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# High serum angiotensin-converting enzyme 2 activity as a biomarker of frailty in nursing home residents

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ABSTRACT

Angiotensin-converting enzyme (ACE) and angiotensin-converting enzyme 2 (ACE2) are two of the main components of the renin–angiotensin system (RAS). Imbalanced RAS showing lower ACE2 has been associated with increased cardiovascular risk, muscular pathologies, sarcopenia, frailty, other age-related pathologies and a poorer health status. However, its role in aging remains unclear. Thus, the aim of this work was to analyze the serum enzymatic activity of ACE and ACE2, the ACE/ACE2 ratio and its association with anthropometric parameters, blood pressure, physical function, dependence and frailty in older people living in nursing homes. This study is a secondary analysis of baseline data from two randomized clinical trials in a population of 228 older individuals living in nursing homes (Spain). Serum ACE and ACE2 enzymatic activities were measured by fluorimetry. Variables linked to cardiovascular risk, physical function, dependence and frailty were measured using validated tests, indexes and scales. Association between ACE, ACE2 serum activities, the ACE/ACE2 ratio and the rest of the quantitative variables were assessed by Pearson's correlations and by partial correlations controlled by age and sex. The association between serum ACE and ACE2 activities, the ACE/ACE2 ratio and frailty scores was analyzed by generalized linear models with and without controlling for sex and age. Differences in enzymatic activities between sexes and between frail and non-frail individuals were analyzed using Student's *t*-test and general linear models to control analysis by age and sex. We found that higher serum ACE2 activity was associated with a higher body mass index, worse physical function, greater dependence and increased frailty. This association is consistent with the elevation of circulating ACE2 in certain pathological conditions and in line with RAS deregulation in muscular dystrophies. Serum ACE2 activity, in combination with other molecules, could be proposed as a biomarker of poor physical function, higher dependence and frailty.

## 1. Introduction

Older people living in nursing homes are not a deeply investigated population group and are more likely to be frequently affected by chronic or acute conditions as compared to community-dwelling older people; they are also, on average, more dependent and frail (Deandrea et al., 2013). Frailty is considered an age-related syndrome—highly prevalent in people living in nursing homes—that refers to an increased vulnerability when facing minor stressors and, consequently, exposes older people to a higher risk of adverse health-related events such as hospitalization, dependence, and even death (Fried et al., 2001; Topinková, 2008).

*Abbreviations:* ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; Ang (1–7), angiotensin (1–7); AngII, angiotensin II; AT1R, angiotensin II type 1 receptor; BMI, body mass index; RAS, renin–angiotensin system.

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ACE2 is a key component of the renin–angiotensin system (RAS), a complex communication system composed of bioactive peptides, enzymes and receptors organized in several branches. The branch comprising angiotensin-converting enzyme (ACE/CD14: EC 3.4.11.1), angiotensin II (AngII) and angiotensin receptor 1 (AT1R) elicits vasoconstriction, oxidative stress, fibrosis and inflammation when activated, whereas counteracted functions—including exerting cardioprotective effects—are linked to ACE2 (EC 3.4.17.23), angiotensin 1–7 (Ang(1–7)) and Mas receptor pathway (Santos et al., 2019).

The main role of the circulating RAS in the organism has been linked to the maintenance of blood pressure, fluid homeostasis and electrolyte balance (Donoghue et al., 2000). In addition, RAS is associated also with inflammation (Warnholtz et al., 1999), oxidative stress (Nickenig and Harrison, 2002) and apoptosis (Wang et al., 2019), which some authors have hypothesized that it can also influence the aging phenotype (Abadir, 2011).

Maintenance of muscle mass and function is crucial during aging (Kostka et al., 2017) and several studies have focused on analyzing the role of RAS in skeletal muscle senescence (Herbert et al., 2008). Regarding the ACE axis, ACE inhibitors (ACEi) and AngII receptor blockers (ARBs) have been investigated in older people not only in relation with cardiovascular disease (Ata et al., 2020; Harper et al., 2020) but also with positive effects on physical function (Harper et al., 2020), sarcopenia (Ata et al., 2020), frailty (Cosardereliogl u et al., 2020) and even COVID-19 (De Spiegeleer et al., 2020). However, published works have shown no association between ACE and physical function (Bustamante-Ara et al., 2010; Kang et al., 2012; Kostka et al., 2017). In animal models, ACE2 deficiency has been related to impaired physical function (Motta-Santos et al., 2016), whereas physical training promotes the activation of ACE2/Ang (1–7)/Mas receptor axis (Nunes-Silva et al., 2017; Gomes-Santos et al., 2014) and ACE2 activity (Gomes-Santos et al., 2014). It has recently been proposed that in young people, physical exercise causes chronic augmentation of muscular ACE2 mRNA and a diminution in ACE2 serum concentrations (Klöting et al., 2020). When other counteracting RAS elements such as AngII and Ang (1–7) are analyzed, they have been proposed as a skeletal muscle waster (Du Bois et al., 2015; Brink et al., 2001) and skeletal muscle enhancer (Aravena et al., 2020; Becker et al., 2018; Cisternas et al., 2015), respectively. Intriguingly, regarding muscle-related pathologies, some authors have proposed a counteractive role for the ACE2 axis, in which it would be enhanced as a compensatory mechanism against the harmful overactivation of the ACE/AngII/AT1R axis (Riquelme et al., 2014; White et al., 2019).

Frailty is considered a reversible condition (Muscedere et al., 2019) and, therefore, its early detection is of the utmost importance. For this reason, the discovery of molecular biomarkers of frailty and frailty-related parameters is a research area attracting increased interest. Given the previously mentioned role of the RAS in functional status and frailty-related variables, it cannot be ruled out that their components may be putative markers for the identification of frail individuals.

Taking into account all the above, the objective of this work was to analyze the serum enzymatic activity of the main component of each RAS branch (ACE and ACE2) and the ACE/ACE2 ratio in older people living in nursing homes and its association with parameters related to cardiovascular risk, physical function, dependence and frailty to ascertain whether they could be proposed as molecular biomarkers in clinical practice.

## 2. Materials and methods

### 2.1. Study design and participants

This is a secondary analysis of the baseline data from three randomized controlled trials whose primary outcomes have been previously published (Arrieta et al., 2019; Rezola-Pardo et al., 2019b; Rezola-Pardo et al., 2020). The first trial aimed to study the effects of a multicomponent physical exercise program (Rodriguez-Larrad et al., 2017) on people living in nursing homes. The second trial, (Rezola-Pardo et al., 2019a) compared the effects of a multicomponent physical exercise program with a dual-task intervention. The third trial compared the effects of a multicomponent physical exercise program and a walking intervention. These studies were conducted at 14 nursing homes in Gipuzkoa, Spain (ACTRN12616001044415, ACTRN12618000536268, NCT03996083), between October 2016 and December 2018. The study adheres to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for cross-sectional studies and Standards for Reporting Enzymology Data (STRENDA, Level 1A) guidelines. These studies were approved by the Ethics Committee for Research in Humans (CEISH: M10/2016/105) and by the Ethics Committee for Research with Biological Agents of the University of the Basque Country (CEIAB: M30/2016/106). Written informed consent was provided by each participant.

The study included a total sample of 228 older people (73 men and 155 women) living in nursing homes who met the following criteria: ≥70 years old; scored ≥50 on the Barthel Index for Activities of Daily Living (0−100) (Wade and Collin, 1988), scored ≥20 on the MEC-35 test (0–35), an adapted and validated version of the Mini Mental State Examination (MMSE) in Spanish (Lobo et al., 1999); and were capable of standing up and walking independently for at least ten meters.

### 2.2. Serum ACE and ACE2 enzymatic activities

Blood samples were collected in the morning following an overnight fast. Following collection, the tubes were centrifuged at 5000 ×*g* for 10 min. The serum obtained from each participant was stored in aliquots at −80 °C for further analysis. Serum enzymatic activities were assayed at 37 °C by fluorimetry in microplates, as previously described by Fernández-Atucha et al. (2017) Estimation of serum ACE activity was based on the fluorescence of the product generated upon hydrolysis by the enzyme of the substrate Abz-Gly-Phe (NO2) Pro (ref. 4003531; Bachem, Bubendorf, Germany). 50 μl of serum sample and 250 μl of substrate with 150 mM Tris-HCl buffer (pH 8.3), 0.3 M NaCl were placed in a microplate reader and incubated for 30 min. Excitation was at 355 nm and emission at 410 nm. The serum ACE2 activity assay was based on the fluorescence of the product generated upon hydrolysis by the enzyme of the substrate Abz-Ser-Pro-3-nitro-Tyr-OH (ref. 4050533; Bachem, Bubendorf, Germany). Reactions were initiated by adding 70 μl of the appropriate incubation mixture (substrate, 50 mM Tris-HCl buffer, pH 7.5, 0.1 mg/ml bovine seroalbumin) to 30 μl of sample during 2 h. After incubation, fluorescence intensity was measured with an excitation filter at 355 nm and an emission filter at 410 nm. The quantification was performed fluorometrically using a FLUOstar OPTIMA Microplate Reader (ThermoFisher Scientific, Waltham, MA, USA) and Optima Control software version 2.20 (BMG, LABTECH, Ortenberg, Germany). Serum samples were measured in triplicate and averaged. Calibration curves with known concentrations of each fluorogenic substrate were performed for each assay. One unit of activity is the amount of enzyme required to release 1 pmol of fluorescent product per minute.

### 2.3. Sociodemographic data, anthropometry and blood pressure

Sociodemographic data (sex and age) were recorded from the nursing home databases. Waist and hip circumference were measured with a non-elastic anthropometric tape and measured to the nearest 0.1 cm. Height was measured with a Holtain stadiometer to the nearest 0.1 cm, and body mass was measured with an Omron digital scale to the nearest 0.1 kg. Body mass index (BMI) was calculated based on mass and height (kg/m$^2$), and the waist-to-hip ratio was based on waist and hip circumferences.

Systolic and diastolic blood pressure (in mmHg) was measured using a clinically validated automatic blood pressure monitor (Omron M6AC) at the beginning of the anthropometrical measurements in the morning.

For blood pressure measurement, participants were asked to remain in the supine decubitus position for several minutes, with their arms resting at their sides. Subsequently, the cuff was positioned on their unclothed right arm, 1–2 cm above the elbow joint, and the cable on the brachial artery. Blood pressure was measured twice, allowing for a rest period between measurements, and both measurements were recorded.

### 2.4. Physical function

Several tests were performed to assess participants' physical function. Handgrip strength was evaluated using a Jamar dynamometer (Fess, 1992); aerobic capacity with the 6-min walk test, and lower limb strength with chair-stand tests from the Senior Fitness Test (Rikli and Jones, 2001). Dynamic balance was assessed with the Timed Up and Go test (Mathias et al., 1986), and static balance with the Berg Balance Scale (Berg et al., 1992). Functional performance of lower limbs (static balance, gait speed, and lower limb strength) was evaluated with the Short Physical Performance Battery (SPPB) (Guralnik et al., 1994).

### 2.5. Activities of daily living and frailty

The Barthel Index reflects autonomy in activities of daily living (ADL), and its score ranges from 0 to 100, with lower scores indicating a higher degree of dependence. Barthel Index scores (Wade and Collin, 1988) were recorded from the nursing home databases.

Frailty status was assessed with the Fried Frailty Index (Fried et al., 2001), the Tilburg Frailty Indicator (Gobbens et al., 2010), and the Clinical Frailty Scale (Rockwood et al., 2005). Frailty, according to the Fried Frailty Index, was identified by the presence of three or more of the following signs/symptoms: unintended weight loss, exhaustion, weakness, slow gait speed, and low physical activity (Fried et al., 2001). The Tilburg Frailty Indicator contains 15 questions on physical, psychological, and social domains of frailty. Participants with a score of 5 or higher were considered frail according to the Tilburg Frailty Indicator (Gobbens et al., 2010). For the Clinical Frailty Scale, frailty status is based on clinical judgment. Possible scores range from 1 to 9 and participants with a score of 6 points or higher were considered frail (Rockwood et al., 2005). All of the tests were performed by accredited and experienced professionals in each area.

### 2.6. Statistical analyses

The normality of quantitative variables was verified using the Kolmogorov–Smirnov test. Continuous variables with non-parametric distributions were log-transformed (ACE, ACE2, age, handgrip, chair-stand test and Timed Up and Go test). Mean and standard deviation were used to describe continuous variables. Ordinal variables (Berg Balance Scale, Short Physical Performance Battery, Barthel Index, Fried Frailty Index, the Tilburg Frailty Indicator, and the Clinical Frailty Scale) were expressed by median and interquartile range. Categorical variables (sex) were expressed as number and percentage (%).

The association between serum ACE and ACE2 enzymatic activities and ACE/ACE2 ratio to the rest of the continuous variables was assessed using Pearson's correlations. Partial correlations were also performed to control associations by age and sex. The association of ACE, ACE2 and ACE/ACE2 with ordinal variables was determined using generalized linear models. Scores were introduced as dependent variables. In all cases, models were constructed using ACE, ACE2 activities or ACE/ACE2 ratio as covariables (Model 1). Models were also controlled by sex and age (Model 2). Differences of serum ACE and ACE2 enzymatic activities between women and men, as well as between frail and non-frail population were analyzed using Student's *t*-test. General linear models (controlled by age in the case of differences between sexes and by sex and age in the case of frailty) were used to control differences between men and women and between frail and non-frail participants. Significance was determined at $p < 0.05$. All statistical analyses were performed by SPSS software v.26.

## 3. Results

### 3.1. Descriptive characteristics of the sample

Participants' descriptive data are shown in Table 1. The mean ± standard deviations of the serum ACE and ACE2 enzymatic activities were 848.4 ± 281.8 and 70.0 ± 30.9 U/ml, respectively, and the mean of the ACE/ACE2 ratio was 13.5 ± 5.6. Of the 228 participants, 155 (68.0%) were women and 73 (32.0%) were men; the mean age was 84.9 ± 6.7 years.

### 3.2. Association of ACE and ACE2 enzymatic activities and ACE/ACE2 with the rest of the variables

Associations of serum ACE and ACE2 enzymatic activities and ACE/ACE2 ratio with the rest of the variables are shown in Tables 2–4, respectively. Tables 2A, 3A and 4A show bivariate Pearson's correlations and partial correlations adjusted by sex and age related to continuous variables. Tables 2B, 3B and 4B, respectively, show generalized linear models for ordinal parameters as dependent variables, and serum ACE and ACE2 enzymatic activities and ACE/ACE2 ratio as covariables (Model 1). Associations were also controlled by age and sex (Model 2). Serum ACE and ACE2 activities were positively associated with one another when Pearson's correlation was performed ($R = 0.305$, $p < 0.001$) and when partial correlation was performed, adjusted by sex and age ($R = 0.307$, $p < 0.001$). ACE/ACE2 was positively associated with ACE ($R = 0.547$, $p < 0.001$) and negatively with ACE2 ($R = -0.618$, $p < 0.001$). This association was maintained when partial correlations were performed ($R = 0.542$, $p < 0.001$ and $R = -0.621$, $p < 0.001$, respectively).

No association was found between serum ACE activity and the rest of the analyzed variables, nor in the Pearson's correlation, nor when partial

**Table 1**
Descriptive data of the analyzed variables. Quantitative variables are shown as mean ± standard deviation (SD). Categorical variables are shown as *n* and %. Ordinal variables were expressed by median and interquartile range (IQR). **U:** the amount of enzyme required to release 1 pmol of fluorescent product per minute.

| Variables | n | Mean<br>N<br>Median | ± SD<br>(%)<br>(IQR) |
|---|---|---|---|
| ACE (U/ml) | 228 | 848.4 | ± 281.8 |
| ACE2 (U/ml) | 228 | 70.0 | ± 30.9 |
| ACE/ACE2 | 228 | 13.5 | ± 5.6 |
| Sociodemographic data | | | |
| Age (years) | 228 | 84.9 | ± 6.7 |
| Sex | 228 | | |
| Women | | 155 | (68) |
| Men | | 73 | (32) |
| Anthropometric parameters and blood pressure | | | |
| Body mass index (kg/m$^2$) | 225 | 28.6 | ± 5.1 |
| Waist-to-hip ratio | 225 | 0.98 | ± 0.07 |
| Systolic blood pressure (mm Hg) | 225 | 148.1 | ± 20.6 |
| Diastolic blood pressure (mm Hg) | 225 | 79.2 | ± 10.9 |
| Physical function | | | |
| Handgrip (kg) | 228 | 19.3 | ± 7.9 |
| 6-min walk test (m) | 227 | 247.6 | ± 104.7 |
| Chair-stand test (n/30s) | 227 | 6.7 | ± 4.1 |
| Timed Up and Go test (m/s) | 226 | 0.35 | ± 0.15 |
| Berg Balance Scale (score 0–56) | 224 | 47 | (41–51) |
| Short Physical Performance Battery (score 0–12) | 224 | 6 | (4–9) |
| Activities of daily living | | | |
| Barthel Index (score 0–100) | 226 | 85 | (70–94) |
| Frailty | | | |
| Fried Frailty Index (score 0–5) | 220 | 3 | (2–4) |
| Tilburg Frailty Indicator (score 0–15) | 218 | 6 | (3–8) |
| Clinical Frailty Scale (score 0–9) | 226 | 5 | (3–6) |

**Table 2**

**(A)** Bivariate Pearson's correlations and partial correlations controlled by sex and age between angiotensin-converting enzyme (ACE) serum enzymatic activity and quantitative variables. **(B)** Generalized linear models for ordinal variables as dependent variable. Model 1: for ACE as covariable. Model 2: for ACE and age and covariables, and sex as factor (*men). **U:** the amount of enzyme required to release 1 pmol of fluorescent product per minute.

| A | N | ACE (U/ml) | | | |
|---|---|---|---|---|---|
| | | Pearson's correlation | | Partial correlation | |
| Variables | | r | p | r | p |
| ACE2 (U/ml) | 228 | 0.305 | < 0.001 | 0.307 | < 0.001 |
| ACE/ACE2 | 228 | 0.547 | < 0.001 | 0.542 | < 0.001 |
| Sociodemographic data | | | | | |
| Age (years) | 228 | 0.025 | 0.702 | −0.074 | 0.267 |
| Anthropometric parameters and blood pressure | | | | | |
| Body mass index (kg/m$^2$) | 225 | 0.023 | 0.733 | 0.007 | 0.923 |
| Waist-to-hip ratio | 225 | −0.060 | 0.371 | −0.039 | 0.566 |
| Systolic blood pressure (mm Hg) | 225 | −0.004 | 0.947 | −0.025 | 0.713 |
| Diastolic blood pressure (mm Hg) | 225 | 0.064 | 0.336 | 0.051 | 0.452 |
| Physical function (continuous variables) | | | | | |
| Handgrip (kg) | 228 | −0.118 | 0.076 | −0.047 | 0.485 |
| 6-min walk test (m) | 227 | −0.009 | 0.889 | 0.021 | 0.759 |
| Chair-stand test (n/30s) | 227 | 0.017 | 0.821 | 0.022 | 0.769 |
| Timed up and go test (m/s) | 226 | 0.028 | 0.675 | 0.053 | 0.432 |

| B | N | Covariables and factors | *Model 1* | | | *Model 2* | | |
|---|---|---|---|---|---|---|---|---|
| Dependent variable | | | β | Wald's $\chi^2$ | *p* | β | Wald's $\chi^2$ | *p* |
| **Physical function** (ordinal variables) | | | | | | | | |
| Berg Balance Scale | 224 | ACE | −0.002 | 1.299 | 0.254 | −0.002 | 0.936 | 0.333 |
| (Score 0–56) | | Age | | | | −0.077 | 0.695 | 0.404 |
| | | Sex* | | | | 2.296 | 2.957 | 0.086 |
| Short Physical Performance Battery | 224 | ACE | < 0.001 | 0.432 | 0.511 | < 0.001 | 0.235 | 0.628 |
| (Score 0–12) | | Age | | | | −0.059 | 3.838 | 0.050 |
| | | Sex* | | | | 0.716 | 2.679 | 0.102 |
| **Activities of daily living** | | | | | | | | |
| Barthel Index | 226 | ACE | −0.005 | 1.804 | 0.179 | −0.004 | 1.180 | 0.277 |
| (score 0–100) | | Age | | | | 0.109 | 0.485 | 0.486 |
| | | Sex* | | | | 5.668 | 6.258 | 0.012 |
| **Frailty** | | | | | | | | |
| Fried Frailty Index | 220 | ACE | < 0.001 | 0.173 | 0.677 | < 0.001 | 0.107 | 0.743 |
| (Score 0–5) | | Age | | | | 0.25 | 4.745 | 0.029 |
| | | Sex* | | | | −0.086 | 0.287 | 0.592 |
| Tilburg Frailty Indicator | 218 | ACE | < 0.001 | 0,017 | 0.897 | < 0.001 | 0.012 | 0.914 |
| (Score 0–11) | | Age | | | | 0.032 | 1.006 | 0.316 |
| | | Sex* | | | | −0.016 | 0.001 | 0.972 |
| Clinical Frailty Scale | 226 | ACE | < 0.001 | 0.360 | 0.549 | < 0.001 | 0.244 | 0.621 |
| (Score 1–9) | | Age | | | | 0.007 | 0.207 | 0.649 |
| | | Sex* | | | | −0.238 | 1.181 | 0.277 |

correlations or generalized linear models were performed (Table 2).

ACE2 was positively associated with BMI when both correlations were performed ($R = 0.151$, $p = 0.023$; $R = 0.146$, $p = 0.030$). Associations for serum ACE2 activity and physical function were similar for both correlation types with the exception of handgrip, which was negatively associated with ACE2 only when sex and age were controlled ($R = -0.146$, $p = 0.028$). Thus, ACE2 was negatively associated with the 6-min walk test ($R = -0.230$, $p < 0.001$; $R = -0.253$, $p < 0.001$) and chair-stand test ($R = -0.145$; $p = 0.047$; $R = -0.146$, $p = 0.046$) (Table 3A). Lower serum ACE2 activity was found in participants with higher scores in the Berg Balance Scale (β = −0.061, W = 10.254, $p = 0.001$; β = −0.061, W = 10.583, p = 0.001), in the total Short Physical Performance Battery score (β = −0.017, W = 7.191, $p = 0.007$; β = −0.017, W = 7.922, $p = 0.005$), and in the Barthel Index (β = −0.095, W = 9.231, $p = 0.002$; β = −0.094, W = 9.237, p = 0.002), reflecting a negative association of serum ACE2 enzymatic activity with balance, physical performance and independence on activities of daily living. Associations between serum ACE2 activity and frailty scores were similar in the generalized linear models without controlling and controlled by sex and age (Table 3B). These associations were positive for the Fried Frailty Index (β = 0.005, W = 5.230, $p = 0.022$; β = 0.006, W = 6.029, $p = 0.014$), and the Clinical Frailty Scale (β = 0.010, W = 10.502, $p = 0.001$; β = 0.010, W = 11.078, p = 0.001). In the case of the Tilburg Frailty Indicator (β = 0.011, W = 2.608, $p = 0.106$; β = 0.011, W = 2.827, $p = 0.093$), no significance was reached.

When correlations were performed for the ACE/ACE2 ratio, the 6-min walk test was the only variable that reached significance ($R = 0.184$, p = 0.005; $R = 0.228$, p = 0.001) (Table 4A). In the generalized linear models (Table 4B), the Barthel Index was positively associated with ACE/ACE2 (β = 0.372, W = 4.504, $p = 0.034$; β = 0.418, W = 5.841, $p = 0.016$), Tilburg Frailty indicator was negatively associated with ACE/ACE2 in Model 2 (β = −0.071, W = 3.881, $p = 0.049$), and Clinical Frailty Scale in both models (1 and 2) (β = −0.034, W = 3.964, $p = 0.046$; β = −0.037, W = 4.758, $p = 0.029$).

### 3.3. Serum ACE and ACE2 activity and ACE/ACE2 depending on sex and in frail and non-frail population

Tables 5–7, respectively, show serum ACE and ACE2 enzymatic activities and ACE/ACE2 depending on sex and for frail and non-frail population. Differences were analyzed by Student's t and general linear models controlled by age (and sex in the case of frailty). No difference was found between women and men. Frail participants according to Clinical Frailty Score had higher levels of serum ACE2 activity

**Table 3**
**(A)** Bivariate Pearson's correlations and partial correlations controlled by sex and age between angiotensin-converting enzyme 2 (ACE2) serum enzymatic activity and quantitative variables. **(B)** Generalized linear models for ordinal variables as dependent variable. Model 1: for ACE2 as covariable. Model 2: for ACE2 and age and covariables, and sex as factor (*men). **U:** the amount of enzyme required to release 1 pmol of fluorescent product per minute.

| A | N | ACE2 (U/ml) | | | |
|---|---|---|---|---|---|
| | | Pearson's correlation | | Partial correlation | |
| Variables | | r | p | r | p |
| ACE (U/ml) | 228 | 0.305 | < 0.001 | 0.307 | < 0.001 |
| ACE/ACE2 | 228 | −0.618 | < 0.001 | −0.618 | < 0.001 |
| Sociodemographic data | | | | | |
| Age (years) | 228 | −0.055 | 0.410 | −0.010 | 0.886 |
| Anthropometric parameters and blood pressure | | | | | |
| Body mass index (kg/m$^2$) | 225 | 0.151 | 0.023 | 0.146 | 0.030 |
| Waist-to-hip ratio | 225 | −0.034 | 0.609 | −0.040 | 0.552 |
| Systolic blood pressure (mm Hg) | 225 | 0.118 | 0.078 | 0.126 | 0.060 |
| Diastolic blood pressure (mm Hg) | 225 | 0.028 | 0.679 | 0.028 | 0.674 |
| Physical function (continuous variables) | | | | | |
| Handgrip (kg) | 228 | −0.101 | 0.130 | −0.146 | 0.028 |
| 6-min walk test (m) | 227 | −0.230 | < 0.001 | −0.253 | < 0.001 |
| Chair-stand test (n/30s) | 227 | −0.145 | 0.047 | −0.146 | 0.046 |
| Timed up and go test (m/s) | 226 | −0.099 | 0.139 | −0.107 | 0.112 |

| B | N | Covariables and factors | *Model 1* | | | *Model 2* | | |
|---|---|---|---|---|---|---|---|---|
| Dependent variable | | | β | Wald's $\chi^2$ | *p* | β | Wald's $\chi^2$ | *p* |
| **Physical function** (ordinal variables) | | | | | | | | |
| Berg Balance Scale | 224 | ACE2 | −0.061 | 10.254 | 0.001 | −0.061 | 10.583 | 0.001 |
| (Score 0–56) | | Age | | | | −0.092 | 1.023 | 0.312 |
| | | Sex* | | | | 2.252 | 2.989 | 0.084 |
| Short physical performance battery | 224 | ACE2 | −0.017 | 7.191 | 0.007 | −0.017 | 7.922 | 0.005 |
| (Score 0–12) | | Age | | | | −0.064 | 4.572 | 0.032 |
| | | Sex* | | | | 0.689 | 2.582 | 0.108 |
| **Activities of daily living** | | | | | | | | |
| Barthel Index | 226 | ACE2 | −0.095 | 9.231 | 0.002 | −0.094 | 9.237 | 0.002 |
| (Score 0–100) | | Age | | | | 0.079 | 0.267 | 0.605 |
| | | Sex* | | | | 5.741 | 6.718 | 0.010 |
| **Frailty** | | | | | | | | |
| Fried Frailty Index | 220 | ACE2 | 0.005 | 5.230 | 0.022 | 0.006 | 6.029 | 0.014 |
| (Score 0–5) | | Age | | | | 0.026 | 5.574 | 0.018 |
| | | Sex* | | | | −0.076 | 0.230 | 0.631 |
| Tilburg Frailty Indicator | 218 | ACE2 | 0.011 | 2.608 | 0.106 | 0.011 | 2.827 | 0.093 |
| (Score 0–11) | | Age | | | | 0.035 | 1.231 | 0.267 |
| | | Sex* | | | | 0.004 | < 0.001 | 0.993 |
| Clinical Frailty Scale | 226 | ACE2 | 0.010 | 10.502 | 0.001 | 0.010 | 11.078 | 0.001 |
| (Score 1–9) | | Age | | | | 0.011 | 0.502 | 0.497 |
| | | Sex* | | | | −0.238 | 1.264 | 0.264 |

than non-frail participants in both analyses ($t = -2.143$, $p = 0.033$; F = 5.016, $p = 0.026$). Despite not reaching statistical significance, serum ACE2 activity was also higher in frail participants according to the Fried Frailty Index and the Tilburg Frailty Indicator.

## 4. Discussion

We have analyzed ACE and ACE2 activity in serum samples, as well as the ACE/ACE2 ratio from a population of older people living in nursing homes. No association was found between ACE and the analyzed parameters. However, serum ACE2 activity was positively associated with BMI, and participants with a higher serum ACE2 activity showed a poorer physical function and were more dependent on activities of daily living. We also found that higher serum ACE2 activity and lower ACE/ACE2 ratio were associated with higher scores on the Clinical Frailty Scale. In addition, frail individuals, according to Clinical Frailty Scale, have higher serum ACE2 activity than non-frail participants.

Studies analyzing RAS in different populations showed a worse general health status associated with elevated levels of elements of the ACE/AngII/AT1R axis; better health status was associated with elevated levels of elements belonging to the ACE2/Ang (1–7)/Mas axis (Santos et al., 2019; Guignabert et al., 2018). However, our results regarding serum ACE2 activity showed the opposite association.

In our study, BMI was positively associated with ACE2. Such association was in opposition to the expected finding, considering the role of this enzyme in health (Santos et al., 2019), despite recent studies that have found a positive association of soluble ACE2 and BMI (Kornilov et al., 2020) in people suffering from metabolic syndrome. Regarding cardiovascular disease, some authors have also described higher levels of plasma ACE2 in men suffering from heart failure (Patel et al., 2016; Sama et al., 2020), or in people with acute heart failure in comparison with ambulatory heart failure patients (Epelman et al., 2008).

Other authors have described a negative association of the ACE axis with physical function and muscle-related parameters in analyses of some RAS elements in older individuals. For example, Abadir et al. (2017) have found that AT1R auto antibodies (reflecting a more active ACE axis) are negatively associated with handgrip strength and gait speed. Accordingly, the use of ACEi and ARB, which inhibit the ACE axis (Harper et al., 2020), has been associated with the enhancement of functional status (Harper et al., 2020) and muscle performance (Vescovo et al., 1998). Positive effects of ACEi and ARB have been also been described regarding sarcopenia (Ata et al., 2020), frailty (Cosarderelioglu et al., 2020), and COVID-19 (De Spiegeleer et al., 2020). In contrast, when serum ACE concentration or activity was analyzed, our

**Table 4**
**(A)** Bivariate Pearson's correlations and partial correlations controlled by sex and age between serum enzymatic activity ratio of angiotensin-converting enzyme and angiotensin-converting enzyme 2 (ACE/ACE2) and quantitative variables. **(B)** Generalized linear models for ordinal variables as dependent variable. Model 1: for ACE/ACE2 as covariable. Model 2: for ACE/ACE2 and age and covariables, and sex as factor (*men). **U:** the amount of enzyme required to release 1 pmol of fluorescent product per minute.

| Variables | N | ACE/ACE2 | | | |
|---|---|---|---|---|---|
| | | Pearson's correlation | | Partial correlation | |
| | | r | p | r | p |
| ACE (U/ml) | 228 | 0.547 | < 0.001 | 0.542 | < 0.001 |
| ACE2 (U/ml) | 228 | −0.618 | < 0.001 | −0.621 | < 0.001 |
| Sociodemographic data | | | | | |
| Age (years) | 228 | 0.066 | 0.323 | −0.049 | 0.467 |
| Anthropometric parameters and blood pressure | | | | | |
| Body mass index (kg/m$^2$) | 225 | −0.108 | 0.108 | −0.117 | 0.080 |
| Waist-to-hip ratio | 225 | −0.025 | 0.709 | −0.003 | 0.970 |
| Systolic blood pressure (mm Hg) | 225 | −0.106 | 0.113 | −0.130 | 0.052 |
| Diastolic blood pressure (mm Hg) | 225 | 0.042 | 0.530 | 0.030 | 0.655 |
| Physical function | | | | | |
| Handgrip (kg) | 228 | −0.004 | 0.946 | 0.097 | 0.148 |
| 6-min walk test (m) | 227 | 0.184 | 0.005 | 0.228 | 0.001 |
| Chair-stand test (n/30s) | 227 | 0.131 | 0.072 | 0.137 | 0.062 |
| Timed up and go test (m/s) | 226 | 0.098 | 0.144 | 0.125 | 0.063 |

| B | N | Covariables and factors | Model 1 | | | Model 2 | | |
|---|---|---|---|---|---|---|---|---|
| Dependent variable | | | β | Wald's $\chi^2$ | *p* | β | Wald's $\chi^2$ | *p* |
| **Physical function** (ordinal variables) | | | | | | | | |
| Berg Balance Scale | | ACE/ACE2 | 0.159 | 2.301 | 0.129 | 0.185 | 3.176 | 0.075 |
| (Score 0–56) | | Age | | | | −0.079 | 0.732 | 0.392 |
| | | Sex* | | | | 2.605 | 3.856 | 0.050 |
| Short physical performance battery (score 0–12) | | ACE/ACE2 | 0.056 | 2.645 | 0.104 | 0.067 | 3.878 | 0.049 |
| | | Age | | | | −0.060 | 4.038 | 0.044 |
| | | Sex* | | | | 0.804 | 3.437 | 0.064 |
| **Activities of daily living** | | | | | | | | |
| Barthel Index | | ACE/ACE2 | 0.372 | 4.504 | 0.034 | 0.418 | 5.841 | 0.016 |
| (score 0–100) | | Age | | | | 0.103 | 0.406 | 0.504 |
| | | Sex* | | | | 6.328 | 8.133 | 0.004 |
| **Frailty** | | | | | | | | |
| Fried Frailty Index | 220 | ACE/ACE2 | −0.012 | 0.936 | 0.333 | −0.015 | 1.499 | 0.221 |
| (Score 0–5) | | Age | | | | 0.025 | 4.952 | 0.026 |
| | | Sex* | | | | −0.107 | 0.448 | 0.504 |
| Tilburg Frailty Indicator | 218 | ACE/ACE2 | −0.067 | 3.507 | 0.061 | −0.071 | 3.881 | 0.049 |
| (Score 0–11) | | Age | | | | 0.034 | 1.153 | 0.283 |
| | | Sex* | | | | −0.097 | 0.045 | 0.832 |
| Clinical Frailty Scale | 226 | ACE/ACE2 | −0.034 | 3.964 | 0.046 | −0.037 | 4.758 | 0.029 |
| (Scosre 1–9) | | Age | | | | 0.008 | 0.264 | 0.182 |
| | | Sex* | | | | −0.289 | 1.778 | 0.608 |

**Table 5**
Enzymatic activity values of serum angiotensin-converting enzyme (ACE) depending on sex and frailty status.

| Variables | N | ACE (U/ml) | | Student's t | p | Fisher's F | p |
|---|---|---|---|---|---|---|---|
| | | Mean | ± SD | | | | |
| Sex | | | | | | | |
| Women | 155 | 866.1 | ± 255.1 | −1.266 | 0.080 | 3.648[a] | 0.057 |
| Men | 73 | 810.7 | ± 330.4 | | | | |
| Fried Frailty Index (score 0–5) | | | | | | | |
| No (< 3) | 83 | 860.9 | ± 286.3 | 0.279 | 0.781 | 0.288[b] | 0.592 |
| Yes (≥ 3) | 137 | 843.7 | ± 285.6 | | | | |
| Tilburg Frailty Indicator (score 0–11) | | | | | | | |
| No (< 5) | 77 | 844.0 | ± 278.4 | −0.262 | 0.794 | 1.146[b] | 0.266 |
| Yes (≥ 5) | 141 | 852.4 | ± 291.8 | | | | |
| Clinical Frailty Scale (score 1–9) | | | | | | | |
| No (< 6) | 118 | 823.8 | ± 285.5 | −1.503 | 0.134 | 0.714[b] | 0.399 |
| Yes (≥ 6) | 108 | 871.6 | ± 278.4 | | | | |

**SD:** standard deviation. **U:** the amount of enzyme required to release 1 pmol of fluorescent product per minute.
[a] Controlled by age.
[b] Controlled by sex and age.

**Table 6**
Angiotensin-converting enzyme 2 (ACE2) serum enzymatic activity values depending on sex and frailty status.

| Variables | N | ACE2 (U/ml) | | Student's t | p | Fisher's F | p |
|---|---|---|---|---|---|---|---|
| | | Mean | ± SD | | | | |
| Sex | | | | | | | |
| Women | 155 | 69.8 | ± 29.2 | 0.028 | 0.977 | 0.092[a] | 0.762 |
| Men | 73 | 70.4 | ± 33.3 | | | | |
| Fried Frailty Index (score 0–5) | | | | | | | |
| No (<3) | 83 | 66.4 | ±25.3 | −0.942 | 0.348 | 1.399[b] | 0.238 |
| Yes (≥3) | 137 | 71.3 | ±32.9 | | | | |
| Tilburg Frailty Indicator (score 0–11) | | | | | | | |
| No (<5) | 77 | 65.2 | ±28.2 | −1.729 | 0.086 | 3.385[b] | 0.067 |
| Yes (≥5) | 141 | 71.4 | ±30.7 | | | | |
| Clinical Frailty Scale (score 1–9) | | | | | | | |
| No (<6) | 118 | 64.9 | ±24.1 | −2.143 | 0.033 | 5.016[b] | 0.026 |
| Yes (≥6) | 108 | 75.0 | ±36.0 | | | | |

**SD:** standard deviation. **U:** the amount of enzyme required to release 1 pmol of fluorescent product per minute.
[a] Controlled by age.
[b] Controlled by sex and age.

**Table 7**
Enzymatic activity values of serum angiotensin-converting enzyme/angiotensin-converting enzyme 2 ratio (ACE/ACE2) values depending on sex and frailty status.

| Variables | N | ACE/ACE2 | | Student's t | p | Fisher's F | p |
|---|---|---|---|---|---|---|---|
| | | Mean | ± SD | | | | |
| Sex | | | | | | | |
| Women | 155 | 13.9 | ± 5.6 | −1.621 | 0.107 | 1.791[a] | 0.182 |
| Men | 73 | 12.6 | ± 5.4 | | | | |
| Fried Frailty Index (score 0–5) | | | | | | | |
| No (< 3) | 83 | 13.9 | ± 5.2 | 0.810 | 0.419 | 1.969[b] | 0.162 |
| Yes (≥ 3) | 137 | 13.3 | ± 5.8 | | | | |
| Tilburg Frailty Indicator (score 0–11) | | | | | | | |
| No (< 5) | 77 | 14.6 | ± 6.6 | 1.959 | 0.051 | 1.013[b] | 0.315 |
| Yes (≥ 5) | 141 | 13.0 | ± 4.8 | | | | |
| Clinical Frailty Scale (score 1–9) | | | | | | | |
| No (< 6) | 118 | 13.6 | ± 5.5 | 0.386 | 0.700 | 1.066[b] | 0.303 |
| Yes (≥ 6) | 108 | 13.3 | ± 5.7 | | | | |

**SD:** standard deviation. **U:** the amount of enzyme required to release 1 pmol of fluorescent product per minute.
[a] Controlled by age.
[b] Controlled by sex and age.

results were in accordance with some of the published results. For instance, serum ACE activity was not related to handgrip strength in people older than 65 (Kang et al., 2012), nor to muscle strength or muscle power in elderly women (Kostka et al., 2017). Thus, research remains inconclusive about the role of the ACE axis in physical function. As proposed by Ata et al. (2020), it can be perhaps due to heterogeneous outcome measures and employed methodologies.

In the current study, serum ACE2 activity was inversely associated with physical function and directly related to dependence on activities of daily living and frailty. Literature on this topic is scarce. In a recent study about SARS-CoV-2 pathogenicity, ACE2 and exercise, Klöting et al. (2020) have shown that physical exercise (which promotes physical function) induces expression of ACE2 in skeletal muscle but leads to lower circulating ACE2 levels. Our results also agree with ACE2 counteraction previously described in muscle-related pathologies. Decline in functional performance and restriction of adaptability in aging are characteristics shared by pathologies that involve muscle wasting, such as muscular dystrophies (Vinciguerra et al., 2010). In animal models, Riquelme et al. have found that ACE2 protein level and activity are augmented in the plasmatic membrane of dystrophic skeletal muscle (Riquelme et al., 2014). These authors suggest that ACE2 activity is enhanced as a compensatory mechanism to produce more Ang (1–7), thereby increasing ACE2/Ang (1–7)/Mas-axis actions. Furthermore, in animal models of limb–girdle muscular dystrophy type 2B, White et al. (2019) have described that losartan (an ARB that blocks the ACE axis, enhancing the ACE2 axis) exacerbates muscle wasting.

In contrast, studies about other elements of ACE2 axis and their association with physical function showed results opposite to ours. For example, Ang (1–7), the product of ACE2-mediated enzymatic reaction, has been associated with positive effects on skeletal muscle wasting (Rivera et al., 2020). Accordingly, other authors have also found incremented serum ACE2 concentration in subjects participating in high-intensity exercise programs (Magalhães et al., 2020). It must be taken into account that previous works on these and other peptidases have demonstrated that serum and tissue activity may not be associated with one another (Larrinaga et al., 2010; Larrinaga et al., 2013; Heffernan and Jae, 2020). In fact, at present it is not fully understood how the balance between tissue and serum ACE2 is maintained (Klöting et al., 2020).

This work has several limitations, such as the lack of knowledge about participants' medications, especially for hypertension, which might have been interesting data for controlling variables in a more robust statistical analysis. In addition, more information could have been obtained with a wider molecular analysis of RAS elements. In our opinion, these limitations must be taken into account when designing similar works in the future. In addition, it should be taken into account that this study has been performed in serum samples of people living in nursing homes and the results presented here cannot be extrapolated to a population not meeting the inclusion criteria of this study.

Despite its limitations, this work also presents several strengths that must be taken into account as well. First, our study reveals an as-yet undescribed association between ACE2 and unfavorable outcomes in a vulnerable and poorly studied population group: people living in nursing homes. Both ACE and ACE2 are serum proteins whose analyses

are well stabilized in routine clinical practice. Thus they could become molecular biomarkers to identify older people with poor physical function and/or frailty.

Our study is the first to describe the association of higher serum ACE2 enzymatic activity with worse physical function and increased dependence in older people living in nursing homes. Additionally, higher serum ACE2 enzymatic activity is associated with frailty, given its positive association with higher frailty scores as measured by the Fried Frailty Index, Tilburg Frailty Indicator and Clinical Frailty Scale. Thus, serum ACE2 activity, in combination with other molecules, may be proposed as a molecular biomarker of poor physical function and physical frailty in this population.

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## CRediT authorship contribution statement

JI and ARL designed the study. HA, CR, and ILD recruited the participants and collected the data from the nursing home databases. HA and CR performed the tests related to physical fitness and frailty. JI, HA, and BS managed the requests for approval from research ethics committees. ILD carried out the collection of the blood samples. BS and JGG analyzed the serum samples and calculated the enzymatic activity. AFA and BS interpreted the data and drafted the manuscript. BS, CR, HA, AFA, ILD, JGG, ARL, and JI have approved the submitted version and agree to be personally accountable for their own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work—even those in which they were not personally involved—are appropriately investigated, resolved, and documented in the literature. All authors have read the journal's authorship agreement.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## References

Abadir, P.M., 2011. The frail renin-angiotensin system. Clin. Geriatr. Med. 27 (1), 53–65. https://doi.org/10.1016/j.cger.2010.08.004.

Abadir, P.M., Jain, A., Powell, L.J., et al., 2017. Discovery and validation of agonistic angiotensin receptor autoantibodies as biomarkers of adverse outcomes. Circulation 135 (5), 449–459. https://doi.org/10.1161/CIRCULATIONAHA.116.022385.

Aravena, J., Abrigo, J., Gonzalez, F., et al., 2020. Angiotensin (1–7) decreases myostatin-induced NF-κB signaling and skeletal muscle atrophy. Int. J. Mol. Sci. 21 (3), 1167. https://doi.org/10.3390/ijms21031167.

Arrieta, H., Rezola-Pardo, C., Gil, S.M., Virgala, J., Iturburu, M., Antón, I., González-Templado, V., Irazusta, J., Rodriguez-Larrad, A., 2019. Effects of multicomponent exercise on frailty in long-term nursing homes: a randomized controlled trial. J. Am. Geriatr. Soc. 67 (6), 1145–1151. https://doi.org/10.1111/jgs.15824.

Ata, A.M., Kara, M., Ekiz, T., et al., 2020. Reassessing sarcopenia in hypertension: STAR and ACE inhibitors excel. Int. J. Clin. Pract., e13800 https://doi.org/10.1111/ijcp.13800.

Becker, L.K., Totou, N., Moura, S., et al., 2018. Eccentric overload muscle damage is attenuated by a novel angiotensin- (1–7) treatment. Int. J. Sports Med. 39 (10), 743–748. https://doi.org/10.1055/a-0633-8892.

Berg, K.O., Wood-Dauphinee, S.L., Williams, J.I., Maki, B., 1992. Measuring balance in the elderly: validation of an instrument. Can. J. Publ. Health 83, S7–S11.

Brink, M., Price, S.R., Chrast, J., et al., 2001. Angiotensin II induces skeletal muscle wasting through enhanced protein degradation and down-regulates autocrine insulin-like growth factor I. Endocrinology 142 (4), 1489–1496. https://doi.org/10.1210/endo.142.4.8082.

Bustamante-Ara, N., Santiago, C., Verde, Z., et al., 2010. ACE and ACTN3 genes and muscle phenotypes in nonagenarians. Int. J. Sports Med. 31 (4), 221–224. https://doi.org/10.1055/s-0030-1247529.

Cisternas, F., Morales, M.G., Meneses, C., et al., 2015. Angiotensin-(1–7) decreases skeletal muscle atrophy induced by angiotensin II through a mas receptor-dependent mechanism. Clin. Sci. 128 (5), 307–319. https://doi.org/10.1042/CS20140215.

Cosardereliogl u, C., Nidadavolu, L.S., George, C.J., et al., 2020. Brain renin-angiotensin system at the intersect of physical and cognitive frailty. Front. Neurosci. 14, 586314 https://doi.org/10.3389/fnins.2020.586314.

De Spiegeleer, A., Bronselaer, A., Teo, J.T., et al., 2020. The effects of ARBs, ACEis, and statins on clinical outcomes of COVID-19 infection among nursing home residents. J. Am. Med. Dir. Assoc. 21 (7), 909–914.e2. https://doi.org/10.1016/j.jamda.2020.06.018.

Deandrea, S., Bravi, F., Turati, F., et al., 2013. Risk factors for falls in older people in nursing homes and hospitals. A systematic review and meta-analysis. Arch. Gerontol. Geriatr. 56 (3), 407–415. https://doi.org/10.1016/j.archger.2012.12.006.

Donoghue, M., Hsieh, F., Baronas, E., et al., 2000. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1–9. Circ. Res. 87 (5), E1–E9. https://doi.org/10.1161/01.res.87.5.e1.

Du Bois, P., Pablo Tortola, C., Lodka, D., et al., 2015. Angiotensin II induces skeletal muscle atrophy by activating TFEB-mediated MuRF1 expression. Circ. Res. 117 (5), 424–436. https://doi.org/10.1161/CIRCRESAHA.114.305393.

Epelman, S., Tang, W.H., Chen, S.Y., Van Lente, F., Francis, G.S., 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 (9), 750–754. https://doi.org/10.1016/j.jacc.2008.02.088.

Fernández-Atucha, A., Izagirre, A., Fraile-Bermúdez, A.B., et al., 2017. Sex differences in the aging pattern of renin-angiotensin system serum peptidases. Biol. Sex Differ. 8, 5. https://doi.org/10.1186/s13293-017-0128-8.

Fess, E.E., 1992. In: Casanova, J.S. (Ed.), Grip strength in clinical assessment recommendations. American Society of Hand Therapists, pp. 41–45.

Fried, L.P., Tangen, C.M., Walston, J., et al., 2001. Frailty in older adults: evidence for a phenotype. J. Gerontol. A Biol. Sci. Med. Sci. 56 (3), M146–M156. https://doi.org/10.1093/gerona/56.3.M146.

Gobbens, R.J., van Assen, M.A., Luijkx, K.G., Wijnen-Sponselee, M.T., Schols, J.M., 2010. The Tilburg frailty indicator: psychometric properties. J. Am. Med. Dir. Assoc. 11 (5), 344–355. https://doi.org/10.1016/j.jamda.2009.11.003.

Gomes-Santos, I.L., Fernantes, T., Couto, G.K., et al., 2014. Effects of exercise training on circulating and skeletal muscle renin-angiotensin system in chronic heart failure rats. PLoS One 9 (5), e98012.

Guignabert, C., de Man, F., Lombès, M., 2018. ACE2 as therapy for pulmonary arterial hypertension: the good outweighs the bad. Eur. Respir. J. 51 (6), 1800848. https://doi.org/10.1183/13993003.00848-2018.

Guralnik, J.M., Simonsick, E.M., Ferrucci, L., et al., 1994. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. J. Gerontol. 49 (2), M85–M94. https://doi.org/10.1093/geronj/49.2.m85.

Harper, S.A., Baptista, L.C., Roberts, L.M., et al., 2020. Enzyme inhibitors combined with exercise for hypertensive seniors (The ACES Trial): study protocol of a randomized controlled trial. Front. Med. 6, 327. https://doi.org/10.3389/fmed.2019.00327.

Heffernan, K.S., Jae, S.Y., 2020. Exercise as medicine for COVID-19: an ACE in the hole? Med. Hypotheses 142, 109835. https://doi.org/10.1016/j.mehy.2020.109835.

Herbert, K.E., Mistry, Y., Hastings, R., Poolman, T., Niklason, L., Williams, B., 2008. Angiotensin II-mediated oxidative DNA damage accelerates cellular senescence in cultured human vascular smooth muscle cells via telomere-dependent and independent pathways. Circ. Res. 102, 201–208. https://doi.org/10.1161/CIRCRESAHA.107.158626.

Kang, H.J., Kim, C.H., Park, D.S., et al., 2012. The impacts of ACE activity according to ACE I/D polymorphisms on muscular functions of people aged 65. Ann. Rehabil. Med. 36 (4), 433–446. https://doi.org/10.5535/arm.2012.36.4.433.

Klöting, N., Ristow, M., Blüher, M., 2020. Effects of exercise on ACE2. Obesity (Silver Spring) 28 (12), 2266–2267. https://doi.org/10.1002/oby.23041.

Kornilov, S.A., Lucas, I., Jade, K., et al., 2020. Plasma levels of soluble ACE2 are associated with sex, metabolic syndrome, and its biomarkers in a large cohort, pointing to a possible mechanism for increased severity in COVID-19. Crit. Care 24 (1), 452. https://doi.org/10.1186/s13054-020-03141-9.

Kostka, J., Sikora, J., Kostka, T., 2017. Relationship of quadriceps muscle power and optimal shortening velocity with angiotensin-converting enzyme activity in older women. Clin. Interv. Aging 12, 1753–1760. https://doi.org/10.2147/CIA.S146494.

Larrinaga, G., Pérez, I., Sanz, B., et al., 2010. Angiotensin-converting enzymes (ACE and ACE2) are downregulated in renal tumors. Regul. Pept. 165 (2–3), 218–223. https://doi.org/10.1016/j.regpep.2010.07.170.

Larrinaga, G., Perez, I., Ariz, U., et al., 2013. Clinical impact of aspartyl aminopeptidase expression and activity in colorectal cancer. Transl. Res. 162 (5), 297–308. https://doi.org/10.1016/j.trsl.2013.07.010.

Lobo, A., Saz, P., Marcos, G., et al., 1999. Revalidación y normalización del mini-examen cognoscitivo (primera versión en castellano del mini-mental status Examination) en la población general geriátrica. (Revalidation and normalization of the mini-cognitive examination (first spanish version of the mini-mental status Examination) in the general geriatric population). Med. Clin. 112, 767–774.

Magalhães, D.M., Nunes-Silva, A., Rocha, G.C., et al., 2020. Two protocols of aerobic exercise modulate the counter-regulatory axis of the renin-angiotensin system. Heliyon 6 (1), e03208. https://doi.org/10.1016/j.heliyon.2020.e03208. Erratum. In: Heliyon 2020;6(10):e05256.

Mathias, S., Nayak, U.S., Isaacs, B., 1986. Balance in elderly patients: the "get-up and go" test. Arch. Phys. Med. Rehabil. 67, 387–389.

Motta-Santos, D., Dos Santos, R.A., Oliveira, M., et al., 2016. Effects of ACE2 deficiency on physical performance and physiological adaptations of cardiac and skeletal muscle to exercise. Hypertens. Res. 39 (7), 506–512. https://doi.org/10.1038/hr.2016.28.

Muscedere, J., Kim, P.M., Afilalo, J., et al., 2019. Proceedings of the Canadian frailty network workshop: identifying biomarkers of frailty to support frailty risk assessment, diagnosis and prognosis. Toronto, January 15, 2018, 8 (3), 106–116.

Nickenig, G., Harrison, D.G., 2002. The AT(1)-type angiotensin receptor in oxidative stress and atherogenesis: part I: oxidative stress and atherogenesis. Circulation 105 (3), 393–396. https://doi.org/10.1161/hc0302.102618.

Nunes-Silva, A., Rocha, G.C., Magalhaes, D.M., et al., 2017. Physical exercise and ACE2-angiotensin-(1–7)-mas receptor axis of the renin angiotensin system. Protein Pept Lett 24 (9), 809–816. https://doi.org/10.2174/0929866524666170728151401.

Patel, V.B., Zhong, J.C., Grant, M.B., Oudit, G.Y., 2016. Role of the ACE2/Angiotensin 1–7 Axis of the renin-angiotensin system in heart failure. Circ. Res. 118 (8), 1313–1326. https://doi.org/10.1161/CIRCRESAHA.116.307708.

Rezola-Pardo, C., Arrieta, H., Gil, S.M., et al., 2019a. A randomized controlled trial protocol to test the efficacy of a dual-task multicomponent exercise program in the attenuation of frailty in long-term nursing home residents: aging-ONDUAL-TASK study. BMC Geriatr. 19 (1), 6. https://doi.org/10.1186/s12877-018-1020-z.

Rezola-Pardo, C., Arrieta, H., Gil, S.M., et al., 2019b. Comparison between multicomponent and simultaneous dual-task exercise interventions in long-term nursing home residents: the ageing-ONDUAL-TASK randomized controlled study. Age Ageing 48 (6), 817–823. https://doi.org/10.1093/ageing/afz105.

Rezola-Pardo, C., Rodriguez-Larrad, A., Gomez-Diaz, J., et al., 2020. Comparison between multicomponent exercise and walking interventions in long-term nursing homes: a randomized controlled trial. Gerontologist 60 (7), 1364–1373. https://doi.org/10.1093/geront/gnz177.

Rikli, R.E., Jones, C.J., 2001. Senior Fitness Test. Champaign, Human Kinetics.

Riquelme, C., Acuña, M.J., Torrejón, J., et al., 2014. ACE2 is augmented in dystrophic skeletal muscle and plays a role in decreasing associated fibrosis. PLoS One 9 (4), e93449. https://doi.org/10.1371/journal.pone.0093449.

Rivera, J.C., Abrigo, J., Tacchi, F., et al., 2020. Angiotensin-(1–7) prevents lipopolysaccharide-induced autophagy via the mas receptor in skeletal muscle. Int. J. Mol. Sci. 21 (24), 9344. https://doi.org/10.3390/ijms21249344.

Rockwood, K., Song, X., MacKnight, C., et al., 2005. A global clinical measure of fitness and frailty in elderly people. CMAJ 173 (5), 489–495. https://doi.org/10.1503/cmaj.050051.

Rodriguez-Larrad, A., Arrieta, H., Rezola, C., et al., 2017. Effectiveness of a multicomponent exercise program in the attenuation of frailty in long-term nursing home residents: study protocol for a randomized clinical controlled trial. BMC Geriatr. 17 (1), 60. https://doi.org/10.1186/s12877-017-0453-0.

Sama, I.E., Ravera, A., Santema, B.T., 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 (19), 1810–1817. https://doi.org/10.1093/eurheartj/ehaa373.

Santos, R.A.S., Oudit, G.Y., Verano-Braga, T., et al., 2019. The renin-angiotensin system: going beyond the classical paradigms. Am. J. Physiol. Heart Circ. Physiol. 316 (5), H958–H970. https://doi.org/10.1152/ajpheart.00723.2018.

Topinková, E., 2008. Aging, disability and frailty. Ann. Nutr. Metab. 52, 6–11. https://doi.org/10.1159/000115340.

Vescovo, G., Dalla Libera, L., Serafini, F., et al., 1998. Improved exercise tolerance after losartan and enalapril in heart failure: correlation with changes in skeletal muscle myosin heavy chain composition. Circulation 98 (17), 1742–1749. https://doi.org/10.1161/01.cir.98.17.1742.

Vinciguerra, M., Musaro, A., Rosenthal, N., 2010. Regulation of muscle atrophy in aging and disease. Adv. Exp. Med. Biol. 694, 211–233. https://doi.org/10.1007/978-1-4419-7002-2_15.

Wade, D.T., Collin, C., 1988. The barthel ADL index: a standard measure of physical disability? Int. Disabil. Stud. 10 (2), 64–67. https://doi.org/10.3109/09638288809164105.

Wang, Y., Fan, Y., Song, Y., et al., 2019. Angiotensin II induces apoptosis of cardiac microvascular endothelial cells via regulating PTP1B/PI3K/Akt pathway. In Vitro Cell Dev. Biol. Anim. 55 (10), 801–811. https://doi.org/10.1007/s11626-019-00395-8.

Warnholtz, A., Nickenig, G., Schulz, E., et al., 1999. Increased NADH-oxidase-mediated superoxide production in the early stages of atherosclerosis: evidence for involvement of the renin-angiotensin system. Circulation 99 (15), 2027–2033. https://doi.org/10.1161/01.cir.99.15.2027.

White, Z., Milad, N., Tehrani, A.Y., et al., 2019. Angiotensin II receptor blocker losartan exacerbates muscle damage and exhibits weak blood pressure-lowering activity in a dysferlin-null model of limb-girdle muscular dystrophy type 2B. PLoS One 14 (8), e0220903. https://doi.org/10.1371/journal.pone.0220903.