R2: Emerging markers in cardiovascular disease: where does ACE2 fit in?

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Disclosure

The authors have no conflict of interest to declare.
SUMMARY

1. The renin angiotensin system plays a major role in the pathophysiology of cardiovascular disease (CVD). The enzyme angiotensin converting enzyme (ACE) converts angiotensin (Ang) I into the vasoconstrictor Ang II, and was thought until recently to be the main effector of the system.

2. The enzyme ACE2, discovered in 2000, can counterbalance the effects of ACE through degradation of Ang II, and generation of Ang 1-7. ACE2 is abundantly expressed in the heart and localised to endothelial cells of coronary vessels and smooth muscle cells. Its catalytically active ectodomain undergoes shedding, resulting in ACE2 in the circulation.

3. There are 10 studies to date that have measured circulating ACE2 activity in man. These included healthy subjects, and those with heart failure, type 1 diabetes, implantable cardioverter/defibrillator, elderly subjects undergoing emergency orthopaedic surgery, and kidney transplant patients.

4. The results suggest that circulating ACE2 activity may be a marker of CVD, with low levels in healthy individuals, and increased levels in those with CV risk factors or disease. Whether increased plasma ACE2 activity reflects increased synthesis from tissue ACE2 mRNA, or increased shedding of tissue ACE2, remains to be determined.

5. ACE2 is located on the X chromosome, and circulating ACE2 levels are higher in men compared to women.

6. Large clinical studies in CVD are needed to more precisely clarify the role of ACE2 as a biomarker of CVD, determine the prognostic significance of circulating ACE2 activity and assess whether the measurement of ACE2 will improve CVD risk prediction.
Keywords: renin angiotensin system, angiotensin converting enzyme 2, cardiovascular disease, coronary artery disease, heart failure.

ABBREVIATIONS
ACE, angiotensin converting enzyme
ACE2, angiotensin converting enzyme 2
Ang, angiotensin
AUC, area under the curve
BNP, B-type natriuretic peptide
CAD, coronary artery disease
CKD, chronic kidney disease
CRT, cardiac resynchronization therapy
CVD, cardiovascular disease
CV, cardiovascular
ICD, implantable cardioverter/defibrillator
LVEF, left ventricular ejection fraction
MI, myocardial infarction
NT-proBNP, N-terminal-pro brain natriuretic peptide
NYHA, New York Heart Association
QFS, quenched fluorescent substrate
RAS, renin angiotensin system

INTRODUCTION
Globally, cardiovascular disease (CVD) is the leading cause of death and disability, due to coronary artery disease (CAD), hypertension, heart failure and stroke. Despite a decline in
mortality in developed countries over the last few decades, CAD remains one of the largest causes of mortality worldwide. The discovery of biomarkers to improve CVD risk prediction has been a rapidly expanding area. The identification of new biomarkers may help to increase our understanding of the pathogenesis of CVD and lead to improved risk prediction and development of novel therapeutic targets.

The renin angiotensin system (RAS) is a major hormonal system involved in the pathophysiology of CVD. Within the RAS, the enzyme angiotensin converting enzyme (ACE) converts angiotensin (Ang) I into the vasoconstrictor Ang II, which mediates its effects predominantly through the angiotensin type 1 receptor. Ang II raises blood pressure through vasoconstriction and salt and water retention, and contributes to cardiac remodelling, fibrosis, inflammation, thrombosis and plaque rupture.

In 2000, angiotensin converting enzyme 2 (ACE2) was discovered by two independent groups, who cloned it from a human cardiac left ventricle cDNA library and a human lymphoma cDNA library. ACE2 acts as a monokarboxypeptidase and removes a single C-terminal amino acid from Ang I to generate Ang 1-9. However, the preferred substrate of ACE2 is Ang II, from which it cleaves the C-terminal amino acid to generate the peptide Ang 1-7, a ligand for the mas receptor, which is reported to have vasodilatory and anti-fibrotic actions. In this way, ACE2 limits the vasoconstrictor action of Ang II through its degradation, and counteracts the actions of Ang II through the formation of Ang 1-7. The substrate-binding pockets of ACE and ACE2 are significantly different, explaining why ACE inhibitors cannot bind and inhibit the activity of ACE2.

ACE2 has been identified in many tissues and is expressed in abundance in the heart, blood vessels, and kidney. The aim of this review is to discuss the measurement of
ACE2 activity, and the evidence regarding circulating ACE2 activity in the 10 studies published to date in healthy subjects and in subjects with cardiovascular (CV) risk factors and disease.

BIOCHEMISTRY AND REGULATION OF ACE2

Ectodomain shedding of membrane bound ACE2

ACE2 is an 805 amino acid type 1 integral-membrane protein consisting of a large N-terminal extracellular catalytically active ectodomain containing a single zinc binding site with a HEXXH motif, a transmembrane region and a short C-terminal cytoplasmic tail.\textsuperscript{4, 5, 26} ACE2 shares approximately 42% homology to the N-terminal ectodomain of ACE\textsuperscript{4} and its active catalytic ectodomain is exposed to circulating vasoactive peptides in the circulation.\textsuperscript{26} The carboxyl end of ACE2 is homologous to collectrin,\textsuperscript{27} a protein that has been shown to regulate renal amino acid uptake.\textsuperscript{28} More recently, Hashimoto \textit{et al.} demonstrated that ACE2 is a key regulator in the uptake of neutral amino acids in the intestine, and genetic deletion of ACE2 in mice results in severe colitis following intestinal injury.\textsuperscript{29}

ACE2 undergoes cleavage or “shedding” to release the catalytically active ectodomain into the extracellular milieu (Figure 1).\textsuperscript{30} The shedding process involves the proteinase ADAM17 (a disintegrin and metalloproteinase), also known as tumor necrosis factor-\(\alpha\) converting enzyme (TACE),\textsuperscript{30, 31} and can be regulated by various stimuli including phorbol esters, calcium ionophores, growth factors and calmodulin.\textsuperscript{30, 31} The shedding mechanism results in ACE2 that can be detected in the circulation.\textsuperscript{32} Western blot analysis in healthy subjects has shown plasma ACE2 protein to be of a smaller fragment size compared to full-length membrane bound ACE2, thus likely to be the result of proteolytic cleavage.\textsuperscript{32}
The cleavage site for ADAM17 mediated ectodomain shedding of human ACE2 has been reported to occur between amino acid positions 716 and 741, which reside in the juxtamembrane region. The use of several mutant and chimeric ACE2 proteins showed that the juxtamembrane stalk region, transmembrane and cytoplasmic domains were not required for constitutive ACE2 shedding. Another group used recombinant human ADAM17 and showed that it was able to cleave an ACE2 peptide mimetic which corresponded to the extracellular juxtamembrane region between the residues Arg and Ser of ACE2. Furthermore, deletion of the juxtamembrane coding region of ACE2 in a mutant cell line attenuates ADAM17 induced ACE2 shedding. Iwata et al. also showed the shedding of two distinct soluble forms of ACE2 with equivalent enzyme activities from cell lines overexpressing ACE2. The deglycosylated molecular masses of the two soluble forms were approximately 80 and 70 kDa. ADAM17 was responsible for shedding of the larger 80 kDa soluble form, but not the constitutive cleavage of the smaller soluble fragment suggesting the involvement of one or more sheddases. This finding has been confirmed by Jia et al. who reported the shedding of two distinct ACE2 soluble forms in primary cultures of human airway epithelial cells. Further studies are needed to characterize the mechanisms involved in ACE2 shedding and to identify the involvement of other sheddases and the actual cleavage sites involved.

We have previously reported that ACE2 is present in both the normal human vasculature, and in diseased internal mammary and radial arteries taken from patients undergoing coronary artery bypass surgery. Others have shown that human carotid atherosclerotic lesions express ACE2 mRNA, with increased ACE2 tissue activity in ruptured atherosclerotic lesions compared to stable advanced atherosclerotic lesions. There are no studies that have simultaneously measured tissue and circulating ACE2 activity in man, but it is possible that
an increase in tissue ACE2 activity will result in increased circulating ACE2 activity. Certainly in the rat myocardial infarction (MI) model, we have previously shown that the increase in cardiac ACE2 post MI is associated with an increase in plasma ACE2 activity.\textsuperscript{36} We have also reported similar results in a rat model of acute renal injury, with increased cardiac ACE2 associated with increased circulating ACE2 activity.\textsuperscript{37}

Thus, ACE2 undergoes shedding to release the catalytically active ectodomain into the circulation, which involves the proteinase ADAM17. Further studies are needed that measure both tissue and circulating ACE2 activity in humans to determine if elevated circulating ACE2 activity is associated with increased tissue ACE2 synthesis from mRNA, and/or increased ACE2 shedding from tissue.

**HUMAN STUDIES OF CIRCULATING ACE2**

**Measurement of ACE2**

There are only 10 studies that have measured circulating ACE2 activity in plasma or serum from human subjects. The studies are summarized in Table 1, and include healthy subjects, subjects with heart failure, type 1 diabetes, implantable cardioverter/defibrillator (ICD), elderly subjects undergoing emergency orthopaedic surgery, and kidney transplant subjects.\textsuperscript{32, 38-46} Not all the published studies include a control group, and to date, there are no studies of ACE2 in subjects with other CVD such as type 2 diabetes, essential hypertension, hyperlipidaemia, CAD, or MI.

All published reports on circulating ACE2 in humans have used an ACE2-specific quenched fluorescent substrate (QFS), based on the initial design of Vickers \textit{et al.},\textsuperscript{6} who demonstrated specific hydrolysis of this substrate with recombinant human ACE2. QFS based assays are
widely used for the detection of proteolytic activity and consist of a short synthetic peptide sequence that is specifically recognized and cleaved by the enzyme of interest and flanked by a quencher and fluorophore (Figure 2). All studies have used the ACE2 QFS (7-methoxycoumarin-4-yl)acetyl (Mca)-Ala-Pro-Lys-2,4-dinitrophenyl (Dnp). ACE2 cleaves the Pro-Lys bond, and the fluorescence of the released Mca-Ala-Pro is measured (Figure 2). This is a specific substrate for ACE2, which has also been widely used in animal studies. In contrast, the substrate used by Soro-Paavonen et al. (o-aminobezoic acid-Ser-Pro-Tyr(NO2)-OH), has only been used in one other animal study and no validation studies have been reported. In all but three studies, the assay sample is set up in parallel with an ACE2 specific inhibitor to determine non-specific activity, and the relative fluorescence of the inhibited sample is then subtracted from the non-inhibited sample. The actual amount of ACE2 enzyme activity in the assay is then determined from a standard curve of relative fluorescent units plotted against either known concentrations of the fluorophore or to recombinant ACE2 enzyme. The inhibitors used in the ACE2 assays include MLN-4760, DX600 and EDTA. Although MLN-4760 has been shown to be the most potent inhibitor of ACE2, it is not readily available commercially. Human ACE2 has been shown to be more sensitive to DX600 compared to rat or mouse ACE2, and Epelman et al. demonstrated a dose dependent reduction in ACE2 activity with DX600. EDTA has previously been shown to be as effective as MLN-4760 in rat tissue, and was initially used by Vickers et al. to quench the assay reactions.

The published ACE2 assays have used modifications in the assay conditions, which include differing temperatures and length of incubation time of the ACE2 QFS with the plasma/serum sample, and different units for quantifying ACE2 activity. All studies report the assay units as ACE2 activity (either mol of ACE2/min/L or mL), except for the studies by
Lehmann et al.\textsuperscript{44} and Soler et al.,\textsuperscript{45} which did not use an ACE2 inhibitor or a standard curve to convert the relative fluorescent units into ACE2 activity concentrations. We have previously reported that ACE2 circulates in human plasma, but its activity is masked by the presence of an endogenous inhibitor.\textsuperscript{32} The addition of neat plasma to recombinant soluble human ACE2 inhibited ACE2 activity in a dose dependent manner, whilst the removal of the endogenous inhibitor using anion exchange chromatography allowed the detection of plasma ACE2 activity using a QFS assay.\textsuperscript{32} Partial purification of the inhibitor suggested it was small, hydrophilic and cationic. The inhibitor was present in similar amounts in plasma samples from healthy volunteers, since the inhibition of recombinant ACE2 by 15 μl of plasma was $37.7 \pm 1.3\%$ (mean ± SEM, n=17).\textsuperscript{32} Hence, in healthy volunteers the difference in ACE2 activity does not reflect a difference in ACE2 inhibitor levels. The studies by Chong et al.,\textsuperscript{43} Roberts et al.,\textsuperscript{46} and data in abstract form in CAD subjects\textsuperscript{56} also removed the endogenous inhibitor before the assay of plasma ACE2 activity. Roberts et al.\textsuperscript{46} determined whether the endogenous inhibitor was affected by reduced kidney function and compared ACE2 plasma activity in a subset of their patient groups (pre-dialysis chronic kidney disease (CKD) n=22; dialysis n=17; transplant n=22) with and without extraction of the endogenous inhibitor. ACE2 plasma activity was substantially lower in the plasma assayed without extraction of the endogenous inhibitor. There was no difference in ACE2 plasma activity between the groups with or without removal of the endogenous inhibitor in this subset. Therefore, endogenous inhibitor levels appear not to be affected by reduced renal function, although further studies will need to be conducted to see if other CV risk factors or diseases affect endogenous inhibitor levels.

Only 6 of the 10 studies have provided assay reproducibility data.\textsuperscript{32,38-40,45,46} Of these, four studies had good intra- and inter-assay variability data (Rice et al.\textsuperscript{38}; inter-assay = 8.8%, intra-
assay = 3.7%; Lew et al.\textsuperscript{32} intra-assay = 7.1%, inter-assay = 13.7%; Soler et al.\textsuperscript{45} intra-assay = 4.7%, inter-assay = 6.2% and Roberts et al.\textsuperscript{46} intra-assay = 5.6%, inter-assay = 11.8%), but 2 studies\textsuperscript{39,40} had a large inter-assay variability (coefficient of variation ± SD = 20.6 ± 5.5%). Thus, the differences in the assay conditions, measurement units and lack of reproducibility data make it difficult to compare circulating ACE2 activity across the subject groups.

**Circulating ACE2 in healthy subjects**

The first study to assess plasma ACE2 activity in man was The Leeds Family study, which consisted of healthy subjects and family members totaling 534 individuals.\textsuperscript{38} Plasma ACE2 activity was detectable in 40 subjects who tended to be older, with greater abdominal adiposity, and higher blood pressure, fasting glucose and lipid levels compared to those with undetectable levels.\textsuperscript{38} In these subjects with CV risk factors, plasma ACE2 levels ranged from 3 to 460 pmol/L (overall mean, 33.0 [95% confidence intervals, 22.1 - 49.4] pmol/L). Furthermore, half of those subjects with detectable ACE2 had at least one other family member with detectable ACE2, with up to 67% of the variability in circulating ACE2 levels being explained by hereditary factors.\textsuperscript{38} Using a similar QFS based assay to Rice et al.,\textsuperscript{38} we have previously reported\textsuperscript{32} that removal of the endogenous inhibitor allowed ACE2 activity to be measured in plasma from healthy volunteers (n = 18); levels were low and lay within a tight range (mean ± SEM = 4.44 ± 0.56, range 1.31 – 8.69 pmol/min/mL). Thus, circulating ACE2 levels are low in healthy subjects and increased in subjects with CV risk factors.

**ACE2 and gender**

The \textit{ACE2} gene is located on the X chromosome and many of the genetic ACE2 studies have shown gender specific associations (for review, see Burrell et al.\textsuperscript{57}). This is of interest, as the
development of CVD is known to display gender specific characteristics. Of the 10 published studies, 3 studies have analysed circulating ACE2 activity according to gender.\textsuperscript{42, 45, 46} These studies have shown increased ACE2 activity in men compared to women in healthy individuals and in subjects with type 1 diabetes\textsuperscript{42} and renal disease.\textsuperscript{45, 46} Future studies should analyse ACE2 activity by gender and determine if associations of ACE2 activity with CV risk or disease vary according to gender.

**Coronary artery disease**

We have previously reported that in a study of high-risk elderly patients undergoing emergency orthopaedic surgery (n = 187), pre-operative plasma ACE2 activity was similar in those with an in-hospital CV event (n = 20) (MI, heart failure, atrial fibrillation, major arrhythmia, cardiac arrest) compared to those with no CV event.\textsuperscript{43} However, post-operative plasma ACE2 activity was significantly increased in those with an in-hospital CV event. Although plasma ACE2 activity was not a significant predictor of CV events in multivariate analysis, in receiver operating characteristics analysis, levels above the cut-off value of 20.3 pmol/min/mL (area under the curve (AUC) = 0.68, P <0.10) gave modest sensitivity and specificity for the prediction of in-hospital CV events.\textsuperscript{43} Further studies in large surgical patient cohorts are needed to confirm these findings. We have previously reported in abstract form that in patients with suspected CAD, plasma ACE2 activity was significantly increased in those with angiographically defined CAD compared to those with normal coronary arteries, suggesting it may be a useful biomarker of cardiac disease.\textsuperscript{56}

**Heart failure**

In a cross-sectional cohort study in subjects suspected of heart failure (n=221),\textsuperscript{39} serum ACE2 activity strongly correlated with a clinical diagnosis of heart failure, left ventricular
ejection fraction (LVEF) and increasing B-type natriuretic (BNP) peptide levels. Higher serum ACE2 activity also reflected the severity of heart failure by the New York Heart Association function classification. Serum ACE2 activity was increased in heart failure of both ischemic and non-ischemic origin, as well as in those with heart failure and preserved LVEF. In a separate cohort of 113 patients with stable chronic systolic heart failure, those with a lower LVEF had higher ACE2 activity and levels correlated with higher plasma N-terminal (NT)-proBNP. Plasma ACE2 activity predicted the combined clinical endpoint (occurred in 29%) of all-cause mortality, heart transplantation and heart failure hospitalization, independently of LVEF and NT-proBNP levels. Similar results have been seen in subjects with Chagas disease, a leading cause of heart disease in Central and South America. Plasma ACE2 activity was significantly higher in those with Chagas disease and heart failure compared to healthy controls, and was predictive of cardiac death and heart transplantation.

As mentioned, in the MI rat model of heart failure, increased cardiac ACE2 is reflected by increased plasma ACE2 activity. The results of increased circulating ACE2 in human heart failure are also consistent with our own study that reported ACE2 immunoreactivity is increased in the explanted ischaemic failing human heart tissue. Others have shown that the ACE2 gene is upregulated in human idiopathic and ischaemic cardiomyopathy, and in myocardial biopsies from subjects with heart failure.

Increased plasma BNP and NT-proBNP levels are powerful independent predictors of morbidity and mortality in heart failure. The current ACE2 activity studies in heart failure subjects suggest a significant positive correlation between circulating ACE2 activity and BNP levels. Epelman et al. reported in 113 heart failure subjects that ACE2 activity
levels above the receiving-operating characteristic derived value of 28.3 ng/mL in combination with NT-proBNP levels above the median value of 1240 pg/mL (AUC = 0.78, P < 0.0001) predicted greater adverse events of all-cause mortality, heart transplantation and heart failure hospitalization compared to ACE2 activity (AUC = 0.66, P < 0.001) and NT-proBNP levels (AUC = 0.71, P < 0.0001) alone. In Chagas disease, the combination of ACE2 activity and BNP levels improved the predictive value of adverse cardiac events. Thus, the assessment of ACE2 activity in heart failure patients together with BNP levels may improve risk prediction in heart failure.

**Ventricular arrhythmia**

A recent study examined serum ACE2 activity in patients with decreased LVEF (≤35%) who had received an ICD/ cardiac resynchronization therapy device (CRT) for primary prevention of sudden cardiac death. The study consisted of 57 patients, mostly male (93%) with ischemic (n = 49) and non-ischemic cardiomyopathy (n = 8) that had received ICD/CRT and were prospectively followed for subsequent arrhythmic episodes and appropriate ICD interventions over a mean follow-up time of 365 ± 90 days. Baseline serum ACE2 activity was significantly increased in patients who developed ventricular arrhythmias and had appropriate ICD intervention (n = 16) compared to subjects without arrhythmia (n = 41). ACE2 was a significant univariate predictor of appropriate ICD intervention (P = 0.015). Interestingly, the proportion of subjects with a previous history of percutaneous coronary intervention was significantly higher in the ICD intervention group (63%) compared to the no ICD group (27%). As increased ACE2 activity has been reported in coronary disease, the increased ACE2 levels in the ICD group may reflect existing coronary disease rather than ventricular arrhythmias. Serum samples for ACE2 measurement were based on a single sample at the time of the ICD surgery or a day before ICD
implantation, and not the ACE2 level at the time of the arrhythmic event. Larger studies are required to assess the prognostic value of plasma ACE2 levels in this group of patients, preferably with repeated sample collection to assess if ACE2 levels increase with each arrhythmic episode and are independent of baseline coronary disease.

**Type 1 diabetes**

In the largest clinical study to date of circulating ACE2 activity, there was no difference in serum ACE2 activity in men and women with type 1 diabetes (n = 859) compared to healthy control subjects (n = 204). The study did suggest that serum ACE2 activity was sex-dependent, with higher levels in males compared with females in control subjects and in subjects with type 1 diabetes. Serum ACE2 was positively correlated with systolic blood pressure and associated with coronary heart disease in both males and females, although no detail is provided as to how the diagnosis of coronary heart disease was made, and the numbers studied were small (males n = 35; females n = 22). The results are however consistent with our previously reported data in a non-diabetic population which showed that plasma ACE2 activity is increased in patients with angiographically proven CAD.

**Kidney disease**

CVD is a major co-morbidity associated with CKD. Our group and others have reported higher levels of ACE2 in men compared to women with renal disease. We have shown that plasma activity of ACE2 is lower in patients undergoing hemodialysis than other forms of CKD. In dialysis patients, ACE2 plasma activity was lower in females than males and most strongly associated with systolic blood pressure. In male dialysis patients, plasma ACE2 activity was most strongly associated with BNP levels. In type 1 diabetes subjects, increased serum ACE2 activity was associated with the diabetic microvascular complication.
of microalbuminuria but not macroalbuminuria, and only in males. In a longitudinal pilot study of serum ACE2 activity in 113 kidney transplant patients,\textsuperscript{45} ACE2 activity was detectable in kidney transplant patients and was increased in those with ischemic heart disease as compared to kidney transplant patients without ischemic heart disease. In addition, ACE2 was increased in male compared to female subjects, and correlated positively with serum creatinine.

**CONCLUSION**

The evidence linking ACE2 with CVD is increasing, but data is limited to 10 studies, and differences in ACE2 assay conditions and lack of reproducibility data must be taken into account in the interpretation of the published data. The results from the ten cross-sectional human studies do suggest that circulating ACE2 activity may be a marker of CVD, with low levels in healthy individuals and increased levels in those with CV risk factors or disease. Whether the increase in plasma ACE2 activity is a reflection of increased synthesis from tissue ACE2 mRNA, or increased shedding of ACE2 from tissue remains to be determined. Data from 3 published studies suggests that circulating ACE2 levels are higher in men compared to women. This issue has not been addressed in the study design of the majority of published reports, with only one study\textsuperscript{42} performing analysis separately for females and males. Further studies examining the gender differences in circulating ACE2 levels are needed. Increased circulating ACE2 is associated with coronary heart disease, adverse CV outcomes in heart failure, and with post-operative cardiac events in elderly orthopaedic surgery patients. In the future, large-scale carefully conducted clinical studies are needed to more precisely clarify the role of ACE2 as a biomarker of CVD, determine the prognostic significance of circulating ACE2 activity and assess whether the measurement of ACE2 will improve CVD risk prediction.
ACKNOWLEDGMENTS

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FIGURE LEGENDS

Figure 1: Cleavage of membrane bound ACE2 by ADAM17. ACE2 is a type 1 integral-membrane protein consisting of a N-terminal extracellular ectodomain, a transmembrane region and a short C-terminal cytoplasmic tail. ACE2 undergoes cleavage or “shedding” to

**Figure 2:** ACE2-specific quenched fluorescent substrate (QFS) assay. The majority of human ACE2 activity studies have used the ACE2 QFS (7-methoxycoumarin-4-yl)acetyl (Mca)-Ala-Pro-Lys-2,4-dinitrophenyl (Dnp). ACE2 cleaves the Pro-Lys bond, and the fluorescence of the released Mca-Ala-Pro is measured. Figure adapted from Smith et al. Reproduced with permission, from Smith AI, Warner FJ, Lew RA, Yarski M, McGrath B, Burrell LM (2009), *Adv. Exp. Med. Biol.*, 611: 419-422. © Springer.

**Table 1: Summary of studies of human circulating ACE2 activity**

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Sample type, assay units</th>
<th>Circulating ACE2 activity</th>
</tr>
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<tbody>
<tr>
<td>Rice <em>et al.</em> 2006&lt;sup&gt;18&lt;/sup&gt;</td>
<td>Healthy individuals from 89 pedigrees, n = 537</td>
<td>Plasma, pmol/L</td>
<td>ACE2 detected in 40 individuals with CV risk factors, Mean ACE2 activity = 33.0 [95% CI 22.1 - 49.4] pmol/L, range = 3.3 to 463.6 pmol/L</td>
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<td>Mean age = 43.2 y</td>
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<td>Lew <em>et al.</em> 2008&lt;sup&gt;45&lt;/sup&gt;</td>
<td>Healthy individuals, n = 18</td>
<td>Plasma&lt;sup&gt;§&lt;/sup&gt;, pmol/min/mL</td>
<td>Mean ACE2 activity ± SEM = 4.4 ± 0.6, range 1.3 to 8.7 pmol/min/mL</td>
</tr>
<tr>
<td></td>
<td>Mean age ± SEM = 35 ± 1 y (range 23-53 y)</td>
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<tr>
<td>Epelman <em>et al.</em></td>
<td>Subjects suspected of having</td>
<td>Plasma, ng/mL</td>
<td>Median ACE2 activity:</td>
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heart failure, n = 221. Of these, 66 subjects showed no biochemical or clinical evidence of heart failure. Mean age = 58 to 64 y‡

Of 221 participants, 66 did not have heart failure. Mean age was 58 to 64 years.

Epelman et al.
2009†
Heart Failure, n = 113 (prospective study, no healthy control group). Mean age = 56 to 58 y‡

Epelman et al. performed a study on 113 patients with heart failure. Mean age was 56 to 58 years.

Wang et al.
2010¶
Chagas disease, n = 111, mean age = 49 to 53 y‡ Healthy controls, n = 40, mean age ± SEM = 52 ± 2 y

Wang et al. studied 111 patients with Chagas disease and 40 healthy controls. Mean ages were 49 to 53 years and 52 ± 2 years, respectively.

Soro-Paavonen et al. 2012**
Type 1 diabetes, n = 859, mean age 27 to 30 y‡ Healthy controls, n = 204, mean age 26 y

Soro-Paavonen et al. investigated Type 1 diabetes in 859 participants and 204 healthy controls. Mean ages were 27 to 30 years and 26 years, respectively.

Chong et al.
2012††
Elderly subjects undergoing emergency orthopaedic surgery, n = 187 Mean age ± SD = 76.7 ± 9.3

Chong et al. examined 187 elderly subjects undergoing orthopaedic surgery. Mean age was 76.7 ± 9.3 years.

Lehmann et al.
2012******
Patients with ICD/CRT-D, n = 58 Mean age = 65 y (range 42 to 82)

Lehmann et al. studied 58 patients with ICD/CRT-D. Mean age was 65 years (range 42 to 82).

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<table>
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<tr>
<th>Soler et al. 2012\textsuperscript{55}</th>
<th>Patients with kidney transplant (KT), n = 113</th>
<th>Serum, relative fluorescent units (RFU)/μL/h</th>
<th>Mean ACE2 activity ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(KT)</td>
<td>KT, male = 105.2 ± 9.1 RFU/μl/h</td>
<td>KT, female = 84.7 ± 6.9</td>
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<tr>
<td></td>
<td>Mean age ± SD = 55 ± 13y</td>
<td>RFU/μL/h</td>
<td>KT, IHD = 105.9 ± 8.7 RFU/μl/h</td>
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<tr>
<td></td>
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<td>KT, no IHD = 84.7 ± 6.9</td>
<td>RFU/μL/h</td>
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<tr>
<th>Roberts et al. 2013\textsuperscript{16}</th>
<th>Pre-dialysis, n = 59, mean age ± SD = 66 ± 13y; Haemodialysis, n = 100, mean age = 62 ± 15y; Kidney transplant, n = 89, mean age = 53 ± 11y.</th>
<th>Plasma\textsuperscript{§}, pmol/min/mL</th>
<th>Median ACE2 activity (interquartile range):</th>
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<tr>
<td></td>
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<td>Pre-dialysis = 15.9 [8.4-26.1], pmol/min/mL</td>
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<td>Dialysis = 9.2 [3.9-21.9], pmol/min/mL</td>
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<td></td>
<td>Transplant = 13.1 [5.7-21.9], pmol/min/mL</td>
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\textsuperscript{†}All assays have used ACE2 specific quenched fluorescent substrates based on the initial design by Vickers \textit{et al.}\textsuperscript{6} \textsuperscript{‡}Overall mean age of the subjects studied were not provided in the publication and therefore the approximate mean age range of the subjects is provided. \textsuperscript{§}Indicates studies in which the endogenous inhibitor was removed from plasma before measurement of ACE2 activity. CI = confidence intervals; NYHA = New York Heart Association functional class; ICD = implantable cardioverter/defibrillator; CRT-D = cardiac resynchronization therapy device; KT = kidney transplant.
![Chemical structure diagram with labels: 7-methoxycumarin-4-acetyl (the fluorophore) and 2,4-dinitrophenyl (the quencher).](image)

**FIGURE 2**
Author/s: Patel, SK; Velkoska, E; Burrell, LM

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