Serelaxin attenuates renal inflammation and fibrosis in a mouse model of dilated cardiomyopathy

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Abstract
Serelaxin has been demonstrated to attenuate renal fibrosis and inflammation in cardiorenal disease. In the present study, we tested the hypothesis that serelaxin can prevent the decline in renal function in dilated cardiomyopathy (DCM) by targeting renal fibrosis and inflammation. Male transgenic mice with DCM (n = 16) and their wild-type littermates (WT; n = 20) were administered either vehicle or serelaxin (500 μg kg⁻¹ day⁻¹; subcutaneous minipumps; 8 weeks). Cardiac function was assessed via echocardiography before and during the eighth week of serelaxin treatment. Renal function and inflammation as well as cardiac and renal fibrosis were assessed at the end of the study. Serelaxin had minimal effect on cardiac function (P > 0.99). Tubulointerstitial and glomerular fibrosis were ∼3-fold greater in vehicle-treated DCM mice compared with vehicle-treated WT mice (P < 0.001). Renal mRNA expression of Tnfα and Il1α were ∼4- and ∼3-fold greater, respectively, in vehicle-treated DCM mice compared with vehicle-treated WT mice (P < 0.05). Tubulointerstitial and glomerular fibrosis were 46 and 45% less, respectively, in serelaxin-treated DCM mice than in vehicle-treated DCM mice (P < 0.01). Renal cortical mRNA expression of Tnfα and Il1α were 56 and 58% less, respectively, in the former group compared with the latter (P < 0.05). The urinary albumin:creatinine ratio was ∼3-fold greater in vehicle-treated DCM mice compared with vehicle-treated WT mice (P = 0.02). The urinary albumin:creatinine ratio was not significantly different between vehicle-treated DCM mice and serelaxin-treated DCM mice (P = 0.38). These data suggest that serelaxin can attenuate renal fibrosis and inflammation and has the potential to exert renoprotective effects in DCM.

Keywords
cardiorenal syndrome, fibrosis, inflammation, serelaxin

1 | INTRODUCTION

Heart failure (HF) patients often develop secondary renal dysfunction, but the precise mechanism by which HF leads to renal injury remains elusive (Lloyd-Jones et al., 2010; Shah & Greaves, 2010). This remains a challenge for development of new treatments to rescue renal function in HF (Rajapakse, Nanayakkara, & Kaye, 2015). Renal dysfunction arising from a primary defect in the heart is termed the cardiorenal syndrome (CRS) type 2 (Shah & Greaves, 2010), and there is an unmet clinical need to develop effective treatment strategies for this disease state.

Our recent data indicate that renal fibrosis and inflammation play a central role in the pathogenesis of experimental CRS type 2 (Giam et al., 2017). This is consistent with previous data indicating that renal inflammation is present in patients with chronic HF and in patients with chronic kidney disease (Colombo et al., 2012). In this context, there is evidence that serelaxin is effective in reducing renal fibrosis and inflammation (Lekgabe et al., 2005; Wang et al., 2017; Yoshida et al., 2012). Serelaxin prevented tumour necrosis factor-α (TNFα) induced endothelial dysfunction in rat aortic endothelial cells (Dschietzig et al., 2012) and interleukin (IL)-1 induced collagen synthesis in human dermal fibroblasts (Unemori & Amento, 1990). Serelaxin also reduced renal fibrosis, inflammation, apoptosis and expression of transforming growth factor beta (TGFβ) in experimental hypertension (Garber et al., 2001; Lekgabe et al., 2005; Yoshida et al., 2012). Furthermore, serelaxin has been demonstrated to restore the imbalance between

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Assessment of fibrosis by Masson's Trichrome staining

New Findings

- What is the central question of this study?
  The aim was to determine the renoprotective effects of serelaxin in the setting of chronic heart failure.
- What are the main findings and its importance?
  Our data indicate that serelaxin can reduce renal fibrosis and inflammation in experimental heart failure. Currently, there are no effective treatments to rescue renal function in heart failure patients, and our data suggest that serelaxin might have the potential to reduce renal fibrosis and inflammation in heart failure.

2 | METHODS

2.1 | Ethical approval

Ethical approval was obtained from the Alfred Medical Research and Education Precinct Animal Ethics Committee (AMREP; E/1641/2016/B). All experiments were conducted in accordance with the Australian Code for Care and Use of Animals for Scientific Purposes (8th edition, 2013) and complied with the principles and standards for reporting animal experiments in The Journal of Physiology and Experimental Physiology (Grundy, 2015).

2.2 | Mice

Eighteen-week-old male mice with dilated cardiomyopathy (DCM; n = 16; AMREP animal facility, Melbourne, Victoria, Australia) and their wild-type (WT) littermates (n = 20; AMREP animal facility) were used in the present study. All mice were allowed ad libitum access to food and water. This DCM model has been described previously (Yamamoto et al., 2003). DCM mice overexpress the mammalian sterile-like 20 kinase 1 (Mst1) gene in a cardiac-specific manner, which induces apoptosis in cardiomyocytes (Yamamoto et al., 2003). By 10 weeks of age, DCM mice display evidence of ventricular dilatation and reduced cardiac contractility (Yamamoto et al., 2003). The severity of HF progresses with age, and renal injury is present by 18 weeks of age in these mice (Giam et al., 2017).

2.3 | Experimental protocol

Cardiac structure and function were assessed by echocardiography in eighteen-week-old mice as previously described (Giam et al., 2016). Forty-eight hours later, osmotic minipumps (Alzet Model 2004; 0.25 μl h⁻¹; Alzet Corporation, Cupertino, CA, USA) were implanted subcutaneously in mice to administer either serelaxin (Novartis, Basel, Switzerland; 500 μg kg⁻¹ day⁻¹; n = 18) or vehicle (20 mM sodium acetate, pH 5.0; n = 18) for a period of 8 weeks. Two minipump implantation surgeries were performed 4 weeks apart in each mouse. Each minipump delivered serelaxin or vehicle for 4 weeks. Briefly, mice were anaesthetized using isoflurane (4–5% induction, 1.5–2% maintenance) delivered through a mask. A subcutaneous injection of lignocaine (2–4 mg kg⁻¹) and carprofen (5 mg kg⁻¹) were administered to provide local anaesthesia and to provide pain relief, respectively. Minipumps were then implanted in mice as previously described by us (Giam et al., 2016). The depth of anaesthesia was monitored throughout the surgery by checking the response of the animal to pedal reflex. During the final week of treatment with serelaxin or vehicle, echocardiography was repeated. Forty-eight hours later, mice were placed in metabolic cages (Tecniplast, Maggio, Italy) to collect a 24 h urine sample. Twenty-four hours later, mice were anaesthetized with isoflurane (in the same manner described above) to perform cardiac catheterization to measure left ventricular function and aortic blood pressure as previously described (Du et al., 2000). A blood sample was obtained via cardiac puncture for later analysis of plasma nitrate and nitrite. Mice were then killed by rapid excision of the heart whilst under deep isoflurane anaesthesia. Heart and kidney tissues were collected for analyses of fibrosis and inflammation.

2.4 | Assessment of fibrosis by Masson’s Trichrome staining

Paraffin sections (4 μm thick) of hearts and kidneys were used for the assessment of fibrosis. Sections were stained with Masson’s Trichrome for the assessment of cardiac and renal fibrosis (Giam et al., 2016). Ten random fields were imaged for each section using the Olympus BH2 microscope (×40 magnification). The extent of fibrosis was quantified with Image Pro-Plus software (Adept Electronic Solutions Pty Ltd; Moorabbin, Victoria, Australia) as previously described (Chu et al., 2011).

2.5 | Assessment of renal function

The urinary albumin concentration was measured according to the manufacturer’s protocol [mouse albumin ELISA kit (E90-134); Bethyl Laboratories Inc., Montgomery, TX, USA; Yu et al., 2016]. Urinary creatinine concentrations were measured using a CREP2 kit (Roche Diagnostics, Meylan, France) according to an established protocol (Badiou, Dupuy, Descomps, & Cristolead, 2003). The urinary albumin:creatinine ratio was then calculated and used as a measure of renal function.
### TABLE 1 Mouse primer sequences for qRT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gapdh</td>
<td>GGGGCTCTCTGCTCCTCCCTG</td>
<td>ACGGCCAAATCCGTTCACACC</td>
</tr>
<tr>
<td>Tnfa</td>
<td>ATCGGTCCCCAAGATGA</td>
<td>TGGTGGTTTGTGAGTGTGAGG</td>
</tr>
<tr>
<td>Il1a</td>
<td>CGCTTGAGTCGGCAAAGAAATC</td>
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<tr>
<td>Ilb</td>
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<td>ATGTGCTGCTGCGAGATTTG</td>
</tr>
<tr>
<td>Il6</td>
<td>TCGTGGAAATGAGAAAAGAGTTGTG</td>
<td>TCCAGTGGTGAGCCATCAT</td>
</tr>
<tr>
<td>Il10</td>
<td>TAATAAGCTCCAAGGCAAGAGTG</td>
<td>TCCAGCAGACTCAATACACACT</td>
</tr>
<tr>
<td>Col1a1</td>
<td>GATGAGAACATCCGCAGCC</td>
<td>TACTCTCCGCTCTTCCAGTCA</td>
</tr>
<tr>
<td>Col3a1</td>
<td>ACAAGCGAAAGCAGATGACT</td>
<td>AAGCAAACAGGCCAAATGTC</td>
</tr>
<tr>
<td>Col4a1</td>
<td>GAGTCCTTCGCCGGTCAATAGG</td>
<td>GCCGATGCTCCAGCAGTAC</td>
</tr>
<tr>
<td>Pai1</td>
<td>TCTTAAATTACCTGGGAGT</td>
<td>GCCGCCGAATAGACAT</td>
</tr>
<tr>
<td>αsma</td>
<td>GACTACTGCGGCAGCGTGAGC</td>
<td>CGGTCAGGCGATGGTCAG</td>
</tr>
<tr>
<td>Tgfβ</td>
<td>GACGGCAACAGCGGATC</td>
<td>CACTGTCCCTGGCAATGGTC</td>
</tr>
<tr>
<td>Mmp2</td>
<td>TACATTTCCTGCGGCAAAGT</td>
<td>GCCAGCAAGGAAATAGCCTAT</td>
</tr>
<tr>
<td>Mmp9</td>
<td>GCCGACTTTTGGTGGCTTC</td>
<td>AGCGGTCAGAATGTCCTTCG</td>
</tr>
</tbody>
</table>

Abbreviations: αsma, alpha smooth muscle actin; Col1a1, collagen type I; Col3a1, collagen type III; Col4a1, collagen type IV; Gapdh, glyceraldehyde 3-phosphate dehydrogenase; Il1a, interleukin-1 alpha; Il6, interleukin-1 beta; Il10, interleukin-10; Mmp2, matrix metalloproteinase-2; Mmp9, matrix metalloproteinase-9; Pai1, plasminogen activator inhibitor-1; Tgfβ, transforming growth factor beta; and Tnfa, tumour necrosis factor alpha.

### 2.6 Renal mRNA expression of profibrotic and inflammation-related genes

The mRNA expression of profibrotic and inflammation-related genes (Table 1) in the renal cortical region was quantified by qPCR as previously described (Marques et al., 2016). Briefly, complementary DNA was generated using the Applied Biosystems High Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific, Waltham, MA, USA). Samples were amplified with SYBR Green PCR master mix and run in duplicate using the QuantStudio 7 Flex Real-Time PCR system (both from Thermo Fisher Scientific). Gapdh was used as the reference transcript.

### 2.7 Assessment of total plasma nitrate and nitrite concentrations

Plasma samples were centrifuged (14,000g; 30 min; 4°C) through a 10 kDa molecular weight cut-off filter [Pall Nanosep 10k Omega (OD010C34); Pall Corporation, Ann Arbor, MI, USA]. Samples were then run in duplicate to measure total nitrate and nitrite concentrations according to the manufacturer’s protocol [nitrate/nitrite fluorometric assay kit (780051); Cayman Chemical, Ann Arbor, MI, USA].

### 2.8 Statistical analysis

GraphPad Prism (v.7; GraphPad Software, San Diego, CA, USA) was used to perform all statistical analyses. Data are means ± SD. The effects of serelaxin were analysed using one-way ANOVA followed by Tukey’s post hoc tests for multiple comparisons. Measurements of cardiac structure and function via echocardiography were repeated in the same mouse, and these data were analysed using a two-way repeated-measures ANOVA followed by Sidak’s post hoc test. Two-tailed $P \leq 0.05$ was considered statistically significant.

### 3 RESULTS

#### 3.1 Organ weights

Heart, lung and liver weights were 34, 53 and 28% greater, respectively, in vehicle-treated DCM mice compared with vehicle-treated WT mice ($P \leq 0.05$; Table 2). Kidney weight was 17% less in vehicle-treated DCM mice compared with their WT counterparts ($P = 0.02$; Table 2). In both genotypes, serelaxin had minimal effect on heart, lung, kidney and liver weights ($P \geq 0.20$; Table 2).

#### 3.2 Serelaxin attenuated renal fibrosis in DCM mice

Renal tubulointerstitial and glomerular fibrosis were ~3-fold greater in vehicle-treated DCM mice compared with vehicle-treated WT mice ($P \leq 0.001$; Figure 1). Renal tubulointerstitial and glomerular fibrosis were less (by 46 and 45%, respectively) in DCM mice treated with serelaxin compared with those treated with vehicle ($P \leq 0.01$; Figure 1).

#### 3.3 Effects of serelaxin on renal function

The urinary albumin:creatinine ratio was ~3-fold greater in vehicle-treated DCM mice compared with vehicle-treated WT mice ($P = 0.02$; Figure 2). In DCM mice, serelaxin had no significant effect on urinary albumin:creatinine ratio ($P = 0.38$; Figure 2).
<table>
<thead>
<tr>
<th>WT vehicle</th>
<th>WT serelaxin</th>
<th>DCM vehicle</th>
<th>DCM serelaxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group size 8</td>
<td>9</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Heart weight/tibia length (mg mm$^{-1}$)</td>
<td>8.0 ± 1.1</td>
<td>7.8 ± 0.5</td>
<td>10.7 ± 2.0**</td>
</tr>
<tr>
<td>Kidney weight/tibia length (mg mm$^{-1}$)</td>
<td>10.8 ± 1.1</td>
<td>10.6 ± 1.0</td>
<td>9.0 ± 0.8&quot;</td>
</tr>
<tr>
<td>Lung weight/tibia length (mg mm$^{-1}$)</td>
<td>8.8 ± 0.4</td>
<td>9.2 ± 1.0</td>
<td>13.5 ± 3.0**</td>
</tr>
<tr>
<td>Liver weight/tibia length (mg mm$^{-1}$)</td>
<td>72.5 ± 11.9</td>
<td>69.8 ± 11.7</td>
<td>92.5 ± 8.4*</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 versus vehicle-treated WT mice. P values were derived from a one-way ANOVA followed by Tukey’s post hoc test. Abbreviations/definitions: DCM, dilated cardiomyopathy; kidney weight = [(left kidney weight + right kidney weight)/2]; and WT, wild-type.

3.4 | Expression of genes relating to fibrosis and inflammation in the renal cortex

### 3.4.1 Effects of serelaxin on renal inflammation

Renal cortical mRNA expression of Tnfα and Il1α was ~4- and ~3-fold greater respectively, in vehicle-treated DCM mice compared with vehicle-treated WT mice (P ≤ 0.05; Figure 3a,b). Importantly, renal cortical mRNA expression of Tnfα and Il1α was 56 and 58% less, respectively, in serelaxin-treated DCM mice compared with vehicle-treated DCM control animals (P ≤ 0.05; Figure 3a,b).

Renal mRNA expression of Il1β, Il6 and Il10 was not significantly different between vehicle-treated WT mice and vehicle-treated DCM mice (P ≥ 0.63; Figure 3c–e). Furthermore, renal mRNA expression of these genes was not significantly different between vehicle-treated DCM mice and serelaxin-treated DCM mice (P ≥ 0.78; Figure 3c–e).

### 3.4.2 Effects of serelaxin on expression of collagen

Renal cortical mRNA expression of Col1α1, Col3α1 and Col4α1 was not significantly different between WT mice administered vehicle and DCM mice administered vehicle (P ≥ 0.62; Figure 4a–c) or between DCM mice administered vehicle and DCM mice administered serelaxin (P ≥ 0.95; Figure 4a–c).

### 3.4.3 Effects of serelaxin on the TGFβ pathway

Renal mRNA expression of Pai1 and asma were ~2-fold greater in vehicle-treated DCM mice compared with vehicle-treated WT mice (P ≤ 0.05; Figure 5a,b). Renal mRNA expression of Pai1 and asma was not significantly different between vehicle-treated DCM mice and serelaxin-treated DCM mice (P ≥ 0.92; Figure 5a,b). Renal cortical mRNA expression of Tgfβ1, Mmp2 and Mmp9 was not significantly different between vehicle-treated WT mice and vehicle-treated DCM mice or between vehicle-treated DCM mice and serelaxin-treated DCM mice (P ≥ 0.06; Figure 5c–e).
3.5 | Serelaxin tended to restore total plasma nitrate and nitrite concentrations

Total nitrate and nitrite concentrations were 53% less in vehicle-treated DCM mice compared with vehicle-treated WT mice \( (P = 0.005; \text{Figure } 6) \). Although not statistically significant, total nitrate and nitrite levels were 43% greater in serelaxin-treated DCM mice compared with vehicle-treated DCM mice \( (P = 0.08; \text{Figure } 6) \).

3.6 | Serelaxin had no effect on cardiac fibrosis

Cardiac interstitial fibrosis and perivascular fibrosis were \( \sim 17\text{- and } \sim 2\text{-fold greater, respectively, in vehicle-treated DCM mice compared with vehicle-treated WT mice} \( (P \leq 0.05; \text{Figure } 7) \). In both genotypes, serelaxin had minimal effect on cardiac fibrosis \( (P \geq 0.18; \text{Figure } 7) \).

3.7 | Cardiovascular effects of serelaxin

At baseline, mean wall thickness and fractional shortening were 28 and 54% less, respectively, in vehicle-treated DCM mice compared with vehicle-treated WT mice \( (P \leq 0.001; \text{Table } 3) \). The left ventricular end-diastolic dimension was 15% greater in vehicle-treated DCM mice compared with vehicle-treated WT mice \( (P = 0.03; \text{Table } 3) \). In DCM mice, treatment with serelaxin had no significant effect on cardiac structure or function \( (P \geq 0.48; \text{Table } 3) \).

Mean arterial pressure and left ventricular systolic pressure were 27 and 31% less, respectively, in vehicle-treated DCM mice compared with vehicle-treated WT mice \( (P \leq 0.01; \text{Table } 4) \). Left ventricular end-diastolic pressure was \( \sim 2\text{-fold greater in vehicle-treated DCM mice compared with vehicle-treated WT mice} \( (P < 0.001; \text{Table } 4) \). In both genotypes, serelaxin had no significant effect on any of these parameters \( (P \geq 0.06; \text{Table } 4) \).

4 | DISCUSSION

Cardioprotective effects of serelaxin have been previously reported in HF \( \text{(Lekgabe et al., 2005; Liu et al., 2016)} \). In the present study,
we hypothesized that serelaxin can rescue renal function in CRS type 2 by restoring NO bioavailability and attenuating renal fibrosis and inflammation. We found that renal tubulointerstitial and glomerular fibrosis were greater in vehicle-treated DCM mice compared with vehicle-treated WT mice. This is consistent with previous studies demonstrating that tubulointerstitial fibrosis is present in renal disease associated with HF (Cruz et al., 2013; Zeisberg & Neilson, 2010). Importantly, tubulointerstitial and glomerular fibrosis were less in serelaxin-treated DCM mice compared with those treated with vehicle. These data are consistent with previous findings, which indicate that serelaxin can halt the progression of renal fibrosis in experimental renal failure (Garber et al., 2001; Yoshida et al., 2012).

Renal expression of TNFα is augmented in renal failure (Therrien et al., 2012). Of note, neutralizing the expression of TNFα attenuates renal inflammation and fibrosis and the development of albuminuria in rats with renal failure (Therrien et al., 2012). Neutralization of TNFα expression in renal failure is also associated with reduced activation of nuclear factor kappa-light-chain-enhancer of activated B cells and infiltration of renal interstitial macrophages (Therrien et al., 2012). In the present study, we found that renal expression of both TNFα and IL-1α was augmented in vehicle-treated DCM mice compared with vehicle-treated WT mice. Renal expression of TNFα and IL-1α was less in DCM mice treated with serelaxin compared with DCM mice treated with vehicle. Taken together with previous data, our present findings suggest that antifibrotic effects of serelaxin in the kidney are likely to be
mediated, at least in part, via a reduction in the expression of inflammatory markers, particularly TNFα. Consistent with our data, serelaxin has been demonstrated to prevent TNFα-induced endothelial dysfunction in rat aortic endothelial cells (Dschietzig et al., 2012). Furthermore, serelaxin has been demonstrated to reverse IL-1-induced collagen production in human dermal fibroblasts (Unemori & Amento, 1990). Together, these data indicate that serelaxin can reduce the expression of inflammatory markers, particularly TNFα, which in turn can attenuate the progression of fibrosis.

There is evidence that reduced NO bioavailability contributes to the progression of renal injury in HF (Ito et al., 2013). Our present data indicate that plasma nitrate and nitrite concentrations were less in vehicle-treated DCM mice compared with vehicle-treated WT mice. Plasma nitrate and nitrite concentrations tended to be greater in serelaxin-treated DCM mice compared with those treated with vehicle. There is considerable evidence that serelaxin mediates its renoprotective effects, at least in part, via increasing NO concentrations in experimental hypertension and diabetes (Ng et al., 2017; Sasser et al., 2014; Segal et al., 2012). Furthermore, it has been demonstrated that exogenous NO improved renal function by reducing serum concentrations of the pro-inflammatory marker TNFα after abdominal aortic surgery in pigs (Lozano et al., 2005), providing evidence that improved NO bioavailability can exert renoprotective effects by inhibiting TNFα-mediated inflammation.

In the present study, serelaxin attenuated renal inflammation and fibrosis in DCM mice, but this did not prevent the loss of renal function. We found that the albumin:creatinine ratio was greater in vehicle-treated DCM mice than in vehicle-treated WT mice, and this ratio was not significantly different between DCM mice administered vehicle and DCM mice administered serelaxin. In this context, previous data indicate that even minimal reductions in renal fibrosis are associated with improvements in renal function in rodent models (Gilbert et al., 2012; Lee, 2003; Li et al., 2014; Mimura et al., 2010). For example,
even minute reductions in tubulointerstitial fibrosis (~2%) were associated with 3-fold increases in glomerular filtration rate in diabetic rats (Gilbert et al., 2012). In the present study, we did not observe an improvement in renal function in DCM mice administered serelaxin, although these mice had 40% less renal fibrosis compared with those treated with vehicle. Our present finding that serelaxin fails to improve renal function in experimental HF is consistent with data indicating that serelaxin does not improve glomerular filtration rate in HF patients (Voors et al., 2014). Mean wall thickness and fractional shortening were less in vehicle-treated DCM mice compared with vehicle-treated WT mice. The left ventricular systolic dimension was greater in vehicle-treated DCM mice compared with vehicle-treated WT mice. Furthermore, cardiac interstitial and perivascular fibrosis were greater in vehicle-treated DCM mice compared with vehicle-treated WT mice. Serelaxin had minimal effect on cardiac structure, function and fibrosis in DCM mice. It might be that serelaxin failed to mitigate the deleterious effects of cardiac-specific overexpression of the Mst1 transgene in these mice. Therefore, it is important to test the cardiac effects of serelaxin in other experimental models of CRS type 2. Alternatively, serelaxin might be able to target only renal and not cardiac-related mechanisms in CRS type 2. It remains to be determined whether different molecular mechanisms are responsible for driving cardiac and renal fibrosis and dysfunction in CRS type 2. Our primary aim in the present study was to test renoprotective effects of serelaxin in the presence of established cardiac dysfunction. Therefore, we used the DCM mouse model because we found previously that these mice have advanced HF and reduced renal function (Giam et al., 2017). We demonstrated that targeting glutathione via administration of N-acetylcysteine reduced both cardiac and renal fibrosis in DCM mice (Giam et al., 2016, 2017). Taken together with our present findings, these data suggest that an imbalance in oxidative stress and NO bioavailability might be central in driving both cardiac and renal fibrosis in CRS type 2. This hypothesis merits further investigation in future.

We found that arterial pressure was lower in vehicle-treated DCM mice than in vehicle-treated WT mice. Serelaxin had minimal effect on arterial pressure in both genotypes, indicating that antibfibratic and anti-inflammatory effects of serelaxin are mediated independently of changes in arterial pressure. This is important, because treatments that reduce arterial pressure may lead to hypotension in HF patients with reduced ejection fraction.

In conclusion, our data provide evidence that serelaxin can reduce renal fibrosis and inflammation in the presence of established cardiac dysfunction. Accordingly, it is of interest to investigate further the renoprotective effects of serelaxin in chronic HF.

**AUTHOR CONTRIBUTIONS**

All experiments were conducted in the Heart Failure Research Laboratory in the Baker Heart and Diabetes Institute and in the Biomedicine Discovery Institute of Monash University. N.W.R. and D.M.K. took part in the conception of the study. B.G. and N.W.R. collected and analysed the data. B.G., P.-Y.C., S.K., D.H., A.M., H.K., X.-J.D. and N.W.R. contributed to conduct of the experiments and data analysis. B.G. and N.W.R. drafted the manuscript, and all authors contributed to revision of the manuscript. All authors approved the final version of the manuscript. All authors contributed to revising the manuscript. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

**COMPETING INTERESTS**

None declared.

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