B-type natriuretic peptide (BNP) and its cleavage equivalent amino terminal pro-B-type natriuretic peptide (NT-proBNP) have rapidly emerged as valuable biomarkers in cardiovascular medicine. Both the diagnostic accuracy as well as the prognostic value of these peptides have been established in patients across the spectrum of cardiovascular diseases, from apparently well patients to those with end-stage heart failure (1–5).

An important prerequisite for use of tests such as the natriuretic peptides is a firm understanding of the physiology that affects their levels. Among the factors that are known to affect both peptides is renal function. Indeed, despite widespread use of natriuretic peptide testing, there is still great uncertainty (and controversy) about the effect of renal function on concentrations of both BNP and NT-proBNP, and whether there is a difference between the 2 peptides in this regard (6,7).
What is known at present is that observational studies suggest that serum concentrations of BNP and NT-proBNP are both inversely related to glomerular filtration rate (GFR) (8–10), and it has therefore been proposed that these peptides accumulate with declining GFR (9). In addition, because the clearance of BNP also depends on neutral endopeptidases and the type-C natriuretic peptide receptor (11,12), the theory is that the NT-proBNP concentration is more affected by renal clearance than is BNP, a theory borne out by some studies suggesting a steeper relationship between GFR and NT-proBNP concentrations compared with BNP, although not all studies confirm this finding (13–16).

On the other hand, it is well acknowledged that there is a strong direct interaction between abnormalities in the cardiac and renal organ systems with prevalent cardiovascular disease among those with chronic kidney disease (CKD) (17,18). Thus, it has also been hypothesized that elevated serum concentrations of natriuretic peptides in patients with impaired renal function may very well reflect increased cardiac production because of cardiac dysfunction (19,20), which bears out their strong prognostic value in those with CKD (15,21).

Although these studies are of interest, they are all observational (and in most cases retrospective), rather than direct examinations of mechanistic relationships between renal function and both BNP and NT-proBNP. Given the widespread and growing use of these peptides, it is clearly necessary at this juncture to move toward a physiologic, mechanistic understanding of this question. Prior studies looking at the dependence on renal clearance of BNP and NT-proBNP failed to show any difference between the 2 peptides (22–27), but these studies were small and often focused on only 1 of the 2 peptides. Accordingly, we performed a prospective, mechanistic study comparing in a head-to-head fashion the renal clearance of BNP and NT-proBNP in a large cohort of patients.

Patients and Methods

Patients. This study was performed in 165 hypertensive patients in whom renal artery stenosis was initially suspected on the basis of 1 or more of the following criteria: treatment-resistant hypertension despite the use of at least 2 adequately dosed antihypertensive agents, overt peripheral vascular disease, the presence of an abdominal bruit, or an increase in serum creatinine during angiotensin-converting enzyme inhibitor treatment. Patients in whom significant renal artery stenosis was proven were not included in this study. Written informed consent was obtained from all patients, and the Medical Ethical Committee of the Maastricht University Hospital approved the study protocol. All antihypertensive medications were stopped 21 days before the catheterization of both renal arteries and veins was performed, with blood samples drawn at the same time for determination of BNP and NT-proBNP concentrations. The blood was centrifuged immediately and frozen at −80°C. Subsequently, mean renal blood flow (RBF) was measured selectively in both kidneys by the 133Xenon washout technique as described (28–30), expressed as ml/min/100 g renal tissue. Routine echocardiography (Sonos 5500, Hewlett Packard, Andover, Massachusetts) was performed according to standard clinical protocol within the 4-month period surrounding angiography. Left ventricular mass index was determined by the formula from Devereux et al. (31), left ventricular ejection fraction was calculated using end-diastolic volume and end-systolic volume measurements from apical 4- and 2-chamber views calculated by the Simpson method. Calculation of the diastolic filling velocity (e velocity) and the peak filling velocity of early atrial contraction (a velocity) were used for the determination of the e/a ratio.

Laboratory tests. The BNP concentrations were measured using an established radioimmunoassay method (Shionoria, Shionogi, Japan). The NT-proBNP concentrations were determined by an immunoelectrochemiluminescence method (Elecsys 2010, Roche Diagnostics, Basel, Switzerland). Creatinine concentrations were assessed by a modified alkaline picate method (Synchron LX 20, Beckman, Fullerton, California). The GFR was calculated using the simplified Modified Diet in Renal Disease formula (32). Calculations and statistics. The fractional extraction (FE) of a molecule is the relative difference between the arterial concentration and the venous concentration of a molecule over a vascular bed or organ. Renal FE of substance X (FEX) was calculated as [(arterial concentration− venous concentration)/arterial concentration]. The serum NT-proBNP/BNP ratio was computed by dividing the arterial NT-proBNP concentration by the arterial BNP concentration. The FENT-proBNP/BNP ratio in each kidney was defined by dividing the FE-NT-proBNP by the FE-NT-proBNP. Renal plasma flow (RPF) was calculated as: RBF × (1 − hematocrit). Total RPF was calculated as the sum of left and right RPF.

For statistical analyses, SPSS 11.5 (SPSS Inc., Chicago, Illinois) software was used. Data are presented as medians with interquartile ranges (IQRs) for nonnormally distributed variables (identified when skewness was more than double its standard error of the mean and compared using the Mann-Whitney U test) and mean ± SD for all normally
distributed continuous variables, which were compared using analysis of variance. Pearson r (r) was used to test correlations when normally distributed. Non-normal variables were log-transformed and/or evaluated with Spearman rho (rs), rather than Pearson r. The 2-tailed independent Student t test was used in normal distributed parameters, whereas the chi-square test was used for categorical variables. Multivariable linear regression analyses identified independent predictors of arterial BNP and NT-proBNP concentrations. Differences or correlations were considered significant when p < 0.05.

**Results**

**Baseline characteristics.** Renal angiography was successfully performed in all 165 subjects. Baseline characteristics are shown in Table 1; 45 subjects (27%) had a GFR < 60 ml/min/1.73 m². Compared with those with better renal function, subjects with a GFR ≤ 60 ml/min/1.73 m² were older, had significantly lower RBF, e/a ratio, and hematocrit, and had higher NT-proBNP and BNP serum concentrations, whereas there were no significant differences in mean arterial blood pressure (MAP) or left ventricular ejection fraction.

**Natriuretic peptide correlations.** Serum concentrations of BNP correlated directly with simultaneous values of NT-proBNP (r = 0.79, p < 0.001) (Fig. 1), whereas correlations between GFR and serum concentrations of BNP and NT-proBNP were significant, although less robust (r = −0.35 and r = −0.30, respectively; p < 0.001 for both).

**Renal clearance of BNP and NT-proBNP.** Both left and right RPF correlated with GFR (r left = 0.46 and r right = 0.45, respectively; both p < 0.001). Despite the stronger correlation between BNP and GFR, we did detect a small but higher increase in NT-proBNP when GFR decreased, reflected by the negative correlation between the serum NT-proBNP/BNP ratio and GFR (r = −0.21, p = 0.008) (Fig. 2).

Median FE BNP was 0.21 (IQR 0.16 to 0.22) for left kidneys and 0.22 (IQR 0.17 to 0.29) for right kidneys (p = 0.43). As shown in Table 2, FE BNP correlated with GFR in both left and right kidneys (r left = 0.26, p = 0.008 in left kidneys and r right = 0.21, p = 0.03 in right kidneys). The median FE NT-proBNP was 0.16 (IQR 0.09 to 0.20) for left kidneys and 0.18 (IQR 0.12 to 0.22) for right kidneys (p = 0.07). The FE NT-proBNP correlated with GFR in both kidneys as well (left: r left = 0.25, p = 0.005; right: r right = 0.20, p = 0.02) (Figs. 3A and 3B).

To compare the renal clearances of BNP and NT-proBNP, we looked at the association between FE of both peptides within the same kidney. The FE BNP and FE NT-proBNP correlated highly in the left kidney as well as in the right kidney..

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**Table 1** Baseline Characteristics of All Patients, Divided According to GFR

<table>
<thead>
<tr>
<th></th>
<th>GFR &gt; 60 ml/min/1.73 m²</th>
<th>GFR ≤ 60 ml/min/1.73 m²</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 120)</td>
<td>(n = 45)</td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>54 ± 12</td>
<td>62 ± 10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>64 (53)</td>
<td>18 (40)</td>
<td>0.26</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27 ± 4</td>
<td>27 ± 5</td>
<td>0.51</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>133 ± 22</td>
<td>139 ± 26</td>
<td>0.17</td>
</tr>
<tr>
<td>e/a ratio</td>
<td>1.01 ± 0.39</td>
<td>0.79 ± 0.22</td>
<td>0.002</td>
</tr>
<tr>
<td>Left ventricular mass index (g/m²)</td>
<td>106 ± 24</td>
<td>116 ± 34</td>
<td>0.09</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (%)</td>
<td>61 ± 7</td>
<td>61 ± 10</td>
<td>0.95</td>
</tr>
<tr>
<td>Serum BNP (pg/ml)</td>
<td>20 (9–40)</td>
<td>65 (26–159)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum NT-proBNP (pg/ml)</td>
<td>129 (44–220)</td>
<td>473 (173–1,266)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>78 ± 17</td>
<td>131 ± 55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>0.44 ± 0.05</td>
<td>0.41 ± 0.07</td>
<td>0.006</td>
</tr>
<tr>
<td>Left renal blood flow (ml/min/100 g)</td>
<td>203 ± 68</td>
<td>147 ± 66</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Right renal blood flow (ml/min/100 g)</td>
<td>242 ± 67</td>
<td>182 ± 44</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are given as mean ± SD for parametric parameters and as median with interquartile range for nonparametric parameters. BNP = B-type natriuretic peptide; GFR = glomerular filtration rate; NT-proBNP = amino terminal pro-B-type natriuretic peptide.
In addition to study differences in changes in FE possibly related to GFR, we calculated the FE_{NT-proBNP}/BNP ratio in each kidney. The median FE_{NT-proBNP}/BNP ratio was 0.69 (IQR 0.52 to 0.95) in the left kidney and 0.77 (IQR 0.61 to 1.00) in the right kidney. Both the left FE_{NT-proBNP}/BNP ratio and the right FE_{NT-proBNP}/BNP ratio did not correlate with GFR, as shown in Table 2 and Figure 4 (r_s = 0.10, p = 0.30 and r_s = −0.08, p = 0.43, respectively).

### Prediction of natriuretic peptide concentrations.

Although univariate analyses showed a correlation between natriuretic peptide levels and FEs or RPF, multivariate analyses showed that FE_{BNP} and FE_{NT-proBNP} were not independent predictors of serum concentrations of these peptides (Table 3). However, left ventricular mass index, MAP, and GFR independently predicted both peptides, whereas RPF independently predicted BNP values and showed a strong trend in predicting NT-proBNP concentrations. The GFR was slightly more strongly associated with NT-proBNP than BNP levels, but RPF was a stronger predictor for BNP concentrations.

### Discussion

In this study, we prospectively compared the renal clearance of BNP and NT-proBNP in 165 hypertensive subjects. We found that both BNP and NT-proBNP are indeed cleared by the kidneys in a moderate fashion, yet interestingly and importantly, we found an equal dependence on renal clearance for both peptides across the range of renal function examined in this study. These results, generated from a mechanistic rather than observational fashion, are robust evidence contra-
dicting prevalent notions about a difference between BNP and NT-proBNP regarding their renal handling (6,14).

Considering the problem from a more scientific point of view, our results may not be surprising, although our data are in stark contrast to what is currently accepted in the literature based on causal assumptions. Indeed, based on their molecular weight, both BNP (3.5 kDa) and NT-proBNP (8.5 kDa) are by definition small molecular weight proteins (SMWPs), which includes all proteins with a molecular weight ranging from 1 to 50 kDa (33–35). The SMWPs are filtered relatively freely by the glomeruli and catabolized by tubular epithelial cells without any other processing such as tubular secretion as in creatinine (0.1 kDa) or active reabsorption and return in the circulation as for albumin (69 kDa). The FE of a SMWP is mostly determined by its molecular weight, whereas steric and electrostatic factors also may play a role (34). When filtration is not influenced at all, the FE equals the filtration fraction, which is usually in the range of 0.20 to 0.25 in healthy subjects (23,36,37), and is approximated by the FE of the FEs we found. Lending further credibility to our results, based on the available theoretical models for renal SMWP clearance, a prior hypothetical study suggested that if clearance was purely based on molecular weight, filtration of BNP molecules would be 1.34 to 1.50 times that of NT-proBNP, which is strikingly consistent with the median FE_{NT-proBNP/BNP} ratios of 0.69 and 0.77 we described (38). In addition, the FE_{NT-proBNP/BNP} ratios did not correlate with GFR, which indicates that the renal clearance of both molecules is equally influenced by renal function.

Renal clearance of a molecule is defined by the FE of the molecule and the RPF of the subject who is clearing the molecule; the latter parameter resembles the proportion of the circulating volume that is directed to the kidneys to be cleared in the manner as determined by the FE. Naturally, RPF is a factor that all molecules that are renally cleared have in common. In our multivariate analyses, FE was not shown to be an independent predictor of serum levels of BNP and NT-proBNP, whereas left ventricular mass index, MAP, GFR, and RPF were found to be independently related to BNP and NT-proBNP concentrations. Although, as mentioned previously, caution is needed when concluding causality merely based on associations, the independent association with RPF might (partially) reflect the molecular nonspecific part of the clearance, whereas left ventricular mass index may reflect cardiac production of BNP and NT-proBNP.

In sum, our data indicate that in most patients tested with BNP or NT-proBNP (described by those with a GFR >30 ml/min/1.73 m²), serum concentrations of BNP and NT-proBNP may be more determined by cardiac production than renal clearance, but there is no difference in the renal clearance between the 2 peptides.

At the lowest extreme of renal function in our study, we did observe an inverse correlation between the NT-proBNP/BNP ratio and GFR, which may be suggestive of a difference in removal from the blood stream between these 2 peptides at a range of GFR below 30 ml/min/1.73 m². Indeed, also in our population, the NT-proBNP/BNP ratio increased when GFR decreased, as previously shown by others (14,39). It is worthwhile to speculate about alternative possibilities that may cause this discrepancy, including increased extrarenal clearance of BNP in the context of declining renal function; indeed, neutral endopeptidases seem to accumulate in the context of renal failure (40), whereas up-regulation of the type-C natriuretic peptide receptor has been observed in more advanced disease states as well (41,42). Both would more reduce concentrations of BNP relative to NT-proBNP in this context, as has also been suggested (43,44). In addition, as suggested in our Figure 2 but also by the same analyses by Vickery et al. (14) and Kemperman et al. (39), heteroskedasticity cannot be ruled out. However, rather than speculation, what is needed now is a mechanistic understanding regarding both the physiology of extrarenal clearance of BNP, as well as NT-proBNP.

While contradicting a widely held notion (albeit based on observational data) that BNP is less dependent than NT-proBNP on renal function for removal from the circulation, we concede that an important limitation of our study is that most subjects had a GFR =30 ml/min/1.73 m². This may

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Univariate Analysis of Correlations Between Serum Concentrations of BNP or NT-proBNP Versus Biochemical, Demographic, Renal, and Echocardiographic Parameters</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>BNP</td>
</tr>
<tr>
<td></td>
<td>Univariate</td>
</tr>
<tr>
<td>Age</td>
<td>r = 0.22</td>
</tr>
<tr>
<td>Body mass index</td>
<td>r = -0.10</td>
</tr>
<tr>
<td>MAP</td>
<td>r = 0.31</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>r = -0.21</td>
</tr>
<tr>
<td>GFR</td>
<td>r = -0.35</td>
</tr>
<tr>
<td>Left RPF</td>
<td>r = -0.31</td>
</tr>
<tr>
<td>Right RPF</td>
<td>r = -0.42</td>
</tr>
<tr>
<td>Total RPF</td>
<td>r = -0.38</td>
</tr>
<tr>
<td>Left FE</td>
<td>r = -0.28</td>
</tr>
<tr>
<td>Right FE</td>
<td>r = -0.26</td>
</tr>
<tr>
<td>LVEF</td>
<td>r = -0.20</td>
</tr>
<tr>
<td>LVMI</td>
<td>r = 0.42</td>
</tr>
<tr>
<td>e/a ratio</td>
<td>r = -0.28</td>
</tr>
</tbody>
</table>

FE = fractional extraction; LVEF = left ventricular ejection fraction; LVMI = left ventricular mass index; MAP = mean arterial pressure; RPF = renal plasma flow; other abbreviations as in Table 1.
be germane; however, the great majority of patients reported in studies of BNP or NT-proBNP have renal function closely parallel to that in our study (1,2). Further, in studies of patients across a much wider range of renal function, correlations between BNP and NT-proBNP remained strong even down to the end stage of CKD (15,16). Another limitation of our study is that we analyzed patients with hypertension, rather than patients with heart failure. Although other much smaller mechanistic studies were not performed in heart failure patients either (22–27), our data actually provide insight to the clearance of BNP or NT-proBNP in a population of patients in which these peptides have been suggested to be of value for detection of cardiac structural abnormalities and provide prognostic value (45–47). The fact that BNP and NT-proBNP concentrations are higher in heart failure patients within the same range of GFR as our patients only strengthens the fact that these concentrations may reflect cardiac production rather than impaired clearance. As the use of both BNP and NT-proBNP expands across the spectrum of the American Heart Association stages of heart failure (from “at risk” to “end stage”), our findings are of value and quite germane to the application of these assays.

Acknowledgments
The authors thank Roche Diagnostics for sponsoring the NT-proBNP kits for this study.

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Key Words: natriuretic peptides • NT-proBNP • BNP • kidney • renal function.