Amelioration of kidney inflammation and apoptotic cell death in an animal model of type 2 diabetes

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Introduction

Diabetic Nephropathy (DN), a progressive renal disease, affects approximately 20-40% of patients with type 2 diabetes mellitus, being the most common cause of end-stage renal disease. This pathology is characterized by excessive accumulation of extracellular matrix, thickening of glomerular basement membrane and cell hypertrophy, which ultimately progress to glomerulosclerosis and tubulointerstitial fibrosis. Increasing evidences suggest that hyperglycemia-induced inflammatory processes and apoptotic cell death play an important role in the development and progression of DN. Previous studies from our group have shown that sitagliptin, an oral antidiabetic agent, corrects the glycemic dysmetabolism, has anti-inflammatory properties, by exerting beneficial effects on the blood-retinal barrier integrity in Zucker Diabetic Fatty (ZDF) rats, a model of type 2 diabetes. Sitagliptin is a dipeptidyl peptidase IV (DPP-IV) inhibitor that has shown to improve glycemic control by stabilizing the active incretin hormones and therefore increasing insulin secretion in T2DM patients.

In this context, the aim of this study was to evaluate the efficacy of sitagliptin in preventing the deleterious effects of diabetes on the kidney of ZDF (*fa/fa*) rat, focusing on anti-inflammatory and antiapoptotic properties.

Animals and Methods

Obese diabetic ZDF (*fa/fa*) at 20 weeks of age were treated with sitagliptin (10 mg/kg bw/day) during 6 wks. Insulin, glucose and HbA_{1c} were evaluated in plasma, serum and total blood, respectively. Kidney function and lesions were evaluated by hematoxylin and eosin staining. Kidney mRNA and/or protein content/distribution of DPP-IV, GLP-1, GLP-1R, TNF-α, IL-1β, BAX, Bcl-2, and Bid were evaluated by RT-PCR and/or western blotting/immunohistochemistry.

Results

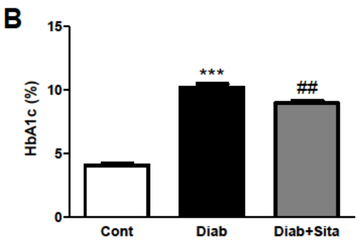
Sitagliptin prevents body weight loss and decreases glucose and HbA1c blood levels in the diabetic animals

A

	Cont		Diab		Diab+Sita
Age (weeks)	20	26	20	26	26
Weight (g)	408±7	438±4	395±7	370±12**	390±16
Blood glucose (mg/dl)	92±3	89±3	305±9***	425±10***	330±24###

Data are expressed as mean SEM (n ≥ 6 per group), *** p<0.001 and **p<0,01 significantly different from age-matched control, ### p<0.001 significantly different from age-matched diabetic. ANOVA followed by Bonferroni's *post hoc* test.

B



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Sitagliptin ameliorates kidney function and lesion of diabetic animals

A

	Cont		Diab		Diab+Sita
Age (weeks)	20	26	20	26	26
BUN (mg/dl)	14.35±0.47	15.05±0.54	18.15±0.84***	18.03±1.20**	15.16±0.61#
Creatinine (mg/dl)	0.55±0.03	0.53±0.03	0.55±0.06	0.54±0.08	0.49±0.04

Data are expressed as mean SEM (n ≥ 6 per group), *** p<0.001 and **p<0,01 significantly different from age-matched control, # p<0.05 significantly different from age-matched diabetic. ANOVA followed by Bonferroni's *post hoc* test.

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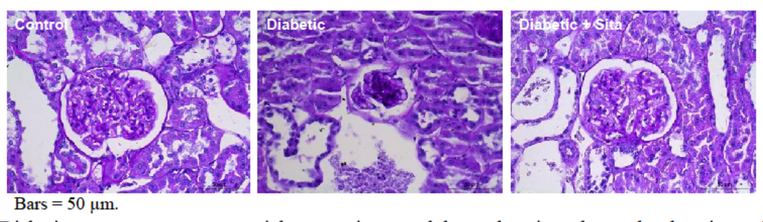
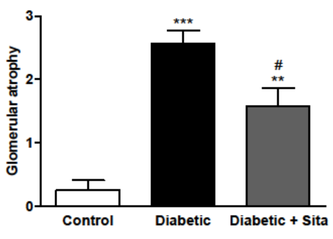


Bars = 50 µm.

Diabetic rats present mesangial expansion, nodular sclerosis, glomerulosclerosis and glomerular atrophy, accompanied by thickening of both glomerular basement membrane and capsule of Bowman, and sitagliptin ameliorate the kidney glomerular lesions, by reduction of fibrosis and global glomerulosclerosis severity.



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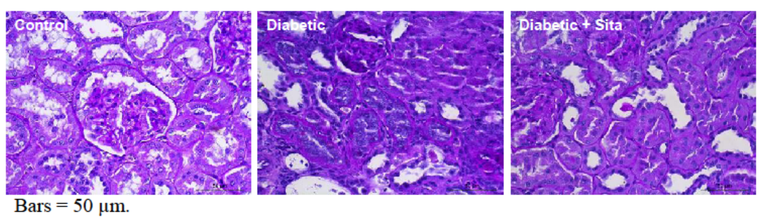
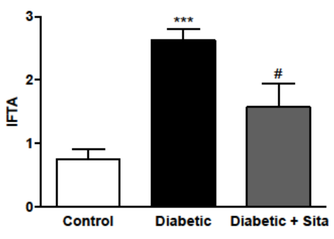


Bars = 50 µm.

Diabetic rats present tubular degeneration, tubular basement membrane irregularity, IFTA and hyaline cylinders. Sitagliptin treatment in the diabetic rats prevented the appearance of hyaline cylinders and decreased basement membrane irregularity, tubular degeneration and IFTA.



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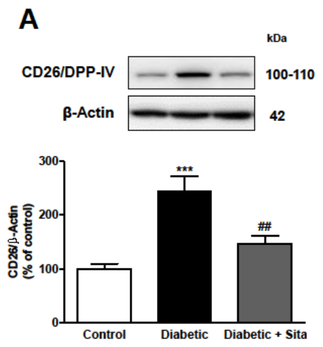
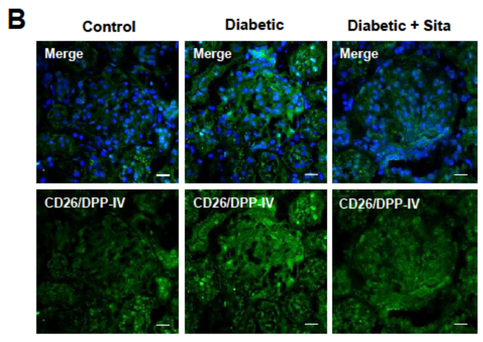
Sitagliptin prevents the upregulation of DPP-IV content in the kidney of diabetic animals

A

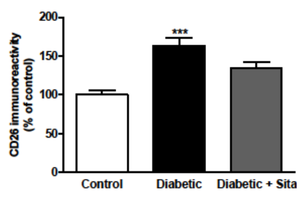


Data are expressed as percentage of control and represent the mean ± SEM (n ≥ 6 per group), *** $P < 0.001$ significantly different from control, ## $P < 0.01$ significantly different from diabetic.

B

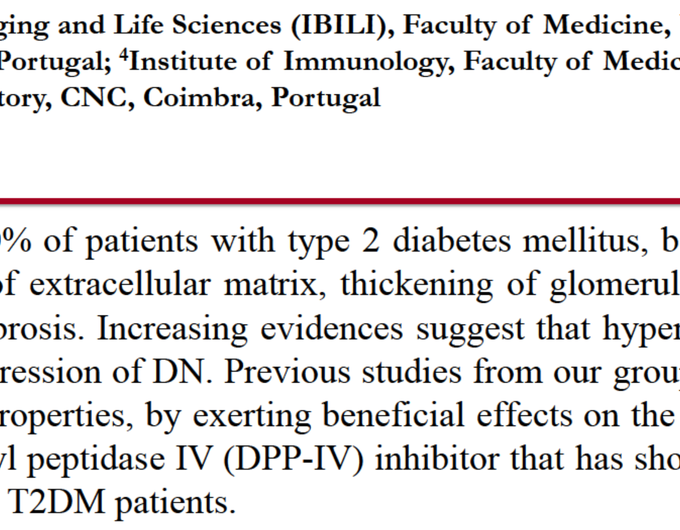


Confocal images for each group of animals showing DPP-IV (green) immunoreactivity and nuclear staining with DAPI (blue) in kidney sections. Bars = 20 µm.



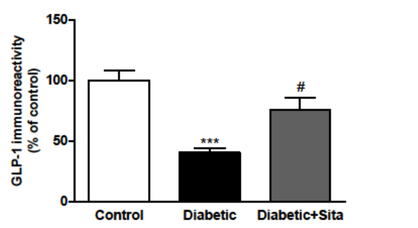
Data are expressed as percentage of control and represent the mean ± SEM (n ≥ 6 per group), *** $P < 0.001$ significantly different from control.

Sitagliptin modulates the incretin axis in the kidney of diabetic animals



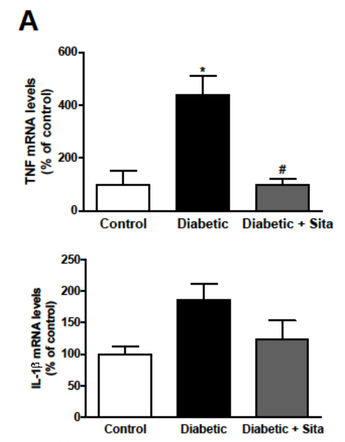
Confocal images of GLP-1 (red) and GLP-1R (green) immunoreactivity, and nuclear staining with DAPI (blue) in kidney sections Bars = 20 µm. Magnification of representative confocal images for the group of ZDF (fa/fa) treated with sitagliptin showing co-localization areas of GLP-1 and GLP-1R (yellow) in kidney sections (6 µm). Bars = 10 µm.

Data are expressed as percentage of control and represent the mean SEM (n ≥ 6 per group), ** $P < 0.01$ and ### $P < 0.001$ significantly different from diabetic.

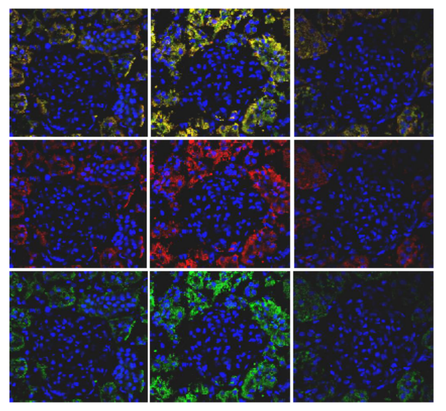


Data are expressed as percentage of control and represent the mean SEM (n ≥ 6 per group*** $P < 0.001$ significantly different from control, # $P < 0.05$ significantly different from diabetic.

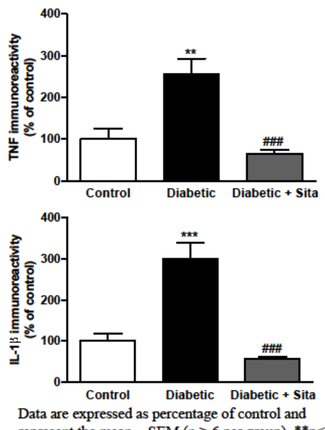
Sitagliptin decreases the inflammatory state in the diabetic kidney



*p<0.05 significantly different from control, #p<0.05 significantly different from diabetic.



Confocal images showing IL-1β immunoreactivity (green), TNF-α immunoreactivity (red) and nuclear staining with DAPI (blue) in kidney sections. Magnification 400 . Legend: G - glomeruli, Arrows - tubular cells.



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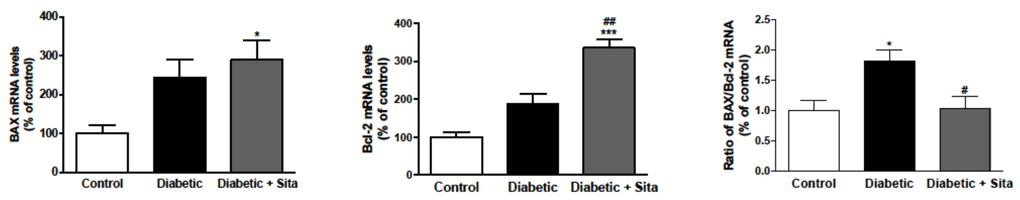
Sitagliptin protects the diabetic kidney against apoptotic cell death induced by diabetes



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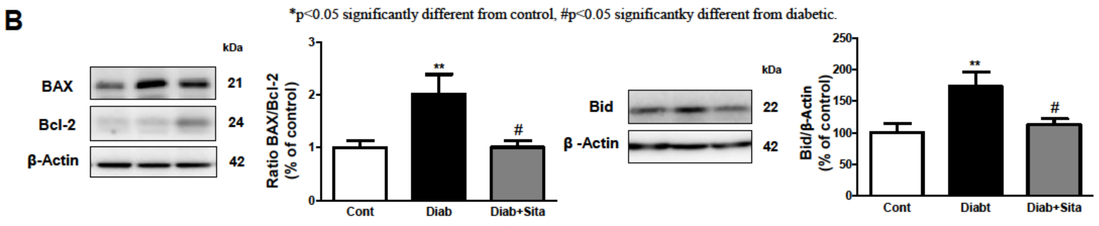
B



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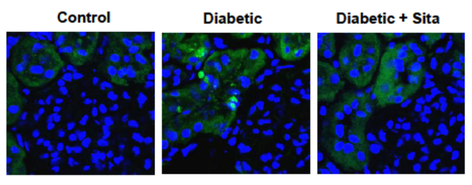
C



Confocal images for each group of animals showing TUNEL-positive (green) cells and nuclear counterstaining with DAPI (blue) in kidney sections (6 µm)

Conclusion

Sitagliptin might have a major role in preventing diabetic nephropathy evolution due to anti-inflammatory and antiapoptotic properties.

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