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Finerenone prevents renal damage by enhancing SDF-1α/CXCR4-mediated stem cell mobilization in experimental type 1 diabetic nephropathy

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ABSTRACT

Finerenone (FIN), a non-steroidal mineralocorticoid receptor antagonist, improves kidney and cardiovascular damage in type 1 diabetic (T1DM) Munich Wistar Frömter (MWF) rats with established chronic kidney disease (CKD). We tested whether renal protection involves stromal cell-derived factor 1 (SDF-1)/CXCR4 chemokine axis, a key regulator of tissue repair and stem cell mobilization. T1DM was induced in sixteen-week-old MWF by streptozotocin (15 mg/Kg, i.p.), combined with high fat/high sucrose (HF/HS) diet for 6 weeks (D). A second group (D-FIN) received FIN (10 mg/Kg/day) via the HF/HS diet. Non-diabetic MWF served as controls (C) (n = 11/group). Renal damage was evaluated by histology, RT-qPCR, ELISA, and zymography for matrix metalloproteinase (MMP) activity. Diabetic kidneys in group D showed enhanced glomerulosclerosis, interstitial inflammation, and elevated MMP-2 and MMP-9 activity. FIN treatment significantly reduced these changes, including tubular necrosis and collagen accumulation. *Timp-1*, *Timp-2* and *Pai-1* expression remained unchanged across groups. Notably, FIN upregulated SDF-1α and its receptor CXCR4, which are crucial for hematopoietic stem cell (HSC) migration. Conversely, SDF-1α (5–67), a truncated, non-functional form that impairs CXCR4 binding, was reduced with FIN. Immunofluorescence revealed co-localization of CXCR4 with CD34, an HSC marker, in the D-FIN group. We conclude that FIN mitigates diabetic kidney injury in MWF rats by promoting

Abbreviations: AFU, Arbitrary fluorescence units; AMC, 7-Amino-4-methylcoumarin; BSA, Bovine serum albumin; CD34, Cluster of differentiation 34; CKD, Chronic kidney disease; CXCR3, C-X-C chemokine receptor type 3; CXCR4, C-X-C chemokine receptor type 4; CXCR7, C-X-C chemokine receptor type 7; D, Diabetic group; D-FIN, Diabetic group treated with finerenone; DAPI, 4′,6-Diamidino-2-phenylindole; DKD, Diabetic kidney disease; DM, Diabetes mellitus; DPP-4, Dipeptidyl peptidase-4; ECM, Extracellular matrix; ELISA, Enzyme-linked immunosorbent assay; FIN, Finerenone; GFR, Glomerular filtration rate; H&E, Hematoxylin and eosin; HIF-1α, Hypoxia-inducible factor 1 alpha; HF/HS, High-fat/high-sucrose; HSC, Hematopoietic stem cell; I.p., Intraperitoneal; KIM-1, Kidney injury molecule-1; MMP, Matrix metalloproteinase; MR, Mineralocorticoid receptor; MRA, Mineralocorticoid receptor antagonist; MWF, Munich Wistar Frömter; NGAL, Neutrophil gelatinase-associated lipocalin; NOX-4, NADPH oxidase 4; PAI-1, Plasminogen activator inhibitor-1; PBS, Phosphate-buffered saline; PCT, Proximal convoluted tubule; RT-qPCR, Quantitative reverse transcription polymerase chain reaction; ROS, Reactive oxygen species; SDF-1α, Stromal cell-derived factor 1 alpha; SDF-1α 5–67, Truncated, non-functional form of SDF-1α; SOD-1, Superoxide dismutase 1; STZ, Streptozotocin; T1DM, Type 1 diabetes mellitus; TGF-β, Transforming growth factor beta; TIMP, Tissue inhibitor of metalloproteinases; VEGF, Vascular endothelial growth factor.

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HSC (CD34⁺) recruitment to the kidney. This is mediated through decreased MMP-2/9 activity, upregulation of the SDF-1α/CXCR4 axis, and reduced expression of the non-functional SDF-1α (5–67) form. These findings support a novel mechanism of FIN-induced renal protection involving stem cell mobilization.

1. Introduction

Chronic kidney disease (CKD) is a common progressive, incurable condition with high morbidity and mortality worldwide [1,2]. The global rise in obesity, metabolic syndrome and diabetes mellitus (DM) leads in parallel to an increasing prevalence of diabetic kidney disease (DKD), the most prevalent form of CKD and the major cause of end-stage kidney disease [3]. Additionally, there is a growing recognition of the interconnected pathophysiology among metabolic risk factors, such as obesity, diabetes, CKD, and cardiovascular disease, which has led to the development of a novel concept known as cardiovascular-kidney-metabolic syndrome [4].

The hallmark of progressive CKD and DKD is kidney fibrosis [5]. Inadequate extracellular matrix (ECM) turnover leads to fibrogenesis, glomerulosclerosis, interstitial chronic inflammation, tubular atrophy, and vascular rarefaction. Matrix metalloproteinases (MMPs) are key players in this deleterious remodelling during the progression of kidney damage [6,7]. MMPs are a large family of endopeptidases which degrade all components of the ECM [8–11] contributing to multiple general and pathological processes such as oxidative stress, inflammation, deleterious remodelling, and apoptosis [9,12]. Among MMPs, classified according to their structure and substrate specificity, MMP-2 and MMP-9 are gelatinases cleaving gelatine, elastin, collagens and other ECM proteins [13]. Moreover, MMP-2 and MMP-9 play critical immunomodulatory roles by proteolytically processing cytokines, chemokines, and their receptors, thereby altering cell signalling pathways by either activating, inactivating, or modulating cytokine and chemokine activities [14].

The stromal cell-derived factor-1 α (SDF-1α) pathway plays an essential role kidney development and kidney homeostasis [15]. SDF-1α is upregulated in response to injury, tissular stress, or inflammation [16]. Its biological activity is primarily mediated through binding with high affinity to its cognate G protein–coupled receptor, CXCR4, mediating angiogenesis and stem cell migration, chemotaxis, apoptosis, proliferation, survival and differentiation [17]. Activation of the SDF-1α /CXCR4 axis recruits immune cells toward sites of injury and contributes to stem cell homing [18]. In the kidney, SDF-1/CXCR4 exerts a protective role [15] preserving microvascular integrity and preventing renal fibrosis [19]. However, evidence in neurons shows that SDF-1α cleavage at its N-terminus by MMP-2 and MMP-9, among other proteases, generates truncated forms, such as SDF-1α (5−67), that reduce its affinity to CXCR4. This proteolytic processing shifts SDF-1α signalling from pro-survival pathways toward deleterious effects [20–22]. We propose that this cleavage also takes place in the kidney and that restoration of this pathway with either novel or existing therapies, may attenuate renal decline in CKD [19].

Finerenone (FIN) is highly selective mineralocorticoid receptor (MR) antagonist (MRA), showing stronger selectivity than for any other steroid hormone receptor. Unlike steroidal MRA, e.g. spironolactone and eplerenone, finerenone acts as a bulky antagonist, preventing the recruitment of transcriptional cofactors that trigger genes linked to cardiorenal damage including inflammation and fibrosis [23,24]. In terms of blocking the MR, finerenone is more potent than eplerenone, at least as potent as spironolactone, and more selective than both [23]. There is robust evidence of both kidney and cardiovascular protection with FIN in patients with a broad spectrum of CKD and type 2 DM (T2DM) [25–27]. However, the potential benefit of FIN in patients with type 1 DM (T1DM) remains to be determined [28]. In this context, our group has induced T1DM in Munich Wistar Frömter (MWF) rat, a genetic model of spontaneous and progressive non-diabetic albuminuria development that mirrors several features observed in CKD patients [29–31]. In this model, FIN has demonstrated a protective effect on the progression of kidney and cardiovascular damage through the reduction of blood pressure, arterial stiffness, and kidney damage markers, as well as diabetes-induced upregulation of proinflammatory, profibrotic, and procalcifying factors in perivascular and perirenal adipose tissue. Interestingly, these beneficial effects of FIN were observed regardless of glycaemic status or other metabolic features associated with diabetes [31].

Since FIN reduces MMP-2 and MMP-9 activity in non-diabetic MWF, we hypothesized that FIN treatment prevents the progression of kidney damage in T1DM MWF rats through i) inhibition of MMP-2 and MMP-9 activities, ii) decrease of SDF-1α (5−67), ii) restoration of the SDF-1/CXCR4 axis and iii) mobilization of stem cells for kidney repair.

2. Material and methods

2.1. Animals and diabetes induction

Sixteen-week-old male MWF rats (Charité, University Medicine Berlin, Germany; n = 24) were housed in individual cages under controlled dark-light cycles (12 h/12 h), temperature conditions, and free access to food and water during the study. Animals were randomly divided into three groups: control (C), diabetic (D) and diabetic treated with FIN (D-FIN). T1DM was induced as previously described [31]. Briefly, MWF rats injected with streptozotocin (STZ 15 mg/kg, i.p.) and were fed a high-fat high-sucrose diet (HF/HS; 45 % fat (61 % saturated; 30 % monounsaturated; 9 % polyunsaturated) and 34 % sucrose; TD08811 ENVIGO, Barcelona, Spain) for 6 weeks without (D; n = 7) or with FIN (D-FIN; 10 mg/kg/day; n = 6) C rats (n = 11) fed standard chow received equivalent vehicle (citrate buffer 0.1 M, pH=4.5) injections as their littermates. Thereafter, animals were anesthetized with i.p. administration of ketamine hydrochloride (80 mg/kg) and xylazine hydrochloride (12 mg/kg) and euthanized by exsanguination. The right kidney was removed and fixed in 4 % paraformaldehyde for histological studies, whereas the left kidney was frozen for protein and mRNA determinations.

All experimental procedures were performed at Instituto Pluridisciplinar and approved by its animal facility (ES-280790000086), as well as by the Institutional Animal Care and Use Committee of the Comunidad de Madrid (PROEX 205/18, approved December 2018 and PROEX_095.3/23, approved in March 2023) in accordance with the guidelines for the ethical care of experimental animals of the European Community. Every effort was made to avoid animal suffering in accordance with the ARRIVE guidelines [32].

2.2. Histology and morphometric analysis

Briefly, kidneys were fixed in 4 % neutral-buffered paraformaldehyde (4 % PFA) for 24 h, cut into 4–5 μm thick sections, stained with standard protocols for haematoxylin and eosin or Masson's trichrome (MT), and scanned into high-resolution digital images. Morphometric analyses included quantification of glomerular tuft and Bowman's space areas, estimation of glomerular volume, and scoring of interstitial inflammation and glomerulosclerosis. Collagen content and tubulo-interstitial changes were assessed through digital image analysis, using mean intensity and area fraction of positive staining in selected regions [33, 34] (Supplementary Methods).

2.3. Immunofluorescence

Briefly, kidney slices were deparaffinized and hydrated (standard protocol). Antigen retrieval was performed with 10 mM Tris buffer (pH 9), followed by permeabilization, blocking with 3 % hydrogen peroxide and goat serum, and overnight incubation with primary antibodies at 4°C; fluorescent secondary antibodies (1:500) were then applied, and sections were mounted with Fluoromount-G with DAPI for fluorescence microscopy (Supplementary Methods)

2.3.1. Determination of MMP-2 and MMP-9 activities by gelatine zymography

Kidneys were homogenized with PBS 1x and 10 μg per well subjected to SDS/PAGE in polyacrylamide gels containing 0.1 % gelatine. Subsequently, gels were renatured and incubated for 24 h at 37 ºC. The colour of the zymography gels was inverted and transformed to a grey-scale image before densitometric analysis. Gelatine zymographies were quantified using absorbance values by ImageJ software.

2.3.2. ELISA for SDF-1α, SDF-1α-(5−67) and CXCR4 protein levels

SDF-1α protein levels were measured by ELISA in homogenates from rat kidneys, following kit guidelines from Antibodies.com laboratory (A77295, Antibodies, Stockholm, Sweden) that employs the sandwich enzyme immunoassay technique for the quantitative measurement of rat SDF-1α. SDF-1α-(5−67) levels were measured using a specific polyclonal antibody (Cristopher Overall Lab) under reducing conditions (Supplementary Methods). For CXCR4 protein estimation by ELISA, we followed the same procedure with primary CXCR4 antibody: 20 μg/mL 24 h (o/n). Anti rabbit secondary antibody: 1:300, 2 h at room temperature and developed following the same abovementioned procedure.

2.4. Determination of caspase-3 activity

Briefly, Caspase-3 activity was measured in 20 μL of kidney extract using a fluorogenic substrate (Ac-DEVD-AMC) in assay buffer at 37 °C for 2–6 h. Released AMC was detected with a spectrofluorometer (excitation 360 nm/emission 460 nm), and activity was expressed as AFU/h/μg protein. Protein concentration was determined using the BioRad Protein Assay with BSA as standard [35] (Supplementary Methods).

2.5. Estimation of superoxide dismutase (SOD-1) activity

Superoxide dismutase activity was measured using the Kono method [36] by monitoring the inhibition of hydroxylamine auto-oxidation in kidney extracts, with absorbance changes at 560 nm recorded over 2 min [37] (Supplementary Methods).

2.6. Nitrite determination

Nitrites were evaluated by a modified protocol [38] incubating kidney extract with 2,3-diaminonaphthalene (1 mg/mL in 0.6 M HCl) for 15 min, stopping the reaction with NaOH, and detecting fluorescence at 340/460 nm; results were expressed as % of pmol/mg tissue.

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

Briefly, total RNA was extracted from kidney tissue using Trizol, quantified with NanoDrop, reverse-transcribed (500 ng) with PrimeScript RT kit, and analyzed by RT-qPCR using SYBR Green, intron-skipping primers (Table 1), and Gapdh and Atpaf-1 as housekeeping genes (Supplementary material).

2.8. Statistical analysis

Continuous 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. Categorical variables were compared using Fisher's exact test. Correlations were calculated using Pearson's correlation coefficient. Data analysis was performed with GraphPad Prism 8 and SPSS. Data are presented as mean ± standard error of the mean (SEM) and statistical significance was considered for $p < 0.05$.

3. Results

3.1. FIN treatment prevented glomerular and tubular damage in diabetic MWF

At 16 weeks of age, no significant differences were detected in body weight or fasting glucose among C, D, and D-FIN rats, indicating comparable baseline conditions (Table 2). By 22 weeks, both diabetic groups exhibited significantly higher body weight and fasting glucose levels compared with age-matched controls ($p < 0.05$), while FIN treatment did not alter these parameters. These findings confirm the development of diabetes and allow the subsequent evaluation of renal structural alterations and the potential protective effects of FIN on glomerular and tubular damage.

A detailed histological examination of the renal glomeruli (Fig. 1) showed that diabetes did not increase all histomorphological parameters analysed. Only the Bowman's space (Fig. 1C) and the glomerulosclerosis index (Fig. 1F) were significantly higher in the D group, indicating that control MWF at 22 weeks of age already exhibit a substantial histomorphological damage, which is not much markedly worsened by diabetes.

Compared to the D group, FIN treatment decreased all histomorphological parameters analyzed (Fig. 1A), i.e. the glomerular tuft

Table 1
Primer sequences.

Gene	Accesion number	Forward (5'-3')	Tm	Reverse (5'-3')	Tm	Product Length
Gadph	NM_017008.4	AAGGCTGAGAATGGGAAGCTG	60.34	CCATTTGATGTTAGCGGGATCT	58.52	81
Atpaf−1	NM_001107959.1	AAGATGACCCCAAGGCATTTTT	58.75	GATCTCTCCAAGAAGCTGCAAG	59.06	111
Kim−1	NM_173149.2	ATTGTTGCCGAGTGGAGAT	56.73	TGTGGTTGTGGGTCTTGTAGT	59.16	125
Ngal	NM_130741.1	GGCCGACACTGACTACGACC	62.54	GCCCCTTGGTTCTTCCGTAC	60.68	101
Timp−1	NM_053819.1	TAAAGCCTGTAGCTGTGCCC	60.04	AGCGTCGAATCCTTTGAGCA	60.04	160
Timp−2	NM_021989.3	CACCCGCAACAGGCGTT	60.99	GGAATCCACCTCCTTCTCGC	60.18	75
Pai−1	NM_012620.2	CATCCTGGAACTGCCCTACC	59.82	TCAGTCATGCCCAGCTTCTC	59.75	225
Nox−2	NM_023965.2	CTTCCTCAGGGGTTCCAGTG	59.67	TTCACACACCACTCCACGTT	59.75	153
Nox−4	NM_031530.1	TCACAGTTGCTGCCTGTAG	60.07	ATGTACTTCTGGACCCATTCC	58.84	111
Sdf−1α	NM_001033882.1	GATGCCCCTGCCGATTCTTT	60.75	GCACACTTGTCTGTTGTTGCT	59.87	128
Cxcr4	NM_022205.3	CCTCGCCTTCTTCCACTGTT	59.96	AAGAGTGTCCACCCCGTTTC	59.89	149
Cxcr3	NM_053415.1	CACCTGCATTGTTGTGTGGG	59.97	GAAGGGGCATCAGGAAACCA	59.96	176
Cxcr7	NM_053352.2	ACCTGGGAACTACTCGGACA	59.89	ACCACGGAGTTGGCAATCAT	59.96	165
Hif−1α	NM_024359.2	GAAACTCCAAAGCCACTTCG	57.31	CTGGCTGATCTTGAATCTGG	55.90	127

Table 2
Body weight and glycemic parameters.

	16 weeks old			22 weeks old		
	C	D	D-FIN	C	D	D-FIN
Weight (g)	386,2 ± 5,5	396,7 ± 8.2	397,8 ± 9.5	400.3 ± 33.2	433.6 ± 14.4*	424.3 ± 23.8*
Fasting glucose (mg/dL)	122.0 ± 5.6	117.0 ± 3.1	112.2 ± 4.6	125.0 ± 3.9	243.0 ± 28.0*	259.0 ± 56.0*

Results are shown as mean ± SEM. *p < 0.05 compared to C 22 weeks old.

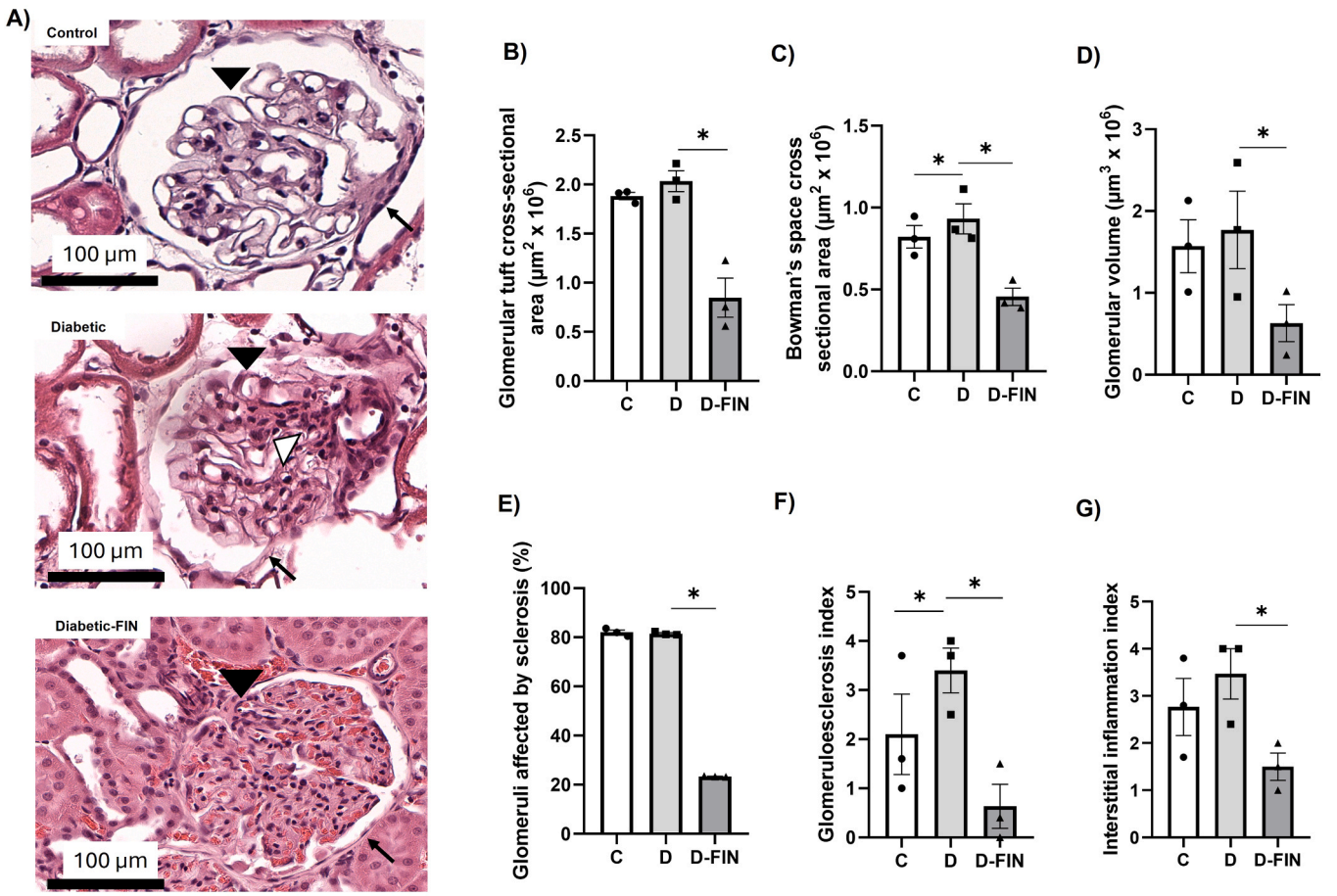

Fig. 1. Effect of FIN on glomerulosclerosis and interstitial inflammation. (A) Representative microphotographs of kidney slices in C (upper panel), D (middle panel) and D-FIN (lower panel) rats. Haematoxylin eosin staining at original magnification 20X. (Upper panel): dilatation of capillaries (black triangle) and a segment of sclerosis (arrow) in C rats. (Middle panel): mesangial cell proliferation (white triangle), dilatation of capillaries forming microaneurysms (black triangle) and a segment of sclerosis (arrow) in D rats. (Lower panel): restoration of capillary diameter (black triangle) in D-FIN rats. (B) Glomerular tuft cross-sectional area, (C) Bowman's space cross sectional area, (D) glomerular volume, (E) percentage of glomeruli affected by sclerosis, (F) glomerulosclerosis index, and (G) interstitial inflammation index in C, D and D-FIN rats. Results are expressed as media ± SEM, n = (3), *p < 0.05 vs D.

(Fig. 1B), the Bowman's space (Fig. 1C), the glomerular volume (Fig. 1D), the percentage of glomeruli affected by sclerosis (Fig. 1E), glomerulosclerosis index (Fig. 1F) and interstitial inflammation index (Fig. 1G). This is in accordance with the reduced proteinuria shown by the D-FIN group, as previously described [31].

Diabetes induced necrosis in the tubuli (Fig. 2A) and collagen density (Fig. 2B). Both parameters were significantly lower in kidneys of D-FIN rats, in accordance with the lower values of kidney damage markers, *Kim-1* and *Ngal*, previously described [31].

3.2. MMP-2 and MMP-9 activities were reduced by FIN treatment

Both MMP-2 and MMP-9 activities were higher ($p < 0.05$) in kidneys of D compared to C rats and were significantly decreased by FIN treatment (Fig. 3A-C). To note, activity of the inducible MMP-9 was completely blunted in the FIN group (Fig. 3A and C).

Changes in levels of TIMP-2 and TIMP-1 which directly inhibit the catalytic domain through a 1:1 stoichiometry, are critical mechanisms controlling MMP-2 y MMP-9 activities, respectively [39]. Both *Timp-2* (Fig. 3D) and *Timp-1* (Fig. 3E) expression were, however, not significantly affected by either diabetes induction or FIN treatment, since similar TIMP levels were detected in all three groups. Expression of *Pai-1*, an indirect activator of pro-MMPs via proteolytic cleavage of MMPs [40], was similar between groups (Fig. 3F).

3.3. FIN reduces oxidative stress and apoptosis markers

SOD-1 activity was higher in kidney of D as compared to C rats ($p < 0.05$) and further increased by FIN treatment (Fig. 4A), whereas nitrite levels were slightly higher in the D-FIN group (Fig. 4B). The renoprotective *Nox-4* [41] was significantly higher in D and no further modified by FIN (Fig. 4C). Caspase-3 activity, a marker of apoptosis

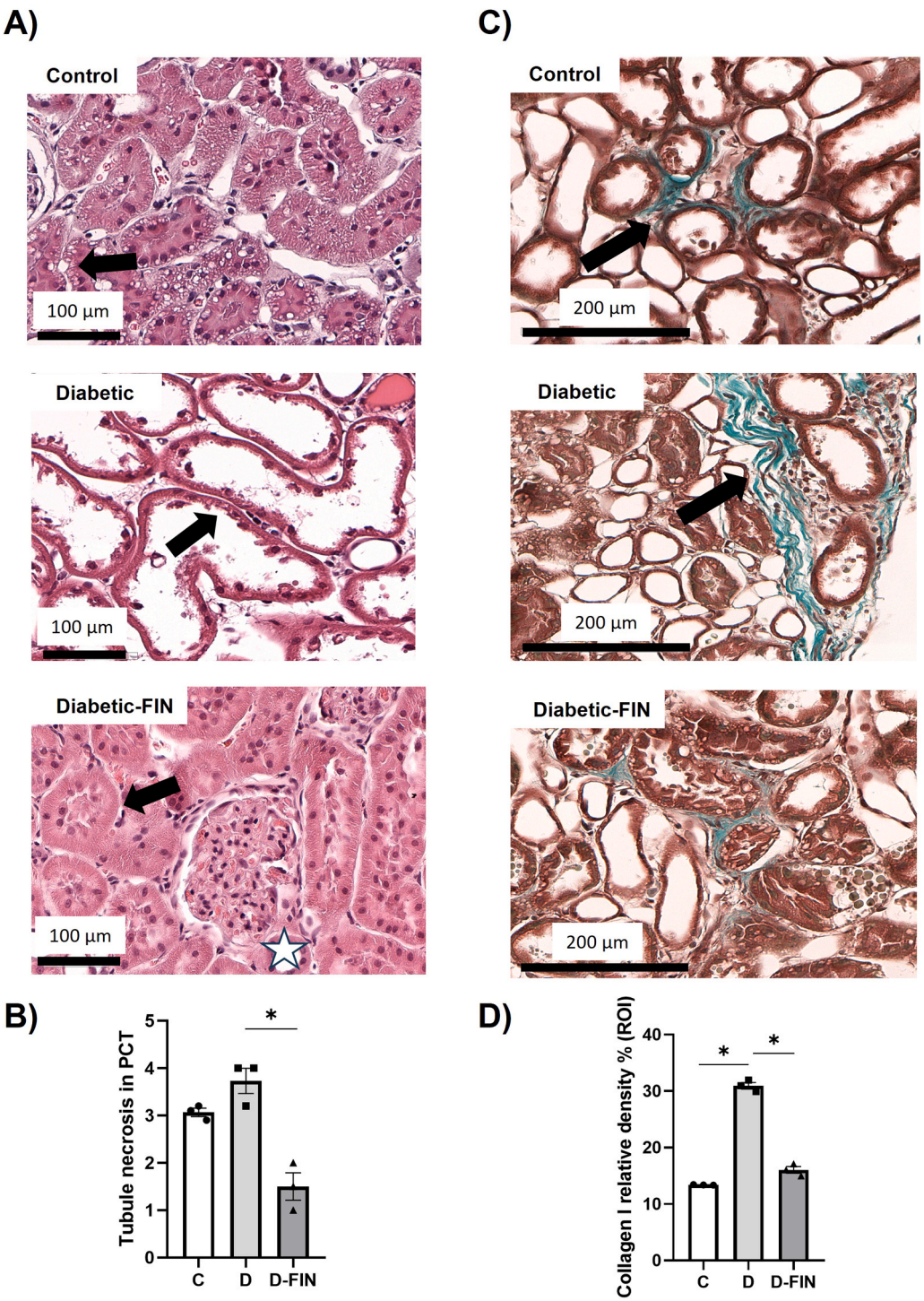

Fig. 2. Effect of FIN on necrosis and collagen. Representative microphotographs of kidney slices in C (upper panel), D (middle panel) and D-FIN rats (lower panel) and their quantification (A) Haematoxylin eosin staining at original magnification 20X. Tubule necrosis in proximal convoluted tubule (PCT) in C, D and D-FIN rats. Upper panel: presence of cytoplasmic vacuoles (arrow) that indicate cellular degeneration, therefore, this is a sign of initial necrosis, indicative of moderate necrosis. Middel panel: necrotic progression of the renal tubules, presence of free cells in the lumen (arrow), as well as (white star) attenuation of the brush border. Lower panel: D-FIN Increased brush border of the renal tubules, absence of vacuoles. Indicative of tissue restoration, and absence of necrotic markers. (B) Masson staining (200) for collagen fibre detection of C, D and D-FIN rats. (D) Collagen relative density % in C, D and D-FIN rats. Results are expressed as media ± SEM, n = (3), *$p < 0.05$ vs D.

[42], was elevated in D compared to C rats, while FIN prevented its upregulation ($p < 0.05$).

3.4. Effect of FIN on SDF-1α level

HIF-1α, a regulator of the expression of SDF-1α and its receptor CXCR4 [43], was upregulated by diabetes and significantly reduced by FIN treatment (Fig. 5A). Renal *Sdf-1α* mRNA expression and SDF-1α

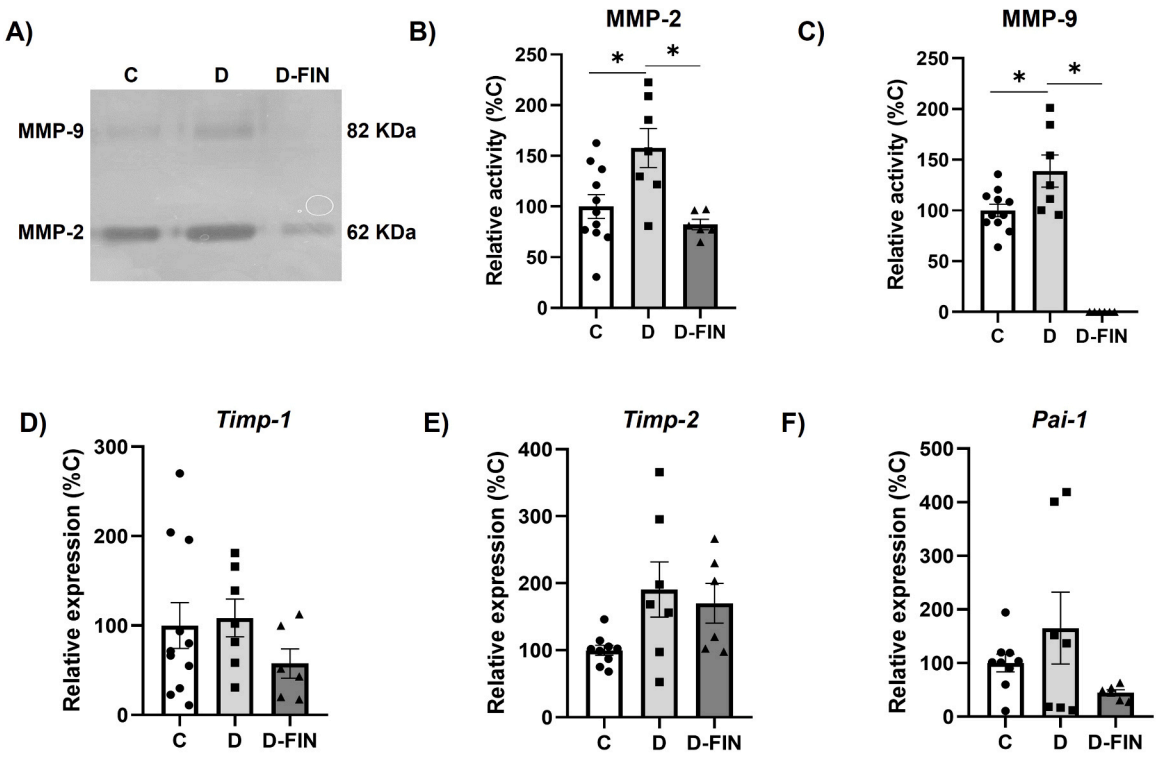

Fig. 3. Effect of FIN treatment on MMP-2 and MMP-9 activity. (A) Representative gel zymography of MMP-2 and MMP-9 activities in C, D and D-FIN rats. Quantification of (B) MMP-2 and (C) MMP-9 activity. (D) *Timp-1*, (E) *Timp-2* and (F) *Pai-1* relative expression in C, D and D-FIN rats. Results are expressed as media ± SEM, n = (6−11), *p < 0.05 vs D.

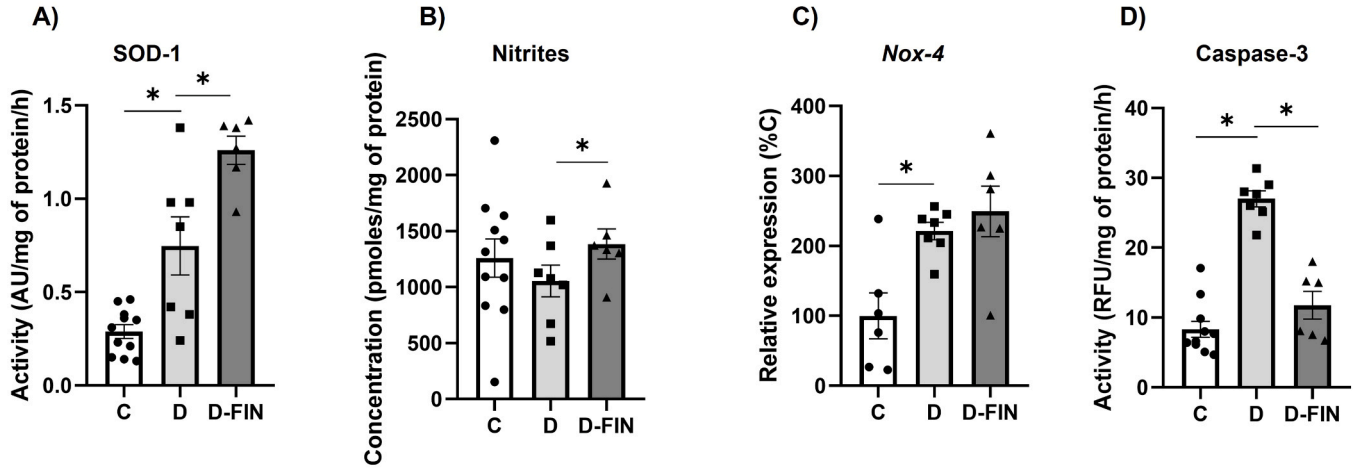

Fig. 4. Effect of FIN on oxidative stress. (A) SOD-1 activity, (B) nitrite level. (C) *Nox-4* expression, and (D) caspase-3 activity in C, D and D-FIN rats. Results are expressed as media ± SEM, n = (6−11), *p < 0.05 vs D.

protein levels were upregulated in the D group compared to C (Fig. 5B and C). In contrast, SDF-1α protein levels were higher in D-FIN kidneys, despite no changes occurred in *Sdf-1α* mRNA expression levels with FIN (Fig. 5B and C). This suggests that increased SDF-1α protein levels in the D do not depend only on the expression, but also on the changes in SDF-1α protein amount, maybe due to proteolytic cleavage to SDF-1α (5−67). Indeed, the level of truncated SDF-1α (5−67) was not modified by diabetes but was significantly lower in the FIN group (Fig. 5D). The ratio SDF-1α/SDF-1α (5−67) clearly shown an upregulation by diabetes and a further increase induced by FIN treatment (Fig. 5E).

Since previous findings in neurons show that MMP-2 and MMP-9 cleave full-length SDF-1α generating biologically active SDF-1α (5–67) [20–22,44], we performed a correlation analysis to assess whether renal levels of SDF-1α (5–67) were associated with MMP-2 and MMP-9 activity. Both MMP-2 (Fig. 5F) and MMP-9 (Fig. 5G) activities positively correlated with renal SDF-1α (5–67) levels.

3.5. Effect of FIN on CXCR4, CXCR7 and CXCR3 receptors

Increased renal *Cxcr4* expression was observed not only in D compared to C rats, and also in D-FIN groups compared to D (Fig. 6A). ELISA data confirmed the enhanced CXCR4 protein levels by FIN treatment in D rats ($p < 0.05$) as compared to C rats ($p < 0.05$) without an effect of diabetes (Fig. 6B). CXCR7 is an alternative receptor for SDF-1α that mainly acts as a scavenger receptor for SDF-1 stimulating its internalisation [45]. Our results show that *Cxcr7* expressions was

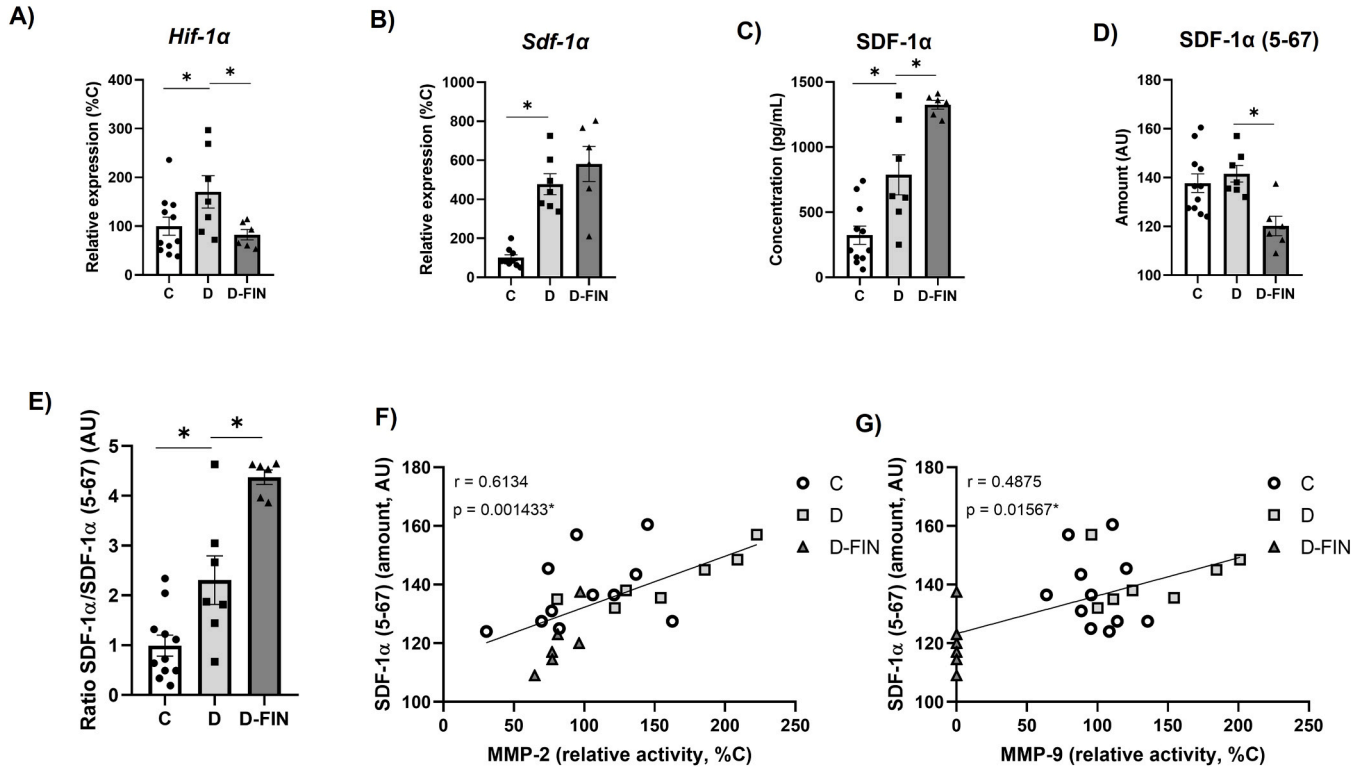

Fig. 5. Effect of FIN treatment on the Sdf-1α. Relative expression of (A) *HIF-1α* and (B) *Sdf-1α* in C, D and D-FIN rats. ELISA determination of (C) SDF-1α and (D) SDF-1α 5–67 in C, D and D-FIN rats. (E) Ratio between SDF-1α / SDF-1α 5–67 in C, D and D-FIN rats. Correlation between (F) MMP-2 activity and SDF-1α 5–67 protein, and (G) MMP-9 activity and SDF-1α 5–67 protein. Results are expressed as media ± SEM, n = (6−11), *p < 0.05 vs D.

significantly enhanced by diabetes ($p < 0.05$) compared to C rats ($p = 0.059$), without any effect of FIN treatment (*Cxcr7*: p = 0,3660) (Fig. 6C). A positive correlation was found between *Cxcr7* and *Cxcr4* expression (Fig. 6D). Moreover, *Sdf-1α* expression levels correlated with both *Cxcr4* (Fig. 6E) and *Cxcr7* (Fig. 6F).

CXCR3 is another alternative receptor, which binds less efficiently to SDF-1α but preferentially to SDF-1α (5–67) [22]. Expressions of *Cxcr3* was significantly enhanced by diabetes ($p < 0.05$) compared to C rats ($p < 0.059$), without any effect of FIN treatment (*Cxcr3*: $p = 0.6679$).

3.6. Effect of FIN on immunofluorescence for CXCR4 and CD34 in the kidney

Immunostaining showed CXCR4 in tubular cells of all groups (Fig. 6I) without staining of the glomeruli. CXCR4 staining was lower in the C and D group, and it was increased in D-FIN groups. The tubules of C and D rat kidneys exhibited a low immunofluorescence for CD34, a marker of hematopoietic stem cells (HSC), while FIN markedly increased CD34 immunofluorescence in the kidney of diabetic rats. There was a colocalization of CXCR4 with CD34 in the D-FIN group.

4. Discussion

DKD is the most prevalent form of CKD and the primary cause of end-stage kidney disease [1]. Despite significant progress in recent years, a broader understanding of the pathogenic mechanisms underlying DKD remains necessary to develop more effective therapies. This study demonstrates a dysregulation of the SDF-1α/CXCR4 axis in MWF diabetic rats, characterized by elevated levels of SDF-1α (5−67) and increased activity of MMP-2 and MMP-9, which are associated with kidney damage. These pathological alterations were effectively prevented by FIN treatment, which led to the downregulation of MMP-2 and MMP-9, upregulation of the SDF-1α/CXCR4 axis, and mobilization of hematopoietic stem cells to the kidney, limiting fibrosis, inflammation, and tubular necrosis. Collectively, these findings support a renoprotective role for FIN in MWF rats with T1DM, i.e. in type 1 diabetic nephropathy, independent of effects on glucose homeostasis.

The glomerular and tubular damage observed in the MWF diabetic group is in accordance with the increased expression of kidney injury marker (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), and collagen I previously described in this model [31]. Moreover, both MMP-2 and MMP-9 activities are markedly increased. This is consistent with previous studies showing MMP-2 and MMP-9 overactivation in kidney of diabetic animal models [46–49]. The observed overactivation of MMP-2 and MMP-9 in diabetic MWF rats appears to occur independently of alterations in PAI-1 expression—an indirect activator of pro-MMPs via proteolytic cleavage—as previously described by [40]. Similarly, levels of TIMP-1 and TIMP-2, key endogenous inhibitors that bind and neutralize MMP catalytic activity [39], remain unaltered, suggesting a dysregulation of MMP activity that is not driven by canonical modulators. Instead, this imbalance may result from excessive generation of reactive oxygen species (ROS) under hyperglycemic conditions, a well-established trigger for enhanced MMP activity and disruption of the MMP/TIMP equilibrium [50]. While our study did not directly quantify oxidative stress markers, previous reports indicate elevated ROS even in non-diabetic MWF kidneys [51], implying a predisposing redox imbalance. In this context, the observed upregulation of SOD-1 and NOX-4 in diabetic MWF rats may reflect a compensatory but ultimately insufficient antioxidant response. Both enzymes are involved in the production of hydrogen peroxide, a signaling molecule capable of activating protective pathways under moderate oxidative conditions [52,53]. However, the concomitant elevation of caspase-3 levels, an executor of apoptosis, suggests that oxidative stress surpasses protective thresholds, leading to increased renal cell death and exacerbation of kidney injury. The above-mentioned findings indicate that dysregulated MMP activities play a significant role in the development and

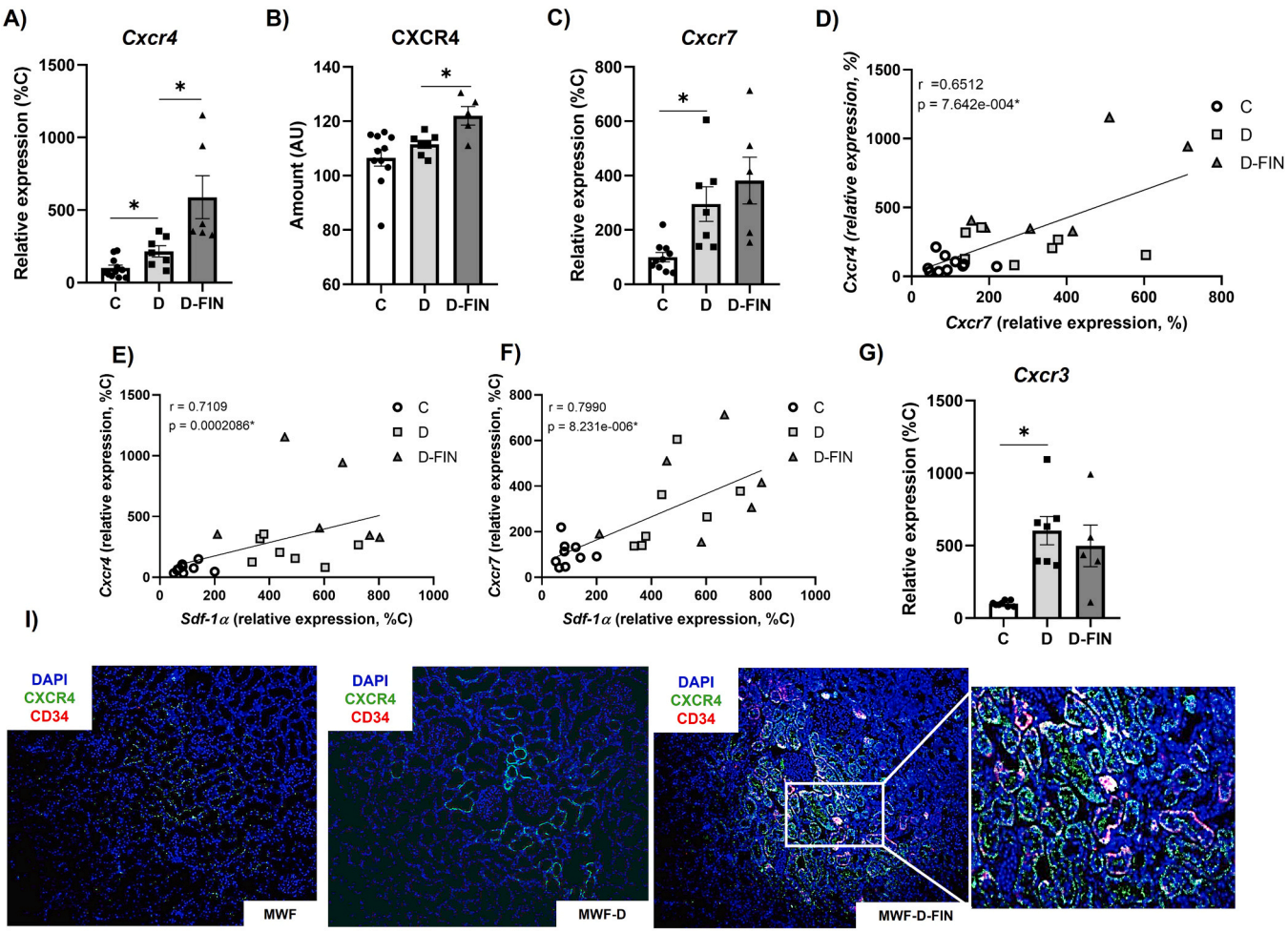

Fig. 6. Effect of FIN treatment on CXCR4, CXCR7 and CXCR3 receptors. (A) *Cxcr4* relative expression, (B) CXCR4 protein, and (C) *Cxcr7* relative expression in C, D and D-FIN rats. Correlation between (D) *Cxcr7* and *Cxcr4* relative expression, (E) *Sdf-1α* and *Cxcr4* relative expression, and (F) *Sdf-1α* and *Cxcr7* relative expression. (G) *Cxcr3* relative expression in C, D and D-FIN rats. Results are expressed as media ± SEM, n = (6−11), *p < 0.05. (I) Immunofluorescence of representative kidney from C, D and D-FIN stained with specific antibody for CXCR4 (green), CD34 (red) and DAPI to visualize the nuclei (blue). Results are expressed as media ± SEM, n = (6−11), *p < 0.05 vs D.

progression of diabetic kidney damage, making MMP-2 and MMP-9 therapeutic targets for managing this condition. Our findings show that FIN effectively suppresses inducible MMP-9 and reduces MMP-2 in diabetic MWF. The renoprotective effect of FIN is confirmed by the renal structural restoration and caspase-3 reduction, despite lack of further upregulation of NOX-4 expression.

Previous work has demonstrated that FIN effectively inhibits MMP-2 and MMP-9 activity in non-diabetic MWF rats, an effect associated with reduced oxidative stress and improved renal function, including decreased albuminuria [51]. These findings suggest that FIN's actions on matrix metalloproteinase regulation are not limited to diabetic conditions but may reflect a broader capacity to modulate proteolytic and redox pathways. Notably, other pharmacological agents known to attenuate MMP-2 and MMP-9 activity, such as the angiotensin II receptor blocker valsartan [46] and the PPARα agonist clofibrate [48], have also been associated with structural and functional improvements in diabetic nephropathy. These parallels reinforce the pathological significance of MMP dysregulation in diabetic kidney disease and highlight the therapeutic potential of MMP-targeting strategies. The consistency of renoprotective outcomes across diverse drug classes further supports the notion that modulating the MMP axis may serve as a convergent mechanism of kidney protection in the context of diabetes.

The protective effects of the CXCR4/SDF-1α axis in DKD may be mediated through multiple downstream signalling pathways [54,55]. Against this background, it appears surprising that CXCR4 immunostaining in the MWF group very low in tubular cells and even absent in glomeruli. The increase of CXCR4 immunoreactivity induced by FIN within tubular compartments, suggests activation of prosurvival signaling pathways involved in tissue repair. This is in agreement with the protective effect on tubular cells associated to CXCR4 activation in diabetic rats [56]. Since CXCR4 antagonism unmasked albuminuria and accelerated renal tubular epithelial cell death [56], the reported upregulation of CXCR4 levels in D-FIN rats suggests a renoprotective effect of FIN. Noteworthy, SDF-1α signalling includes the decoy receptor CXCR7, which terminates CXCR4 signalling through arrestin-mediated CXCR4 downregulation and endocytosis [57].

In this context, the elevated CXCR4 mRNA expression observed in diabetic MWF rats is not mirrored by a corresponding increase in CXCR4 protein levels, suggesting post-transcriptional regulation. One plausible mechanism involves the upregulation of CXCR7, an alternative receptor for SDF-1α, which can act as a decoy by promoting internalization and lysosomal degradation of CXCR4, thereby reducing its availability at the tubular epithelial cell membrane. Notably, CXCR7 expression is transcriptionally regulated by HIF-1α, a key hypoxia-responsive factor that is upregulated in diabetic MWF rats [58]. On the other hand, SDF-1α itself is known to be highly expressed in ischemic tissues, where it plays a critical role in guiding CXCR4-positive progenitor cells through oxygen-dependent chemotactic gradients. This axis is crucial for the

recruitment of endothelial progenitor cells to sites of renal injury [59]. Importantly, the SDF-1α promoter contains hypoxia-responsive elements, indicating that it is likely a downstream target of HIF-1α [43]. Thus, the coordinated regulation of CXCR7 and SDF-1α by hypoxia may contribute to the impaired CXCR4 signaling and limited regenerative cell recruitment observed in the diabetic kidney.

SDF-1α is upregulated in response to injury, tissular stress, or inflammation [16]. Interestingly, SDF-1α levels in neurons are regulated by amino-terminal cleavage by MMP-9 and MMP-2, generating a truncated form, SDF-1α (5−67) [20,21]. The positive correlation between renal MMP-9 and SDF-1α (5−67) suggests that a similar proteolytic mechanism might be active in the kidney. We observe that diabetes increases both SDF-1α mRNA and protein levels, whereas FIN significantly increases SDF-1α protein levels without modifying its expression. This suggests that FIN may preserve full-length SDF-1α by attenuating its cleavage into the truncated SDF-1α (5–67) form through suppression of MMP-2 and MMP-9 activity. Since SDF-1α (5–67) loses its ability to induce CXCR4 signalling [21], its elevation might occur at the expense of the regenerative capacity. Indeed, increased MMP-9, together with impaired CXCR4 activation, abolish prosurvival pathways in renal cells *in vitro* [60].

Further supporting the central role of MMP-9 in the pathogenesis of diabetic nephropathy, genetic deletion of MMP-9 has been shown to attenuate kidney injury by preserving podocyte structure and function and preventing their dedifferentiation [61]. In vitro evidence reinforces this mechanism: treatment of renal tubular cells with recombinant MMP-9 reduces CXCR4 activation under high-glucose conditions [56], suggesting that MMP-9-mediated proteolysis impairs SDF-1α–CXCR4 signaling. This aligns with our findings in diabetic MWF rats, where increased MMP-9 activity correlates with SDF-1α truncation and diminished CXCR4 responsiveness. However, despite FIN-mediated MMP-9 inhibition, a considerable amount of truncated SDF-1α (5–67) remains detectable in the D-FIN group, indicating the likely contribution of additional proteolytic pathways. Enzymes such as dipeptidyl peptidase-4 (DPP-4), MMP-1, MMP-3, cathepsin K, cathepsin G, and elastase have all been implicated in the truncation of SDF-1α [62]. Notably, cleavage of SDF-1α by DPP-4 not only reduces its chemotactic potential but also generates fragments that may act as endogenous agonists of CXCR3, potentially altering cell recruitment dynamics and inflammatory responses. These findings underscore the complexity of chemokine regulation in diabetic nephropathy and highlight the need for broader targeting of proteolytic pathways to fully restore SDF-1α–CXCR4 axis functionality. In neurons, binding of SDF-1α (5–67) to CXCR3 induces neurotoxicity [20,44,63,64]. CXCR3 has been detected in the kidney of renal fibrosis patients [65–67], and its activation promotes the recruitment of leukocyte and T cells into the kidney [65, 68–70]. Therefore, a potential contribution of CXCR3 overexpression to renal injury cannot be excluded in MWF-diabetic rats. In this context, SDF-1α (5−67) might bind to the upregulated CXCR3, thereby contributing to the deleterious effect via the SDF-1α (5−67)/CXCR3 axis. Although FIN does not modify CXCR3 expression, its beneficial effect may involve the reduction of SDF-1α (5−67). Additionally, FIN increases CXCR4 expression, shifting the balance in favour of the SDF-1α/CXCR4 axis. Therefore, the renoprotective effect of FIN is not only due to a reduction of SDF-1α (5−67), but also through an increase in SDF-1α and CXCR4 proteins.

SDF-1α is considered a potent chemoattractant and homing factor involved in tissue repair [71]. It enhances the mobilization of hematopoietic stem cells (HSC: CD34 positive) from the bone marrow following a chemotactic gradient to CXCR4-positive cells in the injured kidney [72]. SDF-1α-dependent gradients influence the number of circulating stem cells [73,74], whereas blockade of CXCR4 abolishes SDF-1α-induced migration of MSCs [75]. In this scenario, FIN enhanced CD34 + immunofluorescence, colocalizing with CXCR4 in renal tubules of diabetic MWF rats. CXCR4 is essential for HSC homing in the damaged kidney [17] and a prerequisite for promoting mobilization, survival, proliferation, and differentiation of HSC [76]. Our results suggest that FIN stimulates the mobilization and homing of CD34 + HSC into the damaged kidney for renal repair, as demonstrated by the histomorphological restoration in MWF-diabetic rats. The regenerative role of SDF-1α/CXCR4 axis has been confirmed by the recruitment of multiple stem cell populations into injured kidney [77–79].

5. Conclusion

In summary, our findings unveil a novel renoprotective mechanism of FIN in MWF rats with T1DM, centered on the preservation of the canonical SDF-1α/CXCR4 signaling axis. FIN treatment effectively reduces levels of the proinflammatory SDF-1α (5–67) fragment, primarily through the inhibition of MMP-2 and MMP-9 activity. This dual action not only limits pathological extracellular matrix remodeling and inflammation but also fosters prosurvival signaling and activates endogenous regenerative pathways. In particular, FIN promotes the mobilization of hematopoietic stem cells ($CD34^+$) to the kidney, supporting tissue repair and functional preservation. Importantly, these renoprotective effects occur independently of changes in glycemic control, underscoring FIN's potential as a disease-modifying therapy for diabetic kidney disease.

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CRediT authorship contribution statement

Fernández-Alfonso María S.: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Marta Sanz-Gómez:** Investigation, Data curation, Formal analysis, Methodology, Writing – review & editing. **Elvira Bragado-García:** Investigation, Conceptualization, Data curation, Formal analysis, Methodology, Resources, Writing – review & editing. **Merino Martin Jose Joaquin:** Conceptualization, Investigation, Supervision, Validation, Writing – original draft, Writing – review & editing. **Esther Durán-Mateos:** Investigation, Data curation, Methodology, Writing – review & editing. **Christopher Overall:** Conceptualization, Methodology, Resources, Writing – review & editing. **Paloma Palma-Guzmán:** Investigation, Conceptualization, Data curation, Formal analysis, Methodology, Resources, Writing – review & editing. **Isabel Aranguez:** Investigation, Methodology, Writing – review & editing. **Elisa Mercado-García:** Methodology, Writing – review & editing. **Gema Ruiz-Hurtado:** Investigation, Methodology, Resources, Writing – review & editing. **Reinhold Kreutz:** Conceptualization, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Adrián Plaza:** Conceptualization, Investigation, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors declare no competing interests except for R. Kreutz and M. S. Fernández-Alfonso. R. Kreutz reports consulting fees and honoraria from AstraZeneca, Bayer, Krka, Menarini, Merck, ProMed, Recor, Servier, and Zentiva, and participation on advisory boards for Krka, ProMed, Servier, and Zentiva. M.S. Fernández-Alfonso reports institutional support from Bayer AG for the work reported in this manuscript.

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Institutional Review Board Statement

The animal study protocol was approved by the Animal Research Committee of Complutense University (PROEX 205/18, approved December 10th, 2018).

Informed Consent Statement

Not applicable

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2025.118727.

Data availability

Data will be made available on request.

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