Renal Hemodynamic Effects of Serelaxin in Patients With Chronic Heart Failure
A Randomized, Placebo-Controlled Study

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Background—Serelaxin is a promising therapy for acute heart failure. The renal hemodynamic effects of serelaxin in patients with chronic heart failure are unknown.

Methods and Results—In this double-blind, randomized, placebo-controlled, multicenter study, patients with New York Heart Association Class II to III chronic heart failure, left ventricular ejection fraction ≤45%, and estimated glomerular filtration rate (GFR) 30 to 89 mL/min per 1.73 m² received intravenous serelaxin 30 μg/kg per day or placebo for 24 hours. Primarily, we assessed the difference between serelaxin and placebo on renal plasma flow (para-aminomipiric acid clearance) and GFR (iothalamate clearance) over 8 to 24 hours. All 22 patients from 1 clinical site were excluded from primary analyses before unblinding because of implausible measurements. The primary analysis comprised 65 patients, mean age was 68 (±10) years, 89% were male, mean estimated GFR was 64 (±19) mL/min per 1.73 m², and 34% had New York Heart Association Class III symptoms. Renal plasma flow increased by 29% with serelaxin and 14% with placebo (13% relative increase with serelaxin; P=0.0386), whereas GFR changes did not differ significantly during 8 to 24 hours. Filtration fraction increased by 36% with serelaxin and 62% with placebo (16% relative decrease with serelaxin; P=0.0019) during 8 to 24 hours. Changes in systolic blood pressure were largely similar, and creatinine clearance did not differ between groups. Adverse event rates were similar with serelaxin (20.5%) and placebo (25.0%).

Conclusions—In patients with chronic heart failure, serelaxin increased renal plasma flow and reduced the increase in filtration fraction compared with placebo, but did not affect GFR. These results suggest beneficial renal hemodynamic effects in patients with chronic heart failure.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT01546532.

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Key Words: clinical trial ■ heart failure ■ hemodynamics ■ kidney

Renal dysfunction is often present in patients with heart failure and is related to a poorer prognosis.1,2 Patients with chronic heart failure (CHF) are often treated with angiotensin-converting enzyme inhibitors and mineralocorticoid receptor antagonists, although they often cause a further deterioration of renal function. Patients with acute heart failure (AHF) are often treated with loop diuretics, which might also further deteriorate renal function. To date, no drugs that are used for treating chronic or AHF have been shown to improve renal function. Serelaxin, a recombinant human relaxin-2, is a promising and novel investigational therapy for the treatment of AHF. In the RELAXin in Acute Heart Failure (RELAX-AHF) study, early infusion of serelaxin was associated with a decrease in dyspnea and lower 180-day all-cause mortality in patients admitted for AHF.3 In addition, the study showed that treatment with serelaxin is associated with lower serum creatinine and plasma cystatin C concentrations compared with placebo.4 Other studies also indicate possible beneficial effects of serelaxin on renal function. A study investigating the renal effects of serelaxin in 11 human volunteers showed a marked increase of renal plasma flow (RPF) by 47% compared with baseline levels, but no significant change was observed in glomerular filtration rate (GFR).5 In models of chronic kidney disease, relaxin showed protective effects on kidney structure.
Renal Hemodynamic Effects of Serelaxin in CHF

Methods

Study Design, Population, and Treatment

This study was a double-blind, randomized, parallel-group, placebo-controlled, multicenter study. Inclusion criteria were CHF with standard oral therapy, including stable oral furosemide 40 to 240 mg/d or equivalent, reduced left ventricular ejection fraction (≤45% within the past 6 months), brain natriuretic peptide ≥400 pg/mL or N-terminal prohormone of brain natriuretic peptide ≥400 pg/mL, New York Heart Association Class II to III, worsening symptoms within the previous 3 months, and mild-to-moderate renal impairment (estimated GFR ≤60 ml/min per 1.73 m²). Key exclusion criteria comprised SBP <110 mm Hg, acute contrast-induced nephropathy (at the time of randomization), administration of intravenous radiographic contrast agent ≤72 hours before randomization or treatment with nonsteroidal anti-inflammatory drugs, and current or planned treatment with any intravenous vasoactive therapies or mechanical support. Furthermore, patients with clinically significant hepatic impairment, defined as any hepatic encephalopathy or total bilirubin >50 μmol/L or spontaneous international normalized ratio >2.0, were not considered for the study. Interventional treatment consisted of serelaxin (30 μg/kg per day) or matching placebo, each provided as a 24-hour intravenous infusion.

A central randomization scheme was used, and patients were randomized using consecutive randomization numbers in blocks of 4 for each of the 2 stratification factors: patients with prescribed daily dose of furosemide <120 mg or ≥120 mg orally, or equivalent dose of other loop diuretics, respectively.

Primary End Points

The 2 primary outcome measures were (1) para-aminomophipuric acid (PAH) plasma clearance for RPF, and (2) iothalamate (IOTH) plasma clearance for GFR. The primary interval of interest was 8 to 24 hours after start of infusion. Renal blood flow (RBF) was defined as a coprimary end point in the study protocol and can be derived from RPF/(1–hematocrit). However, because RPF results are predominately presented in the literature, RPF instead of RBF was treated as the coprimary end point, as specified in the statistical analysis plan that was finalized before database closure.

Secondary outcome variables included filtration fraction (FF), sodium excretion (natriuresis), and changes in SBP and diastolic blood pressure (DBP).

The plasma IOTH and PAH clearances were calculated as (intravenous infusion rate)/(plasma concentration) at each time point. We adjusted the infusion rate for the IOTH and PAH continuous infusion by individual eGFR (using the Modification of Diet in Renal Disease formula). The following formulas were used: infusion rate (mg/h) for IOTH=1.2xGFR (mL/min) and infusion rate (mg/h) for PAH=5.5xGFR (mL/min). For pooled time intervals (8–24 hours, 0–24 hours, and 24–28 hours), plasma concentration in the above formula was replaced by the time-weighted average plasma concentration (area under the curve divided by time).

Baseline plasma clearance was derived using the 0-hour IOTH/PAH concentration measurement.

The urinary PAH and IOTH clearances over each urine collection interval and pooled time intervals were calculated as (amount excreted in urine)/(plasma concentration area under the curve). For baseline (−3 to 0 hours), because plasma concentration was not available for the −3-hour time point, the area under the curve was predicted from a 1-compartment model fitted to the first 3 data points (0, 2, and 4 hours): $C_t = \left( \frac{IV \text{ injection bolus dose}}{V} \right) \times e^{-Kt} + \left( \frac{IV \text{ infusion rate}}{V} \times K \right) \times (1 - e^{-Kt}) + error$

Where $V$ and $K$ are the volume of distribution and elimination rate constant, respectively. Specifically:

AUC = $\int_0^\infty \left( \frac{IV \text{ injection bolus dose}}{V} \right) \times e^{-3K} + \left( \frac{IV \text{ infusion rate}}{V} \times K \right) \times (1 - e^{-3K}) + 3 \times \left( \frac{IV \text{ infusion rate}}{V} \right)$

Plasma clearance of PAH is considered to be the gold standard for RPF, and plasma clearance of IOTH was chosen over urinary clearance as the coprimary end point because fewer than half of the patients had change from baseline values available as a result of the difficulty in estimating the baseline values (the 1-compartment model often failed to converge).

Assessments

To assess renal hemodynamics, patients were placed in the recumbent position, and an intravenous bolus injection PAH and IOTH was administered 3 hours before the start of study drug infusion, followed immediately by constant intravenous infusion (adjusted for individual eGFR) during study drug infusion, and for 4 hours after stopping study drug infusion (31 hours in total). Notably, oral loop diuretics were last to be taken by patients 24 hours before randomization. Parallel to study drug infusion, all patients were converted from their oral loop diuretic dose to a continuous intravenous infusion of furosemide, outlining study drug infusion by 4 hours. The total daily dose of continuous intravenous furosemide was 50% of the prior total daily oral dose of furosemide. All patients were required to be on a stable (≥4 weeks) dose of furosemide 40 to 240 mg/d orally or equivalent dose of other loop diuretic at the time of study enrollment. If a patient had been taking an oral loop diuretic other than furosemide, the equivalent dose of oral furosemide was calculated and used to determine the furosemide dose for continuous intravenous infusion (eg, furosemide 40 mg=torsemide 20 mg=bumetanide 1 mg). All patients were treated with evidence-based medications for treatment of heart failure and had to be on a stable dose, particularly of loop diuretics (≥4 weeks). When domiciled, patients were to be on a standard weight-maintaining diet, ideally with small, caloric/salt equivalent portions approximately every 2 hours during the day until a light dinner at ~7 PM. The total amount of Na/24 hours was ~80 to 100 mmol. Patients could drink water ad libitum to ensure adequate hydration for urine collection and a target fluid intake of ≥240 mL every 4 hours during waking hours was recommended. Study patients were asked to void their bladder before/at the end of each urine collection interval. In general, meals were to be provided when all other study procedures scheduled for that time had been completed.

Serial blood and urine samples were analyzed to assess safety and effects on renin function and biomarkers. Circulatory biomarkers comprised N-terminal prohormone of brain natriuretic peptide and cystatin C.

Uniquely, the effects of serelaxin on RBF and redistribution of blood flow between the cortical and medullary kidney tissue were analyzed by positron emission tomography (PET)/computed tomography scanning with 15O-water in a subset of nine patients. Details of the PET imaging procedure are outlined in the Data Supplement. In brief, patients were scanned using a Biograph mCT PET/computed tomography system (Siemens Medical Systems, Knoxville, TN), with a PET axial field of view of 21 cm. Low-dose computed tomography was used for attenuation correction of the region of interest (abdomen) and for anatomic delineation of the kidney aorta and the abdominal aorta. Renal cortical and medullary blood perfusion of the different regions was estimated by fitting the PET measured time activity curves to a validated 1-compartment model for 15O-water.
Ethics
The study was approved by all local Ethics Committees and complied with the Declaration of Helsinki guidelines. Written informed consent was obtained from all patients.

Power and Sample Size Considerations
Assuming standard deviations (SD) of 40% for RPF and 45% for GFR, and the respective true mean treatment differences of 25.5% and 28.6%, 40 patients per group were predicted to provide at least 80% probability that the estimated treatment difference in percentage change from baseline is at least 17.8% for RPF and 20% for GFR and is statistically significant at the 0.05 level based on a 2-sided test. Assuming a dropout rate of 10%, 44 patients per group needed to be randomized. In a similar trial, it was shown that an adenosine A1 receptor antagonist increased RPF and GFR by 32% and 24%, respectively, relative to placebo treatment, over the first 8 hours after the start of study drug infusion.8 An ±20% lower effect on RPF and an ±20% higher effect on GFR were considered to be clinically relevant and potentially achievable by serelaxin. The true mean treatment difference of 25.5% for RPF and 28.6% for GFR was, therefore, assumed. The respective estimated treatment difference of 17.8% and 20% was based on back calculation given a sample size of 40 patients per treatment arm.

Statistical Analyses
Statistical analyses were performed using SAS software. Relevant baseline variables were summarized and compared between the 2 treatments using methods appropriate for the underlying distribution. Specifically, continuous data were summarized using sample size, mean with SD, geometric mean with 95% confidence interval (biochemical variables only), and compared by a 2-sample t test, whereas categorical data were summarized using sample size, absolute and relative frequency and compared by a Fisher exact test.

ANOVA was performed for time-weighted average (area under the curve divided by time) change from baseline for RPF, GFR, and FF in the log domain over 8 to 24, 0 to 24, and 24 to 28 hours, separately, using treatment and diuretic dose strata as classification factors and the corresponding log-transformed baseline values as covariate. Results were back-transformed to the original scale and expressed as ratio to baseline or percentage change from baseline. In addition, changes from baseline in RPF, GFR, and FF at each time point were analyzed. One patient in the serelaxin group had no PAH and IOTH concentration data at 24 and 26 hours. In addition, a total of 4 measurements from each of the 2 treatment groups (4 patients in the serelaxin group, 2 patients in the placebo group) were taken between 24 and 28 hours after PAH/IOTH infusion had stopped so were set to missing. The last observation carried forward method was used to impute these missing values before any statistical summary and analysis. The least squares mean difference and associated 95% confidence interval, as well as the P value, were obtained from the treatment comparison. Summary of RPF, GFR, and FF, and of the percentage change in RPF, GFR, and FF from baseline based on ratio of geometric means (geomeans), were presented, and the geomeans plotted over time by treatment group.

Further secondary variables included changes in urinary flow rate (diuresis), sodium excretion rate (natriuresis), and creatinine clearance. Brachial SBP and DBP were summarized using descriptive statistics. As a post hoc analysis, an ANCOVA with treatment and diuretic dose stratum as the classification factors, and baseline as a covariate, was performed on change from baseline creatinine clearance in the log domain (measurements including baseline were log-transformed before analysis) for each urinary pooled time interval (8–24, 0–24, and 24–28 hours). For brachial SBP and DBP, a post hoc ANCOVA with treatment as the classification factor, and baseline as a covariate, was performed on change from baseline values. Post hoc analyses were also performed for plasma/serum variables and urine flow rate as well as sodium excretion rate. Safety analyses comprised all patients on study drug infusion with ≥1 postbaseline safety assessment. Patients were analyzed according to treatment received. A 2-sided P<0.05 was considered statistically significant.

The statistical analyses were performed by inVentive Health Clinical, and all results were verified or reviewed by Novartis. The non-Novartis authors did not have direct access to the database. Novartis is ultimately accountable for the accuracy of the analysis results.

Exclusion of Results From One Study Site Before Database Closure
A blinded preliminary data review (before database closure) revealed clinically implausible outlier values for plasma and urine PAH and IOTH data considering the constant rate of administration of PAH and IOTH in this study. These outlier data largely originated from 1 study site (Figures I and II in the Data Supplement). Indeed, 20 of 22 patients from this site were considered outliers for PAH values. The reasons for the implausible PAH/IOTH values could not be identified, and a possible administration/sampling error could not be excluded. Consequently, all data from this study site (n=22 patients) were excluded from the primary pharmacodynamic analyses, and the results presented in the main body of the article refer to the subset of all patients from the other sites. The results of the total per-protocol study cohort, including the data from the site with questionable sampling, are provided in the Data Supplement (Tables I–III in the Data Supplement).

Results

Baseline Characteristics
Overall, 87 patients with CHF were randomized in 4 countries at 13 sites. The primary analysis was performed with data from 65 patients (serelaxin n=28; placebo n=37) (Figure III in the Data Supplement). Details of baseline patient characteristics are shown in Table 1.

Primary Outcome Variables

Renal Plasma Flow
Serelaxin increased RPF from baseline in the 8 to 24 hours of infusion by 29% whereas a 14% increase was reported in the placebo-treated patients (relative increase of 13% with serelaxin; P=0.0386; Figure 1A). In addition, RPF treatment differences were statistically significantly in favor of serelaxin for RPF least square (LS) geometric mean ratios to baseline over 0 to 24 hours and 24 to 28 hours, with treatment differences of 16% for both (P=0.0042 and 0.0115, respectively; Table 2). The maximum RPF change from baseline was 56% at 4 hours in the serelaxin group and 20% at 4 and 24 hours in the placebo group (data shown in Table 1 in the Data Supplement).

Glomerular Filtration Rate
There was no statistically significant treatment difference for the coprimary end point of GFR change from baseline over 8 to 24 hours, determined by plasma IOTH clearance (Figure 1B). Treatment differences in GFR change from baseline over 0 to 24 hours and 24 to 28 hours were also nonsignificant (Table 2). However, steady state of IOTH did not seem to have been achieved on start of the study drug infusion, which resulted in an underestimation of GFR during the first hours of infusion for both treatments.

To verify that the cause of this rising GFR over time was a methodological issue and not a true effect, we also determined GFR by urinary IOTH clearance and creatinine clearance. The results support the primary analysis showing no treatment difference comparing serelaxin with placebo, but do not show a rise in GFR (Figure 2) during study drug infusion in either treatment group.
Secondary Outcome Variables

**Filtration Fraction**

FF increased from baseline over 8 to 24 hours, 0 to 24 hours, and 24 to 28 hours in both the serelaxin and placebo groups. However, the FF changes from baseline over these time points were lower in serelaxin-treated patients compared with placebo-treated patients. Over 8 to 24 hours, serelaxin caused a relative decrease of FF by 16% compared with placebo.
Similar relative decreases in FF were found over 0 to 24 hours and 24 to 28 hours by 16% (P=0.0004) and 22% (P<0.0001), respectively (Figure 3).

**Blood Pressure**

From mean baselines of 133.0±18.8 mmHg in the serelaxin group and 125.3±14.5 mmHg in the placebo group, changes in brachial SBP during the infusion period were largely similar between patients treated with either serelaxin or placebo (Figure 4A). Mean SBP remained below baseline after the end of the infusion. Baseline brachial DBP was also similar between the serelaxin group and the placebo group (Figure 4B). Only at the 8-hour time point were changes from baseline in brachial SBP and DBP significantly greater in serelaxin-treated patients compared with placebo. It is of note that changes in SBP and DBP were adjusted for baseline blood pressure differences.

**Other Secondary Outcome Variables**

No treatment differences were observed in sodium, potassium, creatinine, urea levels, and creatinine clearance (Table 3). N-terminal prohormone of brain natriuretic peptide baseline values were similar in the 2 treatment groups, and no significant change from baseline was seen in either treatment group at any time point. Cystatin C baseline values were also similar in the 2 treatment groups and increased with time in the placebo group, but not in the serelaxin group. Both urinary flow rate and sodium excretion rate were significantly higher in the placebo-treated patients (Table 3). The mean equivalent oral furosemide dose during the 24-hour infusion period was 60.1 (±6.85) mg in serelaxin-treated patients and 75.2 (±5.96) mg in placebo-treated patients (Table 3).

**PET/Computed Tomography Data**

On day 1 (baseline), tissue blood perfusion as estimated from the K1 rate constant appeared to be ≈20% greater in the renal medulla than in the cortex of serelaxin-treated patients, whereas no difference was observed in the placebo group (Figure 5). On day 2, K1 values remained stable in placebo patients, however increased by ≈35% with serelaxin, in both cortex and medulla. Accordingly, pre- and post-treatment values of the corresponding medulla/cortex ratios remained ≈1.2 and ≈1.0 with serelaxin and placebo, respectively. For both groups, the volume of distribution also appeared to be well below 1.0 and stable over the 24-hour treatment period (ie, ≈0.6 and ≈0.8 mL plasma/mL tissue for the cortex and the medulla, respectively), most likely indicative of a relatively strong rediffusion of H215O from the renal tissue into the circulation (ie, k2 rate constant, Figure IV in the Data Supplement). In line with this,

![Figure 1. Hemodynamic results. Primary outcome variables renal plasma flow (A) and glomerular filtration rate (B) over time.](http://circheartfailure.ahajournals.org/)
Serelaxin induced an ≈30% increase in the reverse transport of H\textsubscript{2}\textsuperscript{15}O both in the cortex and the medulla (Figure IV in the Data Supplement) whereas no change in k\textsubscript{2} was detected in placebo-treated patients. Finally, V\textsubscript{blood} data (Figure V in the Data Supplement) showed that the blood volume contributed to only ≈20% of the PET signal (ie, the PET signal emanates from the tissue), was similar in both cortex, and medulla and was unaffected by treatment.

Safety Parameters
Details on safety data refer to the safety analysis set of all 87 randomized patients. Eight serelaxin-treated patients (20.5%) and 12 placebo-treated patients (25.0%) reported ≥1 adverse event. The majority of adverse events were mild in nature (serelaxin: seven patients [17.9%]; placebo: nine patients [18.8%]) or moderate (serelaxin: 1 patient [2.6%]; placebo: 2 patients [4.2%]) in severity. Overall, five serious adverse events were reported in 4 patients, and 2 had suspected relationship to study drug. One patient treated with serelaxin (2.1%) reported flushing, possibly caused by inadvertent administration of IOTH/PAH bolus, and 3 patients treated with placebo (6.3%) reported nausea or hypotension, hydrothorax, or an ischemic stroke. In 2 of the patients (1 patient in each group) who met the blood pressure safety criterion (decrease in SBP to <90 mmHg), the study drug was stopped prematurely and permanently. In 1 serelaxin-treated patient, the infusion rate was halved based on the predefined criteria.

Discussion
In this double-blind study, using the gold standard methods for measuring renal hemodynamics, serelaxin increased RPF and reduced the increase in FF compared with placebo, but did not affect GFR.

The increase in RPF in serelaxin-treated patients is of interest and may be of clinical relevance. Central hemodynamic studies of serelaxin in patients with AHF showed that serelaxin decreased the mean pulmonary artery pressure and pulmonary capillary wedge pressure, indicating cardiac unloading.\textsuperscript{9,10} However, in these studies, serelaxin did not increase cardiac index. An increase in cardiac index as an explanation for the increase in RPF with serelaxin in this study is, therefore, unlikely. Another explanation for the increase in RBF in this study might be related to a possible reduction in venous congestion with serelaxin, which has been...
related to renal perfusion in several studies.\textsuperscript{11–14} However, as we did not measure venous pressures, we cannot support this potential explanation. Third, the increase in RPF in the absence of an increase in GFR might be explained by a direct renal vasorelaxant effect of serelaxin, probably by both renal afferent and efferent vasorelaxation, leading to unloading of the glomerulus.

Several studies have shown that RBF (and, in turn, RPF) is the main driver of preservation of renal function, both in AHF and CHF.\textsuperscript{11–14} It could, therefore, be expected that an increase in RPF results in an improvement in GFR. Correspondingly, a recent biomarker analysis of the RELAX-AHF trial showed that serelaxin-treated patients had lower serum creatinine and cystatin C values compared with placebo-treated patients.\textsuperscript{4} However, GFR remained unchanged in the present study, and results may, therefore, seem contradictory. Because we could confirm the absence of a treatment effect of serelaxin on urinary IOTH and creatinine clearances, the treatment comparison for plasma IOTH clearance is considered valid, despite the methodological limitation with regards to steady state attainment of IOTH at baseline and during the early hours of study drug infusion for both serelaxin and placebo treatments.

Most importantly, it needs to be emphasized that this study included patients with CHF whereas RELAX-AHF included patients with AHF.\textsuperscript{3} Patients with CHF were chosen for the current study as large volume shifts occurring in patients with AHF may mask treatment effects in a relatively small study like this. However, it is reasonable to assume that renal function is more seriously compromised during acute hemodynamic deterioration in patients with acute decompensated heart failure.\textsuperscript{1} Interestingly, both Ljungman et al.\textsuperscript{11} and Smilde et al.\textsuperscript{14} showed that in patients with CHF, GFR remains stable even when renal blood pressure dropped. As a result, FF increases, probably by efferent vasoconstriction, to maintain glomerular filtration. However, this increase in FF is associated with an increase in intraglomerular pressures, potentially leading to renal damage in the long term. In the current study, serelaxin resulted in a relative reduction of FF, thereby unloading the glomerulus, potentially leading to preservation of renal function over time.

The absence of improvement of GFR in our study is supported by the lack of marked differences in serum creatinine, blood urea nitrogen, and cystatin C values between the serelaxin- and placebo-treated CHF patients.

The increase in RPF in this study is further supported by an independent assessment, namely a PET study in a subset of patients. In the serelaxin-treated patients, serelaxin substantially increased RBF, both in the cortex and medulla, whereas no changes were found in the placebo-treated patients. These findings indicate that serelaxin increases renal perfusion and support the hypothesis that the effects of serelaxin on renal function can be explained by a direct intrarenal effect.

How could the observed change in renal hemodynamics support long-term preservation of renal function? The increase in RBF without change in GFR leads to a reduction in FF. In animal experiments in remnant kidney models, an elevated FF, indicating elevated glomerular pressure, consistently predicts a more pronounced future renal function decline, whereas reduction of FF, by renin–angiotensin–aldosterone system-blockade, which induces a rise in RBF with unchanged GFR, results in protection against renal function loss, assumed to be mediated predominantly by the decrease in filtration pressure.\textsuperscript{15} This renal hemodynamic pattern, which is elicited by afferent and predominantly efferent vasodilation, displays an attractive similarity to the current findings with serelaxin. However, no such data are available in heart failure, and substantial differences in renal hemodynamics between heart failure, where overall RBF is decreased, and remnant kidney, where neophron blood flow is increased, preclude straightforward extrapolation. In humans, moreover, longitudinal data on the prognostic effects of renal hemodynamics on outcome are virtually absent, because of the demanding nature of renal hemodynamic studies, which
conflits with the sample size needed for hard end point studies. Nevertheless, we found previously that a higher FF (low RBF relative to GFR) is an independent predictor of future renal function loss in human transplant recipients. To the best of our knowledge, this is the only human study that provides a direct analysis of the predictive effect of renal hemodynamics on renal and overall outcome. Interestingly, in this study in renal transplant recipients, a higher FF was also a predictor of mortality, and approximately half of the mortality was cardiovascular. A possible mechanism linking higher FF to worse overall outcome is the effect on renal sodium handling: elevated FF blunts renal sodium excretion by its effects on the peritubular Starling force. Of note, such a renal hemodynamic profile is not assumed to lead to an abrupt rise in sodium excretion, but rather to a gradual loss of sodium and water that evolves over several days.

In spite of the consistent data from the renal field, however, it is a limitation to the current study that the prognostic value of a particular renal hemodynamic pattern in heart failure patients has not been substantiated.

Serelaxin did not seem to have natriuretic and diuretic effects in this study because urinary flow rate and sodium excretion rate were even higher in patients treated with placebo. Given the observation that GFR was unchanged, and RPF increased, a reduction in sodium excretion and urinary flow rate is difficult to explain. Finally, similar to previous studies with serelaxin, the drug was well tolerated in this CHF population with a safety profile comparable to placebo.

This study has some limitations. First, the implausible outlier values of IOTH and PAH occurred in patients treated at 1 study site. Meticulous efforts were performed to find out the origin of these outliers, unfortunately without success. Before unblinding, a decision was taken, therefore, to exclude all 22 patients from this single site for the primary analysis, hence seriously compromising the statistical power of the study. Second, steady state of IOTH had not been reached by the start of study drug infusion, which may in part be explained by the physiological circadian rhythm in GFR. However, for the primary end point, the between-treatment difference was of interest and change between 8 and 24 hours was prespecified in this study. We do not feel that this lack of steady state altered the findings related to the changes between serelaxin and placebo groups, which were further corroborated by urinary IOTH and creatinine clearance results. Finally, serelaxin is currently under investigation in patients with AHF, whereas this study was performed in patients with CHF.

In conclusion, in patients with CHF, serelaxin increased RPF and reduced glomerular FF relative to placebo, but did not increase GFR. These data indicate a direct renal vasorelaxant effect of serelaxin.

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**CLINICAL PERSPECTIVE**

Worsening renal function complicates the treatment of acute and chronic heart failure and might lead to less aggressive treatments with life-saving therapies and poorer clinical outcomes. There is, therefore, a need for heart failure treatments that improve renal function as well. Serelaxin, recombinant human relaxin-2, is a promising and novel investigational therapy for the treatment of acute heart failure and has been associated with an improvement in clinical outcomes in randomized trials to date. In addition, treatment with serelaxin has been associated with lower serum creatinine and plasma cystatin C concentrations compared with placebo. However, prospective controlled studies on the renal hemodynamic effects of serelaxin in patients with heart failure, under controlled conditions using the gold standard methods for measuring glomerular filtration rate and renal plasma flow, are lacking. In this double-blind, randomized, placebo-controlled multicenter study in 65 systolic chronic heart failure patients, intravenous serelaxin 30 μg/kg per day for 24 hours increased renal plasma flow by 29%, compared with 14% on placebo (13% relative increase with serelaxin; P=0.04), whereas glomerular filtration rate did not differ significantly during 8 to 24 hours. Filtration fraction increased by 36% with serelaxin and 62% with placebo (16% relative decrease with serelaxin; P=0.002). Changes in systolic blood pressure were largely similar, and creatinine clearance did not differ between groups. Adverse event rates were similar with serelaxin (20.5%) and placebo (25.0%). The combination of an increased renal blood flow, unchanged glomerular filtration rate, and decreased filtration fraction suggests that serelaxin may have favorable effects on parameters of kidney function in patients with heart failure.
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Renal hemodynamic effects of serelaxin in patients with chronic heart failure: A randomized, placebo-controlled study

Voors. Renal hemodynamic effects of serelaxin in CHF

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5 Novartis Pharmaceuticals, East Hanover, NJ, USA
SUPPLEMENTAL MATERIALS

Supplementary Methods

Positron Emission Tomography/Computed Tomography

Positron Emission Tomography Tracer and Image Acquisition

Subjects were all scanned on a Biograph mCT-64 positron emission tomography/computed tomography (PET/CT) system (Siemens Medical Systems, TN, USA) with a PET axial field of view of 21 cm. A low-dose CT (LDCT) was used to correct for the PET signal attenuation, which is typically greater in deep areas or areas that are surrounded by relatively dense structure. This LDCT scan was also used for anatomical delineation of the kidneys and the abdominal aorta. Settings for the LDCT scan are based on the weight of the subject with default settings of 100 kV and 30 mAs.

At the imaging session day, approximately 800 MBq H$_2^{15}$O was produced on-line using a Scanditronix MC17 cyclotron and was administrated as an intravenous bolus of approximately 8.3 mL. Each subject had two tracer injections, the first one at baseline and the second one 24 hours later (placebo or serelaxin). A 10-minute dynamic PET was initiated immediately before the injection of H$_2^{15}$O and acquired in list-mode. Blood pressure and heart rate were measured during PET acquisition.

Image Reconstruction

For the kinetic analysis, dynamic PET images were reconstructed into 33 frames per scan with durations of 12x5s, 12x10s, 4x30s, and 5x60s, using an iterative Siemens uHD reconstruction approach with three iterations, 21 subsets, 5 mm Gaussian post-smoothing, and zoom 1 to a matrix of 400*400 with all corrections. For PET co-registration purposes, the LDCT scan (64 slices) with a transaxial scan field of view of 78 cm was reconstructed with
an axial field of view of 21 cm, matrix size of 512x512 (pixel size 0.98 mm), and a slice thickness of 2 mm using filtered back projection and noise filtered with a standard CT filter. Upon completion of the reconstruction, all images were reviewed for artifacts and motion.

**Image Processing and Analysis**

Before image processing, all scans were inspected to check alignment between the PET and CT images. The LDCT was fused with the H$_2^{15}$O PET scan using Syngo workstation software (version WinNT 5.2 Service Pack 2; Siemens Medical Systems) at baseline and after treatment administration separately. If appropriate, coordinates of fusion were registered to overcome misregistration during further analysis. The H$_2^{15}$O PET scan obtained after treatment administration was also fused with the baseline H$_2^{15}$O PET scan to ensure exactly aligned positions of the kidneys at the different time points.

Regions of interest were drawn on the fused H$_2^{15}$O PET/CT scans at baseline using Inveon Research Workplace (version 3.0; Siemens Medical Systems). Time activity curves for the cortex and medulla were provided by kidney (left, right, both) and anatomical location (upper, middle, and lower region, including all region analysis, all in 3D axis view). The same regions were copied into the H$_2^{15}$O PET/CT scans obtained after treatment administration to ensure the same regions and volumes will be analyzed.

For kinetic modeling, the arterial tracer input function was determined from dynamic PET measurements of the abdominal aortic activity. This method has been shown to be a suitable alternative to arterial blood sampling.¹ A small region of interest (5 voxels) was defined in the center of the aorta on the summed PET image, where all of the dynamic image frames are overlaid to generate one static image. Drawing of the region of interest was performed on the transaxial slices. On the caudal side, the region of interest is bounded one slice above the
aortic bifurcation. On the cranial side only the most upper slice was excluded. The time-activity curve of this region of interest was used as the arterial input function.

Calculation of Renal Perfusion

The selected regions of the kidneys were further analyzed in the modeling program PMOD (version 3.1, PMOD technologies Ltd, Zurich, Switzerland). Renal cortical and medullary blood perfusion (mL·min⁻¹·g⁻¹) of the different regions were estimated by fitting the PET measured time-activity curves to a validated one-compartment model for H₂¹⁵O² with the tracer concentration in the blood of the abdominal aorta as arterial input. The fitting program assumes a very high diffusion speed of H₂¹⁵O (making the tissue net uptake of H₂¹⁵O limited by perfusion) and yields the kinetic parameter K₁, which is indicative of tracer clearance from blood to tissue and in the case of H₂¹⁵O equals tissue perfusion (or here renal blood flow). Other parameters generated from this model are k₂, the rate constant describing the reverse transport of H₂¹⁵O, and V_blood the apparent blood volume. The blood volume gives the fraction of the measured volume, which has the same time-activity curve as plasma. In most tissues this is the capillary fraction. For the kidneys this may include (part of) the nephrons. Finally the distribution volume, calculated as V_T = K₁/k₂, gives the ratio of tissue to plasma concentration when tissue and plasma concentration are in equilibrium.
References


Supplementary Results

Table S1. Renal Plasma Flow (mL/min) Change from Baseline – All Sites and Excluding Site 3004

<table>
<thead>
<tr>
<th>Time point/interval (hours)</th>
<th>All sites</th>
<th>Excluding site 3004</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serelaxin LS-geomean ratio to baseline (SE)</td>
<td>Placebo LS-geomean ratio to baseline (SE)</td>
</tr>
<tr>
<td>2</td>
<td>1.56 (1.19)</td>
<td>0.99 (1.16)</td>
</tr>
<tr>
<td>4</td>
<td>1.76 (1.16)</td>
<td>0.94 (1.13)</td>
</tr>
<tr>
<td>6</td>
<td>1.67 (1.16)</td>
<td>0.87 (1.13)</td>
</tr>
<tr>
<td>8</td>
<td>1.51 (1.15)</td>
<td>0.91 (1.13)</td>
</tr>
<tr>
<td>20</td>
<td>1.11 (1.16)</td>
<td>1.19 (1.14)</td>
</tr>
<tr>
<td>22</td>
<td>1.11 (1.18)</td>
<td>1.20 (1.15)</td>
</tr>
<tr>
<td>24</td>
<td>1.53 (1.15)</td>
<td>1.20 (1.13)</td>
</tr>
<tr>
<td>26</td>
<td>1.57 (1.19)</td>
<td>1.11 (1.16)</td>
</tr>
<tr>
<td>28</td>
<td>1.62 (1.22)</td>
<td>1.16 (1.19)</td>
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<tr>
<td>0–24</td>
<td>1.06 (1.14)</td>
<td>0.97 (1.12)</td>
</tr>
<tr>
<td>8–24</td>
<td>1.03 (1.14)</td>
<td>1.01 (1.12)</td>
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<tr>
<td>24–28</td>
<td>1.50 (1.16)</td>
<td>1.09 (1.14)</td>
</tr>
</tbody>
</table>

All sites: N=39 (serelaxin); N=48 (placebo); Excluding site 3004: N=28 (serelaxin); N=37 (placebo).
LS-geomean* geometric least-squares mean.
* Serelaxin to placebo.
<table>
<thead>
<tr>
<th>Time point/interval (hours)</th>
<th>Serelaxin LS-geomean ratio to baseline (SE)</th>
<th>Placebo LS-geomean ratio to baseline (SE)</th>
<th>Ratio of LS-geomean ratios* (95% CI)</th>
<th>p-value</th>
<th>Serelaxin LS-geomean ratio to baseline (SE)</th>
<th>Placebo LS-geomean ratio to baseline (SE)</th>
<th>Ratio of LS-geomean ratios* (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
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<td>4</td>
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<td>2.13 (1.05)</td>
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<td>0.1371</td>
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</table>

All sites: N=39 (serelaxin); N=48 (placebo); Excluding site 3004: N=28 (serelaxin); N=37 (placebo).

LS-geomean, geometric least-squares mean.

* Serelaxin to placebo.
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<th>All sites</th>
<th>Excluding site 3004</th>
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</thead>
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<tr>
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<td>Serelaxin</td>
<td>Placebo</td>
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<td>LS-geomean ratio to baseline (SE)</td>
<td>LS-geomean ratio to baseline (SE)</td>
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<td>2</td>
<td>0.85 (1.12)</td>
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<td>1.58 (1.08)</td>
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<td>1.45 (1.10)</td>
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<td>1.83 (1.14)</td>
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<td>28</td>
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<td>1.79 (1.14)</td>
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<tr>
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<td>1.64 (1.10)</td>
<td>1.61 (1.08)</td>
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<tr>
<td>8–24</td>
<td>1.95 (1.11)</td>
<td>1.75 (1.09)</td>
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<tr>
<td>24–28</td>
<td>1.55 (1.13)</td>
<td>1.70 (1.11)</td>
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All sites: N=39 (serelaxin); N=48 (placebo); Excluding site 3004: N=28 (serelaxin); N=37 (placebo).
LS-geomean, geometric least-squares mean.

* Serelaxin to placebo.
Table S4. Creatinine Clearance – Excluding Site 3004

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Serelaxin geomean ratio to baseline (95% CI)</th>
<th>Placebo geomean ratio to baseline (95% CI)</th>
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<tbody>
<tr>
<td>2</td>
<td>1.05 (0.73, 1.51)</td>
<td>1.05 (0.84, 1.30)</td>
</tr>
<tr>
<td>4</td>
<td>1.06 (0.82, 1.37)</td>
<td>0.88 (0.70, 1.11)</td>
</tr>
<tr>
<td>6</td>
<td>0.87 (0.64, 1.18)</td>
<td>0.82 (0.69, 0.97)</td>
</tr>
<tr>
<td>8</td>
<td>1.06 (0.84, 1.34)</td>
<td>0.96 (0.83, 1.11)</td>
</tr>
<tr>
<td>20</td>
<td>0.86 (0.68, 1.10)</td>
<td>0.77 (0.67, 0.88)</td>
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<tr>
<td>22</td>
<td>1.30 (0.88, 1.92)</td>
<td>1.13 (0.89, 1.44)</td>
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<td>24</td>
<td>1.12 (0.88, 1.42)</td>
<td>0.98 (0.81, 1.19)</td>
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<tr>
<td>26</td>
<td>0.93 (0.65, 1.32)</td>
<td>1.02 (0.86, 1.22)</td>
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<td>28</td>
<td>1.40 (1.08, 1.80)</td>
<td>1.03 (0.84, 1.26)</td>
</tr>
<tr>
<td>0–24</td>
<td>1.04 (0.86, 1.26)</td>
<td>0.94 (0.81, 1.09)</td>
</tr>
<tr>
<td>8–24</td>
<td>1.01 (0.82, 1.23)</td>
<td>0.88 (0.76, 1.01)</td>
</tr>
<tr>
<td>24–28</td>
<td>1.14 (0.94, 1.39)</td>
<td>0.99 (0.81, 1.22)</td>
</tr>
</tbody>
</table>
Figure S1. Overlaying individual patient plasma para-aminohippuric acid profiles, all sites (A), and excluding site 3004 (B).
**Figure S2.** Overlaying individual patient plasma iothalamate profiles, all sites (A), and excluding site 3004 (B).
Figure S3. CONSORT flow of patient disposition.

IOTH, iothalamate; PAH, para-aminohippuric acid; PD, pharmacodynamic; PK, pharmacokinetic.
Figure S4. Reverse transport of $\text{H}_2^{15}\text{O}$ from blood to renal tissue (k2 in L/min), in medulla (A) and, cortex (B).
Figure S5. Cortical and medullary blood volume ($V_{\text{blood}}$ in L), in medulla (A) and, cortex (B).