Urotensin II Immunoreactivity in the Human Circulation: Evidence for Widespread Tissue Release

Yen-Hsing Chen,1 Timothy G. Yandle,1 A. Mark Richards,1 and Suetonia C. Palmer1*

BACKGROUND: The sources of secretion and clearance of plasma urotensin II (UII) in the human circulation remain uncertain and may be relevant to understanding the role of UII in human physiology and cardiovascular disease.

METHODS: In 94 subjects undergoing clinically indicated cardiac catheterization, we collected blood samples from arterial and multiple venous sites to measure transorgan gradients of plasma UII immunoreactivity.

RESULTS: Net UII release occurred (in descending order of proportional transorgan gradient) across the heart, kidney, head and neck, liver, lower limb, and pulmonary circulations ($P < 0.01$). Although no specific clearance site was localized, the absence of an overall subdiaphragmatic aorto-caval peptide gradient indicated that there were lower body segment sites of UII clearance as well as secretion. The proportional increase in UII immunoreactivity was significantly correlated across all sites of net peptide release within an individual ($P \leq 0.05$). In univariate analyses, mixed venous UII concentrations were correlated with diagnosis of acute coronary syndrome and femoral artery oxygen tension and inversely with systolic blood pressure and body mass index. Diagnosis of acute coronary syndrome and body mass index were independent predictors of mixed venous UII immunoreactivity in multivariate analysis. No correlates of net cardiac UII release were identified.

CONCLUSIONS: UII is secreted from the heart and multiple other tissues into the circulation. Related increments in UII immunoreactivity across multiple tissue sites suggest that peptide release occurs via a shared mechanism. Increased UII immunoreactivity is observed in subjects with acute coronary syndrome.

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Materials and Methods

SUBJECTS

We enrolled 94 unselected consecutive adults [mean age 63.6 (SD 8.8) years] undergoing clinically indicated...
cardiac catheterization for coronary angiography (n = 33), percutaneous coronary intervention (n = 59), or right and left heart study (n = 2) for additional sampling of arterial and venous blood to yield multiple transtissue gradients for UII immunoreactivity. For subjects admitted with an acute coronary syndrome, blood sampling occurred during the index admission after symptom onset. Exclusion criteria included age <18 years, anemia, end-stage kidney disease, or systolic blood pressure <100 mmHg. The protocol was conducted under approval from a regional ethics committee (Ministry of Health, New Zealand) in compliance with the Declaration of Helsinki; all subjects provided written and informed consent. We compared peripheral venous plasma UII concentrations (femoral vein) with those in 40 age- and sex-matched healthy individuals (age 46–79 years) recruited randomly from the local electoral roll.

STUDY DESIGN
The right femoral artery and vein were cannulated (6F sheaths) under local anesthesia. As a multipurpose diagnostic catheter (6F) was advanced to the pulmonary artery, blood samples were drawn sequentially from the femoral, renal, and hepatic veins, high inferior vena cava, internal jugular vein, and pulmonary artery. The position of the catheter tip at the time of sampling was at least 1–2 cm within the vessel of interest, as confirmed by a small-volume retrograde radio-contrast injection. Blood sampling was performed after aspiration of approximately 4 times the dead-space volume of the catheter (2 mL). We then use an Amplatz left-1 diagnostic catheter (6F) to cannulate and draw blood samples from the coronary sinus (n = 77). Blood samples were obtained from the femoral artery before and after completion of venous sampling to assess stability of plasma UII levels over the time required for multisite venous sampling (20 min). Intravenous saline, up to 500 mL, was administered throughout the procedure.

We measured femoral and pulmonary arterial pressures concurrently with blood sampling and measured cardiac output by the Fick technique. A 6F pigtail catheter was advanced to the left ventricle for measurement of left ventricular end-diastolic pressure (LVEDP). A cardiologist (A.M. Richards) adjudicated the ischemic burden of coronary artery disease using the Brandt score of myocardial jeopardy (14). Trans-thoracic echocardiography was obtained in 83 (88%) patients within 3 weeks of sampling according to a standardized imaging protocol (15).

PEPTIDE ASSAYS
Blood samples were drawn into prechilled tubes containing EDTA and centrifuged within 20 min at 3000 g, 4 °C for 10 min. The plasma supernatant was separated and stored at −80 °C until extraction and RIA for UII using established methods (9). Within- and between-assay CVs were 12.0% and 11.7%, respectively, at 3.1 pmol/L. Cross reactivities with human UII (100%) in our assay, using commercial UII antiserum (Phoenix Pharmaceuticals), were UII (rat) 69%, UII (mouse) 10%, urotensin-related peptide 1.06%, urocortin 1 (human) 0%, somatostatin 0%, angiotensin II 0%, and endothelin I 0% (data supplied by Phoenix Pharmaceuticals). We assayed all samples from a single individual within the same assay to reduce intraindividual assay variability.

CALCULATIONS
To derive peptide gradients across tissue beds, UII immunoreactivity in the venous bed of interest (Cv) was paired with the femoral artery concentration (Ca) obtained closest in time to the venous sample. We calculated the transorgan UII gradient from the formula Cv − Ca and the proportional change (%) in UII concentration across a tissue bed using the formula:

\[
\text{Proportional UII release} = \frac{C_v - C_a}{C_a} \times 100.
\]

We estimated regional blood flows as a proportion of resting cardiac output (assumed as 22%, 27%, 14%, 26%, and 4% to renal, hepatic, head and neck, musculoskeletal, and cardiac tissues, respectively) (16). We used directly measured cardiac output, hematocrit, and transorgan peptide gradients together with these assumed proportional flows to estimate regional plasma UII secretion (in pmol/min):

\[
\text{Estimated UII secretion} = (C_a - C_v) \times \left[ \frac{\text{regional blood flow}}{(1 - \text{hematocrit})} \right].
\]

STATISTICAL ANALYSIS
We calculated descriptive variables as mean (SD) for all normally distributed variables or median (interquartile range) for non-normally distributed data. We compared matched arterial and venous UII concentrations using paired t tests and determined univariate Pearson correlation coefficients for predictors of mixed venous (pulmonary artery) UII immunoreactivity. We used multivariate regression analysis to determine independent predictors of mixed venous UII concentrations incorporating variables with univariate association significant at the P < 0.10 level. Statistical significance was assumed at P < 0.05. Investigators and echocardiography and assay technicians were blinded to patient status and UII concentrations during data analysis. Data analysis was conducted using SPSS version 13 (SPSS Inc.).
Results

Clinical and cardiac characteristics for the cohort are shown in Tables 1 and 2, respectively. During the hospital admission in which cardiac catheterization and sampling were performed, 54 of 94 individuals (57%) were investigated for an acute coronary syndrome. The mean arterial pressure decreased slightly [99.4 (15.2) mmHg to 97.5 (14.7) mmHg (P < 0.04)] over the 20 min required for sampling. Femoral arterial plasma UII immunoreactivity increased across the sampling period [mean increment 0.12 (0.37) pmol/L, P < 0.01]; this increment was correlated with duration of sampling (n = 92, r = 0.29, P < 0.01). Twenty-four (2.9%) of the UII concentrations were below the plasma assay limit of detection (0.51 pmol/L) and were not available for analysis.

The mean UII immunoreactivity in the pulmonary artery (mixed venous) was 1.40 (0.43) pmol/L. Peripheral venous (femoral vein) UII concentrations were higher in patients undergoing cardiac catheterization [1.50 (0.46) pmol/L] than in 40 age- and sex-matched controls [1.35 (0.25) pmol/L], P < 0.07. In addition, peripheral venous UII concentrations were significantly increased in subjects admitted with an acute coronary syndrome [1.63 (0.41) pmol/L] compared with those without an acute ischemic presentation [1.35 (0.38) pmol/L], P < 0.001. In subjects with an acute coronary syndrome, mean systolic blood pressure was significantly lower [128.0 (16.7) mmHg] than in the remainder of the cohort [139.7 (21.6) mmHg], P < 0.004.

As shown in Fig. 1 and Table 3, a significant increase in UII immunoreactivity was observed from arterial to venous plasma (in descending order of magnitude) across the heart, kidney, head and neck, liver, lower limb, and pulmonary circulations, consistent with net tissue UII release (P < 0.01 for all). No change in UII immunoreactivity was observed between the high inferior vena cava and arterial plasma (P = 0.21). When we analyzed the net percentage secretion of UII from total UII secretion, taking into account the estimated plasma flow and UII arteriovenous gradient across each organ, approximately 33% was derived from the kidney, 20% from the lungs, 15% each from musculoskeletal and hepatic tissues, and 10% each from the

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**Table 1. Baseline characteristics (n = 94).**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>63.6 (8.8)</td>
</tr>
<tr>
<td>Male gender</td>
<td>69 (73)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29.9 (5.0)</td>
</tr>
<tr>
<td>Estimated glomerular filtration rate, ml·min⁻¹·(1.73 m²)⁻¹</td>
<td>73.7 (15.5)</td>
</tr>
<tr>
<td>Former or current smoker</td>
<td>55 (59)</td>
</tr>
</tbody>
</table>

**Baseline medical conditions**

- Angina                                  | 59 (63)        |
- Prior myocardial infarction             | 36 (38)        |
- Heart failure                           | 12 (13)        |
- Cerebrovascular disease                 | 7 (7)          |
- Coronary artery bypass grafting         | 8 (9)          |
- Diabetes                               | 12 (13)        |
- Peripheral vascular disease             | 6 (6)          |

**NYHA class**

- I                                      | 36 (38)        |
- II                                     | 32 (34)        |
- III                                    | 20 (21)        |
- IV                                     | 6 (6)          |

**Hemoglobin, g/L**                      | 143.4 (10.3)   |

**Arterial partial pressure oxygen, mmHg** | 85.0 (22.0)   |

**Medication**

- Diuretic                               | 18 (19)        |
- ACE inhibitor or ARB                   | 41 (44)        |
- Beta blocker                           | 77 (82)        |
- Aspirin                                | 85 (90)        |
- Nitrate                                | 23 (25)        |
- Lipid-lowering therapy                 | 80 (85)        |

*Data are mean (SD) or n (%).*

**Table 2. Baseline cardiac characteristics (n = 94).**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, min⁻¹</td>
<td>66.2 (11.8)</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>132.9 (19.5)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>74.7 (12.3)</td>
</tr>
<tr>
<td>Brandt score</td>
<td>4.4 (1.8–7.0)</td>
</tr>
<tr>
<td>Peak troponin I ≥0.03 μg/L</td>
<td>50 (53)</td>
</tr>
</tbody>
</table>

**Cardiac catheterization**

- Mean pulmonary artery pressure, mmHg | 18.1 (6.4)     |
- Left ventricular end diastolic pressure, mmHg | 6.0 (3.5) |
- Cardiac output index, L/min/m²        | 2.6 (0.8)      |

**Echocardiography**

- Left ventricular ejection fraction, % | 62.0 (54.1–67.4) |
- Left ventricular systolic volume, mL  | 43.7 (32.5–61.6) |
- E to E'                                | 10.4 (8.3–13.3) |
- Deceleration time, ms                  | 235 (193.8–282.3) |

*Data are mean (SD), median (interquartile range), or n (%)."
limbs and the heart (Table 3). The proportional increment in UII immunoreactivity was significantly correlated across all sites of net peptide release within an individual ($P < 0.05$ for all comparisons) (Table 4).

In univariate analyses, mixed venous UII immunoreactivity correlated with a diagnosis of acute coronary syndrome and femoral arterial oxygen tension and inversely with body mass index (BMI) and systolic

<table>
<thead>
<tr>
<th>Tissue bed</th>
<th>n</th>
<th>Arteriovenous gradient, pmol/L</th>
<th>Proportional arteriovenous change, %</th>
<th>Estimated UII secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>pmol/min</td>
</tr>
<tr>
<td>Cardiac</td>
<td>74</td>
<td>0.78 (0.05)</td>
<td>52.7 (3.4)</td>
<td>0.09 (0.01)</td>
</tr>
<tr>
<td>Renal</td>
<td>90</td>
<td>0.49 (0.08)</td>
<td>35.7 (5.8)</td>
<td>0.35 (0.03)</td>
</tr>
<tr>
<td>Head and neck</td>
<td>90</td>
<td>0.40 (0.05)</td>
<td>29.2 (3.6)</td>
<td>0.15 (0.02)</td>
</tr>
<tr>
<td>Hepatic</td>
<td>89</td>
<td>0.19 (0.06)</td>
<td>13.9 (4.3)</td>
<td>0.15 (0.04)</td>
</tr>
<tr>
<td>Lower limb</td>
<td>92</td>
<td>0.16 (0.04)</td>
<td>10.2 (2.9)</td>
<td>0.10 (0.02)</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>89</td>
<td>0.09 (0.02)</td>
<td>6.0 (1.3)</td>
<td>0.23 (0.09)</td>
</tr>
</tbody>
</table>

* Data are mean (SE) unless noted otherwise.
blood pressure ($P < 0.05$ for all) (Table 5). In multivariate regression analysis, presence of acute coronary syndrome and BMI were independent predictors of mixed venous UII immunoreactivity (Table 5). No significant correlations of the cardiac arteriovenous UII gradient were identified in univariate analysis. Net release of UII immunoreactivity by cardiac or any other tissue (renal, hepatic, limb, head and neck, or pulmonary) was similar in individuals with an acute coronary syndrome compared with subjects with stable coronary disease ($P \geq 0.3$ for all).

**Discussion**

We provide the first evidence for widespread tissue UII secretion in the human circulation, observing an increase in UII immunoreactivity (in descending order of magnitude) across the heart, kidneys, head and neck, liver, lower limb, and lungs. Taking into account estimated plasma flow, of total measured UII secretion approximately one-third was derived from the renal circulation, with contributions of 20% from the lungs and 10%–15% each from the remaining organ sites measured (head and neck, hepatic, limb, and cardiac). Correlated increments in UII immunoreactivity observed across organ sites where peptide release occurred suggested a common stimulus for UII secretion across multiple tissues.

No specific clearance site for UII was observed accordant with generalized intracirculatory degradation. The absence of an arteriovenous UII gradient across the lower body segment (femoral artery $\rightarrow$ high inferior vena cava) together with significant increases in UII immunoreactivity across other lower body tissues (limb, entero-hepatic, and renal) suggests 1 or more subdiaphragmatic sites of net UII clearance that were

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**Table 4. Correlation matrix of regional proportional increments for plasma UII immunoreactivity.**

<table>
<thead>
<tr>
<th></th>
<th>Skeletal</th>
<th>Renal</th>
<th>Hepatic</th>
<th>Head and neck</th>
<th>Cardiac</th>
<th>Pulmonary</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r^*$</td>
<td>1</td>
<td>0.79</td>
<td>0.82</td>
<td>0.76</td>
<td>0.56</td>
<td>0.33</td>
</tr>
<tr>
<td>$P$</td>
<td>$&lt;0.001$</td>
<td>$&lt;0.001$</td>
<td>$&lt;0.001$</td>
<td>$&lt;0.001$</td>
<td>$&lt;0.001$</td>
<td>0.002</td>
</tr>
<tr>
<td>n</td>
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<td>90</td>
<td>90</td>
<td>91</td>
<td>75</td>
<td>90</td>
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<td></td>
<td></td>
<td></td>
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<td>r</td>
<td>0.79</td>
<td>1</td>
<td>0.74</td>
<td>0.75</td>
<td>0.52</td>
<td>0.21</td>
</tr>
<tr>
<td>$P$</td>
<td>$&lt;0.001$</td>
<td>$&lt;0.001$</td>
<td>$&lt;0.001$</td>
<td>$&lt;0.001$</td>
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<td>n</td>
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<td>89</td>
<td>73</td>
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</tr>
<tr>
<td><strong>Hepatic</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
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<td>0.74</td>
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<td>0.74</td>
<td>0.44</td>
<td>0.32</td>
</tr>
<tr>
<td>$P$</td>
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<td>$&lt;0.001$</td>
<td>$&lt;0.001$</td>
<td>$&lt;0.001$</td>
<td>$&lt;0.001$</td>
<td>0.002</td>
</tr>
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<td>89</td>
<td>74</td>
<td>88</td>
</tr>
<tr>
<td><strong>Head and neck</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
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<td>0.75</td>
<td>0.74</td>
<td>1</td>
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<td>0.31</td>
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<tr>
<td>$P$</td>
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<td>89</td>
<td>89</td>
<td>91</td>
<td>74</td>
<td>89</td>
</tr>
<tr>
<td><strong>Cardiac</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>r</td>
<td>0.56</td>
<td>0.52</td>
<td>0.44</td>
<td>0.50</td>
<td>1</td>
<td>0.26</td>
</tr>
<tr>
<td>$P$</td>
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<td>$&lt;0.001$</td>
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<td>$&lt;0.05$</td>
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<tr>
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<td>73</td>
<td>74</td>
<td>74</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td><strong>Pulmonary</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.33</td>
<td>0.21</td>
<td>0.32</td>
<td>0.31</td>
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<td>1</td>
</tr>
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<td>$P$</td>
<td>0.002</td>
<td>$&lt;0.05$</td>
<td>0.002</td>
<td>0.003</td>
<td>$&lt;0.05$</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>90</td>
<td>88</td>
<td>88</td>
<td>89</td>
<td>75</td>
<td>90</td>
</tr>
</tbody>
</table>

* Pearson correlation coefficient.
not measured in the protocol. These potential sites of UII degradation include adipose, pancreatic, adrenal, and urogenital tissues.

The physiological relevance of UII remains uncertain, largely because heterogeneous responses to UII administration in vivo and in vitro are observed in differing vascular beds and between species (17). Despite variable tissue responses to administered UII, a physiological role for the peptide is suggested by its highly conserved sequence across species (invertebrates to mammals) and a more potent tissue response to ligand–receptor binding than other vasoactive peptides, including endothelin I (3). Profound circulatory collapse with intense coronary artery vasospasm follows intravenous UII administration in anesthetized nonhuman primates (3). In addition, increased UII concentrations are observed in human disease including myocardial infarction (11), heart failure (9), hypertension (18, 19), diabetes mellitus (20), cirrhosis (21), and end-stage kidney disease (22), suggesting that UII secretion may be a common tissue response to injury.

Generalized tissue release of UII immunoreactivity observed in the present analysis is consistent with prior studies showing diverse tissue expression of mature UII and its ligand receptor in cardiomyocytes, fibroblasts, arterial endothelium, vascular smooth muscle, kidney, skeletal muscle, thyroid, and neural tissues (3, 23, 24). Similarly, prepro-UII mRNA has been demonstrated, although more variably, in human right atrium, spinal cord, kidney, spleen, small intestine, thymus, prostate, pituitary gland, adrenal gland, stomach, pancreas, ovary, and liver, suggesting widespread UII gene expression (25–27). Although cellular sources of circulating UII are yet to be fully elucidated, generalized UII expression and release, as seen in the present study, indicate that UII may be secreted by the endothelium. This observation is consistent with UII immunoreactivity previously identified in human endothelial cells and in inflammatory cells colocalized with atherosclerotic lesions in human arteries (24, 28).

The current report is the first evidence of cardiac secretion of UII. Net release of UII from the human cardiopulmonary circulation has been suggested in a previous clinical study showing an increase in UII immunoreactivity between pulmonary artery and aorta (13); that this gradient between pulmonary artery and

<table>
<thead>
<tr>
<th>Table 5. Univariate and multivariate predictors of mixed venous plasma UII immunoreactivity.*</th>
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<tbody>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Univariate</strong></td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Male sex</td>
</tr>
<tr>
<td>Acute coronary syndrome</td>
</tr>
<tr>
<td>BMI</td>
</tr>
<tr>
<td>Estimated glomerular filtration rate</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
</tr>
<tr>
<td>Femoral arterial oxygen tension</td>
</tr>
<tr>
<td>Hemoglobin</td>
</tr>
<tr>
<td>Left ventricular ejection fraction</td>
</tr>
<tr>
<td>Mean pulmonary artery pressure</td>
</tr>
<tr>
<td>LVEDP</td>
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<td>Brandt score</td>
</tr>
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<td>Peak troponin ( =0.03 ) ( \mu)g/L</td>
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<td><strong>Multivariate</strong></td>
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<tr>
<td>Acute coronary syndrome</td>
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</tr>
<tr>
<td>Femoral artery oxygen tension</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
</tr>
<tr>
<td>Mean pulmonary artery pressure</td>
</tr>
</tbody>
</table>

\* \( n = 91 \); adjusted \( R^2 = 0.19 \).
\( \text{b} \) Pearson correlation.
\( \text{c} \) Statistically significant.
aortic root was greatest in subjects with cardiomyopathy—compared with healthy controls—indicated that increased plasma UII concentrations observed in individuals with heart failure might result from increased cardiopulmonary UII release (13). In direct contrast, Dschietzig et al. (12) identified no significant differences between UII immunoreactivity in the pulmonary artery, left ventricle, coronary sinus, and antecubital vein in subjects with and without heart failure. In that study, UII concentrations were similar at all sites measured and between subject groups regardless of heart failure status. Given the discordance between these 2 earlier analyses, the present data provide some clarification by showing both significant cardiac and pulmonary UII release in subjects under investigation for cardiovascular disorders.

UII mRNA and mature protein expression in the human heart is observed predominantly in myocardial cells and, to a lesser extent, in vascular smooth muscle cells, endothelial cells, and myofibroblasts, suggesting that cardiomyocytes may be the predominant cardiac source for UII (10). Notably, UII immunoreactivity and UII receptor expression is greatest in viable cardiomyocytes surrounding ischemic tissue, suggesting that upregulated UII expression may be a response to ischemic myocardial injury (10). This observation is consistent with the increased circulating UII concentrations in the present and previous studies in subjects with acute coronary injury (11). However, although individuals in the present cohort who were admitted with an acute coronary syndrome had higher circulating UII concentrations, net cardiac or pulmonary release of UII was not greater in these individuals; therefore, increased cardiopulmonary secretion alone does not explain the higher circulating UII concentrations in these patients (8, 9, 13). Notably also in the present analysis, the net transcardiac UII gradient was unrelated to cardiac function, including left ventricular ejection fraction or cardiac filling pressures, suggesting that upregulated cardiac UII release is unrelated to myocardial wall tension. This contrasts with the previous analysis of human heart tissue showing that UII tissue expression correlated with left ventricular end diastolic dimensions and inversely with left ventricular ejection fraction, consistent with augmented cardiac tissue UII expression with increasing ventricular dysfunction (10).

Our finding of increased circulating UII immunoreactivity in people with an acute coronary syndrome is consistent with a recent report of increased UII concentrations in subjects after acute myocardial infarction compared with controls (11) but is in conflict with an earlier report of UII immunoreactivity in subjects with acute coronary syndrome, stable ischemic heart disease, and healthy controls (29). In that study by Joyal et al., subjects with an acute coronary syndrome reportedly had lower UII levels in plasma compared with subjects with stable disease and controls in univariate analysis. Further, the authors found lower plasma UII immunoreactivity in subjects with acute coronary syndrome and systolic dysfunction compared to patients with acute coronary syndrome and preserved ventricular function. This latter observation is discordant with antecedent studies demonstrating higher UII immunoreactivity in plasma (9) and cardiac tissue (10) of patients with heart failure, including those with ischemic heart disease. The reason for the differences between the present study and these other analyses of Joyal et al. are unclear.

In vitro UII stimulation of cultured cardiomyocytes expressing the UII receptor activates a hypertrophic phenotype, suggesting that UII may stimulate myocardial hypertrophy in response to injury (5, 30). Specific UII receptor antagonism after experimental myocardial infarction abrogates ventricular dilation and cardiac hypertrophy, further lending support for a pathophysiological role for UII in cardiac remodeling after myocardial ischemia (31). Conversely, however, increased UII concentrations have been associated with better event-free survival after myocardial infarction (11) and in patients receiving dialysis (22), consistent with UII secretion as a protective response to cellular injury in these patients. Given these apparently conflicting reports between preclinical and clinical studies, the role of UII in myocardial recovery following cardiac injury and subsequent patient survival awaits further characterization.

We confirm net hepatic release of UII first reported by Heller et al. (32), who showed a rise in UII immunoreactivity from portal venous to central venous blood in patients with cirrhosis. In that study, increased circulating UII immunoreactivity in subjects with impaired liver function suggested either increased hepatic UII secretion or impaired UII degradation by the diseased liver. The results of the present study showing an increase in UII immunoreactivity in subjects with normal liver function, combined with previously reported data, suggest that entero-hepatic tissue may be a source of UII.

Increased UII concentrations have been demonstrated in subjects receiving dialysis compared with healthy volunteers, consistent with increased UII secretion or reduced clearance of UII associated with impaired glomerular filtration (33, 34). Highly prevalent left ventricular hypertrophy in people requiring dialysis (35), however, suggests that increased circulating UII immunoreactivity may in fact be due to augmented cardiac expression in response to concomitant cardiac dysfunction rather than to impaired UII degradation due to impaired kidney function. The present data suggest that the kidney is a major contributor to circulating UII levels. Additionally, the absence of any correlation between kidney function and UII levels observed in regression analyses suggests that circulating UII lev-
els are unrelated to glomerular filtration. A direct association shown between UII levels and a measure of left ventricular hypertrophy (mean wall thickness) in people receiving dialysis (36) is also consistent with cardiac rather than renal function as a primary predictor of UII levels in patients with chronic kidney disease.

We observed a negative correlation between systolic blood pressure and circulating UII immunoreactivity, which did not remain significant when adjusted for other covariates, including acute coronary syndrome. The inverse relationship between blood pressure and UII immunoreactivity seen here is discordant with 2 case-control studies in individuals with stable hypertension that found higher UII immunoreactivity in subjects with hypertension and positive correlations between UII and systolic blood pressure (18, 19). The difference between the findings in these studies and the present analysis is likely to reflect the differing cohort characteristics. Unlike the previous studies in which subjects had stable cardiovascular function, in our study nearly 60% of subjects had experienced a recent acute coronary syndrome. These individuals had higher UII concentrations and significantly lower blood pressure. It is likely, therefore, that the inverse relationship between blood pressure and UII we observed was confounded by myocardial ischemia; relative hypotension was associated with increased plasma UII immunoreactivity via the presence of myocardial ischemia. Indeed, when blood pressure was adjusted for the presence of an acute coronary syndrome, it no longer remained a significant predictor of UII immunoreactivity.

In the present multivariate analysis, diagnosis of acute coronary syndrome and BMI were independent predictors of mixed venous immunoreactive UII. The inverse relationship of UII immunoreactivity with BMI suggests that a higher BMI may be associated with increased UII clearance (or reduced secretion). It should be noted that the multivariate regression model in the current study predicts only 19% of the variance in UII immunoreactivity; therefore, the major influences on circulating UII levels remain unidentified.

**Limitations**

The significant increase in UII immunoreactivity over time, which correlated with duration of sampling, was also seen in a similar experimental protocol (37). Increasing peptide immunoreactivity may relate to the duration of supine posture via increased peptide release because of redistribution of circulating volume to the thorax and changes in intracardiac volumes and wall tension. Only 12 subjects in the cohort had antecedent heart failure; the study was therefore unable to determine the contribution of cardiac UII secretion to increased UII immunoreactivity associated with cardiac impairment. Although cardiac output (and therefore pulmonary blood flow) was measured directly, values for blood flow to other organs were necessarily estimates only. In addition, we are unable to comment on the net production or clearance of UII by a number of organs for which it was not practical to obtain venous samples.

**Conclusions**

We conclude that UII is released from multiple sites (heart, kidney, head and neck, hepatosplenic, and musculoskeletal tissues) into the human circulation (and is also subject to clearance at one or more subdiaphragmatic sites not further localized at this time). Increased UII immunoreactivity is observed in subjects with an acute coronary syndrome. Related increments in UII immunoreactivity across multiple tissue sites suggest peptide release by a shared mechanism.

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