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ARTICLE

Correlation between the 24-h urinary angiotensinogen or aldosterone level and muscle mass: Japan shimanami health promoting program study

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Abstract

Our previous report indicated that sarcopenia is associated with arterial stiffness and cardiovascular death. The renin–angiotensin system (RAS) plays an important role in cardiovascular disease and its activation may be correlated with sarcopenia according to basic research. However, few clinical studies have assessed the correlation between skeletal muscle loss and RAS component concentrations in healthy subjects. The purpose of this study was to investigate the relationships between the excretion of angiotensinogen (AGT) and aldosterone (Ald) in 24-h urine samples and clinical and sarcopenic indices. A total of 344 people participated in a voluntary medical check-up program, "Anti-Aging Doc", and underwent measurement of their sarcopenia-related indices. Urine samples were collected for 24-h within 8 weeks after a medical check-up using a partition cup and a proportional sampling method. Urine AGT and Ald levels were evaluated by enzyme-linked immunosorbent assay (ELISA). After compensating for possible confounding parameters, including baPWV, the 24-h urinary excretion of AGT was independently and negatively associated with the thigh muscle cross-sectional area. On the other hand, urinary Ald excretion was not associated with sarcopenia-related indices after compensation, even though it showed a modest but significantly positive association with sarcopenic indices in single regression analysis. Urinary AGT was related to sarcopenic indices and may be involved in the pathogenesis of sarcopenia. On the other hand, urinary Ald was not related to sarcopenic indices when considering other risk factors.

Introduction

The renin–angiotensin system (RAS) plays an important role in the regulation of the hydro-mineral balance and blood pressure in mammals. Moreover, recent reports have indicated that dysregulation of RAS might be related to body composition, such as in obesity, adipose tissue dysfunction [1–3], and skeletal muscle loss, which is known as sarcopenia [4–7]. Sarcopenia, age-related loss of skeletal muscle mass and muscle strength, is related to physical dysfunction, a decline in activities of daily living, and frailty in the elderly [8, 9]. Furthermore, sarcopenia has been associated with arterial stiffness and cardiovascular death [10, 11]. RAS is involved in the pathophysiology of cardiovascular disease. Therefore, RAS activation may be correlated with sarcopenia and could play a role in skeletal muscle mass.

Evaluation of RAS activation in serum is difficult because of changes in the circadian rhythm, position, physical activity, medication, and other factors, whereas a spot urine sample reflects the effects of the above-mentioned factors. Moreover, a 24-h urine sample reliably reflects the average daily hormonal activity, such as RAS activation. Particularly, the 24-h urinary level of angiotensinogen (AGT) or aldosterone (Ald) is usually used to assess RAS activation. However, it is relatively difficult to collect a sufficient number of 24-h urine samples to accurately assess the urine levels of these markers,

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and the relationships between the 24-h urinary RAS component levels and skeletal muscle mass have not been investigated.

Here, we investigated the correlations between 24-h urinary excretion of AGT or Ald and clinical markers, including blood pressure and muscle mass.

Methods

Study participants

Study participants were middle-aged to elderly community residents recruited from 1501 participants in the medical check-up program provided by the Anti-Aging Center of Ehime University Hospital during July 2011 to February 2013. They participated in a medical check-up program, "Anti-Aging Doc", a program provided to residents of Ehime Prefecture, Japan.

Among them, we analyzed 344 people who completed 24-h urine sampling, gave written consent to participate in the study and had no history of symptomatic cardiovascular events, including peripheral arterial disease, stroke, coronary heart disease, and congestive heart failure. All participants were functionally independent in their daily lives. The series of studies to which the present study belongs was approved by the Ethics Committee of Ehime University Graduate School of Medicine.

Urine samples

Urine samples were collected for 24-h within 8 weeks after a medical check-up (15.8 ± 10.3 days) using a partition cup with a proportional sampling method [12], which collected 1/50th of the urine in every urination (U-Container, Sumitomo Bakelite Co., Ltd., Tokyo, Japan). Urinary Na and K levels were determined using an automated analyzer (Architect c8000; Toshiba, Inc., Ohtawara, Japan). The 24-h excretion of Na and K was calculated.

Measurement of 24-h urinary excretion of angiotensinogen and aldosterone

AGT and Ald in 24-h urine samples were measured by ELISA systems for AGT (Immuno-Biological Laboratories, Co., Ltd., Gunma, Japan) and Ald (Immuno-Biological Laboratories, Co., Ltd., Gunma, Japan). According to the manufacturers' data, the precision, recovery, and linearity of these assays were as follows: for the human AGT ELISA, the intra-assay coefficient of variation (CV) was 4.4–5.5%, the inter-assay CV was 0.9–5.8%, and the recovery range was 84.8–95.7%; for the human Ald ELISA, the intra-assay CV was 4.1–10.4%, the inter-assay CV was 9.4–9.7%, the recovery range was 91.3–123.7%, and the linearity was ~96.88% of expected.

Measurement of sarcopenia-related indices

Hand grip strength

Hand grip strength was measured using a digital hand dynamometer (T.K.K. 5410; Takei Scientific Instruments Co. Ltd., Niigata, Japan), performed once on each side; the highest value was used for analysis [13]. The dynamometer's minimum detectable hand grip strength was 0.1 kg. All participants who performed hand grip strength measurements were free of any condition (such as arthralgia) that might interfere with measurement.

Bioelectric impedance analysis

The total skeletal muscle ratio was evaluated by the bioelectrical impedance method using a commercially available body composition analyzer (body scan HBF-701; Omron Healthcare Co. Ltd., Kyoto, Japan). The manufacturer's built-in preprogrammed algorithms were used to calculate the percentage of skeletal muscle. Skeletal muscle mass (in kg) was calculated as body weight (kg)*skeletal muscle percentage [12]. Skeletal muscle mass measurements were available for 343 subjects (152 men and 191 women).

Thigh muscle cross-sectional area

Thigh muscle cross-sectional area (CSA) was measured using computed tomography (LightSpeed VCT; GE Healthcare, Tokyo, Japan) at the mid-thigh, defined as the midpoint from the inguinal crease to the proximal pole of the patella [11, 14]. Muscle CSA (in cm^2), excluding intramuscular fat, was computed using an attenuation range of 0–100 Hounsfield units.

Measurement of visceral fat area

Visceral fat area was measured using CT at the level of the umbilicus, with attenuation in the range of 150–50 Hounsfield units. Images were obtained with a minimum slice width of 5 mm and were analyzed using OsiriX software (OsiriX Foundation, Geneva, Switzerland) [11, 14, 15]. Thigh muscle CSA and visceral fat area measurements were only available for 295 subjects (136 men and 159 women).

Pulse wave velocity

Pulse wave velocity (PWV) was measured using a volume-plethysmograph (PWV/ankle brachial index; Omron

Table 1 Characteristics of study participants

	$n = 344$
Male, *n* (%)	152 (44)
Age, years	66.6 ± 10.3
Body height, cm	158.7 ± 8.6
Body weight, kg	58.6 ± 11.2
Body mass index, kg/m^2	23.2 ± 3.3
Visceral fat area, cm^2 ($n = 295$)	102.9 ± 62.2
Systolic blood pressure, mmHg	127.2 ± 17.8
Diastolic blood pressure, mmHg	71.5 ± 10.5
Heart rate, beats/min	65.5 ± 10.2
Triglyceride, mg/dl	105.8 ± 58.4
Total cholesterol, mg/dl	209.3 ± 34.7
High-density lipoprotein cholesterol, mg/dl	62.2 ± 14.7
Fasting glucose, mg/dl	102.4 ± 18.6
Insulin, μU/ml	6.5 ± 5.7
Serum sodium, mEq/L	140.5 ± 1.9
Serum potassium, mEq/L	4.3 ± 0.4
Serum chloride, mEq/L	103.5 ± 2.0
Creatinine, mg/dl	0.76 ± 0.18
Blood urea nitrogen, mg/dl	14.8 ± 3.5
Estimated-glomerular filtration rate, ml/min/1.73 m^2	69.7 ± 12.7
Use of antihypertensive drugs, *n* (%)	109 (32)
Use of RAS inhibitor (ACE inhibitor + ARB), *n*	66 (2 + 64)
Use of CCB, *n*	74
Use of diuretics, *n*	11
Use of antidyslipidemia drugs, *n* (%)	115 (33)
Use of antidiabetic drugs, *n* (%)	18 (5)
Smoking status, current/past/never	19/102/223
Physical activity, every day/sometimes/not often/never	53/196/82/13
Hand grip strength, kg ($n = 344$)	29.3 ± 9.2
Skeletal muscle mass, kg ($n = 343$)	15.1 ± 4.3
Thigh muscle cross sectional area, cm^2 ($n = 295$)	111.9 ± 25.9
Brachial-ankle pulse wave velocity, cm/s ($n = 343$)	1576 ± 316
Urine volume, ml/day	1556 ± 674
Urinary Na excretion, mEq/day	142.6 ± 64.3
Urinary K excretion, mEq/day	51.5 ± 20.9
Urinary angiotensinogen excretion, μg/day	2.80 ± 5.92
Urinary aldosterone excretion, μg/day	2.86 ± 0.25

Values are mean ± SD

RAS renin–angiotensin system, *ACE* angiotensin-converting enzyme, *ARB* angiotensin receptor blocker, *CCB* calcium channel blocker

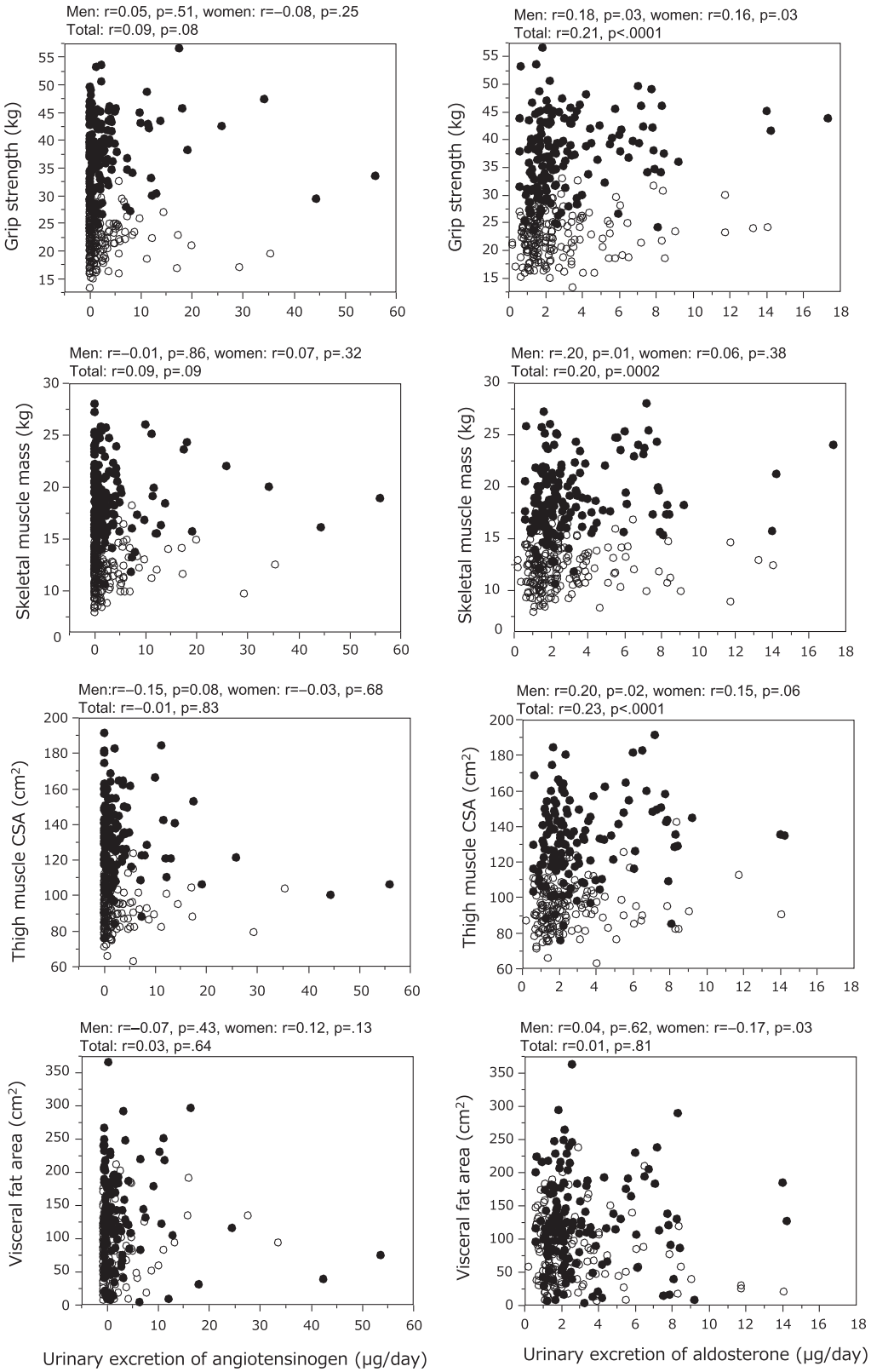
Table 2 Simple correlation coefficient between urinary angiotensinogen excretion and several parameters examined

	Urinary angiotensinogen excretion (μg/day)		Urinary aldosterone excretion (μg/day)	
	r	*p*	*r*	*p*
Age, years	0.07	0.18	−0.29	<0.0001
Body height, cm	0.06	0.24	0.20	0.0002
Body weight, kg	0.09	0.11	0.13	0.01
Body mass index, kg/m^2	0.07	0.18	0.02	0.70
Visceral fat area, cm^2 ($n = 295$)	0.03	0.64	0.01	0.81
Systolic BP, mmHg	0.10	0.054	−0.02	0.75
Diastolic BP, mmHg	0.00	0.99	0.07	0.19
Heart rate, bpm	0.08	0.15	0.05	0.38
Triglyceride, mg/dl	0.05	0.33	−0.04	0.50
Total cholesterol, mg/dl	−0.06	0.24	−0.08	0.14
HDL cholesterol, mg/dl	−0.08	0.16	−0.11	0.04
Fasting glucose, mg/dl	0.24	<0.0001	0.02	0.65
Insulin, μU/ml	0.02	0.65	0.05	0.34
Serum sodium, mEq/L	0.00	0.96	−0.21	<0.0001
Serum potassium, mEq/L	0.07	0.23	−0.10	0.06
eGFR, ml/min/1.73 m^2	−0.14	0.01	0.19	0.0003
Use of antihypertensive drugs, yes = 1	0.05	0.39	−0.00	0.99
Use of RAS inhibitors, yes = 1	0.07	0.17	−0.06	0.29
Use of antidyslipidemia drugs, yes = 1	0.01	0.86	−0.17	0.001
Use of antidiabetic drugs, yes = 1	0.23	<0.0001	−0.03	0.60
Current smoking, yes = 1	−0.06	0.30	−0.00	0.94
Physical activity[a], 1–4	0.01	0.79	0.08	0.13
baPWV, cm/s ($n = 343$)	0.09	0.08	−0.15	0.006
Urinary Na excretion, mEq/day	0.15	0.006	0.13	0.02
Urinary K excretion, mEq/day	0.12	0.03	0.27	<0.0001

BP blood pressure, *HDL* high-density lipoprotein, *Na* sodium, *K* potassium, *eGFR* estimated-glomerular filtration rate, *RAS* renin–angiotensin system, *baPWV* brachial-ankle pulse wave velocity

[a] Physical activity (every day = 1, sometimes = 2, not often = 3, never = 4)

Healthcare Co. Ltd., Kyoto, Japan). A detailed explanation of this device, as well as the validity and reproducibility of its measurements, have been provided elsewhere [16]. Brachial-to-ankle PWV (baPWV) was calculated from the time interval between the wave fronts of the brachial and ankle waveforms (ΔTba) and the path length from the brachium to the ankle. The path lengths from the suprasternal notch to the brachium (Lb) and ankle (La) were obtained using the following formulae: $Lb = 0.2195 \times height + 2.0734$; $La = 0.8129 \times height + 12.328$. Then, baPWV was obtained using the following equation: (La−Lb)/ΔTba. The intra-measurement reproducibility of baPWV in our laboratory was 2.1% ± 1.8%, and the reproducibility between measurements was 2.2% ± 1.5%. baPWV measurements were available for 343 subjects (152 men and 191 women).


Men: r=0.05, p=.51, women: r=–0.08, p=.25
Total: r=0.09, p=.08
Grip strength (kg)
Men: r=0.18, p=.03, women: r=0.16, p=.03
Total: r=0.21, p<.0001
Grip strength (kg)
Men: r=–0.01, p=.86, women: r=0.07, p=.32
Total: r=0.09, p=.09
Skeletal muscle mass (kg)
Men: r=.20, p=.01, women: r=0.06, p=.38
Total: r=0.20, p=.0002
Skeletal muscle mass (kg)
Men:r=–0.15, p=0.08, women: r=–0.03, p=.68
Total: r=–0.01, p=.83
Thigh muscle CSA (cm²)
Men: r=0.20, p=.02, women: r=0.15, p=.06
Total: r=0.23, p<.0001
Thigh muscle CSA (cm²)
Men: r=–0.07, p=.43, women: r=0.12, p=.13
Total: r=0.03, p=.64
Visceral fat area (cm²)
Men: r=0.04, p=.62, women: r=–0.17, p=.03
Total: r=0.01, p=.81
Visceral fat area (cm²)
Urinary excretion of angiotensinogen (μg/day)
Urinary excretion of aldosterone (μg/day)

◀ **Fig. 1** Scatter plots between 24-h urinary excretion of angiotensinogen [left] and aldosterone [right] and hang grip strength [top], skeletal muscle mass [second from the top], thigh muscle cross sectional area (CSA) [third from the top], and visceral fat area [bottom]. Closed circles indicate men, and open circles indicate women. The correlation coefficient (r) and p values for men, women and the total population are shown in each figure. The numbers of subjects were 344 (men = 152) for hand grip strength, 343 (men = 152) for skeletal muscle mass, and 295 (men = 136) for thigh muscle CSA and visceral fat area

Evaluation of risk factors

Lifestyle, medical history, and prescribed drugs were evaluated by questionnaire. Anthropometric measurements were performed by a trained nurse. Venous blood was collected in the morning after >11 h fasting for measurement of electrolytes, creatinine, serum lipids, and plasma insulin and glucose concentrations. Estimated-glomerular filtration rate (eGFR (mL/min/1.73 m^2)) was calculated with the formula: 194 × Serum creatinine–1.094 × Age–0.287 (×0.739 if female)[17].

Statistical analysis

Pearson's' correlation coefficients between 24-h urinary excretion of AGT or Ald and various clinical parameters were obtained. Differences in frequency were assessed by the χ^2 test. Covariate adjusted analysis was performed by multiple linear regression analyses for sarcopenia indices with possible independent parameters, including age, body height, body weight, systolic BP, total cholesterol, high-density lipoprotein cholesterol, triglyceride, fasting blood glucose, immune-reactive insulin, serum Na, K, eGFR, current smoking, physical activity, use of antihypertensive drugs, anti-dyslipidemic drugs, anti-diabetic medication, and baPWV. Statistical analyses were conducted using commercially available statistical software (JMP version 10.0.2; SAS Institute Inc., Cary, NC), with $p < 0.05$ considered statistically significant.

Results

Relationships between 24-h urinary excretion of AGT or Ald and clinical parameters

The clinical characteristics of the studied population are summarized in Table 1. Table 2 summarizes simple correlation coefficients between 24-h urinary excretion of AGT or Ald and several clinical parameters. The 24-h urinary excretion of AGT was positively associated with plasma glucose, urinary Na excretion, and urinary K excretion and negatively associated with eGFR. On the other hand, 24-h Ald excretion was positively associated with male gender, body height, body weight, eGFR, urinary Na excretion, and urinary K excretion and negatively associated with age, serum Na, and baPWV.

Relationships between 24-h urinary excretion of AGT or Ald and sarcopenic indices

Scatter plots between 24-h urinary excretion of AGT or Ald and sarcopenic indices and visceral fat area are shown in Fig. 1. The 24-h urinary AGT excretion was not associated with these parameters. On the other hand, urinary Ald excretion was modestly but significantly associated with all sarcopenic indices. In sex-separated analyses, men showed a significant positive association between urinary Ald excretion and all sarcopenic indices, while in women, urinary excretion of Ald was associated only with hand grip strength (Fig. 1).

Further, we evaluated whether 24-h urinary excretion of AGT or Ald was independently correlated with sarcopenic indices by two models of multiple linear regression (Tables 3 and 4). After inclusion of possible confounding parameters, including baPWV, 24-h urinary excretion of AGT showed a significant negative association with thigh muscle CSA (Table 3, model 1 and model 2).

Discussion

We demonstrated that urinary excretion of AGT was negatively correlated with thigh muscle CSA using 24-h urine samples. Thus, it is possible that AGT plays a role in the pathogenesis of sarcopenia.

Previous reports indicate the roles of RAS in sarcopenia, focusing on drug targets using angiotensin-converting enzyme (ACE) inhibitors or angiotensin II type 1 receptor blockers (ARBs) as a pharmacological option. For example, Burks et al. demonstrated that an ARB, losartan, prevents fibrosis and impairment of muscle function after cardiotoxin-induced injury by differentially regulating the transforming growth factor β (TGF-β) and insulin-like growth factor 1 (IGF-1)/Akt/mammalian target of rapamycin (mTOR) signaling cascades [18]. Thus, RAS activation is thought to play a role in muscle wasting or muscle loss. Moreover, Motta-Santos demonstrated that ACE2-deficient mice had diminished physical performance and impaired skeletal muscle adaptations to exercise due to an increase in angiotensin II type 1 receptor gene expression in skeletal muscle [4]. However, plasma and urine Ang II levels are not reliable because Ang II is a small peptide that readily undergoes degradation. On the other hand, a direct correlation between AGT and skeletal muscle has not been reported. In the present study, we demonstrated that urinary excretion of AGT was negatively and independently correlated with thigh muscle CSA.

Table 3 Multiple linear regression analyses for sarcopenic indices: model 1

	Hand grip strength ($n = 343$)		Skeletal muscle mass ($n = 342$)		Thigh muscle CSA ($n = 294$)	
	β	p	β	p	β	p
Sex, male = 1	0.65	<0.0001	0.29	<0.0001	0.42	<0.0001
Age, years	−0.24	<0.0001	−0.14	<0.0001	−0.21	<0.0001
Body height, cm	0.10	0.03	0.25	<0.0001	−0.00	0.97
Body weight, kg	0.19	<0.0001	0.52	<0.0001	0.52	<0.0001
Systolic BP, mmHg	0.04	0.17	0.01	0.39	0.06	0.04
Triglyceride, mg/dl	−0.05	0.09	0.01	0.52	0.01	0.75
Total cholesterol, mg/dl	0.05	0.08	0.00	0.93	−0.04	0.23
HDL cholesterol, mg/dl	−0.01	0.74	0.00	0.86	0.03	0.38
Fasting glucose, mg/dl	0.01	0.87	0.01	0.42	0.05	0.14
Insulin, μU/ml	0.02	0.58	−0.01	0.54	−0.04	0.21
Na, mEq/L	0.05	0.054	−0.00	0.95	−0.05	0.053
K, mEq/L	0.02	0.40	0.00	0.77	0.01	0.72
eGFR, ml/min/1.73 m^2	−0.07	0.01	0.00	0.78	−0.20	<0.0001
Use of RAS inhibitors, yes = 1	−0.03	0.20	0.01	0.57	−0.06	0.04
Use of antihypertensive drugs other than RAS inhibitors, yes = 1	0.00	0.97	0.00	0.96	−0.08	0.79
Use of antidyslipidemia drugs, yes = 1	0.01	0.63	0.00	0.89	−0.01	0.68
Use of antidiabetic drugs, yes = 1	−0.13	<0.0001	−0.01	0.37	−0.05	0.11
Current smoking, yes = 1	0.02	0.59	0.00	0.95	0.01	0.85
Physical activity[a], 1–4	−0.02	0.61	−0.00	0.86	−0.07	0.08
baPWV, cm/s	−0.01	0.86	−0.05	0.004	−0.10	0.006
Urinary AGT, μg/day	0.04	0.11	0.01	0.29	−0.06	0.02
Urinary Ald, μg/day	0.03	0.29	−0.01	0.64	0.05	0.07

CSA cross-sectional area, *BP* blood pressure, *HDL* high-density lipoprotein, *Na* sodium, *K* potassium, *eGFR* estimated-glomerular filtration rate, *RAS* renin–angiotensin system, *baPWV* brachial-ankle pulse wave velocity, *AGT* angiotensinogen, *Ald* aldosterone

[a] Physical activity (every day = 1, sometimes = 2, not often = 3, never =4

This result is compatible with previous reports. However, there was no correlation between AGT level and other parameters of sarcopenia, namely skeletal muscle mass and grip strength. Thus, it is difficult to conclude there is any causative relationship between sarcopenia and urinary AGT. Although the apparently discrepant results about AGT and muscle loss might be due to methodological differences, our previous study suggested that thigh muscle CSA is negatively associated with carotid intima media thickness and baPWV in men but not in women [11]. Therefore, thigh muscle CSA may reflect atherosclerosis, and RAS activation, reflected by AGT level, may be a key player linking vascular dysfunction with skeletal muscle atrophy.

In the present study, the simple correlations between 24-h urinary excretion of AGT or Ald and sarcopenic indices indicated that urinary Ald excretion was modestly but significantly associated with all sarcopenic indices, but 24-h urinary AGT excretion was not associated with these parameters. However, after inclusion of possible confounding parameters, including baPWV, 24-h urinary excretion of AGT showed a significant negative association with thigh muscle CSA. Moreover, further inclusion of urinary parameters eliminated the association between thigh muscle CSA and urinary excretion of Ald. These results suggest that urinary Ald level is affected by various cardiovascular risk factors or the use of antihypertensive drugs. Considering that urinary AGT level demonstrated an association with thigh muscle CSA after adjustment for baPWV and other variables, urinary AGT level is an independent predictor of sarcopenia that is related to multiple parameters.

Activation of mediators that stimulate the ATP-dependent ubiquitin–proteasome system (UPS) is involved in the mechanism that stimulates loss of skeletal muscle [19]. Angiotensin II activates the UPS via generation of reactive oxygen species and via inhibition of the insulin-like growth factor-1 signaling pathway [20]. Kobori et al. demonstrated that urinary AGT level reflects intrarenal RAS activation [21, 22]. Because the circadian rhythm of the intrarenal RAS clearly exists [23], 24-h urinary excretion of

Table 4 Multiple linear regression analyses for sarcopenic indices: model 2

	Hand grip strength ($n = 343$)		Skeletal muscle mass ($n = 342$)		Thigh muscle CSA ($n = 294$)	
	β	p	β	p	β	p
Sex, male = 1	0.65	<0.0001	0.29	<0.0001	0.41	<0.0001
Age, years	−0.24	<0.0001	−0.14	<0.0001	−0.22	<0.0001
Body height, cm	0.10	0.03	0.25	<0.0001	−0.01	0.91
Body weight, kg	0.19	<0.0001	0.52	<0.0001	0.52	<0.0001
Systolic BP, mmHg	0.04	0.17	0.01	0.42	0.07	0.03
Triglyceride, mg/dl	−0.05	0.10	0.01	0.37	0.02	0.62
Total cholesterol, mg/dl	0.05	0.08	0.00	0.91	−0.04	0.25
HDL cholesterol, mg/dl	−0.01	0.75	0.00	0.83	0.03	0.33
Fasting glucose, mg/dl	0.01	0.86	0.01	0.35	0.05	0.13
Insulin, μU/ml	0.01	0.62	−0.01	0.47	−0.04	0.14
Na, mEq/L	0.05	0.054	0.00	0.97	−0.05	0.08
K, mEq/L	0.02	0.44	0.00	0.97	0.00	0.96
eGFR, ml/min/1.73 m^2	−0.07	0.001	0.01	0.63	−0.20	<0.0001
Use of RAS inhibitors, yes = 1	−0.03	0.21	0.01	0.57	−0.06	0.04
Use of antihypertensive drugs other than RAS inhibitors, yes = 1	−0.00	0.99	0.00	0.76	−0.01	0.64
Use of antidyslipidemia drugs, yes = 1	0.01	0.64	0.00	0.89	−0.01	0.63
Use of antidiabetic drugs, yes = 1	−0.13	<0.0001	−0.01	0.40	−0.05	0.14
Current smoking, yes = 1	0.02	0.57	0.00	0.84	0.01	0.74
Physical activity[a], 1–4	−0.02	0.65	0.00	0.94	−0.06	0.12
baPWV, cm/s	−0.01	0.87	−0.05	0.0006	−0.09	0.008
Na excretion, mEq/day	−0.00	0.96	−0.03	0.054	−0.00	0.91
K excretion, mEq/day	0.01	0.71	0.03	0.03	0.05	0.11
Urinary AGT, μg/day	0.04	0.12	0.01	0.30	−0.07	0.01
Urinary Ald, μg/day	0.02	0.37	−0.01	0.31	0.04	0.20

CSA cross-sectional area, *BP* blood pressure, *HDL* high-density lipoprotein, *Na* sodium, *K* potassium, *eGFR* estimated-glomerular filtration rate, *RAS* renin–angiotensin system, *baPWV* brachial-ankle pulse wave velocity, *AGT* angiotensinogen, *Ald* aldosterone

[a] Physical activity (every day = 1, sometimes = 2, not often = 3, never =4

AGT is more reliable to evaluate intrarenal RAS activation than spot urine analysis. Interestingly, thigh muscle CSA is also negatively correlated with eGFR. Therefore, we hypothesized that changes over time in sarcopenic indices are enhanced by basal renal function or basal urinary AGT level. However, there was no correlation between basal eGFR, basal urine albumin level, or basal urinary AGT level and change over time (average, 2 years) in any sarcopenic index (data not shown). Because there are few reports on the effect of intrarenal RAS on sarcopenia, it is not well known whether the negative correlation between urinary AGT level and thigh muscle CSA is induced by intrarenal RAS activation involving the UPS. Moreover, 2 years of follow-up may be too short to detect an impairment of sarcopenic indices; thus, further analysis is necessary to evaluate this hypothesis.

Mineralocorticoid receptors are present in skeletal muscle and may be a potential therapeutic target [24]. Lowe et al. showed that spironolactone and eplerenone have the potential to treat skeletal muscle in Duchenne muscular dystrophy [25]. However, in the present study, we did not observe a correlation between urinary Ald level and any skeletal muscle parameter. As shown in Fig. 1, Ald level showed a wider distribution than did AGT level. Moreover, 32% of study patients were using antihypertensive drugs. Therefore, the background of the study participants may have affected hormonal changes and modified the Ald excretion.

In conclusion, the 24-h urinary AGT level is correlated with thigh muscle CAS. Thus, the urinary AGT level may act as a clinical indicator to predict sarcopenia. Future investigations should use a longer-term follow up to assess the time course of changes in urinary AGT and the concentrations of other RAS components, such as ACE2 and renin.

Author contributions Conceptualization and methodology of the experiments: MM, KK, YT. Performance of the experiments: KK, YT,

KT, MI. Formal analysis of the data: MM, KK, KT. Resources: KK, MM, MH. Writing the paper (original draft preparation): MM, KK. Writing (review and editing): YT, MH.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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