

# Maximum renal responses to renin inhibition in healthy subjects: VTP-27999 versus aliskiren

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## ABSTRACT

*Background:* Renin inhibition with aliskiren induced the largest increases in renal plasma flow (RPF) in salt-depleted healthy volunteers of all renin-angiotensin system (RAS) blockers. However, given its side effects at doses >300 mg, no maximum effect of renin inhibition could be established. We hypothesized that VTP-27999, a novel renin inhibitor without major side effects at high doses, would allow us to establish this.

*Methods and Results:* The effects of escalating VTP-27999 doses (75-600 mg) on RPF, glomerular filtration rate (GFR), and plasma RAS components were compared with those of 300 mg aliskiren in 22 normal volunteers on a low-sodium diet. VTP-27999 dose-dependently increased RPF and GFR; its effects on both parameters at 600 mg (increases of  $18\pm 4\%$  and  $20\pm 4\%$ , respectively) were equivalent to those at 300 mg, indicating that a maximum had been reached. The effects of 300 mg aliskiren (increases of  $+13\pm 5\%$  and  $+8\pm 6\%$ , respectively;  $P < 0.01$  versus 300 and 600 mg VTP-27999) resembled those of 150 mg VTP-27999. VTP-27999 dose-dependently increased renin, and lowered plasma renin activity and angiotensin II to detection limit levels. The effects of aliskiren on RAS components were best comparable to those of 150 mg VTP-27999.

*Conclusion:* Maximum renal renin blockade in healthy, salt-depleted volunteers, requires aliskiren doses >300 mg, but can be established with 300 mg VTP-27999. To what degree such maximal effects (exceeding those of ACE inhibitors and AT<sub>1</sub> receptor blockers) are required in patients with renal disease, given the potential detrimental effects of excessive RAS blockade, remains to be determined.

## INTRODUCTION

The effects of renin-angiotensin system (RAS) blockers in the kidney, i.e., with regard to renal hemodynamics, albuminuria and renal function, require higher doses than their blood pressure effects.<sup>1-3</sup> This most likely reflects the fact that renin, angiotensin-converting enzyme (ACE) and angiotensin (Ang) II type 1 (AT<sub>1</sub>) receptors in the kidney are less easily accessible to drugs taken orally than in blood or the vascular wall.<sup>4-10</sup> In addition, their levels at renal tissue sites, in particular those of renin, are much higher than in blood<sup>9, 11</sup>, therefore requiring even higher doses to obtain sufficient blockade. A well-known model to test the efficacy of blockade of RAS activity in the kidney is to measure the renal vasodilator responses to RAS blockade in subjects in whom the RAS has been activated by restriction of sodium intake.<sup>12-14</sup> We have wide experience with this model, both in healthy volunteers and in (diabetic) patients under carefully standardized conditions (e.g., receiving a fixed sodium diet) during a multiple day-stay in our clinical research center.

Remarkably, when using this model, we observed that the renal plasma flow (RPF) responses to aliskiren exceeded those seen with ACE inhibitors or AT<sub>1</sub> receptor blockers.<sup>13</sup> Doses of 300 mg and 600 mg aliskiren were tested, and the effects of 600 mg were ≈20% larger than those of 300 mg. Unfortunately, 600 mg of aliskiren leads to diarrhea; therefore, aliskiren is clinically used at a maximum dose of 300 mg/day. Consequently, at this stage, we do not know to what degree the effects of 600 mg aliskiren, which were twice as large as those observed with captopril (25 mg), and 40% larger than those observed with AT<sub>1</sub> receptor blockers (300 mg irbesartan, 16 mg candesartan, or 600 mg eprosartan)<sup>12, 14, 15</sup>, resembled the maximum effects of RAS blockade in the kidney.

A new renin inhibitor, VTP-27999, has recently been compared with aliskiren in healthy volunteers.<sup>16</sup> The drug was safe and well tolerated. Since VTP-27999 is a potent renin inhibitor with an oral bioavailability that is about 10-fold higher than that of aliskiren<sup>17, 18</sup>, we hypothesized that, with the help of this new and well-tolerated renin inhibitor, we would be able to establish the maximum effect of renin inhibition in the kidney. Therefore, in the present study, we compared the acute renal effects of escalating VTP-27999 doses (75-600 mg) with those of 300 mg aliskiren in healthy, salt-depleted volunteers.

## MATERIAL AND METHODS

### Study protocol

This single-center, prospective randomized, double-blinded, placebo-controlled study was performed in healthy volunteers between the age of 18 and 75 years of both sexes. Ethical approval was obtained by expedited review through the Brigham and Women's

Hospital/Partner Healthcare Human Research Committee. Female subjects were required to be postmenopausal or surgically sterilized to participate. Subjects were free of hypertension, diabetes, or any significant medical condition. After an outpatient evaluation, which included history, physical examination, screening chemistry, and hematology laboratory tests, all subjects were studied during a 7-day admission to a metabolic ward, at the Brigham and Women's Hospital General Clinical Research Center (GCRC) (see Table S1). Written informed consent was obtained from each subject, and the protocol was approved by the Human Subjects Committee of the institution. Subjects were placed on a controlled low sodium diet (10 mmol sodium daily, the first several days as outpatient) and randomly assigned to 2 groups. The diet did not affect blood pressure ( $126\pm 5/76\pm 4$  mm Hg versus  $120\pm 5/73\pm 3$  mm Hg in group 1, and  $125\pm 4/75\pm 3$  mm Hg versus  $116\pm 4/71\pm 2$  mm Hg in group 2;  $P=NS$  for both). The first group received 75 mg VTP-27999, placebo, and 300 mg aliskiren, respectively. The second group received VTP-27999 in 3 doses, 150 mg, 300 mg, and 600 mg. All drugs were given in single doses on separate study days.

Twenty four-hour urine samples were collected daily; when urinary sodium matched sodium intake (usually on day 5), the first study was initiated. Each subject was tested on three separate study days (Monday, Wednesday, and Friday), separated by a rest interval of 48 hours; drug/placebo was only administered on study days. Phlebotomy limitations prevented subjects from undergoing more than three studies each. Studies began at 6 AM. Subjects had been recumbent and fasting overnight and remained recumbent throughout the study. RPF was measured by the clearance of paraaminohippurate (PAH; Clinalfa, Laufelfingen, Switzerland) and glomerular filtration rate (GFR) by the clearance of inulin (Inutest Polyfructosan, Fresenius Pharma, Linz, Austria) by autoanalyzer methods described previously.<sup>19</sup>

After a 60-minute control period to establish basal RPF, placebo/drug was dosed by mouth. Over the next four (treatment day 2) or five (treatment days 1 and 3) hours, blood pressure was checked every 15 minutes by an automatic recording device (Dinamap, Critikon Inc, Tampa, FL, USA) or as deemed necessary by study staff. Blood samples were collected on ice at the start of the PAH infusion, at 60-minute intervals throughout, and at the end of each study (after five hours). Due to limited PAH stock, on treatment day 2, the PAH clearance measurements stopped at four hours. Samples were spun immediately, and plasma was stored at  $-80^{\circ}\text{C}$  until the time of assay.

### Biochemical measurements

PRA was determined by measuring Ang I generation during incubation of plasma at  $37^{\circ}\text{C}$  and pH 7.4 using an in-house assay.<sup>16</sup> PRC was measured with an immunoradiometric kit (Renin III, Cisbio, Gif-sur-Yvette, France).<sup>13</sup> Plasma prorenin was measured with a direct prorenin enzyme-linked immunosorbent assay (Molecular Innovations, Novi, MI, USA).<sup>20</sup>

Plasma Ang II was measured by radioimmunoassay after SepPak extraction as described before.<sup>21</sup>

### Statistical analysis

RPF and GFR are presented as percent change from baseline on each respective study day. An F-test was applied to the mean percent change of each group to assess overall significance of the linear mixed model. In this analysis, we assumed non-independence of observations across the protocol assignment and the intervention dosings. All other parameters are presented as absolute values or changes (mean±SEM), and have been analyzed by Student's t-test or one-way ANOVA, followed by post-hoc evaluation according to Bonferroni.  $P < 0.05$  was considered statistically significant. All authors had full access to and take responsibility for the data.

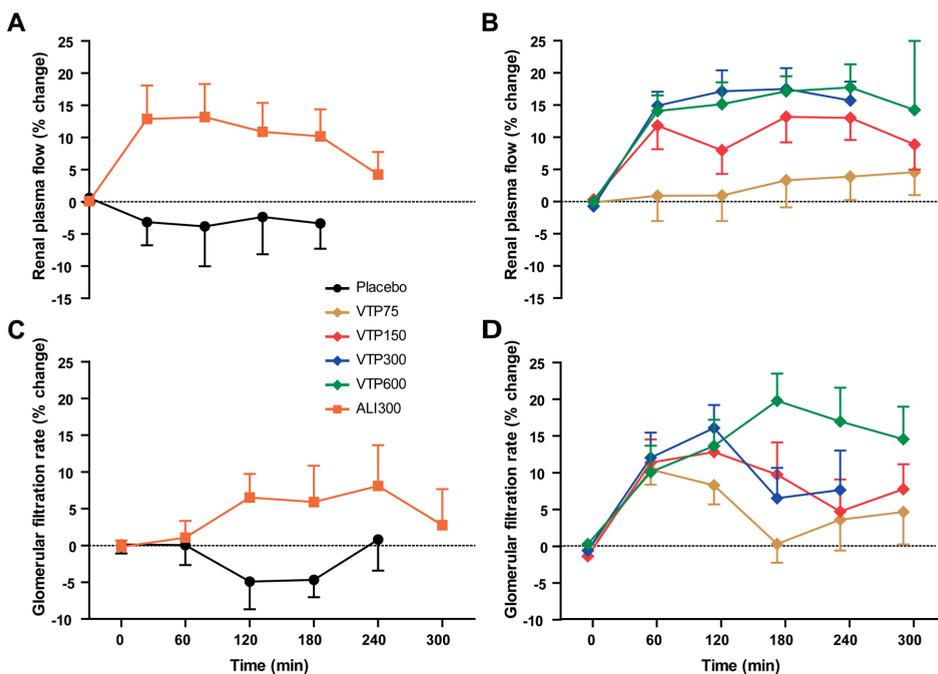
## RESULTS

Baseline characteristics of the two groups of healthy volunteers that participated in this study were equivalent (Table 1), and thus all subjects were evaluated together. VTP-27999 dose-dependently increased RPF (Fig. 1A&B), its maximum effect reached at a dose of 300

**Table 1.** Baseline characteristics (mean±SEM) of the 2 treatment groups under low-sodium conditions. Group 1 received 75 mg VTP-27999, 300 mg aliskiren, and placebo, respectively. Group 2 received VTP-27999 in 3 doses, 150 mg, 300 mg, and 600 mg. BMI, body mass index; SBP, DBP, systolic, diastolic blood pressure; PRA, plasma renin activity; Ang, angiotensin.

Parameter	Group 1 (n=10)	Group 2 (n=12)
Age (years)	48±5	43±5
Male, %	70%	100%
Race (Caucasian/Hispanic), %	90/10%	92/8%
BMI (kg/m <sup>2</sup> )	25±1	28±1
SBP (mm Hg)	126±5	125±4
DBP (mm Hg)	76±4	75±3
Heart rate	73±5	72±3
Hematocrit (%)	44±0.9	45±1.0
Serum glucose (mg/dL)	79±4	81±4
Blood urea nitrogen (mg/dL)	14±1	16±1
Serum creatinine (mg/dL)	1.0±0.1	1.0±0.0
Serum Sodium (mmol/L)	140±0	140±1
Serum Potassium (mmol/L)	4.3±0.2	4.3±0.1
Plasma renin (ng/L)	26±5	31±3
Plasma prorenin (ng/L)	82±16	85±10
PRA (nmol Ang I/L.hr)	2.36±0.49	3.12±0.30
Plasma Ang II (pmol/L)	7.6±1.1	8.9±0.8
Renal plasma flow (mL/min.1.73 m <sup>2</sup> )	432±17	436±18

mg, given the fact that the effects observed at 600 mg were equivalent to those at 300 mg. Aliskiren 300 mg effects on RPF were similar to those obtained with 150 mg VTP-27999, and significantly lower than those obtained at 300 and 600 mg VTP-27999 ( $P < 0.01$ ). Renin inhibitor-induced GFR changes paralleled this pattern (Fig. 1C&D), although significant increases in fact only occurred at the highest VTP-27999 dose ( $P < 0.05$  versus placebo). Both renin inhibitors induced small, non-significant decreases in blood pressure (Table 2). No serious adverse effects were observed (Table S2).

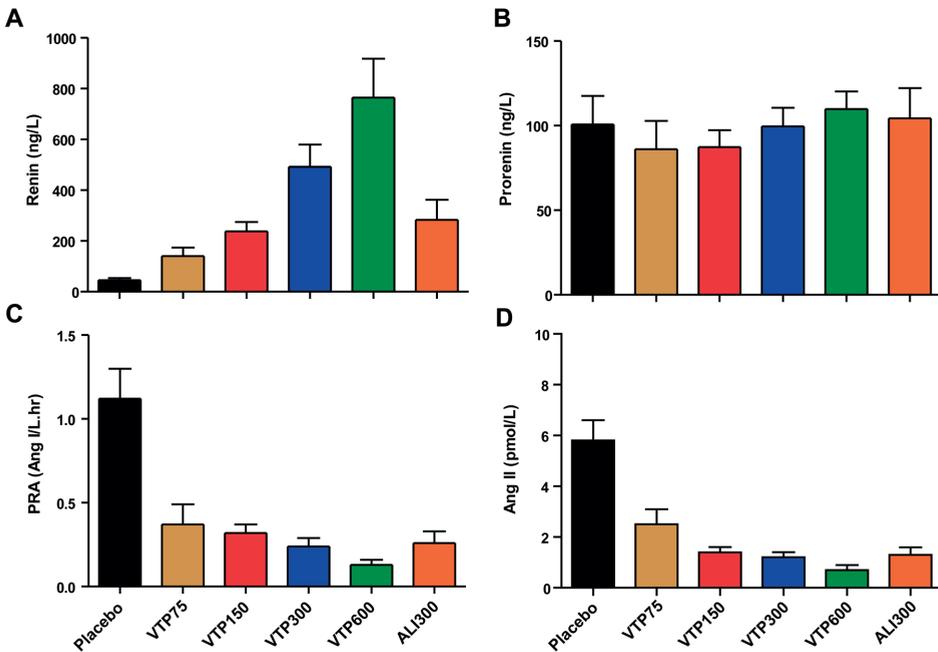


**Figure 1.** Percent change in renal plasma flow (panels A&B) and glomerular filtration rate (panels C&D) in healthy, salt-depleted volunteers over a 5-hour period following oral intake of placebo, VTP-27999 (VTP; 75, 150, 300 or 600 mg) or 300 mg aliskiren (ALI). Data are mean $\pm$ SEM of n=9-12. See text for statistical analysis.

**Table 2.** Change in systolic blood pressure (SBP) and mean arterial pressure (MAP) at 5 hours after placebo/drug intake. Data are mean $\pm$ SEM of n=9-12. No significant changes vs. placebo were noted.

Treatment	$\Delta$ SBP (mm Hg)	MAP (mm Hg)
Placebo	4.1 $\pm$ 4.3	112 $\pm$ 4
VTP-27999 75 mg	-5.9 $\pm$ 3.3	110 $\pm$ 4
VTP-27999 150 mg	0.0 $\pm$ 3.6	114 $\pm$ 2
VTP-27999 300 mg	-5.2 $\pm$ 4.1	108 $\pm$ 3
VTP-27999 600 mg	-5.0 $\pm$ 4.4	112 $\pm$ 2
Aliskiren 300 mg	-4.8 $\pm$ 4.1	100 $\pm$ 5

VTP-27999 dose-dependently increased renin, and lowered PRA and Ang II at 5 hours after dosing ( $P < 0.01$  for all renin inhibitor groups versus placebo), without significantly affecting prorenin (Fig. 2A-D). The effects of aliskiren on RAS components again were best comparable to those of 150 mg VTP-27999.



**Figure 2.** Plasma levels of renin (A) and prorenin (B), plasma renin activity (C) and the plasma level of angiotensin (Ang II) (D) in healthy, salt-depleted volunteers at 5 hours after oral intake of placebo, VTP-27999 (VTP; 75, 150, 300 or 600 mg) or 300 mg aliskiren (ALI). Data are mean $\pm$ SEM of n=9-12. See text for statistical analysis.

Importantly, when determining RPF, renin, and Ang II at  $t=0$  on each specific treatment day, it became clear that RPF and Ang II, but not renin, at 48 hours after the previous treatment day had returned to baseline (pre-treatment) levels. Baseline renin levels in group 1, receiving 75 mg VTP-27999, placebo and 300 mg aliskiren on Monday, Wednesday and Friday, respectively, were  $27 \pm 5$ ,  $70 \pm 14$  ( $P < 0.05$  versus Monday) and  $57 \pm 13$  pg/mL ( $P < 0.05$  versus Monday), while in group 2, receiving 150, 300 and 600 mg VTP-27999 on Monday, Wednesday and Friday, respectively, they were  $31 \pm 3$ ,  $130 \pm 15$  ( $P < 0.01$  versus Monday), and  $238 \pm 28$  pg/mL ( $P < 0.01$  versus Monday). For RPF, these values were  $432 \pm 17$ ,  $442 \pm 16$  and  $442 \pm 26$  versus  $436 \pm 18$ ,  $448 \pm 19$  and  $456 \pm 23$  mL/min.1.73 m<sup>2</sup>, respectively, and for Ang II  $7.6 \pm 1.1$ ,  $9.0 \pm 1.3$  and  $9.4 \pm 1.5$  versus  $8.9 \pm 0.8$ ,  $8.7 \pm 0.8$  and  $7.9 \pm 0.9$  pmol/L, respectively. These data demonstrate that not all drug had been washed away at 48 hours, but that the original state of renal hemodynamics and RAS activity had been restored at that time point due to

the rise in renin. This observation has been made before<sup>13</sup>, and is in full agreement with the fact that the half life of both aliskiren and VTP-27999 is  $\approx 24$  hours.<sup>16</sup>

## DISCUSSION

This study revealed a maximum effect of oral renin inhibition on RPF and GFR, which was reached at VTP-27999 doses of 300 mg and higher, but not at an aliskiren dose of 300 mg. The maximum effect of VTP-27999 was roughly 30% higher than the effect of 300 mg aliskiren. This difference is close to the  $\approx 20\%$  larger effect observed with 600 mg aliskiren versus 300 mg aliskiren in our previous study.<sup>13</sup> Therefore, retrospectively, the effects of 600 mg aliskiren most likely did resemble the maximum effects that can be established with renin inhibition. Yet, such high aliskiren doses are not clinically recommended, and only now, with VTP-27999, could we establish that indeed larger RPF increases cannot be accomplished with renin inhibition.

In the current acute study we did not make a comparison with other RAS blockers. Selected comparisons have been made in the past, and, taken together, suggested that the maximum effects of renin inhibition with aliskiren are double those of captopril (25 mg), and 40% larger than those observed with the AT<sub>1</sub> receptor blockers eprosartan (600 mg), irbesartan (300 mg) and candesartan (16 mg).<sup>12-15, 22</sup> Yet, since none of these studies titrated the doses of all three types of RAS blockers up to a maximum effect in a parallel fashion, it is still likely that similar maximal renal effects can be achieved with all types of RAS blockade. Therefore, our data at most indicate that maximum renal effects of renin inhibition are reached at doses that are in or slightly above the normal clinical range, whereas for the other types of RAS blockers much higher doses are required to induce maximum renal effects.<sup>1-3</sup> A likely explanation of this observation is that renin inhibitors, and VTP-27999 in particular, more easily accumulate in the kidney.<sup>23-26</sup> Although it seems logical to attribute this to the fact that renin is abundantly present and stored in the kidney, studies in renin knockout animals showed that this accumulation is in fact unrelated to the presence of renin.<sup>25</sup> However, since accumulation was selective for the kidney, it must involve a kidney-specific uptake system, possibly the organic anion-transporting polypeptide 2B1 (OATP2B1).<sup>27</sup> Interestingly, due to its accumulation at renal tissue sites, the renin inhibitor can still be demonstrated in renal tissue several weeks after stopping treatment.<sup>24, 28</sup> Yet, as a consequence, particularly following treatment with very high doses of VTP-27999, renal RAS inhibition may exceed RAS inhibition in the circulation after stopping drug intake. This will paradoxically cause a rise in circulating Ang II and aldosterone, due to the fact that renal RAS inhibition continues to stimulate renin release, while after stopping drug intake the VTP-27999 levels in plasma are no longer sufficient to block this renin.<sup>16</sup>

In the present study, volunteers received placebo, aliskiren or VTP-27999 on 3 different treatment days with a 48-hour rest interval in between. Clearly, given the half life of aliskiren and VTP-27999 ( $\approx 24$  hours for both)<sup>6</sup>, not all drug will have been washed away on each subsequent treatment day. Indeed, renin levels were still elevated at 48 hours following a dose of either aliskiren or VTP-27999, like in our previous study.<sup>13</sup> Nevertheless, Ang II and RPF at that time had returned to pre-drug baseline values, suggesting that the renin rise was sufficient to overcome the blocking effects of any remaining renin inhibitor still being present. Importantly therefore, baseline hemodynamics and RAS activity were identical at each occasion. Moreover, changes in RPF and GFR have been expressed as a percentage of the baseline value on each respective study day, to correct for any carry-over effect.

RAS inhibitor-induced RPF changes reflect renal vascular function, and may thus serve as an indication of the efficacy on renal vasculature of these drugs. Obviously, the observed acute physiologic response is only a surrogate of the acute drug effect on the kidney that does not by definition indicate meaningful clinical responses in the real world in terms of the drug benefit, when the drug is given on a continuous basis. For instance, under the latter condition, multiple compensatory mechanisms may come into play, which may alter the net effect of renin inhibition. The most important of these is the rise in renin release. Nevertheless, given our current and earlier findings, it seems that maximum beneficial effects of RAS blockade in the kidney may be achieved more easily with a renin inhibitor (i.e., at relatively low doses) than with other types of RAS blockers. Importantly, to obtain beneficial effects, renin inhibitors should be dosed optimally rather than maximally.<sup>29</sup> The combination of a renin inhibitor with other RAS blockers might rapidly tip the balance (i.e., induce too much RAS blockade), as has become apparent in the ALTITUDE trial.<sup>30</sup> Other trials investigating dual or triple RAS blockade<sup>31</sup> also yielded the typical consequences of RAS annihilation: hypotension, renal dysfunction and hyperkalaemia.

Consequently, the optimal dose of VTP-27999 is not necessarily 600 mg, but rather 150-300 mg/day.<sup>16</sup> In the present study, the renal and hormonal effects of VTP-27999 at a dose of 150 mg most closely resembled those of 300 mg aliskiren. Neither of the two renin inhibitors affected prorenin, due to the fact that changes in prorenin require de novo synthesis, because prorenin, unlike renin, is not stored in the kidney. Only VTP-27999, at its highest dose, significantly increased GFR. Since changes in GFR, at least in healthy volunteers, directly correlate with changes in RPF<sup>32</sup>, this simply reflects the larger effects of VTP-27999 on RPF. The mechanism underlying this phenomenon involves a change in intravascular oncotic pressure along the glomerular capillary, resulting in greater surface area available for filtration.<sup>32</sup> No significant effects on blood pressure were observed. This is most likely related to the fact that this is an acute study, evaluating a single dose only in a small number of healthy subjects. Similarly, in our previous study, evaluating different

aliskiren doses in healthy, salt-depleted volunteers, we also did not detect significant decreases in blood pressure.<sup>13</sup> Certainly, when dosing repetitively, blood pressure decreases are more likely to occur – particularly in patients with an activated RAS, since such activation will limit their compensatory capacity. Indeed, blood pressure responses to RAS blockers are generally the highest in patients with the highest degree of RAS activation.<sup>33</sup>

In conclusion, of all RAS blockers, renin inhibitors require relatively the lowest doses to inhibit renal RAS activity completely. This may relate to their capacity to selectively accumulate in the kidney. It may also reflect the fact that renin inhibition is the most efficient way to block the RAS, hampered the least by counteracting mechanisms (PRA rise, ACE upregulation) and/or the appearance of multiple angiotensin metabolites acting on non-AT<sub>1</sub> receptors.<sup>34</sup> On the one hand, this implies that maximal beneficial renal effects can be achieved easily with a single RAS blocker, i.e., a renin inhibitor, instead of combining 2 or more alternative RAS blockers at varying (and often high!) doses. However, selective renal accumulation also implies that the degree of renal RAS blockade may rapidly become too high. Therefore, studies are warranted to carefully determine the renin inhibitor dose required for optimal rather than maximal renal RAS blockade. Ultimately, the effects of these doses will need to be tested in large clinical trials specifically in patients with diabetes and nephropathy, where there is a large unmet need.

Unfortunately, an extensive preclinical comparison of the 3 types of RAS blockers is virtually impossible because (human) renin inhibitors are highly species-specific and do not act in rodents, except at high doses.<sup>35</sup> The only model that is available for this purpose is the so-called double transgenic rat, a hypertensive rat model expressing both human renin and human angiotensinogen.<sup>36</sup> Yet, whether this artificial model truly mimics all aspects of hypertension is questionable to date. Furthermore, animal models may also help to shed light on the contribution of the (pro)renin receptor, if at all, to the beneficial effects of RAS blockers. This receptor binds and activates prorenin at high concentrations *in vitro*.<sup>37</sup> However, recent studies questioned the physiological relevance of these findings, since in reality, prorenin concentrations are many orders of magnitude below the levels that are required to interact with this receptor, even during treatment with RAS blockers.<sup>38</sup> Indeed, the (pro)renin receptor has now also been linked to functions beyond the RAS.<sup>39</sup> To what degree its concentrations vary in relation with salt intake still needs to be investigated further.

Finally, an important aspect of future trials might be whether the effects of RAS blockade are sex-specific. Currently, there are no sex-specific recommendations for RAS blocker therapy, given the lack of strong evidence that men and women may respond differentially to RAS blockers.<sup>40, 41</sup> Animal studies do, however, support the existence of such differences.<sup>42</sup> Moreover, men display higher renin levels than women<sup>43</sup>, and thus there is a reason to believe that men and women may respond differently, e.g., with regard to renal effects, when exposed to renin inhibitors.

## DISCLOSURES

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