrison SC. Structure of sars coronavirus spike receptor-binding domain complexed with receptor. *Science*. 2005;309:1864-1868.
32. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS. Cryo-em structure of the 2019-ncov spike in the prefusion conformation. *Science*. 2020;367:1260-1263.
33. Perlman S, Netland J. Coronaviruses post-sars: Update on replication and pathogenesis. *Nat Rev Microbiol*. 2009;7:439-450.
34. Kunkel F, Herrler G. Structural and functional analysis of the surface protein of human coronavirus oc43. *Virology*. 1993;195:195-202.

35. McIntosh K, Dees JH, Becker WB, Kapikian AZ, Chanock RM. Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease. *Proc Natl Acad Sci U S A*. 1967;57:933-940.
36. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in wuhan, china: A descriptive study. *Lancet*. 2020;395:507-513.
37. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, Zhao Y, Li Y, Wang X, Peng Z. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in wuhan, china. *JAMA*. Feb 7, 2020. doi:10.1001/jama.2020.1585. [epub ahead of print]
38. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in wuhan, china. *Lancet*. 2020;395:497-506.
39. Ding Y, He L, Zhang Q, Huang Z, Che X, Hou J, Wang H, Shen H, Qiu L, Li Z, Geng J, Cai J, Han H, Li X, Kang W, Weng D, Liang P, Jiang S. Organ distribution of severe acute respiratory syndrome (sars) associated coronavirus (sars-cov) in sars patients: Implications for pathogenesis and virus transmission pathways. *J Pathol*. 2004;203:622-630.
40. Gu J, Gong E, Zhang B, Zheng J, Gao Z, Zhong Y, Zou W, Zhan J, Wang S, Xie Z, Zhuang H, Wu B, Zhong H, Shao H, Fang W, Gao D, Pei F, Li X, He Z, Xu D, Shi X, Anderson VM, Leong AS. Multiple organ infection and the pathogenesis of sars. *J Exp Med*. 2005;202:415-424.
41. Wu A, Peng Y, Huang B, Ding X, Wang X, Niu P, Meng J, Zhu Z, Zhang Z, Wang J, Sheng J, Quan L, Xia Z, Tan W, Cheng G, Jiang T. Genome composition and divergence of the novel coronavirus (2019-ncov) originating in china. *Cell Host Microbe*. 2020;27:325-328.
42. Shang J YG, Shi K, Wan Y, Luo C, Aihara H, Geng Q, Auerbach A, Li F. Structural basis of receptor recognition by sars-cov-2. *Nature*. Mar 30, 2020. doi:10.1038/s41586-020-2179-y. [epub ahead of print]
43. Millet JK, Whittaker GR. Host cell proteases: Critical determinants of coronavirus tropism and pathogenesis. *Virus Res*. 2015;202:120-134.
44. da Silva JS, Gabriel-Costa D, Wang H, Ahmad S, Sun X, Varagic J, Sudo RT, Ferrario CM, Dell Italia LJ, Sudo GZ, Groban L. Blunting of cardioprotective actions of estrogen in female rodent heart linked to altered expression of cardiac tissue chymase and ace2. *J Renin Angiotensin Aldosterone Syst*. 2017;18:1470320317722270.
45. Liu Y, Yang Y, Zhang C, Huang F, Wang F, Yuan J, Wang Z, Li J, Li J, Feng C, Zhang Z, Wang L, Peng L, Chen L, Qin Y, Zhao D, Tan S, Yin L, Xu J, Zhou C, Jiang C, Liu L. Clinical and biochemical indexes from 2019-ncov infected patients linked to viral loads and lung injury. *Sci China Life Sci*. 2020;63:364-374.
46. Oudit GY, Kassiri Z, Jiang C, Liu PP, Poutanen SM, Penninger JM, Butany J. Sars-coronavirus modulation of myocardial ace2 expression and inflammation in patients with sars. *Eur J Clin Invest*. 2009;39:618-625.
47. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, Du B, Li LJ, Zeng G, Yuen KY, Chen RC, Tang CL, Wang T, Chen PY, Xiang J, Li SY, Wang JL, Liang ZJ, Peng YX, Wei L, Liu Y, Hu YH, Peng P, Wang JM, Liu JY, Chen Z, Li G, Zheng ZJ, Qiu SQ, Luo J, Ye CJ, Zhu SY, Zhong NS, China Medical Treatment Expert Group for Covid-19. Clinical characteristics of coronavirus disease 2019 in china. *N Engl J Med*. Feb 28, 2020. doi:10.1056/NEJMoa2002032. [epub ahead of print]
48. Kuster GM, Pfister O, Burkard T, Zhou Q, Twerenbold R, Haaf P, Widmer AF, Osswald S. Sars-cov2: Should inhibitors of the renin-angiotensin system be withdrawn in patients with covid-19? *Eur Heart J*. Mar 20, 2020. doi:10.1093/eurheartj/ehaa235. [epub ahead of print]
49. Inciardi RM LL, Zaccone G, Italia L, Raffo M, Tomasoni D, Cani DS, Cerini M, Farina D, Gavazzi E, Maroldi R, Adamo M, Ammirati E, Sinagra G, Lombardi CM, Metra M. Cardiac involvement in a patient with coronavirus disease 2019 (covid-19). *JAMA Cardiol*. Mar 27, 2020. doi:10.1001/jamacardio.2020.1096. [epub ahead of print]

50. Shi S QM, Shen B, Cai Y, Liu T, Yang F, Gong W, Liu X, Liang J, Zhao Q, Huang H, Yang B, Huang C. Association of cardiac injury with mortality in hospitalized patients with covid-19 in wuhan, china. *JAMA Cardiol*. Mar 25, 2020. doi:10.1001/jamacardio.2020.0950. [epub ahead of print]
51. Clerkin KJ, Fried JA, Raikhelkar J, Sayer G, Griffin JM, Masoumi A, Jain SS, Burkhoff D, Kumaraiah D, Rabbani L, Schwartz A, Uriel N. Coronavirus disease 2019 (covid-19) and cardiovascular disease. *Circulation*. Mar 21, 2020. doi:10.1161/CIRCULATIONAHA.120.046941. [epub ahead of print]
52. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, Wang H, Wan J, Wang X, Lu Z. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (covid-19). *JAMA Cardiol*. Mar 27, 2020. doi:10.1001/jamacardio.2020.1017. [epub ahead of print]
53. Chan JF-W, Yuan S, Kok K-H, To KK-W, Chu H, Yang J, Xing F, Liu J, Yip CC-Y, Poon RW-S, Tsoi H-W, Lo SK-F, Chan K-H, Poon VK-M, Chan W-M, Ip JD, Cai J-P, Cheng VC-C, Chen H, Hui CK-M, Yuen K-Y. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: A study of a family cluster. *The Lancet*. 2020;395:514-523.
54. Cheng PKC, Wong DA, Tong LKL, Ip S-M, Lo ACT, Lau C-S, Yeung EYH, Lim WWL. Viral shedding patterns of coronavirus in patients with probable severe acute respiratory syndrome. *The Lancet*. 2004;363:1699-1700.
55. Leung WK, To K-F, Chan PKS, Chan HLY, Wu AKL, Lee N, Yuen KY, Sung JJY. Enteric involvement of severe acute respiratory syndrome–associated coronavirus infection. *Gastroenterology*. 2003;125:1011-1017.
56. Zhou J, Li C, Zhao G, Chu H, Wang D, Yan HH, Poon VK, Wen L, Wong BH, Zhao X, Chiu MC, Yang D, Wang Y, Au-Yeung RKH, Chan IH, Sun S, Chan JF, To KK, Memish ZA, Corman VM, Drosten C, Hung IF, Zhou Y, Leung SY, Yuen KY. Human intestinal tract serves as an alternative infection route for middle east respiratory syndrome coronavirus. *Science advances*. 2017;3:eaao4966.
57. Santisteban MM, Kim S, Pepine CJ, Raizada MK. Brain-gut-bone marrow axis: Implications for hypertension and related therapeutics. *Circulation research*. 2016;118:1327-1336.
58. Wu Y, Guo C, Tang L, Hong Z, Zhou J, Dong X, Yin H, Xiao Q, Tang Y, Qu X, Kuang L, Fang X, Mishra N, Lu J, Shan H, Jiang G, Huang X. Prolonged presence of sars-cov-2 viral rna in faecal samples. *Lancet Gastroenterol Hepatol*. Mar 19, 2020. doi:10.1016/S2468-1253(20)30083-2. [epub ahead of print]
59. Yeoa C, Kaushala S, Yeoa D. Enteric involvement of coronaviruses: Is faecal–oral transmission of sars-cov-2 possible? *The Lancet Gastroenterology & Hepatology*. 2020;5:335-337.
60. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ace2 protein, the functional receptor for sars coronavirus. A first step in understanding sars pathogenesis. *J Pathol*. 2004;203:631-637.
61. Beli E, Yan Y, Moldovan L, Vieira CP, Gao R, Duan Y, Prasad R, Bhatwadekar A, White FA, Townsend SD, Chan L, Ryan CN, Morton D, Moldovan EG, Chu FI, Oudit GY, Derendorf H, Adorini L, Wang XX, Evans-Molina C, Mirmira RG, Boulton ME, Yoder MC, Li Q, Levi M, Busik JV, Grant MB. Restructuring of the gut microbiome by intermittent fasting prevents retinopathy and prolongs survival in db/db mice. *Diabetes*. 2018;67:1867-1879.
62. Vallianou NG, Stratigou T, Tsagarakis S. Microbiome and diabetes: Where are we now? *Diabetes research and clinical practice*. 2018;146:111-118.
63. Budden KF, Gellatly SL, Wood DL, Cooper MA, Morrison M, Hugenholtz P, Hansbro PM. Emerging pathogenic links between microbiota and the gut-lung axis. *Nat Rev Microbiol*. 2017;15:55-63.
64. Iyer SN, Lu D, Katovich MJ, Raizada MK. Chronic control of high blood pressure in the spontaneously hypertensive rat by delivery of angiotensin type 1 receptor antisense. *Proceedings of the National Academy of Sciences of the United States of America*. 1996;93:9960-9965.

65. Qi Y, Goel R, Kim S, Richards EM, Carter CS, Pepine CJ, Raizada MK, Buford TW. Intestinal permeability biomarker zonulin is elevated in healthy aging. *J Am Med Dir Assoc*. 2017;18:810 e811-810 e814.
66. Sharma RK, Yang T, Oliveira AC, Lobaton GO, Aquino V, Kim S, Richards EM, Pepine CJ, Sumners C, Raizada MK. Microglial cells impact gut microbiota and gut pathology in angiotensin ii-induced hypertension. *Circulation research*. 2019;124:727-736.
67. Cole-Jeffrey CT, Liu M, Katovich MJ, Raizada MK, Shenoy V. Ace2 and microbiota: Emerging targets for cardiopulmonary disease therapy. *Journal of cardiovascular pharmacology*. 2015;66:540-550.
68. Oliveira AC, Richards EM, Raizada MK. Pulmonary hypertension: Pathophysiology beyond the lung. *Pharmacol Res*. 2020;151:104518.
69. Syvanen M. Churning out safer microbes for drug delivery. *Nat Biotechnol*. 2003;21:758-759.
70. Steidler L. Genetically engineered probiotics. *Best Pract Res Clin Gastroenterol*. 2003;17:861-876.
71. Bron PA, Kleerebezem M. Lactic acid bacteria for delivery of endogenous or engineered therapeutic molecules. *Front Microbiol*. 2018;9:1821.
72. Black RA, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF, Castner BJ, Stocking KL, Reddy P, Srinivasan S, Nelson N, Boiani N, Schooley KA, Gerhart M, Davis R, Fitzner JN, Johnson RS, Paxton RJ, March CJ, Cerretti DP. A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells. *Nature*. 1997;385:729-733.
73. Moss ML, Jin SL, Milla ME, Bickett DM, Burkhart W, Carter HL, Chen WJ, Clay WC, Didsbury JR, Hassler D, Hoffman CR, Kost TA, Lambert MH, Leesnitzer MA, McCauley P, McGeehan G, Mitchell J, Moyer M, Pahel G, Rocque W, Overton LK, Schoenen F, Seaton T, Su JL, Becherer JD, et al. Cloning of a disintegrin metalloproteinase that processes precursor tumour-necrosis factor-alpha. *Nature*. 1997;385:733-736.
74. Black RA, White JM. Adams: Focus on the protease domain. *Curr Opin Cell Biol*. 1998;10:654-659.
75. Gooz M. Adam-17: The enzyme that does it all. *Crit Rev Biochem Mol Biol*. 2010;45:146-169.
76. Fan D, Takawale A, Shen M, Wang W, Wang X, Basu R, Oudit GY, Kassiri Z. Cardiomyocyte adam17 is essential in post-myocardial infarction repair by regulating angiogenesis. *Circulation. Heart failure*. 2015;8:970-979.
77. Patel VB, Clarke N, Wang Z, Fan D, Parajuli N, Basu R, Putko B, Kassiri Z, Turner AJ, Oudit GY. Angiotensin ii induced proteolytic cleavage of myocardial ace2 is mediated by tace/adam-17: A positive feedback mechanism in the ras. *J Mol Cell Cardiol*. 2014;66:167-176.
78. Shen M, Hu M, Fedak PWM, Oudit GY, Kassiri Z. Cell-specific functions of adam17 regulate the progression of thoracic aortic aneurysm. *Circulation research*. 2018;123:372-388.
79. Epelman S, Tang WH, Chen SY, Van Lente F, Francis GS, Sen S. Detection of soluble angiotensin-converting enzyme 2 in heart failure: Insights into the endogenous counter-regulatory pathway of the renin-angiotensin-aldosterone system. *Journal of the American College of Cardiology*. 2008;52:750-754.
80. Scott AJ, O'Dea KP, O'Callaghan D, Williams L, Dokpesi JO, Tatton L, Handy JM, Hogg PJ, Takata M. Reactive oxygen species and p38 mitogen-activated protein kinase mediate tumor necrosis factor alpha-converting enzyme (tace/adam-17) activation in primary human monocytes. *J Biol Chem*. 2011;286:35466-35476.
81. Xu P, Derynck R. Direct activation of tace-mediated ectodomain shedding by p38 map kinase regulates egf receptor-dependent cell proliferation. *Mol Cell*. 2010;37:551-566.
82. Xu J, Sriramula S, Xia H, Moreno-Walton L, Culicchia F, Domenig O, Poglitsch M, Lazartigues E. Clinical relevance and role of neuronal at1 receptors in adam17-mediated ace2 shedding in neurogenic hypertension. *Circ Res*. 2017;121:43-55.
83. Wang H, Yang P, Liu K, Guo F, Zhang Y, Zhang G, Jiang C. Sars coronavirus entry into host cells through a novel clathrin- and caveolae-independent endocytic pathway. *Cell Res*. 2008;18:290-301.
84. Haga S, Yamamoto N, Nakai-Murakami C, Osawa Y, Tokunaga K, Sata T, Yamamoto N, Sasazuki T, Ishizaka Y. Modulation of tnf-alpha-converting enzyme by the spike protein of sars-cov and

ace2 induces tnf-alpha production and facilitates viral entry. *Proceedings of the National Academy of Sciences of the United States of America*. 2008;105:7809-7814.
85. Hofmann H, Pyrc K, van der Hoek L, Geier M, Berkhout B, Pohlmann S. Human coronavirus nl63 employs the severe acute respiratory syndrome coronavirus receptor for cellular entry. *Proceedings of the National Academy of Sciences of the United States of America*. 2005;102:7988-7993.
86. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by novel coronavirus from wuhan: An analysis based on decade-long structural studies of sars. *J Virol*. Jan 29, 2020. doi:10.1128/JVI.00127-20. [epub ahead of print]
87. Kuba K, Imai Y, Ohto-Nakanishi T, Penninger JM. Trilogy of ace2: A peptidase in the renin-angiotensin system, a sars receptor, and a partner for amino acid transporters. *Pharmacology & therapeutics*. 2010;128:119-128.
88. Jia H. Pulmonary angiotensin-converting enzyme 2 (ace2) and inflammatory lung disease. *Shock*. 2016;46:239-248.
89. Ye R, Liu Z. Ace2 exhibits protective effects against lps-induced acute lung injury in mice by inhibiting the lps-tlr4 pathway. *Exp Mol Pathol*. 2020;113:104350.
90. Zhong J, Basu R, Guo D, Chow FL, Byrns S, Schuster M, Loibner H, Wang XH, Penninger JM, Kassiri Z, Oudit GY. Angiotensin-converting enzyme 2 suppresses pathological hypertrophy, myocardial fibrosis, and cardiac dysfunction. *Circulation*. 2010;122:717-728.
91. Zhong J, Guo D, Chen CB, Wang W, Schuster M, Loibner H, Penninger JM, Scholey JW, Kassiri Z, Oudit GY. Prevention of angiotensin ii-mediated renal oxidative stress, inflammation, and fibrosis by angiotensin-converting enzyme 2. *Hypertension*. 2011;57:314-322.
92. Mercure C, Yogi A, Callera GE, Aranha AB, Bader M, Ferreira AJ, Santos RA, Walther T, Touyz RM, Reudelhuber TL. Angiotensin(1-7) blunts hypertensive cardiac remodeling by a direct effect on the heart. *Circulation research.* 2008;103:1319-1326.
93. Oudit GY, Penninger JM. Recombinant human angiotensin-converting enzyme 2 as a new renin-angiotensin system peptidase for heart failure therapy. *Curr Heart Fail Rep*. 2011;8:176-183.
94. Alenina N, Xu P, Rentzsch B, Patkin EL, Bader M. Genetically altered animal models for mas and angiotensin-(1-7). *Exp. Physiol.* 2008;93:528-537.
95. Bader M. Ace2, angiotensin-(1–7), and mas: The other side of the coin. *Pflügers Archiv - European Journal of Physiology*. 2012;465:79-85.
96. Santos RAS, Oudit GY, Verano-Braga T, Canta G, Steckelings UM, Bader M. The renin-angiotensin system: Going beyond the classical paradigms. *Am J Physiol Heart Circ Physiol*. 2019;316:H958-H970.
97. Chappell MC. Emerging evidence for a functional angiotensin-converting enzyme 2-angiotensin-(1-7)-mas receptor axis: More than regulation of blood pressure? *Hypertension*. 2007;50:596-599.
98. Iyer SN, Ferrario CM, Chappell MC. Angiotensin-(1-7) contributes to the antihypertensive effects of blockade of the renin-angiotensin system. *Hypertension*. 1998;31:356-361.
99. Dell'Italia LJ, Collawn JF, Ferrario CM. Multifunctional role of chymase in acute and chronic tissue injury and remodeling. *Circulation research*. 2018;122:319-336.
100. Sampaio WO, Souza dos Santos RA, Faria-Silva R, da Mata Machado LT, Schiffrin EL, Touyz RM. Angiotensin-(1-7) through receptor mas mediates endothelial nitric oxide synthase activation via akt-dependent pathways. *Hypertension*. 2007;49:185-192.
101. Santos RA, Simoes e Silva AC, Maric C, Silva DM, Machado RP, de Buhr I, Heringer-Walther S, Pinheiro SV, Lopes MT, Bader M, Mendes EP, Lemos VS, Campagnole-Santos MJ, Schultheiss HP, Speth R, Walther T. Angiotensin-(1-7) is an endogenous ligand for the g protein-coupled receptor mas. *Proc. Natl. Acad. Sci. U. S. A.* 2003;100:8258-8263.
102. Tallant EA, Ferrario CM, Gallagher PE. Angiotensin-(1-7) inhibits growth of cardiac myocytes through activation of the mas receptor. *American journal of physiology. Heart and circulatory physiology*. 2005;289:H1560-1566.
103. Zhang Y, Li B, Wang B, Zhang J, Wu J, Morgan T. Alteration of cardiac ace2/mas expression and cardiac remodelling in rats with aortic constriction. *Chin. J. Physiol.* 2014;57:335-342.

104. Flores-Munoz M, Godinho BM, Almalik A, Nicklin SA. Adenoviral delivery of angiotensin-(1-7) or angiotensin-(1-9) inhibits cardiomyocyte hypertrophy via the mas or angiotensin type 2 receptor. *PloS one*. 2012;7:e45564.
105. Flores-Munoz M, Work LM, Douglas K, Denby L, Dominiczak AF, Graham D, Nicklin SA. Angiotensin-(1-9) attenuates cardiac fibrosis in the stroke-prone spontaneously hypertensive rat via the angiotensin type 2 receptor. *Hypertension*. 2012;59:300-307.
106. Ocaranza MP, Lavandero S, Jalil JE, Moya J, Pinto M, Novoa U, Apablaza F, Gonzalez L, Hernandez C, Varas M, Lopez R, Godoy I, Verdejo H, Chiong M. Angiotensin-(1-9) regulates cardiac hypertrophy in vivo and in vitro. *J Hypertens*. 2010;28:1054-1064.
107. Ocaranza MP, Rivera P, Novoa U, Pinto M, Gonzalez L, Chiong M, Lavandero S, Jalil JE. Rho kinase inhibition activates the homologous angiotensin-converting enzyme-angiotensin-(1-9) axis in experimental hypertension. *J. Hypertens.* 2011;29:706-715.
108. Oudit GY, Crackower MA, Backx PH, Penninger JM. The role of ace2 in cardiovascular physiology. *Trends Cardiovasc. Med.* 2003;13:93-101.
109. Lo J, Patel VB, Wang Z, Levasseur J, Kaufman S, Penninger JM, Oudit GY. Angiotensin-converting enzyme 2 antagonizes angiotensin ii-induced pressor response and nadph oxidase activation in wistar-kyoto rats and spontaneously hypertensive rats. *Exp Physiol*. 2013;98:109-122.
110. Oudit GY, Kassiri Z, Patel MP, Chappell M, Butany J, Backx PH, Tsushima RG, Scholey JW, Khokha R, Penninger JM. Angiotensin II-mediated oxidative stress and inflammation mediate the age-dependent cardiomyopathy in ace2 null mice. *Cardiovascular research*. 2007;75:29-39.
111. Patel VB, Bodiga S, Fan D, Das SK, Wang Z, Wang W, Basu R, Zhong J, Kassiri Z, Oudit GY. Cardioprotective effects mediated by angiotensin II type 1 receptor blockade and enhancing angiotensin 1-7 in experimental heart failure in angiotensin-converting enzyme 2-null mice. *Hypertension*. 2012;59:1195-1203.
112. Danilczyk U, Penninger JM. Angiotensin-converting enzyme ii in the heart and the kidney. *Circ. Res.* 2006;98:463-471.
113. Patel VB, Takawale A, Ramprasath T, Das SK, Basu R, Grant MB, Hall DA, Kassiri Z, Oudit GY. Antagonism of angiotensin 1-7 prevents the therapeutic effects of recombinant human ace2. *J Mol Med (Berl)*. 2015;93:1003-1013.
114. Zhong JC, Zhang ZZ, Wang W, McKinnie SMK, Vederas JC, Oudit GY. Targeting the apelin pathway as a novel therapeutic approach for cardiovascular diseases. *Biochim Biophys Acta Mol Basis Dis*. 2017;1863:1942-1950.
115. Pitkin SL, Maguire JJ, Bonner TI, Davenport AP. International union of basic and clinical pharmacology. Lxxiv. Apelin receptor nomenclature, distribution, pharmacology, and function. *Pharmacological reviews*. 2010;62:331-342.
116. Chong KS, Gardner RS, Morton JJ, Ashley EA, McDonagh TA. Plasma concentrations of the novel peptide apelin are decreased in patients with chronic heart failure. *Eur J Heart Fail*. 2006;8:355-360.
117. Chen MM, Ashley EA, Deng DX, Tsalenko A, Deng A, Tabibiazar R, Ben-Dor A, Fenster B, Yang E, King JY, Fowler M, Robbins R, Johnson FL, Bruhn L, McDonagh T, Dargie H, Yakhini Z, Tsao PS, Quertermous T. Novel role for the potent endogenous inotrope apelin in human cardiac dysfunction. *Circulation*. 2003;108:1432-1439.
118. Przewlocka-Kosmala M, Kotwica T, Mysiak A, Kosmala W. Reduced circulating apelin in essential hypertension and its association with cardiac dysfunction. *J Hypertens*. 2011;29:971-979.
119. Kuba K, Zhang L, Imai Y, Arab S, Chen M, Maekawa Y, Leschnik M, Leibbrandt A, Markovic M, Schwaighofer J, Beetz N, Musialek R, Neely GG, Komnenovic V, Kolm U, Metzler B, Ricci R, Hara H, Meixner A, Nghiem M, Chen X, Dawood F, Wong KM, Sarao R, Cukerman E, Kimura A, Hein L, Thalhammer J, Liu PP, Penninger JM. Impaired heart contractility in apelin gene-deficient mice associated with aging and pressure overload. *Circulation research*. 2007;101:e32-42.
120. Wang W, McKinnie SM, Patel VB, Haddad G, Wang Z, Zhabyeyev P, Das SK, Basu R, McLean B, Kandalam V, Penninger JM, Kassiri Z, Vederas JC, Murray AG, Oudit GY. Loss of apelin

exacerbates myocardial infarction adverse remodeling and ischemia-reperfusion injury: Therapeutic potential of synthetic apelin analogues. *J Am Heart Assoc*. 2013;2:e000249.
121. Sato T, Suzuki T, Watanabe H, Kadowaki A, Fukamizu A, Liu PP, Kimura A, Ito H, Penninger JM, Imai Y, Kuba K. Apelin is a positive regulator of ace2 in failing hearts. *J Clin Invest*. 2013;123:5203-5211.
122. Siddiquee K, Hampton J, McAnally D, May L, Smith L. The apelin receptor inhibits the angiotensin ii type 1 receptor via allosteric trans-inhibition. *Br J Pharmacol*. 2013;168:1104-1117.
123. Zhang ZZ, Wang W, Jin HY, Chen X, Cheng YW, Xu YL, Song B, Penninger JM, Oudit GY, Zhong JC. Apelin is a negative regulator of angiotensin II-mediated adverse myocardial remodeling and dysfunction. *Hypertension*. 2017;70:1165-1175.
124. Wang W, Shen M, Fischer C, Basu R, Hazra S, Couvineau P, Paul M, Wang F, Toth S, Mix DS, Poglitsch M, Gerard NP, Bouvier M, Vederas JC, Penninger JM, Kassiri Z, Oudit GY. Apelin protects against abdominal aortic aneurysm and the therapeutic role of neutral endopeptidase resistant apelin analogs. *Proc Natl Acad Sci U S A*. 2019;116:13006-13015.
125. Wang W, McKinnie SM, Farhan M, Paul M, McDonald T, McLean B, Llorens-Cortes C, Hazra S, Murray AG, Vederas JC, Oudit GY. Angiotensin-converting enzyme 2 metabolizes and partially inactivates pyr-apelin-13 and apelin-17: Physiological effects in the cardiovascular system. *Hypertension*. 2016;68:365-377.
126. Camargo SM, Singer D, Makrides V, Huggel K, Pos KM, Wagner CA, Kuba K, Danilczyk U, Skovby F, Kleta R, Penninger JM, Verrey F. Tissue-specific amino acid transporter partners ace2 and collectrin differentially interact with hartnup mutations. *Gastroenterology*. 2009;136:872-882.
127. Patel VB, Zhong JC, Fan D, Basu R, Morton JS, Parajuli N, McMurtry MS, Davidge ST, Kassiri Z, Oudit GY. Angiotensin-converting enzyme 2 is a critical determinant of angiotensin II-induced loss of vascular smooth muscle cells and adverse vascular remodeling. *Hypertension*. 2014;64:157-164.
128. Patel VB, Mori J, McLean BA, Basu R, Das SK, Ramprasath T, Parajuli N, Penninger JM, Grant MB, Lopaschuk GD, Oudit GY. Ace2 deficiency worsens epicardial adipose tissue inflammation and cardiac dysfunction in response to diet-induced obesity. *Diabetes*. 2016;65:85-95.
129. Santos RA, Castro CH, Gava E, Pinheiro SV, Almeida AP, Paula RD, Cruz JS, Ramos AS, Rosa KT, Irigoyen MC, Bader M, Alenina N, Kitten GT, Ferreira AJ. Impairment of in vitro and in vivo heart function in angiotensin-(1-7) receptor mas knockout mice. *Hypertension*. 2006;47:996-1002.
130. Sampaio WO, Henrique de Castro C, Santos RA, Schiffrin EL, Touyz RM. Angiotensin-(1-7) counterregulates angiotensin ii signaling in human endothelial cells. *Hypertension*. 2007;50:1093-1098.
131. Iwata M, Cowling RT, Gurantz D, Moore C, Zhang S, Yuan JX, Greenberg BH. Angiotensin-(1-7) binds to specific receptors on cardiac fibroblasts to initiate antifibrotic and antitrophic effects. *American journal of physiology. Heart and circulatory physiology*. 2005;289:H2356-2363.
132. Patel SK, Velkoska E, Freeman M, Wai B, Lancefield TF, Burrell LM. From gene to protein-experimental and clinical studies of ace2 in blood pressure control and arterial hypertension. *Front Physiol*. 2014;5:227.
133. Gheorghiade M, Sopko G, De Luca L, Velazquez EJ, Parker JD, Binkley PF, Sadowski Z, Golba KS, Prior DL, Rouleau JL, Bonow RO. Navigating the crossroads of coronary artery disease and heart failure. *Circulation*. 2006;114:1202-1213.
134. Burrell LM, Risvanis J, Kubota E, Dean RG, MacDonald PS, Lu S, Tikellis C, Grant SL, Lew RA, Smith AI, Cooper ME, Johnston CI. Myocardial infarction increases ace2 expression in rat and humans. *Eur. Heart J.* 2005;26:369-375.
135. Kassiri Z, Zhong J, Guo D, Basu R, Wang X, Liu PP, Scholey JW, Penninger JM, Oudit GY. Loss of angiotensin-converting enzyme 2 accelerates maladaptive left ventricular remodeling in response to myocardial infarction. *Circulation. Heart failure*. 2009;2:446-455.
136. Qi YF, Zhang J, Wang L, Shenoy V, Krause E, Oh SP, Pepine CJ, Katovich MJ, Raizada MK. Angiotensin-converting enzyme 2 inhibits high-mobility group box 1 and attenuates cardiac dysfunction post-myocardial ischemia. *Journal of molecular medicine*. 2016;94:37-49.

137. Wang Y, Qian C, Roks AJ, Westermann D, Schumacher SM, Escher F, Schoemaker RG, Reudelhuber TL, van Gilst WH, Schultheiss HP, Tschope C, Walther T. Circulating rather than cardiac angiotensin-(1-7) stimulates cardioprotection after myocardial infarction. *Circulation Heart failure*. 2010;3:286-293.
138. Wang W, Patel VB, Parajuli N, Fan D, Basu R, Wang Z, Ramprasath T, Kassiri Z, Penninger JM, Oudit GY. Heterozygote loss of ace2 is sufficient to increase the susceptibility to heart disease. *J Mol Med (Berl)*. 2014;92:847-858.
139. Patel VB, Shah S, Verma S, Oudit GY. Epicardial adipose tissue as a metabolic transducer: Role in heart failure and coronary artery disease. *Heart failure reviews*. 2017;22:889-902.
140. Packer M. Epicardial adipose tissue may mediate deleterious effects of obesity and inflammation on the myocardium. *Journal of the American College of Cardiology*. 2018;71:2360-2372.
141. Patel VB, Basu R, Oudit GY. Ace2/ang 1-7 axis: A critical regulator of epicardial adipose tissue inflammation and cardiac dysfunction in obesity. *Adipocyte*. 2016;5:306-311.
142. Mori J, Patel VB, Abo Alrob O, Basu R, Altamimi T, Desaulniers J, Wagg CS, Kassiri Z, Lopaschuk GD, Oudit GY. Angiotensin 1-7 ameliorates diabetic cardiomyopathy and diastolic dysfunction in db/db mice by reducing lipotoxicity and inflammation. *Circulation Heart failure*. 2014;7:327-339.
143. Mori J, Patel VB, Ramprasath T, Alrob OA, DesAulniers J, Scholey JW, Lopaschuk GD, Oudit GY. Angiotensin 1-7 mediates renoprotection against diabetic nephropathy by reducing oxidative stress, inflammation, and lipotoxicity. *American journal of physiology. Renal physiology*. 2014;306:F812-821.
144. Morimoto H, Mori J, Nakajima H, Kawabe Y, Tsuma Y, Fukuhara S, Kodo K, Ikoma K, Matoba S, Oudit GY, Hosoi H. Angiotensin 1-7 stimulates brown adipose tissue and reduces diet-induced obesity. *American journal of physiology. Endocrinology and metabolism*. 2018;314:E131-E138.
145. Kawabe Y, Mori J, Morimoto H, Yamaguchi M, Miyagaki S, Ota T, Tsuma Y, Fukuhara S, Nakajima H, Oudit GY, Hosoi H. Ace2 exerts anti-obesity effect via stimulating brown adipose tissue and induction of browning in white adipose tissue. *Am J Physiol Endocrinol Metab*. 2019;317:E1140-E1149.
146. Diez-Freire C, Vazquez J, Correa de Adjounian MF, Ferrari MF, Yuan L, Silver X, Torres R, Raizada MK. Ace2 gene transfer attenuates hypertension-linked pathophysiological changes in the shr. *Physiological genomics*. 2006;27:12-19.
147. Rentzsch B, Todiras M, Iliescu R, Popova E, Campos LA, Oliveira ML, Baltatu OC, Santos RA, Bader M. Transgenic angiotensin-converting enzyme 2 overexpression in vessels of shrsp rats reduces blood pressure and improves endothelial function. *Hypertension*. 2008;52:967-973.
148. Wysocki J, Ye M, Rodriguez E, Gonzalez-Pacheco FR, Barrios C, Evora K, Schuster M, Loibner H, Brosnihan KB, Ferrario CM, Penninger JM, Batlle D. Targeting the degradation of angiotensin ii with recombinant angiotensin-converting enzyme 2: Prevention of angiotensin ii-dependent hypertension. *Hypertension*. 2010;55:90-98.
149. Lambert DW, Yarski M, Warner FJ, Thornhill P, Parkin ET, Smith AI, Hooper NM, Turner AJ. Tumor necrosis factor-alpha convertase (adam17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome-coronavirus (sars-cov) receptor, angiotensin-converting enzyme-2 (ace2). *The Journal of biological chemistry*. 2005;280:30113-30119.
150. Xia H, Sriramula S, Chhabra KH, Lazartigues E. Brain angiotensin-converting enzyme type 2 shedding contributes to the development of neurogenic hypertension. *Circulation research*. 2013;113:1087-1096.
151. Thomas MC, Pickering RJ, Tsorotes D, Koitka A, Sheehy K, Bernardi S, Toffoli B, Nguyen-Huu TP, Head GA, Fu Y, Chin-Dusting J, Cooper ME, Tikellis C. Genetic ace2 deficiency accentuates vascular inflammation and atherosclerosis in the apoe knockout mouse. *Circulation research*. 2010;107:888-897.
152. Chen Y, Wang X, Yang C, Su X, Yang W, Dai Y, Han H, Jiang J, Lu L, Wang H, Chen Q, Jin W. Decreased circulating catestatin levels are associated with coronary artery disease: The emerging anti-inflammatory role. *Atherosclerosis*. 2019;281:78-88.

153. Oudit GY, Liu GC, Zhong J, Basu R, Chow FL, Zhou J, Loibner H, Janzek E, Schuster M, Penninger JM, Herzenberg AM, Kassiri Z, Scholey JW. Human recombinant ace2 reduces the progression of diabetic nephropathy. *Diabetes*. 2010;59:529-538.
154. Patel VB, Bodiga S, Basu R, Das SK, Wang W, Wang Z, Lo J, Grant MB, Zhong J, Kassiri Z, Oudit GY. Loss of angiotensin-converting enzyme-2 exacerbates diabetic cardiovascular complications and leads to systolic and vascular dysfunction: A critical role of the angiotensin ii/at1 receptor axis. *Circulation research*. 2012;110:1322-1335.
155. Tikellis C, Pickering R, Tsorotes D, Du XJ, Kiriazis H, Nguyen-Huu TP, Head GA, Cooper ME, Thomas MC. Interaction of diabetes and ace2 in the pathogenesis of cardiovascular disease in experimental diabetes. *Clinical science*. 2012;123:519-529.
156. Patel VB, Parajuli N, Oudit GY. Role of angiotensin-converting enzyme 2 (ace2) in diabetic cardiovascular complications. *Clinical science*. 2014;126:471-482.
157. Cooper ME, Johnston CI. Optimizing treatment of hypertension in patients with diabetes. *Jama*. 2000;283:3177-3179.
158. Wong DW, Oudit GY, Reich H, Kassiri Z, Zhou J, Liu QC, Backx PH, Penninger JM, Herzenberg AM, Scholey JW. Loss of angiotensin-converting enzyme-2 (ace2) accelerates diabetic kidney injury. *The American journal of pathology*. 2007;171:438-451.
159. Bae EH, Fang F, Williams VR, Konvalinka A, Zhou X, Patel VB, Song X, John R, Oudit GY, Pei Y, Scholey JW. Murine recombinant angiotensin-converting enzyme 2 attenuates kidney injury in experimental alport syndrome. *Kidney international*. 2017;91:1347-1361.
160. Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, Remuzzi G, Snapinn SM, Zhang Z, Shahinfar S, Investigators RS. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *The New England journal of medicine*. 2001;345:861-869.
161. Tikellis C, Johnston CI, Forbes JM, Burns WC, Burrell LM, Risvanis J, Cooper ME. Characterization of renal angiotensin-converting enzyme 2 in diabetic nephropathy. *Hypertension*. 2003;41:392-397.
162. Verma A, Shan Z, Lei B, Yuan L, Liu X, Nakagawa T, Grant MB, Lewin AS, Hauswirth WW, Raizada MK, Li Q. Ace2 and ang-(1-7) confer protection against development of diabetic retinopathy. *Molecular therapy : the journal of the American Society of Gene Therapy*. 2012;20:28-36.
163. Dominguez JM, 2nd, Hu P, Caballero S, Moldovan L, Verma A, Oudit GY, Li Q, Grant MB. Adeno-associated virus overexpression of angiotensin-converting enzyme-2 reverses diabetic retinopathy in type 1 diabetes in mice. *The American journal of pathology*. 2016;186:1688-1700.
164. Busik JV, Tikhonenko M, Bhatwadekar A, Opreanu M, Yakubova N, Caballero S, Player D, Nakagawa T, Afzal A, Kielczewski J, Sochacki A, Hasty S, Li Calzi S, Kim S, Duclas SK, Segal MS, Guberski DL, Esselman WJ, Boulton ME, Grant MB. Diabetic retinopathy is associated with bone marrow neuropathy and a depressed peripheral clock. *J Exp Med*. 2009;206:2897-2906.
165. Hazra S, Jarajapu YP, Stepps V, Caballero S, Thinschmidt JS, Sautina L, Bengtsson N, Licalzi S, Dominguez J, Kern TS, Segal MS, Ash JD, Saban DR, Bartelmez SH, Grant MB. Long-term type 1 diabetes influences haematopoietic stem cells by reducing vascular repair potential and increasing inflammatory monocyte generation in a murine model. *Diabetologia*. 2013;56:644-653.
166. Hinojosa AE, Garcia-Bueno B, Leza JC, Madrigal JL. Ccl2/mcp-1 modulation of microglial activation and proliferation. *J Neuroinflammation*. 2011;8:77.
167. Skuljec J, Sun H, Pul R, Benardais K, Ragancokova D, Moharregh-Khiabani D, Kotsiari A, Trebst C, Stangel M. Ccl5 induces a pro-inflammatory profile in microglia in vitro. *Cell Immunol*. 2011;270:164-171.
168. Semple BD, Kossmann T, Morganti-Kossmann MC. Role of chemokines in cns health and pathology: A focus on the ccl2/ccr2 and cxcl8/cxcr2 networks. *J Cereb Blood Flow Metab*. 2009;30:459-473.
169. Tsou CL, Peters W, Si Y, Slaymaker S, Aslanian AM, Weisberg SP, Mack M, Charo IF. Critical roles for ccr2 and mcp-3 in monocyte mobilization from bone marrow and recruitment to inflammatory sites. *J Clin Invest*. 2007;117:902-909.

170. Huang D, Wujek J, Kidd G, He TT, Cardona A, Sasse ME, Stein EJ, Kish J, Tani M, Charo IF, Proudfoot AE, Rollins BJ, Handel T, Ransohoff RM. Chronic expression of monocyte chemoattractant protein-1 in the central nervous system causes delayed encephalopathy and impaired microglial function in mice. *FASEB J*. 2005;19:761-772.
171. Schilling M, Strecker JK, Ringelstein EB, Schabitz WR, Kiefer R. The role of cc chemokine receptor 2 on microglia activation and blood-borne cell recruitment after transient focal cerebral ischemia in mice. *Brain Res*. 2009;1289:79-84.
172. Jarajapu YP, Bhatwadekar AD, Caballero S, Hazra S, Shenoy V, Medina R, Kent D, Stitt AW, Thut C, Finney EM, Raizada MK, Grant MB. Activation of the ace2/angiotensin-(1-7)/mas receptor axis enhances the reparative function of dysfunctional diabetic endothelial progenitors. *Diabetes*. 2013;62:1258-1269.
173. Reich HN, Oudit GY, Penninger JM, Scholey JW, Herzenberg AM. Decreased glomerular and tubular expression of ace2 in patients with type 2 diabetes and kidney disease. *Kidney international*. 2008;74:1610-1616.
174. Xie X, Chen J, Wang X, Zhang F, Liu Y. Age- and gender-related difference of ace2 expression in rat lung. *Life sciences*. 2006;78:2166-2171.
175. Yilin Z, Yandong N, Faguang J. Role of angiotensin-converting enzyme (ace) and ace2 in a rat model of smoke inhalation induced acute respiratory distress syndrome. *Burns*. 2015;41:1468-1477.
176. Treml B, Neu N, Kleinsasser A, Gritsch C, Finsterwalder T, Geiger R, Schuster M, Janzek E, Loibner H, Penninger J, Loeckinger A. Recombinant angiotensin-converting enzyme 2 improves pulmonary blood flow and oxygenation in lipopolysaccharide-induced lung injury in piglets. *Crit Care Med*. 2010;38:596-601.
177. Rigatto K, Casali KR, Shenoy V, Katovich MJ, Raizada MK. Diminazene aceturate improves autonomic modulation in pulmonary hypertension. *European journal of pharmacology*. 2013;713:89-93.
178. Ishiyama Y, Gallagher PE, Averill DB, Tallant EA, Brosnihan KB, Ferrario CM. Upregulation of angiotensin-converting enzyme 2 after myocardial infarction by blockade of angiotensin ii receptors. *Hypertension*. 2004;43:970-976.
179. Ferrario CM, Jessup J, Chappell MC, Averill DB, Brosnihan KB, Tallant EA, Diz DI, Gallagher PE. Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. *Circulation*. 2005;111:2605-2610.
180. Ferrario CM, Jessup J, Gallagher PE, Averill DB, Brosnihan KB, Ann Tallant E, Smith RD, Chappell MC. Effects of renin-angiotensin system blockade on renal angiotensin-(1-7) forming enzymes and receptors. *Kidney Int*. 2005;68:2189-2196.
181. Igase M, Strawn WB, Gallagher PE, Geary RL, Ferrario CM. Angiotensin ii at1 receptors regulate ace2 and angiotensin-(1-7) expression in the aorta of spontaneously hypertensive rats. *Am J Physiol Heart Circ Physiol*. 2005;289:H1013-1019.
182. Jessup JA, Gallagher PE, Averill DB, Brosnihan KB, Tallant EA, Chappell MC, Ferrario CM. Effect of angiotensin ii blockade on a new congenic model of hypertension derived from transgenic ren-2 rats. *Am J Physiol Heart Circ Physiol*. 2006;291:H2166-2172.
183. Ocaranza MP, Godoy I, Jalil JE, Varas M, Collantes P, Pinto M, Roman M, Ramirez C, Copaja M, Diaz-Araya G, Castro P, Lavandero S. Enalapril attenuates downregulation of angiotensin-converting enzyme 2 in the late phase of ventricular dysfunction in myocardial infarcted rat. *Hypertension*. 2006;48:572-578.
184. Soler MJ, Ye M, Wysocki J, William J, Lloveras J, Batlle D. Localization of ace2 in the renal vasculature: Amplification by angiotensin II type 1 receptor blockade using telmisartan. *Am J Physiol Renal Physiol*. 2009;296:F398-405.
185. Jin HY, Song B, Oudit GY, Davidge ST, Yu HM, Jiang YY, Gao PJ, Zhu DL, Ning G, Kassiri Z, Penninger JM, Zhong JC. Ace2 deficiency enhances angiotensin ii-mediated aortic profilin-1 expression, inflammation and peroxynitrite production. *PLoS One*. 2012;7:e38502.

186. Gembardt F, Sterner-Kock A, Imboden H, Spalteholz M, Reibitz F, Schultheiss HP, Siems WE, Walther T. Organ-specific distribution of ace2 mrna and correlating peptidase activity in rodents. *Peptides*. 2005;26:1270-1277.
187. Lo CS, Liu F, Shi Y, Maachi H, Chenier I, Godin N, Filep JG, Ingelfinger JR, Zhang SL, Chan JS. Dual ras blockade normalizes angiotensin-converting enzyme-2 expression and prevents hypertension and tubular apoptosis in akita angiotensinogen-transgenic mice. *Am J Physiol Renal Physiol*. 2012;302:F840-852.
188. Koka V, Huang XR, Chung AC, Wang W, Truong LD, Lan HY. Angiotensin ii up-regulates angiotensin i-converting enzyme (ace), but down-regulates ace2 via the at1-erk/p38 map kinase pathway. *Am J Pathol*. 2008;172:1174-1183.
189. Takeda Y, Zhu A, Yoneda T, Usukura M, Takata H, Yamagishi M. Effects of aldosterone and angiotensin ii receptor blockade on cardiac angiotensinogen and angiotensin-converting enzyme 2 expression in dahl salt-sensitive hypertensive rats. *Am J Hypertens*. 2007;20:1119-1124.
190. Yamamuro M, Yoshimura M, Nakayama M, Abe K, Sumida H, Sugiyama S, Saito Y, Nakao K, Yasue H, Ogawa H. Aldosterone, but not angiotensin ii, reduces angiotensin converting enzyme 2 gene expression levels in cultured neonatal rat cardiomyocytes. *Circ J*. 2008;72:1346-1350.
191. Keidar S, Gamliel-Lazarovich A, Kaplan M, Pavlotzky E, Hamoud S, Hayek T, Karry R, Abassi Z. Mineralocorticoid receptor blocker increases angiotensin-converting enzyme 2 activity in congestive heart failure patients. *Circulation research*. 2005;97:946-953.
192. Harada E, Yoshimura M, Yasue H, Nakagawa O, Nakagawa M, Harada M, Mizuno Y, Nakayama M, Shimasaki Y, Ito T, Nakamura S, Kuwahara K, Saito Y, Nakao K, Ogawa H. Aldosterone induces angiotensin-converting-enzyme gene expression in cultured neonatal rat cardiocytes. *Circulation*. 2001;104:137-139.
193. Robert V, Heymes C, Silvestre JS, Sabri A, Swynghedauw B, Delcayre C. Angiotensin at1 receptor subtype as a cardiac target of aldosterone: Role in aldosterone-salt-induced fibrosis. *Hypertension*. 1999;33:981-986.
194. Fu GX, Xu CC, Zhong Y, Zhu DL, Gao PJ. Aldosterone-induced osteopontin expression in vascular smooth muscle cells involves mr, erk, and p38 mapk. *Endocrine*. 2012;42:676-683.
195. Walczak C, Gaignier F, Gilet A, Zou F, Thornton SN, Ropars A. Aldosterone increases vegf-a production in human neutrophils through pi3k, erk1/2 and p38 pathways. *Biochim Biophys Acta*. 2011;1813:2125-2132.
196. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ace2) as a sars-cov-2 receptor: Molecular mechanisms and potential therapeutic target. *Intensive Care Med*. 2020;46:586-590.
197. Vaduganathan M, Vardeny O, Michel T, McMurray JJV, Pfeffer MA, Solomon SD. Renin-angiotensin-aldosterone system inhibitors in patients with covid-19. *N Engl J Med*. Mar 30, 2020. doi:10.1056/NEJMsr2005760. [epub ahead of print]
198. Minnesota Uo. Losartan for patients with covid-19 requiring hospitalization. 2020;ClinicalTrials.gov Identifier: NCT04312009.
199. Minnesota Uo. Losartan for patients with covid-19 not requiring hospitalization. 2020;ClinicalTrials.gov Identifier: NCT04311177.
200. Augusto Di Castelnuovo NI. Ace inhibitors, angiotensin ii type-i receptor blockers and severity of covid-19 (covid-ace). 2020;ClinicalTrials.gov Identifier: NCT04318418.
201. Verma A, Xu K, Du T, Zhu P, Liang Z, Liao S, Zhang J, Raizada MK, Grant MB, Li Q. Expression of human ace2 in lactobacillus and beneficial effects in diabetic retinopathy in mice. *Mol Ther Methods Clin Dev*. 2019;14:161-170.

FIGURE LEGENDS

Figure 1. Historical Timeline of discovery of the major RAS components, including ACE2. Renin was the first component of the RAS discovered following the finding that extracts from rabbit kidney produced pressor effects (Tigerstedt & Bergman, 1898). Constriction of the renal artery was then found to lead to hypertension (HTN), thus driving the discovery of hypertensin and angiotonin (and later termed angiotensin) (Goldblatt *et al*, 1934; Page & Helmer, 1940). Angiotensin was subsequently purified, and two forms were resolved: Ang I and Ang II. Therefore, the existence of a converting enzyme was predicted (ACE) and subsequently isolated and characterized (Skeggs *et al*, 1956). The counter-regulatory axis of RAS was then described, pioneered with the discovery of ACE2 by two independent research groups (Donoghue *et al*, 2000; Tipnis *et al*, 2000). The cardioprotective effects of ACE2 were discovered shortly after (Crackower *et al*, 2002). Studies have identified the ACE2 protease domain as the receptor for SARS-CoV (Li *et al*, 2003) and, more recently, as the SARS-CoV-2 receptor (Walls *et al*, 2020; Yan *et al*, 2020).

Figure 2. ACE2 expression throughout the body and schematic of ACE2 primary domains. (**A**) ACE2 is expressed in the vascular system (endothelial cells, migratory angiogenic cells, and vascular smooth muscle cells), heart (cardiofibroblasts, cardiomyocytes, endothelial cells, pericytes, and epicardial adipose cells) and kidneys (glomerular endothelial cells, podocytes and proximal tubule epithelial cells). ACE2 is also expressed and functions in the local RAS of the liver (cholangiocytes and hepatocytes), retina (pigmented epithelial cells, rod and cone photoreceptor cells and Müller glial cells), enterocytes of the intestines, circumventricular organs of the central nervous system, upper airway (goblet and ciliated epithelial cells), and alveolar (Type II) epithelial cells of the lungs and pulmonary vasculature. (**B**) ACE2 has an extracellular facing N-terminal domain and a C-terminal transmembrane domain with a cytosolic tail. The N-terminal portion of the protein contains the claw-like protease domain (PD), while the C-terminal domain is referred to as the Collectrin-like domain (CLD). The receptor-binding domain (RBD) of SARS-CoV-2 binds with the PD of ACE2, forming the RBD-PD complex distinct from the ACE2 catalytic site.

Figure 3. Role of ACE2 in the pathogenesis of COVID-19 and the inflammatory response. ACE2-mediated cardiovascular protection is lost following endocytosis of the enzyme along with SARS-CoV-2 viral particles. Ang II levels elevate with increased activity of angiotensin 1 receptors (AT_1R) at the cost of ACE2/Ang 1–7 driven pathways leading to adverse fibrosis, hypertrophy, increased reactive oxygen species (ROS), vasoconstriction, and gut dysbiosis. ADAM17 mediated proteolytic cleavage of ACE2 is upregulated by endocytosed SARS-CoV-2 spike proteins. Activation of the AT_1R by elevated Ang II levels also further increases ADAM17 activity. ADAM17 correspondingly also cleaves its primary substrate releasing soluble TNF-α into the extracellular region where it has auto- and paracrine functionality. TNF-α activation of its Tumor Necrosis Factor Receptor (TNFR) represents a third pathway elevating ADAM17 activity. TNF-α along with systemic cytokines released due to SARS-CoV-2 infection and in conjunction with comorbidities such as diabetes and hypertension can lead to a cytokine storm.

Figure 4. Link between ACE2, gut dysbiosis and cardiovascular disease. Loss of ACE2 on the luminal surface of the gut alters microbiota profiles facilitating dysbiosis and disruption of the integrity of the epithelial barrier. Epithelial dysfunction provides a conduit for both gastrointestinal metabolites and bacterium passage into the vascular bed driving local and systemic inflammation which may cumulate in hypertension and septic shock. ACE2 deficiency and gut dysbiosis predisposes to the development of pulmonary hypertension through the gut-lung axis. SARS-CoV-2 enteric or pulmonary infection can further worsen the pathophysiology of the gut-lung axis through increased bacterial infiltration and inflammation in addition to worsened pulmonary function.

Figure 5. ACE2 role in the RAS peptide cascade and its interaction with the apelinergic peptide system. (**A**) Angiotensinogen is processed by renin into angiotensin I (Ang I) which is further cleaved by angiotensin-converting enzyme (ACE) or mast cell chymase into angiotensin II (Ang II). Ang II can go on to affect the cardiovascular system predominantly through the angiotensin type 1 receptor (AT_1R) or via

the angiotensin type 2 receptor (AT_2R). Alternatively, Ang II can be degraded by the carboxypeptidase angiotensin-converting enzyme 2 (ACE2) or the prolyl carboxypeptidase (PCP) into angiotensin 1–7 (Ang 1–7). Ang 1–7 mediates protective effects throughout tissues which host the Mas receptor (MasR). Ang 1–7 can be further formed through the activity of ACE2 on Ang I forming Ang 1-9 which is then cleaved by either ACE or neprilysin (NEP). (**B**) Stimulation of the apelin receptor by apelin peptides leads to cardiovascular protective effects while disrupting Ang II signaling by sequestration of the AT_1R through receptor heterodimerization. Apelin is inactivated by ACE2 cleavage of its C-terminal phenylalanine while stimulation of the apelin receptor promotes ACE2 mRNA transcription presenting apelin's role as a positive regulator of ACE2.

Figure 6. Loss of ACE2 exacerbates diabetic cardiovascular complications via a multitude of disease mechanisms. The loss of ACE2 action in diabetic states elevates Ang II and lowers Ang 1–7 levels in tissues and systemically. Increased Ang II/AT_1R signaling drives multiple pathologies in various end-organs elevating reactive oxygen species (ROS) and promoting fibrosis, hypertrophy, and inflammation aggravated by the loss of the protective effects of Ang 1–7. Ang II stimulation also systemically alters metabolic profiles and modulates insulin sensitivity in affected tissues.

Table. Pharmacological regulation of the renin-angiotensin system (RAS) and their effects on RAS components, ACE2 gene expression, protein levels and cellular activity

Pharmacological Agent	Experimental Model/Subject	Tissues	Observation
Angiotensin converting enzyme Inhibitors			
Lisinopril	Lewis rats	Heart	Decrease in plasma Ang II, increase in plasma Ang 1–7 and *Ace2* mRNA, but not cardiac ACE2 activity[179]
Enalapril	Coronary artery ligation in Sprague Dawley rats	Heart	Increased plasma and cardiac ACE2 activity, and cardiac *Ace2* mRNA levels 8 weeks post surgery[183]
Lisinopril	Transgenic Ren2 rats	Heart/Kidney	Decrease in plasma Ang II, increase in plasma Ang 1–7, cardiac and renal *Ace2* mRNA and activity[182]
Lisinopril	Lewis rats	Kidney	No change in kidney *Ace2* mRNA, but increased ACE2 activity[180]
Angiotensin receptor blockers			
Losartan/Olmesartan	Coronary artery ligation in Lewis rats	Heart	Increase in plasma Ang II, Ang 1–7 and *Ace2* mRNA 28 days post surgery[178]
Losartan	Lewis rats	Heart	Increase in plasma Ang II, Ang 1–7 levels, *Ace2* mRNA and cardiac ACE2 activity[179]
Irbesartan	C57BL/6 mice	Heart	Increase in cardiac *Ace2* mRNA, Irbesartan prevented Ang II induced decrease in ACE2 protein levels[77]
Losartan	Transgenic Ren2 rats	Heart/Kidney	Increase in plasma Ang II, Ang 1–7, cardiac and renal *Ace2* mRNA and activity[182]

Telmisartan	C57BLKS/J mice	Kidney	Following 2 weeks administration, increased ACE2 protein levels, and *Ace2* mRNA expression[184]
Irbesartan	C57BL/6 mice	Aorta	Treatment with irbesartan significantly augmented ACE2 protein levels and *Ace2* mRNA expression[185]
Olmesartan	Spontaneously hypertensive rats	Aorta	Increased plasma Ang II and Ang 1–7 levels, *Ace2* mRNA expression and ACE2 protein levels[181]
Mineralocorticoid receptor blockers			
Spironolactone	Heart failure patients	Monocyte-derived Macrophage	Increase in ACE2 activity and *ACE2* mRNA expression one-month post therapy[191]
Eplerenone	Balb/C mice	Heart/Kidney	Increase in cardiac ACE2 activity and *Ace2* mRNA expression, but non-significant increase in the kidneys[191]
Eplerenone	Wistar rats	Heart	Prevented aldosterone induced reduction in cardiac *Ace2* mRNA expression[190]
Eplerenone	Dahl salt-sensitive hypertensive rats	Heart	No effect on cardiac *Ace2* mRNA expression and protein levels observed in DS rats[189]

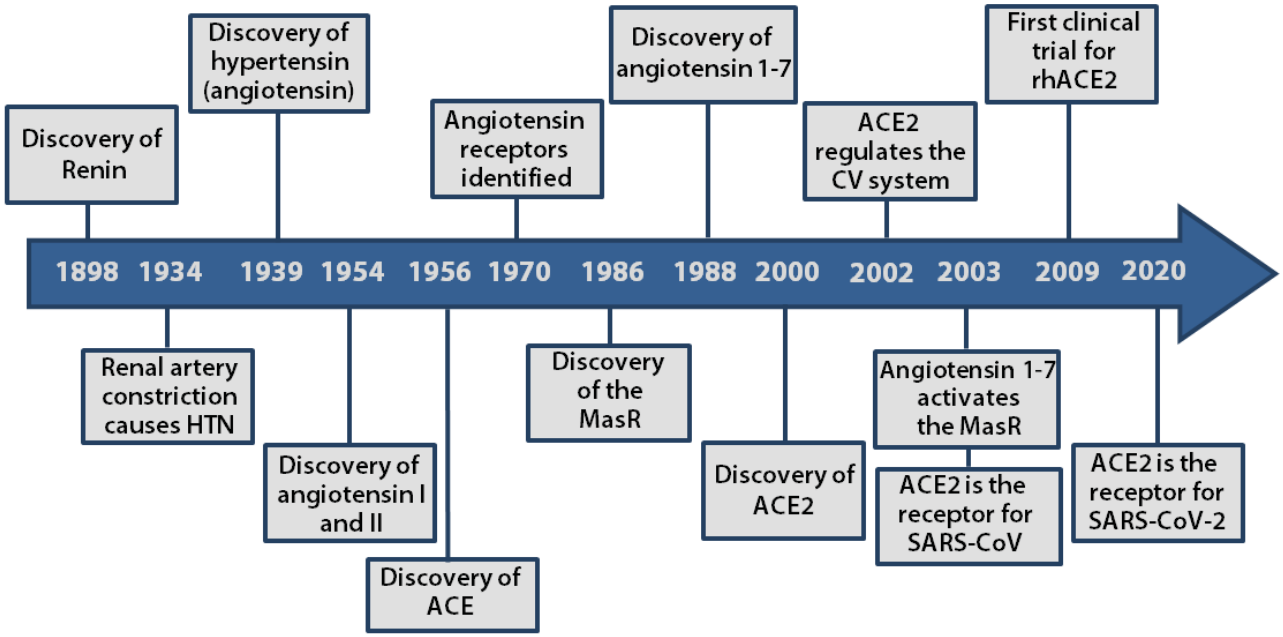


Figure 1.

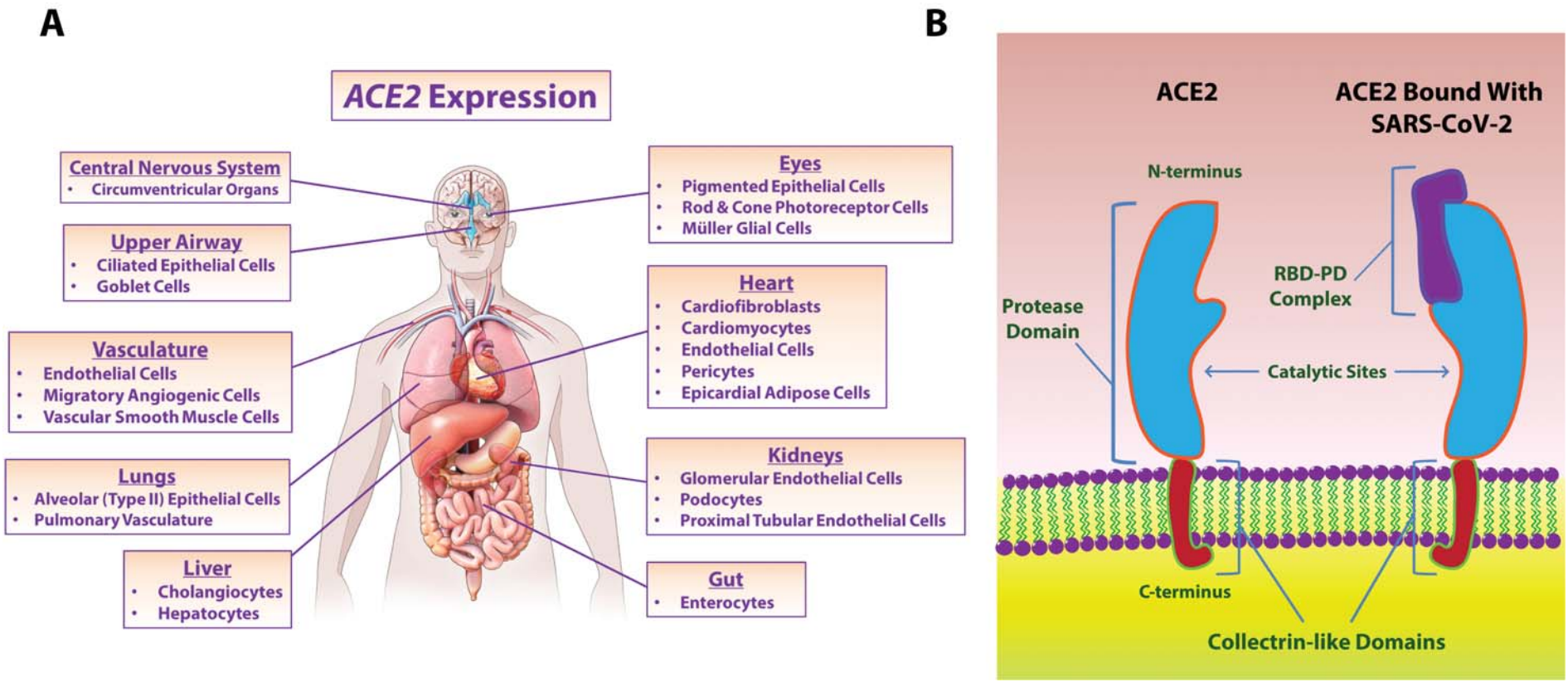


Figure 2.

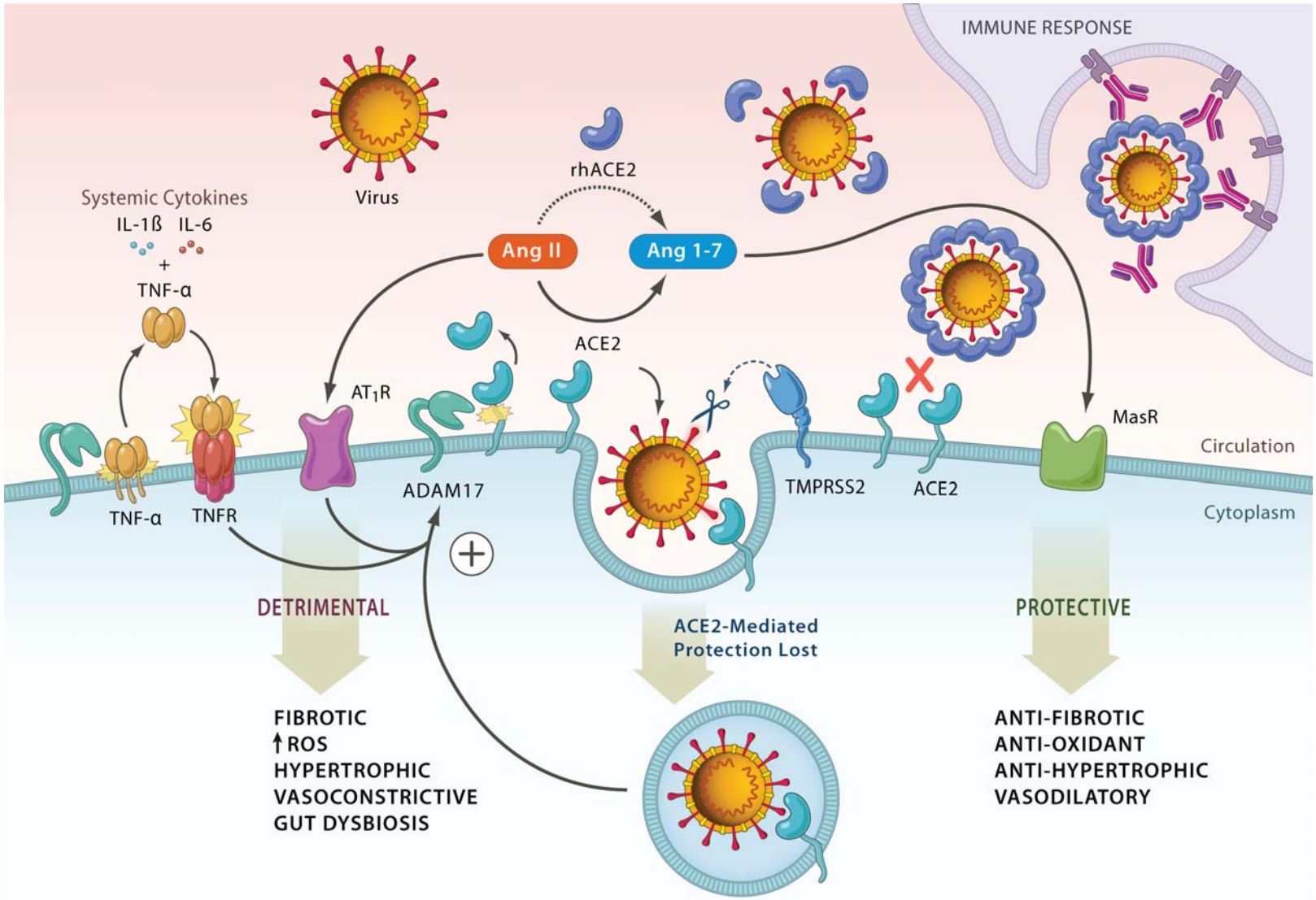


Figure 3.

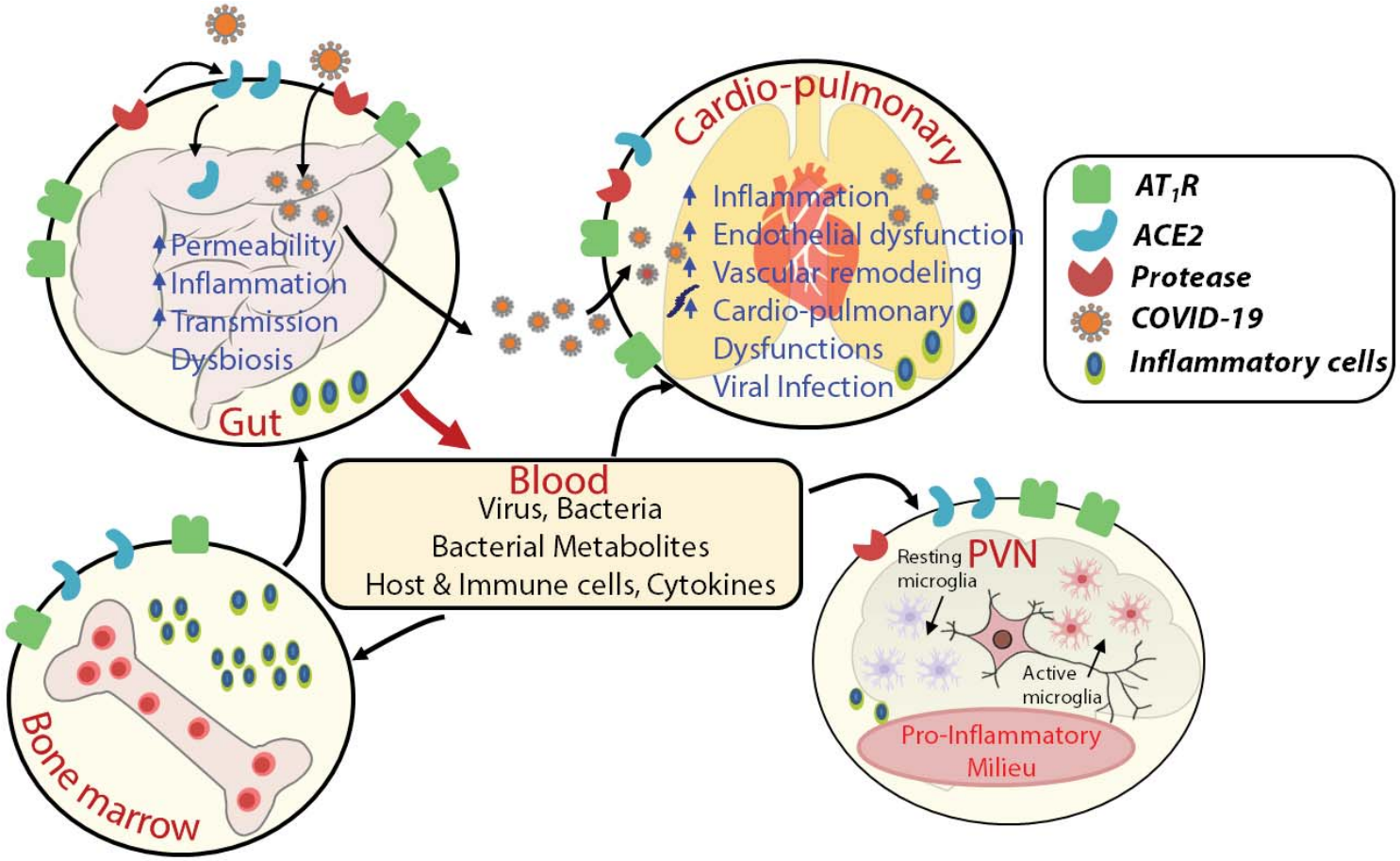
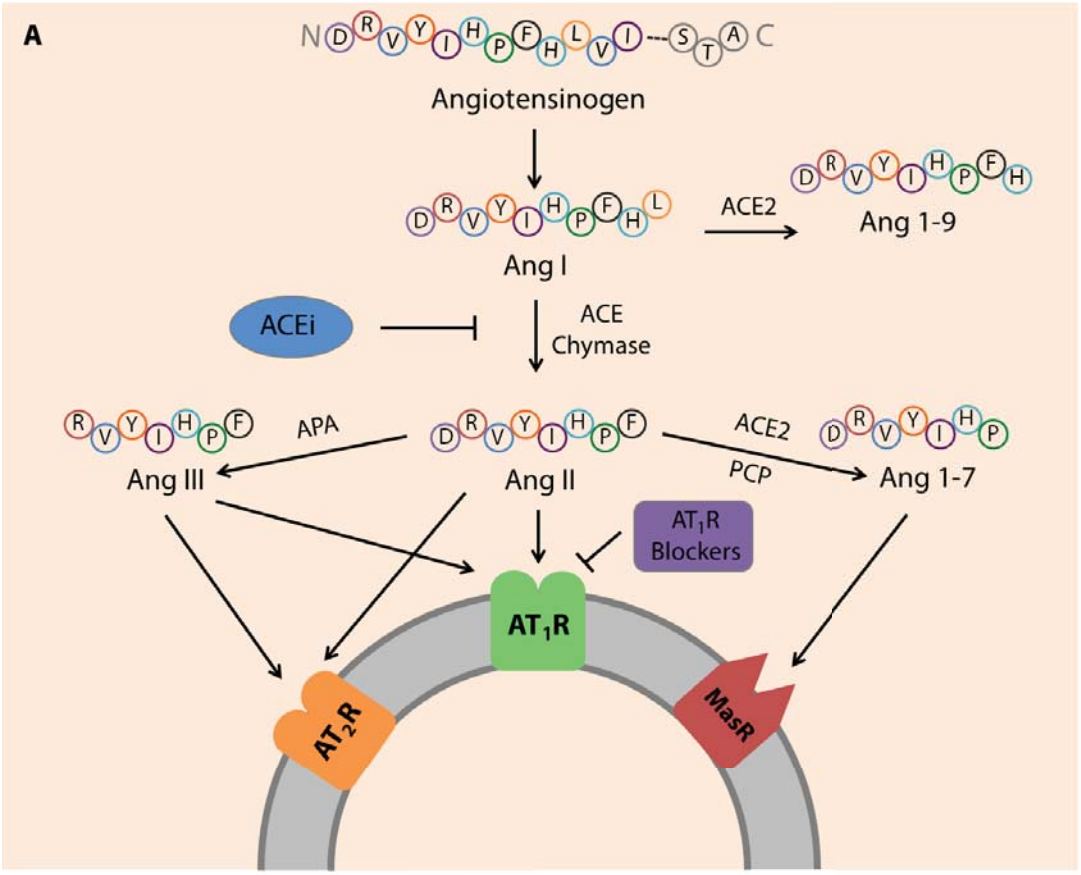


Figure 4.



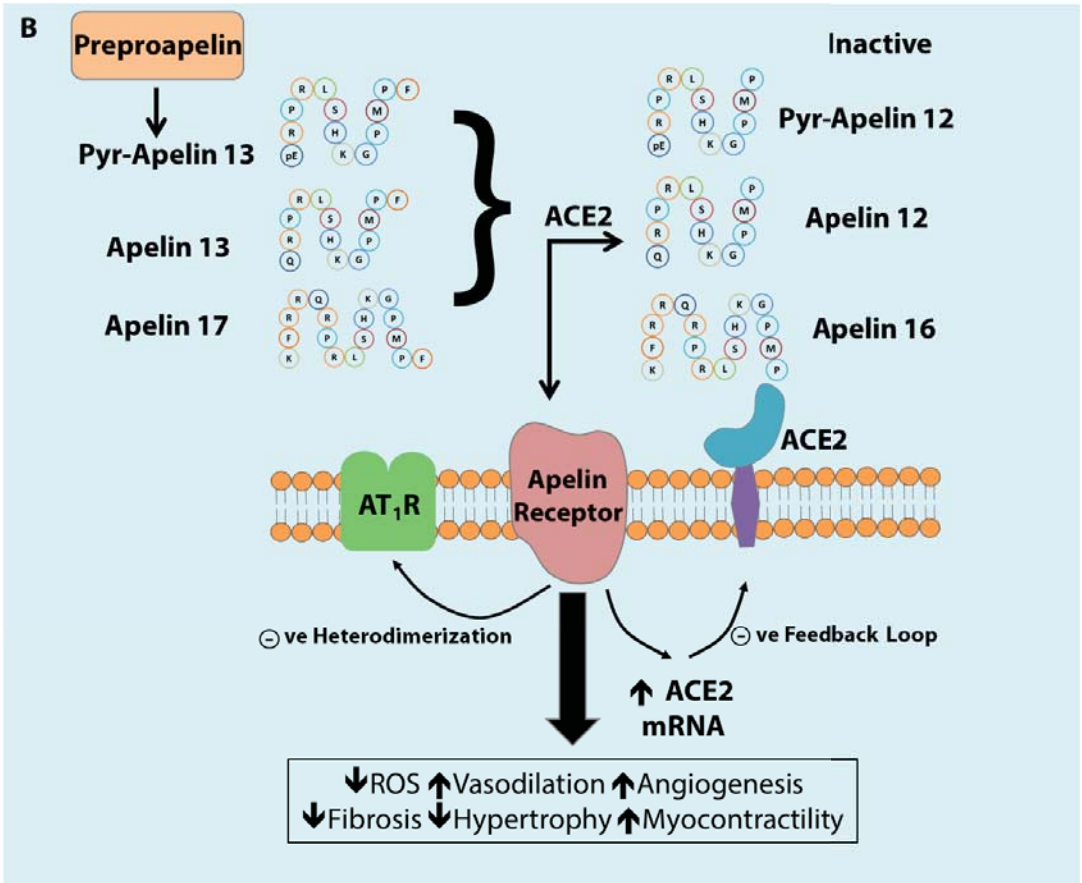


Figure 5.

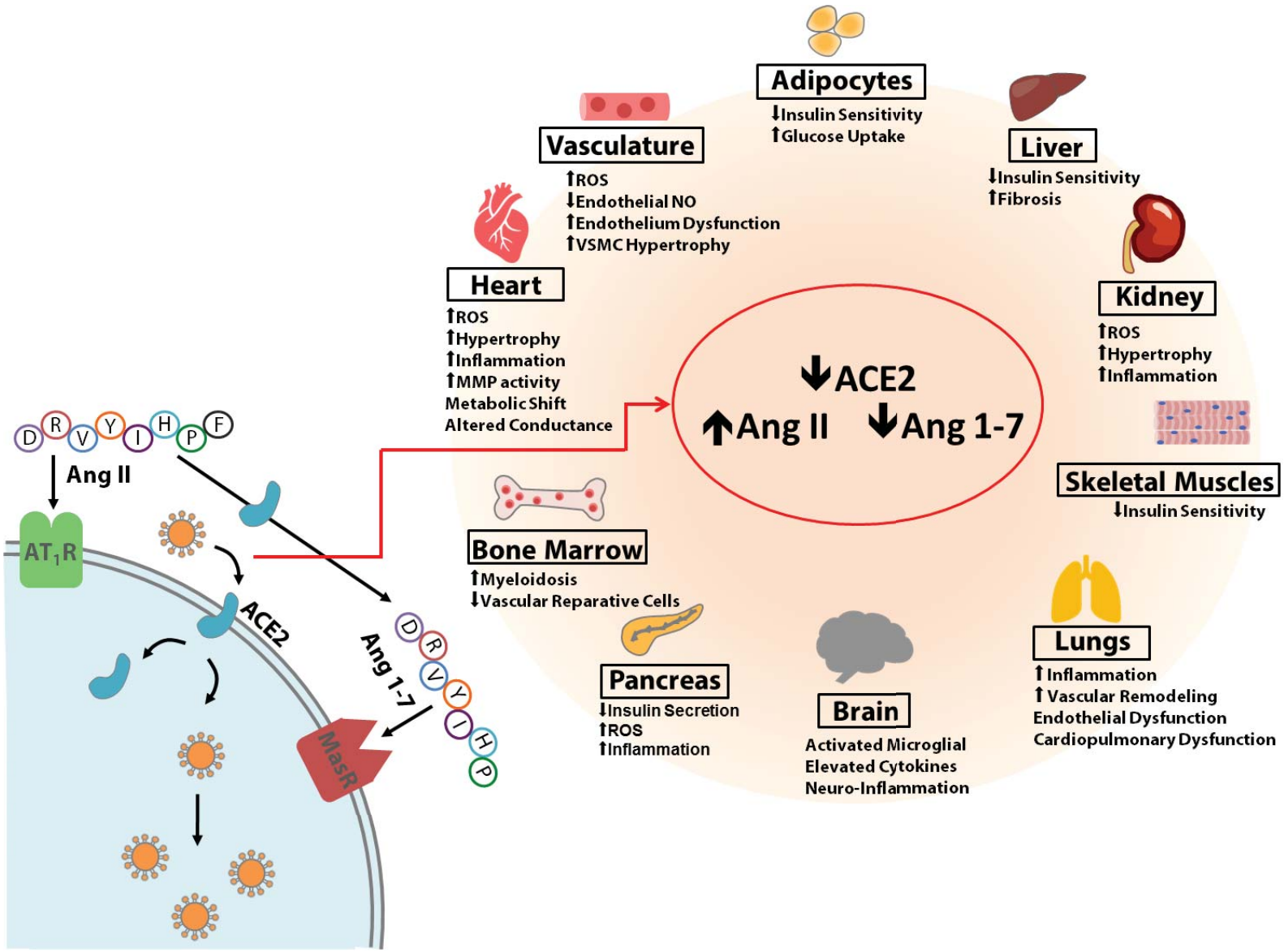


Figure 6.