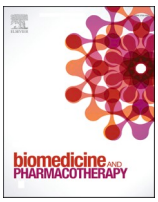
Biomedicine & Pharmacotherapy 168 (2023) 115661

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha



Finerenone protects against progression of kidney and cardiovascular damage in a model of type 1 diabetes through modulation of proinflammatory and osteogenic factors

M. Sanz-Gómez [a,1], F.J. Manzano-Lista [a,1], E. Vega-Martín [a], D. González-Moreno [b], M. Alcalá [c], M. Gil-Ortega [b], B. Somoza [b], C. Pizzamiglio [a], L.M. Ruilope [d], I. Aránguez [a], P. Kolkhof [e], R. Kreutz [f,*], M.S. Fernández-Alfonso [a,**]

[a] Instituto Pluridisciplinar and Facultad de Farmacia, Universidad Complutense de Madrid, Spain
[b] Departamento de Ciencias Farmacéuticas y de la Salud, Facultad de Farmacia, Universidad CEU-San Pablo, CEU Universities, 28925 Madrid, Spain
[c] Departamento de Química y Bioquímica, Facultad de Farmacia, Universidad CEU-San Pablo, CEU Universities, 28925 Madrid, Spain
[d] Unidad de Hipertensión, Instituto de Investigación Imas12, Hospital Universitario 12 de Octubre, Madrid, Spain
[e] Cardiovascular Precision Medicines, Research & Early Development, Pharmaceuticals, Bayer AG, Wuppertal, Germany
[f] Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Department of Clinical Pharmacology and Toxicology, Germany

ARTICLE INFO

Keywords:
Chronic kidney disease
Type 1 diabetes
Streptozotocin
Finerenone
Bone morphogenetic proteins
Perivascular adipose tissue
Perirenal adipose tissue
Vascular disease

ABSTRACT

The non-steroidal mineralocorticoid receptor antagonist (MRA) finerenone (FIN) improves kidney and cardiovascular outcomes in patients with chronic kidney disease (CKD) in type 2 diabetes (T2D). We explored the effect of FIN in a novel model of type 1 diabetic Munich Wistar Frömter (MWF) rat (D) induced by injection of streptozotocin (15 mg/kg) and additional exposure to a high-fat/high-sucrose diet. Oral treatment with FIN (10 mg/kg/day in rat chow) in diabetic animals (D-FIN) was compared to a group of D rats receiving no treatment and a group of non-diabetic untreated MWF rats (C) ($n = 7–10$ animals per group). After 6 weeks, D and D-FIN exhibited significantly elevated blood glucose levels (271.7 ± 67.1 mg/dl and 266.3 ± 46.8 mg/dl) as compared to C (110.3 ± 4.4 mg/dl; $p < 0.05$). D showed a 10-fold increase of kidney damage markers *Kim-1* and *Ngal* which was significantly suppressed in D-FIN. Blood pressure, pulse wave velocity (PWV) and arterial collagen deposition were lower in D-FIN, associated to an improvement in endothelial function due to a reduction in procontractile prostaglandins, as well as reactive oxygen species (ROS) and inflammatory cytokines (IL-1, IL-6, TNFα and TGFβ) in perivascular and perirenal adipose tissue (PVAT and PRAT, respectively). In addition, FIN restored the imbalance observed in CKD between the procalcifying BMP-2 and the nephroprotective BMP-7 in plasma, kidney, PVAT, and PRAT. Our data show that treatment with FIN improves kidney and vascular damage in a new rat model of DKD with T1D associated with a reduction in inflammation, fibrosis and osteogenic factors independently from changes in glucose homeostasis.

Abbreviations: Ach, acetylcholine; AT, 3-Amino-1,2,4-triazole; BMP, bone morphogenetic protein; BLGLAP, osteocalcin; CKD, Chronic Kidney Disease; Col1A1, collagen 1A1; CVD, Cardiovascular Disease; DKD, Diabetic Kidney Disease; ECM, Extracellular Matrix; FIN, Finerenone; H_2O_2, Hydrogen Peroxide; IL, interleukin; kim-1, kidney injury molecule 1; L-NAME, N_G-nitro-L-arginine methyl ester; MRA, Mineralocorticoid-receptor Antagonists; MWF, Munich Wistar Frömter; NA, Noradrenaline; Ngal, Neutrophil Gelatinase-Associated Lipocalin; NO, Nitric Oxide; NOXi, NOX inhibitor; $O_2^{\cdot-}$, Superoxide Anion; PSS, Physiological Salt Solution; PWV, Pulse Wave Velocity; ROS, reactive oxygen species; Runx2, RUNX family transcription factor 2; SBP, Systolic Blood Pressure; TGF β, Transforming growth factor β; TNFα, tumor necrosis factor α.

* Correspondence to: Department of Clinical Pharmacology and Toxicology Charité, Universitätsmedizin Berlin, 10117 Berlin, Germany.
** Correspondence to: Instituto Pluridisciplinar, Paseo de Juan XXIII, 1, 28040 Madrid, Spain.
E-mail addresses: reinhold.kreutz@charite.de (R. Kreutz), marisolf@ucm.es (M.S. Fernández-Alfonso).
[1] Equal contribution.

https://doi.org/10.1016/j.biopha.2023.115661
Received 7 July 2023; Received in revised form 28 September 2023; Accepted 4 October 2023
Available online 11 October 2023

1. Introduction

Chronic kidney disease (CKD) is a progressive, chronic, and incurable disease especially prevalent in the hypertensive and diabetic population [1]. Patients with CKD exhibit an elevated cardiovascular (CV) risk, which increases exponentially with stage and severity [2–4]. In diabetic patients, the most prevalent form of CKD is diabetic kidney disease (DKD), the major cause of end-stage kidney disease, but also of an increased CV risk and CV mortality due to diabetic cardiomyopathy or stroke [5].

Vascular damage in CKD includes endothelial dysfunction [6,7] through and increased oxidative stress [8,9], arterial stiffness [10–12] and calcification [13–15] leading to increased cardiac workload, reduced coronary artery perfusion, and chronic cardiac dysfunction [16,17]. In this scenario, we [18] have recently described an increase of the procalcifying bone morphogenetic protein (BMP)− 2 [19,20] together with a decrease of the renoprotective BMP-7 [21–24] in plasma of both CKD patients and the Munich Wistar Frömter (MWF) rat model of CKD. This imbalance is associated with the severity of CKD, 25-OH-cholecalciferol deficiency, and blood pressure. Moreover, plasma BMP-2 and BMP-7 reflect the expression level of these proteins in kidney of MWF rats, associated with increased kidney damage, profibrotic, and calcification factors [18].

Other mechanisms associated with kidney and vascular damage in CKD involve a possible paracrine effect of several adipose tissue depots, such as perivascular adipose tissue (PVAT), adjacent to renal [25] and non-renal arteries [18]. PVAT from MWF rats exhibits a reduced *Bmp-7* expression associated with an increase in transforming growth factor β (*Tgfβ*) and collagen1A1 *(Col1A1)* expression [18]. In parallel, there is an upregulation of *BMP-2* expression together with the osteogenic and calcification factors RUNX family transcription factor 2 (*Runx2),* alkaline phosphatase *(Alp)*, and osteocalcin (*Bglap)* [18]. Moreover, recent studies in CKD patients have highlighted the key role of perirenal adipose tissue (PRAT) thickness as a novel independent risk factor for CKD [26–30]. A deeper characterization of pathophysiological mechanisms in PRAT and PVAT associated to nondiabetic and diabetic CKD is warranted.

Finerenone (FIN) is a next-generation, oral, selective, non-steroidal mineralocorticoid receptor antagonist (MRA) which is as least as potent as spironolactone and even more selective as eplerenone. Its non-steroidal structure confers a different binding mode to the mineralocorticoid receptor (MR) as well as different physicochemical properties, which have an impact on distribution and tissue penetration [31]. FIN reduced cardiac hypertrophy, pro-B-type natriuretic peptide (BNP) and proteinuria more efficiently than eplerenone when directly comparing equinatriuretic doses [32]. More importantly, once-daily oral administration of 10 mg of FIN was at least as effective as spironolactone (25–50 mg/day) in decreasing BNP, NT-pro-BNP and albuminuria, but was associated with significantly lower increases in serum potassium, a significantly lower incidence of hyperkalemia as well as a lower incidence of worsening kidney function [33]. The recent results of two phase III trials, FIDELIO-DKD [34] and FIGARO-DKD [35] together with their combined analysis in FIDELITY [36] provides robust evidence of both cardiovascular and kidney protection with FIN vs. placebo in patients with a broad spectrum of CKD and type 2 diabetes (T2DM), all treated with an optimized dose of an angiotensin-converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB).

Preclinical studies from our group in the MWF model have further analysed the mechanisms underlying the protective effects of FIN [12,37]. Treatment with FIN reduces albuminuria, endothelial dysfunction, high blood pressure [37] and arterial stiffness [12] associated with a reduction of kidney and vascular oxidative stress, thus supporting the functional link between the extra kidney normalization of vascular dysfunction and the improvement of glomerular permeability dysfunction. We have now created a new DKD model trough induction of T1DM in the MWF rat with established CKD. Animals develop glucose intolerance and insulin resistance, an aggravated kidney damage and cardiac hypertrophy with preserved ejection fraction, associated to proinflammatory and profibrotic alterations in PRAT which might contribute in a paracrine way to the progression of DKD. We thus suggest that the MWF diabetic might represent a valuable model for uncovering novel pathophysiological mechanisms as well as testing of pharmacologic intervention.

The hypothesis of this work is that FIN treatment of T1DM MWF (D) rats prevents the progression of kidney, cardiovascular, and metabolic damage through a modulation of the proinflammatory and procalcifying environment, potentially including the modulation of BMP-2 and BMP-7.

2. Material and methods

2.1. Animal treatments and experimental protocol

Sixteen-week-old male MWF rats (Charité – University Medicine Berlin, Germany; n = 27) housed in individual cages under controlled dark-light cycles (12 h/12 h) and temperature conditions had free access to water and food for the duration of the study. Only male rats were used, as female MWF do not develop the albuminuria phenotype [38]. Animals were randomly divided in three groups: control (C), diabetic (D) and diabetic treated with FIN (D-FIN).

The control group (C; n = 10) was fed standard chow during the study (6 weeks). Another group (n = 17) was fed a high-energy diet in combination with streptozotocin (STZ) administration. At the beginning of the dietary treatment, rats received 3 doses of STZ (15 mg/kg, i.p.) to reach blood glucose values above 200 mg/dL, considered as hyperglycemia. C rats fed standard chow received equivalent vehicle injections as their littermates (citrate buffer 0.1 M, pH=4.5). Simultaneously, rats were fed a high-fat high-sucrose diet (HF/HS, TD08811 ENVIGO, Barcelona, Spain) for 6 weeks without (D; n = 10) or with FIN (D-FIN; n = 7). FIN was added to the HF/HS diet in a concentration of 0.27 g/Kg of whole diet. This concentration was estimated based on the previous determination of individual food intake for each rat to reach a dose of 10 mg/kg/day.

All experimental procedures were approved by the Animal Facility of Instituto Pluridisciplinar UCM (ES-280790000086), as well as by Institutional Animal Care and Use Committee of the Comunidad de Madrid (PROEX 205/18, approved December 2018) in accordance with the guidelines for the ethical care of experimental animals of the European Community. Experiments were performed at Instituto Pluridisciplinar. Every effort was made to avoid animal suffering in accordance with the ARRIVE guidelines (Animal Research: Reporting of in Vivo Experiments [39].

2.2. Determination of glycemic parameters and urinary albumin excretion

Fasting blood glucose and insulin were monitored once a week 6 h after food removal. Blood was drawn from the tail vein of conscious rats for glucose determination with a Contour XT glucometer (Barcelona, Spain). Insulin levels were determined by means of a specific enzyme immunoassay kit (Mercodia, Denmark) (2.2% intra-assay variation, 4.9% inter-assay variation). HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) index was calculated with the following formula: [fasting blood glucose (mmol/L) × fasting plasma insulin (μU/mL)]/22.5. Postprandial blood glucose was determined before euthanasia at 8:30 a.m. in rats which had access to food and water the night before.

Urine was collected by placing the animals in metabolic cages for 24 h after a 1-day adaptation period. After 6 weeks of treatment urinary albumin excretion (UAE), urinary creatinine, proteinuria and protein/creatinine ratio and creatinine clearance were determined as previously described [8,20,40]. Urine glucose was determined by glucose Trinder Method (Roche Applied Science, Barcelona, Spain).

2.3. Invasive hemodynamic measurements and tissue obtention

On the last day, both carotid and femoral arteries were catheterized under anesthesia (80 mg/kg ketamine hydrochloride and 12 mg/kg xylazine hydrochloride, ip) and blood pressure waves were recorded in a PowerLab system (ADInstruments) [12]. The following formula was used to calculate pulse wave velocity (PWV): D (meters)/Δt (seconds). D is the distance between carotid and femoral arteries and Δt is the time delay, determined by using the displacement between the carotid and femoral pressure waves.

After hemodynamic measurements, animals were euthanized by exsanguination, blood was collected in heparinized tubes and plasma was obtained after a 10 min centrifugation at 4000 rpm. Kidneys, thoracic aorta, first branch mesenteric arteries, perirenal adipose tissue (PRAT), and periaortic perivascular adipose tissue (PVAT) were excised. Aorta was used fresh for vascular function experiments. Other tissues were frozen for further experiments.

2.4. Plasma determinations

Potassium, calcium, phosphate, 25-OH cholecalciferol, and creatinine were determined on a sample of heparin-anticoagulated plasma by the Megalab® laboratory following standard procedures. Determination of plasma atrial natriuretic peptide (ANP), BMP-2 and BMP-7 levels was performed using specific ELISA kits according to the manufacturer's specifications (Quantikine®, R&D Systems Inc, Minnesota, USA), as previously described [18].

2.5. Vascular function study in the isolated thoracic aorta

Vascular function was assessed as previously described [41]. Briefly, thoracic aorta cleaned of PVAT was carefully isolated, placed in oxygenated physiological salt solution (PSS: 115 mmol/L NaCl, 4.6 mmol/L KCl, 2.5 mmol/L $CaCl_2$, 25 mmol/L $NaHCO_3$, 1.2 mmol/L KH_2PO_4, 1.2 mmol/L $MgSO_4$, 0.01 mmol/L EDTA, 5.5 mmol/L glucose), and cleaned of blood. Vascular rings (3-mm-long) were suspended on two intraluminal parallel wires, introduced in an organ bath containing PSS and connected to a Piodem strain gauge. Isometric tension was recorded in a BioPac AcQKnowledge 3.9 (BioPac Systems, EE UU). Segments were given an optimal resting tension of 1.5 g, which is then readjusted every 15 min during a 60 min equilibration period. Thereafter, the vessels were exposed to 75 mmol/L KCl to check their contractility. Concentration-response curves to acetylcholine (Ach, 10^{-9}-10^{-4} mol/L) and noradrenaline (NA, 10^{-10}-10^{-6} mol/L) were performed. Cyclooxygenase (COX) inhibitor indomethacin (INDO, 3 $\times 10^{-6}$ mol/L), nitric oxide synthase (NOS) inhibitor, N_G-nitro-L-arginine methyl ester (L-NAME, 10^{-4} mol/L), the NOX-1 inhibitor, ML171 (Calbiochem-Merck (10^{-5} mol/L) or the catalase inhibitor, 3-amino-1,2,4-triazole (3-AT, 5 $\times 10^{-3}$ mol/L) were incubated 30 min prior to addition of the agonists.

2.6. Determination of collagen deposition in mesenteric artery by immunofluorescence

First branch mesenteric arteries were incubated in sodium nitroprusside (SNP, 10^{-6} mol/L) for 1 h for them to dilate and immediately fixed in 4% paraformaldehyde [60 min; room temperature (RT)], washed with 0.2% Tween-20 in PBS (15 min; RT) and incubated with blocking solution (3% BSA in PBS; 30 min; RT). Mesenteric arteries were incubated thereafter with collagen type I and type III antibodies (1:50; Abcam, Germany; overnight; 4ºC) in immunofluorescence buffer (1% BSA, 0.2% Triton and 0.05% Tween-20 in PBS). Segments were washed and incubated with the secondary antibody Alexa-Fluor 555 (1:200; Invitrogen, USA; 60 min; RT) and with the nuclear dye TO-PRO®− 3 (642−661) (1:500; Invitrogen, USA; 10 min; RT; light protected). Arterial segments were mounted as previously described [12] and visualized by confocal microscopy (Leica TCS SP5). Stacks of serial optical sections were captured from 3 randomly chosen regions with a x63 objective at zoom 3, under identical conditions of laser intensity, brightness and contrast [12]. Fluorescence intensity was quantified with ImageJ software (NIH, USA, Version 1.50 f).

2.7. RNA extraction and real-time PCR (RT-qPCR)

Total RNA was isolated from the kidney, PRAT and PVAT using Qiazol Reagent (Qiagen). The samples were processed using a RNA Spin illustraTM (GE Healthcare) and the concentration and purity of RNA were assessed with NanoDropTM 2000/c (Fisher Scientific). Reverse transcription was performed on 500 ng of RNA with iScript cDNA synthesis kit (BioRad) using random hexamer primers. Optimal annealing temperature and amplicon sizes were checked for each pair of primers. RT-qPCR analyses were performed in a CFX96 Instrument (BioRad). 10.00 ng of cDNA from six to ten samples of each group were run in duplicate and the mRNA levels were determined using a 1:1 mix of forward and reverse intron-skipping primers at a concentration of 3 μM, *Gapdh* and *Atpaf-1* were used as housekeeping genes and 2x SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA). All primer sequences are detailed in Table 1.

2.8. Statistical analysis

Variables were compared by Student's t test or one-way ANOVA with the Newman-Keuls test, and nonparametric variables were compared by the Kruskal-Wallis test. Data analysis was performed with GraphPad Prism 9. Data are presented as mean ± standard error of the mean and statistical significance was considered for $p < 0.05$.

3. Results

3.1. Effect of FIN treatment on body and tissue weights

As shown in Table 2, caloric intake was significantly higher in D animals which exhibited an increased body weight at the end of treatment compared to C. PRAT, PVAT, and mesenteric adipose tissue amounts were significantly increased compared to the C group. FIN significantly reduced caloric intake compared to D group, although it was still significantly higher compared to the C group. No effect of FIN was observed on other parameters (Table 2).

3.2. Effect of FIN treatment on glycemic parameters

D rats showed a significant increase in fasting and postprandial blood glucose and significantly lower levels in postprandial insulin with no difference in fasting insulin levels compared to the C group (Table 3). An increase in HOMA-IR was detected in D rats compared to C rats, showing that this group is not only glucose intolerant but also insulin resistant. The D group also showed higher water intake, urine volume, and increased glucosuria, classical features of diabetes. No significant effect of FIN was observed on none of these parameters (Table 3).

3.3. FIN prevents the progression of kidney damage in diabetic MWF

UAE (Fig. 1A), urinary creatinine (Table 4), urinary protein/creatinine ratio (UPCR) (Table 4), creatinine clearance (Table 4) and plasma creatinine values (Fig. 1B) were similar in the D compared with the C group with no significant effect of FIN on these parameters compared with the D group (Figs. 1A and 1B, Table 4). FIN significantly reduced urinary protein excretion vs. the C group and by trend vs. the D group (Table 4). Kidneys of D rats exhibited an upregulation of the kidney damage markers *Kim-1* and *Ngal* as well as profibrotic *Col1A1* compared to the C group (Fig. 1C-E) which was prevented by FIN treatment. No difference in *Tgfβ* expression was detected between groups (Fig. 1F).

Table 1
Primer sequences.

Gene	Accesion number	Forward (5′−3′)	Reverse (5′−3′)
Rn *Kim-1*	NM_173149.2	ATTGTTGCCGAGTGGAGAT	TGTGGTTGTGGGTCTTGTAGT
Rn *Ngal*	NM_130741.1	GGCCGACACTGACTACGACC	GCCCCTTGGTTCTTCCGTAC
Rn *Bmp-2*	NM_017178.2	CCCCTATATGCTCGACCTGTACC	TGAAAGTTCCTCGATGGCTTCT
Rn *Bmp-7*	NM_001191856.2	GAGGGCTGGTTGGTATTTGACA	AACTTGGGGTTGATGCTCTGC
Rn *Il-6*	NM_012589.2	CCTGGAGTTTGTGAAGAACAACTT	TGGAAGGTGGGGTAGGAAGGAC
Rn *Il-10*	NM_012854.2	CAGTGGAGCAGGTGAAGAATGA	CATTCATGGCCTTGTAGACACC
Rn *Il-1β*	NM_031512.2	TGACAGACCCCAAAAGATTAAGGA	CGAGATGCTGCTGTGAGATTTG
Rn *Col1A1*	NM_053304.1	GGATGCCATCAAGGTCTACTGC	TGAGTGGGGAACACACAGGTCT
Rn *Tgfβ*	NM_021578.2	ATGGTGGACCGCAACAAC	CAGCAATGGGGGTTCTGG
Rn *Gapdh*	NM_017008.4	AAGGCTGAGAAATGGGAAGCTC	CCATTTGATGTTAGCGGGATCT
Rn *Atpaf-1*	NM_001107959.1	GATCTCTCCAAGAAGCTGCAAG	AAGATGACCCCAAGGCATTTTT

Table 2
Caloric intake, body weight and tissue amount.

	C	D	D-FIN
Caloric intake (kcal/day)	47.9 ± 4.9	70.0 ± 17.3 *	59.3 ± 14.6 *#
Final weight (g)	400.3 ± 33.2	433.6 ± 14.4 *	424.3 ± 23.8 *
Tibial length (mm)	4.3 ± 0.03	4.3 ± 0.08	4.2 ± 0.001
PRAT/tibial length (g/mm)	0.90 ± 0.01	0.145 ± 0.02 *	0.152 ± 0.01 *
PVAT/tibial length (mg/mm)	7 ± 2	9 ± 2 *	10 ± 2 *
Mesenteric AT/tibial length (mg/mm)	73 ± 5	116 ± 2 *	117 ± 3 *

Results are shown as mean ± SEM. *p < 0.05 compared to C; #p < 0.05 compared to D.

Table 3
Glycemic parameters.

	C	D	D-FIN
Fasting glucose (mg/dL)	125.0 ± 3.9	243.0 ± 28 *	259.0 ± 56 *
Fasting insulin (ng/mL)	2.6 ± 0.4	3.1 ± 0.4	3.3 ± 0.2
Postprandial glucose (mg/dL)	302.3 ± 25	481.2 ± 33 *	497.5 ± 68 *
Postprandial insulin (mg/dL)	5.8 ± 0.3	3.4 ± 0.5 *	3.0 ± 0.2 *
HOMA-IR	17.4 ± 2.1	40.0 ± 5.3 *	50.0 ± 12.5 *
Water intake (mL)	26.2 ± 6.1	44.0 ± 25.6 *	57.5 ± 31.1 *
Urine excretion (mL)	8.5 ± 2.5	27.7 ± 22.5 *	36.8 ± 27.1 *
Glucosuria (mg/24 h)	14.7 ± 6.6	241.0 ± 70.4 *	157.9 ± 42.2.4 *

Results are shown as mean ± SEM. *p < 0.05 compared to C.

As shown in Table 5, potassium, calcium, or phosphate plasma levels were similar between groups. 25-OH cholecalciferol levels were higher in the MWF-D group and not modified by FIN.

3.4. *FIN reduced blood pressure, pulse wave velocity and collagen deposition in diabetic MWF*

Diabetes induction did not further increase SBP (Fig. 2A), DBP (Fig. 2B) or PWV (Fig. 2C) compared to the C group. However, D-FIN rats showed significantly lower values of SBP, DBP and PWV (Fig. 2A-C). No differences in HR were observed between the groups (Fig. 2D). Both heart weight (Fig. 2E) and plasma ANP levels (Fig. 2F) were significantly higher in the D group and reduced by FIN treatment.

Since increased PWV in MWF is related to an increased collagen amount in mesenteric arteries [12], we ought to determine if the FIN-induced decrease of PWV was associated to a decreased collagen content in these vessels. As shown in Figs. 2G and 2H, D rats showed a significant increase in collagen content compared to the C group, which was significantly reduced to control levels by FIN treatment.

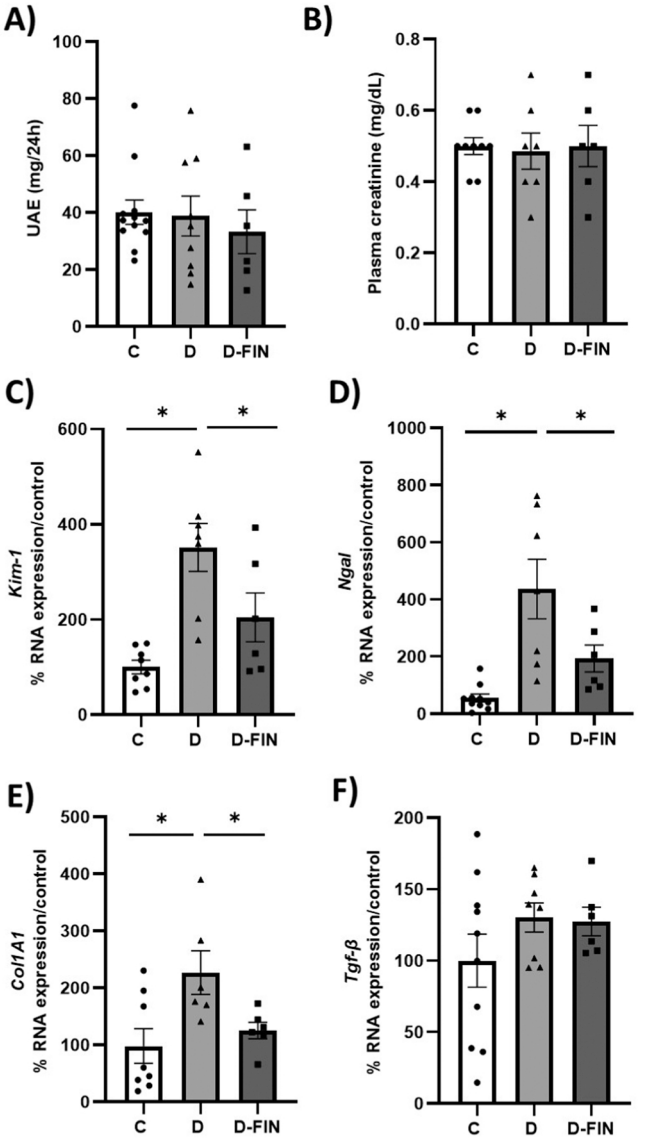


Fig. 1. Effect of FIN treatment on MWF kidney damage markers. (A) Urinary albumin excretion (UAE) and (B) plasma creatinine in C, D and D-FIN rats. Kidney mRNA expression of tubular damage markers (C) *Kim-1* and (D) *Ngal* and fibrosis markers (E) *Col1A1* and (F) *Tgf-β* in C, D and D-FIN rats. Expression values are represented as the percentage of basal expression, considering the C group as basal. Values are expressed as means ± SEM. *p < 0.05; n = 6–10 animals.

Table 4
Albumin, creatinine and total protein urine excretion, plasmatic creatinine concentration and creatinine clearance.

	C	D	D-FIN
Urine creatinine excretion (mg/24 h)	1.89 ± 0.29	0.14 ± 0.45 *	0.28 ± 0.17 *
Urine protein excretion (mg/24 h)	73.89 ± 7.18	65.33 ± 12.61	39.10 ± 9.75 *
Urine protein/creatinine ratio (UPCR)	6532 ± 666.7	5934 ± 750.8	5648 ± 993.8
Plasma creatinine (mg/dL)	0.50 ± 0.23	0.59 ± 0.11	0.50 ± 0.06
Creatinine clearance (mL/min)	1.64 ± 0.05	1.87 ± 0.24	1.91 ± 0.24

Results are shown as mean ± SEM. *p < 0.05 compared to C.

Table 5
Calcification parameters.

	C	D	D-FIN
Calcium (mg/dl)	9.73 ± 0,2	9.9 ± 0.4	9.7 ± 0.4
Potassium (mEq/l)	5.9 ± 0.2	5.8 ± 0.3	6.0 ± 0.2
Phosphate (mg/dl)	7.2 ± 0.3	8.4 ± 0.3	8.2 ± 0.1 *
25-OH cholecalciferol (ng/mL)	13.6 ± 0.8	17.1 ± 1.0 *	16.8 ± 0.7

Values are expressed as means ± SEM. *p < 0.05 compared with C.

3.5. FIN treatment improves vascular function through reduction of vasoconstrictor prostanoids and reactive oxygen species (ROS)

C rats exhibited an aortic endothelial dysfunction, characterized by a reduced relaxation to Ach (10^{-9}-10^{-4} mol/L). This response was not further aggravated in the diabetic group, but significantly improved in D-FIN (Fig. 3A). Aortic contractions elicited by NA (10^{-9}-10^{-6} mol/L) were significantly higher in the diabetic compared to the C group (Fig. 3B) and returned to control levels in the FIN-treated group (Fig. 3B).

Since hypersensitivity of vascular smooth muscle to contractile factors in diabetes activates the production of cyclooxygenase (COX)-derived vasoconstrictor prostanoids [42,43] aortic segments were pre-incubated with the cyclooxygenase (COX) inhibitor, INDO (3×10^{-6} mol/L). NA-induced contractions were significantly reduced by INDO in the D (Fig. 3D) but not in the C group (Fig. 3C) or FIN-D group (Fig. 3E), suggesting that FIN prevents production of COX-derived contractile prostanoids induced by diabetes.

Next, we analyzed the contribution of NO and ROS to increased contractions. Nitric oxide synthase (NOS) inhibitor L-NAME (10^{-4} mol/L) significantly increased NA-induced aortic contractions in both the C (Fig. 3F) and the D (Fig. 3G) but not in the D-FIN group (Fig. 3H). NA-induced contractions were significantly reduced by the catalase inhibitor 3-AT (5×10^{-3} mol/L) the aorta from D (Fig. 3J) but not from either C (Fig. 3I), or D-FIN rats (Fig. 3K). Preincubation with the NADPH oxidase 1 (NOX-1) inhibitor ML171 (10^{-5} mol/L) significantly reduced aortic NA-induced contractions in the C (Fig. 3L) and D-FIN (Fig. 3N) groups. However, contractions to NA were significantly increased in the D group (Fig. 3M).

3.6. FIN prevents diabetes-induced alterations in PVAT and PRAT

Next, we aimed at evaluating the impact of diabetes and the effect of FIN on aortic PVAT expression of inflammatory and fibrotic factors. The expression of the inducible NOS (*iNos*) in PVAT from D rats was almost abolished by FIN treatment (Fig. 4A). Expression of *Il-1β, Il-6, IL-10, Tnfα, Tgfβ*, and *Col1A1* were significantly higher in the D group and reduced to control levels by FIN treatment (Fig. 4B, C, E, F, G). No difference between groups was observed in expression levels of the anti-inflammatory *Il-10* (Fig. 4D).

Since the increase in PRAT amount is a novel independent risk factor for CKD [26–30], we ought to determine the effect of FIN on the expression of proinflammatory and profibrotic factors in this tissue. Expression of *Il-1β* and *Il-6* was significantly lower in PRAT from the D-FIN groups as compared to C and D groups (Fig. 5A and B). *Il-10* expression was downregulated by diabetes and not modified by FIN (Fig. 5C). *Tnfα* and *Tgfβ* were significantly upregulated in the D group and reduced to control levels by FIN treatment (Fig. 5C, D). Diabetes increased *Col1A1* (Fig. 5E) which was not modified by FIN.

3.7. FIN prevents diabetes-induced imbalance of BMP-2 and BMP-7

Based on the imbalance between BMP-2 and BMP-7 levels in CKD associated to vascular fibrosis and increased PWV [18], we aimed at evaluating the impact of diabetes and the effect of FIN on these proteins in plasma, kidney, PVAT and PRAT. BMP-2 was significantly lower in plasma, kidney and PVAT from the D-FIN group compared to C and D groups (Fig. 6 A, C, E). FIN significantly increased BMP-7 levels in plasma and BMP-7 expression in the kidney to control levels (Fig. 6B and D). In PRAT, no differences were observed between groups in BMP-2, whereas BMP-7 expression was higher in both D and D-FIN groups (Fig. 6G and H).

4. Discussion

Our study shows a protective effect of FIN on the progression of kidney and cardiovascular damage in a novel model of T1DM in established CKD through the reduction of i) kidney damage markers, ii) blood pressure, heart weight, PWV and vascular collagen content, and iii) vascular prostanoids and reactive oxygen species (ROS). Moreover, here we show for the first time that FIN prevents diabetes-induced alterations in PVAT and PRAT, such as the upregulation of proinflammatory and profibrotic factors. In addition, FIN restores the imbalance observed in CKD between the procalcifying BMP-2 and the nephroprotective BMP-7 in plasma, kidney, PVAT and PRAT. All these beneficial effects of FIN are observed independently of changes in glycaemia other characteristics of diabetes.

The FIDELIO and FIGARO studies showed a reduced risk of CKD progression and cardiovascular events in patients with CKD and T2DM treated with FIN [34,35] independent of baseline glycemia [44], HbA1c levels or insulin use [45]. In the present preclinical study, FIN was started concurrent with diabetes induction in 16-week-old rats with overt albuminuria, kidney and vascular damage [12,37,41]. This intervention is, thus, a prevention study to assess the efficacy of FIN on kidney and cardiovascular damage progression in a T1DM model. Treatment with FIN did not prevent any of the metabolic parameters directly related to diabetes. Conversely, FIN prevented the upregulation of kidney damage markers *Kim-1*, *Ngal*, *Bmp-2*, and *Col1A1*, as well as the downregulation of *Bmp-7* after induction of diabetes. BMP-2 is a well-known procalcifying factor, which inversely correlates with eGFR in the CKD population [20] and which is increased in stage III CKD patients and MWF rats [18]. In addition, the reduction of *Bmp-7* in kidney of MWF rats is associated with a progression of kidney and vascular damage [18]. BMP-7 is a physiological antagonist of TGFβ, a key bystander in kidney fibrosis implicated in CKD progression [46,47]. In this line, decreased BMP-7 levels in T2DM patients who subsequently develop major kidney events, might predict these better than the best currently available risk marker, i.e. albuminuria, serum creatinine or cystatin C [48]. In fact, the reduction of plasma BMP-7 already in CKD stage I patients suggests it might be an early event in CKD development, reflecting a reduction in kidney tubular BMP-7 expression [18]. In a mouse model of T1DM [24], administration of BMP-7 partially reversed diabetic-induced kidney hypertrophy, reduced albuminuria and restored GFR and glomerular histology toward normal.

Although diabetes induction does not further increase BP in the D group, FIN significantly reduced both SBP and DBP associated to an

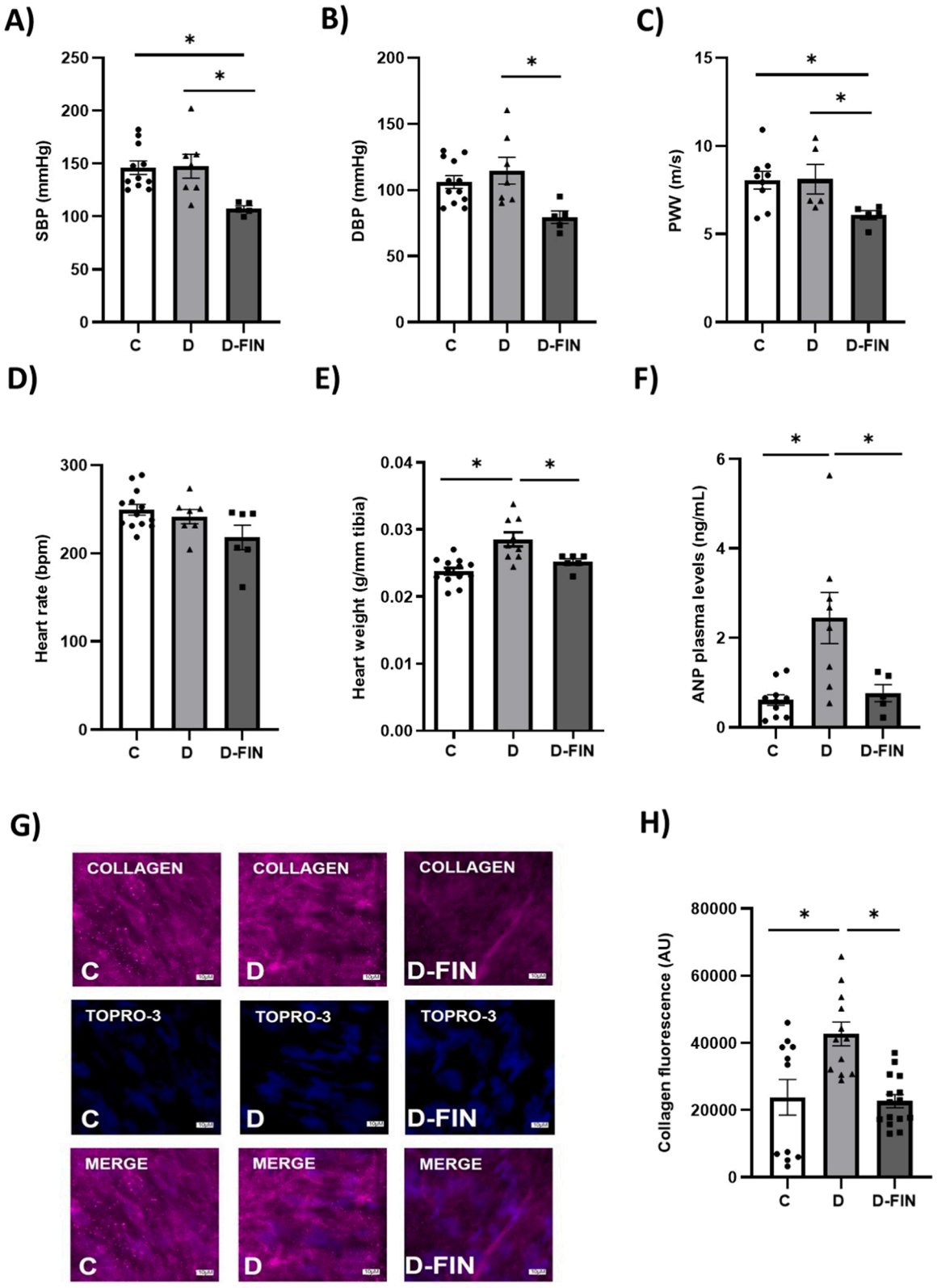
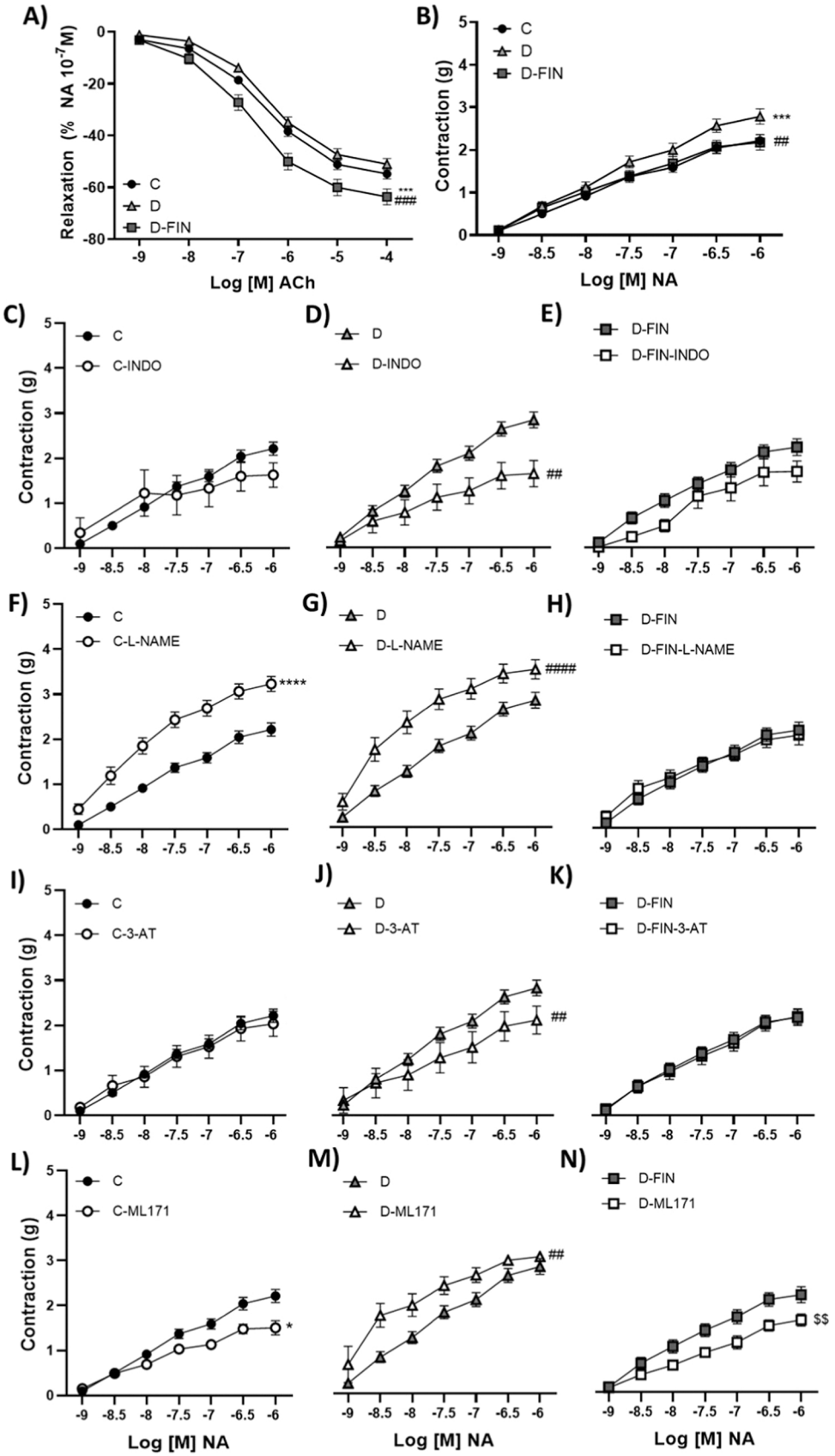


Fig. 2. Effect of FIN treatment on cardiovascular hemodynamics and fibrosis. (A) Systolic blood pressure (SBP), (B) diastolic blood pressure (DBP), (C) pulse wave velocity (PWV) and (D) heart rate in anesthetized C, D and D-FIN rats. (E) Whole heart weight normalized by respective tibial length (F) and ANP plasma level. (G) Representative images of first-branch mesenteric artery immunofluorescence showing collagens 1 and 3 content detected with primary antibodies marked with Alexa Fluor 555 (magenta, upper row), nuclei marked with TOPRO-3 (blue, middle row), and merged images (bottom row). (H) Immunofluorescence quantification. Values are expressed as means ± SEM. *$p < 0.05$; $n = 6–10$ animals.



(caption on next page)

Fig. 3. Effect of FIN on vascular function. Cumulative concentration-response curves to (A) Ach (10^{-9} to 10^{-4} M) and (B) NA (10^{-9} to 10^{-6} M) in thoracic aorta with intact endothelium from C, D and D-FIN rats. (C-N) Cumulative dose-response curves to NA (10^{-9} to 10^{-6} M) in presence or absence of: (C-E) the cyclooxygenase (COX) inhibitor indomethacin (INDO, 3×10^{-6} mol/L); (F-G) the NOS inhibitor L-NAME (10^{-4} mol/L); (I-K)); the catalase inhibitor 3-AT (5×10^{-3} mol/L); (L-N) the NADPH oxidase 1 (NOX-1) inhibitor ML171 (10^{-5} mol/L in thoracic aorta with intact endothelium from C, D and D-FIN rats. Values are expressed as means ± SEM. ***p < 0.001 compared with C; #p < 0.05 compared with D; ##p < 0.01 compared with D; n = 6–10 animals.

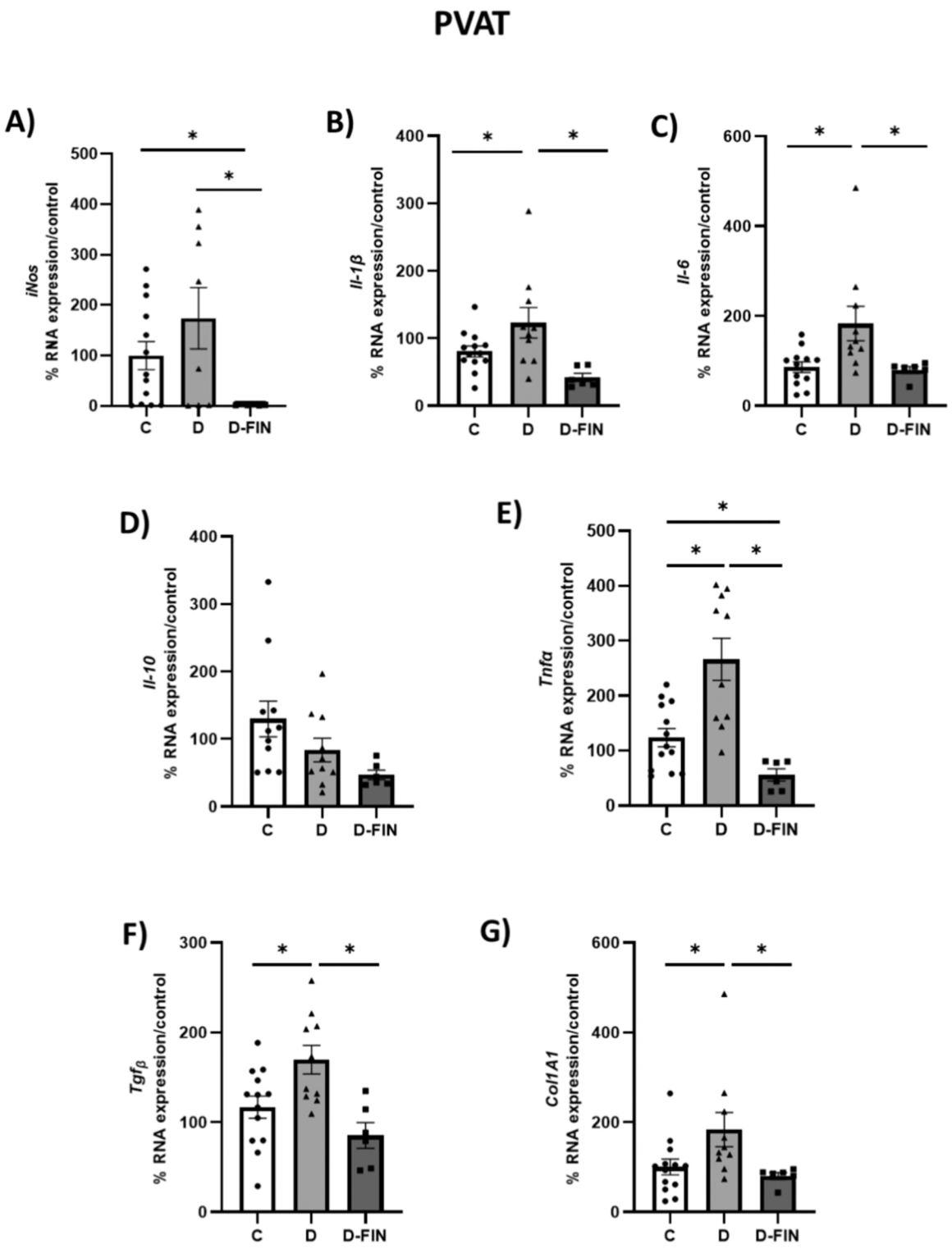


Fig. 4. Effect of FIN treatment on inflammatory and fibrotic factor expression in periaortic adipose tissue (PVAT). Adipose mRNA expression of (A) *iNos*, (B) *Il-1β*, (C) *Il-6*, (D) *Il-10*, (E) *Tnfα*, (F) *Tgfβ* and (G) *Col1A1* from C, D and D-FIN rats. Expression values are represented as the percentage of basal expression, considering the C group as basal. Values are expressed as means ± SEM. *p < 0.05;(n = 6–10 animals).

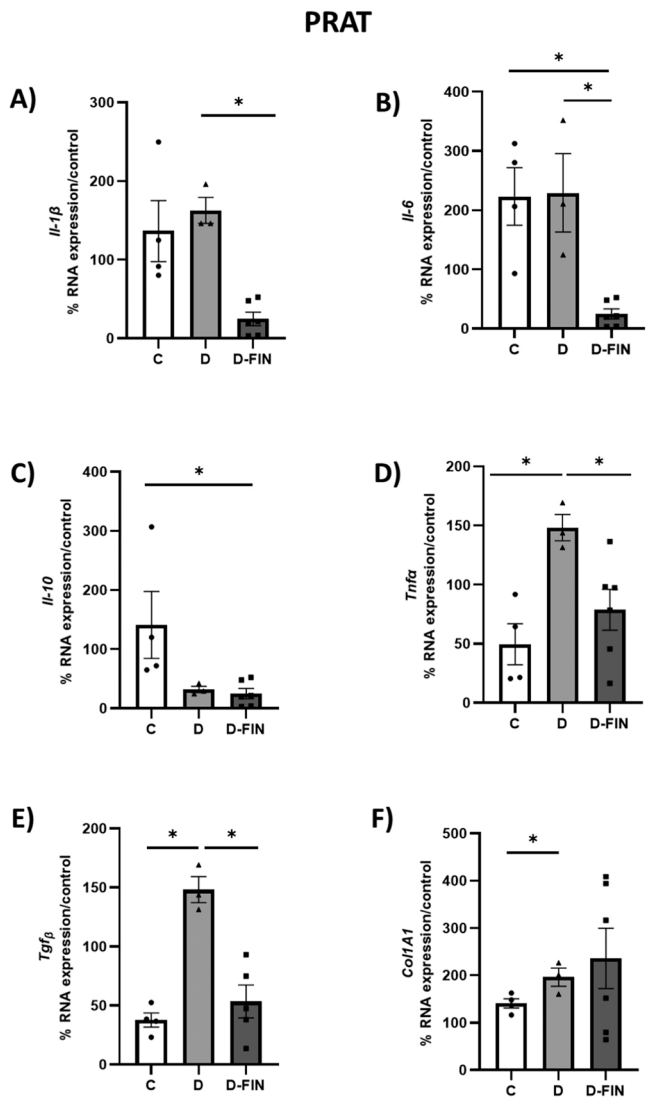


Fig. 5. Effect of FIN treatment on inflammatory and fibrotic factor expression in perirenal adipose tissue (PRAT). Adipose mRNA expression of (A) *Il-1β*, (B) *Il-6*, (C) *Il-10*, (D) *Tnfα*, (E) *Tgfβ* and (F) *Col1A1*. Expression values are represented as the percentage of basal expression, considering the C group as basal. Values are expressed as means ± SEM. *p < 0.05 compared with C; * **p < 0.001 compared with C; #p < 0.05 compared with D; n = 6–10 animals.

amelioration of endothelial function shown by the increased relaxations to Ach and the reduced contractions to NA. The effect of the non-selective COX inhibitor INDO used in this study suggests an increased prostanoid production in the aorta of the diabetic group, which is prevented by FIN treatment. In accordance, an upregulation of the constitutive COX-1 and inducible COX-2 isoform in conductance arteries from T1D rats [43] catalyse prostanoid formation which are important inflammatory mediators leading to increased contractions [49]. Upregulation of COX is, in part, due to excess production of ROS [50,51] and NOX1 is considered the major source of vascular ROS in diabetes [52]. Indeed, impairment of endothelial function in the MWF rat is related to increased oxidative stress due to abnormal ROS formation [41]. Moreover, H_2O_2 plays an essential role in pathological conditions compensating for vasorelaxation in large vessels [53]. In this study, aortas from the D group showed an increased production of H_2O_2 eliciting vasodilatation, as observed in presence of 3-AT. Moreover, increased NA-induced contractions in presence of ML171 might reflect the abolishment of NOX1-dependent superoxide production and subsequent H_2O_2 formation, unmasking the increased prostanoid-dependent vasoconstriction. Thus, increased H_2O_2 production might counterbalance enhanced prostanoid-induced contractions, as observed after NOX1 inhibition. In this scenario, FIN efficiently reduces both vasoconstrictor prostanoid formation and ROS generation in the vascular wall, as previously demonstrated in nondiabetic MWF rats [37]. To note, ROS stimulates the procalcifying effects of the BMP-2 in human endothelial cells [19,20] thus suggesting that increased BMP-2 is likely to contribute to impaired endothelial function.

The increase in heart size and ANP plasma levels in the D group despite no BP increase is noteworthy. Heart enlargement is related to one of the cardiac complications of diabetes such as heart failure with preserved ejection fraction, which is also related to increased vascular stiffness. A pressure-related effect on the prevention of cardiac alterations by FIN might not be excluded. However, even in CKD with well-controlled BP, the administration of MRA reduces left ventricular hypertrophy, as well as PWV [54]. Accordingly, FIN reversed increased PWV associated to a decrease in diabetes-induced collagen deposition in mesenteric resistance arteries due to a probable reduction of arterial stiffness. In fact, mesenteric arteries from MWF rats have an increased intrinsic arterial stiffness due to alterations in the elastin/collagen balance and increased MMP-9 activity, all reduced by FIN [12].

Other mechanisms associated with kidney and vascular damage in CKD involve a dysfunction of different adipose tissue depots. PVAT is a source of multiple vasoactive factors with physiological paracrine effects on both vascular function and structure [55,56]. In pathophysiological conditions, such as hypertension, obesity or diabetes, alteration in the expression pattern of these factors lead to PVAT dysfunction, creating a contractile, pro-oxidant, inflammatory, and pro-fibrotic environment leading to vascular dysfunction, an increase in SBP and DBP, and to higher PWV [18,55,56]. Moreover, PRAT is a possible bystander in CKD due to its anatomical location surrounding the kidney. Recent studies have highlighted the key role of PRAT thickness as a novel independent risk factor for CKD in patients associated with a decrease of the estimated glomerular filtration rate (eGFR) [27,57], with abdominal obesity and albuminuria [30,58], as well as with CKD progression [26,28,30]. Expansion of the adipose tissue is tightly associated with adipose inflammation and dysfunctional adipocytes. The imbalance between pro-inflammatory TNFα, IL-1, IL-6, and the anti-inflammatory IL-10, as well as the upregulation of TGFβ and Col1A1 suggests a net inflammatory and profibrotic environment in both PVAT and PRAT from MWF-D. Reductions in kidney and vascular fibrosis along with amelioration of concomitant inflammation have been shown with FIN in different experimental models by decreasing interstitial fibrosis, proinflammatory macrophages myofibroblasts and collagen deposition at non hypotensive doses [59]. However, this is the first report demonstrating an anti-inflammatory and antifibrotic effect of FIN on both PVAT and PRAT.

There are several limitations of the study. First, neither albuminuria, proteinuria, urinary creatinine, protein/creatinine ratio, creatinine clearance nor plasma creatinine were modified by the additional induction of T1 diabetes in albuminuric MWF rats. Accordingly, there was no significant effect on these parameters by FIN treatment vs. the D group. However, FIN significantly reduced urinary protein excretion vs. the albuminuric C group and by trend vs. the D group (Table 4) as observed with urinary albumin excretion in previous studies using non-diabetic albuminuric Wistar Frömter rats [12,37]. Therefore, 16 weeks old MWF animals seem to be already maximally albuminuric in the non-diabetic state while a rather short-term additional exposure to diabetes does not further increase albuminuria. Furthermore, we observed a substantial deviation in albuminuria and other kidney function values per group. Other potential reasons for a non-significant UAE reduction by finerenone could be a too short treatment time. This observation is in strong contrast to the congruent UACR reduction by finerenone in all phase II and phase III studies conducted so far [33–35,60,61]. Second, the L-NAME-induced abolishment of NO oxide

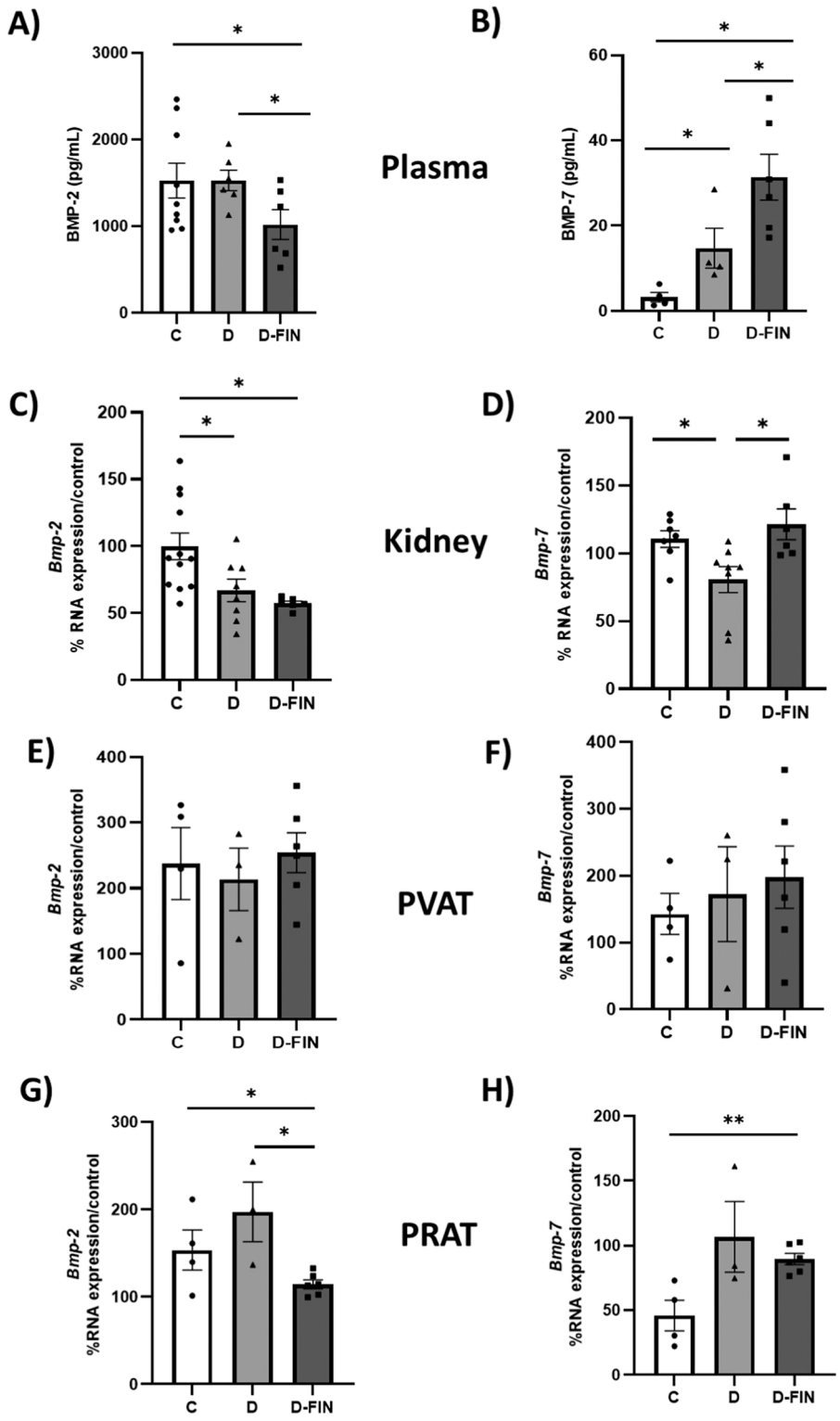


Fig. 6. Effect of FIN treatment on BMP-2 and BMP-7 levels in: (A, B) plasma, (C, D) kidney, (E, F) periaortic adipose tissue (PVAT), and (G,H) perirenal adipose tissue (PRAT). mRNA expression values are represented as the percentage of basal expression, considering the C group as basal. Values are expressed as means ± SEM. *$p < 0.05$; n = 6–10 animals.

availability in the D-FIN group might be attributed to a possible reduction of iNOS expression in the vascular wall, as observed in PVAT, thus contributing to a reduction of vascular inflammation. However, this is an indirect interpretation since, unfortunately, there was no aortic tissue available for assessing iNOS expression.

In conclusion, our study shows that treatment with FIN improves kidney and vascular damage in a rat model of DKD with T1D. We show for the first time that FIN prevents diabetes-induced alterations in PVAT and PRAT, such as the upregulation of proinflammatory and profibrotic factors, and restores the imbalance observed in CKD between the pro-calcifying BMP-2 and the nephroprotective BMP-7 in plasma, kidney, PVAT and PRAT. All these beneficial effects of FIN are observed

independent of changes in glycaemia other characteristics of diabetes.

CRediT authorship contribution statement

M. Sanz-Gómez: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. **F.J. Manzano-Lista:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. **E. Vega-Martín:** Methodology, Formal analysis, Investigation. **D. González-Moreno:** Methodology, Formal analysis, Investigation. **M. Alcalá:** Formal analysis, Investigation. **M. Gil-Ortega:** Formal analysis, Investigation. **B. Somoza:** Formal analysis, Investigation. **C. Pizzamiglio:** Formal analysis, Investigation. **L.M. Ruilope:** Conceptualization. **I. Aránguez:** Conceptualization. **P. Kolkhof:** Conceptualization, Supervision, Resources, Writing – review & editing. **R. Kreutz:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding adquisition. **M.S. Fernández-Alfonso:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding adquisition.

Declaration of Competing Interest

P. Kolkhof is a full-time employee of BAYER AG**; R. Kreutz** has received support of research and personal honoraria from BAYER AG, Berlin-Chemie Menarini, Daiichi Sankyo, Ferrer, Merck, Sanofi, and Servier; **M. S. Fernández-Alfonso** has received support of research by BAYER AG as principal investigator.

Data Availability

Data will be made available on request.

Acknowledgement

This work was supported by Bayer AG, by UCM Grupos Santander, and by the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement MINDSHIFT (ID 954798). Marta Sanz is recipient of a fellowship from Universidad Complutense de Madrid. Francisco Javier Manzano-Lista is fellow from Ministerio de Educación, Cultura y Deporte (FPU17/03075).

References

[1] World Health Organization. Mortality and global health estimates: Causes of death; Projections for 2015–2030; Projection of death rates. Available online: http://apps.who.int/gho/data/node.main.PROJRATEWORLD?lang=en (accessed on September 13th, 2022).
[2] K. Kalantar-Zadeh, T.H. Jafar, D. Nitsch, B.L. Neuen, V. Perkovic, Chronic kidney disease, Lancet (Lond., Engl.) 398 (10302) (2021) 786–802, https://doi.org/10.1016/S0140-6736(21)00519-5.
[3] S. Thompson, M. James, N. Wiebe, B. Hemmelgarn, B. Manns, S. Klarenbach, M. Tonelli, Alberta Kidney Disease Network, Cause of death in patients with reduced kidney function, J. Am. Soc. Nephrol.: JASN 26 (10) (2015) 2504–2511, https://doi.org/10.1681/ASN.2014070714.
[4] J. Jankowski, J. Floege, D. Fliser, M. Böhm, N. Marx, Cardiovascular disease in chronic kidney disease: pathophysiological insights and therapeutic options, Circulation 143 (11) (2021) 1157–1172, https://doi.org/10.1161/CIRCULATIONAHA.120.050686.
[5] S.D. Navaneethan, J.D. Schold, S.E. Jolly, S. Arrigain, W.C. Winkelmayer, J. V. Nally, Jr, Diabetes control and the risks of ESRD and mortality in patients with CKD, Am. J. Kidney Dis.: Off. J. Natl. Kidney Found. 70 (2) (2017) 191–198, https://doi.org/10.1053/j.ajkd.2016.11.018.
[6] M. Satoh, Endothelial dysfunction as an underlying pathophysiological condition of chronic kidney disease, Clin. Exp. Nephrol. 16 (4) (2012) 518–521, https://doi.org/10.1007/s10157-012-0646-y.
[7] N.D. Vaziri, Z. Ni, F. Oveisi, K. Liang, R. Pandian, Enhanced nitric oxide inactivation and protein nitration by reactive oxygen species in renal insufficiency, Hypertens. (Dallas, Tex.: 1979) 39 (1) (2002) 135–141, https://doi.org/10.1161/hy0102.100540.
[8] H. Pulido-Olmo, C.F. García-Prieto, G. Álvarez-Llamas, M.G. Barderas, F. Vivanco, I. Aranguez, B. Somoza, J. Segura, R. Kreutz, M.S. Fernández-Alfonso, L. M. Ruilope, G. Ruiz-Hurtado, Role of matrix metalloproteinase-9 in chronic kidney disease: a new biomarker of resistant albuminuria, Clin. Sci. (Lond., Engl.: 1979) 130 (7) (2016) 525–538, https://doi.org/10.1042/CS20150517.
[9] G. Ruiz-Hurtado, L. Condezo-Hoyos, H. Pulido-Olmo, I. Aranguez, M. Del Carmen Gónzalez, S. Arribas, C. Cerezo, J. Segura, M. Praga, M.S. Fernández-Alfonso, L. M. Ruilope, Development of albuminuria and enhancement of oxidative stress during chronic renin-angiotensin system suppression, J. Hypertens. 32 (10) (2014) 2082–2091, https://doi.org/10.1097/HJH.0000000000000292.
[10] X. Kong, X. Jia, Y. Wei, M. Cui, Z. Wang, L. Tang, W. Li, Z. Zhu, P. Chen, D. Xu, Association between microalbuminuria and subclinical atherosclerosis evaluated by carotid artery intima-media in elderly patients with normal renal function, BMC Nephrol. 13 (2012) 37, https://doi.org/10.1186/1471-2369-13-37.
[11] M. Gil-Ortega, C.F. García-Prieto, G. Ruiz-Hurtado, C. Steireif, M.C. González, A. Schulz, R. Kreutz, M.S. Fernández-Alfonso, S. Arribas, B. Somoza, Genetic predisposition to albuminuria is associated with increased arterial stiffness: role of elastin, Br. J. Pharmacol. 172 (17) (2015) 4406–4418, https://doi.org/10.1111/bph.13223.
[12] M. Gil-Ortega, E. Vega-Martín, M. Martín-Ramos, R. González-Blázquez, H. Pulido-Olmo, G. Ruiz-Hurtado, A. Schulz, L.M. Ruilope, P. Kolkhof, B. Somoza, R. Kreutz, M.S. Fernández-Alfonso, Finerenone reduces intrinsic arterial stiffness in munich wistar frömter rats, a genetic model of chronic kidney disease, Am. J. Nephrol. 51 (4) (2020) 294–303, https://doi.org/10.1159/000506275.
[13] C.M. Giachelli, The emerging role of phosphate in vascular calcification, Kidney Int. 75 (9) (2009) 890–897, https://doi.org/10.10dido/ki.2008.644.
[14] A.P. Guérin, G.M. London, S.J. Marchais, F. Metivier, Arterial stiffening and vascular calcifications in end-stage renal disease, Nephrol., Dial., Transplant.: Off. Publ. Eur. Dial. Transpl. Assoc. - Eur. Ren. Assoc. 15 (7) (2000) 1014–1021, https://doi.org/10.1093/ndt/15.7.1014.
[15] M.R. Davies, K.A. Hruska, Pathophysiological mechanisms of vascular calcification in end-stage renal disease, Kidney Int. 60 (2) (2001) 472–479, https://doi.org/10.1046/j.1523-1755.2001.060002472.x.
[16] J. Blacher, A.P. Guerin, B. Pannier, S.J. Marchais, M.E. Safar, G.M. London, Impact of aortic stiffness on survival in end-stage renal disease, Circulation 99 (18) (1999) 2434–2439, https://doi.org/10.1161/01.cir.99.18.2434.
[17] L. Zanoli, P. Lentini, M. Briet, P. Castellino, A.A. House, G.M. London, L. Malatino, P.A. McCullough, D.P. Mikhailidis, P. Boutouyrie, Arterial Stiffness in the Heart Disease of CKD, J. Am. Soc. Nephrol.: JASN 30 (6) (2019) 918–928, https://doi.org/10.1681/ASN.2019020117.
[18] F.J. Manzano-Lista, M. Sanz-Gómez, D. González-Moreno, E. Vega-Martín, M. Gil-Ortega, A. Schulz, M.Á. Rubio, G. Ruiz-Hurtado, L.M. Ruilope, I. Aránguez, R. Kreutz, M.S. Fernández-Alfonso, Imbalance in bone morphogenic proteins 2 and 7 is associated with renal and cardiovascular damage in chronic kidney disease, Int. J. Mol. Sci. 24 (1) (2022) 40, https://doi.org/10.3390/ijms24010040.
[19] G. Dalfino, S. Simone, S. Porreca, C. Cosola, C. Balestra, C. Manno, F.P. Schena, G. Grandaliano, G. Pertosa, Bone morphogenetic protein-2 may represent the molecular link between oxidative stress and vascular stiffness in chronic kidney disease, Atherosclerosis 211 (2) (2010) 418–423, https://doi.org/10.1016/j.atherosclerosis.2010.04.023.
[20] S. Rong, X. Zhao, X. Jin, Z. Zhang, L. Chen, Y. Zhu, W. Yuan, Vascular calcification in chronic kidney disease is induced by bone morphogenetic protein-2 via a mechanism involving the Wnt/β-catenin pathway, Cell. Physiol. Biochem.: Int. J. Exp. Cell. Physiol., Biochem., Pharmacol. 34 (6) (2014) 2049–2060, https://doi.org/10.1159/000366400.
[21] S. Vukicevic, V. Basic, D. Rogic, N. Basic, M.S. Shih, A. Shepard, D. Jin, B. Dattatreyamurty, W. Jones, H. Dorai, S. Ryan, D. Griffiths, J. Maliakal, M. Jelic, M. Pastorcic, A. Stavljenic, T.K. Sampath, Osteogenic protein-1 (bone morphogenetic protein-7) reduces severity of injury after ischemic acute renal failure in rat, J. Clin. Investig. 102 (1) (1998) 202–214, https://doi.org/10.1172/JCI2237.
[22] K.A. Hruska, G. Guo, M. Wozniak, D. Martin, S. Miller, H. Liapis, K. Loveday, S. Klahr, T.K. Sampath, J. Morrissey, Osteogenic protein-1 prevents renal fibrogenesis associated with ureteral obstruction, Am. J. Physiol. Ren. Physiol. 279 (1) (2000) F130–F143, https://doi.org/10.1152/ajprenal.2000.279.1.F130.
[23] H. Sugimoto, G. Grahovac, M. Zeisberg, R. Kalluri, Renal fibrosis and glomerulosclerosis in a new mouse model of diabetic nephropathy and its regression by bone morphogenic protein-7 and advanced glycation end product inhibitors, Diabetes 56 (7) (2007) 1825–1833, https://doi.org/10.2337/db06-1226.
[24] S. Wang, R. Hirschberg, BMP7 antagonizes TGF-beta -dependent fibrogenesis in mesangial cells, Am. J. Physiol. Ren. Physiol. 284 (5) (2003) F1006–F1013, https://doi.org/10.1152/ajprenal.00382.2002.
[25] C.B.A. Restini, A. Ismail, R.K. Kumar, R. Burnett, H. Garver, G.D. Fink, S.W. Watts, Renal perivascular adipose tissue: form and function, Vasc. Pharmacol. 106 (2018) 37–45, https://doi.org/10.1016/j.vph.2018.02.004.
[26] X. Chen, Y. Mao, J. Hu, S. Han, L. Gong, T. Luo, S. Yang, H. Qing, Y. Wang, Z. Du, M. Mei, L. Zheng, X. Lv, Y. Tang, Q. Zhao, Y. Zhou, J.C. He, Q. Li, Z. Wang, Perirenal fat thickness is significantly associated with the risk for development of chronic kidney disease in patients with diabetes, Diabetes 70 (10) (2021) 2322–2332, https://doi.org/10.2337/db20-1031.
[27] Y. Fang, Y. Xu, Y. Yang, C. Liu, D. Zhao, J. Ke, The relationship between perirenal fat thickness and reduced glomerular filtration rate in patients with type 2 diabetes, J. Diabetes Res. 2020 (2020) 6076145, https://doi.org/10.1155/2020/6076145.
[28] G. Geraci, M.M. Zammuto, A. Mattina, L. Zanoli, C. Geraci, A. Granata, E. Nardi, P. M. Fatuzzo, S. Cottone, G. Mulè, Para-perirenal distribution of body fat is associated with reduced glomerular filtration rate regardless of other indices of

adiposity in hypertensive patients, J. Clin. Hypertens. (Greenwich, Conn.) 20 (10) (2018) 1438–1446, https://doi.org/10.1111/jch.13366.
[29] B.X. Liu, W. Sun, X.Q. Kong, Perirenal fat: a unique fat pad and potential target for cardiovascular disease, Angiology 70 (7) (2019) 584–593, https://doi.org/10.1177/0003319718799967.
[30] F.C. Shen, B.C. Cheng, J.F. Chen, Peri-renal fat thickness is positively associated with the urine albumin excretion rate in patients with type 2 diabetes, Obes. Res. Clin. Pract. 14 (4) (2020) 345–349, https://doi.org/10.1016/j.orcp.2020.06.006.
[31] P. Kolkhof, A. Joseph, U. Kintscher, Nonsteroidal mineralocorticoid receptor antagonism for cardiovascular and renal disorders - New perspectives for combination therapy, Pharmacol. Res. 172 (2021), 105859, https://doi.org/10.1016/j.phrs.2021.105859.
[32] P. Kolkhof, M. Delbeck, A. Kretschmer, W. Steinke, E. Hartmann, L. Bärfacker, F. Eitner, B. Albrecht-Küpper, S. Schäfer, Finerenone, a novel selective nonsteroidal mineralocorticoid receptor antagonist protects from rat cardiorenal injury, J. Cardiovasc. Pharmacol. 64 (1) (2014) 69–78, https://doi.org/10.1097/FJC.0000000000000091.
[33] B. Pitt, L. Kober, P. Ponikowski, M. Gheorghiade, G. Filippatos, H. Krum, C. Nowack, P. Kolkhof, S.Y. Kim, F. Zannad, Safety and tolerability of the novel non-steroidal mineralocorticoid receptor antagonist BAY 94-8862 in patients with chronic heart failure and mild or moderate chronic kidney disease: a randomized, double-blind trial, Eur. Heart J. 34 (31) (2013) 2453–2463, https://doi.org/10.1093/eurheartj/eht187.
[34] G.L. Bakris, R. Agarwal, S.D. Anker, B. Pitt, L.M. Ruilope, P. Rossing, P. Kolkhof, C. Nowack, P. Schloemer, A. Joseph, G. Filippatos, FIDELIO-DKD Investigators, Effect of finerenone on chronic kidney disease outcomes in type 2 diabetes, New Engl. J. Med. 383 (23) (2020) 2219–2229, https://doi.org/10.1056/NEJMoa2025845.
[35] B. Pitt, G. Filippatos, R. Agarwal, S.D. Anker, G.L. Bakris, P. Rossing, A. Joseph, P. Kolkhof, C. Nowack, P. Schloemer, L.M. Ruilope, FIGARO-DKD Investigators, Cardiovascular events with finerenone in kidney disease and type 2 diabetes, New Engl. J. Med. 385 (24) (2021) 2252–2263, https://doi.org/10.1056/NEJMoa2110956.
[36] R. Agarwal, G. Filippatos, B. Pitt, S.D. Anker, P. Rossing, A. Joseph, P. Kolkhof, C. Nowack, M. Gebel, L.M. Ruilope, G.L. Bakris, FIDELIO-DKD and FIGARO-DKD investigators, Cardiovascular and kidney outcomes with finerenone in patients with type 2 diabetes and chronic kidney disease: the FIDELITY pooled analysis, Eur. Heart J. 43 (6) (2022) 474–484, https://doi.org/10.1093/eurheartj/ehab777.
[37] R. González-Blázquez, B. Somoza, M. Gil-Ortega, M. Martín Ramos, D. Ramiro-Cortijo, E. Vega-Martín, A. Schulz, L.M. Ruilope, P. Kolkhof, R. Kreutz, M. S. Fernández-Alfonso, Finerenone attenuates endothelial dysfunction and albuminuria in a chronic kidney disease model by a reduction in oxidative stress, Front. Pharmacol. 9 (2018) 1131, https://doi.org/10.3389/fphar.2018.01131.
[38] L. Herlan, J. Unland, S. Langer, L. Schulte, S. Schütten, C.F. García-Prieto, P. Kossmehl, M.S. Fernández-Alfonso, A. Schulz, R. Kreutz, Development of progressive albuminuria in male Munich Wistar Frömter rats is androgen dependent, Physiol. Genom. 47 (7) (2015) 281–289, https://doi.org/10.1152/physiolgenomics.00008.2015.
[39] N. Percie du Sert, V. Hurst, A. Ahluwalia, S. Alam, M.T. Avey, M. Baker, W. J. Browne, A. Clark, I.C. Cuthill, U. Dirnagl, M. Emerson, P. Garner, S.T. Holgate, D.W. Howells, N.A. Karp, S.E. Lazic, K. Lidster, C.J. MacCallum, M. Macleod, E. J. Pearl, H. Würbel, The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, PLoS Biol. 18 (7) (2020), e3000410, https://doi.org/10.1371/journal.pbio.3000410.
[40] Ó.J. Pellicer-Valero, G.A. Massaro, A.G. Casanova, M. Paniagua-Sancho, I. Fuentes-Calvo, M. Harvat, J.D. Martín-Guerrero, C. Martínez-Salgado, F.J. López-Hernández, Neural network-based calculator for rat glomerular filtration rate, Biomedicines 10 (3) (2022) 610, https://doi.org/10.3390/biomedicines10030610.
[41] C. Steireif, C.F. García-Prieto, G. Ruiz-Hurtado, H. Pulido-Olmo, I. Aranguez, M. Gil-Ortega, B. Somoza, G. Schönfelder, A. Schulz, M.S. Fernández-Alfonso, R. Kreutz, Dissecting the genetic predisposition to albuminuria and endothelial dysfunction in a genetic rat model, J. Hypertens. 31 (11) (2013) 2203–2212, https://doi.org/10.1097/HJH.0b013e3283642384.
[42] Y. Alvarez, A.M. Briones, G. Balfagón, M.J. Alonso, M. Salaices, Hypertension increases the participation of vasoconstrictor prostanoids from cyclooxygenase-2 in phenylephrine responses, J. Hypertens. 23 (4) (2005) 767–777, https://doi.org/10.1097/01.hjh.0000163145.12707.63.
[43] Y. Shi, P.M. Vanhoutte, Oxidative stress and COX cause hyper-responsiveness in vascular smooth muscle of the femoral artery from diabetic rats, Br. J. Pharmacol. 154 (3) (2008) 639–651, https://doi.org/10.1038/bjp.2008.110.
[44] D. Patoulias, C. Papadopoulos, A. Karagiannis, V. Vassilikos, M. Doumas, Cardiovascular outcomes with finerenone according to glycemic status at baseline and prior treatment with newer antidiabetics among patients with type 2 diabetes mellitus, Endocrinol. Metab. (Seoul., Korea) 37 (1) (2022) 170–174, https://doi.org/10.3803/EnM.2021.1296.
[45] P. Rossing, E. Burgess, R. Agarwal, S.D. Anker, G. Filippatos, B. Pitt, L.M. Ruilope, P. Gillard, R.J. MacIsaac, J. Wainstein, A. Joseph, M. Brinker, L. Roessig, C. Scott, G.L. Bakris, FIDELIO-DKD Investigators, Finerenone in patients with chronic kidney disease and type 2 diabetes according to baseline HbA1c and insulin use: an analysis from the FIDELIO-DKD study, Diabetes care 45 (4) (2022) 888–897, https://doi.org/10.2337/dc21-1944.
[46] Y. Liu, Cellular and molecular mechanisms of renal fibrosis, Nat. Rev. Nephrol. 7 (12) (2011) 684–696, https://doi.org/10.1038/nrneph.2011.149.
[47] M.R. Boon, G. van der Horst, G. van der Pluijm, J.T. Tamsma, J.W. Smit, P. C. Rensen, Bone morphogenetic protein 7: a broad-spectrum growth factor with multiple target therapeutic potency, Cytokine Growth Factor Rev. 22 (4) (2011) 221–229, https://doi.org/10.1016/j.cytogfr.2011.08.001.
[48] M.G. Wong, V. Perkovic, M. Woodward, J. Chalmers, Q. Li, G.S. Hillis, D. Yaghobian Azari, M. Jun, N. Poulter, P. Hamet, B. Williams, B. Neal, G. Mancia, M. Cooper, C.A. Pollock, Circulating bone morphogenetic protein-7 and transforming growth factor-β1 are better predictors of renal end points in patients with type 2 diabetes mellitus, Kidney Int. 83 (2) (2013) 278–284, https://doi.org/10.1038/ki.2012.383.
[49] F.E. Ramos-Alves, D.B. de Queiroz, J. Santos-Rocha, G.P. Duarte, F.E. Xavier, Increased cyclooxygenase-2-derived prostanoids contributes to the hyperreactivity to noradrenaline in mesenteric resistance arteries from offspring of diabetic rats, PloS One 7 (11) (2012), e50593.
[50] R.P. Brandes, K. Schröder, Differential vascular functions of Nox family NADPH oxidases, Curr. Opin. Lipidol. 19 (5) (2008) 513–518, https://doi.org/10.1097/MOL.0b013e32830c91e3.
[51] M.E. Armitage, K. Wingler, H.H. Schmidt, M. La, Translating the oxidative stress hypothesis into the clinic: NOX versus NOS, J. Mol. Med. (Berl., Ger.) 87 (11) (2009) 1071–1076, https://doi.org/10.1007/s00109-009-0544-2.
[52] M. Christ, J. Bauersachs, C. Liebetrau, M. Heck, A. Günther, M. Wehling, Glucose increases endothelial-dependent superoxide formation in coronary arteries by NAD (P)H oxidase activation: attenuation by the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor atorvastatin, Diabetes 51 (8) (2002) 2648–2652, https://doi.org/10.2337/diabetes.51.8.2648.
[53] H. Langbein, C. Brunssen, A. Hofmann, P. Cimalla, M. Brux, S.R. Bornstein, A. Deussen, E. Koch, H. Morawietz, NADPH oxidase 4 protects against development of endothelial dysfunction and atherosclerosis in LDL receptor deficient mice, Eur. Heart J. 37 (22) (2016) 1753–1761, https://doi.org/10.1093/eurheartj/ehv564.
[54] N.C. Edwards, R.P. Steeds, P.M. Stewart, C.J. Ferro, J.N. Townend, Effect of spironolactone on left ventricular mass and aortic stiffness in early-stage chronic kidney disease: a randomized controlled trial, J. Am. Coll. Cardiol. 54 (6) (2009) 505–512, https://doi.org/10.1016/j.jacc.2009.03.066.
[55] M.S. Fernández-Alfonso, B. Somoza, D. Tsvetkov, A. Kuczmanski, M. Dashwood, M. Gil-Ortega, Role of perivascular adipose tissue in health and disease, Compr. Physiol. 8 (1) (2017) 23–59, https://doi.org/10.1002/cphy.c170004.
[56] M. Gil-Ortega, B. Somoza, Y. Huang, M. Gollasch, M.S. Fernández-Alfonso, Regional differences in perivascular adipose tissue impacting vascular homeostasis, Trends Endocrinol. Metab.: TEM 26 (7) (2015) 367–375, https://doi.org/10.1016/j.tem.2015.04.003.
[57] O. Lamacchia, V. Nicastro, D. Camarchio, U. Valente, R. Grisorio, L. Gesualdo, M. Cignarelli, Para- and perirenal fat thickness is an independent predictor of chronic kidney disease, increased renal resistance index and hyperuricaemia in type-2 diabetic patients, Nephrol., Dial., Transplant.: Off. Publ. Eur. Dial. Transpl. Assoc. - Eur. Ren. Assoc. 26 (3) (2011) 892–898, https://doi.org/10.1093/ndt/gfq522.
[58] X. Sun, F. Han, W. Miao, N. Hou, Z. Cao, G. Zhang, Sonographic evaluation of para- and perirenal fat thickness is an independent predictor of early kidney damage in obese patients, Int. Urol. Nephrol. 45 (6) (2013) 1589–1595, https://doi.org/10.1007/s11255-013-0404-4.
[59] K. Droebner, M. Pavkovic, M. Grundmann, E. Hartmann, L. Goea, J. Nordlohne, J. Klar, F. Eitner, P. Kolkhof, Direct blood pressure-independent anti-fibrotic effects by the selective nonsteroidal mineralocorticoid receptor antagonist finerenone in progressive models of kidney fibrosis, Am. J. Nephrol. 52 (7) (2021) 588–601, https://doi.org/10.1159/000518254.
[60] G. Filippatos, S.D. Anker, R. Agarwal, B. Pitt, L.M. Ruilope, P. Rossing, P. Kolkhof, P. Schloemer, I. Tornus, A. Joseph, G.L. Bakris, FIDELIO-DKD Investigators, Finerenone and cardiovascular outcomes in patients with chronic kidney disease and type 2 diabetes, Circulation 143 (6) (2021) 540–552, https://doi.org/10.1161/CIRCULATIONAHA.120.051898.
[61] G.L. Bakris, R. Agarwal, J.C. Chan, M.E. Cooper, R.T. Gansevoort, H. Haller, G. Remuzzi, P. Rossing, R.E. Schmieder, C. Nowack, P. Kolkhof, A. Joseph, A. Pieper, N. Kimmeskamp-Kirschbaum, L.M. Ruilope, Mineralocorticoid Receptor Antagonist Tolerability Study–Diabetic Nephropathy (ARTS-DN) Study Group, Effect of finerenone on albuminuria in patients with diabetic nephropathy: a randomized clinical trial, JAMA 314 (9) (2015) 884–894, https://doi.org/10.1001/jama.2015.10081.