Clinical Science (2020) **134** 3047–3062
https://doi.org/10.1042/CS20200486

PORTLAND PRESS

Review Article

ACE2, angiotensin 1-7 and skeletal muscle: review in the era of COVID-19

Koichi Yamamoto, Hikari Takeshita and Hiromi Rakugi

Department of Geriatric and General Medicine, Osaka University Graduate School of Medicine, Suita, Osaka, Japan

Correspondence: Koichi Yamamoto (kyamamoto@geriat.med.osaka-u.ac.jp) or Hiromi Rakugi (rakugi@geriat.med.osaka-u.ac.jp)

Angiotensin converting enzyme-2 (ACE2) is a multifunctional transmembrane protein recently recognised as the entry receptor of the virus causing COVID-19. In the renin–angiotensin system (RAS), ACE2 cleaves angiotensin II (Ang II) into angiotensin 1-7 (Ang 1-7), which is considered to exert cellular responses to counteract the activation of the RAS primarily through a receptor, Mas, in multiple organs including skeletal muscle. Previous studies have provided abundant evidence suggesting that Ang 1-7 modulates multiple signalling pathways leading to protection from pathological muscle remodelling and muscle insulin resistance. In contrast, there is relatively little evidence to support the protective role of ACE2 in skeletal muscle. The potential contribution of endogenous ACE2 to the regulation of Ang 1-7-mediated protection of these muscle pathologies is discussed in this review. Recent studies have suggested that ACE2 protects against ageing-associated muscle wasting (sarcopenia) through its function to modulate molecules outside of the RAS. Thus, the potential association of sarcopenia with ACE2 and the associated molecules outside of RAS is also presented herein. Further, we introduce the transcriptional regulation of muscle ACE2 by drugs or exercise, and briefly discuss the potential role of ACE2 in the development of COVID-19.

Introduction

In 2020, the global pandemic of COVID-19 has shed light on Angiotensin converting enzyme-2 (ACE2) as the functional receptor of SARS-CoV2, the causal virus of COVID-19 [1]. ACE2 was identified as a homologue of ACE in 2000, and numerous reports during the last two decades have suggested that ACE2 serves as a protective molecule maintaining physiological homoeostasis and preventing the development of multiple pathologies [2]. The binding of SARS-Cov2 to ACE2 not only initiates virus entry into the human body but also impairs the protective action of ACE2 in affected organs. Therefore, it is currently important to introduce the function of ACE2 in modulating organ function in physiological and pathological conditions, and re-evaluate the net impact of ACE2 on human health in the era of COVID-19. Among organs supposed to be functionally affected by endogenous ACE2, this review focuses on ACE2 in skeletal muscle. While a majority of previous studies have investigated the role of ACE2 and its associated pathway within the renin–angiotensin system (RAS) in skeletal muscle, our recent studies have suggested the potential involvement of ACE2 in modulating muscle function through pathways outside of the RAS [3–5]. To clarify this point herein, we introduce the association between ACE2 and skeletal muscle within and outside of the RAS. Additionally, we review the pharmacological or non-pharmacological modulation of muscle ACE2 expression and/or activity, and briefly discuss the potential involvement of ACE2 in muscle symptoms or inflammation in COVID-19. The papers reviewed in this article that investigated the role of ACE2-Mas-Angiotensin 1-7 (Ang 1-7) in the skeletal muscle of rodents are summarised in the Supplementary Table S1.

Received: 17 July 2020
Revised: 30 October 2020
Accepted: 03 November 2020

Version of Record published:
24 November 2020

Clinical Science (2020) **134** 3047–3062
https://doi.org/10.1042/CS20200486

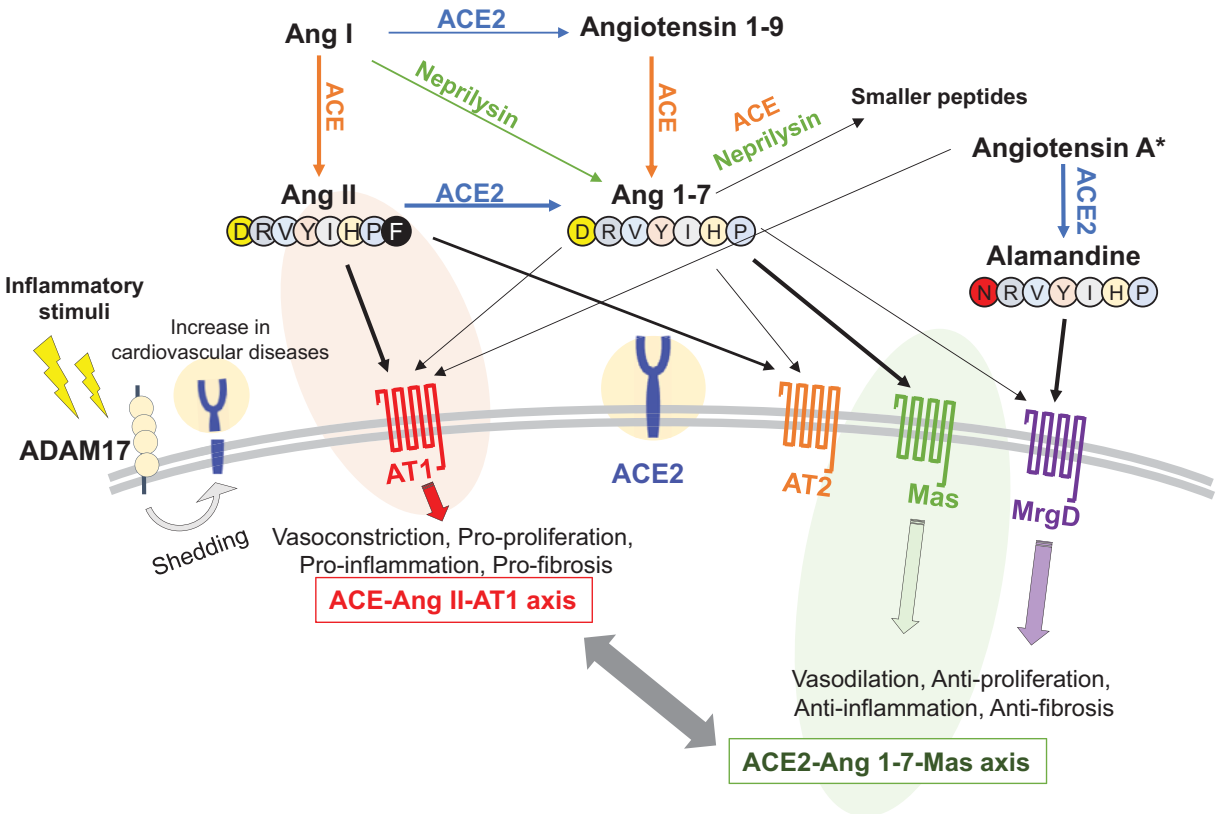

Figure 1. Cascades of the RAS and ACE2

*Produced with enzymatic decarboxylation of Ang II. Abbreviation: Ang I, angiotensin I.

ACE2 and associated molecules in skeletal muscle in the RAS

ACE2-Ang 1-7 pathway as a counter-regulating system of the RAS

Figure 1 *ACE2*, located on chromosome Xp22, is transcribed into a type-I integral membrane peptidase with 40% identity and 61% similarity with ACE [6]. A recent structural analysis revealed that ACE2 forms a homodimer, at least in the presence of the amino acid transporter B0AT1, for which ACE2 functions as a chaperone protein [7]. In contrast with ACE, which functions as a peptidyl-dipeptidase, the ectodomain of ACE2 cleaves angiotensin II [1–8] (Ang II) into Ang 1-7 as a monocarboxypeptidase. Human ACE2 also cleaves angiotensin I to angiotensin 1-9 with a lower catalytic efficacy than that of Ang II to Ang 1-7; however, the effect is absent from mice ACE2 [6,8]. In 2003, Santos et al. demonstrated that the binding of Ang 1-7 to kidneys was abolished in mice deficient in Mas, a G protein-coupled receptor that had been formerly regarded as a proto-oncogene, and identified Mas as a functional receptor of Ang 1-7 [9]. While the biological activity of Ang 1-7 was reported during the 1980s [10–12], the identification of the primary enzyme and receptor to produce and bind the peptide, respectively, has prompted studies to clarify the role of Ang 1-7 in pathological and physiological conditions [2,13]. Ang 1-7 mediates multiple intracellular signalling pathways, including the synthesis of nitric oxide primarily via the AKT-eNOS pathway, inhibition of MAP kinase signalling (ERK1/2, p38, and JNK), inhibition of reactive oxygen species (ROS) production by NADPH oxidases, inhibition of TGF-β-SMAD signalling, and modulation of cAMP signalling response [13–17]. These signalling pathways exert the vasodilating, anti-proliferative, anti-inflammatory, and anti-fibrotic actions of Ang 1-7 [13–17]. While Ang 1-7 has been shown to exert its effect by binding to Mas in several studies using genetic deletion of Mas or Mas-specific antagonists (i.e., A779) [13,15,16,18–20], there are several other receptors that could bind to Ang 1-7 and exert biologically relevant cellular and organ responses, including MrgD, a member of Mas-related G-protein coupled receptors [21,22] and Ang II type2 receptors [17,23,24]. Ang 1-7 also binds to Ang II type 1 receptors (AT1) and functions as a biased agonist of AT1 [25,26]. In addition, it has been recently reported that alamandine (Ala–Arg–Val–Tyr–Ile–His–Pro–Phe), an endogenous peptide that differs from Ang 1-7

(Asp–Arg–Val–Tyr–Ile–His–Pro–Phe) in one N-terminal amino acid and is cleaved from angiotensin A by ACE2, binds to MrGD and provokes a protective organ response similar to Ang 1-7 [27–30]. To date, no study has been reported on the function of alamandine in skeletal muscle. While ACE2 is a major determinant in regulating the tissue level of Ang 1-7, neprilysin, a type-II integral membrane protein that cleaves angiotensin I to Ang 1-7 also participates in the regulation of the tissue and plasma levels of Ang 1-7 [30–32]. Neprilysin also cleaves Ang 1-7 to small peptides as well as ACE [33,34], and angiotensin 1-2, the proteolytic product of neprylisin, has been shown to be relevant in pancreatic insulin secretion [35]. Collectively, while the ACE2-Ang 1-7-Mas axis represents a major pathway to counteract the activation of the ACE-Ang II-AT1 axis, recent developments in research have revealed multiple alternative pathways and diversely widened the regulatory network of the RAS [30].

Regarding the organ-specific activity of ACE2, the ectodomain shedding of ACE2 by a disintegrin and metalloproteinase 17 (ADAM17), also known as TNFα-converting enzyme (TACE), is an important biological reaction that degrades tissue ACE2 activity under multiple pathological conditions [36–39]. The activity of ADAM17 increases in response to various inflammatory stimuli [40,41], and circulating soluble ACE2 produced by shedding with ADAM17 has been shown to be a potential biomarker of human cardiovascular diseases, including heart failure [42–44], atrial fibrillation [45], chronic kidney disease [46], atherosclerosis [47], myocardial infarction [48] and stroke [49]. Petel et al. reported that Ang II increases the myocardial activity of ADAM17, whereas myocardial ACE2 protein levels and activity are substantially decreased with a corresponding increase in plasma ACE2 activity [37]. Further, it has been reported that skeletal muscle ADAM17 increases in several conditions, including increased adiposity [50] and type 2 diabetes [51], and that a PPARγ agonist reduces the muscle ADAM17 activity [52]; however, the relationship between ADAM17 and ACE2 in skeletal muscle has not yet been investigated. In addition, studies on the alteration of circulating ACE2 levels in patients with muscle disorders are not currently available.

ACE2-Ang 1-7 in skeletal muscle

Skeletal muscle is a central player in regulating not only the motor system but also metabolic homoeostasis by modulating insulin sensitivity [53,54]. Activation of the classical RAS pathway: the ACE-Ang II-AT1 axis has been proven to participate in muscle pathogenesis to promote disorder in the motor system or insulin sensitivity, that is, muscle wasting accompanied by pathological muscle remodelling [55–57] or insulin resistance [58,59], respectively. Regarding the ACE2-Ang 1-7 pathway as a counter-regulatory system of the ACE-Ang II-AT1 axis, previous studies in skeletal muscle have primarily focused on the protective role of Ang 1-7 in pathological muscle remodelling and insulin resistance, and direct evidence linking ACE2 to these pathologies are relatively small. The role of Ang 1-7 in muscle disorders has been extensively reviewed previously [13,60–63]. In this section, we briefly introduce a vital evidence in support of the protective role of Ang 1-7 in muscle disorders and discuss the plausible role of ACE2 in the muscle RAS within the scope of the available evidence.

Muscle remodelling

Figure 2 Muscle wasting is a morbidity caused by multiple factors including those outside of skeletal muscle, such as disorders in motor neurons, abnormal nutrition, systemic inflammation, physical immobility and impaired oxygen supply [64]. Herein, we discuss only pathologies that could directly cause muscle remodelling, leading to muscle wasting. Pathological muscle remodelling involves two primary alterations in skeletal muscle including atrophy and fibrosis that could occur independently but synergistically modulate the progression of functional disorders in multiple pathological conditions with muscle wasting. The process of muscle remodelling could also involve endothelial dysfunction in muscle microcirculation, and the influence of the RAS on endothelial dysfunction in skeletal muscle is reviewed in the next section. As reviewed previously, the activation of the classical RAS pathway plays a pivotal role in the development of pathological muscle remodelling by promoting atrophy and fibrosis in skeletal muscle [60,63]. Briefly, muscle atrophy is caused by an imbalance in protein synthesis and degradation. Ang II impairs muscle protein synthesis primarily by inhibiting the IGF-1-AKT-mTOR pathway. Protein degradation is also promoted by Ang II via the induction of multiple cell signalling pathways including the up-regulation of atrogenes, such as atrogin-1 and MuRF-1, which induce ubiquitin-proteasome-dependent protein breakdown and caspase-dependent myonuclear apoptosis [60,63]. This imbalance of protein synthesis and degradation caused by Ang II is primarily attributed to NOX2-dependent ROS production and subsequent cellular phenomena including NfκB-dependent inflammation and mitochondrial damage [60,63].

Muscle fibrosis can occur when damaged or atrophied muscle fibres are replaced with connective tissues, and Ang II plays a pivotal role in promoting muscle fibrosis [60,63]. NOX2-dependent ROS production is also an upstream signalling pathway of Ang II-induced fibrosis, and TGF-β and TGF-β-induced connective tissue growth factor are considered key players in this process [60,63,65]. In addition, the process of Ang II-induced fibrosis could involve

Clinical Science (2020) **134** 3047–3062
https://doi.org/10.1042/CS20200486

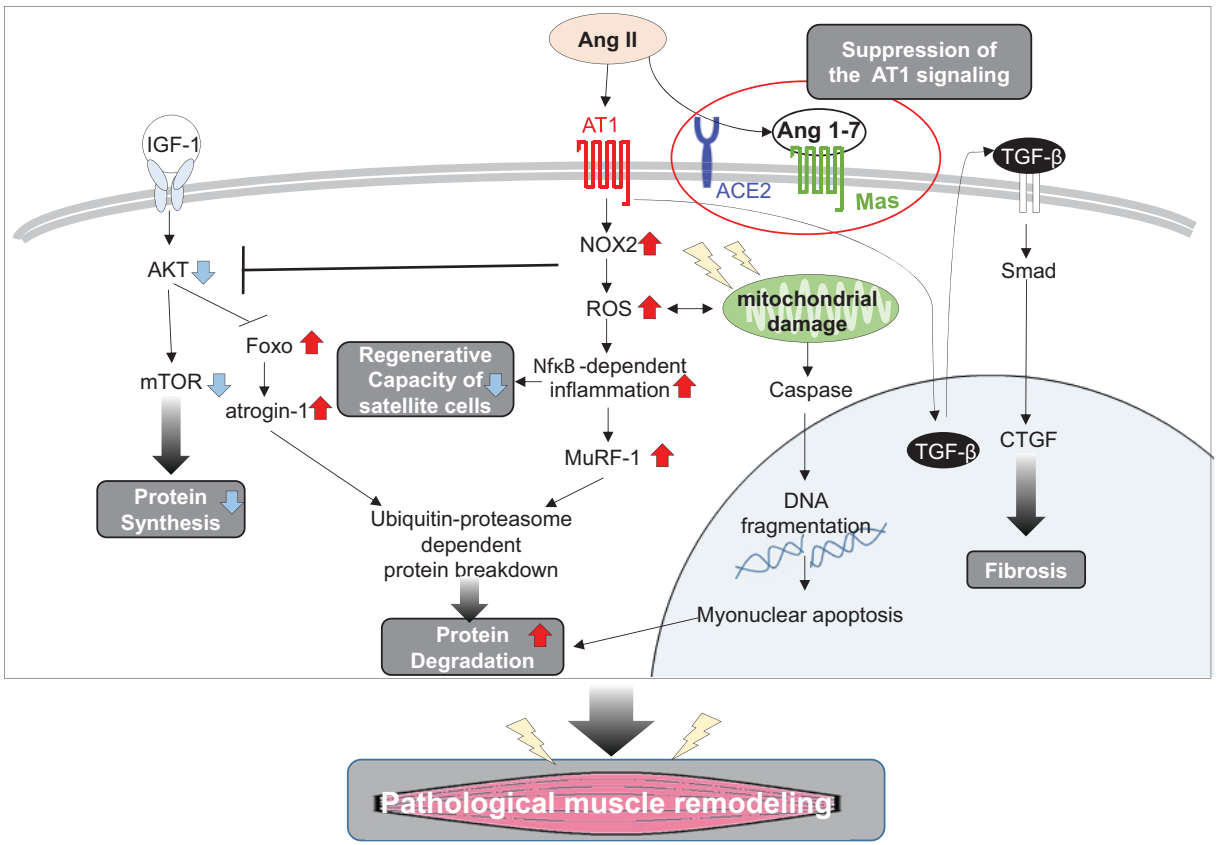

Figure 2. The ACE2-Ang 1-7-Mas axis counter-regulating the action of the ACE-Ang II-AT1 axis in the development of pathological muscle remodelling

NfκB-induced tissue inflammation and the impaired regenerative capacity of muscle progenitor cells and satellite cells, while controversy exists due to competing studies on the effect of Ang II on muscle regeneration [60,66,67].

Current understanding of the beneficial effect of Ang 1-7 on muscle remodelling is primarily explained by the inhibition of Ang II-associated phenomenon to induce atrophy and fibrosis. The biological role of Ang 1-7 signalling through Mas has been suggested by a study that showed that pathological conditions inducing muscle remodelling, including immobilisation and infusion of Ang II and LPS, increased the muscle Mas expression [68]. In line with this, we found that muscle *Mas* was 3.7-fold up-regulated in 15-month-old Tsukuba hypertensive mice carrying human renin and angiotensinogen, suggesting that Mas could increase as a compensation for the chronic overload of Ang II [4]. Ang 1-7 infusion alleviated muscle dysfunction in multiple pathological conditions, including Ang II infusion [69–71], muscular dystrophy [72,73], disuse-induced atrophy [74,75], chronic liver disease [76], exhaustive swimming exercise [77] and cancer cachexia [78] in rodents. Most of these studies used genetic deletion [72,75] or pharmacological inhibition [69–72] of Mas to show the dependency of Mas in the Ang 1-7-induced effects on pathological muscle remodelling, while Murphy et al. used muscle-specific Mas overexpression mice or a Mas agonist to show the protective effects of Mas in cancer-induced muscle wasting [78].

In contrast with the multiple lines of evidence for Ang 1-7 and Mas, only one study investigating the potential role of ACE2 in pathological muscle remodelling currently exists [79]. In this study, Riquelme et al. reported that ACE2 activity and protein levels in skeletal muscle were increased by the genetic induction of muscular dystrophy or chronic injury with local $BaCl_2$ injection. This could imply a compensation mechanism to up-regulate the protective pathway of the RAS against muscle injury, along with an increase in Mas in several muscle pathologies [4,68]. They also showed that muscle injection of adenovirus encoding human ACE2 decreased collagen I levels and macrophage infiltration in the affected muscles, suggesting that the enhanced Ang 1-7 production by ACE2 overexpression contributes to pathology reduction in muscle dystrophy [79]. Nevertheless, the biological relevance of endogenous ACE2 in muscle remodelling is yet to be determined, as it remains unknown whether the tissue Ang 1-7 level potentially produced

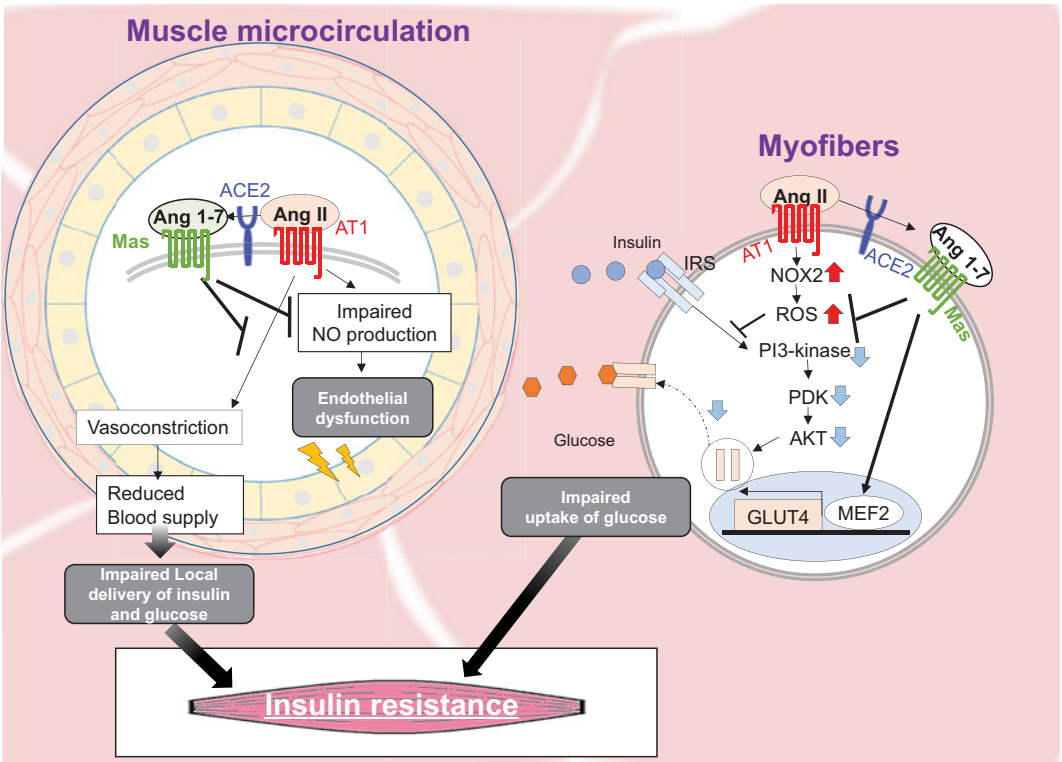

Figure 3. The ACE2-Ang 1-7-Mas axis counter-regulating the action of the ACE-Ang II-AT1 axis in the development of insulin resistance

Abbreviation: MEF2, myocyte enhancer factor.

by endogenous ACE2 is sufficient to contribute to the protection from the development of pathological muscle remodelling. Further, Acuna et al. reported that the genetic deletion or antagonist A779 of Mas deteriorated muscular architecture and increased fibrosis and TGF-β signalling with diminished muscle strength in dystrophic MDX mice, suggesting that endogenous Mas in skeletal muscle is relevant for protection against pathological muscle remodelling [72]. However, given the alternative pathway of Ang 1-7 production by neprilysin [30–32], it remains undetermined whether the proposed role of endogenous Mas in muscle remodelling is completely linked to the catalytic activity of ACE2. Studies using ACE2-function-deficient animals may be helpful in drawing conclusions about this subject.

Muscle insulin resistance

Figure 3 Accumulating evidence has suggested that insulin resistance in skeletal muscle caused by the activation of the classical RAS pathway primarily involves two independent mechanisms: haemodynamic disorder in the local delivery of insulin and glucose to skeletal muscle and dysregulation of insulin-mediated cell signalling for the uptake of glucose in skeletal muscle [58–60]. The former is associated with reduced blood supply potentially caused by Ang II-mediated vasoconstriction and endothelial dysfunction in muscle microcirculation. Ang II-mediated endothelial dysfunction involves multiple signalling pathways, eventually leading to the decreased production of nitric peroxide [80–82]. The latter is a reduced translocation of the cytoplasmic glucose transporter 4 (GLUT4) to the plasma membrane in response to insulin binding to insulin receptors in skeletal muscle cells. This process is primarily caused by ROS generated by the Ang II-induced activation of muscle NOX2 [83] which interferes with intracellular signalling cascades of insulin, including the insulin receptor substrate, phosphatidylinositol-3-kinase, 3-phosphoinositide-dependent kinases and AKT [58–60]. Previous studies have indicated that Ang 1-7 antagonises these cellular mechanisms of Ang II in mediating insulin resistance primarily through a receptor, Mas. First, multiple lines of evidence have confirmed that Ang 1-7 could restore endothelial dysfunction in various organs by counteracting Ang II-induced impairment of NO production [84–91]. The involvement of Mas in Ang 1-7-induced endothelial protection has been shown by endothelial dysfunction in rodents with genetic [92–94] or pharmacological blockade of Mas [95]. Interestingly, it

Clinical Science (2020) **134** 3047–3062
https://doi.org/10.1042/CS20200486

was recently reported that skeletal muscle angiogenesis and endothelial cell tube formation were induced by Ang 1-7 and inhibited by either genetic deletion of AT1a or pharmacological blockade of Mas in Dahl salt-sensitive rats [96], supporting a previous finding that the interaction between AT1 and Mas is pivotal in the role of Ang 1-7 in endothelial function [97]. Consistently, the potential production of Ang 1-7 by ACE2 has also been shown to contribute to endothelial protection. Overexpression or pharmacological activation of ACE2 improves endothelial function in systemic [98–100] and pulmonary arteries [101]. Nevertheless, there is no direct evidence supporting that the potential protective roles of ACE2, Ang 1-7 and Mas in insulin sensitivity are truly attributed to the improvement of microcirculation in skeletal muscle. The direct action of Ang 1-7 primarily through Mas in insulin-mediated translocation of GLUT4 has also been shown by several lines of evidence in adipose tissue [102,103], heart [104], liver [103] and skeletal muscle [103,105,106]. More recently, it was reported that the insulin-sensitising effect of exercise was abolished by the selective Mas agonist A779 in skeletal muscle, suggesting that Ang 1-7, acting through Mas, participates in exercise-induced enhancement of insulin sensitivity [107]. Regarding the association of ACE2 with muscle glucose metabolism, we previously found that deficiency of ACE2 exaggerated insulin resistance and glucose tolerance in response to a high-fat diet and Ang II infusion [108].

Whereas we unexpectedly did not find any alteration of insulin-mediated AKT activation in soleus muscle of ACE2-deficient mice, we found that the protein expressions of GLUT4 and MEF2A, a major transcriptional factor involved in GLUT4 transcription, were markedly reduced in soleus muscle in standard-diet fed ACE2-deficient mice [108]. The differences between the expression levels of these proteins in ACE2-deficient mice compared with those in wild-type mice were abolished by either ANG 1-7 or the Mas agonist A779, suggesting that this phenomenon depends on the role of ACE2 in the RAS [108]. This observation is in contrast with a work by Bernardi et al., which reported that ACE2-deficient mice showed glucose intolerance with impaired pancreatic insulin production but preserved or rather enhanced insulin sensitivity [109]. They showed that *GLUT-4* mRNA was rather elevated in vastus lateralis muscle in standard diet-fed ACE2-deficient mice. While these contrast findings might not be easy to explain, the difference in the study period (4 weeks high-fat, high-sucrose diet from 8 weeks old in [108] and 12 weeks high-fat diet from 8 weeks old in [109]) or different sources of ACE2-deficient mice could have influenced the phenotypes of the experimental animals (both had a C57BL6J background, but generated separately) [110]. Further, the pathogenesis of muscle insulin resistance by ACE2 deletion has been demonstrated by Cao et al., who reported that ACE2-deficient mice exhibited lipid accumulation in skeletal muscle with elevated levels of ER stress and mitochondrial dysfunction [111]. Another study reported that ACE2-deficient mice fed with a high-fat diet from weaning to 6 months of age exhibited glucose intolerance with epicardial adipose tissue inflammation and cardiac dysfunction accompanied by cardiac insulin resistance, which were ameliorated by Ang 1-7 treatment [112].

ACE2 and associated molecules in skeletal muscle outside of the RAS

While numerous studies have established the pivotal role of ACE2 in the RAS during the last two decades of discovery, some studies have focused on the multiple functions of ACE2 outside of the RAS. Although these studies were primarily conducted in organs not including skeletal muscles, our recent findings have shown the pivotal role of ACE2 in maintaining muscle function during ageing that appears to be independent of its role in the RAS. In this section, we briefly summarise the multiple roles of ACE2 and discuss its relevance independent of the RAS in skeletal muscle during ageing.

The multiple actions of ACE2 outside of the RAS

Figure 4 Multiple actions of ACE2 are mediated by either a peptidase-dependent or -independent pathway [113,114]. The peptidase-dependent pathway depends on the catalytic activity of ACE2, and the known target peptides of ACE2 in addition to angiotensin-related peptides include apelin-13, des-Arg[9]-bradykinin, and dynorphin A-13 [113,115,116]. The peptidase-independent pathway of ACE2 includes its function as a receptor of integrins [117,118] and a chaperon protein of the amino-acid transporter based on its homology to collectrin [119–123], in addition to its role as an entry receptor of SARS-CoV and SARS-CoV2 [113,114,124]. The C-terminus domain, including the transmembrane and intracellular domains, is homologous to collectrin, while the N-terminus extracellular domain is responsible for the other functions of ACE2 [113] (Figure 4).

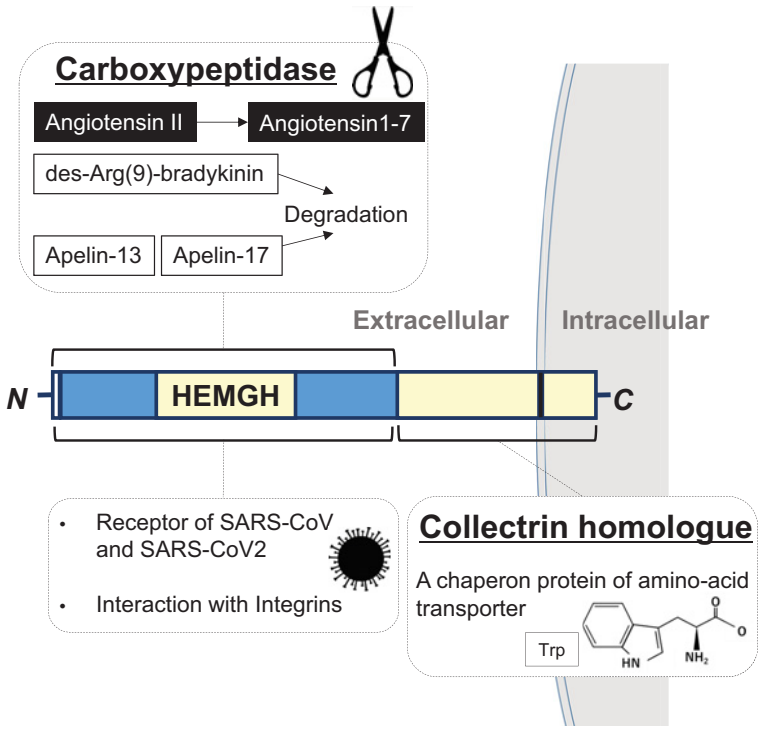

Figure 4. The structure and multiple actions of ACE2

The potential role of ACE2 in sarcopenia through pathways outside of the RAS

Age-associated loss of muscle mass and function has recently been recognised as sarcopenia, and the underlying molecular mechanisms in the development of sarcopenia are at least partly considered distinct from those of the above-mentioned pathological muscle remodelling [125,126]. We recently reported that ACE2-knockout mice exhibit early ageing-associated muscle weakness with signatures of ageing including the induction of *p16INK4a*, a senescence-associated gene, and increased numbers of centrally nucleated fibres (CNFs), a hallmark of either regenerated or aged muscle, in skeletal muscle at 25 months [5]. Thereafter, we confirmed the ageing-associated acceleration of muscle weakness and reduction in muscle mass in ACE2-knockout mice with contrasting findings in old Mas-knockout mice that exhibited muscle strength, architecture, and other ageing-related changes equivalent to those of the wild-type mice [3]. Interestingly, we also found that Ang 1-7 infusion alleviated muscle weakness in 25-month-old wild-type and ACE2-knockout mice, while the effects were absent from Mas-knockout mice [3] (Figure 4). Collectively, these findings suggest that Ang 1-7-Mas signalling is not responsible for the impact of ACE2 on sarcopenia, while Ang 1-7 has a beneficial effect on ageing-associated muscle weakness through Mas. The RAS-independent mechanism in the sarcopenic phenotypes of old ACE2-knockout mice was further supported by comparison with mice with chronic activation of the RAS [4]. We found that 15-month-old Tsukuba hypertensive mice carrying human renin and angiotensinogen did not exhibit increased CNF and *p16INK4a* expressions, while ACE2-knockout mice of the same age did. These findings suggest that the potential chronic activation of the RAS does not explain the muscle phenotypes in ACE2-knockout mice, whereas chronic inhibition of the classical RAS was shown to preserve late-life muscle function in the report that AT1a-knockout mice exhibited a significant decrease in muscle strength with ageing compared with wild-type mice [127]. Taken together, ACE2 appears to contribute to the prevention of sarcopenia through pathways independent of the RAS, while it remains undetermined whether this is attributed to known or unidentified mechanisms of ACE2. Nevertheless, previous studies have provided hints to connect the known RAS-independent actions of ACE2 and the development of sarcopenia. In the next section, we introduce the plausible roles of the ACE2-associated molecules in the development of sarcopenia, and discuss the possibility of these roles to explain the sarcopenic phenotypes of ACE2-knockout mice.

Clinical Science (2020) **134** 3047–3062
https://doi.org/10.1042/CS20200486

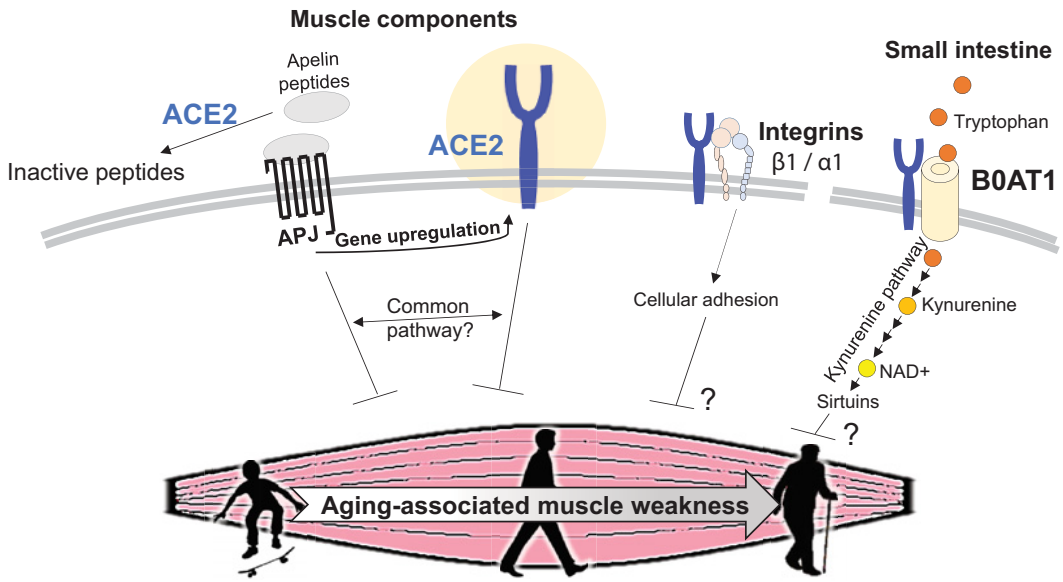

Figure 5. The plausible RAS-independent actions of ACE2 and the associated-molecules in the ageing-associated muscle weakness

The RAS-independent functions of ACE2 and associated molecules in skeletal muscle

Figure 5 Apelin peptides are cleaved from the 77-amino-acid prepropeptide and bind to the G protein-coupled receptor APLNR to mediate biological responses involved in many physiological processes [128]. As a carboxypepidase, ACE2 has been shown to cleave two biologically active forms of apelin peptides, pyr-apelin 13 and apelin 17, into inactive peptides, pyr-apelin 12 and 16, respectively [129]. Further, Wang et al. [129] recently reported that the inactivation of these apelin peptides by ACE2 can blunt the hypotensive effect of apelin in mice, suggesting that the apelin catalytic activity of ACE2 is physiologically relevant. However, the relationship between ACE2 and apelin is not unidirectional, as it was reported that apelin increases gene transcription of ACE2 through APLNR, and Ang 1-7 rescued hypertrophy and heart dysfunctions in apelin-knockout mice, suggesting that ACE2 is a downstream effector of the apelin-APLNR system [130].

Interestingly, apelin is an exercise-induced peptide [131] and has been recently reported to have anti-senescent properties [35,132]. Vinel et al. recently reported that apelin production is reduced by ageing and the deletion of either apelin or APLNR accelerated, and that supplementation of apelin rescued ageing-associated muscle weakness with signatures of ageing in mice [132]. Given the close relationship between apelin and ACE2, it is conceivable that mice deficient in ACE2, apelin and APLNR share the same mechanisms to exhibit accelerated sarcopenic phenotypes.

Based on its abundance in small intestinal enterocytes, ACE2, as a collectrin homologue, plays an important role in absorbing amino acids by interacting with the neutral amino-acid transporter B0AT1 [119–123]. ACE2-knockout mice exhibited marked reduction, particularly in the essential amino acid tryptophan (Trp) in plasma and organs, including skeletal muscle [122,123]. Importantly, Trp and its metabolites, including kynurenine, are closely associated with age-related diseases and lifespan [133]. Additionally, Ninomiya et al. recently reported that serum concentration of Trp is positively correlated with the volume of skeletal muscles in patients with diffuse large B-cell lymphoma [134]. They also showed that fibre diameters in the tibialis anterior of C57BL/6 mice fed with a Trp-deficient diet were smaller than those in mice fed with a standard diet, suggesting a critical role of Trp in regulating muscle mass. In addition, Trp is a starting material of the *de novo* synthetic pathway of NAD^+, which is known to protect from ageing through pathways including sirtuin regulation [135,136]. It was recently reported that *de novo* NAD^+ synthesis enhanced the lifespan of *C. elegans* and alleviated disorders in the kidney and liver [137]. Finally, ACE2 also binds to integrin β1 and integrin α5 [117,138], and was shown in an *in vitro* study to induce cellular adhesion, probably by interacting with integrin [117]. Integrins are important molecules for adhesion, and Rozo et al. recently reported

that β1-integrin enhanced regeneration in aged and dystrophic mice [139]. Further investigation is required to clarify whether these potential interactions between ACE2 and molecules outside of the RAS could contribute to the sarcopenic phenotype in ACE2-knockout mice.

Pharmacological or non-pharmacological modulation of ACE2 in skeletal muscle

Given the potential benefit of ACE2 in regulating organ function, including that of skeletal muscles, attention has also been paid to the modulation of ACE2 expression or activity via pharmacological and non-pharmacological interventions. Regarding pharmacological modulation, while inhibitors of RAS, AT1 blockers (ARB), and ACE inhibitors have been shown to increase the expression or activity of several organs including the heart, kidney and arteries [2,140], no previous report supports the increase in ACE2 by these drugs in skeletal muscle. A study reported that pioglitazone, a PPARγ agonist, increased the protein expression of ACE2 in the liver, adipose tissue, and skeletal muscle in rats fed with a high-fat diet [141]. Diminazene aceturate (DIZE) is a potent ACE2 activator and has been shown to exert beneficial effects in experimental models of cardiovascular diseases [142]. Bruce et al. reported that DIZE reduced adiposity but preserved lean mass with increased serum ACE2 activity in young and old rats [143]. Accumulating evidence suggests that exercise is a promising non-pharmacological intervention to modulate the RAS [144,145]. Gomes-Santos et al. reported that exercise training did not alter the activity and protein expression of ACE2 but increased the concentration of Ang 1-7 in the skeletal muscle of Wistar rats with chronic heart failure induced by left coronary artery ligation [146]. Further, Frantz et al. reported that a high-fat diet increased the protein expression ratio of ACE to ACE2, and that the ratio was reversed by a high volume of exercise in Wistar–Kyoto rats [147]. In both reports, exercise training increased Mas expression in skeletal muscles [146,147]. It has also been reported that exercise training increases Mas in the left ventricle [148] or vascular endothelium of spontaneous hypertensive rats [149,150]. These findings suggest that exercise training could shift the RAS from the ACE-AngII-AT1 axis to the ACE2-Ang 1-7-Mas axis in the muscle and muscle environment. Nevertheless, it remains unknown whether the potential modulation of ACE2 by exercise could be, at least partially, involved in the diverse effects of exercise on muscle function. Motta-Santos et al. reported that ACE2-knockout mice ran less than wild-type mice in voluntary wheel running, which reduced the fat mass and increased the muscle mass in wild-type mice but not in ACE2-knockout mice [151]. This report suggests that ACE2 affects physical performance and could imply a positive feedback loop in which ACE2 increases exercise performance and exercise activates the ACE2-Ang 1-7-Mas axis.

ACE2 and skeletal muscle in COVID-19

In the COVID-19 pandemic, it has been shown that the clinical and laboratory manifestations of organ damage besides pneumonia are associated with poor clinical course after infection with SARS-CoV2 [152–154]. Mao et al. reported that COVID-19 patients with more severe infection had a higher prevalence of skeletal muscle injury with myalgia and elevated serum creatine kinase levels compared with those with non-severe infection (19.3 vs. 4.8%, $P<0.01$) [154]. COVID-19 has also been reported to induce rhabdomyolysis [155,156]. While the mechanisms of individual organ damage in COVID-19 could involve a systemic inflammatory response that parallels the severity of the disease [157], interest has focused on the presence or absence of organ injury by direct virus invasion [158–162]. It has been reported that SARS-CoV2 virus particles were detected in organs including the heart, liver and kidney in autopsy analysis [163–165]. It has also been shown that SARS-CoV2 could directly infect engineered human blood vessel organoids and human kidney organoids, which can be inhibited by human recombinant soluble ACE2, suggesting ACE2-dependent virus entry into each organ [166]. In addition, several studies have indicated that mice harbouring human ACE2 showed extra-pulmonary infection of SARS-CoV-2 in tissues expressing human ACE2 [155,160,161]. As skeletal muscles express ACE2 and TMPRSS2, a protease that facilitates the virus–cell fusion [158], several studies have postulated that skeletal muscle could be invaded by SARS-CoV-2 through resident ACE2-expressing cells [158–162]. Nevertheless, there has been no evidence in support of the direct invasion of SARS-CoV2 into skeletal muscle. A study reported that no virus particle was detected in the skeletal muscle of four patients who died from SARS, in which SARS-CoV utilised ACE2 for invasion [138]. Further, myositis with muscle atrophic changes has been previously observed in a patient with infection by MERS-CoV that utilises dipeptidyl peptidase-4 for invasion, while tissue tropism in skeletal muscle has not been examined in the literature [167]. Given the limited evidence, further investigation is required to elucidate whether skeletal muscle injury in COVID-19 is attributed to direct virus invasion through ACE2 or just secondary to systemic inflammation.

Clinical Science (2020) **134** 3047–3062
https://doi.org/10.1042/CS20200486

Perspectives

Abundant evidence during the last two decades appears to have established the pivotal role of the non-classical axis of the RAS, the ACE2-Ang 1-7-Mas axis of skeletal muscle in protecting against the development of insulin resistance or muscle wasting accompanied by muscle remodelling. However, recent analysis of angiotensin-related peptides and receptors revealed that the protective pathways of the RAS are more diverse than previously thought, and that re-evaluation of the simple concept of the ACE2-Ang 1-7-Mas axis is needed. In addition, previous studies on skeletal muscle primarily focused on the function of Ang 1-7 through Mas, and there was relatively little evidence in support of the role of endogenous ACE2 in producing sufficient levels of Ang 1-7 to exert biologically relevant functions in skeletal muscle. Finally, recent findings support the function of ACE2 independent of the RAS in regulating ageing-associated muscle weakness. Thus, there is still a need to identify the net effects of ACE2 in regulating muscle homoeostasis during a lifetime. Given the new adverse role of ACE2 in the invasion of SARS-CoV2, the biological significance of ACE2 in the human body needs to be re-evaluated in the era of COVID-19.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

This work was supported by the Grant-in-Aid for Scientific Research [grant number J550703552]; and the JSPS KAKENHI [grant number JP 20H03576].

Abbreviations

ACE2, angiotensin converting enzyme-2; ADAM, a disintegrin and metalloproteinase; Ang 1-7, angiotensin 1-7; Ang II, angiotensin II; ARB, Angiotensin II Receptor Blocker; AT1, Ang II type 1 receptor; CNF, centrally nucleated fibre; COVID-19, Coronavirus disease 2019; DIZE, diminazene aceturate; eNOS, Endothelial nitric oxide synthase; ER, Endoplasmic reticulum; GLUT4, glucose transporter 4; IGF1, Insulin-like growth factor-1; LPS, Lipopolysaccharide; MERS-CoV, Middle East respiratory syndrome coronavirus; MrGD, Mas-related G protein-coupled receptor member D; MuRF-1, Muscle RING-Finger Protein-1; NfκB, Nuclear factor-kappa B; NOX, NADPH oxidase; RAS, renin–angiotensin system; ROS, reactive oxygen species; SARS-CoV2, Severe acute respiratory syndrome coronavirus 2; Trp, tryptophan.

References

1 Zhou, P., Yang, X.L., Wang, X.G., Hu, B., Zhang, L., Zhang, W. et al. (2020) A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* **579**, 270–273, https://doi.org/10.1038/s41586-020-2012-7

2 Gheblawi, M., Wang, K., Viveiros, A., Nguyen, Q., Zhong, J.C., Turner, A.J. et al. (2020) Angiotensin-converting enzyme 2: SARS-CoV-2 receptor and regulator of the renin-angiotensin system: celebrating the 20th Anniversary of the Discovery of ACE2. *Circ. Res.* **126**, 1456–1474, https://doi.org/10.1161/CIRCRESAHA.120.317015

3 Nozato, S., Yamamoto, K., Takeshita, H., Nozato, Y., Imaizumi, Y., Fujimoto, T. et al. (2019) Angiotensin 1-7 alleviates aging-associated muscle weakness and bone loss, but is not associated with accelerated aging in ACE2-knockout mice. *Clin. Sci. (Lond.)* **133**, 2005–2018, https://doi.org/10.1042/CS20190573

4 Takeshita, H., Yamamoto, K., Mogi, M., Nozato, S., Horiuchi, M. and Rakugi, H. (2020) Different effects of the deletion of angiotensin converting enzyme 2 and chronic activation of the renin-angiotensin system on muscle weakness in middle-aged mice. *Hypertens. Res.* **43**, 296–304, https://doi.org/10.1038/s41440-019-0375-7

5 Takeshita, H., Yamamoto, K., Nozato, S., Takeda, M., Fukada, S.I., Inagaki, T. et al. (2018) Angiotensin-converting enzyme 2 deficiency accelerates and angiotensin 1-7 restores age-related muscle weakness in mice. *J. Cachexia. Sarcopenia Muscle* **9**, 975–986, https://doi.org/10.1002/jcsm.12334

6 Tipnis, S.R., Hooper, N.M., Hyde, R., Karran, E., Christie, G. and Turner, A.J. (2000) A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J. Biol. Chem.* **275**, 33238–33243, https://doi.org/10.1074/jbc.M002615200

7 Yan, R., Zhang, Y., Li, Y., Xia, L., Guo, Y. and Zhou, Q. (2020) Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science* **367**, 1444–1448, https://doi.org/10.1126/science.abb2762

8 Ye, M., Wysocki, J., Gonzalez-Pacheco, F.R., Salem, M., Evora, K., Garcia-Halpin, L. et al. (2012) Murine recombinant angiotensin-converting enzyme 2: effect on angiotensin II-dependent hypertension and distinctive angiotensin-converting enzyme 2 inhibitor characteristics on rodent and human angiotensin-converting enzyme 2. *Hypertension* **60**, 730–740, https://doi.org/10.1161/HYPERTENSIONAHA.112.198622

9 Santos, R.A., Simoes e Silva, A.C., Maric, C., Silva, D.M., Machado, R.P., de Buhr, I. et al. (2003) Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 8258–8263, https://doi.org/10.1073/pnas.1432869100

10 Greene, L.J., Spadaro, A.C., Martins, A.R., Perussi De Jesus, W.D. and Camargo, A.C. (1982) Brain endo-oligopeptidase B: a post-proline cleaving enzyme that inactivates angiotensin I and II. *Hypertension* **4**, 178–184, https://doi.org/10.1161/01.HYP.4.2.178

11 Kono, T., Taniguchi, A., Imura, H., Oseko, F. and Khosla, M.C. (1986) Biological activities of angiotensin II-(1-6)-hexapeptide and angiotensin II-(1-7)-heptapeptide in man. *Life Sci.* **38**, 1515–1519, https://doi.org/10.1016/0024-3205(86)90565-5

12 Santos, R.A., Brosnihan, K.B., Chappell, M.C., Pesquero, J., Chernicky, C.L., Greene, L.J. et al. (1988) Converting enzyme activity and angiotensin metabolism in the dog brainstem. *Hypertension* **11**, I153–7, https://doi.org/10.1161/01.HYP.11.2˙Pt˙2.I153
13 Santos, R.A.S., Sampaio, W.O., Alzamora, A.C., Motta-Santos, D., Alenina, N., Bader, M. et al. (2018) The ACE2/Angiotensin-(1-7)/MAS axis of the renin-angiotensin system: focus on angiotensin-(1-7). *Physiol. Rev.* **98**, 505–553, https://doi.org/10.1152/physrev.00023.2016
14 Gironacci, M.M. (2015) Angiotensin-(1-7): beyond its central effects on blood pressure. *Ther. Adv. Cardiovasc. Dis.* **9**, 209–216, https://doi.org/10.1177/1753944715599875
15 Santos, R.A. (2014) Angiotensin-(1-7). *Hypertension* **63**, 1138–1147, https://doi.org/10.1161/HYPERTENSIONAHA.113.01274
16 Chappell, M.C. and Al Zayadneh, E.M. (2017) Angiotensin-(1-7) and the regulation of anti-fibrotic signaling pathways. *J. Cell Signal.* **2**, https://doi.org/10.4172/2576-1471.1000134
17 Karnik, S.S., Singh, K.D., Tirupula, K. and Unal, H. (2017) Significance of angiotensin 1-7 coupling with MAS1 receptor and other GPCRs to the renin-angiotensin system: IUPHAR Review 22. *Br. J. Pharmacol.* **174**, 737–753, https://doi.org/10.1111/bph.13742
18 Santos, R.A., Ferreira, A.J. and Simoes, ESAC (2008) Recent advances in the angiotensin-converting enzyme 2-angiotensin(1-7)-Mas axis. *Exp. Physiol.* **93**, 519–527, https://doi.org/10.1113/expphysiol.2008.042002
19 Touyz, R.M. and Montezano, A.C. (2018) Angiotensin-(1-7) and vascular function: the clinical context. *Hypertension* **71**, 68–69, https://doi.org/10.1161/HYPERTENSIONAHA.117.10406
20 Jiang, T., Gao, L., Lu, J. and Zhang, Y.D. (2013) ACE2-Ang-(1-7)-Mas axis in brain: a potential target for prevention and treatment of ischemic stroke. *Curr. Neuropharmacol.* **11**, 209–217, https://doi.org/10.2174/1570159X11311020007
21 Tetzner, A., Gebolys, K., Meinert, C., Klein, S., Uhlich, A., Trebicka, J. et al. (2016) G-protein-coupled receptor MrgD is a receptor for Angiotensin-(1-7) involving adenylyl cyclase, cAMP, and Phosphokinase A. *Hypertension* **68**, 185–194, https://doi.org/10.1161/HYPERTENSIONAHA.116.07572
22 Gembardt, F., Grajewski, S., Vahl, M., Schultheiss, H.P. and Walther, T. (2008) Angiotensin metabolites can stimulate receptors of the Mas-related genes family. *Mol. Cell. Biochem.* **319**, 115–123, https://doi.org/10.1007/s11010-008-9884-4
23 Ohshima, K., Mogi, M., Nakaoka, H., Iwanami, J., Min, L.J., Kanno, H. et al. (2014) Possible role of angiotensin-converting enzyme 2 and activation of angiotensin II type 2 receptor by angiotensin-(1-7) in improvement of vascular remodeling by angiotensin II type 1 receptor blockade. *Hypertension* **63**, e53–9, https://doi.org/10.1161/HYPERTENSIONAHA.113.02426
24 Walters, P.E., Gaspari, T.A. and Widdop, R.E. (2005) Angiotensin-(1-7) acts as a vasodepressor agent via angiotensin II type 2 receptors in conscious rats. *Hypertension* **45**, 960–966, https://doi.org/10.1161/01.HYP.0000160325.59323.b8
25 Galandrin, S., Denis, C., Boularan, C., Marie, J., M'Kadmi, C., Pilette, C. et al. (2016) Cardioprotective Angiotensin-(1-7) peptide acts as a natural-biased ligand at the angiotensin II type 1 receptor. *Hypertension* **68**, 1365–1374, https://doi.org/10.1161/HYPERTENSIONAHA.116.08118
26 Teixeira, L.B., Parreiras, ESLT, Bruder-Nascimento, T., Duarte, D.A., Simoes, S.C., Costa, R.M. et al. (2017) Ang-(1-7) is an endogenous beta-arrestin-biased agonist of the AT1 receptor with protective action in cardiac hypertrophy. *Sci. Rep.* **7**, 11903, https://doi.org/10.1038/s41598-017-12074-3
27 Hrenak, J., Paulis, L. and Simko, F. (2016) Angiotensin A/Alamandine/MrgD axis: another clue to understanding cardiovascular pathophysiology. *Int. J. Mol. Sci.* **17**, 1098, https://doi.org/10.3390/ijms17071098
28 Lautner, R.Q., Villela, D.C., Fraga-Silva, R.A., Silva, N., Verano-Braga, T., Costa-Fraga, F. et al. (2013) Discovery and characterization of alamandine: a novel component of the renin-angiotensin system. *Circ. Res.* **112**, 1104–1111, https://doi.org/10.1161/CIRCRESAHA.113.301077
29 Mendoza-Torres, E., Oyarzun, A., Mondaca-Ruff, D., Azocar, A., Castro, P.F., Jalil, J.E. et al. (2015) ACE2 and vasoactive peptides: novel players in cardiovascular/renal remodeling and hypertension. *Ther. Adv. Cardiovasc. Dis.* **9**, 217–237, https://doi.org/10.1177/1753944715597623
30 Santos, R.A.S., Oudit, G.Y., Verano-Braga, T., Canta, G., Steckelings, U.M. and Bader, M. (2019) The renin-angiotensin system: going beyond the classical paradigms. *Am. J. Physiol. Heart Circ. Physiol.* **316**, H958–H70, https://doi.org/10.1152/ajpheart.00723.2018
31 Pavo, N., Wurm, R., Goliasch, G., Novak, J.F., Strunk, G., Gyongyosi, M. et al. (2016) Renin-angiotensin system fingerprints of heart failure with reduced ejection fraction. *J. Am. Coll. Cardiol.* **68**, 2912–2914, https://doi.org/10.1016/j.jacc.2016.10.017
32 Domenig, O., Manzel, A., Grobe, N., Konigshausen, E., Kaltenecker, C.C., Kovarik, J.J. et al. (2016) Neprilysin is a mediator of alternative renin-angiotensin-system activation in the murine and human kidney. *Sci. Rep.* **6**, 33678, https://doi.org/10.1038/srep33678
33 Rice, G.I., Thomas, D.A., Grant, P.J., Turner, A.J. and Hooper, N.M. (2004) Evaluation of angiotensin-converting enzyme (ACE), its homologue ACE2 and neprilysin in angiotensin peptide metabolism. *Biochem. J.* **383**, 45–51, https://doi.org/10.1042/BJ20040634
34 Yamada, K., Iyer, S.N., Chappell, M.C., Ganten, D. and Ferrario, C.M. (1998) Converting enzyme determines plasma clearance of angiotensin-(1-7). *Hypertension* **32**, 496–502, https://doi.org/10.1161/01.HYP.32.3.496
35 Brar, G.S., Barrow, B.M., Watson, M., Griesbach, R., Choung, E., Welch, A. et al. (2017) Neprilysin is required for angiotensin-(1-7)'s ability to enhance insulin secretion via its proteolytic activity to generate angiotensin-(1-2). *Diabetes* **66**, 2201–2212, https://doi.org/10.2337/db16-1318
36 Patel, V.B., Zhong, J.C., Grant, M.B. and Oudit, G.Y. (2016) Role of the ACE2/Angiotensin 1-7 axis of the renin-angiotensin system in heart failure. *Circ. Res.* **118**, 1313–1326, https://doi.org/10.1161/CIRCRESAHA.116.307708
37 Patel, V.B., Clarke, N., Wang, Z., Fan, D., Parajuli, N., Basu, R. et al. (2014) 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.* **66**, 167–176, https://doi.org/10.1016/j.yjmcc.2013.11.017
38 Mukerjee, S., Gao, H., Xu, J., Sato, R., Zsombok, A. and Lazartigues, E. (2019) ACE2 and ADAM17 interaction regulates the activity of presympathetic neurons. *Hypertension* **74**, 1181–1191, https://doi.org/10.1161/HYPERTENSIONAHA.119.13133
39 Pedersen, K.B., Chodavarapu, H., Porretta, C., Robinson, L.K. and Lazartigues, E. (2015) Dynamics of ADAM17-mediated shedding of ACE2 applied to pancreatic islets of male db/db mice. *Endocrinology* **156**, 4411–4425, https://doi.org/10.1210/en.2015-1556
40 Zunke, F. and Rose-John, S. (2017) The shedding protease ADAM17: physiology and pathophysiology. *Biochim. Biophys. Acta Mol. Cell Res.* **1864**, 2059–2070, https://doi.org/10.1016/j.bbamcr.2017.07.001

41 Arribas, J. and Esselens, C. (2009) ADAM17 as a therapeutic target in multiple diseases. *Curr. Pharm. Des.* **15**, 2319–2335, https://doi.org/10.2174/138161209788682398
42 Epelman, S., Tang, W.H., Chen, S.Y., Van Lente, F., Francis, G.S. and Sen, S. (2008) Detection of soluble angiotensin-converting enzyme 2 in heart failure: insights into the endogenous counter-regulatory pathway of the renin-angiotensin-aldosterone system. *J. Am. Coll. Cardiol.* **52**, 750–754, https://doi.org/10.1016/j.jacc.2008.02.088
43 Epelman, S., Shrestha, K., Troughton, R.W., Francis, G.S., Sen, S., Klein, A.L. et al. (2009) Soluble angiotensin-converting enzyme 2 in human heart failure: relation with myocardial function and clinical outcomes. *J. Card. Fail.* **15**, 565–571, https://doi.org/10.1016/j.cardfail.2009.01.014
44 Sama, I.E., Ravera, A., Santema, B.T., van Goor, H., Ter Maaten, J.M., Cleland, J.G.F. et al. (2020) Circulating plasma concentrations of angiotensin-converting enzyme 2 in men and women with heart failure and effects of renin-angiotensin-aldosterone inhibitors. *Eur. Heart J.* **41**, 1810–1817, https://doi.org/10.1093/eurheartj/ehaa373
45 Walters, T.E., Kalman, J.M., Patel, S.K., Mearns, M., Velkoska, E. and Burrell, L.M. (2017) Angiotensin converting enzyme 2 activity and human atrial fibrillation: increased plasma angiotensin converting enzyme 2 activity is associated with atrial fibrillation and more advanced left atrial structural remodelling. *Europace* **19**, 1280–1287
46 Roberts, M.A., Velkoska, E., Ierino, F.L. and Burrell, L.M. (2013) Angiotensin-converting enzyme 2 activity in patients with chronic kidney disease. *Nephrol. Dial. Transplant.* **28**, 2287–2294, https://doi.org/10.1093/ndt/gft038
47 Anguiano, L., Riera, M., Pascual, J., Valdivielso, J.M., Barrios, C., Betriu, A. et al. (2016) Circulating angiotensin converting enzyme 2 activity as a biomarker of silent atherosclerosis in patients with chronic kidney disease. *Atherosclerosis* **253**, 135–143, https://doi.org/10.1016/j.atherosclerosis.2016.08.032
48 Ortiz-Perez, J.T., Riera, M., Bosch, X., De Caralt, T.M., Perea, R.J., Pascual, J. et al. (2013) Role of circulating angiotensin converting enzyme 2 in left ventricular remodeling following myocardial infarction: a prospective controlled study. *PLoS ONE* **8**, e61695, https://doi.org/10.1371/journal.pone.0061695
49 Mogi, M., Kawajiri, M., Tsukuda, K., Matsumoto, S., Yamada, T. and Horiuchi, M. (2014) Serum levels of renin-angiotensin system components in acute stroke patients. *Geriatr. Gerontol. Int.* **14**, 793–798, https://doi.org/10.1111/ggi.12167
50 Timmerman, K.L., Connors, I.D., Deal, M.A. and Mott, R.E. (2016) Skeletal muscle TLR4 and TACE are associated with body fat percentage in older adults. *Appl. Physiol. Nutr. Metab.* **41**, 446–451, https://doi.org/10.1139/apnm-2015-0567
51 Monroy, A., Kamath, S., Chavez, A.O., Centonze, V.E., Veerasamy, M., Barrentine, A. et al. (2009) Impaired regulation of the TNF-alpha converting enzyme/tissue inhibitor of metalloproteinase 3 proteolytic system in skeletal muscle of obese type 2 diabetic patients: a new mechanism of insulin resistance in humans. *Diabetologia* **52**, 2169–2181, https://doi.org/10.1007/s00125-009-1451-3
52 Tripathy, D., Daniele, G., Fiorentino, T.V., Perez-Cadena, Z., Chavez-Velasquez, A., Kamath, S. et al. (2013) Pioglitazone improves glucose metabolism and modulates skeletal muscle TIMP-3-TACE dyad in type 2 diabetes mellitus: a randomised, double-blind, placebo-controlled, mechanistic study. *Diabetologia* **56**, 2153–2163, https://doi.org/10.1007/s00125-013-2976-z
53 DeFronzo, R.A. and Tripathy, D. (2009) Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care* **32**, S157–S163, https://doi.org/10.2337/dc09-S302
54 Abdul-Ghani, M.A. and DeFronzo, R.A. (2010) Pathogenesis of insulin resistance in skeletal muscle. *J. Biomed. Biotechnol.* **2010**, 476279, https://doi.org/10.1155/2010/476279
55 Cabello-Verrugio, C., Cordova, G. and Salas, J.D. (2012) Angiotensin II: role in skeletal muscle atrophy. *Curr. Protein Pept. Sci.* **13**, 560–569, https://doi.org/10.2174/138920312803582933
56 Sukhanov, S., Semprun-Prieto, L., Yoshida, T., Michael Tabony, A., Higashi, Y., Galvez, S. et al. (2011) Angiotensin II, oxidative stress and skeletal muscle wasting. *Am. J. Med. Sci.* **342**, 143–147, https://doi.org/10.1097/MAJ.0b013e318222e620
57 Yoshida, T., Tabony, A.M., Galvez, S., Mitch, W.E., Higashi, Y., Sukhanov, S. et al. (2013) Molecular mechanisms and signaling pathways of angiotensin II-induced muscle wasting: potential therapeutic targets for cardiac cachexia. *Int. J. Biochem. Cell Biol.* **45**, 2322–2332, https://doi.org/10.1016/j.biocel.2013.05.035
58 Olivares-Reyes, J.A., Arellano-Plancarte, A. and Castillo-Hernandez, J.R. (2009) Angiotensin II and the development of insulin resistance: implications for diabetes. *Mol. Cell. Endocrinol.* **302**, 128–139, https://doi.org/10.1016/j.mce.2008.12.011
59 Henriksen, E.J. and Prasannarong, M. (2013) The role of the renin-angiotensin system in the development of insulin resistance in skeletal muscle. *Mol. Cell. Endocrinol.* **378**, 15–22, https://doi.org/10.1016/j.mce.2012.04.011
60 Cabello-Verrugio, C., Morales, M.G., Rivera, J.C., Cabrera, D. and Simon, F. (2015) Renin-angiotensin system: an old player with novel functions in skeletal muscle. *Med. Res. Rev.* **35**, 437–463, https://doi.org/10.1002/med.21343
61 Cabello-Verrugio, C., Rivera, J.C. and Garcia, D. (2017) Skeletal muscle wasting: new role of nonclassical renin-angiotensin system. *Curr. Opin. Clin. Nutr. Metab. Care* **20**, 158–163, https://doi.org/10.1097/MCO.0000000000000361
62 Powers, S.K., Morton, A.B., Hyatt, H. and Hinkley, M.J. (2018) The renin-angiotensin system and skeletal muscle. *Exerc. Sport Sci. Rev.* **46**, 205–214, https://doi.org/10.1249/JES.0000000000000158
63 Winslow, M.A. and Hall, S.E. (2019) Muscle wasting: a review of exercise, classical and non-classical RAS axes. *J. Cell. Mol. Med.* **23**, 5836–5845, https://doi.org/10.1111/jcmm.14412
64 Cohen, S., Nathan, J.A. and Goldberg, A.L. (2015) Muscle wasting in disease: molecular mechanisms and promising therapies. *Nat. Rev. Drug Discov.* **14**, 58–74, https://doi.org/10.1038/nrd4467
65 Murphy, A.M., Wong, A.L. and Bezuhly, M. (2015) Modulation of angiotensin II signaling in the prevention of fibrosis. *Fibrogenesis Tissue Rep.* **8**, 7, https://doi.org/10.1186/s13069-015-0023-z
66 Laurino, A., Spinelli, V., Gencarelli, M., Balducci, V., Dini, L., Diolaiuti, L. et al. (2019) Angiotensin-II drives human satellite cells toward hypertrophy and myofibroblast trans-differentiation by two independent pathways. *Int. J. Mol. Sci.* **20**, 4912, https://doi.org/10.3390/ijms20194912

67 Murphy, K.T., Allen, A.M., Chee, A., Naim, T. and Lynch, G.S. (2012) Disruption of muscle renin-angiotensin system in AT1a-/- mice enhances muscle function despite reducing muscle mass but compromises repair after injury. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **303**, R321–31, https://doi.org/10.1152/ajpregu.00007.2012
68 Morales, M.G., Abrigo, J., Meneses, C., Cisternas, F., Simon, F. and Cabello-Verrugio, C. (2015) Expression of the Mas receptor is upregulated in skeletal muscle wasting. *Histochem. Cell Biol.* **143**, 131–141, https://doi.org/10.1007/s00418-014-1275-1
69 Morales, M.G., Abrigo, J., Meneses, C., Simon, F., Cisternas, F., Rivera, J.C. et al. (2014) The Ang-(1-7)/Mas-1 axis attenuates the expression and signalling of TGF-beta1 induced by AngII in mouse skeletal muscle. *Clin. Sci. (Lond.)* **127**, 251–264, https://doi.org/10.1042/CS20130585
70 Cisternas, F., Morales, M.G., Meneses, C., Simon, F., Brandan, E., Abrigo, J. et al. (2015) Angiotensin-(1-7) decreases skeletal muscle atrophy induced by angiotensin II through a Mas receptor-dependent mechanism. *Clin. Sci. (Lond.)* **128**, 307–319, https://doi.org/10.1042/CS20140215
71 Meneses, C., Morales, M.G., Abrigo, J., Simon, F., Brandan, E. and Cabello-Verrugio, C. (2015) The angiotensin-(1-7)/Mas axis reduces myonuclear apoptosis during recovery from angiotensin II-induced skeletal muscle atrophy in mice. *Pflugers Arch.* **467**, 1975–1984, https://doi.org/10.1007/s00424-014-1617-9
72 Acuna, M.J., Pessina, P., Olguin, H., Cabrera, D., Vio, C.P., Bader, M. et al. (2014) Restoration of muscle strength in dystrophic muscle by angiotensin-1-7 through inhibition of TGF-beta signalling. *Hum. Mol. Genet.* **23**, 1237–1249, https://doi.org/10.1093/hmg/ddt514
73 Sabharwal, R., Cicha, M.Z., Sinisterra, R.D., De Sousa, F.B., Santos, R.A. and Chapleau, M.W. (2014) Chronic oral administration of Ang-(1-7) improves skeletal muscle, autonomic and locomotor phenotypes in muscular dystrophy. *Clin. Sci. (Lond.)* **127**, 101–109, https://doi.org/10.1042/CS20130602
74 Marquez-Miranda, V., Abrigo, J., Rivera, J.C., Araya-Duran, I., Aravena, J., Simon, F. et al. (2017) The complex of PAMAM-OH dendrimer with Angiotensin (1-7) prevented the disuse-induced skeletal muscle atrophy in mice. *Int. J. Nanomed.* **12**, 1985–1999, https://doi.org/10.2147/IJN.S125521
75 Morales, M.G., Abrigo, J., Acuna, M.J., Santos, R.A., Bader, M., Brandan, E. et al. (2016) Angiotensin-(1-7) attenuates disuse skeletal muscle atrophy in mice via its receptor, Mas. *Dis. Model Mech.* **9**, 441–449, https://doi.org/10.1242/dmm.023390
76 Aguirre, F., Abrigo, J., Gonzalez, F., Gonzalez, A., Simon, F. and Cabello-Verrugio, C. (2020) Protective effect of Angiotensin 1-7 on sarcopenia induced by chronic liver disease in mice. *Int. J. Mol. Sci.* **21**, https://doi.org/10.3390/ijms21113891
77 Becker, L.K., Totou, N.L., Oliveira, M.F., Coelho, D.B., de Oliveira, E.C., Motta-Santos, D. et al. (2019) Lifetime overproduction of circulating angiotensin-(1-7) in rats attenuates the increase in skeletal muscle damage biomarkers after exhaustive exercise. *Chin. J. Physiol.* **62**, 226–230
78 Murphy, K.T., Hossain, M.I., Swiderski, K., Chee, A., Naim, T., Trieu, J. et al. (2019) Mas receptor activation slows tumor growth and attenuates muscle wasting in cancer. *Cancer Res.* **79**, 706–719, https://doi.org/10.1158/0008-5472.CAN-18-1207
79 Riquelme, C., Acuna, M.J., Torrejon, J., Rebolledo, D., Cabrera, D., Santos, R.A. et al. (2014) ACE2 is augmented in dystrophic skeletal muscle and plays a role in decreasing associated fibrosis. *PLoS ONE* **9**, e93449, https://doi.org/10.1371/journal.pone.0093449
80 Shatanawi, A., Romero, M.J., Iddings, J.A., Chandra, S., Umapathy, N.S., Verin, A.D. et al. (2011) Angiotensin II-induced vascular endothelial dysfunction through RhoA/Rho kinase/p38 mitogen-activated protein kinase/arginase pathway. *Am. J. Physiol. Cell Physiol.* **300**, C1181–92, https://doi.org/10.1152/ajpcell.00328.2010
81 Watanabe, T., Barker, T.A. and Berk, B.C. (2005) Angiotensin II and the endothelium: diverse signals and effects. *Hypertension* **45**, 163–169, https://doi.org/10.1161/01.HYP.0000153321.13792.b9
82 Loot, A.E., Schreiber, J.G., Fisslthaler, B. and Fleming, I. (2009) Angiotensin II impairs endothelial function via tyrosine phosphorylation of the endothelial nitric oxide synthase. *J. Exp. Med.* **206**, 2889–2896, https://doi.org/10.1084/jem.20090449
83 Ferreira, L.F. and Laitano, O. (2016) Regulation of NADPH oxidases in skeletal muscle. *Free Radic. Biol. Med.* **98**, 18–28, https://doi.org/10.1016/j.freeradbiomed.2016.05.011
84 Raffai, G., Durand, M.J. and Lombard, J.H. (2011) Acute and chronic angiotensin-(1-7) restores vasodilation and reduces oxidative stress in mesenteric arteries of salt-fed rats. *Am. J. Physiol. Heart Circ. Physiol.* **301**, H1341–52, https://doi.org/10.1152/ajpheart.00202.2011
85 Yuan, L., Li, Y., Li, G., Song, Y. and Gong, X. (2013) Ang(1-7) treatment attenuates beta-cell dysfunction by improving pancreatic microcirculation in a rat model of Type 2 diabetes. *J. Endocrinol. Invest.* **36**, 931–937
86 Grace, J.A., Klein, S., Herath, C.B., Granzow, M., Schierwagen, R., Masing, N. et al. (2013) Activation of the MAS receptor by angiotensin-(1-7) in the renin-angiotensin system mediates mesenteric vasodilatation in cirrhosis. *Gastroenterology* **145**, 874.e5–884.e5, https://doi.org/10.1053/j.gastro.2013.06.036
87 Raffai, G. and Lombard, J.H. (2016) Angiotensin-(1-7) selectively induces relaxation and modulates endothelium-dependent dilation in mesenteric arteries of salt-fed rats. *J. Vasc. Res.* **53**, 105–118, https://doi.org/10.1159/000448714
88 Xiao, X., Zhang, C., Ma, X., Miao, H., Wang, J., Liu, L. et al. (2015) Angiotensin-(1-7) counteracts angiotensin II-induced dysfunction in cerebral endothelial cells via modulating Nox2/ROS and PI3K/NO pathways. *Exp. Cell Res.* **336**, 58–65, https://doi.org/10.1016/j.yexcr.2015.06.010
89 Paz Ocaranza, M., Riquelme, J.A., Garcia, L., Jalil, J.E., Chiong, M., Santos, R.A.S. et al. (2020) Counter-regulatory renin-angiotensin system in cardiovascular disease. *Nat. Rev. Cardiol.* **17**, 116–129, https://doi.org/10.1038/s41569-019-0244-8
90 Liu, M.L., Xing, S.J., Liang, X.Q., Luo, Y., Zhang, B., Li, Z.C. et al. (2020) Reversal of hypoxic pulmonary hypertension by hypoxia-inducible overexpression of angiotensin-(1-7) in pulmonary endothelial cells. *Mol. Ther. Methods Clin. Dev.* **17**, 975–985, https://doi.org/10.1016/j.omtm.2020.04.008
91 Beyer, A.M., Guo, D.F. and Rahmouni, K. (2013) Prolonged treatment with angiotensin 1-7 improves endothelial function in diet-induced obesity. *J. Hypertens.* **31**, 730–738, https://doi.org/10.1097/HJH.0b013e32835ecbe5
92 Alenina, N., Xu, P., Rentzsch, B., Patkin, E.L. and Bader, M. (2008) Genetically altered animal models for Mas and angiotensin-(1-7). *Exp. Physiol.* **93**, 528–537, https://doi.org/10.1113/expphysiol.2007.040345
93 Rabelo, L.A., Xu, P., Todiras, M., Sampaio, W.O., Buttgereit, J., Bader, M. et al. (2008) Ablation of angiotensin (1-7) receptor Mas in C57Bl/6 mice causes endothelial dysfunction. *J. Am. Soc. Hypertens.* **2**, 418–424, https://doi.org/10.1016/j.jash.2008.05.003

94 Xu, P., Costa-Goncalves, A.C., Todiras, M., Rabelo, L.A., Sampaio, W.O., Moura, M.M. et al. (2008) Endothelial dysfunction and elevated blood pressure in MAS gene-deleted mice. *Hypertension* **51**, 574–580, https://doi.org/10.1161/HYPERTENSIONAHA.107.102764

95 Murugan, D., Lau, Y.S., Lau, C.W., Mustafa, M.R. and Huang, Y. (2015) Angiotensin 1-7 protects against angiotensin II-induced endoplasmic reticulum stress and endothelial dysfunction via Mas Receptor. *PLoS ONE* **10**, e0145413, https://doi.org/10.1371/journal.pone.0145413

96 Exner, E.C., Geurts, A.M., Hoffmann, B.R., Casati, M., Stodola, T., Dsouza, N.R. et al. (2020) Interaction between Mas1 and AT1RA contributes to enhancement of skeletal muscle angiogenesis by angiotensin-(1-7) in Dahl salt-sensitive rats. *PLoS ONE* **15**, e0232067, https://doi.org/10.1371/journal.pone.0232067

97 Hoffmann, B.R., Stodola, T.J., Wagner, J.R., Didier, D.N., Exner, E.C., Lombard, J.H. et al. (2017) Mechanisms of Mas1 receptor-mediated signaling in the vascular endothelium. *Arterioscler. Thromb. Vasc. Biol.* **37**, 433–445, https://doi.org/10.1161/ATVBAHA.116.307787

98 Lovren, F., Pan, Y., Quan, A., Teoh, H., Wang, G., Shukla, P.C. et al. (2008) Angiotensin converting enzyme-2 confers endothelial protection and attenuates atherosclerosis. *Am. J. Physiol. Heart Circ. Physiol.* **295**, H1377–84, https://doi.org/10.1152/ajpheart.00331.2008

99 Rentzsch, B., Todiras, M., Iliescu, R., Popova, E., Campos, L.A., Oliveira, M.L. et al. (2008) Transgenic angiotensin-converting enzyme 2 overexpression in vessels of SHRSP rats reduces blood pressure and improves endothelial function. *Hypertension* **52**, 967–973, https://doi.org/10.1161/HYPERTENSIONAHA.108.114322

100 Fraga-Silva, R.A., Costa-Fraga, F.P., Murca, T.M., Moraes, P.L., Martins Lima, A., Lautner, R.Q. et al. (2013) Angiotensin-converting enzyme 2 activation improves endothelial function. *Hypertension* **61**, 1233–1238, https://doi.org/10.1161/HYPERTENSIONAHA.111.00627

101 Li, G., Zhang, H., Zhao, L., Zhang, Y., Yan, D. and Liu, Y. (2017) Angiotensin-converting enzyme 2 activation ameliorates pulmonary endothelial dysfunction in rats with pulmonary arterial hypertension through mediating phosphorylation of endothelial nitric oxide synthase. *J. Am. Soc. Hypertens.* **11**, 842–852, https://doi.org/10.1016/j.jash.2017.10.009

102 Santos, S.H., Fernandes, L.R., Mario, E.G., Ferreira, A.V., Porto, L.C., Alvarez-Leite, J.I. et al. (2008) Mas deficiency in FVB/N mice produces marked changes in lipid and glycemic metabolism. *Diabetes* **57**, 340–347, https://doi.org/10.2337/db07-0953

103 Munoz, M.C., Giani, J.F., Burghi, V., Mayer, M.A., Carranza, A., Taira, C.A. et al. (2012) The Mas receptor mediates modulation of insulin signaling by angiotensin-(1-7). *Regul. Pept.* **177**, 1–11, https://doi.org/10.1016/j.regpep.2012.04.001

104 Giani, J.F., Gironacci, M.M., Munoz, M.C., Pena, C., Turyn, D. and Dominici, F.P. (2007) Angiotensin-(1 7) stimulates the phosphorylation of JAK2, IRS-1 and Akt in rat heart in vivo: role of the AT1 and Mas receptors. *Am. J. Physiol. Heart Circ. Physiol.* **293**, H1154–63, https://doi.org/10.1152/ajpheart.01395.2006

105 Prasannarong, M., Santos, F.R. and Henriksen, E.J. (2012) ANG-(1-7) reduces ANG II-induced insulin resistance by enhancing Akt phosphorylation via a Mas receptor-dependent mechanism in rat skeletal muscle. *Biochem. Biophys. Res. Commun.* **426**, 369–373, https://doi.org/10.1016/j.bbrc.2012.08.093

106 Echeverria-Rodriguez, O., Del Valle-Mondragon, L. and Hong, E. (2014) Angiotensin 1-7 improves insulin sensitivity by increasing skeletal muscle glucose uptake in vivo. *Peptides* **51**, 26–30, https://doi.org/10.1016/j.peptides.2013.10.022

107 Echeverria-Rodriguez, O., Gallardo-Ortiz, I.A., Del Valle-Mondragon, L. and Villalobos-Molina, R. (2020) Angiotensin-(1-7) participates in enhanced skeletal muscle insulin sensitivity after a bout of exercise. *J. Endocr. Soc.* **4**, bvaa007, https://doi.org/10.1210/jendso/bvaa007

108 Takeda, M., Yamamoto, K., Takemura, Y., Takeshita, H., Hongyo, K., Kawai, T. et al. (2013) Loss of ACE2 exaggerates high-calorie diet-induced insulin resistance by reduction of GLUT4 in mice. *Diabetes* **62**, 223–233, https://doi.org/10.2337/db12-0177

109 Bernardi, S., Tikellis, C., Candido, R., Tsorotes, D., Pickering, R.J., Bossi, F. et al. (2015) ACE2 deficiency shifts energy metabolism towards glucose utilization. *Metabolism* **64**, 406–415, https://doi.org/10.1016/j.metabol.2014.11.004

110 Gurley, S.B. and Coffman, T.M. (2008) Angiotensin-converting enzyme 2 gene targeting studies in mice: mixed messages. *Exp. Physiol.* **93**, 538–542, https://doi.org/10.1113/expphysiol.2007.040014

111 Cao, X., Lu, X.M., Tuo, X., Liu, J.Y., Zhang, Y.C., Song, L.N. et al. (2019) Angiotensin-converting enzyme 2 regulates endoplasmic reticulum stress and mitochondrial function to preserve skeletal muscle lipid metabolism. *Lipids Health Dis.* **18**, 207, https://doi.org/10.1186/s12944-019-1145-x

112 Patel, V.B., Mori, J., McLean, B.A., Basu, R., Das, S.K., Ramprasath, T. et al. (2016) ACE2 deficiency worsens epicardial adipose tissue inflammation and cardiac dysfunction in response to diet-induced obesity. *Diabetes* **65**, 85–95

113 Kuba, K., Imai, Y. and Penninger, J.M. (2013) Multiple functions of angiotensin-converting enzyme 2 and its relevance in cardiovascular diseases. *Circ. J.* **77**, 301–308, https://doi.org/10.1253/circj.CJ-12-1544

114 Lambert, D.W., Clarke, N.E. and Turner, A.J. (2010) Not just angiotensinases: new roles for the angiotensin-converting enzymes. *Cell. Mol. Life Sci.* **67**, 89–98, https://doi.org/10.1007/s00018-009-0152-x

115 Warner, F.J., Smith, A.I., Hooper, N.M. and Turner, A.J. (2004) Angiotensin-converting enzyme-2: a molecular and cellular perspective. *Cell. Mol. Life Sci.* **61**, 2704–2713, https://doi.org/10.1007/s00018-004-4240-7

116 Vickers, C., Hales, P., Kaushik, V., Dick, L., Gavin, J., Tang, J. et al. (2002) Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. *J. Biol. Chem.* **277**, 14838–14843, https://doi.org/10.1074/jbc.M200581200

117 Clarke, N.E., Fisher, M.J., Porter, K.E., Lambert, D.W. and Turner, A.J. (2012) Angiotensin converting enzyme (ACE) and ACE2 bind integrins and ACE2 regulates integrin signalling. *PLoS ONE* **7**, e34747, https://doi.org/10.1371/journal.pone.0034747

118 Lin, Q., Keller, R.S., Weaver, B. and Zisman, L.S. (2004) Interaction of ACE2 and integrin beta1 in failing human heart. *Biochim. Biophys. Acta* **1689**, 175–178, https://doi.org/10.1016/j.bbadis.2004.05.005

119 Camargo, S.M., Singer, D., Makrides, V., Huggel, K., Pos, K.M., Wagner, C.A. et al. (2009) Tissue-specific amino acid transporter partners ACE2 and collectrin differentially interact with hartnup mutations. *Gastroenterology* **136**, 872–882, https://doi.org/10.1053/j.gastro.2008.10.055

120 Fairweather, S.J., Broer, A., Subramanian, N., Tumer, E., Cheng, Q., Schmoll, D. et al. (2015) Molecular basis for the interaction of the mammalian amino acid transporters B0AT1 and B0AT3 with their ancillary protein collectrin. *J. Biol. Chem.* **290**, 24308–24325, https://doi.org/10.1074/jbc.M115.648519

121 Kowalczuk, S., Broer, A., Tietze, N., Vanslambrouck, J.M., Rasko, J.E. and Broer, S. (2008) A protein complex in the brush-border membrane explains a Hartnup disorder allele. *FASEB J.* **22**, 2880–2887, https://doi.org/10.1096/fj.08-107300
122 Hashimoto, T., Perlot, T., Rehman, A., Trichereau, J., Ishiguro, H., Paolino, M. et al. (2012) ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature* **487**, 477–481, https://doi.org/10.1038/nature11228
123 Singer, D., Camargo, S.M., Ramadan, T., Schafer, M., Mariotta, L., Herzog, B. et al. (2012) Defective intestinal amino acid absorption in Ace2 null mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* **303**, G686–95, https://doi.org/10.1152/ajpgi.00140.2012
124 Kuba, K., Imai, Y., Ohto-Nakanishi, T. and Penninger, J.M. (2010) Trilogy of ACE2: a peptidase in the renin-angiotensin system, a SARS receptor, and a partner for amino acid transporters. *Pharmacol. Ther.* **128**, 119–128, https://doi.org/10.1016/j.pharmthera.2010.06.003
125 Ryall, J.G., Schertzer, J.D. and Lynch, G.S. (2008) Cellular and molecular mechanisms underlying age-related skeletal muscle wasting and weakness. *Biogerontology* **9**, 213–228, https://doi.org/10.1007/s10522-008-9131-0
126 Larsson, L., Degens, H., Li, M., Salviati, L., Lee, Y.I., Thompson, W. et al. (2019) Sarcopenia: aging-related loss of muscle mass and function. *Physiol. Rev.* **99**, 427–511, https://doi.org/10.1152/physrev.00061.2017
127 Yabumoto, C., Akazawa, H., Yamamoto, R., Yano, M., Kudo-Sakamoto, Y., Sumida, T. et al. (2015) Angiotensin II receptor blockade promotes repair of skeletal muscle through down-regulation of aging-promoting C1q expression. *Sci. Rep.* **5**, 14453, https://doi.org/10.1038/srep14453
128 Wysocka, M.B., Pietraszek-Gremplewicz, K. and Nowak, D. (2018) The role of apelin in cardiovascular diseases, obesity and cancer. *Front. Physiol.* **9**, 557, https://doi.org/10.3389/fphys.2018.00557
129 Wang, W., McKinnie, S.M., Farhan, M., Paul, M., McDonald, T., McLean, B. et al. (2016) Angiotensin-converting enzyme 2 metabolizes and partially inactivates Pyr-Apelin-13 and Apelin-17: physiological effects in the cardiovascular system. *Hypertension* **68**, 365–377, https://doi.org/10.1161/HYPERTENSIONAHA.115.06892
130 Sato, T., Suzuki, T., Watanabe, H., Kadowaki, A., Fukamizu, A., Liu, P.P. et al. (2013) Apelin is a positive regulator of ACE2 in failing hearts. *J. Clin. Invest.* **123**, 5203–5211, https://doi.org/10.1172/JCI69608
131 Zhang, J., Ren, C.X., Qi, Y.F., Lou, L.X., Chen, L., Zhang, L.K. et al. (2006) Exercise training promotes expression of apelin and APJ of cardiovascular tissues in spontaneously hypertensive rats. *Life Sci.* **79**, 1153–1159, https://doi.org/10.1016/j.lfs.2006.03.040
132 Vinel, C., Lukjanenko, L., Batut, A., Deleruyelle, S., Pradere, J.P., Le Gonidec, S. et al. (2018) The exerkine apelin reverses age-associated sarcopenia. *Nat. Med.* **24**, 1360–1371, https://doi.org/10.1038/s41591-018-0131-6
133 van der Goot, A.T. and Nollen, E.A. (2013) Tryptophan metabolism: entering the field of aging and age-related pathologies. *Trends Mol. Med.* **19**, 336–344, https://doi.org/10.1016/j.molmed.2013.02.007
134 Ninomiya, S., Nakamura, N., Nakamura, H., Mizutani, T., Kaneda, Y., Yamaguchi, K. et al. (2020) Low levels of serum tryptophan underlie skeletal muscle atrophy. *Nutrients* **12**, https://doi.org/10.3390/nu12040978
135 Imai, S.I. and Guarente, L. (2016) It takes two to tango: NAD(+) and sirtuins in aging/longevity control. *NPJ Aging Mech. Dis.* **2**, 16017, https://doi.org/10.1038/npjamd.2016.17
136 Grabowska, W., Sikora, E. and Bielak-Zmijewska, A. (2017) Sirtuins, a promising target in slowing down the ageing process. *Biogerontology* **18**, 447–476, https://doi.org/10.1007/s10522-017-9685-9
137 Katsyuba, E., Mottis, A., Zietak, M., de Franco, F., van der Velpen, V., Gariani, K. et al. (2018) De novo NAD(+) synthesis enhances mitochondrial function and improves health. *Nature* **563**, 354–359, https://doi.org/10.1038/s41586-018-0645-6
138 Ding, Y., He, L., Zhang, Q., Huang, Z., Che, X., Hou, J. et al. (2004) Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways. *J. Pathol.* **203**, 622–630, https://doi.org/10.1002/path.1560
139 Rozo, M., Li, L. and Fan, C.M. (2016) Targeting beta1-integrin signaling enhances regeneration in aged and dystrophic muscle in mice. *Nat. Med.* **22**, 889–896, https://doi.org/10.1038/nm.4116
140 Shibata S, A.H., Asayama, K., Hoshide, S., Ichihara, A., Ishimitsu, T., Kario, K. et al. (2020) Hypertension and related diseases in the era of COVID-19: a report from the Japanese Society of Hypertension Task Force on COVID-19. *Hypertens. Res.* **43**, https://doi.org/10.1038/s41440-020-0515-0
141 Zhang, W., Xu, Y.Z., Liu, B., Wu, R., Yang, Y.Y., Xiao, X.Q. et al. (2014) Pioglitazone upregulates angiotensin converting enzyme 2 expression in insulin-sensitive tissues in rats with high-fat diet-induced nonalcoholic steatohepatitis. *Scientific World J.* **2014**, 603409
142 Qaradakhi, T., Gadanec, L.K., McSweeney, K.R., Tacey, A., Apostolopoulos, V., Levinger, I. et al. (2020) The potential actions of angiotensin-converting enzyme II (ACE2) activator diminazene aceturate (DIZE) in various diseases. *Clin. Exp. Pharmacol. Physiol.* **47**, 751–758, https://doi.org/10.1111/1440-1681.13251
143 Bruce, E.B., Sakarya, Y., Kirichenko, N., Toklu, H.Z., Sumners, C., Morgan, D. et al. (2018) ACE2 activator diminazene aceturate reduces adiposity but preserves lean mass in young and old rats. *Exp. Gerontol.* **111**, 133–140, https://doi.org/10.1016/j.exger.2018.07.008
144 Zucker, I.H., Schultz, H.D., Patel, K.P. and Wang, H. (2015) Modulation of angiotensin II signaling following exercise training in heart failure. *Am. J. Physiol. Heart Circ. Physiol.* **308**, H781–H791, https://doi.org/10.1152/ajpheart.00026.2015
145 Frantz, E.D.C., Prodel, E., Braz, I.D., Giori, I.G., Bargut, T.C.L., Magliano, D.C. et al. (2018) Modulation of the renin-angiotensin system in white adipose tissue and skeletal muscle: focus on exercise training. *Clin. Sci. (Lond.)* **132**, 1487–1507, https://doi.org/10.1042/CS20180276
146 Gomes-Santos, I.L., Fernandes, T., Couto, G.K., Ferreira-Filho, J.C., Salemi, V.M., Fernandes, F.B. et al. (2014) Effects of exercise training on circulating and skeletal muscle renin-angiotensin system in chronic heart failure rats. *PLoS ONE* **9**, e98012, https://doi.org/10.1371/journal.pone.0098012
147 Frantz, E.D.C., Giori, I.G., Machado, M.V., Magliano, D.C., Freitas, F.M., Andrade, M.S.B. et al. (2017) High, but not low, exercise volume shifts the balance of renin-angiotensin system toward ACE2/Mas receptor axis in skeletal muscle in obese rats. *Am. J. Physiol. Endocrinol. Metab.* **313**, E473–E482, https://doi.org/10.1152/ajpendo.00078.2017

148 da Costa, T.S.R., Masson, G.S., Eichler, R., Silva, J.C.S., Lacchini, S. and Michelini, L.C. (2020) Training-induced deactivation of the AT1 receptor pathway drives autonomic control and heart remodeling during the transition from the pre- to hypertensive phase in spontaneously hypertensive rats. *Circ. J.* **84**, 1294–1303, https://doi.org/10.1253/circj.CJ-19-1161

149 Silva, D.M., Gomes-Filho, A., Olivon, V.C., Santos, T.M., Becker, L.K., Santos, R.A. et al. (2011) Swimming training improves the vasodilator effect of angiotensin-(1-7) in the aorta of spontaneously hypertensive rat. *J. Appl. Physiol. (1985)* **111**, 1272–1277, https://doi.org/10.1152/japplphysiol.00034.2011

150 Gu, Q., Wang, B., Zhang, X.F., Ma, Y.P., Liu, J.D. and Wang, X.Z. (2014) Contribution of renin-angiotensin system to exercise-induced attenuation of aortic remodeling and improvement of endothelial function in spontaneously hypertensive rats. *Cardiovasc. Pathol.* **23**, 298–305, https://doi.org/10.1016/j.carpath.2014.05.006

151 Motta-Santos, D., Dos Santos, R.A., Oliveira, M., Qadri, F., Poglitsch, M., Mosienko, V. et al. (2016) Effects of ACE2 deficiency on physical performance and physiological adaptations of cardiac and skeletal muscle to exercise. *Hypertens. Res.* **39**, 506–512, https://doi.org/10.1038/hr.2016.28

152 Shi, S., Qin, M., Shen, B., Cai, Y., Liu, T., Yang, F. et al. (2020) Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. *JAMA Cardiol.* **5**, https://doi.org/10.1001/jamacardio.2020.0950

153 Inciardi, R.M., Lupi, L., Zaccone, G., Italia, L., Raffo, M., Tomasoni, D. et al. (2020) Cardiac involvement in a patient with coronavirus disease 2019 (COVID-19). *JAMA Cardiol.*, https://doi.org/10.1001/jamacardio.2020.1096

154 Mao, L., Jin, H., Wang, M., Hu, Y., Chen, S., He, Q. et al. (2020) Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. *JAMA Neurol.*, https://doi.org/10.1001/jamaneurol.2020.1127

155 Jin, M. and Tong, Q. (2020) Rhabdomyolysis as potential late complication associated with COVID-19. *Emerg. Infect. Dis.* **26**, 1618–1620, https://doi.org/10.3201/eid2607.200445

156 Suwanwongse, K. and Shabarek, N. (2020) Rhabdomyolysis as a presentation of 2019 novel coronavirus disease. *Cureus* **12**, e7561

157 Liu, J., Li, S., Liu, J., Liang, B., Wang, X., Wang, H. et al. (2020) Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. *EBioMedicine* **55**, 102763, https://doi.org/10.1016/j.ebiom.2020.102763

158 Disser, N.P., De Micheli, A.J., Schonk, M.M., Konnaris, M.A., Piacentini, A.N., Edon, D.L. et al. (2020) Musculoskeletal consequences of COVID-19. *J. Bone Joint Surg. Am.* **102**, 1197–1204, https://doi.org/10.2106/JBJS.20.00847

159 Lahiri, D. and Ardila, A. (2020) COVID-19 pandemic: a neurological perspective. *Cureus* **12**, e7889

160 Ferrandi, P.J., Alway, S.E. and Mohamed, J.S. (2020) The interaction between SARS-CoV-2 and ACE2 may have consequences for skeletal muscle viral susceptibility and myopathies. *J. Appl. Physiol. (1985)* **129**, 864–867, https://doi.org/10.1152/japplphysiol.00321.2020

161 Gavriatopoulou, M., Korompoki, E., Fotiou, D., Ntanasis-Stathopoulos, I., Psaltopoulou, T., Kastritis, E. et al. (2020) Organ-specific manifestations of COVID-19 infection. *Clin. Exp. Med.* **20**, https://doi.org/10.1007/s10238-020-00648-x

162 Azizi, S.A. and Azizi, S.A. (2020) Neurological injuries in COVID-19 patients: direct viral invasion or a bystander injury after infection of epithelial/endothelial cells. *J. Neurovirol.* **26**, 631–641, https://doi.org/10.1007/s13365-020-00903-7

163 Puelles, V.G., Lutgehetmann, M., Lindenmeyer, M.T., Sperhake, J.P., Wong, M.N., Allweiss, L. et al. (2020) Multiorgan and renal tropism of SARS-CoV-2. *N. Engl. J. Med.* **383**, https://doi.org/10.1056/NEJMc2011400

164 Farkash, E.A., Wilson, A.M. and Jentzen, J.M. (2020) Ultrastructural evidence for direct renal infection with SARS-CoV-2. *J. Am. Soc. Nephrol.* **31**, https://doi.org/10.1681/ASN.2020040432

165 Wichmann, D., Sperhake, J.P., Lutgehetmann, M., Steurer, S., Edler, C., Heinemann, A. et al. (2020) Autopsy findings and venous thromboembolism in patients with COVID-19. *Ann. Intern. Med.* **173**, https://doi.org/10.7326/M20-2003

166 Monteil, V., Kwon, H., Prado, P., Hagelkruys, A., Wimmer, R.A., Stahl, M. et al. (2020) Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. *Cell* **181**, 905.e7–913.e7, https://doi.org/10.1016/j.cell.2020.04.004

167 Alsaad, K.O., Hajeer, A.H., Al Balwi, M., Al Moaiqel, M., Al Oudah, N., Al Ajlan, A. et al. (2018) Histopathology of Middle East respiratory syndrome coronovirus (MERS-CoV) infection - clinicopathological and ultrastructural study. *Histopathology* **72**, 516–524, https://doi.org/10.1111/his.13379

Supplemental Table. The summary of papers investigating the role of ACE2-MAS-Angiotensin 1-7 (Ang 1-7) in skeletal muscle in rodents.

Pathology	Animals	Model	Treatment of angiotensin 1-7 (Ang 1-7)	Modulation of Mas or ACE2	Main findings in skeletal muscle	Ref.
Muscle wasting	Male MDX mice and MDX/Mas knockout(KO) mice.	Mice model of Duchenne muscular dystrophy (DMD)	Ang 1-7 infusion	Gene knockout of Mas or Mas antagonist (A779)	Ang 1-7 infusion normalized skeletal muscle architecture, decreased local fibrosis and improved muscle function. MDX mice with A779 or MDX/Mas KO mice showed highly deteriorated muscular architecture, increased fibrosis and TGFβ signaling with diminished muscle strength.	72
	Male C57BL/6J mice and Mas KO mice	Unilateral cast immobilization of the hind limb	Ang 1-7 infusion	Gene knockout of Mas	Ang 1-7 prevented decreased muscle strength and reduced myofiber diameter, myosin heavy chain levels, and the induction of atrogin-1 and MuRF-1 expressions, all of which normally occur during immobilization. The anti-atrophic effects of Ang 1-7 were not observed in Mas KO mice.	75
	C57BL/6J mice	Angiotensin II (Ang II) infusion	Ang 1-7 infusion	A779	Ang 1-7 prevented the increase in TGF-beta1 expression induced by Ang II, ROS production dependent on NOX and the early phase of p38 MAPK phosphorylation. The effects of Ang 1-7 were reversed by the administration of A779.	69

	C57BL/6J mice	Ang II infusion	Ang 1-7 infusion	A779	Ang 1-7 prevents the effects induced by Ang II in muscle gastrocnemius: the decrease in the fiber diameter, muscle strength, MHC levels and the increase in atrogin-1 and MuRF-1. Ang 1-7 induced AKT phosphorylation. The effects of Ang 1-7 were reversed by the administration of A779.	70
	Male C57BL/6J mice	Ang II infusion	Ang 1-7 infusion	A779	Ang 1-7 prevents the effects induced by Ang II in the diaphragm muscles and decreases several events associated with apoptosis in the diaphragm. The effects of Ang 1-7 were reversed by the administration of A779.	71
	C57BL/6J and Sgcd KO mice	Mice model of Limb Girdle Muscular Dystrophy 2F	Ang 1-7 infusion	N/A	Ang 1-7 treatment decreased oxidative stress and fibrosis in skeletal muscle, increased locomotor activity, and prevented autonomic dysfunction in Sgcd KO mice.	73
	Male C57BL/10J mice	Cast immobilization in the lower hind limb	non-cytotoxic hydroxyl-terminated poly(amidoamine) (PAMAM-OH) dendrimer as an Ang-(1-7) carrier	N/A	The Ang 1-7/PAMAM-OH complex, but not Ang 1-7 alone, had an anti-atrophic effect when administered intraperitoneally, as evaluated by muscle strength, fiber diameter, myofibrillar protein levels, and atrogin-1 and MuRF-1 expressions.	74

	Male mice C57BL/6J	Chronic liver disease induced by the 5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) hepatotoxin	Ang 1-7 infusion	N/A	Ang 1-7 prevented the decline of the function and strength of muscle and increased the fatigue detected in the DDC-fed mice. Decreased fiber diameter and MHC levels, as well as the transition of fiber types, were all abolished by Ang 1-7 in mice fed with DDC.	76
	Male Sprague-Dawley (SD) and transgenic rats TGR(A1-7)3292 (TR)	Exhaustive swimming exercise	TR which overproduce Ang 1-7 (2.5-fold increase)	N/A	There was no difference in time to exhaustion between SD and TR. Lactate dehydrogenase and α-actinin values were significantly lower in TR. There was a significant decrease in the range of blood glucose levels in SD rats. Muscle and hepatic glycogen in TR were higher.	77
	CD2F1 mice	The Colon-26 (C-26) mouse model of cancer cachexia	N/A	Plasmid overexpression of Mas or Mas agonist AVE 0991	Plasmid overexpression of Mas or AVE 0991 did not affect healthy muscle fiber size. In mice with cancer cachexia, AVE 0991 slowed tumor development, reduced weight loss, improved locomotor activity, and attenuated muscle wasting, with the majority of these effects dependent on the orexigenic and not anti-tumor properties of AVE 0991.	78
	MDX mice	Mice model of DMD or chronic injury with muscle BaCl2 injection	N/A	Adenovirus encoding human ACE2	ACE2 activity and protein levels are increased in dystrophic skeletal muscle. ACE2 overexpression reduced the fibrosis associated with the dystrophic tibialis anterior muscles.	79

	Male C57BL6J and ACE2 KO mice	Physiological aging	Ang 1-7 infusion	Gene knockout of ACE2	Grip strength of ACE2 KO mice was reduced at 6 months and was persistently lower than that of wild-type mice. Ang 1-7 improved grip strength in both types of 24-month old mice, with larger effects observed in ACE2 KO mice.	5
	Male C57BL6J, ACE2 KO, and Mas KO mice	Physiological aging	Ang 1-7 infusion	Gene knockout of ACE or Mas	ACE2 KO mice exhibited decreased grip strength after 6 months of age, while grip strength of Mas KO mice was similar to that of wild-type mice. Ang 1-7 improved grip strength in 24-month old ACE2 KO and wild-type mice, but not in Mas KO mice.	3
Insulin resistance	Male SD rat	Ang II infusion or fructose diet	Ang 1-7 infusion	A779	Ang 1-7 attenuates acute Ang II-mediated inhibition of insulin signaling components in normal rats via a Mas receptor-dependent mechanism. Ang 1-7 exerted beneficial effects on the phosphorylation of crucial insulin signaling mediators in liver, skeletal muscle and adipose tissue of fructose-fed rats. A779 reversed the phenomenon.	103
	Female Zucker rats	Ang II infusion	Ang 1-7 infusion	A779	Ang II decreased insulin-stimulated glucose transport activity and co-administration of Ang 1-7 improved the glucose transport activity in muscle. Ang 1-7 treatment increased Akt phosphorylation. The dependence of Ang 1-7 action on the positive	105

					effects of Ang 1-7 were completely prevented in presence of A779.	
	Male Wistar rats	The hyperinsulinemic euglycemic clamp	Ang 1-7 infusion	A779	The euglycemic clamp exhibited that Ang 1–7 did not promote glucose transport, but increased insulin-stimulated glucose disposal in the rat. Captopril (an ACE inhibitor) enhanced insulin-induced glucose uptake and this effect was blocked by A779.	106
	Male Wistar rats	Forced swimming for 2.5 hours	N/A	A779	Prior exercise enhanced insulin tolerance and insulin-mediated 2-deoxyglucose disposal in soleus muscle, and the effects were abolished by A779.	107
	Male C57BL6J mice and ACE2 KO mice	Ang II infusion or high-fat, high-sucrose (HFHS) diet	Ang 1-7 infusion	A779 or gene knockout of ACE2	Ang II infusion or HFHS diet impaired glucose tolerance and insulin sensitivity more severely in ACE2 KO mice. The strain difference in glucose tolerance was not eliminated by an ARB but was eradicated by Ang 1-7 or an ARB combined with A779.	108
	Male C57BL6J mice and ACE2KO mice	High-fat diet (HFD)	N/A	Gene knockout of ACE2	ACE2KO mice showed a pancreatic β-cell defect associated with low insulin. On the other hand, ACE2 deficiency increased the respiratory exchange ratio, reduced palmitate oxidation and PCG-1α expression in the skeletal muscle, where it up-regulated glucose transport proteins.	109

	Male ACE2KO mice and wild-type littermates db/db mice	N/A	N/A	Gene knockout of ACE2 or Adenovirus (AD)-ACE2	ACE2 deficiency is associated with increased lipid accumulation, an elevated level of ER stress and mitochondrial dysfunctions in skeletal muscle. AD-ACE2 can ameliorate ER stress and mitochondrial function, which slightly accompanied by reduced TG content and down-regulated the expression of skeletal muscle lipogenic proteins in the db/db mice.	111