

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 homeostasis 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.

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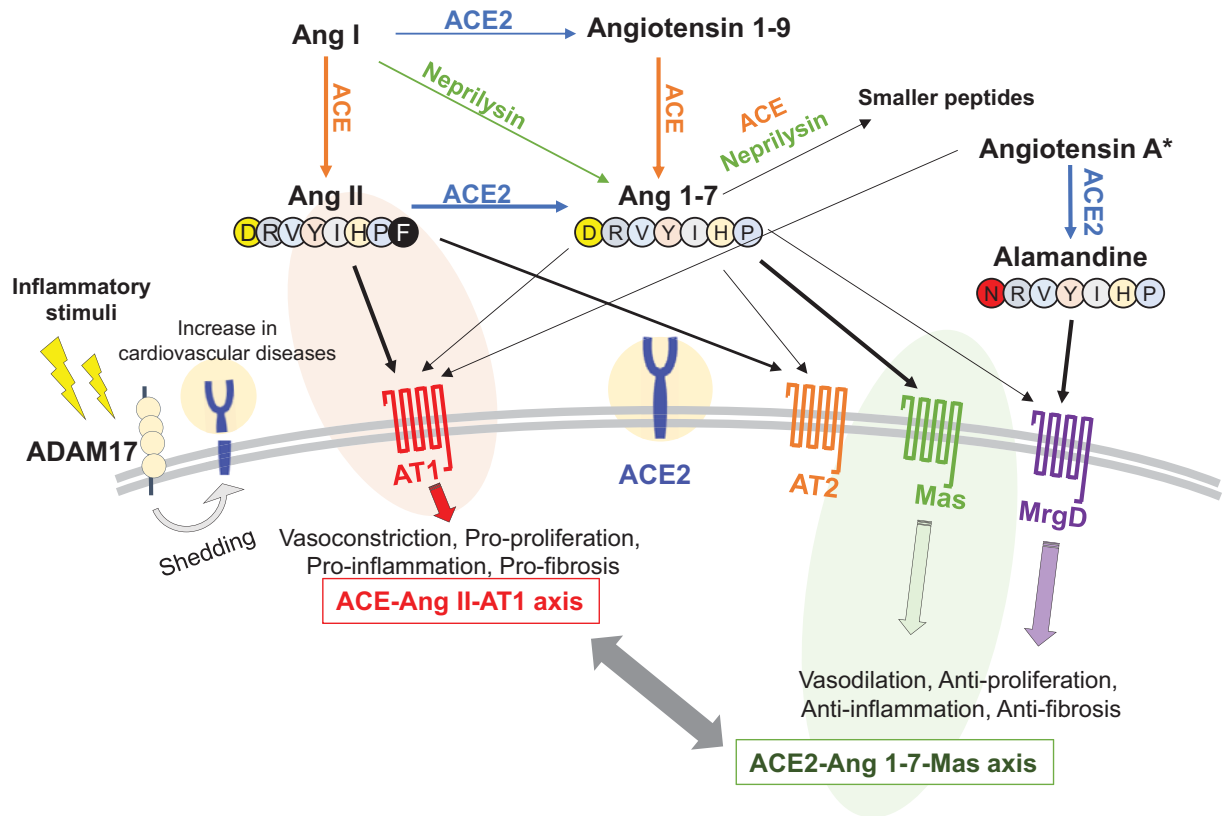


Figure 1. Cascades of the RAS and ACE2

*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 neprilysin, 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 TNF α -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 PPAR γ 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 homeostasis 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 atrogen-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 Nf κ B-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

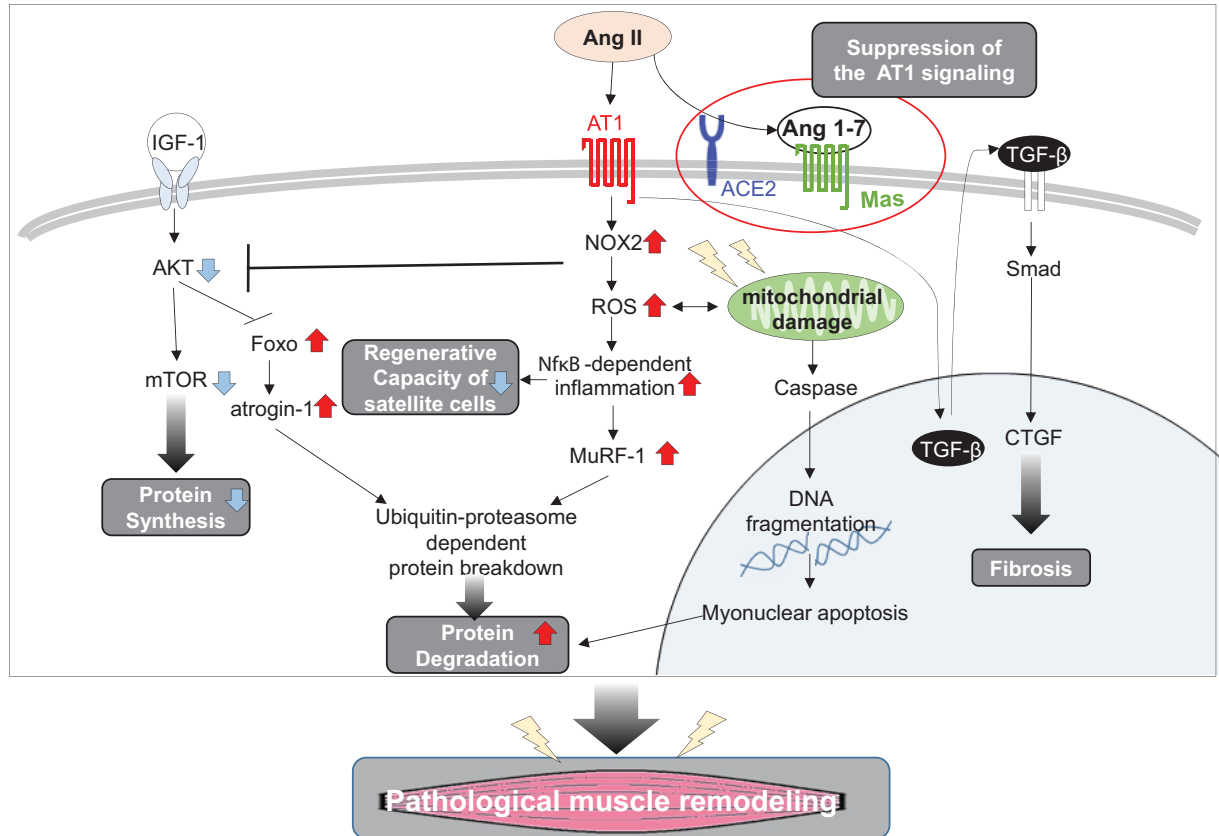


Figure 2. The ACE2-Ang 1-7-Mas axis counter-regulating the action of the ACE-Ang II-AT1 axis in the development of pathological muscle remodelling

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 BaCl₂ 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

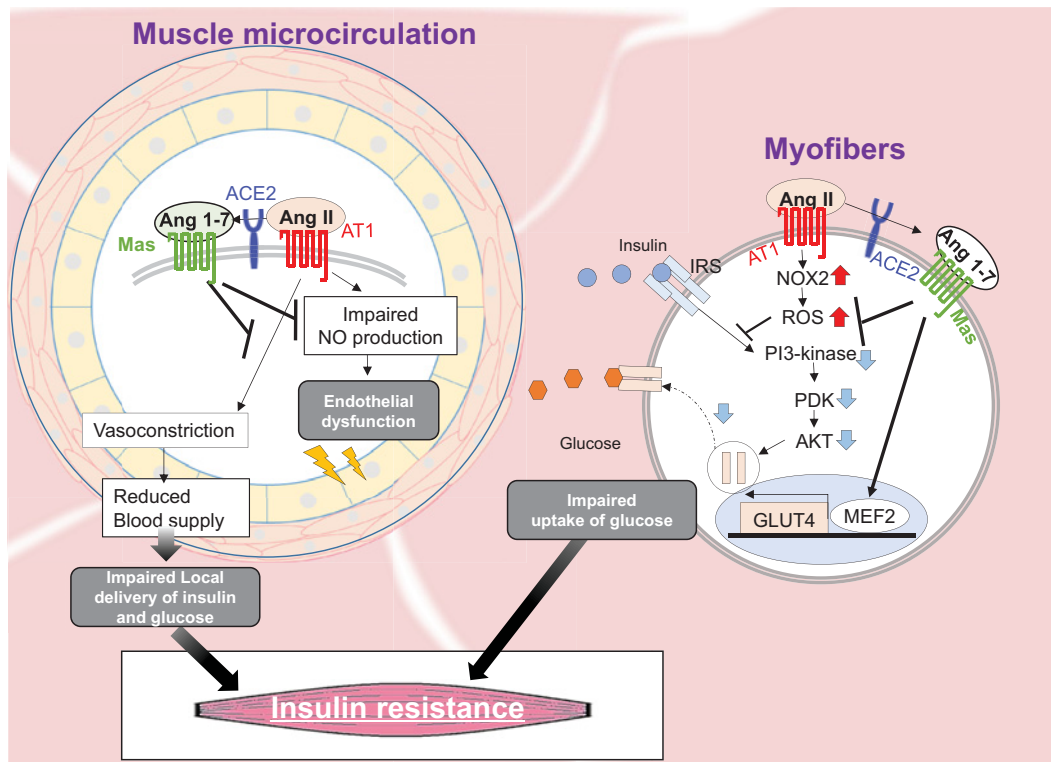


Figure 3. The ACE2-Ang 1-7-Mas axis counter-regulating the action of the ACE-Ang II-AT1 axis in the development of insulin resistance

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

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).

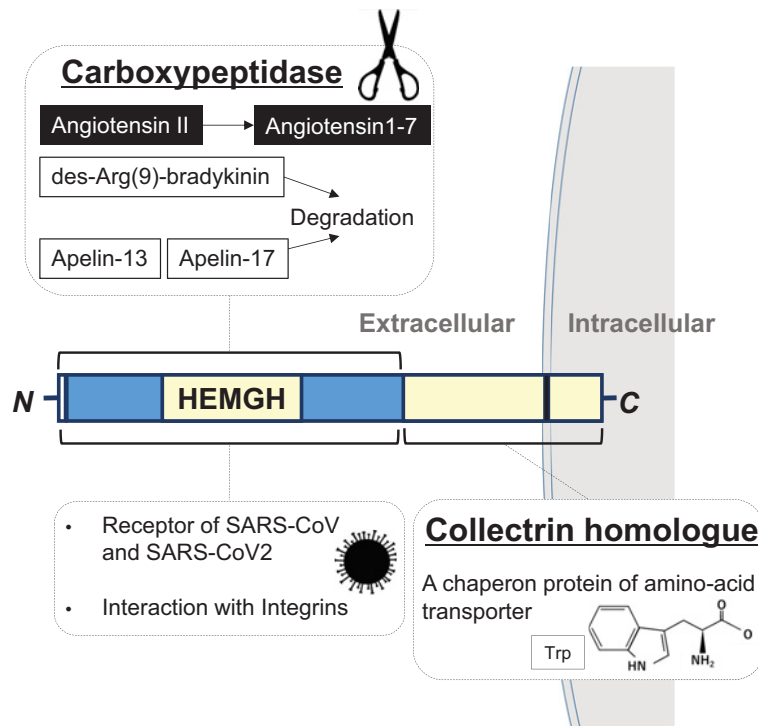


Figure 4. The structure and multiple actions of ACE2

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.

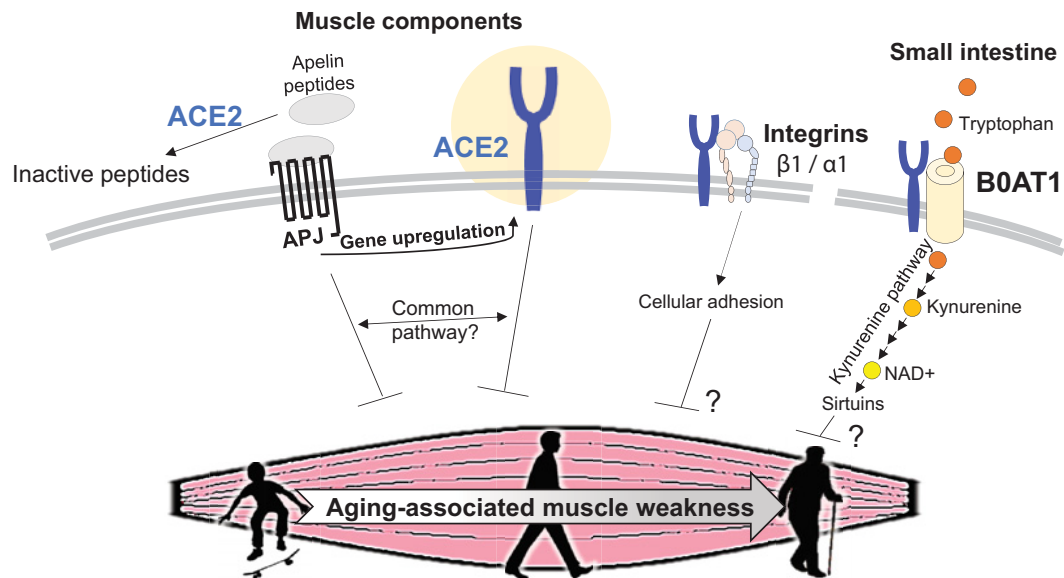


Figure 5. The plausible RAS-independent actions of ACE2 and the associated-molecules in the ageing-associated muscle weakness

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 carboxypeptidase, 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 β 1-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 PPAR γ 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.

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 homeostasis 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.

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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.

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