Kidneys extract BNP and NT-proBNP in healthy young men

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METHODS

The study was carried out in 10 healthy young men [age: 25 (median) yr (range: 21–28), height: 182 cm (175–195), weight: 81 kg (69–90)], i.e., negative history of cardiovascular and kidney diseases with physical examination being normal, and none of the subjects took medication at the time of the study. The experimental protocol was approved by the Ethics Committee of Copenhagen (KF 11-110/03), and written, informed consent was obtained according to the Declaration of Helsinki.

Each subject was investigated on 1 experimental day in three sessions including a 30-min baseline in the supine position, 20 min semirecumbent seated on a bicycle with moderate exercise from minutes 5 to 20, and 30-min recovery in the supine position. Arterial and venous blood samples were drawn at the end of each session. No complications occurred.

Exercise. Exercise was performed on a modified cycle ergometer in a semisupine position with the workload adjusted to elicit a target metabolism of NT-proBNP (10). Renal excretion is suggested, because plasma concentrations of NT-proBNP correlate with plasma concentrations of creatinine, and NT-proBNP is detectable in urine (9, 27, 28), whereas the correlation is weak between plasma concentrations of BNP and creatinine (24). In addition, plasma concentrations of NT-proBNP are more affected by age than that of BNP, which may be explained by an age-dependent decrease in the glomerular filtration rate (GFR) (24). However, nonsignificantly different renal extraction ratios [arterial concentrations − venous concentrations]/arterial concentrations] of BNP and NT-proBNP have been reported in cardiac patients undergoing catheterization, suggesting that the kidneys extract BNP and NT-proBNP to a similar extent in patients with elevated levels of BNP and NT-proBNP owing to cardiac disease (10). Renal extraction ratios of BNP and NT-proBNP may, however, be changed during pathophysiological conditions. It is therefore important to know the magnitudes of renal extraction ratios in healthy humans, as they are presently unknown.

Regional extraction ratios of BNP have been described for most organs (8, 10, 17, 33), but the cerebral extraction ratios of BNP have not yet been reported. In the brain, BNP may be metabolized by endopeptidases and/or clearance receptors in the vascular epithelium, which has been shown to be the case in the limb (10, 17, 33).

Therefore, the magnitudes of renal extraction ratios of BNP and NT-proBNP were determined in young healthy men. In addition, we evaluated cerebral extraction ratios and the effect of short-time moderate exercise on all parameters.

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heart rate of ~140 beats/min and rating of perceived exertion (scale from 6 to 20) (1) of 14–15, corresponding to “moderately hard.”

**Catheterization.** A catheter (Super Turque Plus, Cordis, Miami, FL; 6-Fr, 0.97 mm) was advanced through a sheath in the left femoral vein (7-Fr, Cordis), placed in the right or left renal vein under fluoroscopy guidance, and verified with bolus injection of contrast medium (Omnipaque, Amersham Health, Oslo, Norway; 240 mg/ml). Also, a catheter was placed in the brachial artery of the nondominant arm (1.1 mm; 20 gauge). Venous blood from the brain was obtained by a catheter advanced through the right internal jugular vein (2.2 mm; 14 gauge) with the tip in the superior venous bulb.

**Cardiovascular variables.** Mean arterial pressure (MAP) was measured in the brachial artery with a Bentley transducer (Uden, The Netherlands) positioned at the level of the right atrium and connected to a pressure-monitoring system (Hewlett-Packard M1275A, Loveland, CO). Beat-to-beat data acquisitions of cardiovascular variables were collected by a personal computer with customized software. The heart rate (HR), cardiac output (CO), and stroke volume (SV) were calculated from the blood pressure waveform using the Modelflow software (Scope version 1.0 software, TNO-TPD, Biomedical Instrumentation, Amsterdam, The Netherlands). MAP, CO, SV, and HR were determined at the end of each session.

**NT-proBNP, BNP, and creatinine assays.** Plasma concentrations of NT-proBNP and BNP in plasma were measured by highly sensitive and specific immunoassays (26). Plasma concentrations of creatinine were measured by an enzymatic calorimetric method (11).

**Extraction ratios and estimated GFR.** Renal and cerebral extraction ratios of BNP and NT-proBNP were determined as (arterial concentration BNP/NT-proBNP – renal vein concentration BNP/NT-proBNP)/arterial concentration BNP/NT-proBNP and (arterial concentration BNP/NT-proBNP – jugular vein concentration BNP/NT-proBNP)/arterial concentration BNP/NT-proBNP, respectively. Estimated GFR was determined by the formula of Gault and Crockoft: (140 – age) × weight in kg/serum concentrations in creatinine measured in micromol/l × a constant (men: 1.25; women: 1.03) (3a). Statistics.** Data are presented as means ± SE, and the level of significance was chosen at P < 0.05. A two-way ANOVA for repeated measures with a variable as the dependent factor and session and subjects as explanatory factors was used to evaluate the effects of the sessions on the variable. Differences were evaluated by a post hoc multiple-range test (Tukey). Differences between values at selected points in time were evaluated by a paired two-sided test (parametric) and by a Wilcoxon’s signed-rank test (nonparametric). For data that were not normally distributed (plasma concentrations of BNP and NT-proBNP), log transformation was performed.

**RESULTS**

**Natriuretic peptides.** Arterial concentrations of BNP and NT-proBNP did not increase during exercise (P > 0.05; Fig. 1). The values during the three sessions of NT-proBNP were 1.2 ± 0.19 pmol/l (Baseline), 1.4 ± 0.19 pmol/l (Exercise), and 1.5 ± 0.23 pmol/l (Recovery), and values were 14.0 ± 2.4 pmol/l (Baseline), 15.4 ± 2.7 pmol/l (Exercise), and 13.6 ± 2.1 pmol/l (Recovery) for BNP. Arterial concentrations of NT-proBNP were higher than renal venous concentrations during Baseline and Exercise (1.2 ± 0.19 vs. 1.1 ± 0.19 pmol/l and 1.4 ± 0.19 vs. 1.2 ± 0.19 pmol/l) (P < 0.05) but did not reach significance during recovery (1.5 ± 0.23 vs. 1.4 ± 0.20 pmol/l) (P > 0.05). Arterial concentrations of BNP were higher than renal venous concentrations during all sessions (14.0 ± 2.4 vs. 10.0 ± 2.0 pmol/l, 15.4 ± 2.7 vs. 11.7 ± 2.3 pmol/l, and 13.6 ± 2.1 vs. 11.0 ± 1.9 pmol/l) (P < 0.05; Fig. 1). The renal extraction ratio values of NT-proBNP were 0.19 ± 0.05, 0.21 ± 0.06, and 0.12 ± 0.03 during the three sessions (P > 0.05), and renal extraction ratio of BNP values were 0.18 ± 0.07, 0.15 ± 0.11, and 0.14 ± 0.06 (P > 0.05). Renal extraction ratios of BNP and NT-proBNP did not differ (P > 0.05; Fig. 2). Arterial concentrations of BNP and NT-proBNP were not different from jugular venous concentrations during the three sessions (NT-proBNP: 1.2 ± 0.19 vs. 1.2 ± 0.22 pmol/l, 1.4 ± 0.19 vs. 1.4 ± 0.22 pmol/l, and 1.5 ± 0.24 vs. 1.5 ± 0.23 pmol/l; BNP: 14.0 ± 2.4 vs. 14.1 ± 2.1 pmol/l, 15.4 ± 2.7 vs. 12.7 ± 2.7 pmol/l and 13.6 ± 2.1 vs. 13.9 ± 2.2 pmol/l) (P > 0.05), and cerebral extraction ratios varied insignificantly between −0.21 ± 0.15 and 0.11 ± 0.08 for NT-proBNP and between −0.02 ± 0.04 and 0.05 ± 0.06 for BNP (Table 1).

**Cardiovascular variables and estimated glomerular filtration.** During exercise CO increased by 13 ± 1 l/min, SV by 36 ± 6 ml, HR by 73 ± 7 beats/min, and MAP by 11 ± 3 mmHg (P < 0.05) (see Table 2). Calculated total peripheral resistance (TPR) decreased by 9 ± 1 mmHg·min⁻¹·l⁻¹ (P < 0.05). Estimated GFRs were not affected by exercise and varied insignificantly between 167 ± 6 and 175 ± 8 ml/min during the three sessions (P > 0.05).

**DISCUSSION**

The main finding of this experiment is that the renal extraction ratio of both BNP and NT-proBNP is ~0.15–0.20. There is no cerebral extraction, and short-term moderate exercise does not affect these values.
Renal extraction ratios of BNP and NT-proBNP. Arterial concentrations of BNP and NT-proBNP were higher than renal venous concentrations, indicating that both peptides are extracted by the kidneys, but renal extraction ratios of BNP and NT-proBNP did not differ. In agreement with our results, Hunt et al. (10) did not detect differences between renal extraction ratios of BNP and NT-proBNP in ~60-yr-old cardiac patients, and the magnitudes of renal extraction ratios were ~0.13 for BNP and 0.22 for NT-proBNP. We determined comparable renal extraction ratios in young healthy subjects. Taken together, the results by Hunt et al. along with our data suggest that renal extractions of BNP and NT-proBNP do not differ and appear to be independent of age and cardiac disease. However, changes below the detection limits (5–10%) in renal extraction ratios may induce biological differences, thereby posing a risk of type II error. Additionally, the amount of BNP and NT-proBNP metabolized by the kidneys may decrease with age and cardiac disease, because renal clearance (extraction ratio × renal blood flow) depends on renal blood flow, which declines with age and cardiac disease (18, 19).

Renal extraction ratio of BNP was determined by Lainchbury et al. (17) and Richards et al. (33) to be ~0.20 in patients referred for cardiac catheterization, which is in accordance with our results in healthy young men and the results by Hunt et al. (10). Seemingly, ~15–20% of the BNP delivered to the kidneys is extracted. The renal extraction of BNP and NT-proBNP arises as a combination of glomerular filtration, tubular reabsorption, and renal secretion (22) and other renal processes e.g., clearance by endopeptidases in the glomeruli (34) or clearance by natriuretic peptide C receptor (NPR-C) receptors in the renal vascular endothelium (23). The present study does not identify the responsible mechanisms. The kidneys can produce BNP during pathophysiological conditions (12) and mRNA for BNP is expressed in cultured human proximal tubular cells (25). Thus peptide secreted into the bloodstream may affect the renal extraction ratios. However, whether the kidneys produce BNP in healthy humans is unknown. Metabolism of BNP and NT-proBNP by endopeptidases could also affect the magnitude of renal extraction ratios (34), but Lainchbury et al. did not observe an effect after administration of an endopeptidase inhibitor. Renal extraction ratios of BNP and NT-proBNP in healthy young men may therefore reflect the sum of glomerular filtration, tubular reabsorption, and clearance by NPR-C receptors.

Cerebral extraction ratios of BNP and NT-proBNP. Cerebral extraction ratios of BNP and NT-proBNP are, to our knowledge, unknown in humans. They varied insignificantly between −0.02 ± 0.04 and 0.05 ± 0.06 and −0.21 ± 0.15 and 0.11 ± 0.09, respectively, and arterial and jugular venous concentrations were not different, indicating that the peptides are not metabolized in the brain. However, mRNA of natriuretic peptides is localized in the brain (5), and it can therefore not be excluded that BNP is secreted and metabolized to a similar extent in the brain.

Cardiovascular and endocrine response to exercise. Exercise was performed to stress the cardiovascular system. Previous experiments in healthy subjects have shown that venous plasma concentrations of BNP are unaffected by such a stimulus (14, 29, 36), but the effects on arterial concentrations and regional extraction ratios of BNP and NT-proBNP are unknown. We did not observe an increase in arterial concentrations of BNP and NT-proBNP or a change in renal and cerebral extraction ratios of BNP and NT-proBNP, indicating that BNP and NT-proBNP release and renal and cerebral extraction ratios were unaffected by moderate exercise. Other conditions of tachycardia and dilatation of the atria and ventricles raise plasma concentrations of the natriuretic peptides (2, 30, 37), but this seems not to be the case for dynamic exercise in healthy subjects (14, 29, 36). However, with longer duration and/or strenuous exercise imposing a greater stress on the cardiovascular system than inferred by 15 min of moderate exercise as in the present study, plasma concentrations of BNP and NT-proBNP may increase and/or renal and cerebral extraction ratios may change. Calculated TPR decreased during exercise. The unaffected release of BNP combined with the large decrease in TPR indicates that factors other than increased BNP release initiate the vasodilatation during physical exercise.

Plasma concentrations of BNP and NT-proBNP. In heart failure patients and elderly individuals, plasma concentrations of NT-proBNP are normally higher than plasma concentrations

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**Table 1. Cerebral extraction ratios of BNP and NT-proBNP**

<table>
<thead>
<tr>
<th>Ext-Cer BNP</th>
<th>Baseline</th>
<th>Exercise</th>
<th>Recovery</th>
</tr>
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<tbody>
<tr>
<td>0.05±0.06</td>
<td></td>
<td>−0.02±0.04</td>
<td>−0.01±0.03</td>
</tr>
<tr>
<td>Ext-Cer NT-proBNP</td>
<td>−0.21±0.15</td>
<td>0.11±0.08</td>
<td>−0.07±0.09</td>
</tr>
</tbody>
</table>

Values are means ± SE. Ext-Cer BNP; cerebral extraction ratio of brain natriuretic peptide (BNP); Ext-Cer NT-proBNP; cerebral extraction ratio of NH₂-terminal-proBNP (NT-proBNP).

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**Table 2. Cardiovascular variables and estimated GFR**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Exercise</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>98±2</td>
<td>108±3†</td>
<td>94±2</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>6.9±0.3</td>
<td>19.9±1.2†</td>
<td>6.6±0.3</td>
</tr>
<tr>
<td>TPR, mmHg/min−1</td>
<td>14±1</td>
<td>6±0.3†</td>
<td>14±1</td>
</tr>
<tr>
<td>SV, ml</td>
<td>107±6.7</td>
<td>144±6.6†</td>
<td>88±6.6†</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>66±3</td>
<td>139±5†</td>
<td>78±3†</td>
</tr>
<tr>
<td>eGFR, ml/min</td>
<td>175±8</td>
<td>167±6</td>
<td>174±8</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 subjects. MAP, mean arterial pressure; CO, cardiac output; TPR, calculated total peripheral resistance; SV, stroke volume; HR, heart rate; eGFR, estimated glomerular filtration rate. *Significant difference (P < 0.05) between exercise and recovery. †Significant difference (P < 0.05) compared with baseline.
of BNP (16, 17, 26). In this experiment we observed the opposite in young healthy test subjects. The measured plasma concentrations are in accordance with the values in young healthy blood donors reported by Abbott (BNP) and Roche Diagnostics (NT-proBNP), and the observed extraction ratios and response to exercise are in accordance with previous experiments (10, 14, 17, 29, 33, 36). Therefore, our results seem reasonable. It could therefore be hypothesized that metabolism of BNP and NT-proBNP changes with age. However, the relationship between BNP and NT-proBNP in healthy humans should be further elucidated with different assays in similar subjects before any firm conclusions regarding plasma levels of brain natriuretic peptides in young healthy individuals are made.

Limitations. We did not measure renal and cerebral blood flows in this experiment. Therefore, we cannot calculate the amounts of BNP and NT-proBNP metabolized by the kidneys and brain and cannot determine the renal extraction ratios of BNP and NT-proBNP in these persons.

Perspectives. Our data suggest that the kidneys clear (renal clearance × renal blood flow) BNP and NT-proBNP to a similar extent. Total body clearances of BNP and NT-proBNP are unknown. If total body clearance of BNP is larger than total body clearance of NT-proBNP, as indicated by the shorter half-life of BNP (30), renal clearance of NT-proBNP may be relatively more important than renal clearance of BNP for total body clearance. Total body clearances and renal clearances of BNP and NT-proBNP should therefore be determined in younger and older healthy subjects, obese people, and women and during pathophysiological conditions, e.g., during mild, moderate, and severe renal failure and heart failure. Furthermore, NT-proBNP's affinity for NPR-C receptors and endopeptidases should be investigated, because decreased affinity for those compared with BNP rather than decreased renal clearance may explain the increased half-life of NT-proBNP (30) and the age-, sex-, and weight-dependent differences (32, 38).

In conclusion, the renal extraction ratio of both BNP and NT-proBNP is ~0.15–0.20. There is no cerebral extraction, and short-term moderate exercise does not affect these values. Our findings suggest that the kidneys extract BNP and NT-proBNP to a similar extent in healthy young men.

ACKNOWLEDGMENTS

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REFERENCES


