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# Plasma Pro-Endothelin-1 Peptide Concentrations Rise in Chronic Kidney Disease and Following Selective Endothelin A Receptor Antagonism

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**Background**—Endothelin 1 (ET-1) contributes to chronic kidney disease (CKD) development and progression, and endothelin receptor antagonists are being investigated as a novel therapy for CKD. The proET-1 peptides, endothelin-like domain peptide (ELDP) and C-terminal pro-ET-1 (CT-proET-1), are both potential biomarkers of CKD and response to therapy with endothelin antagonists.

**Methods and Results**—We assessed plasma and urine ELDP and plasma CT-proET-1 in CKD patients with minimal comorbidity. Next, in a randomized double-blind crossover study of 27 subjects with proteinuric CKD, we examined the effects of 6 weeks of treatment with placebo, sitaxentan (endothelin A antagonist), and nifedipine on these peptides alongside the primary end points of proteinuria, blood pressure, and arterial stiffness. Plasma ELDP and CT-proET-1 increased with CKD stage (both  $P < 0.0001$ ), correlating inversely with estimated glomerular filtration rate (both  $P < 0.0001$ ). Following intervention, placebo and nifedipine did not affect plasma and urine ELDP or plasma CT-proET-1. Sitaxentan increased both plasma ELDP and CT-proET-1 (baseline versus week 6  $\pm$  SEM: ELDP,  $11.8 \pm 0.5$  versus  $13.4 \pm 0.6$  fmol/mL; CT-proET-1,  $20.5 \pm 1.2$  versus  $23.3 \pm 1.5$  fmol/mL; both  $P < 0.0001$ ). Plasma ET-1 was unaffected by any treatment. Following sitaxentan, plasma ELDP and CT-proET-1 correlated negatively with 24-hour urinary sodium excretion.

**Conclusions**—ELDP and CT-proET-1 increase in CKD and thus are potentially useful biomarkers of renal injury. Increases in response to endothelin A antagonism may reflect *EDN1* upregulation, which may partly explain fluid retention with these agents.

**Clinical Trial Registration**—URL: [www.clinicaltrials.gov](http://www.clinicaltrials.gov) Unique identifier: NCT00810732 (*J Am Heart Assoc.* 2015;4:e001624 doi: 10.1161/JAHA.114.001624)

**Key Words:** antagonists • CKD • endothelin • fluid retention

Chronic kidney disease (CKD) is common and affects 6% to 11% of the population globally.<sup>1</sup> It is strongly associated with incident cardiovascular disease.<sup>2</sup> As glomerular filtration rate (GFR) declines, the risk of cardiovascular

disease increases.<sup>3</sup> Consequently, early detection of CKD is important to reduce morbidity and mortality. Current measures of renal function (eg, using serum creatinine) are often inadequate because substantial renal tissue damage must occur before function is impaired to a detectable extent.<sup>4</sup> An unmet need exists for more sensitive biomarkers of renal injury that will allow earlier detection of CKD and that will potentially reflect efficacy of therapy.

Endothelin 1 (ET-1) is a potent endogenous vasoconstrictor. It is implicated in both the development and progression of CKD<sup>5</sup> and plays an important role in renal salt and water handling.<sup>6</sup> Its effects are mediated via 2 receptors, the endothelin A (ET<sub>A</sub>) and endothelin B (ET<sub>B</sub>) receptors, with the major pathological effects mediated by the ET<sub>A</sub> receptor.<sup>5</sup> Several selective ET<sub>A</sub> and mixed ET<sub>A/B</sub> receptor antagonists are now available and licensed for the treatment of pulmonary arterial hypertension and scleroderma digital ulcers. Endothelin receptor antagonists are also being investigated as a novel therapeutic strategy in CKD.<sup>7</sup> Although urinary ET-1

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excretion is well correlated with renal ET-1 production,<sup>8,9</sup> plasma ET-1 is an unreliable measure of vascular ET-1 production because of the predominantly abluminal release of ET-1,<sup>10</sup> its rapid receptor-mediated uptake,<sup>5</sup> and technical limitations in measurement.<sup>11</sup> Endothelin-like domain peptide (ELDP; preproET-1[93–166])<sup>12</sup> and C-terminal pro-ET-1 (CT-proET-1; preproET-1[169–212]) are both cosynthesized with ET-1 from the *EDN1* gene (Figure 1). They are more stable in the circulation and may be alternative markers of ET-1 synthesis.<sup>12</sup>

We previously investigated novel cardiovascular disease risk factors in CKD patients across a wide range of renal function<sup>13,14</sup> and showed that plasma and urine ET-1 increase as GFR declines.<sup>15</sup> We showed recently that chronic selective ET<sub>A</sub> receptor antagonism using the orally active drug sitaxentan reduces proteinuria, blood pressure (BP), and arterial stiffness—effects that are potentially renoprotective—in patients with proteinuric CKD.<sup>16</sup> We hypothesized that in these same cohorts of patients, the proET-1 peptides ELDP and CT-proET-1 would increase as GFR declined. Whether sitaxentan treatment would alter proET-1 peptide levels was unclear, but we hypothesized that any changes would relate to changes in urine sodium excretion.

## Methods

Both studies were performed with the approval of the local research ethics committee and the written informed consent of each subject. The investigations conformed to the principles outlined in the Declaration of Helsinki.

### Observational Study: Patients With Varying Degrees of CKD and Minimal Comorbidity

The rationale and study design have been reported in detail elsewhere.<sup>13</sup> In brief, subjects were recruited from the renal outpatient clinic at the Royal Infirmary of Edinburgh and categorized into the 5 stages of CKD on the basis of the Kidney Disease Outcome Quality Initiative (K/DOQI) classifi-

cation.<sup>17</sup> Age-matched controls were recruited from the community. Creatinine clearance, as an estimate of GFR (eGFR), was calculated according to the Cockcroft and Gault equation.<sup>18</sup> This equation was selected to assess renal function in this study because it is more accurate than the Modification of Diet in Renal Disease (MDRD) equation if used to assess mild renal insufficiency.<sup>19</sup> It was further corrected by body surface area. Blood and urine samples were obtained from subjects after 12 hours of overnight fasting.

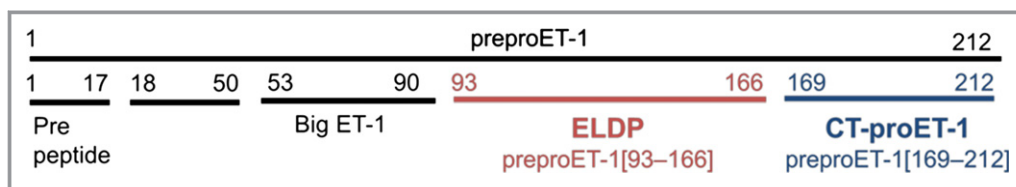
### Interventional Study: Selective ET<sub>A</sub> Receptor Antagonism in CKD

The rationale and design for this study have been reported elsewhere.<sup>16</sup> In brief, in a randomized, double-blind, 3-way crossover study, 27 subjects on recommended renoprotective treatment received 6 weeks of placebo, sitaxentan 100 mg once daily, and nifedipine LA 30 mg once daily. 24-hour proteinuria; urine protein:creatinine ratio; 24-hour ambulatory BP; and pulse wave velocity, as an index of arterial stiffness, were measured at baseline, week 3, and week 6 of each treatment period. Plasma and urine ELDP and ET-1 and plasma CT-proET-1 were also assessed at these same time points.

### Sample Collection and Analysis

ELDP, CT-proET-1, and ET-1 venous blood samples were collected in EDTA tubes and were immediately centrifuged at 2500g for 20 minutes at 4°C. For urine ELDP, a 20-mL aliquot of urine was collected into plain tubes. For urine ET-1, a 20-mL aliquot of urine was collected into plain tubes with 2.5 mL of 50% acetic acid. Samples were stored at –80°C until analysis.

ELDP and CT-proET-1 were measured by sandwich ELISA (Figure 1) using previously described methodologies.<sup>20</sup> A well-established format was followed using specific IgG that had been affinity purified from polyclonal sheep antisera raised against the N- and C-terminal sequences of each peptide. Assays were performed in 96-well plates coated with capture



**Figure 1.** Schematic outline of the amino acid structure of preproET-1 indicating the peptides generated by post-translational processing. Positions of ELDP (preproET-1[93–166]) and CT-proET-1 (preproET-1[169–212]) are shown. ET-1 is produced from big ET-1 by endothelin-converting enzyme. CT-proET-1 indicates C-terminal pro-endothelin-1; ELDP, endothelin-like domain peptide; ET-1, endothelin 1; preproET-1, prepro-endothelin-1.

IgG (1 µg/mL) specific for ELDP (anti-preproET-1[93–109] [ALENLLPTKATDRENRC]) or CT-proET-1 (anti-preproET-1[169–186] [SSEEHLRQTRSETMRNSV]). Following overnight incubation (25 µL of plasma or 100 µL urine), detection of bound peptide was achieved with biotinylated IgG for ELDP (preproET-1[155–166] [CIYQQLVGRKII]) or CT-proET-1 (preproET-1[204–212] [YVTHNRAHW]), respectively. This was in conjunction with NeutrAvidin HRP (Pierce; Thermo Fisher Scientific) and chemiluminescent substrate. Synthetic peptides were used as assay standards.<sup>12</sup> The lower limit of detection for ELDP was 0.09 fmol/mL in urine and 0.30 fmol/mL in plasma. The detection limit for CT-proET-1 in plasma was 0.60 fmol/mL. Urine CT-proET-1 could not be measured reliably using either a double-recognition-site sandwich ELISA or a single-site ELISA directed at the C-terminal sequence of CT-proET-1.

After extraction,<sup>21</sup> ET-1 was determined by radioimmunoassay.<sup>22</sup> The mean recovery of ET-1, from extraction to assay, was >90% for both plasma and urine. The intra- and interassay variations were 6.3% and 7.2%, respectively. The cross-reactivity of the antibody was 100% with ET-1, 7% for both ET-2 and ET-3, and 10% with big ET-1.

## Statistical Analysis

Data were statistically analyzed using GraphPad Prism version 5. Descriptive data are given as mean±SD. Statistical analysis was performed on untransformed data. Data were compared using repeated-measures, 1-way ANOVA with Bonferroni

correction for multiple comparisons. For the observational study, stepwise linear regression was used to identify factors that predicted eGFR. Correlation coefficients were calculated using the Pearson method. Statistical significance was taken at the 5% level.

## Results

### Observational Study

#### Plasma and urine proET-1 peptides levels across CKD stages

Subject characteristics are shown in Table 1. Plasma ELDP concentration increased with CKD stage ( $P<0.0001$  for trend), with control subjects having plasma ELDP of  $6.4\pm 0.7$  fmol/mL compared with  $12.4\pm 2.5$  fmol/mL for stage 5 CKD subjects (Figure 2A). Although plasma ELDP did not differ between control subjects and CKD patients in stages 1–3, patients in stages 4 and 5 had higher plasma ELDP compared with controls ( $P<0.001$  for both). Plasma ELDP correlated inversely with eGFR ( $r=0.51$ ,  $P<0.0001$ ) (Figure 2B). This correlation was similar using both Cockcroft and Gault and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations. Mean urine ELDP was  $1.07\pm 0.11$  fmol/mL with a  $\approx 75$ -fold difference between the minimum and maximum values of 0.09 and 6.65 fmol/L; however, there were no differences in urine ELDP among CKD stages and no correlation with eGFR (Figure 3).

**Table 1.** Subject Characteristics

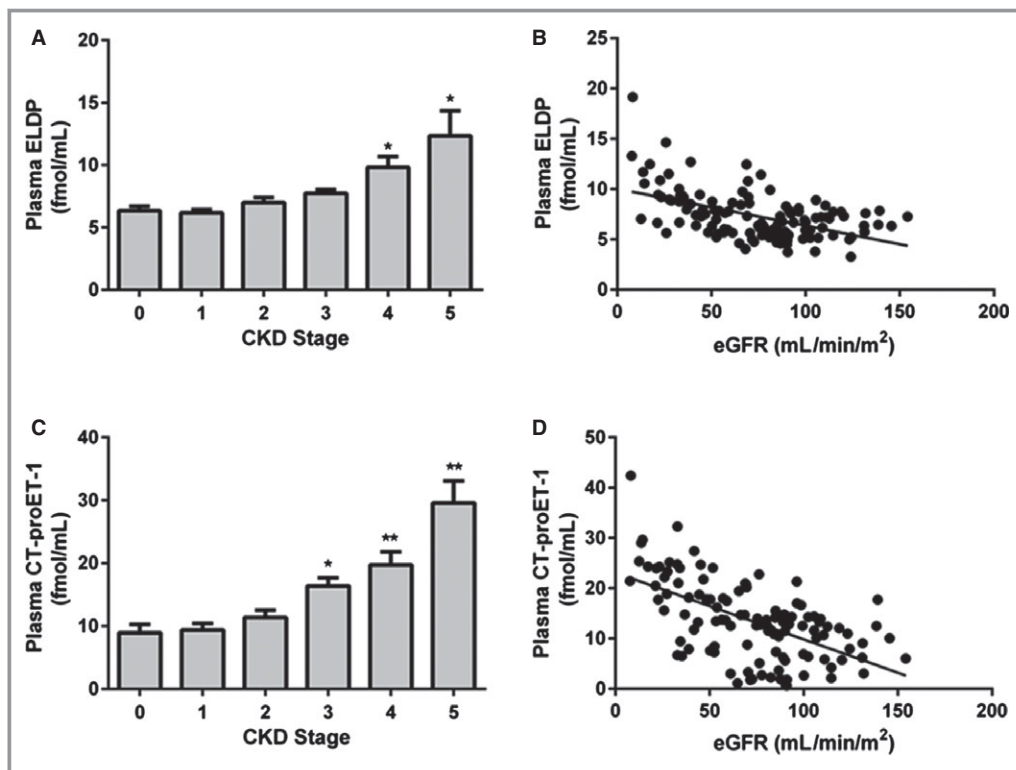
	Controls	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	P Value
n	23	27	30	29	20	7	—
Male/female, n	13/10	16/11	18/12	21/8	16/4	4/3	—
Smokers/nonsmokers, n	2/21	9/18	5/25	5/24	4/16	0/7	—
Serum creatinine,* mg/dL	0.9±0.2	0.9±0.2	1.1±0.2	2.0±0.6	4.1±1.2	7.1±2.0	<0.05
CrCl, mL/min per 1.73 m <sup>2</sup>	97±19	108±17	77±9	46±9	23±4	11±3	<0.05
Age, y	47±8	43±11	49±9	50±10	45±9	51±12	NS
SBP, mm Hg	113±16	113±15	116±13	119±13	121±12	132±16	<0.05
DBP, mm Hg	71±10	71±10	74±9	77±10	76±7	76±7	NS
MAP, mm Hg	85±12	85±11	88±10	91±10	91±8	95±8	<0.05
PP, mm Hg	42±9	42±8	41±7	42±10	45±9	56±17	<0.05
Body mass index, kg/m <sup>2</sup>	26±6	29±5	28±4	29±6	27±5	25±7	NS
Plasma glucose,† mg/dL	90±9	89±9	91±8	90±9	87±12	91±14	NS
Total cholesterol,‡ mg/dL	189±30	180±37	186±32	174±31	170±32	181±31	NS

Values are given as mean±SD. P values are for ANOVA by chronic kidney disease stage. CrCl indicates creatinine clearance; DBP, diastolic blood pressure; MAP, mean arterial pressure; NS, not significant; PP, pulse pressure; SBP, systolic blood pressure.

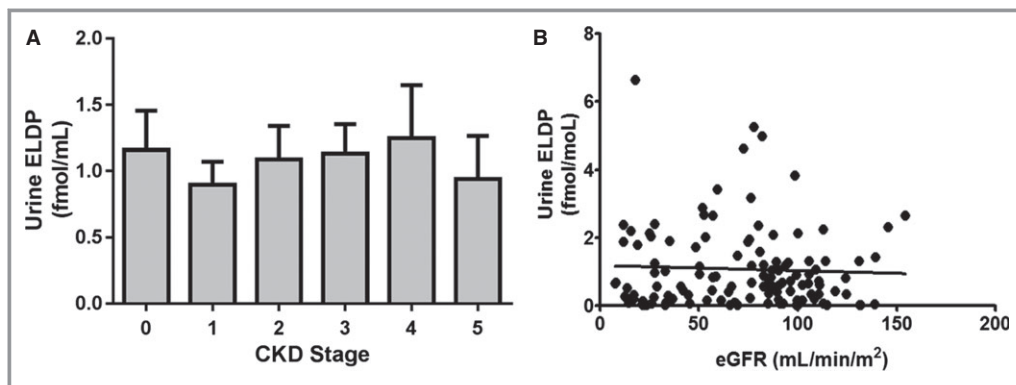
\*To convert to µmol/L, multiply by 88.4.

†To convert to mmol/L, multiply by 0.0555.

‡To convert to mmol/L, multiply by 0.0259.



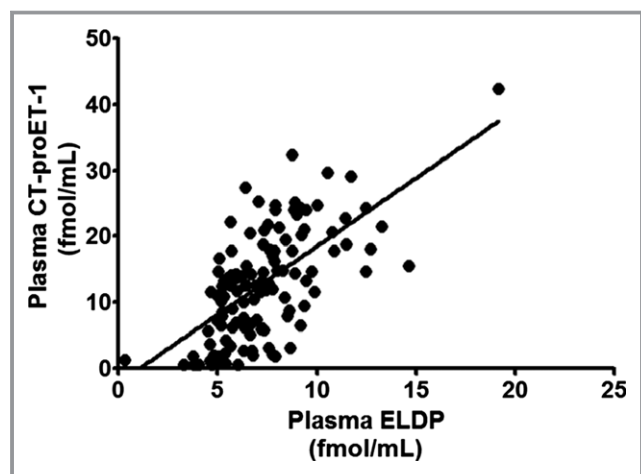
**Figure 2.** Plasma ELDP (A) and CT-proET-1 (C) by stage of CKD and relationship between plasma ELDP (B) and CT-proET-1 (D) and eGFR. \* $P < 0.001$ , \*\* $P < 0.0001$  compared with non-CKD control subjects (stage 0). For correlation between plasma ELDP and eGFR,  $r = 0.51$ ,  $P < 0.0001$ . For correlation between plasma CT-proET-1 and eGFR,  $r = 0.54$ ,  $P < 0.0001$ . CKD indicates chronic kidney disease; CT-proET-1, C-terminal pro-endothelin-1; eGFR, estimated glomerular filtration rate; ELDP, endothelin-like domain peptide.



**Figure 3.** Urine ELDP by stage of CKD (A) and relationship between urine ELDP and eGFR (B). CKD indicates chronic kidney disease; eGFR, estimated glomerular filtration rate; ELDP, endothelin-like domain peptide.

Plasma CT-proET-1 concentration also increased with CKD stage ( $P < 0.0001$  for trend), with patients in stages 3, 4, and 5 having higher plasma CT-proET-1 than controls ( $P < 0.001$ ,  $< 0.0001$ , and  $< 0.0001$ , respectively) (Figure 2C). Plasma CT-proET-1 correlated negatively with eGFR ( $r = 0.57$ ,  $P < 0.0001$ ) (Figure 2D). Again, this correlation was similar using both Cockcroft and Gault and CKD-EPI equations.

Consistent with their cosynthesis, plasma ELDP correlated with CT-proET-1 ( $r = 0.63$ ,  $P < 0.0001$ ) (Figure 4). Plasma ELDP and CT-proET-1 showed no correlations with components of BP or arterial stiffness in this cohort of CKD subjects. Previously published data from this study showed that plasma and urine ET-1 increased as eGFR declined.<sup>13,15</sup>



**Figure 4.** Correlation of plasma ELDP with CT-proET-1 ( $r=0.63$ ,  $P<0.0001$ ). CT-proET-1 indicates C-terminal pro-endothelin-1; ELDP, endothelin-like domain peptide.

We performed stepwise linear regression to identify factors that predicted eGFR. We added plasma ELDP and CT-proET-1 to the previously reported factors associated with worsening CKD in this patient population (plasma ET-1, pulse wave velocity, flow-mediated dilatation, asymmetric dimethylarginine, mean arterial pressure, pulse pressure, and systolic BP).<sup>13</sup> In order of strongest correlation, plasma CT-proET-1, pulse wave velocity, plasma ET-1, and mean arterial pressure were predictors of eGFR. If plasma CT-proET-1 was not included in the model, the predictors of eGFR in order of strongest correlation were plasma ELDP, plasma ET-1, and pulse wave velocity (Table 2).

## Interventional Study

### Effect of selective $ET_A$ receptor antagonism on plasma proET-1 peptides

Baseline subject characteristics of those participating in the interventional study are shown in Table 3. Data from this study have been published previously and show that after 6 weeks of dosing, there were no significant differences between sitaxentan and nifedipine in the reductions from baseline in BP parameters.<sup>16</sup> Despite this, sitaxentan reduced proteinuria to a significantly greater extent than nifedipine. Pulse wave velocity as a measure of arterial stiffness fell to a similar degree with nifedipine as with sitaxentan. Placebo did not affect proteinuria, BP, or pulse wave velocity (summary data are shown in Table 4).

Baseline ELDP and CT-proET-1 concentrations were the same for all 3 phases of the study (Table 4). Whereas placebo and nifedipine treatments did not affect ELDP or CT-proET-1, sitaxentan increased both peptides by  $\approx 15\%$  at both weeks 3 and 6 of the study phase (Figures 5A and 5B). Urine ELDP did not change in any of the 3 phases of the study.

**Table 2.** Multivariable Analysis of Predictors for Estimated Glomerular Filtration Rate

Predictor	Model 1 (n=120)	Model 2 (n=120; CT-proET-1 Excluded)
Plasma ELDP	-0.16	-0.33*
Plasma CT-proET-1	-0.44*	—
Plasma ET-1	-0.19 <sup>†</sup>	-0.31*
PWV	-0.16 <sup>†</sup>	-0.24*
FMD	-0.05	0.01
ADMA	-0.04	-0.07
SBP	0.20	-0.10
PP	0.09	0.01
MAP	-0.16 <sup>†</sup>	-0.13
$r^2$	0.46	0.41

The table gives standardized regression coefficients ( $\beta$ -values). ADMA indicates asymmetric dimethylarginine; CT-proET-1, C-terminal pro-endothelin-1; ELDP, endothelin-like domain peptide; FMD, flow-mediated dilation of the brachial artery; MAP, mean arterial pressure; PP, pulse pressure; PWV, pulse wave velocity;  $r^2$ , multiple coefficient of determination; SBP, systolic blood pressure.

\* $P<0.01$ .

<sup>†</sup> $P<0.05$ .

### Plasma proET-1 peptides and urinary sodium excretion

Following 6 weeks treatment with sitaxentan, both plasma ELDP and CT-proET-1 concentrations correlated inversely with 24-hour urine sodium excretion ( $r=0.46$ ,  $P=0.01$  and  $r=0.47$ ,  $P=0.01$ , respectively) (Figures 6A and 6B), whereas placebo and nifedipine showed no such associations (Figure 7). Changes in ELDP and CT-proET-1 did not correlate with changes in components of BP, proteinuria, or arterial stiffness in any of the 3 phases of the study.

### Plasma and urine ET-1

Plasma ET-1 concentrations were similar at baseline in all 3 phases of the study and were not affected by any of the interventions (Table 4). Baseline urine ET-1 levels were also similar in all 3 phases of the study. Although placebo and nifedipine had no impact on urine ET-1, sitaxentan reduced this by  $\approx 20\%$  (Table 4).

## Discussion

The increases in plasma ELDP and CT-proET-1 as GFR declined support the concept that the endothelin system contributes to CKD progression. In agreement with previous studies of plasma ET-1 in CKD, both plasma ELDP and CT-proET-1 were inversely correlated with GFR.<sup>13,15</sup> These increases are likely due in part to reduced renal clearance from the circulation and/or to increases in synthesis. Serum creatinine, currently the most widely used measure of renal

**Table 3.** Baseline Subject Characteristics.

Parameter	n=27
<b>Demographic</b>	
Age, y	48±12
Sex, male (%)	23 (85)
White (%)	27 (100)
<b>Clinical</b>	
Body mass index, kg/m <sup>2</sup>	29.3±4.6
24-hour BP, mm Hg	
Systolic	125±12
Diastolic	78±7
Mean	94±8
Creatinine,* mg/dL	1.73±0.85
eGFR, mL/min per 1.73 m <sup>2</sup>	54±26
Hemoglobin, g/L	136±18
Serum potassium, mmol/L	4.6±0.4
Cholesterol,† mg/dL	178±32
Urinary protein excretion	
Grams per 24 hours	2.03±1.7
PCR, mg/mmol	156±143
Arterial stiffness	
PWV, m/s	8.3±2.4
cAix, %	28±12
Medications, n (%)	
ACE inhibitor	18 (67)
ARB	11 (41)
ACE inhibitor plus ARB	5 (19)
No ACE inhibitor or ARB	3 (11)
α-Blocker	6 (22)
β-Blocker	8 (30)
Calcium channel blocker	3 (11)
Diuretic	2 (7)
Statin	18 (67)

Values are given as mean of 3 baseline pretreatment periods±SD. ACE indicates angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BP, blood pressure; cAix, central augmentation index; eGFR, estimated glomerular filtration rate; PCR, protein:creatinine ratio; PWV, pulse wave velocity.

\*To convert to μmol/L, multiply by 88.4.

†To convert to mmol/L, multiply by 0.0259.

function, has a nonlinear relationship with GFR and begins to rise only when GFR falls below ≈60 mL/min. In contrast, our data show that ELDP and CT-proET-1 have a linear relationship with GFR and begin to rise much earlier in the CKD trajectory. Although this may make these peptides potentially useful biomarkers of CKD, any utility beyond that provided by serum creatinine needs to be investigated further in much larger clinical studies with more diverse CKD populations.

Furthermore, studies that relate to the time course of CKD progression and risk of clinical outcomes would be of particular interest.

The CKD patients studied had minimal comorbidity, so repeating these studies in other cohorts of patients will be important. Our assessment of urine ELDP showed no clear relationship with degree of CKD, but this merits further investigation. ELDP is a recently identified peptide derived from proET-1 that potentiates ET-1-induced vasoconstriction<sup>12</sup>; therefore, data on its potential role as a biomarker or its physiological actions are limited. By comparison, previous studies suggest that CT-proET-1 may be a useful prognostic biomarker in various groups of patients, including those with pulmonary arterial hypertension,<sup>23</sup> type 2 diabetes,<sup>24</sup> and the metabolic syndrome.<sup>25</sup> Our data are the first report of raised levels in CKD patients. Currently, there are no data relating to the actions of these proET-1 peptides in CKD, which should be an area of future research.

In addition to the important evidence of potentially renoprotective effects on proteinuria, BP, and arterial stiffness, the current data show that selective ET<sub>A</sub> receptor antagonism increases the proET-1 peptides ELDP and CT-proET-1. There may be a number of explanations for this finding. Although 6 weeks of sitaxentan treatment was not associated with any change in serum creatinine, actual GFR (measured by inulin clearance) fell from 57±8 to 48±8 mL/min. Consequently, the rise in ELDP and CT-proET-1 may relate to a fall in their clearance and/or an increase in production as a result of the reduction in GFR; however, in this phase of the study, the change in the proET-1 peptides from baseline to week 6 did not correlate with the change in GFR. This may be due to small sample size, but the lack of rise in serum creatinine despite the ≈15% fall in real GFR highlights the poor sensitivity of this test as a measure of GFR.

A more likely explanation for the increases in ELDP and CT-proET-1 following sitaxentan is that ET<sub>A</sub> receptor antagonism interferes with the physiological negative feedback effects of ET-1 on *EDN1* gene expression.<sup>26</sup> In support of this explanation, early preclinical data suggested that mixed ET<sub>A/B</sub> receptor antagonism increased plasma ET-1 to a greater extent than selective ET<sub>B</sub> blockade.<sup>27</sup> Furthermore, plasma ET-1 increased in a study administering high doses of the ET<sub>A</sub>-selective antagonist atrasentan to healthy volunteers.<sup>28</sup> In the current study, plasma ET-1 did not increase after sitaxentan, but this is recognized as a poor marker of vascular ET-1 production.<sup>5,10,11</sup> An additional explanation for the lack of rise in plasma ET-1 may be an increase in ET<sub>B</sub> receptor-mediated clearance in the presence of a blocked ET<sub>A</sub> receptor. Importantly, this study provides the first clinical evidence that increases in plasma ET-1 seen in previous studies<sup>27,28</sup> may be due to upregulation of its synthesis. It would be interesting to see if other studies of selective ET<sub>A</sub>

**Table 4.** Main Study Data At Baseline and Week 6 of Each Study Period

	Placebo		Sitaxentan		Nifedipine	
	Baseline	Week 6	Baseline	Week 6	Baseline	Week 6
24-hour proteinuria, g/day	2.06±0.38	2.00±0.33	2.07±0.34	1.46±0.26*	1.95±0.30	1.99±0.33
PCR, mg/mmol	155±31	153±27	157±28	114±23 <sup>†</sup>	155±27	152±29
MAP, mm Hg	94.6±2.2	94.3±1.7	94.4±1.8	90.7±1.8 <sup>§</sup>	95.5±2.0	91.7±1.7 <sup>§</sup>
SBP, mm Hg	125.4±2.7	124.2±1.9	124.3±2.2	120.7±1.9 <sup>§</sup>	125.7±2.4	120.7±1.6 <sup>§</sup>
DBP, mm Hg	77.9±1.5	77.5±1.2	77.9±1.3	74.3±1.3*	78.9±1.5	75.7±1.2*
PWV, m/s	7.7±0.3	8.0±0.4	8.0±0.3	7.6±0.3 <sup>§</sup>	7.9±0.3	7.6±0.3 <sup>§</sup>
cAlx, %	20±2	20±2	20±2	15±2*	19±2	17±2
Plasma ELDP, fmol/mL	12.0±0.6	11.2±0.5	11.8±0.5	13.4±0.6 <sup>‡</sup>	11.3±0.6	11.6±0.5
Urine ELDP, fmol/mL	0.81±0.1	0.94±0.2	0.78±0.1	0.83±0.2	0.89±0.1	0.79±0.1
Plasma CT-proET-1, fmol/mL	20.2±0.9	20.2±1.1	20.5±1.2	23.3±1.5 <sup>‡</sup>	19.3±1.2	20.3±1.4
Plasma ET-1, pg/mL	3.6±0.5	3.7±0.6	3.6±0.5	3.7±0.5	3.5±0.5	3.5±0.5
Urine ET-1/creatinine, pg/mmol	761±95	758±93	783±84	613±81*	824±97	734±89

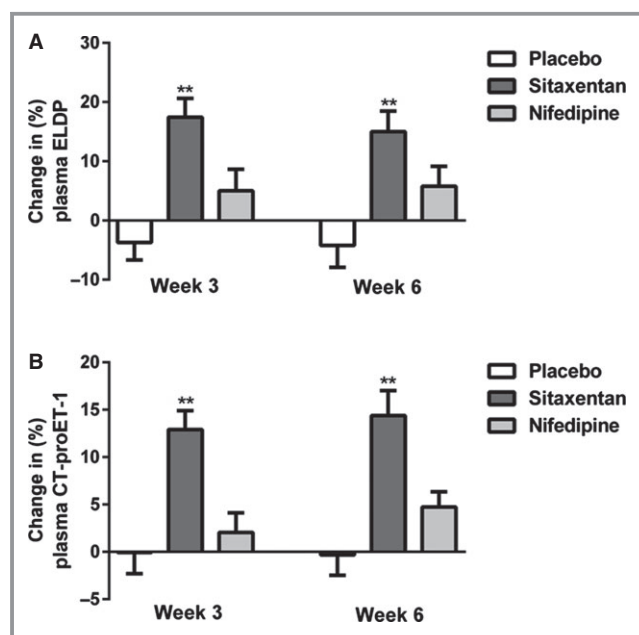
Values are given as predosing baseline±SEM. cAlx indicates central augmentation index; CT-proET-1 indicates C-terminal pro-endothelin-1; DBP, diastolic blood pressure; ELDP, endothelin-like domain peptide; ET-1, endothelin 1; MAP, mean arterial pressure; PCR, protein:creatinine ratio; PWV, pulse wave velocity; SBP, systolic blood pressure.

\* $P<0.01$ .

<sup>†</sup> $P=0.01$ .

<sup>‡</sup> $P<0.0001$ .

<sup>§</sup> $P<0.05$  for week 6 vs baseline.



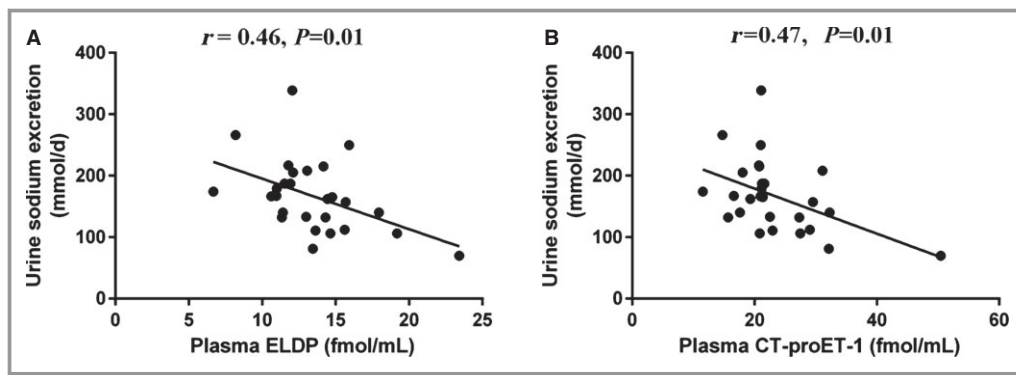
**Figure 5.** Percentage change from baseline in plasma ELDP (A) and plasma CT-proET-1 (B) following 3 and 6 weeks of treatment with placebo (open bar), sitaxentan (dark grey bar), and nifedipine (light grey bar) (mean±SEM, \* $P<0.0001$  for sitaxentan at 3 or 6 weeks vs baseline). CT-proET-1 indicates C-terminal pro-endothelin-1; ELDP, endothelin-like domain peptide.

receptor blockade<sup>29,30</sup> also showed a similar rise in ELDP and CT-proET-1. If such were the case, then this rise may be a useful biomarker of selective ET<sub>A</sub> receptor blockade, similar to

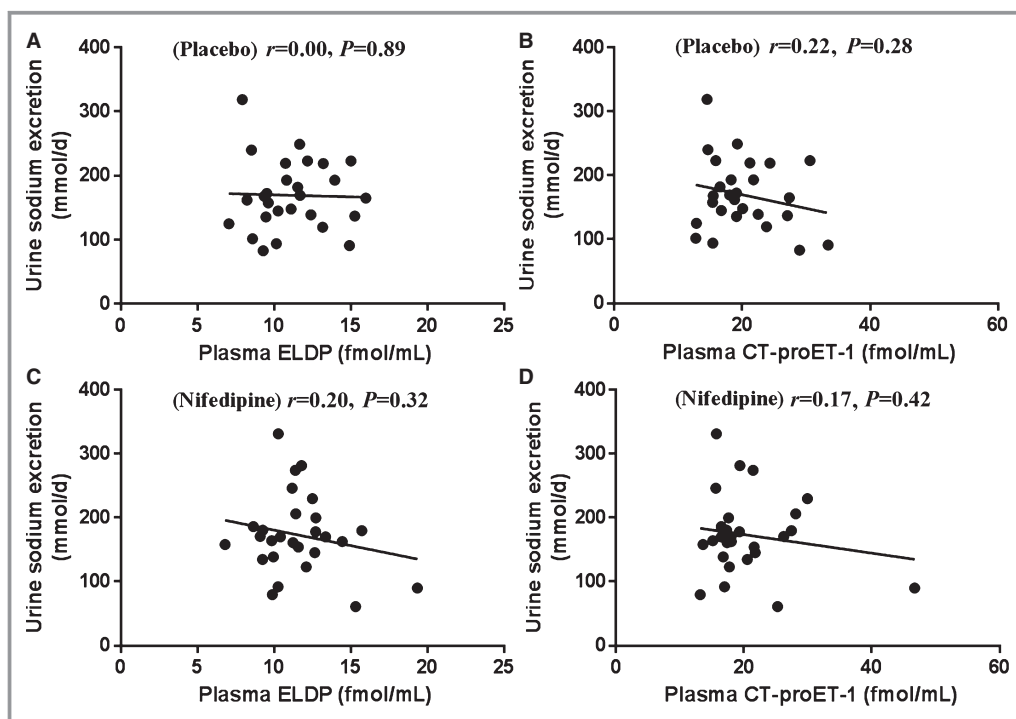
the rise in plasma ET-1 reflecting effective ET<sub>B</sub> receptor antagonism.<sup>31</sup>

Blocking ET<sub>A</sub> receptor-mediated negative feedback of *EDN1* expression may have important consequences. Edema is a recognized side effect of both selective ET<sub>A</sub> and mixed ET<sub>A/B</sub> receptor antagonists and has led to increased morbidity in clinical trials.<sup>32,33</sup> No good biomarkers currently exist for this effect. If the increases in ELDP and CT-proET-1 are due to upregulation of ET-1 synthesis, that could be implicated in the renal regulation of sodium and water.<sup>6</sup> In this study, the correlations between plasma proET-1 peptides and urinary sodium excretion (following dosing with sitaxentan) support this explanation. The fall in sodium excretion with sitaxentan will result in part from the observed fall in GFR (−9 mL/min). Nevertheless, following 6 weeks treatment with an ET<sub>A</sub> receptor antagonist, higher plasma ELDP and CT-proET-1 concentrations were associated with lower 24-hour urine sodium excretion. This association suggests that as proET-1 peptide concentration rises, there is renal conservation of both salt and water. Although a rise in plasma ET-1 may be considered to promote natriuresis and diuresis, our data also show that urine ET-1 fell following treatment with sitaxentan. Urine ET-1 is a measure of renal ET-1 production, so our findings suggest that with concomitant ET<sub>A</sub> receptor blockade, less intrarenal ET-1 may be available to act on the unblocked tubular ET<sub>B</sub> receptor to promote salt and water excretion. There was no correlation between the changes in proET-1 peptides and urine ET-1, although, again, this may be related





**Figure 6.** Relationships between plasma ELDP (A) and plasma CT-proET-1 (B) and urine sodium excretion following 6 weeks of treatment with sitaxentan. CT-proET-1 indicates C-terminal pro-endothelin-1; ELDP, endothelin-like domain peptide.



**Figure 7.** Relationships between plasma ELDP and plasma CT-proET-1 and urine sodium excretion following 6 weeks treatment with placebo (A and B) and nifedipine (C and D). CT-proET-1 indicates C-terminal pro-endothelin-1; ELDP, endothelin-like domain peptide.

to the small sample size of this study. Furthermore, free water clearance was not measured, but one may postulate that this might show a similar trend to ELDP and CT-proET-1. Our current data suggest that ELDP and CT-proET-1 merit further investigation in patients experiencing edema with an endothelin receptor antagonist because these biomarkers may have utility in tailoring the optimal dose to reduce this side effect. They may also encourage drug companies to continue development of endothelin-converting enzyme inhibitors that would effectively act as mixed  $ET_{A/B}$  receptor blockers without affecting  $ET_B$  receptor-mediated ET-1 clearance or

perturbing the negative feedback regulation of *EDN1* gene expression mediated by  $ET_A$  receptors.

## Conclusions

Plasma concentrations of the proET-1 peptides ELDP and CT-proET-1 increase as GFR declines in patients with CKD. Measurement of ELDP and CT-proET-1 may have advantages over the limitations associated with plasma ET-1. These peptides may have utility in the early diagnosis of CKD and in

assessing response to treatment. Furthermore, these peptides may serve as potentially useful biomarkers of selective ET<sub>A</sub> receptor antagonism and provide insights into fluid retention that is a recognized side effect in clinical trials of ET receptor antagonists. These preliminary data need further exploration in larger and longer clinical studies of endothelin receptor antagonists in CKD.

## Addendum

Sitaxentan has been voluntarily withdrawn by Pfizer, Ltd due to unacceptable structure-related liver side effects. However, the findings in this study are likely to be representative of the effects of the class of selective endothelin A receptor antagonists.

## Authors Contributions

Dr Dhaun, Dr Goddard, Dr Corder, and Dr Webb were involved in the design of the study. Dr Corder, Dr Yuzugulen, and Wood developed the ELDP and CT-proET-1 assays. Dr Dhaun, Dr Chariyavilaskul, Dr Yuzugulen, Dr MacIntyre, Wood, and Kimmitt undertook the study. Dr Dhaun, Dr Yuzugulen, Dr Goddard, Dr Webb, and Kimmitt analyzed the samples and the data. All authors were involved in the writing and critique of the manuscript.

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## Disclosures

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