Angiotensin-converting enzyme 2 (ACE2), a type I transmembrane glycoprotein, is a recently discovered homologue of angiotensin-converting enzyme (ACE). The action of ACE2 as a monocarboxypeptidase preferentially removes carboxy-terminal amino acids from various substrates, and ACE2 converts angiotensin (Ang) II to Ang-(1–7) and Ang I to Ang-(1–9), although the effect of ACE2 is not suppressed by ACE inhibitors. ACE2 is an important modulator of the renin-angiotensin system (RAS) by reducing Ang II levels and increasing the generation of Ang-(1–7). ACE2 is highly expressed in the kidney and converts angiotensin (Ang) II to Ang-(1–7), a renoprotective peptide. Urinary ACE2 has been shown to be elevated in patients with chronic kidney disease. However, the effects of antihypertensive agents on urinary ACE2 remain unclear.

METHODS
Of participants in the Tanno-Sobetsu cohort study in 2011 (n = 617), subjects on no medication (n = 101) and hypertensive patients treated with antihypertensive agents, including the calcium channel blockers amlodipine and long-acting nifedipine; the ACE inhibitor enalapril; and the Ang II receptor blockers losartan, candesartan, valsartan, telmisartan, and olmesartan, for more than 1 year (n = 100) were enrolled, and urinary ACE2 level was measured.

RESULTS
Glucose and hemoglobin A1c were significantly higher in patients treated with enalapril, telmisartan or olmesartan than in the control subjects. Urinary ACE2 level was higher in patients treated with enalapril than in the control subjects. Urinary ACE2 level was higher in the olmesartan-treated group, but not the other treatment groups, than in the control group. Urinary ACE2 level was positively correlated with systolic blood pressure (r = 0.211; P = 0.003), UACR (r = 0.367; P < 0.001), and estimated salt intake (r = 0.260; P < 0.001). Multivariable regression analysis after adjustment of age, sex, and the correlated indices showed that the use of olmesartan was an independent predictor of urinary ACE2 level.

CONCLUSIONS
In contrast with other antihypertensive drugs, olmesartan may uniquely increase urinary ACE2 level, which could potentially offer additional renoprotective effects.

Keywords: angiotensin-converting enzyme 2; angiotensin II receptor blocker; blood pressure; hypertension; olmesartan, urine, urinary protein.

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glomerular injury in rodent models. Furthermore, ACE2 delivery by a recombinant protein or virus ameliorated the progression of diabetes-related complications, such as nephropathy and retinopathy. Taken together, the earlier findings indicate that ACE2 is an endogenous protector against the progression of chronic kidney disease.

ACE2 is detectable in urine, which most likely reflects its release from proximal tubules. It has recently been suggested that urinary ACE2 could be a potential biomarker of kidney disease. Urinary ACE2 level was elevated in patients with renal diseases, including diabetic nephropathy and in patients with had undergone renal transplantation, indicating a possible role of urinary ACE2 as a noninvasive disease biomarker. However, alterations in urinary concentrations of ACE2 caused by drugs have not been determined. We examined whether different types of antihypertensive agents, including a calcium channel blocker (CCB), ACE inhibitor, and Ang II receptor blocker (ARB), have distinct effects on urinary ACE2 levels.

METHODS

Study population

In this study, 617 Japanese subjects (men/women: 260/357; mean age = 66 ± 13 years) were recruited from participants in annual health examinations in the Tanno-Sobetsu Study in 2011. The Tanno-Sobetsu Study is a prospective study following up a cohort consisting of residents of 2 rural towns, Tanno and Sobetsu, in Hokkaido, the northernmost island of Japan. Of the 617 subjects in Sobetsu Town, 101 subjects and 100 hypertensive subjects (men/women: 42/58) who had been treated with a CCB, an ACE inhibitor, or an ARB for >1 year were enrolled in this study. The exclusion criteria included combination therapy using a CCB and any other antihypertensive drugs except for an ACE inhibitor and ARB to make groups of CCB alone as a control of drug treatment compared with RAS inhibitors. Because it has been reported that urinary ACE2 is significantly increased in chronic kidney disease, subjects with estimated glomerular filtration rate (eGFR) <30 ml/min/1.73m² were also excluded. This study conformed to the principles outlined in the Declaration of Helsinki and was performed with the approval of the Ethical Committee of Sapporo Medical University. Written informed consent was received from all of the subjects.

Measurements

Medical examination was performed between 6:00 AM and 9:00 AM after an overnight fast. After measuring anthropometric parameters, blood pressure was measured twice consecutively on the upper arm using an automated sphygmomanometer (HEM-907; Omron, Kyoto, Japan) with subjects in a seated resting position, and average blood pressure was used for analysis. Body mass index was calculated as body weight in kilograms divided by the square of body height in meters. Urine and peripheral venous blood samples were obtained after physical examination for urinary examination and biochemical analyses of the serum. The urine and serum samples were analyzed immediately or stored at −80 °C until biochemical analyses.

Concentrations of ACE2 in urine samples were measured using a commercially available enzyme-linked immunosorbent assay kit (AdipoGen, Seoul, Korea). The accuracy, precision and reproducibility of the kit have been described previously. The intra- and interassay coefficient variances in the kit were <10%. Urinary ACE2 level was normalized by urinary creatinine level (μg/gCr).

Creatinine and lipid profiles, including total cholesterol, high-density lipoprotein cholesterol, and triglycerides, were determined by enzymatic methods. Low-density lipoprotein cholesterol level was calculated by the Friedewald equation. HOMA-R (homeostasis model assessment of insulin resistance), an index of insulin resistance, was calculated by the previously reported formula: insulin (μU/ml) × glucose (mg/dl) / 405. Hemoglobin A1c (HbA1c) was determined by a latex coagulation method and expressed in national glycohemoglobin standardization program scale. Brain natriuretic peptide was measured using an assay kit (Shionogi, Osaka, Japan). High-sensitivity C-reactive protein was measured by a nephelometry method. As an index of renal function, eGFR (ml/min/1.73m²) was calculated by an equation for Japanese:

\[
194 \times \frac{\text{Cr}^{-1.094}}{\text{age}^{0.287}} \times \frac{0.739}{(\text{if female})}.
\]

Urinary albumin-to-creatinine ratio (UACR; mg/gCr) was used as an index of microalbuminuria. Estimated salt intake (g/day) was calculated from spot urine sample data by using the following equation:

\[
21.98 \times ((U-\text{Na (mEq/l)/U-Cr (mg/l)}) \times (-2.04 + \text{age} + 14.89 \times \text{body weight (kg)} + 16.14 \times \text{height (cm)} - 2.244.45))^{0.392} \times 0.0585
\]

Statistical analysis

Numeric variables are expressed as means ± SD and as medians (interquartile ranges) in normal and non-normal distributions of the data, respectively. The distribution of each parameter was tested for its normality using the Shapiro–Wilk test, and non-normally distributed parameters were logarithmically transformed for comparison and regression analyses. One-way analysis of variance and Tukey–Kramer post hoc test were used for detecting significant differences in data between groups. The correlation between 2 variables was evaluated using Pearson’s correlation coefficient. Multivariable regression analysis was performed to identify independent determinants of urinary ACE2 level using the variables with a significant and nonconfounding correlation in a simple regression analysis and the use of olmesartan as independent predictors, showing the t ratio calculated as the ratio of regression coefficient and standard error of regression coefficient and the percentage of variance in the urinary ACE2 level that they explained (R²). P < 0.05 was considered statistically significant. All data were analyzed by using JMP 9 for Macintosh (SAS Institute, Cary, NC).

RESULTS

Antihypertensive agents in the hypertensive subjects were the CCBs amlodipine (n = 22), and long-acting nifedipine (n = 7); the ACE inhibitor enalapril (n = 6); and the ARBs...
Losartan (n = 5), candesartan (n = 19), valsartan (n = 15), telmisartan (n = 13), and olmesartan (n = 13). Basal characteristics of the subjects are shown in Table 1. Because of exclusion criteria, subjects in CCB treatment groups, who had received amlopidine or long-acting nifedipine, did not have any combination therapies with other hypertensive drugs. Some of the patients treated with enalapril or ARBs had combination therapy with other antihypertensive drugs: CCBs (42.1%–84.6%), diuretics (0%–80%), α-blockers (0%–16.7%) and β-blockers (0%–30%). There were no significant differences between the control and treatment groups in clinical parameters except for glucose, HbA1c, UACR, and urinary ACE2. Patients treated with enalapril, telmisartan, or olmesartan had significantly higher levels of glucose and HbA1c than did the control subjects with no medication. UACR was significantly higher in patients treated with enalapril than in the control subjects. Urinary ACE2 level was significantly higher in hypertensive patients treated with olmesartan than in the control subjects (Figure 1), whereas there was no significant difference in urinary ACE2 level between the control and the other hypertensive groups. Frequency of subjects treated with antihypertensive drugs in quartile of urinary ACE2 levels is shown in Figure 2. In the 4th quartile (Q4) of urinary ACE2 level, 7 of 13 patients took olmesartan (53.9%) compared with 14.3%–33.3% in the other groups. In a simple regression analysis using all of the subjects, urinary ACE2 level was positively correlated with systolic blood pressure (r = 0.211; P = 0.003), UACR (r = 0.367; P < 0.001), and estimated salt intake (r = 0.260; P < 0.001) but not with glucose, HbA1c, or eGFR (Table 2). Multivariable regression analysis after adjustment of age, sex, and the correlated indices in a simple regression analysis showed that the use of olmesartan was an independent predictor for urinary ACE2 level (t = 3.38; P < 0.001), totally explaining 23.6% of the variance in this measure (R² = 0.236) (Table 3).

**DISCUSSION**

This is the first report showing that olmesartan may uniquely increase urinary ACE2 level, although other antihypertensive drugs, including CCBs and other RAS inhibitors, have no such effect on urinary ACE2. Earlier studies have not shown significant effects of ARBs on urinary ACE2 level, but the effect was analyzed for multiple ARBs and not specific to each ARB in the negative studies. It has been indicated that there are many functional differences among ARBs that are not mediated by Ang II type 1 receptor blockade. The clinical significance of the effect of olmesartan on urinary ACE2 is still unclear, but several lines of evidence suggest that it affords renoprotection in addition to the class effect of ARBs. It has been shown that ACE2 plays a crucial role in protection against renal damage and cardiovascular disease. Deletion or inhibition of ACE2 was associated with albuminuria in diabetic mice or the development of Ang II–dependent renal damage. Conversely, delivery of ACE2 by a recombinant protein or virus ameliorated the progression of diabetic nephropathy, indicating that ACE2 is a renoprotective target in diabetes and chronic kidney disease. As was found in a previous study, urinary ACE2 was positively correlated with UACR, implying that urinary ACE2 expression is a compensatory response of renal tissue to insults.

Previous studies showed an inverse relationship between urinary ACE2 level and renal function. However, we found no significant correlation between urinary ACE2 and eGFR in this study. A possible reason for the discrepancy is different background characteristics of the recruited subjects. In our study, half of the subjects were untreated and possibly healthy subjects, and patients with low eGFR (<30 ml/min/1.73m²) were excluded. It has been reported that ACE2 is mainly a tissue enzyme and that its levels in circulation are relatively low. ACE2 is unlikely to be filtered through the glomerulus because the full-length sequence for human ACE2 encodes an 805–amino acid protein with a calculated mass of 92.4 kDa, and the glycosylated form of ACE2 is 120 kDa. Moreover, infusion of recombinant ACE2 in mice markedly increased ACE2 activity in the serum but not in the urine. These observations suggest that the elevated urinary ACE2 is derived from the kidney rather than glomerular filtration from serum ACE2. Collectively, our findings and reported findings support the notion that olmesartan induces ACE2 expression in the kidney, affording renoprotection by its pleiotropic effects.

Alterations in renal ACE2 by inhibition of the RAS have been studied by using rodent models of hypertension. Ramipril increased ACE2 protein levels in streptozotocin-induced diabetic rat kidneys, and renal cortex ACE2 activity was significantly augmented in rats treated with lisinopril or losartan. Furthermore, lisinopril and losartan induced elevation of ACE2 mRNA levels in both the kidney and heart of mRen2.Lewis hypertensive rats. Olmesartan also significantly increased the mRNA expression level of ACE2 in rat hearts with myocardial infarction, both the kidney and heart of stroke-prone spontaneously hypertensive rats, the cuff–injured femoral artery of mice, and mouse hearts with cardiac hypertrophy. These findings suggest a potential augmentation of urinary ACE2 by RAS blockade in rodent models. However, urinary ACE2 in mice was not changed by either chronic administration of telmisartan or captopril. Hence, the effect of RAS blockade on renal expression of ACE2 and urinary ACE2 excretion appears to be dependent on the type of coexisting disease and pharmacological profile of the agent. This issue clearly needs further investigation.

Long-term treatment of hypertensive patients with olmesartan reduced plasma Ang II level, whereas several other types of ARBs have been reported to increase plasma Ang II level in hypertensive patients. In stroke-prone spontaneously hypertensive rats, coadministration of olmesartan and an Ang-(1–7) antagonist significantly increased plasma Ang II level compared with the effect of olmesartan alone. These findings suggest that increased ACE2 activity by olmesartan contributes to reduction in the plasma Ang II level by upregulation of Ang-(1–7) formation. However, in mRen2.Lewis congenic rats, another hypertensive rat model, effect of olmesartan on plasma Ang II level was not observed, although olmesartan increased ACE2 in the kidney. There might be differences in findings that may be related to use of different rat models. Although we did not determine plasma level of Ang-(1–7) in our study, opposing effects of olmesartan and other ARBs on plasma Ang II level

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Table 1. Characteristics of the study subjects treated with and without antihypertensive drugs

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control</th>
<th>Amlodipine</th>
<th>L-Nifedipine</th>
<th>Enalapril</th>
<th>Losartan</th>
<th>Candesartan</th>
<th>Valsartan</th>
<th>Telmisartan</th>
<th>Olmesartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (men/women)</td>
<td>101 (40/61)</td>
<td>22 (6/16)</td>
<td>7 (3/4)</td>
<td>6 (4/2)</td>
<td>5 (3/2)</td>
<td>19 (10/9)</td>
<td>15 (5/10)</td>
<td>13 (5/8)</td>
<td>13 (6/7)</td>
</tr>
<tr>
<td>Age, y</td>
<td>72 ± 7</td>
<td>72 ± 5</td>
<td>77 ± 10</td>
<td>76 ± 10</td>
<td>73 ± 5</td>
<td>73 ± 5</td>
<td>73 ± 7</td>
<td>73 ± 7</td>
<td>71 ± 9</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>22.7 ± 3.1</td>
<td>23.3 ± 3.9</td>
<td>24.3 ± 3.1</td>
<td>24.3 ± 2.2</td>
<td>22.0 ± 2.9</td>
<td>23.9 ± 2.5</td>
<td>23.9 ± 1.9</td>
<td>24.6 ± 3.8</td>
<td>24.6 ± 2.6</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>83 ± 9</td>
<td>83 ± 13</td>
<td>90 ± 8</td>
<td>89 ± 5</td>
<td>84 ± 7</td>
<td>87 ± 7</td>
<td>88 ± 8</td>
<td>87 ± 12</td>
<td>90 ± 8</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>142 ± 22</td>
<td>147 ± 18</td>
<td>142 ± 17</td>
<td>159 ± 15</td>
<td>148 ± 25</td>
<td>152 ± 21</td>
<td>151 ± 19</td>
<td>142 ± 21</td>
<td>142 ± 24</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>78 ± 12</td>
<td>76 ± 11</td>
<td>77 ± 8</td>
<td>80 ± 13</td>
<td>83 ± 16</td>
<td>80 ± 12</td>
<td>81 ± 11</td>
<td>74 ± 12</td>
<td>72 ± 10</td>
</tr>
</tbody>
</table>

**Biochemical data**

| Total cholesterol, mg/dl                  | 206 ± 31  | 206 ± 24   | 187 ± 35     | 201 ± 36  | 195 ± 18  | 204 ± 25    | 199 ± 30  | 198 ± 26    | 197 ± 49   |
| HDL cholesterol, mg/dl                   | 66 ± 18   | 71 ± 17    | 61 ± 13      | 60 ± 8    | 63 ± 19   | 64 ± 12     | 71 ± 17   | 64 ± 20     | 62 ± 19    |
| LDL cholesterol, mg/dl                   | 128 ± 28  | 123 ± 21   | 111 ± 27     | 129 ± 33  | 116 ± 25  | 129 ± 27    | 118 ± 32  | 122 ± 20    | 118 ± 38   |
| Triglycerides, mg/dl                     | 85 (64–108) | 79 (63–110) | 108 (79–134) | 125 (67–175) | 64 (44–193) | 86 (70–95) | 86 (74–124) | 96 (67–128) | 83 (70–118) |
| Glucose, mg/dl                           | 96 ± 12   | 96 ± 12    | 103 ± 15     | 116 ± 26* | 93 ± 11   | 97 ± 13     | 103 ± 25  | 108 ± 14*   | 112 ± 27* |
| Insulin, µU/ml                           | 4.5 (3.2–6.6) | 4.5 (3.1–7.1) | 5.9 (4.8–10.9) | 4.4 (2.4–6.3) | 5.0 (3.2–10.5) | 4.7 (4.0–7.6) | 4.6 (3.4–8.0) | 4.3 (3.6–6.2) | 6.5 (3.0–7.6) |
| HOMA-R                                   | 1.0 (0.7–1.6) | 1.0 (0.7–1.8) | 1.6 (1.1–2.7) | 1.2 (1.0–1.6) | 1.1 (0.7–2.6) | 1.0 (0.9–1.9) | 1.1 (0.9–2.0) | 1.2 (0.9–1.7) | 1.7 (0.8–2.3) |
| HbA1c, %                                 | 5.5 ± 0.4 | 5.5 ± 0.4  | 5.4 ± 0.3    | 6.3 ± 1.2* | 5.4 ± 0.4 | 5.6 ± 0.5   | 5.7 ± 0.7 | 6.2 ± 0.5*  | 6.1 ± 1.2* |
| Creatinine, mg/dl                        | 0.74 ± 0.15 | 0.72 ± 0.15 | 0.78 ± 0.17  | 0.79 ± 0.15 | 0.79 ± 0.11 | 0.78 ± 0.12 | 0.74 ± 0.13 | 0.74 ± 0.10 | 0.76 ± 0.17 |
| eGFR, ml/min/1.73m²                      | 68 ± 12   | 68 ± 11    | 64 ± 12      | 68 ± 11   | 66 ± 8    | 66 ± 11     | 66 ± 11   | 67 ± 8      | 68 ± 11    |
| UACR, mg/gCr                              | 9 (5–17)  | 10 (6–18)  | 15 (5–30)    | 67 (18–107)* | 6 (4–16)  | 11 (7–15)   | 14 (8–26) | 15 (9–51)   | 18 (8–68)  |
| hsCRP, mg/dl                             | 0.04 (0.02–0.08) | 0.04 (0.03–0.12) | 0.07 (0.01–0.10) | 0.06 (0.04–0.08) | 0.04 (0.01–0.22) | 0.05 (0.02–0.09) | 0.05 (0.03–0.11) | 0.08 (0.03–0.15) | 0.04 (0.03–0.09) |
| Estimated salt intake, g/day              | 9.3 ± 2.0 | 8.7 ± 2.3  | 9.4 ± 2.9    | 10.2 ± 3.6 | 9.8 ± 2.7 | 10.6 ± 3.2  | 9.5 ± 2.2 | 10.1 ± 1.9  | 11.3 ± 3.9 |
| U-ACE2, µg/gCr                            | 1.9 (0.9–3.4) | 1.6 (0.6–3.9) | 2.4 (0.8–3.9) | 2.7 (1.8–4.8) | 1.0 (0.5–4.3) | 1.1 (0.9–3.3) | 2.4 (0.9–3.9) | 2.2 (1.5–5.4) | 5.8 (1.6–32.1)* |

**Diagnosis**

- Diabetes mellitus
- Dyslipidemia
- Ischemic heart disease

**Combination therapy**

- Calcium channel blockers
- Diuretics
- α-Blockers
- β-Blockers

Variables are expressed as no. (%), means ± SD, or medians (interquartile range).

Abbreviations: BNP, brain natriuretic peptide; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; HOMA-R, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; L-Nifedipine, long-acting nifedipine; U-ACE2, urinary angiotensin-converting enzyme 2; UACR, urinary albumin-to-creatinine ratio.

*P < 0.05 vs. control.
Urinary ACE2 and Olmesartan in previous human studies are consistent with our finding that urinary ACE2 level was significantly higher in the olmesartan-treated group than in the groups treated with other ARBs (Figure 1). The precise mechanism of the increase in urinary ACE2 by olmesartan is unclear. As a possible explanation, olmesartan has a double-chain domain consisting of carboxyl and hydroxyl groups, which can strongly combine with Ang II type 1 receptor and block the action of Ang II. In a clinical study, olmesartan was more effective than other ARBs in reducing proteinuria. Collectively, the results indicate that the strong action of olmesartan as an ARB may lead to upregulation of urinary ACE2.

There have been few studies in which the impact of salt intake on urinary ACE2 level was determined. Wysocki et al. showed in previous human studies are consistent with our finding that urinary ACE2 level was significantly higher in the olmesartan-treated group than in the groups treated with other ARBs (Figure 1).

The precise mechanism of the increase in urinary ACE2 by olmesartan is unclear. As a possible explanation, olmesartan has a double-chain domain consisting of carboxyl and hydroxyl groups, which can strongly combine with Ang II type 1 receptor and block the action of Ang II. In a clinical study, olmesartan was more effective than other ARBs in reducing proteinuria. Collectively, the results indicate that the strong action of olmesartan as an ARB may lead to upregulation of urinary ACE2.

There have been few studies in which the impact of salt intake on urinary ACE2 level was determined. Wysocki et al. showed

![Figure 1](https://academic.oup.com/ajh/article-fig/28/1/15/2743217)

**Figure 1.** Urinary angiotensin-converting enzyme 2 (ACE2) levels in subjects treated with antihypertensive drugs. Urinary ACE2 (U-ACE2) levels were determined in subjects on no medication and those treated with antihypertensive agents for >1 year. Results are shown for each treatment: nontreatment control (Cont; n = 101), amlodipine (Aml; n = 22), long-acting nifedipine (L-Nif; n = 7), enalapril (Ena; n = 6), losartan (Los; n = 5), candesartan (Can; n = 19), valsartan (Val; n = 15), telmisartan (Tel; n = 13), and olmesartan (Olm; n = 13). Data are shown as logarithmically transformed U-ACE2 in box plots. Untransformed values of U-ACE2 (µg/gCr) are also shown as medians (interquartile ranges) in the bottom. *P < 0.001 vs. control.

![Table 2](https://academic.oup.com/ajh/article-fig/28/1/15/2743217)

**Table 2.** Simple regression analysis for log urinary angiotensin-converting enzyme 2

<table>
<thead>
<tr>
<th>Parameters</th>
<th>r</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.105</td>
<td>0.14</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.037</td>
<td>0.61</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>0.047</td>
<td>0.51</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.211</td>
<td>0.003</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.079</td>
<td>0.26</td>
</tr>
</tbody>
</table>

**Biochemical data**

Total cholesterol: -0.065, 0.36
HDL cholesterol: 0.021, 0.77
LDL cholesterol: -0.070, 0.32
log Triglycerides: -0.091, 0.20
Glucose: 0.009, 0.90
log Insulin: -0.100, 0.16
log HOMA-R: -0.090, 0.20
HbA1c: 0.110, 0.12
Creatinine: -0.043, 0.55
eGFR: 0.009, 0.90
log UACR: 0.367, <0.001
log BNP: 0.085, 0.23
log hsCRP: -0.017, 0.82
Estimated salt intake: 0.260, <0.001

Abbreviations: BNP, brain natriuretic peptide; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; HOMA-R, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; U-ACE2, urinary angiotensin-converting enzyme 2; UACR, urinary albumin-to-creatinine ratio.
Table 3. Multivariable regression analysis for log urinary angiotensin-converting enzyme 2

<table>
<thead>
<tr>
<th>Parameters</th>
<th>t</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.56</td>
<td>0.57</td>
</tr>
<tr>
<td>Male sex</td>
<td>-0.75</td>
<td>0.45</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>1.69</td>
<td>0.09</td>
</tr>
<tr>
<td>log UACR</td>
<td>3.51</td>
<td>0.001</td>
</tr>
<tr>
<td>Estimated salt intake</td>
<td>2.21</td>
<td>0.03</td>
</tr>
<tr>
<td>Use of olmesartan</td>
<td>3.38</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

R² = 0.236.
Abbreviation: UACR, urinary albumin-to-creatinine ratio.

that switching from a normal diet to a very high–salt diet resulted in an increase in urinary ACE2 activity in both diabetic and non-diabetic mice. Conversely, a low-salt diet induced a fall in the level of urinary ACE2 activity in both groups of mice. Consistent with the findings in mice, we found that urinary ACE2 was positively correlated with estimated salt intake in humans.

This study has some limitations. First, the study was an observational study in which selection of the antihypertensive agent for each patient was not randomized. Second, urine sample for ACE2 determination was collected only once from each subject, and thus the extent of change in ACE2 by each antihypertensive agent could not be critically assessed. Third, sample sizes in treatment groups were relatively small, limiting statistical powers. Fourth, because we analyzed spot urine samples, urinary creatinine was used as a marker of daily excretion from kidney for normalization of urinary ACE2 level in this study, although level of urinary ACE2, which is mainly derived from proximal tubules, should ideally be normalized by another tubular marker. Finally, we did not measure plasma levels of Ang-(1–7), Ang II, and ACE2, as well as urinary ACE2 activity in this study, and thus changes in ACE2 activity and their impacts on the RAS have not been fully characterized. Larger interventional studies are needed to confirm that olmesartan, but not other ARBs, increases urinary ACE2 and to determine whether the elevation of urinary ACE2 level by olmesartan is associated with preservation or improvement of renal functions.

In conclusion, the results of this study suggest that olmesartan is unique among ARBs and other antihypertensive drugs and increases renal expression and urinary excretion of ACE2, potentially leading to additional renal protection by pleiotropic effects.

DISCLOSURE
The authors declared no conflict of interest.

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