Kobe J. Med. Sci., Vol. 64, No. 3, pp. E119-E125, 2018

Deletion Polymorphism of Angiotensin-Converting Enzyme Gene Is Associated with Low Muscle Mass in Elderly People in Jakarta, Indonesia

MARIA DARA NOVI HANDAYANI[1,2*], AHMAD HAMIM SADEWA[3], ARTA FARMAWATI[3], and WASILAH ROCHMAH[4]

[1]*Doctorate Program on Medical and Health Science, Faculty of Medicine Universitas Gadjah Mada,Yogyakarta, Indonesia*

[2]*Department of Biochemistry, Faculty of Medicine Atma Jaya University, Jakarta, Indonesia*

[3]*Department of Biochemistry, Faculty of Medicine Universitas Gadjah Mada, Yogyakarta, Indonesia*

[4]*Department of Internal Medicine, Faculty of Medicine Universitas Gadjah Mada Yogyakarta, Indonesia*

** Corresponding author*

Received 26 January 2018/ Accepted 31 May 2018

Key words: ACE Polymorphism, Muscle Mass, Elderly.

Decline in muscle mass due to aging is a growing public health problem as it contributes to a decreased capacity for independent living among elderly people. A clear understanding of genetic factors is important, as it is known that angiotensin-converting enzyme insertion/deletion (ACE I/D) polymorphism affects muscle mass, although the findings are frequently heterogeneous. This study was conducted to determine the association between ACE I/D polymorphism and muscle mass in elderly people. A total of 130 elderly people were recruited from nursing homes in Jakarta. Anthropometric components affecting the muscle mass were examined. Cross-sectional analyses were performed to compare data using *t*-test, ANOVA and ANCOVA, and linear regression. Genotyping of the ACE I/D polymorphisms was performed by PCR methods, and muscle mass was evaluated by BIA. Genotype distribution counts II 65.38%, ID 13.85%, and DD 20.77% were not consistent with the Hardy–Weinberg equilibrium (χ^2 = 22.2, df = 2; p < 0.01). Individuals with the DD genotype showed lower muscle mass that was significantly different compared to the muscle mass in individuals with the II/ID genotype (II 16.14 ± 0.38, ID 15.71 ± 0.59; DD 13.95 ± 0.61 kg), after adjusting for % fat as a covariate. The linear regression analysis showed that age, gender, weight, height, nutritional status, protein content, and waist, hip, and calf circumference were significant contributors to muscle mass. In the multivariate analysis, adjusted age and gender significantly correlated with muscle mass, with r^2 = 0.98, by the likelihood ratio test (p < 0.01). The genotype variability accounted for 2.65% of the DD genotype. This study showed that in an elderly population in Jakarta, the DD genotype was associated with low muscle mass. This result suggests the role of nutritional status as a potential mediator in the association between ACE gene and muscle mass.

INTRODUCTION

Muscle mass of elderly people is very important to their activities of daily living and vital prognosis for some disease like diabetes mellitus, dyslipidemia, etc. The decline in muscle mass due to aging is one of the contributors to sarcopenia and results in a decrease in the capacity for independent living and an increase in the risk of falls and death among the elderly people, thereby leading to important socioeconomic consequences (1). Several factors have been reported to contribute to the decrease in muscle mass during the aging process in the elderly people, including genetic factors such as the angiotensin-converting enzyme (ACE) gene (2,3).

ACE is a key enzyme in the renin–angiotensin system (RAS), which converts angiotensin I (Ang I) into angiotensin II (Ang II) (4,5). Angiotensin II appears to influence the decline in muscle mass among the elderly people through a number of mechanisms that increase protein breakdown; reduce protein synthesis, regenerative processes, and insulin resistance in the skeletal muscle; and also decrease the appetite (5,6).

Polymorphism of the human ACE gene has been identified to be related to either the presence (insertion, I allele) or the absence (deletion, D allele) of a 287-bp segment in intron 16. Homozygosity for the D allele of ACE I/D polymorphism has been suggested as a marker for higher ACE activity and Ang II concentration (3-5,7). The homozygous deletion (DD) of the ACE I/D polymorphism has been identified to be associated with lower muscle mass (8). However, the results are still conflicting (7,9,10), involving heterogeneous factors, including ethnic differences, subject characteristics, and a complex interaction between genetics and nutritional status (1,2,3).

Phone: +62 8128284307 Fax: +62 21 6606122/3 Email: maria.dara@atmajaya.ac.id

Several studies on the relationship between polymorphism of ACE gene and muscle mass have been published in Europe and the U.S., but no study has been conducted among Indonesian elderly people living in subsidized nursing homes, considering the subject characteristics of lower education and socioeconomic levels. This study was conducted to clarify the role of the ACE I/D polymorphisms that have been identified to have potential influences contributing to the variations in the skeletal muscle. We also investigated the association between the ACE I/D polymorphism and the muscle mass of elderly people in Jakarta. In addition, we examined the role of nutritional status in the association between ACE I/D polymorphism gene and declining muscle mass in an elderly at-risk population.

METHODS

Subjects and study design

This cross-sectional study conducted in October 2016 examined a total of 130 elderly people aged > 60 years living in a government nursing home in Jakarta, Indonesia. The inclusion criteria for the subjects were being healthy, able to perform basic activities, able to communicate well, and no history of drug consumption continuously that could affect muscle mass at least for 6 months period and willing to undergo several examinations of anthropometric components that are involved in the status of muscle mass.

Anthropometric measurements

Body mass index (BMI), calf circumference (CC), hip circumference (HC), and waist circumference (WC) were measured using standardized equipment and procedures. BMI was measured in kilograms divided by height in square meters. Subjects were considered as malnourished if their BMI was <18.5 kg/m^2, normal when their BMI ranged from 18.5 to 24.9 kg/m^2, overweight if their BMI was 25.029.9 kg/m^2, and obese when their BMI was >30.0 kg/m^2. Subjects with a WC of 94.0–101.9 or >102.0 cm for men and 80.0–87.9 or >88.0 cm for women were classified as overweight or obese, respectively. Subjects with a waist hip ratio (WHR) of >0.85 for women and >1.00 for men were classified as overweight (11). The total lean body mass, total fat mass, and protein (kg) measurements were analyzed using the Bioscan 916 body composition analyzer with an operating frequency of 50 kHz at 0.7 mA (Maltron, UK) (12).

Genotyping

DNA was isolated from the buffy coat obtained from peripheral blood samples of the subjects following a standard protocol using a Wizard Genomic DNA Purification kit (Promega). The ACE I/D gene polymorphisms were identified by polymerase chain reaction (PCR) assay. PCR was performed at a final reaction mixture of 25 µl containing genomic DNA, PCR master mix (Promega), and oligonucleotide of the ACE gene with the following sequences: two primers were used in each PCR, F: 5-CTG GAG ACC ACT CCC ATC CTT TCT3 and R: 5′-GAT GTG GCC ACT ACA TTC GTC AGAT-3. The samples were initially denatured at 94°C for 4 min, followed by 30 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min. A final extension for 7 min at 72°C completed the PCR (Bio-Rad, Thermal Cycler C1000). The PCR products of the two alleles of 490 and 190 bp were separated on 1.5% agarose gel and visualized by ethidium bromide staining (13).

The study was approved by the Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine Universitas Gadjah Mada (Ref: KE/FK/599/EC/2016).

Statistical analysis

Statistical analyses were performed using Stata (Ver. 12.0). The ACE genotype distribution was evaluated for conformity with the Hardy–Weinberg (HW) equilibrium using the test with one degree of freedom. Differences in nutritional status and anthropometric components among subjects with II vs ID vs DD genotypes were analyzed using analysis of variance (ANOVA), while those among subjects with II vs ID/DD genotypes were analyzed using unpaired *t*-tests. Muscle mass was compared among the genotype groups using analysis of covariance (ANCOVA) models adjusted by fat as a covariate. Robust linear regression was analyzed for assessing the contribution of anthropometric components and elderly people's characteristics to muscle mass. Multiple linear regression, including likelihood ratio (LR) tests, Akaike information criterion (AIC), Bayesian information criterion (BIC), and effect size among model 1 and model 2, were analyzed to estimate the degree of variance in the ACE genotype attributable to muscle mass. Values were expressed as mean ± SD; $p < 0.05$ was considered to be statistically significant. Effect sizes (ES) were reported as Cohen's d based on pooled standard deviations that provide partial eta squared values of 0.0099, 0.0588, and 0.1379 as references for small, medium, and large effect sizes, respectively (14). The genetic advance as a percentage of mean was calculated using the Falconer (1989) formula (15).

RESULTS

All the 130 elderly people (52 males and 78 females) were recruited from four nursing homes located at North, South, East, and West Jakarta, Indonesia. The mean age of the subjects was 69.25 ± 9.82 years, and 67.07% of them had an education level of < 6 years

Genotyping of the ACE I/D polymorphisms performed by the PCR method detected the presence of a PCR DNA product band of 490 bp for genotype II and/or 190 bp for the DD genotype and bands of 490 and 190 bp for the ID genotype (Figure 1). Sequences with the largest frequency among the II, DD, and ID genotypes comprised 78 (66.1%), 23 (19.49%), and 17 (14.41%), respectively (Table I). Genotype frequencies for the ACE I/D gene distributions among the subject population were not consistent with the HW equilibrium (χ^2 = 22.2, df = 2, p = 0.00). A deviation of the ACE genotypes from the HW equilibrium observed in this population may potentially hamper the generalizability of the results.

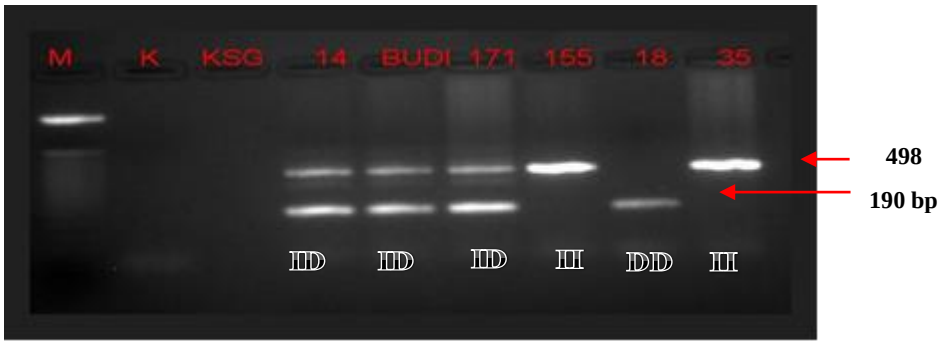

Figure 1. The ACE I/D genotype, number 171 ID genotype 2 band, number 155 II genotype 1 band, and number 18 DD genotype 1 band.

The results showed that the ACE I/D genotypes were significant differences to muscle mass level with the DD genotype was lower (Table I). We found protein as predictors and confounding factor to muscle mass, but fat was considered as covariate, whereas other anthropometric components like weight, height, protein content, CC, and HC had no significant differences.

Table I. Characteristics of the subjects according to the ACE genotype

Variable	II		ID		DD		ID & DD		*p value*	*p value*
	X	+SD/ SEM	X	+SD/ SEM	X	+SD/ SEM	X	+SD/ SEM	IIvsIDvsDD	ID/DD vsII
Muscle (kg)**	16.1	3.36/ 0.38*	15.71	2.43/ 0.58*	13.95	2.93 0.61*	14.7	2.83/ 0.45*	0.02	0.02
Weight (cm)	51.4	10.44	49.46	7.1	47.37	6.64	48.26	6.83	0.18	0.09
Height (cm)	151	8.78	150.47	6.88	149.3 9	9.07	149.85	8.13	0.76	0.53
CC (cm)	31.6	4.29	30.15	2.67	30.67	2.56	30.45	2.58	0.27	0.12
HC (cm)	81.4	11.15	80.76	11.36	79.28	10.57	79.91	10.79	0.73	0.5
Fat (kg)	13.4	7.57	12.13	5.07	12.64	6.76	12.43	6.03	0.75	0.47
Protein (kg)	7.3	1.51	7.15	1.03	6.34	1.26	6.68	1.22	0.02	0.03
WHR	0.9	0.07	0.89	0.09	0.89	0.07	0.89	0.07	0.94	0.73

*standard error of the mean (SEM), ** Adjusted for fat by ANCOVA

Therefore, we statistically adjusted muscle mass with fat as a covariate, and this analysis revealed that the muscle mass of the subjects with the DD genotype was significantly lower than the muscle mass of the subjects with the II and DD genotypes (p < 0.05).

Linear regression and multiple regression analyses were conducted to determine the ACE I/D polymorphisms that contributed to muscle mass in the elderly subjects (Table II). Linear regression analyses showed that older age, female gender, DD genotype, and overweight (BMI) significantly contributed to a decrease in muscle mass (coef = −5.21, −3.53, −2.18, and −1.92, respectively), whereas protein content, CC, HC, weight, height, and hip

circumference significantly contributed to an increase in muscle mass in the elderly subjects (coef = +2.24, +0.43, +0.25, +0.22, and +0.10, respectively).

Further tests using multiple regression analysis determined the direction and magnitude of the contribution of the ACE I/D polymorphisms to muscle mass, showing that in model I (adjusted for age and gender, older age, and female gender), the DD genotype had a significant contribution to the decline in muscle mass (coef = −3.76, −2.70, −1.85, respectively). In model II (anthropometric components + model I adjusted), it was observed that the DD genotype had no significant contribution to the decline in muscle mass; however, only older age, female gender, and protein content had a significant contribution to the increase in muscle mass (coef = −1.03, +0.2, and +2.15, respectively). Table II shows the association between muscle mass and ACE polymorphism, adjusted by gender, age, and anthropometric components. The linear and multiple regression analyses showed that the ACE polymorphisms contributed to muscle mass after adjusting for anthropometric components. These models resulted in r^2 values (0.36; 0.98), which implies that models I and II could predict the muscle mass by 36% and 98%, respectively.

Table II. Linear and multiple regression analyses of ACE polymorphisms

Muscle mass	Linier regression (Coef.)	Multple regression (coef.)	
		Model I	Model II
Genotypes ACE: II-reff	-	-	-
ID	-0.42	0.003	-0.14
DD	-2.18*	-1.85*	-0.57
Gender: man-reff	-	-	-
Woman	-3.53*	-2.70*	0.20*
Age: <60 year-reff	-	-	-
60-69 year	-1.61	-0.83	-0.31*
70-79 year	-3.64*	-2.07*	-0.67*
≥80	-5.20*	-3.76*	-1.03*
Nutritional status: normal-reff	-		-
Overweight	-1.59*		-0.03
Obesity	1.40		0.05
WHR: normal-reff	-		-
Moderate risk	1.92*		-0.06
High risk	0.65		-0.03
Protein	2.24*		2.15*
Weight	0.25*		0.02
Height	0.22*		0.01
Calf circumference	0.43*		-0.02
Hip circumference	0.10*		-.0.01
r^2		0.36	0.98
LR test (*p-value*)		-	0.00
AIC		573.69	147.02
BIC		593.09	190.23
Effect size	0.06	0.07	0.012
Variability attributable to genotype effect	Gen ACE II=0.49%, ID= I/D= 0.25%	3.46%, DD= 2.65%,	ID/DD= 1.12%,

Models I and II were shown to significantly contribute to muscle mass after adjusting for anthropometric components determined by the LR test ($p < 0.05$). The AIC and BIC values showed that model II was a good model to predict muscle mass compared with model I. The effect sizes of the ACE I/D genotypes II vs ID vs DD are summarized in detail in Table II. The effect sizes of ACE II vs ID vs DD genotypes in the linear regression and models I and II were small (partial eta squared values <0.09). Percentage coefficient variation of genotype ACE II was counted lower than the ID/DD genotypes and the variability attributable to the ACE was up to 3.46% of the muscle mass variance elderly in Jakarta.

DISCUSSION

In this study, we examined the hypothesis that the ACE I/D polymorphism may contribute to muscle mass in elderly people and that it was partially supported by the association with anthropometric components. We

analyzed the relationship between ACE I/D polymorphisms and muscle mass in elderly people with different races and educational and socioeconomic levels, which has not been previously investigated in the Indonesian population. Our study clearly demonstrated significant associations between ACE I/D polymorphisms and muscle mass in elderly people from Jakarta.

The primary result of our study showed that the DD genotype was significantly associated with low muscle mass. The correlation of DD genotype with the declining muscle mass may be consistent with the hypothesis regarding the correlation of DD genotype with higher Ang II levels in muscle atrophy (4-7). The mechanisms whereby Ang II induces muscle atrophy are complex. Several studies in mice have explained that angiotensin II functions as an atrophic factor to skeletal muscle by decreasing capillary perfusion, oxygen utilization, protein synthesis, and regenerative processes and enhancing apoptosis, protein breakdown, especially in fast-twitch muscle fiber, and insulin resistance. Studies have shown that Ang II was associated with weight loss, anorexigenic effects on the central nervous system (7,16-19). Another study showed that Ang II levels locally in the adipose tissue cause further adipose tissue growth associated with adipocyte hypertrophy and an increase in lipogenesis and triglyceride accumulation, thus affecting the volume of intermuscular adipose tissue (IMAT) in the muscular system (20). Based on previous studies, our results suggest that individuals with the DD genotype have lower muscle mass, which is further supported by studies showing that individuals with the ID/DD genotype have lower muscle mass than those with the II genotype (10) and individuals with the II genotype who have lower ACE activity have better blood flow and oxygen utilization than those with the DD genotype (4,8,21,22). Another study reported the use of an inhibitor of ACE in elderly who exhibited a larger of lower extremity muscle mass (23) while administering ACE inhibitor in mice indicated significant loss of fat mass (24) and protect tissues from oxidative damage by increasing antioxidant activity at various systems (25,26). Nevertheless, some studies have also demonstrated an association between the DD genotype and skeletal muscle hypertrophy and higher values of muscle mass (7,9,27), whereas other studies found no such association (10).

Other results support our primary findings that individuals with the DD genotype had significantly lower total protein content in the body than that in individuals with the ID/II genotype. Protein is the largest component of muscle mass. Low protein levels in individuals with the DD genotype in addition to those associated with increased Ang II protein breakdown and reduced protein synthesis (7,17,18) are also influenced by the nature of Ang II, which might play a pathogenetic role in the development of proteinuria-induced renal structural damage as shown in the studies of Bos and Tang (4,28,29). Another related study showed a correlation between proteinuria and plasma ACE levels (30).

In this study, we also found that age, gender, and nutritional status (weight, height, BMI, WHR, CC, HC, and protein) were important factors for muscle mass in the elderly subjects. This result is also consistent with that of other studies showing a relationship between muscle mass and age (31), gender (32), and anthropometric components (20,32,33). This study also showed that a possible interaction exists between ACE I/D polymorphisms and risk factors for muscle mass (age, gender, and protein level). This finding suggests the presence of an interaction between genes and environmental factors affecting muscle mass variability (2,3).

As we know, it was the first study that investigated the role of ACE I/D polymorphisms in an elderly population in South East Asian population, concerning muscle mass and this results may have possibility of development of the sarcopenia treatment. Some limitations exist in this study. Notably, angiotensin II levels were not examined, only elderly retirement homes were considered, and the distributions of the ACE genotypes were not in agreement with the HW equilibrium. Further research could be improved by examining angiotensin II levels using a significantly larger sample of elderly people in other areas of Indonesia to better clarify and confirm the results.

In conclusion, the ACE gene polymorphisms were found to be associated with muscle mass and protein levels in an elderly population living in nursing homes in Jakarta, Indonesia, with nutritional status being identified as a potential mediator in the association between ACE I/D polymorphisms and muscle mass. This study may provide additional evidence for the role of DD genotype in the declining muscle mass, which is of significant clinical importance. ACE polymorphism is an important consideration for future studies aiming to determine how muscle mass can be preserved in older people.

ACKNOWLEDGMENTS

We would like to thank the volunteers who gave their time and DNA for this research. The authors are indebted to Akmal Towe, Tuti Sulistyaningsih, and Mardjito; the elderly retirement homes management in Jakarta, Sofia Mubarika and Mohammad Juffrie; the Director of the Doctorate Program Faculty of Medicine Universitas Gadjah Mada Yogyakarta, who assisted in various areas of this study. We extend our gratitude to Dr. Donal for his valuable guidance in the statistical analysis. This research was supported by a grant from the RISBIN IPTEKDOK (HK.02.03/1.2/1072/2016).

REFERENCES

1. **Iannuzzi-Sucich, M., Prestwood, K.M., and Kenny, A.M.** 2002. Prevalence of sarcopenia and predictor of skeletal muscle mass in healthy, older men and women. J. Gerontol. A Biol. Sci. Med. Sci. **57**(12): M772-777.
2. **Pescatello, L.S., Devaney, J.M., Hubal, M.J., Thompson, P.D., and Hoffman, E.P.** 2013. Highlights from the functional single nucleotide polymorphisms associated with human muscle size and strength or FAMuSS study. In Hindawi Publising Corporation, Biomed. Res. Int. 2013. Article ID 643575, http://dx.doi.org/10.1155/2013/643575
3. **Garatachea, N., and Lucia, A.** 2013. Genes and the ageing muscle: a review on genetic association studies. AGE **35**(1):207-233.
4. **Carter, C.S., Onder, G., Kristchevsky, S.B., and Pahor, M.** 2005. Angiotensin-converting enzyme inhibition intervention in elderly persons: Effects on body composition and physical performance. J. Gerontol. A Biol. Sci. Med. Sci. **60**(11):1437-1446.
5. **Sayed-Tabatabaei, F.A., Oostra, B.A., Isaacs, A., van Duijn, C.M., and Witteman, J.C.** 2006. ACE polymorphisms. Circ Res. **98**:1123-1133.
6. **Yoshida, T., Tabony, A.M., Galvez, S., Mitch, W.E., Higashi, Y., Sukhanov, S., and Delafontaine, P.** 2013. Molecular mechanisms and signaling pathways of angiotensin II-induced muscle wasting: Potential therapeutic targets for cardiac cachexia. Int. J. Biochem. Cell Biol. **45**(10)2322-2332.
7. **Kang, H.J., Kim, C.H., Park, D.S., Choi, S.Y., Lee, D.H., Nam, H.S, Hur, J.G., and Woo, J.H.** 2012. The impacts of ACE activity according to ACE I/D polymorphisms on muscular functions of people aged 65. Ann. Rahabil. Med. **36**:433-446.
8. **Montgomery, H., Clarkson, P., Barnard, M., Bell, J., Brynes, A., Dollery, C., Hajnal, J., Hemingway, H., Mercer, D., Jarman, P., Marshall, R., Prasad, K., Rayson, M., Saeed, N., Talmud, P., Thomas, L., Jubb, M., World, M., and Humphries, S.** 1999. Angiotensin-converting-enzyme gene insertion/deletion polymorphism and response to physical training. Lancet **13:** 353(9152):541-545.
9. **Charbonneau, D.E., Hanson, E.D., Ludlow, A.T., Delmonico, M.J., Hurley, B.F., and Roth, S.M.** 2008. ACE genotype and the muscle hypertrophic and strength responses to strength training. Med Sci Sports Exerc. **40**(d den4):677-683.
10. **Vigano, A., Trutschnigg, B., Kilgour, R.D., Hamel, N., Hornby, L., Lucar, E., Foulkes, W., Tremblay, M.L., and Morais, J.A.** 2009. Relationship between angiotensin-converting enzyme gene polymorphism and body composition, functional performance, and blood biomarkers in advanced cancer patients. Clin. Cancer Res. **15**(7):2442-2447.
11. **Sanchez-Garcia, S., Garcia-Pena, C., Duque-Lopez, M.X., Juarez-Cedillo, T., Cortes-Nunez, A.R., and Reyes-Beaman, S.** 2007. Anthropometric measures and nutritional status in a healthy elderly population. BMC Public Health. **72**:2
12. **Chen, M., Sun, J., Bai, H., Wang, Y., Xu, D., Zhu, X., Chen, J., Bap, Z.** 2015. Muscle mass reference standard for sarcopenia using bioelectrical impedance analysis. Asian J. Gerontol. Geriatr. **10**:16-21.
13. **Sasongko, T.H., Sadewa, A.H., Kusuma, P.A., Damanik, M.P., Lee, M.J., Ayaki, H., Nozu, K., Goto, A., Matsuo, M., and Nishio, H.** 2005. ACE gene polymorphism in children with nephrotic syndrome in the Indonesia population. Kobe J. Med. Sci. **51**:41-47
14. **Cohen, J.** 1988. Statistical power analysis for the behavioral sciences. Lawrence Erlbaum Associates. 2nd ed. Hillsdale, NJ, USA.
15. **Ahsan, M.Z., Majidano, M.S., Bhutto, H., Soomro, A.W., Panhwar, F.H., Channa, A.R., and Sial, K.B.** 2015. Genetic variability, coefficient of variance, heritability and genetic advance of some gossypium hirsutum L. Accessions. J. Agric. Sci. **7(2)**:147-151.
16. **Csibi, A., Communi, D., Muller, N., and Bottari, S.P.** 2010. Angiotensin II inhibits insulin-stimulated GLUT4 translocation and Akt activation through tyrosine nitration-dependent mechanisms. PLoS ONE. **5(4)**:e10070.
17. **Sanders, P.M., Russell, S.T., and Tisdale, M.J.** 2005. Angiotensin II directly induces muscle protein catabolism through the ubiquitin-proteasome proteolytic pathway and may play a role in cancer cachexia. Br. J. Cancer **22**:425-434.
18. **Brink, M, Price, S.R., Chrast, J., Bailey, J.L., Anwar, A., Mitch, W.E., and Delafontaine, P.** 2001. Angiotensin II induces skeletal muscle wasting through enhanced protein degradation and down-regulates autocrine insulin-like growth factor I. Endocrinology **142**:1489-1496.
19. **Cassis, L., Helton,M., English,V., and Burke,G.** 2002. Angiotensin II regulates oxygen consumption. Am. J. Physiol. Regul. Integr. Comp. Physiol. **282**:R445-453.
20. **Song, M. Y., Ruts, E., Janumala, I., Heymsfiled, S., and Gallagher, D.** 2004. Sarcopenia and increased adipose tissue infiltration of muscle in elderly african american women. Am. J. Clin. Nutr. **79**(5): 874-880.

21. **Williams, A.G., Rayson, M.P., Jubb, M., World, M., Woods, D.R., Hayward, M., Martin, J., Humphries, S.E., and Montgomery, H.E.** 2000. The ACE gene and muscle performance. Nature **403**:614.
22. **Danser, A.H., Schalekamp, M.A., Bax, W.A., van den Brink, AM., Saxena, P.R., Riegger, G.A., and Schunkert, H.** 1995. Angiotensin-converting enzyme in the human heart. Effect of the deletion/insertion polymorphism. Circulation. **92**:1387-1388.
23. **Di Bari, M., van de Poll-Franse, L.V., Onder, G., Kirtchevsky, S.B., Newman, A., Harris, T.B., Williamson, J.D., Marchionni, N., and Pahor, M.** 2004. Antihypertensive medications and differences in muscle mass in older persons: the Health, Aging and Body Composition study. J. Am Geriatr. Soc. **52**:961-966.
24. **Carter, C.S., Cesari, M., Ambrosius, W.T., Hu, N., Diz, D., Oden, S., Sonntaq, W.E., and Pahor, M.** 2004. Angiotensin-converting enzyme inhibition, body composition, and physical performance in aged rats. J. Gerontol A Biol. Sci. Med. Sci. **59(5)**:416-423.
25. **de Cavanagh, E.M., Fraga, C.G., Ferder, L., and Inserra, F.** 1997. Enalapril and captopril enhance antioxidant defenses in mouse tissues. Am. J. Physiol. **272**(2 Pt 2):R514-R518.
26. **de Cavanagh, E.M., Inserra, F., Ferder, L., and Fraga, C.G.** 2000. Enalapril and captopril enhance glutathione-dependent antioxidant defenses in mouse tissues. Am. J. Physiol. Regul. Integr. Comp. Physiol. **278:** R572-R577.
27. **Massidda, M., Corrias, L., Scorcu, M., Vona, G., and Calo, M.C.** 2013. ACTN-3 and ACE genotypes in elite male Italian athletes. A. Rev. Anthropol. **75**(1):51-59.
28. **Bos, H., Laverman, G.D., Henning, R.H., Tiebosch, A.T., de Jong, P.E., de Zeeuw, D., and Navis, G.** 2003. Involvement of renal ACE activity in proteinuriaassociated renal damage in untreated and treated adriamycin nephrotic rats. J Renin Angiotensin Aldosterone Syst. **4(2)**:106-112.
29. **Tang, S.C., Leung, J.C., Chan, L.Y., Eddy, A.A., and Lai, K.N.** 2008. Angiotensin converting enzyme inhibitor but not angiotensin receptor blockade or statin ameliorates murine adriamycin nephropathy. Kidney Int. **73**(3):288-299.
30. **Huang, W., Gallois, Y., Bouby, N., Bruneval, P., Heudes, D., Belair, M.F., Krege, J.H., Meneton, P., Marre, M., Smithies, O., and Alhenc-Gelas, F.** 2001. Genetically increased angiotensin I-converting enzyme level and renal complications in the diabetic mouse. Proc. Natl. Acad. Sci USA. **98**:13330-13334.
31. **Czarkowska-Paczek, B., and Milezarczyk, S.** 2006. Age-related muscle mass loss. Przegl Lek. **63**(8): 658-661.
32. **Tseng, L.A., Delmonico, M.J., Visser, M., Boudreau, R.M., Goodpaster, B.H., Schwartz, A.V., Simonsick, E.M., Satterfield, S., Harris, T., and Newman, A.B.** 2014. Body composition explains sex differential in physical performance among older adults. J. Gerontol. A Biol. Sci. Med. Sci. **69**(1):93-100.
33. **Rolland, Y., Lauwers-Cances, V., Cournot, M., Nourhashemi, F., Reynish, W., Riviere, D., Vellas, B., and Grandjean, H.** 2003 Sarcopenia, calf circumference, and physical function of elderly women: a cross-sectional study. J. Am. Geriatr. Soc. **51**(8):1120-1124.