High Protein Aggravates, and Mineralocorticoid Antagonism Ameliorates Renal Injury in the BTBR ob/ob Mouse Model of Diabetic Nephropathy

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Background

Diabetic nephropathy is a rapidly increasing health issue worldwide with a large unmet medical need. One of the challenges in the development of new treatments is the lack of relevant and translatable rodent models.

Introduction

- The obese and type 2 diabetic BTBR ob/ob mouse has been shown to mimic key features of human Diabetic Nephropathy, including progressive proteinuria and glomerular lesions (1).
- The introduction of high protein diet to the model results in faster, aggravated and more robust disease progression.
- A benchmarking study to assess the renoprotective effect of the mineralocorticoid receptor (MR) antagonist eplerenone was performed in the model.

Methods

- Regular or high protein diet (HPD, 30%, Research Diets, New Brunswick, NJ, US) with or without admixture of eplerenone (100 mg/kg/day) was provided to female BTBR ob/ob mice from 8 to 18 weeks of age, or were fed regular diet or 40% HPD up to 24 weeks of age.
- Albuminuria expressed as UAE (urinary albumin excretion) or UACR (albumin to creatinine ratio) was measured along with parameters of glycemic and metabolic control (plasma glucose, insulin, HbA1c, triglycerides (TG), non esterified fatty acids (NEFA)).
- Histopathological scoring focusing on mesangial proliferation and interstitial fibrosis was performed on kidney sections.
- Gene expression analysis was performed on renal cortex and isolated glomeruli and multivariate analysis was performed on gene expression data from isolated glomeruli.

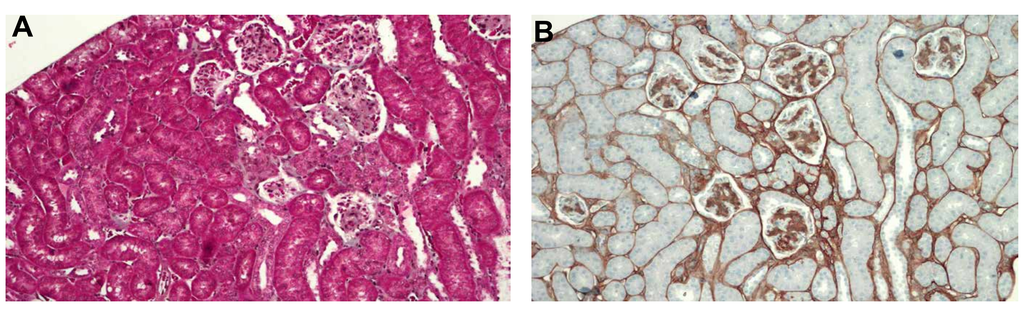
Results

HPD results in increased proteinuria and metabolic disturbances

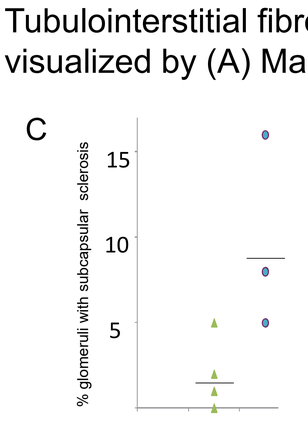
	Glucose (mM)	HbA1c (%)	TG (mM)	NEFA (mM)	UAE (µg/13h)
Regular diet (n=5)	21.2 (7.0, 33.3)	10.5 (8.8, 12.5)	1.0 (0.7, 1.7)	0.39(0.24, 0.55)	499 (238, 1864)
HPD (n=3)	33.3 (30.3, 33.3)	14.0 (13.1, 14.0)	2.8(1.5, 2.9)	0.44(0.44, 0.45)	2041 (1448, 3478)

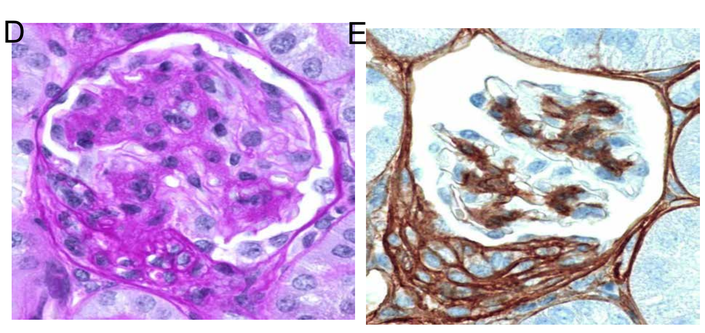
Metabolic parameters at 24 weeks of age in female BTBR ob/ob mice on regular diet or 40% HPD.
Median(min, max)

HPD results in aggravated renal pathology



Tubulointerstitial fibrosis in HPD treated BTBR ob/ob mice visualized by (A) Massons trichrome and (B) collagen IV staining.





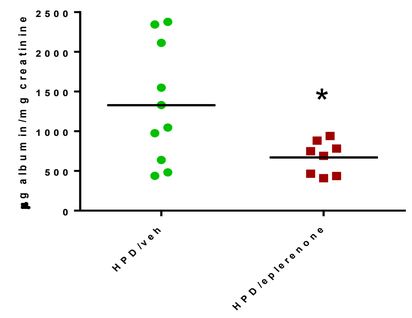
(C) Subcapsular glomerular sclerosis in BTBR ob/ob mice +/- HPD visualized by (D) PAS, (E) coll.IV staining in BTBR ob/ob mice on HPD.

References

1. Hudkins KL et al. 2010, BTBR ob/ob Mutant Mouse Model of Diabetic Nephropathy , J Am Soc Nephrol 21: 1533-1541

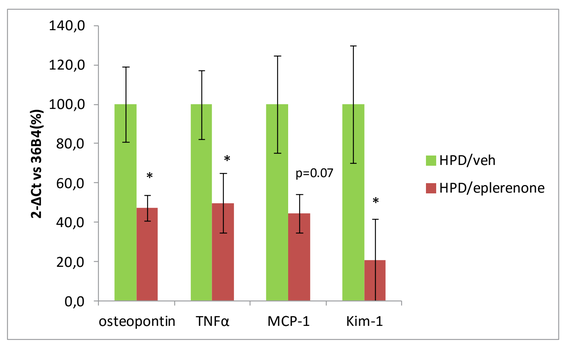
Results cont.

Treatment with MR antagonist eplerenone reduces UACR



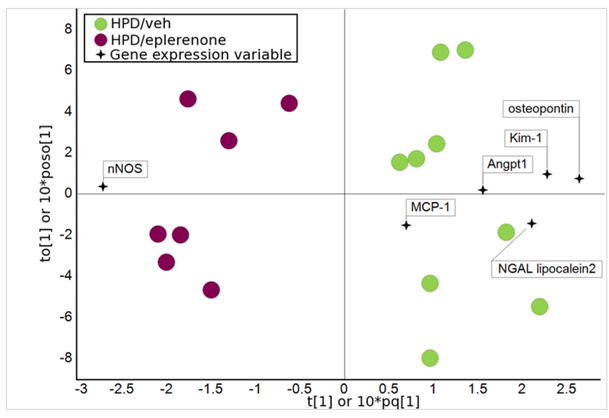
Albuminuria measured as UACR was significantly decreased after 10 weeks treatment with eplerenone in BTBR ob/ob mice on HPD
mean±SEM
*p<0.05, HPD/veh n=9, HPD/eplerenone n=7 in all following results unless otherwise stated

Treatment with MR antagonist eplerenone reduces markers of kidney injury and inflammation in kidney cortex and in isolated glomeruli.



Real time quantitative PCR gene expression analysis of renal cortex from BTBR ob/ob mice on HPD revealed significant effects on markers of kidney injury and inflammation after eplerenone treatment for 10 weeks.
mean±SEM

Multivariate analysis was used to investigate genes responsible for separating vehicle and eplerenone treated BTBR ob/ob mice on HPD. 45 genes measured from isolated glomeruli were used in an orthogonal projections to latent structures-discriminant analysis (OPLS-DA).



All individual mice were assigned to either HPD/vehicle or HPD/eplerenone groups and OPLS-DA was used to maximize the class separation. All variables were log transformed. The score plot shows a clear separation between vehicle- and eplerenone-treated mice. Each dot represents an individual animal. A selection of the most influential genes responsible for the separation from the loading plot are highlighted. The Y-axis display the orthogonal (non-predictive) variation.

Treatment with MR antagonist eplerenone have beneficial effects on renal pathology

Interstitial fibrosis (0=nil/minimal, 1= minimal, focal, 2= slight, focal)	Individuals						Average
HPD/veh	2	2	1	1	1	1	1,33
HPD/eplerenone	1	1	1	1	0	-	0,80
Subcapsular sclerosis (%; 100 glomeruli counted)	Individuals						Average
HPD/veh	15	5	2	0	0	0	3,33
HPD/eplerenone	0	0	0	0	0	-	0
Mesangial matrix expansion (ave 100 glom/mouse, 0=nil/minimal, 1=slight/moderate, <4 cells/segment, 2=moderate, 4-6 cells/segment, 3=moderate/severe, >6 cells/segment, 4=severe+nodularity±mesangiolysis)	Individuals						Average
HPD/veh	2.0	1.9	1.9	1.9	1.9	1.8	1.7	1.7	1.4	1.8
HPD/eplerenone	1.6	1.5	1.4	1.4	1.3	1.3	1.0	-	-	1.4

Conclusions

- Introduction of a high protein diet to BTBR ob/ob mice results in faster disease progression with more aggravated and robust renal damage
- The declining renal function in BTBR ob/ob mice on a high protein diet can be effectively ameliorated by treatment with an MR antagonist
- These results suggests the BTBR ob/ob model as a translatable model to test and validate novel therapies aimed at treating diabetic nephropathy

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