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Relaxin elicits renoprotective actions accompanied by increasing bile acid levels in streptozotocin-induced diabetic mice

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ABSTRACT

Background: The peptide hormone relaxin has potent anti-fibrotic and anti-inflammatory properties in various organs, including the kidneys. However, the protective effects of relaxin in the context of diabetic kidney complications remain controversial. Here, we aimed to evaluate the effects of relaxin treatment on key markers of kidney fibrosis, oxidative stress, and inflammation and their subsequent impact on bile acid metabolism in the streptozotocin-induced diabetes mouse model.

Methods and results: Male mice were randomly allocated to placebo-treated control, placebo-treated diabetes or relaxin-treated diabetes groups (0.5 mg/kg/d, final 2 weeks of diabetes). After 12 weeks of diabetes or sham, the kidney cortex was harvested for metabolomic and gene expression analyses. Diabetic mice exhibited significant hyperglycaemia and increased circulating levels of creatine, hypoxanthine and trimethylamine N-oxide in the plasma. This was accompanied by increased expression of key markers of oxidative stress (*Txnip*), inflammation (*Ccl2 and Il6*) and fibrosis (*Col1a1, Mmp2 and Fn1*) in the diabetic kidney cortex. Relaxin treatment for the final 2 weeks of diabetes significantly reduced these key markers of renal fibrosis, inflammation, and oxidative stress in diabetic mice. Furthermore, relaxin treatment significantly increased the levels of bile acid metabolites, deoxycholic acid and sodium glycodeoxycholic acid, which may in part contribute to the renoprotective action of relaxin in diabetes.

Conclusion: In summary, this study shows the therapeutic potential of relaxin and that it may be used as an adjunctive treatment for diabetic kidney complications.

1. Introduction

The global prevalence of diabetes has been rising steadily for many years. According to the most recent estimates from the International Diabetes Federation Diabetes Atlas, more than 536 million individuals worldwide have diabetes, which is projected to affect 783 million people by 2045 [1]. It is well-established that hyperglycaemia leads to the activation of detrimental cellular signalling pathways, causing damage to cells, tissues and whole organ systems, contributing to the pathogenesis of diabetic complications [2,3]. Among the deleterious complications of diabetes, nearly 40% of patients with long-standing type 1 and type 2 diabetes suffer from diabetic kidney disease (DKD) which is the leading cause of end-stage renal disease [4,5]. In addition to appropriate glycaemic control, current management and therapies that limit DKD progression include angiotensin II receptor blockers, angiotensin-converting enzyme inhibitors, and more recently sodium–glucose co-transporter 2 inhibitors, glucagon-like peptide-1 receptor agonists and mineralocorticoid receptor antagonists [6–9]. Despite the standard of care pharmacotherapeutics, the residual risk of DKD progression remains high as these current treatments often do not

Abbreviations: DKD, diabetic kidney disease; ROS, reactive oxygen species; TGF-β, transforming growth factor-β; RXFP1, relaxin/insulin-like family peptide receptor 1; NO, nitric oxide; qPCR, Quantitative real-time PCR; STZ, streptozotocin.

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consistently prevent progression to end-stage renal failure in patients with DKD [6–9]. Thus, the identification of new molecular pathways with an opportunity for therapeutic targeting to reverse DKD progression would address this major unmet clinical need.

There is a strong appreciation for the role of chronic low-grade inflammation and oxidative stress in the pathogenesis of diabetic complications [10–12]. Specifically, the overproduction of reactive oxygen species (ROS) and increased levels of inflammatory markers (including TNF-α, IL-1, IL-6, and monocyte chemoattractant protein 1) are reported in patients with diabetes [10,11]. These factors activate several signalling pathways, leading to the increased activation of growth factors such as transforming growth factor-β (TGF-β), and accumulation of excess collagen, contributing to pathological fibrosis [13–15]. One of the emerging therapies is the peptide hormone, relaxin, which mediates its actions through its major receptor, relaxin/insulin-like family peptide receptor 1 (RXFP1). Relaxin has pleiotropic effects in several organs including the heart, vasculature, liver and kidney [16–20]. Mechanistic studies have shown that relaxin treatment diminishes the effects of pro-fibrotic stimuli such as TGF-β1 through the inhibition of Smad2/3 phosphorylation, which is dependent at least in part on the pERK1/2, nitric oxide (NO) and Notch-1 signalling pathways [13,14]. This leads to a decrease in the rate of collagen deposition and increased collagen degradation, which promotes relaxin-induced degradation of matrix/-collagen and eventually reduction in tissue fibrosis [13,14].

The anti-fibrotic actions of relaxin are well-reported to inhibit excessive collagen accumulation and fibrosis in cells and in organs of different types of animal models such as hypertension and diabetes [14, 21–24]. In the kidney of hypertensive rats, chronic infusion of relaxin ameliorates renal inflammation, collagen content and lipid accumulation, leading to the overall reduction in fibrosis and improvement in renal function [25]. Despite the renoprotective effects of relaxin in hypertension, the anti-fibrotic effects of relaxin remain controversial in diabetes [21,22,26–28]. For instance, earlier studies demonstrated that relaxin treatment did not exhibit any beneficial effects in the kidneys of streptozotocin (STZ)-treated endothelial nitric oxide synthase knock-out (eNOS-KO) mice or STZ-treated transgenic mRen-2 rats [27,28]. In comparison to other diabetic models, relaxin treatment reverses insulin resistance in the muscle and increases endothelium-dependent relaxation in the aorta of type 2 diabetic mice [26]. Similarly, relaxin treatment for 2 weeks reverses vascular dysfunction in the mesenteric artery and aorta, as well as ameliorates cardiac remodelling in the STZ-induced diabetes mouse model [21,22]. Taken together, the inconsistencies in the reported renoprotective effects of relaxin in diabetes may be at least in part attributed to the induction of STZ in eNOS-KO or transgenic mRen-2 rats. Given there is a lack of concrete evidence that relaxin treatment is effective in reversing diabetes-induced kidney complications, particularly in an animal model of type 1 diabetes, we evaluated the effects of relaxin treatment on kidney oxidative stress, inflammation and genetic markers of fibrosis in the STZ-induced diabetes mouse model.

2. Materials and methods

2.1. Animal model

This study used male C57BL/6 J mice aged 19 weeks old ($n = 37$) bred in the Alfred Medical Research and Education Precinct (AMREP, now known as the Alfred Research Alliance) Animal Centre. The mice were housed and bred at room temperature of 18–22 °C under 12 h-day/night cycles and given free access to adequate standard rodent chow (Barastoc, VIC, Australia) and water. At 6 weeks of age, the mice were randomly allocated to either control ($n = 13$) or diabetic groups ($n = 24$). After overnight fasting, mice from the diabetic group were given daily intraperitoneal injections of 55 mg/kg body weight doses of streptozotocin (STZ, AdipoGen Life Sciences Cat# AG-CN2–0046-G001) over 5 consecutive days to induce type 1 diabetes [29]. Mice from the control group were injected with equal volumes of 0.1 M citrate vehicle (control). Blood glucose levels were monitored fortnightly after 2 weeks from the initial STZ/citrate buffer injections using a glucometer (Accu-Chek Performa, Roche, Basel, Switzerland). Mice with blood glucose levels above 25 mM were considered diabetic. Eleven weeks following the STZ/citrate buffer injections, diabetic mice were further assigned into 2 groups to receive either a placebo (20 mM sodium acetate, $n = 13$) or relaxin treatment (0.5 mg/kg/day, $n = 11$). Recombinant human relaxin-2 was kindly provided by Novartis Pharma AG, Basel, Switzerland. Both the placebo and relaxin were administered subcutaneously using sterile osmotic pumps (ALZET Model 1002, CA, USA) for 2 weeks. Relaxin treatment for two weeks in diabetic mice yielded plasma relaxin levels of 93 ± 16 ng/ml, whereas relaxin levels are below the detection limit in the control mice and placebo-treated diabetic mice [22]. All procedures for animal care and usage were as humane as possible and approved by the AMREP Animal Ethic Committee (E/1535/2015/B) and complied with the Australian Code of Practice & National Health and Medical Research Council guidelines and the ARRIVE guidelines.

2.2. Tissue collection

After two weeks of treatment with either placebo or relaxin, mice were anaesthetised by a cocktail of ketamine (85 mg/kg) and xylazine (8.5 mg/kg) via intraperitoneal injections followed by cardiac puncture for blood collection. Two μl whole blood aliquots were used to measure HbA1c using a Cobas b 101 POC system (Roche, Basel, Switzerland). Concentrations of relaxin in the blood plasma at study end point were measured using a Human Relaxin-2 Quantikine ELISA kit (Cat# DRL200; R&D systems, Minneapolis, MN, USA) following the manufacturer's protocol, with a threshold level of sensitivity of 4.57 pg/ml. The whole kidney was isolated and immediately placed in ice-cold Krebs bicarbonate solution (120 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 11.1 mM D-glucose, 2.5 mM $CaCl_2$) and then cleared of fat, connective tissues and separated into cortex and medulla region. The kidney cortex and liver were snapped frozen and stored at − 80 °C for subsequent molecular analysis.

2.3. Quantitative real-time PCR (qPCR)

The frozen kidney cortex was pulverised using a BeadBug homogenizer (Sigma Aldrich, Singapore). Total RNA from the kidney cortex was extracted using the Bio-Rad Aurum Total RNA Mini Kit (Cat# 732–6820). Quality and quantity of RNA were analysed using a NanoDrop ND1000 Spectrophotometer (Thermo Fischer Scientific, Singapore) with A_{260}:A_{280} ratios > 1.8 indicating sufficient quality for qPCR analysis [30]. First-strand cDNA synthesis used 1 μg of total RNA in a 20 μl reaction using the Bio-Rad iScript cDNA Synthesis Kit (Biorad, Singapore).

The comparative cycle threshold ($2^{-\Delta Ct}$) method was used to analyse the quantitative real-time polymerase chain reaction (qPCR) data as described previously [31–33]. Briefly, monocyte chemoattractant protein-1 (*Ccl2*), collagen type 1 alpha 1 (*Col1a1*), fibronectin 1 (*Fn1*), interleukin-6 (*Il6)*, G protein-coupled bile acid receptor 1 (*Gpbar1*), matrix metalloproteinase 2 (*Mmp2*), endothelial NOS (*Nos3*), farnesoid X receptor (*Nr1h4*), SMAD family member 2 (*Smad2*), transforming growth factor β1 (*Tgfb1*), tissue inhibitor of metalloproteinase 1 (*Timp1*), and thioredoxin interacting protein (*Txnip*) gene expression was analysed in the mouse kidney cortex. Mouse-specific forward and reverse primers (Supplementary Table S1) were purchased from Sigma Aldrich (Singapore). Real-time PCR reaction was determined by SYBR green chemistry using the Bio-Rad CFX96 Real-time PCR system with triplicate samples containing SYBR Green PCR Master Mix (Biorad, Singapore) and 10 μM (gene of interest) of primers. β-actin (*Actb*) was used as the reference gene. The mean C_T value of control mice was used as the internal calibrator and subtracted from the mean C_T value of the gene of

interest, and then analysed using the $2^{-\Delta Ct}$ method to reduce unwanted sources of variation, as described previously [34,35]. These normalised data (ΔC_T) were expressed as fold change relative to means of controls (non-diabetic) and presented as mean ± SEM.

2.4. Liquid chromatography-mass spectrometry (LC-MS)

Additional frozen kidney cortex and liver (22–28 mg) were homogenised in chloroform: methanol (3:1) using the BeadBug homogenizer (Sigma Aldrich, Singapore) and centrifuged at 10,000 rpm for 1 min. The resulting supernatant was dried in the Eppendorf Concentrator Plus vacuum centrifuge machine at 45 °C for 50 min. For plasma samples, proteins were precipitated using methanol and centrifuged at 10,000 rpm for 5 min. Methanol was added to the pellet after separating the supernatant to extract the remaining lipids in the sample. Supernatants obtained from the kidney cortex, liver and plasma were used to run LC-MS for the analysis of metabolites. Briefly, dried lipid samples from the kidney cortex and liver were resuspended in 1 ml of methanol in glass vials and 200 µl of the extracted lipid samples from the plasma were aliquoted into glass vials with vial inserts to be injected into the LC-MS system (SHIMADZU LCMS-8050). Selected Ion Monitoring (SIM) mode was used for kidney cortex and liver samples, whereas full scan mode was used for plasma samples. The product ions from 100 to 1000 were collected for kidney cortex and liver samples, and 50–800 were collected for plasma samples. Chromatographs produced were analysed to identify various metabolites present in the samples. This was achieved by comparing the specific retention times of each respective elution peak in the chromatograph to known compounds. Chromatographs collected from LC-MS were integrated to get the retention times and peak areas of each mass-to-charge ratio (m/z) which were compared with known retention times and peak areas of standard compounds. These standards were obtained from previous studies [36] which are used to identify compounds detected in the samples (Supplementary Table S2-S3). Peak areas were normalised to be relative to the sum of all peak areas for each sample [37]. The normalised data were presented in the soft independent modelling of class analogy (SIMCA) software to classify the data points and observe the overall effect with Orthogonal Projections to Latent Structures-Discriminant Analysis (OPLS-DA) score plots [38,39].

2.5. Statistical analyses

Data were expressed as mean ± SEM of normalised peak areas and $2^{\hat{}-\Delta\Delta Ct}$ values which is fold change relative to the mean of the control group, for LC-MS and qPCR respectively [40]. Data were analysed using the GraphPad Prism software (Prism version 6.0, GraphPad Software, San Diego, CA, USA) to perform either One-way ANOVA with Tukey's post-hoc test or Kruskal-Wallis test with Dunn's post-hoc test as appropriate to determine any significant differences between the groups. Results with $P < 0.05$ were considered statistically significant. *n* refers to the number of animals or independent experiments.

3. Results

3.1. Systemic characteristics following 12 weeks of STZ-induced diabetes

At the end of the 12 weeks of STZ-induced diabetes, blood glucose levels and glycated haemoglobin (HbA1c) of the diabetic mice were significantly ($F_{2,26} = 62.11$, $P < 0.0001$) higher compared to the control mice. Relaxin treatment for 2 weeks in the diabetic mice did not affect blood glucose or HbA1c levels (Fig. 1**A-B**). Similarly, body weight gained was significantly ($F_{2,26} = 30.93$, $P < 0.0001$) lower in diabetic mice compared to control mice, and relaxin treatment had no impact on the body weight (Fig. 1 **C**) or kidney weight (Fig. 1**D-E**).

OPLS-DA analyses were performed on plasma samples to measure metabolite concentrations in the control, placebo-treated diabetic and relaxin-treated diabeticmice. A clear separation in metabolite profile was observed between the control and placebo-treated diabetic groups, indicating a marked alteration of metabolites in the plasma of the STZ-induced diabetic model (Supplementary Fig. S1A). Similarly, relaxin treatment in diabetic mice affected the metabolite variations but in a different direction. Specifically, OPLS-DA analysis showed a clear separation between the diabetic+relaxin group and the control group in the plasma (Supplementary Fig. S1A). However, the separation of metabolite profile between relaxin treatment in the diabetic mice was less

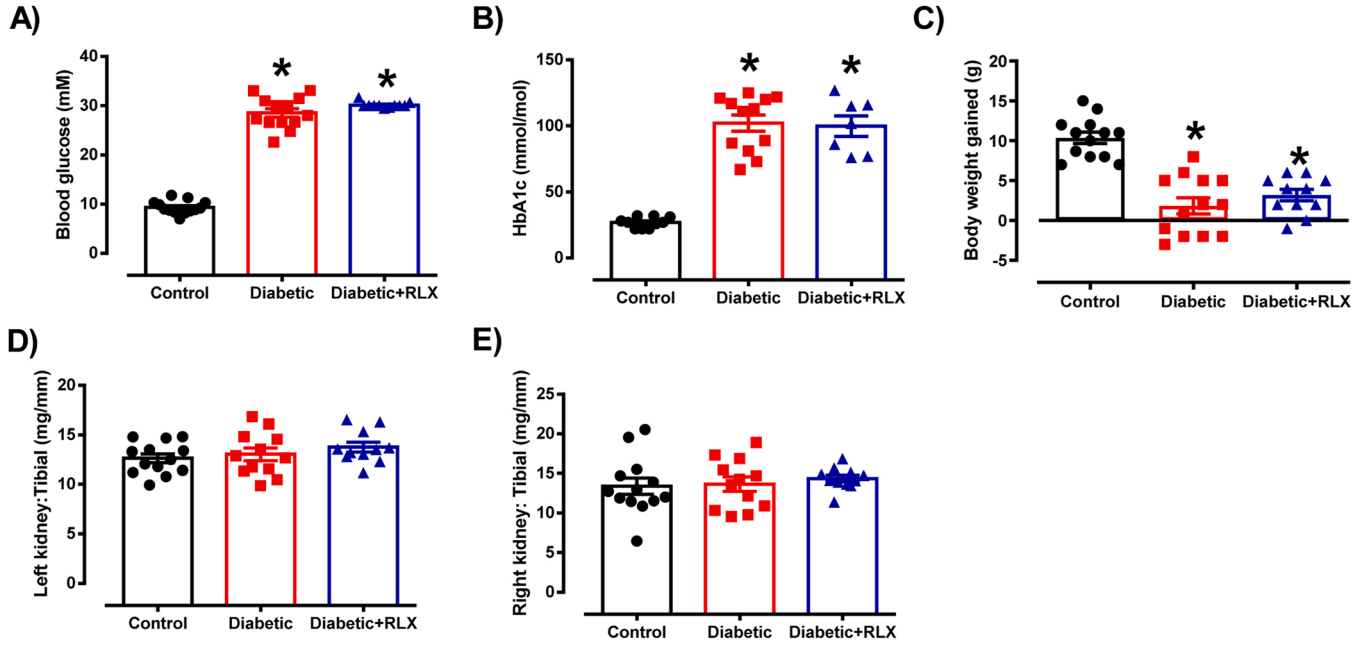

Fig. 1. Systemic characteristics of placebo-treated control, placebo-treated diabetic or relaxin-treated diabetic mice, including A) blood glucose level (Kruskal-Wallis test with Dunn's post-hoc test), B) glycated haemoglobin level, C) body weight gained, D) normalised left kidney weight and E) normalised right kidney weight. Values are expressed as mean ± SEM; number of mice per group as shown by individual data points. *Significantly different to placebo-treated control mice (One-way ANOVA with Tukey's post-hoc test, $P < 0.05$).

apparent compared to the diabetic mice administered placebo, suggesting that relaxin treatment has minimal impact on modulating the plasma metabolite profile in diabetic mice.

In this study, we identified several metabolites that are potentially important characteristics of diabetes-induced kidney disease (Fig. 2). Specifically, diabetes caused a significant ($F_{2,26} = 9.192$, $P = 0.001$) increase in creatine levels and a decrease ($F_{2,26} = 8.802$, $P = 0.001$) in carnitine levels in the plasma when compared to control (Fig. 2**A-B**). Furthermore, the circulating levels of trimethylamine N-oxide (TMAO) and hypoxanthine were also significantly increased ($F_{2,26} = 11.85$, $P = 0.0002$) in the plasma of diabetic mice (Fig. 2**C-D**). Relaxin treatment had no effect on the circulating levels of creatine, carnitine, TMAO and hypoxanthine in the diabetic mice (Fig. 2**A-D**). Uric acid was identified in the LC-MS analysis, but was not altered between the treatment groups (Fig. 2**E**).

3.2. Effects of relaxin treatment on markers of fibrosis, oxidative stress, and inflammation in the diabetic kidney cortex

In the kidney cortex, diabetes significantly ($F_{2,16} = 6.624$, $P = 0.008$) increased the expression of collagen type I α1 (*Col1a1*) and fibronectin 1 (*Fn1*) ($F_{2,19} = 22.41$, $P < 0.0001$) compared to controls (Fig. 3**A-B**). However, the diabetes-induced increase in collagen and fibronectin expression in the kidney cortex was not accompanied by changes in transforming growth factor β1 (*Tgfb1*) or Smad2 (*Smad2*) mRNA expression (Fig. 3**C-D**). We found that relaxin treatment for 2 weeks significantly reduced the expression of *Col1a1*, *Fn1*, *Tgfb1* and *Smad2* in the diabetic kidney cortex in comparison to placebo-treated diabetic mice (Fig. 3**A-D**). Similarly, the mRNA expression of matrix metalloproteinase 2 (*Mmp2*) in the kidney cortex was significantly ($F_{2,16} = 10.32$, $P = 0.0013$) upregulated by diabetes and was significantly reduced by relaxin treatment (Fig. 3**E**). Diabetes also significantly ($F_{2,15} = 13.78$, $P = 0.0004$) increased the mRNA expression of tissue inhibitors of metalloproteinases 1 (*Timp1*) in the kidney cortex; however, relaxin treatment did not affect *Timp1* expression (Fig. 3**F**). In addition to markers of fibrosis, we evaluated several markers of oxidative stress and inflammation in the kidney cortex. Diabetes significantly ($F_{2,16} = 6.112$, $P = 0.0107$) increased the expression of thioredoxin-interacting protein (*Txnip*) and endothelial NOS (*Nos3*) ($F_{2,17} = 20.18$, $P < 0.0001$) compared to controls (Fig. 3**A-B**). Similarly, mRNA expression of the inflammatory cytokines, *Il6* ($F_{2,17} = 10.04$, $P = 0.0013$) and *Ccl2* was also significantly ($F_{2,18} = 13.72$, $P = 0.0002$) upregulated in the diabetic kidney cortex (Fig. 4**C-D**). Importantly, we found that all four genes (*Txnip, Nos3, Il6 and Ccl2*) were significantly downregulated by relaxin treatment in the diabetic kidney cortex when compared to placebo-treated mice (Fig. 4**A-D**).

3.3. Effects of relaxin treatment on bile acid metabolites in the kidney cortex and liver

A distinct alteration of metabolites in the kidney cortex and liver of the STZ-induced diabetic model was observed as there was a clear separation in the metabolite profiles between the control and diabetic groups (Supplementary Fig. S1B-S1C). Interestingly, relaxin treatment also affected the metabolite levels, demonstrated by a clear separation from the diabetic group. However, the separation of metabolite profiles between relaxin-treated diabetic mice and diabetic mice treated with placebo was less obvious, suggesting that relaxin treatment has little impact on modulating the overall metabolite profile in the diabetic kidney cortex and liver (Supplementary Fig. S1B-S1C). Several biomarkers of metabolic stress, such as bile acid metabolites are recognised as important modulators of kidney function. Hence, we measured several key bile acid metabolites in the kidney cortex. Specifically, we found that the level of deoxycholic acid and cholic acid were comparable between diabetic and control kidney cortex (Fig. 5**A-B**). Interestingly, relaxin treatment for 2 weeks significantly ($F_{2,23} = 67.98$, $P < 0.0001$) increased the level of deoxycholic acid and concomitantly decreased ($F_{2,23} = 16.94$, $P < 0.0001$) the level of cholic acids in the diabetic kidney cortex (Fig. 5**A-B**) when compared to either diabetic or control mice. This suggests that the effects of relaxin on bile acid metabolites in the kidney are independent of diabetes. Levels of deoxycholic acid and cholic acids were not detected in the liver from all groups of mice. However, diabetes significantly ($F_{2,23} = 84.53$, $P < 0.0001$) increased the level of glycosylated deoxycholic acid, which was further increased in the kidney cortex of diabetic mice treated with relaxin (Fig. 5**C**). This effect was also observed in the liver where the level of glycodeoxycholic acid was significantly increased only with relaxin treatment in diabetic mice when compared to control (Supplementary Fig. S2A). Neither

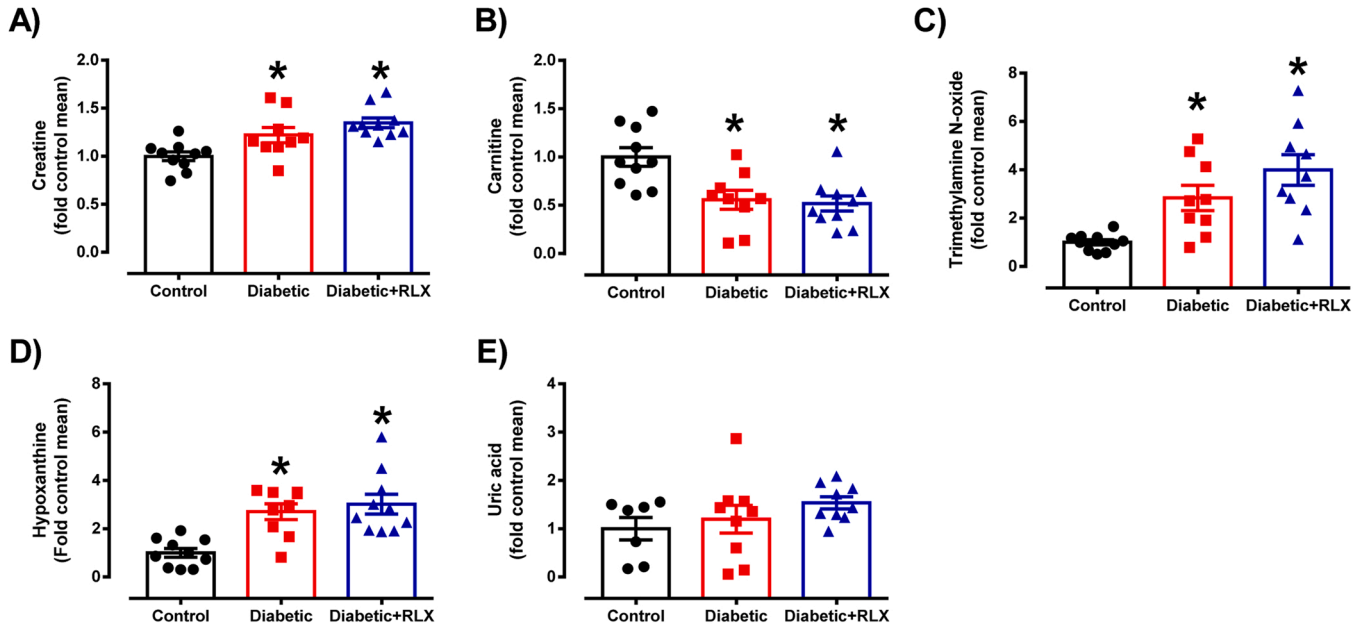

Fig. 2. Circulating levels of A) creatine, B) carnitine, C) trimethylamine N-oxide, D) hypoxanthine and E) uric acid in the plasma of placebo-treated control, placebo-treated diabetic or relaxin-treated diabetic mice. Values are expressed as mean ± SEM; number of mice per group as shown by individual data points. *Significantly different to placebo-treated control mice (One-way ANOVA with Tukey's post-hoc test, $P < 0.05$).

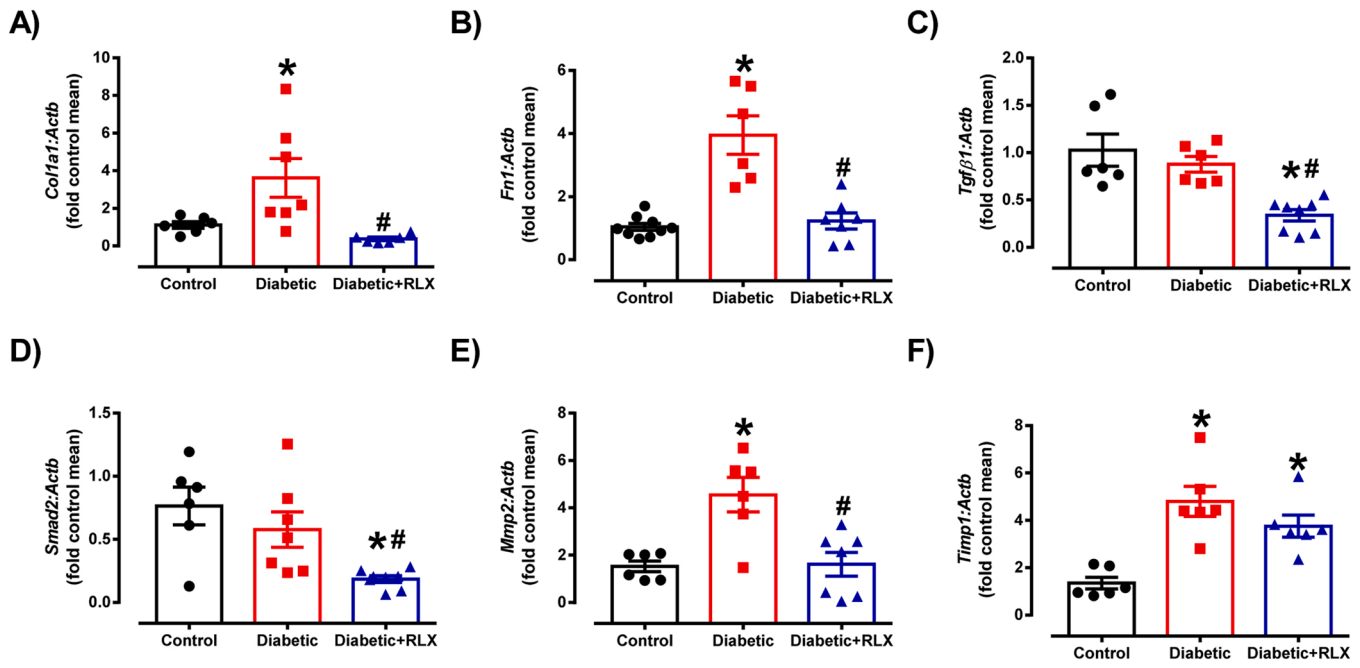

Fig. 3. Fibrotic gene expression in placebo-treated control, placebo-treated diabetic or relaxin-treated diabetic mice, including A) collagen type I α1 (*Col1a1*), B) fibronectin 1 (*Fn1*), C) transforming growth factor beta 1 (*Tgfb1*), D) *Smad2*, E) matrix metalloproteinases 2 (*Mmp2*) and F) tissue inhibitor of metalloproteinases 1 (*Timp1*) in the kidney cortex. Values are expressed as mean ± SEM; number of mice per group as shown by individual data points. Gene expression is normalised to the reference gene (*Actb*) and presented as fold control mean. *Significantly different to placebo-treated control mice (One-way ANOVA with Tukey's post-hoc test, $P < 0.05$). #Significantly different to untreated diabetic mice (One-way ANOVA with Tukey's post-hoc test, $P < 0.05$).

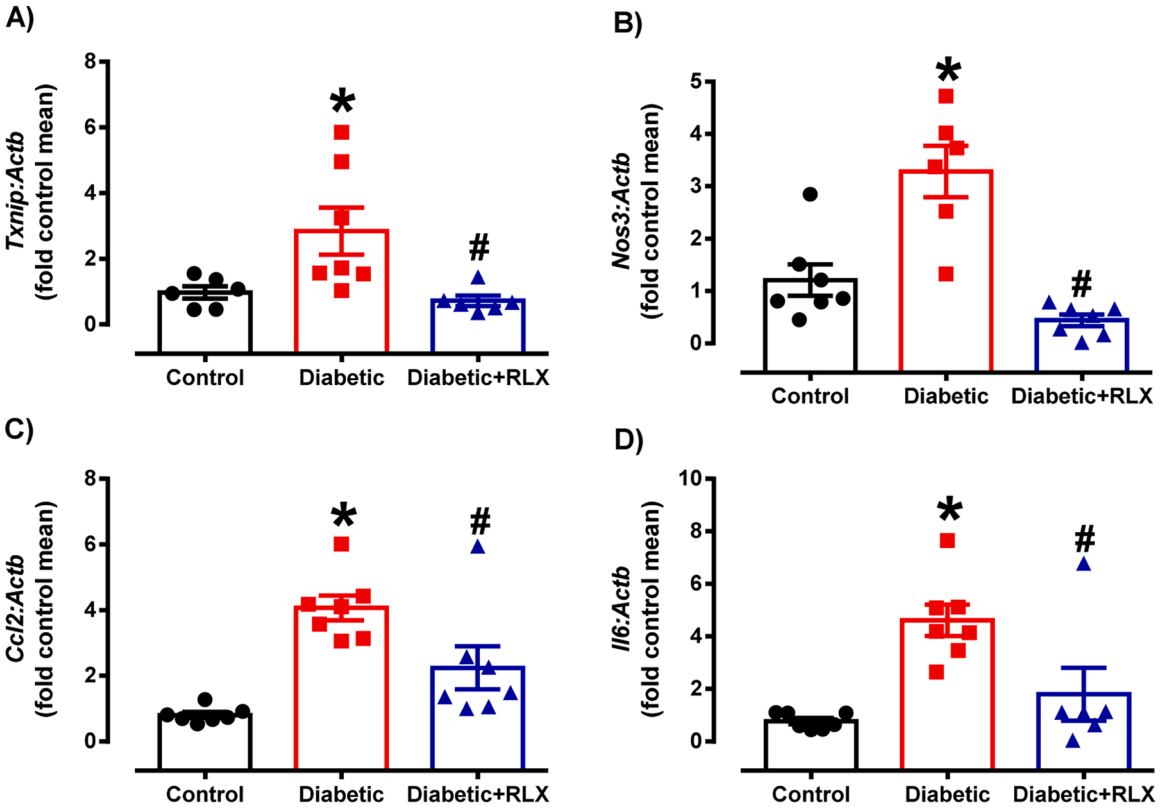

Fig. 4. Gene expression of A) thioredoxin interacting protein (*Txnip*), B) endothelial nitric oxide synthase (*Nos3*), C) monocyte chemoattractant protein-1 (*Ccl2*) and D) interleukin 6 (*Il6*) in the kidney cortex from placebo-treated control, placebo-treated diabetic or relaxin-treated diabetic mice. Gene expression is normalised to the reference gene (*Actb*) and presented as fold control mean. Values are expressed as mean ± SEM; number of mice per group as shown by individual data points. *Significantly different to placebo-treated control mice (One-way ANOVA with Tukey's post-hoc test, $P < 0.05$). #Significantly different to untreated diabetic mice (One-way ANOVA with Tukey's post-hoc test, $P < 0.05$).

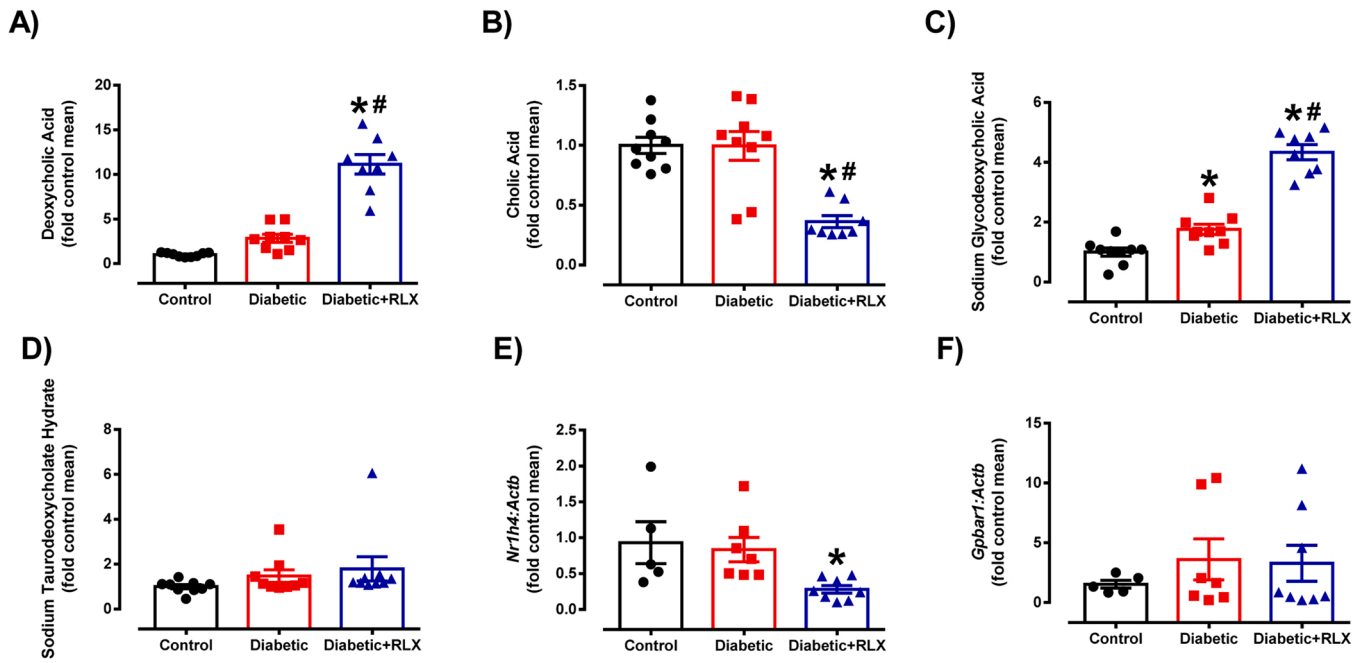

Fig. 5. Levels of A) deoxycholic acid, B) cholic acid, C) sodium glycodeoxycholic acid, D) sodium taurodeoxycholate hydrate, and gene expression of E) farnesoid X receptor (*Nr1h4*) and F) G protein-coupled bile acid receptor 1 (*Gpbar1)* in the kidney cortex from placebo-treated control, placebo-treated diabetic or relaxin-treated diabetic mice. Values are expressed as mean ± SEM; number of mice per group as shown by individual data points. Gene expression is normalised to the reference gene (*Actb*) and presented as fold control mean. *Significantly different to placebo-treated control mice (One-way ANOVA with Tukey's post-hoc test, $P < 0.05$). #Significantly different to untreated diabetic mice (One-way ANOVA with Tukey's post-hoc test, $P < 0.05$).

diabetes nor relaxin had any effect on the level of taurodeoxycholate in the kidney cortex (Fig. 5**D**). In contrast, in the liver of diabetic mice treated with either placebo or relaxin, the level of taurodeoxycholate was significantly ($F_{2,32} = 6.19$, $P = 0.0054$) increased in comparison to control mice (Supplementary Fig. S2B). Furthermore, diabetes did not have any effect on the expression of bile acid receptors, FXR (*Nr1h4*) and *Gpbar1* when compared to controls (Fig. 5**E-F**). Relaxin treatment appears to only reduce ($F_{2,17} = 4.735$, $P = 0.0232$) the expression of FXR (Fig. 5**E**), but not *Gpbar1* (Fig. 5**F**) in the diabetic kidney cortex.

Although the level of choline was reduced in the diabetic kidney cortex (Fig. 6**A**) and liver (Supplementary Fig. S2C), it failed to reach statistical significance. However, the downstream metabolite of choline metabolism, betaine, was significantly ($F_{2,24} = 6.987$, $P = 0.0041$) reduced in the diabetic kidney cortex when compared with the control (Fig. 6**B**). Neither choline nor betaine levels were affected by relaxin treatment in the diabetic kidney cortex (Fig. 6**A-B**). Furthermore, the precursor of the pro-inflammatory 20-hydroxyeicosatetraenoic acid (20-HETE), 17–19-HETE was significantly ($F_{2,24} = 12.33$, $P = 0.0002$) upregulated in the diabetic kidney cortex, but was not affected by relaxin treatment (Fig. 6**C**).

4. Discussion

This study demonstrated that relaxin treatment for two weeks increased the production of bile acids in the kidney cortex of STZ-induced diabetic mice, and is accompanied by a reduction in diabetes-induced oxidative stress and inflammation. Specifically, relaxin treatment ameliorated the upregulation of markers of oxidative stress and inflammatory cytokines, leading to the downregulation of the expression of collagen and fibronectin in the diabetic kidney cortex.

Many previous studies of relaxin have demonstrated its potent anti-fibrotic, anti-inflammatory and vasoprotective actions in the heart, blood vessels and kidneys of various animal models including those with hypertension and diabetes [14]. Despite the reported promising anti-fibrotic effects of relaxin in many organs, these are not evident in the kidneys of two diabetic animal models, namely the hypertensive mRen-2 rats [28] and the eNOS knockout mice [27]. This might be explained by the genetic background of the animal models used. For

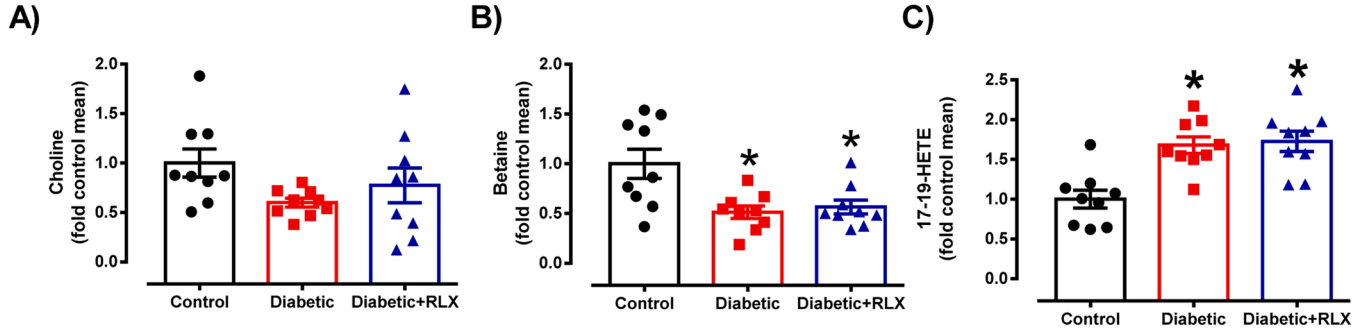

Fig. 6. Levels of A) choline, B) betaine and C) 17–19 HETE in the kidney cortex from placebo-treated control, placebo-treated diabetic or relaxin-treated diabetic mice. Values are expressed as mean ± SEM; number of mice per group as shown by individual data points. *Significantly different to placebo-treated control mice (One-way ANOVA with Tukey's post-hoc test, $P < 0.05$).

example, relaxin evokes a NO-mediated signalling pathway [19,20,41], so the absence of eNOS might have negated the ability of relaxin to alter NO signalling and reduce fibrosis in the eNOS knockout mice. Our study used a STZ-induced type 1 diabetic mouse model to evaluate relaxin's anti-fibrotic effects in the kidney cortex.

Consistent with earlier studies, relaxin treatment did not affect blood glucose levels or systemic markers of diabetes in this type 1 diabetic mice [22,27,28]. However, these mice had increased expression of extracellular matrix (ECM) components and inflammatory cytokines in the kidney cortex, suggesting inflammation and the induction of fibrosis. In contrast to earlier studies, our study demonstrated that relaxin treatment reduced TGF-β1 and Smad2 expression in the diabetic kidney cortex. We suggest that the downregulation in TGF-β1/Smad2 signalling will likely suppress myofibroblast proliferation and differentiation, leading to reduced ECM synthesis [14]. Indeed, we showed that relaxin treatment also decreased the enhanced collagen I and fibronectin expression in the diabetic kidney cortex, indicative of a reduction in fibrosis. Although most studies report that relaxin enhances MMP-2 activity and promotes ECM degradation [14], MMP-2 expression was significantly reduced by relaxin treatment in our study. Furthermore, we also observed that diabetes caused an increased expression of MMP-2 expression in the kidney cortex; this may be a result of compensatory mechanisms in diabetic animals to counteract fibrosis. Consistent with the reported anti-fibrotic effects of relaxin in other rodent models [14], our study confirmed that relaxin acts by reducing expression of TGF-β1 and markers of ECM components in STZ-induced diabetic mice. One of the limitations to our study is our inability to demonstrate direct histological evidence of fibrosis due to the lack of viable tissues from this study. Therefore, we cannot report the exact location of the fibrosis within the kidney cortex. But our data are a step-change from previous work because we have identified positive effects of relaxin in the diabetic kidney.

Oxidative stress and inflammation are well-known contributors to diabetic complications [29,34,42,43]. TXNIP is an endogenous inhibitor of the antioxidant thioredoxin, which is a redox protein that controls the ROS levels in cells and suppresses damage caused by oxidative stress. It is a critical molecule in the in vivo regulation of glucose [44]. It is well-established that sustained diabetes-induced overexpression of TXNIP is one of the important contributors to the development of diabetic nephropathy because it triggers increased production of ROS [44]. Consistent with earlier studies, TXNIP expression was significantly increased in the diabetic kidney cortex [44]. Notably, relaxin treatment reduced TXNIP expression, indicating indirect antioxidant effects of relaxin in the diabetic kidney cortex. Our findings could be further substantiated by a more direct measure of oxidative stress in the tissues, such as 4-Hydroxy-2-Nonenal (4-HNE) or 8-hydroxy-deoxyguanosine (8OHdG). However, we did not have sufficient tissue for such analysis.

Interestingly, relaxin treatment downregulated eNOS expression in the diabetic kidney cortex, which is in contrast to earlier reports that show relaxin increases NOS expression and/or activity, leading to enhanced NO production [19,20,41]. In diabetes, there can be a compensatory upregulation of NOS expression and NOS uncoupling, contributing to oxidative stress [45]. So it is possible that relaxin causes NOS-recoupling [31] or a downregulation of NOS expression, which resulted in the reduction of oxidative stress [46] in the diabetic kidney cortex.

CCL2 and IL6 are pro-inflammatory cytokines that contribute to chronic inflammatory conditions in diabetes. In this study, we showed that relaxin treatment reduced CCL2 and IL6 expression in the kidney cortex; this is consistent with many reported anti-inflammatory effects of relaxin [21,22,24]. Interestingly, the protective effects of relaxin were only observed in the kidney cortex but not in the medulla (data not shown). A plausible explanation for this observation could be due to the differential expression and localisation of RXFP1 within the kidney cortex and medulla [28].

Bile acids are synthesised from cholesterol in the liver, and are the glycine and taurine conjugates of chenodeoxycholic acid, deoxycholic acid, and cholic acid [47]. Traditionally, bile acids are regulators of biliary cholesterol secretion and transportation, and metabolism of endogenous cholesterol [47]. Bile acids are increasingly recognised as signalling molecules because they can regulate metabolic and cellular functions through interaction with their endogenous bile acid receptors, FXR and GPBAR-1 [47–49]. In the context of renal physiology, bile acids activate transcription factors relevant for lipid, cholesterol and carbohydrate metabolism, as well as genes involved in inflammation and renal fibrosis [48,49]. Specifically, alteration in bile acid metabolism and abnormal bile acid receptor expression is associated with metabolic disorders including diabetes and obesity, cardiovascular diseases, and kidney disease [48–52]. Furthermore, previous studies show that activation of bile acid receptors decreases kidney inflammation, oxidative stress, and fibrosis in the diabetic kidneys, suggesting that activation of bile acid receptors has renoprotective effects in diabetes [48–52]. Although diabetes per se had no effect on bile acid production in the kidney cortex in our study, relaxin-treated mice had reduced primary bile acid and cholic acid levels, but increased secondary bile acid and deoxycholic acid levels in the kidney cortex. Cholic acid is metabolised into deoxycholic acid in normal physiology, suggesting the possibility that relaxin treatment may increase the metabolism of cholic acid to deoxycholic acid, which has a much higher potency for FXR [47]. Interestingly, increased levels of deoxycholic acid in the kidney of relaxin-treated mice were associated with a compensatory decrease in FXR. Several recent studies have demonstrated that relaxin treatment alters the lipidome and metabolome profile in the liver and cardiac tissues of normal mice [53,54]. Specifically, relaxin treatment caused significant changes to membrane lipid bilayer components such as glycerophospholipids, sphingolipids and amino acids, and to a much lesser extent, the bile acids [53]. Using a metabolomic approach, these recent findings suggest that relaxin could regulate lipid metabolites in various tissues [53,54], independent of diabetes. In our study, we were unable to identify the precise mechanisms of relaxin action that led to the increase levels of bile acids in the kidneys. We speculate that relaxin increased deoxycholic acid levels by decreasing FXRs, and this led to a reduction in renal inflammation and oxidative stress.

In conclusion, our study demonstrated that relaxin treatment for the final 2 weeks of diabetes increased the level of bile acid, which may have contributed to the attenuation of key markers of renal fibrosis, inflammation, and oxidative stress in STZ-induced diabetic mice. These data indicate the potential role of relaxin as an adjunctive therapy for the treatment of diabetic kidney complications.

CRediT authorship contribution statement

Chen Huei Leo: Conceptualization, Project administration, Methodology, Formal analysis, Visualisation, Writing – original draft. **Jamie Li Min Ou**: Investigation, Validation, Writing – review & editing. **Eng Shi Ong**: Conceptualization, Methodology, Supervision, Visualisation, Writing – review & editing. **Cheng Xue Qin**: Project administration, Writing – review & editing. **Rebecca H. Ritchie**: Resources, Conceptualization, Supervision, Project administration, Writing – review & editing. **Laura J. Parry**: Conceptualization, Resources, Supervision, Funding acquisition, Writing – review & editing. **Hooi Hooi Ng**: Investigation, Conceptualization, Methodology, Formal analysis, Visualisation, Funding acquisition, Writing – review & editing.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2023.114578.

References

[1] H. Sun, P. Saeedi, S. Karuranga, M. Pinkepank, K. Ogurtsova, B.B. Duncan, C. Stein, A. Basit, J.C.N. Chan, J.C. Mbanya, M.E. Pavkov, A. Ramachandaran, S.H. Wild, S. James, W.H. Herman, P. Zhang, C. Bommer, S. Kuo, E.J. Boyko, D.J. Magliano, I. D.F. Diabetes, Atlas: global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045, Diabetes Res. Clin. Pract. 183 (2022), 109119.

[2] J.M. Forbes, M.E. Cooper, Mechanisms of diabetic complications, Physiol. Rev. 93 (2013) 137–188.

[3] R.H. Ritchie, E.D. Abel, Basic mechanisms of diabetic heart disease, Circ. Res. 126 (2020) 1501–1525.

[4] H. Fu, S. Liu, S.I. Bastacky, X. Wang, X.J. Tian, D. Zhou, Diabetic kidney diseases revisited: a new perspective for a new era, Mol. Metab. 30 (2019) 250–263.

[5] R.Z. Alicic, M.T. Rooney, K.R. Tuttle, Diabetic kidney disease: challenges, progress, and possibilities, Clin. J. Am. Soc. Nephrol. 12 (2017) 2032–2045.

[6] A.D. Kaze, M. Zhuo, S.C. Kim, E. Patorno, J.M. Paik, Association of SGLT2 inhibitors with cardiovascular, kidney, and safety outcomes among patients with diabetic kidney disease: a meta-analysis, Cardiovasc. Diabetol. 21 (2022) 47.

[7] J. Barrera-Chimal, I. Lima-Posada, G.L. Bakris, F. Jaisser, Mineralocorticoid receptor antagonists in diabetic kidney disease - mechanistic and therapeutic effects, Nat. Rev. Nephrol. 18 (2022) 56–70.

[8] T.A. Zelniker, S.D. Wiviott, I. Raz, K. Im, E.L. Goodrich, R.H.M. Furtado, M. P. Bonaca, O. Mosenzon, E.T. Kato, A. Cahn, D.L. Bhatt, L.A. Leiter, D.K. McGuire, J.P.H. Wilding, M.S. Sabatine, Comparison of the effects of glucagon-like peptide receptor agonists and sodium-glucose cotransporter 2 inhibitors for prevention of major adverse cardiovascular and renal outcomes in type 2 diabetes mellitus, Circulation 139 (2019) 2022–2031.

[9] G. Schernthaner, N. Shehadeh, A.S. Ametov, A.V. Bazarova, F. Ebrahimi, P. Fasching, A. Janež, P. Kempler, I. Konrāde, N.M. Lalić, B. Mankovsky, E. Martinka, D. Rahelić, C. Serafinceanu, J. Škrha, T. Tankova, Ž. Visockienė, Worldwide inertia to the use of cardiorenal protective glucose-lowering drugs (SGLT2i and GLP-1 RA) in high-risk patients with type 2 diabetes, Cardiovasc. Diabetol. 19 (2020) 185.

[10] K.I. Alexandraki, C. Piperi, P.D. Ziakas, N.V. Apostolopoulos, K. Makrilakis, V. Syriou, E. Diamanti-Kandarakis, G. Kaltsas, A. Kalofoutis, Cytokine secretion in long-standing diabetes mellitus type 1 and 2: associations with low-grade systemic inflammation, J. Clin. Immunol. 28 (2008) 314–321.

[11] J.M. Forbes, M.T. Coughlan, M.E. Cooper, Oxidative stress as a major culprit in kidney disease in diabetes, Diabetes 57 (2008) 1446–1454.

[12] N. Kahlberg, C.X. Qin, J. Anthonisz, E. Jap, H.H. Ng, M. Jelinic, L.J. Parry, B. K. Kemp-Harper, R.H. Ritchie, C.H. Leo, Adverse vascular remodelling is more sensitive than endothelial dysfunction to hyperglycaemia in diabetic rat mesenteric arteries, Pharmacol. Res. 111 (2016) 325–335.

[13] H.H. Ng, M. Shen, C.S. Samuel, J. Schlossmann, R.G. Bennett, Relaxin and extracellular matrix remodeling: mechanisms and signaling pathways, Mol. Cell Endocrinol. 487 (2019) 59–65.

[14] C.S. Samuel, S.G. Royce, T.D. Hewitson, K.M. Denton, T.E. Cooney, R.G. Bennett, Anti-fibrotic actions of relaxin, Br. J. Pharmacol. 174 (2017) 962–976.

[15] Y. Liu, Cellular and molecular mechanisms of renal fibrosis, Nat. Rev. Nephrol. 7 (2011) 684–696.

[16] R.A. Bathgate, M.L. Halls, E.T. van der Westhuizen, G.E. Callander, M. Kocan, R. J. Summers, Relaxin family peptides and their receptors, Physiol. Rev. 93 (2013) 405–480.

[17] K.P. Conrad, Unveiling the vasodilatory actions and mechanisms of relaxin, Hypertension 56 (2010) 2–9.

[18] M. Jelinic, S.A. Marshall, C.H. Leo, L.J. Parry, M. Tare, From pregnancy to cardiovascular disease: lessons from relaxin-deficient animals to understand relaxin actions in the vascular system, Microcirculation 26 (2019), e12464.

[19] C.H. Leo, M. Jelinic, H.H. Ng, L.J. Parry, M. Tare, Recent developments in relaxin mimetics as therapeutics for cardiovascular diseases, Curr. Opin. Pharmacol. 45 (2019) 42–48.

[20] C.H. Leo, M. Jelinic, H.H. Ng, M. Tare, L.J. Parry, Serelaxin: a novel therapeutic for vascular diseases, Trends Pharmacol. Sci. 37 (2016) 498–507.

[21] H.H. Ng, C.H. Leo, L.J. Parry, R.H. Ritchie, Relaxin as a therapeutic target for the cardiovascular complications of diabetes, Front. Pharmacol. 9 (2018) 501, https://doi.org/10.3389/fphar.2018.00501.

[22] H.H. Ng, C.H. Leo, D. Prakoso, C. Qin, R.H. Ritchie, L.J. Parry, Serelaxin treatment reverses vascular dysfunction and left ventricular hypertrophy in a mouse model of Type 1 diabetes, Sci. Rep. 7 (2017) 39604, https://doi.org/10.1038/srep39604.

[23] Q. Xu, A. Chakravorty, R.A. Bathgate, A.M. Dart, X.J. Du, Relaxin therapy reverses large artery remodeling and improves arterial compliance in senescent spontaneously hypertensive rats, Hypertension 55 (2010) 1260–1266.

[24] H.H. Ng, C.H. Leo, L.J. Parry, Serelaxin (recombinant human relaxin-2) prevents high glucose-induced endothelial dysfunction by ameliorating prostacyclin production in the mouse aorta, Pharmacol. Res. 107 (2016) 220–228.

[25] D. Wang, Y. Luo, K. Myakala, D.J. Orlicky, E. Dobrinskikh, X. Wang, M. Levi, Serelaxin improves cardiac and renal function in DOCA-salt hypertensive rats, Sci. Rep. 7 (2017) 9793, https://doi.org/10.1038/s41598-017-09470-0.

[26] J.S. Bonner, L. Lantier, K.M. Hocking, L. Kang, M. Owolabi, F.D. James, D.P. Bracy, C.M. Brophy, D.H. Wasserman, Relaxin treatment reverses insulin resistance in mice fed a high-fat diet, Diabetes 62 (2013) 3251–3260.

[27] T.B. Dschietzig, K. Krause-Relle, M. Hennequin, K. von Websky, J. Rahnenführer, J. Ruppert, H.J. Grön, F.P. Armbruster, R.A. Bathgate, J.R. Aschenbach, W. G. Forssmann, B. Hocher, Relaxin-2 does not ameliorate nephropathy in an experimental model of type-1 diabetes, Kidney Blood Press Res. 40 (2015) 77–88.

[28] S.E. Wong, C.S. Samuel, D.J. Kelly, Y. Zhang, G.J. Becker, T.D. Hewitson, The anti-fibrotic hormone relaxin is not reno-protective, despite being active, in an experimental model of type 1 diabetes, Protein Pept. Lett. 20 (2013) 1029–1038.

[29] S.A. Marshall, C.X. Qin, M. Jelinic, K. O'Sullivan, M. Deo, J. Walsh, M. Li, L. J. Parry, R.H. Ritchie, C.H. Leo, The novel small-molecule annexin-a1 mimetic, compound 17b, elicits vasoprotective actions in streptozotocin-induced diabetic mice, Int. J. Mol. Sci. 21 (2020) 1384, https://doi.org/10.3390/ijms21041384.

[30] M. Jelinic, K.L. Jackson, K. O'Sullivan, J. Singh, T. Giddy, M. Deo, L.J. Parry, R. H. Ritchie, O.L. Woodman, G.A. Head, C.H. Leo, C.X. Qin, Endothelium-dependent relaxation is impaired in Schlager hypertensive (BPH/2J) mice by region-specific mechanisms in conductance and resistance arteries, Life Sci. (2023), https://doi.org/10.1016/j.lfs.2023.121542.

[31] M. Jelinic, C.H. Leo, S.A. Marshall, S.N. Senadheera, L.J. Parry, M. Tare, Short-term (48 h) intravenous serelaxin infusion has no effect on myogenic tone or vascular remodeling in rat mesenteric arteries, Microcirculation 24 (2017), https://doi.org/10.1111/micc.12371.

[32] M. Jelinic, C.H. Leo, E.D. Post Uiterweer, S.L. Sandow, J.H. Gooi, M.E. Wlodek, K. P. Conrad, H. Parkington, M. Tare, L.J. Parry, Localization of relaxin receptors in arteries and veins, and region-specific increases in compliance and bradykinin-mediated relaxation after in vivo serelaxin treatment, FASEB J. 28 (2014) 275–287.

[33] C.H. Leo, M. Jelinic, H.C. Parkington, M. Tare, L.J. Parry, Acute intravenous injection of serelaxin (recombinant human relaxin-2) causes rapid and sustained bradykinin-mediated vasorelaxation, J. Am. Heart Assoc. 3 (2014), e000493, https://doi.org/10.1161/JAHA.113.000493.

[34] M. Jelinic, N. Kahlberg, C.H. Leo, H.H. Ng, S. Rosli, M. Deo, M. Li, S. Finlayson, J. Walsh, L.J. Parry, R.H. Ritchie, C.X. Qin, Annexin-A1 deficiency exacerabates pathological remodelling of the mesenteric vasculature in insulin-resistance but not insulin-deficiency, Br. J. Pharmacol. 177 (2020) 1677–1691.

[35] S.A. Marshall, C.H. Leo, S.N. Senadheera, J.E. Girling, M. Tare, L.J. Parry, Relaxin deficiency attenuates pregnancy-induced adaptation of the mesenteric artery to angiotensin II in mice, Am. J. Physiol. Regul. Integr. Comp. Physiol. 310 (2016) R847–R857.

[36] E.S. Ong, L. Zou, S. Li, P.Y. Cheah, K.W. Eu, Metabolic profiling in colorectal cancer reveals signature metabolic shifts during tumorigenesis, Mol. Cell Proteom. (2010), https://doi.org/10.1074/mcp.M900551-MCP200.

[37] C.H. Leo, S.Y. Foo, J.C.W. Tan, U.X. Tan, C.K. Chua, E.S. Ong, Green extraction of orange peel waste reduces TNFα-induced vascular inflammation and endothelial dysfunction, Antioxidants 11 (2022) 1768, https://doi.org/10.3390/antiox11091768.

[38] E.S. Ong, J. Low, J.C.W. Tan, S.Y. Foo, C.H. Leo, Valorization of avocado seeds with antioxidant capacity using pressurized hot water extraction, Sci. Rep. 12 (2022) 13036, https://doi.org/10.1038/s41598-022-17326-5.

[39] E.S. Ong, C.L.Y. Oh, J.C.W. Tan, S.Y. Foo, C.H. Leo, Pressurized hot water extraction of okra seeds reveals antioxidant, antidiabetic and vasoprotective activities, Plants 10 (2021) 1645, https://doi.org/10.3390/plants10081645.

[40] C.H. Leo, H.H. Ng, S.A. Marshall, M. Jelinic, T. Rupasinghe, C. Qin, U. Roessner, R. H. Ritchie, M. Tare, L.J. Parry, Relaxin reduces endothelium-derived vasoconstriction in hypertension: revealing new therapeutic insights, Br. J. Pharmacol. 177 (2020) 217–233.

[41] C.H. Leo, M. Jelinic, H.H. Ng, M. Tare, L.J. Parry, Time-dependent activation of prostacyclin and nitric oxide pathways during continuous i.v. infusion of serelaxin (recombinant human H2 relaxin), Br. J. Pharmacol. 173 (2016) 1005–1017.

[42] C.X. Qin, J. Anthonisz, C.H. Leo, N. Kahlberg, A. Velagic, M. Li, E. Jap, O. L. Woodman, L.J. Parry, J.D. Horowitz, B.K. Kemp-Harper, R.H. Ritchie, NO• resistance, induced in the myocardium by diabetes is circumvented by the NO redox sibling, nitroxyl, Antioxid. Redox Signal 32 (2020) 60–77.

[43] J.C. Li, A. Velagic, C.X. Qin, M. Li, C.H. Leo, B.K. Kemp-Harper, R.H. Ritchie, O. L. Woodman, Diabetes attenuates the contribution of endogenous nitric oxide but

not nitroxyl to endothelium dependent relaxation of rat carotid arteries, Front. Pharmacol. 11 (2021), 585740.
[44] C.Y.R. Tan, W. Qi, Y. Zhang, A.J. Cox, D.J. Kelly, R.G. Langham, Thioredoxin-interacting protein: a potential therapeutic target for treatment of progressive fibrosis in diabetic nephropathy, Nephron 129 (2015) 109–127.
[45] C.H. Leo, J.L. Hart, O.L. Woodman, Impairment of both nitric oxide-mediated and EDHF-type relaxation in small mesenteric arteries from rats with streptozotocin-induced diabetes, Br. J. Pharmacol. 162 (2011) 365–377.
[46] C.H. Leo, D.T. Fernando, L. Tran, H.H. Ng, S.A. Marshall, L.J. Parry, Serelaxin treatment reduces oxidative stress and increases aldehyde dehydrogenase-2 to attenuate nitrate tolerance, Front. Pharmacol. 8 (2017) 141, https://doi.org/10.3389/fphar.2017.00141.
[47] A. Di Ciaula, G. Garruti, R. Lunardi Baccetto, E. Molina-Molina, L. Bonfrate, D.Q. H. Wang, P. Portincasa, Bile acid physiology, Ann. Hepatol. 16 (2017) S4–S14.
[48] M. Herman-Edelstein, T. Weinstein, M. Levi, Bile acid receptors and the kidney, Curr. Opin. Nephrol. Hypertens. 27 (2017) 56–62.
[49] C. Masaoutis, T. S, The Farnesoid X Receptor: a potential target for expanding the therapeutic arsenal against kidney disease, Expert Opin. Ther. Targets 23 (2019) 107–116.
[50] T. Jiang, X.X. Wang, P. Scherzer, P. Wilson, J. Tallman, H. Takahashi, J. Li, M. Iwahashi, E. Sutherland, L. Arend, M. Levi, Farnesoid X Receptor modulates renal lipid metabolism, fibrosis, and diabetic nephropathy, Diabetes 56 (2007) 2485–2493.
[51] X.X. Wang, M.H. Edelstein, U. Gafter, L. Qiu, Y. Luo, E. Dobrinskikh, S. Lucia, L. Adorini, V.D. D'Agati, J. Levi, A. Rosenberg, J.B. Kopp, D.R. Gius, M.A. Saleem, M. Levi, G protein-coupled bile acid receptor TGR5 activation inhibits kidney disease in obesity and diabetes, J. Am. Soc. Nephrol. 27 (2016) 1362–1378.
[52] Z. Yang, F. Xiong, Y. Wang, W. Gong, J. Huang, C. Chen, P. Liu, H. Huang, TGR5 activation suppressed S1P/S1P2 signaling and resisted high glucose-induced fibrosis in glomerular mesangial cells, Pharmacol. Res. 111 (2016) 226–236.
[53] A. Aragón-Herrera, S. Feijóo-Bandín, V. Abella, L. Álvarez, E. Roselló-Lletí, M. Portolés, E. Tarazón, M. Bigazzi, D. Bani, O. Gualillo, J.R. González-Juanatey, F. Lago, Serelaxin (recombinant human relaxin-2) treatment affects the endogenous synthesis of long chain poly-unsaturated fatty acids and induces substantial alterations of lipidome and metabolome profiles in rat cardiac tissue, Pharmacol. Res. 144 (2019) 51–65.
[54] A. Aragón-Herrera, S. Feijóo-Bandín, S. Moraña-Fernández, L. Anido-Varela, E. Roselló-Lletí, M. Portolés, E. Tarazón, R. Lage, I. Moscoso, L. Barral, D. Bani, M. Bigazzi, O. Gualillo, J.R. González-Juanatey, F. Lago, Relaxin has beneficial effects on liver lipidome and metabolic enzymes, FASEB J. 35 (2021), e21737.