Supplementary Material

A Hypercaloric Diet Induces Early Podocyte Damage in Aged, Non-Diabetic Rats

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Supplementary Table 1. Gender differences in HFHCD-induced renal injury – Glomerular parameters.

HFHCD = high-fat-high-carbohydrate diet, n = number, SD = standard deviation,

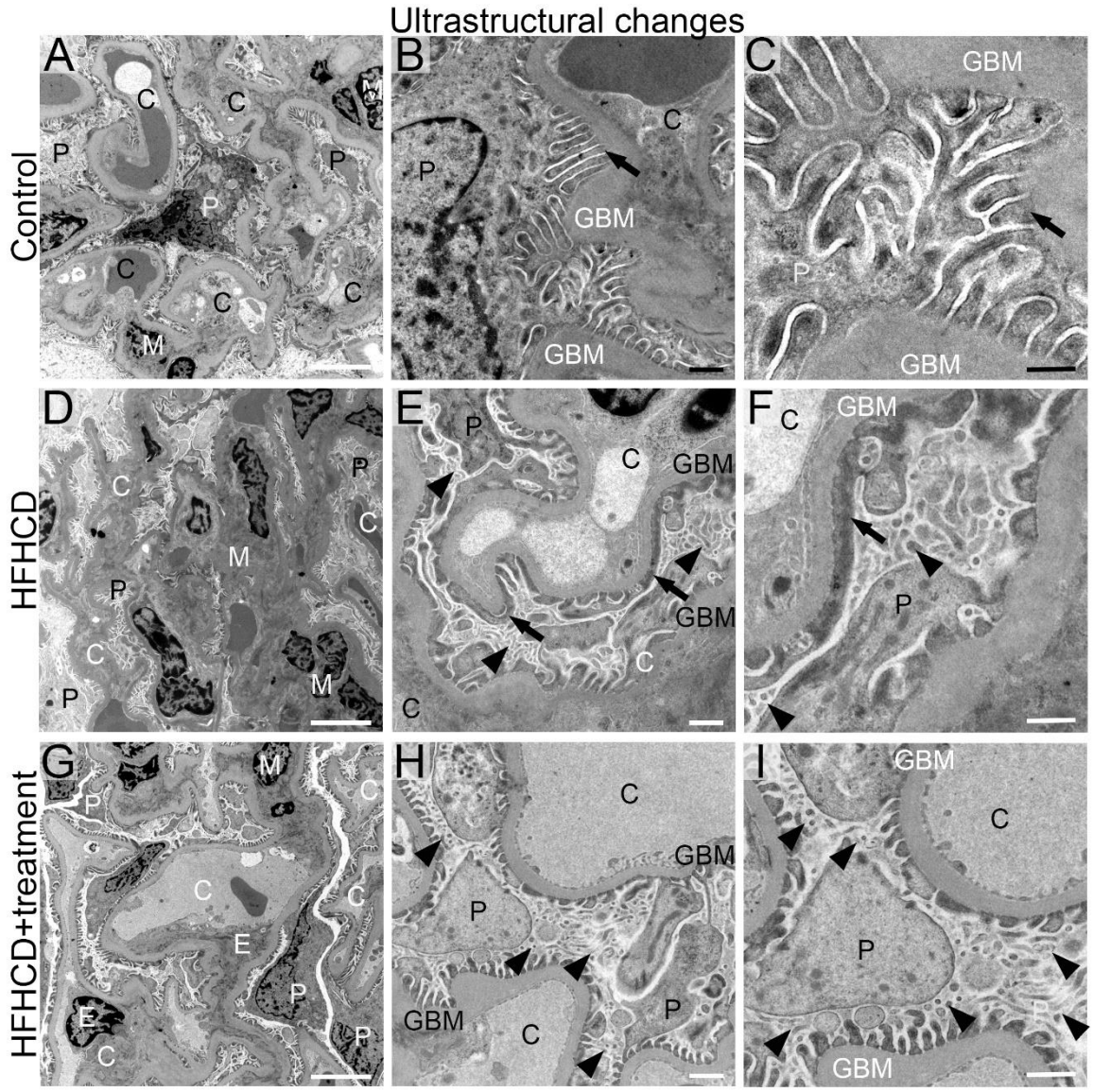
* p<0.05, ** p<0.01 versus HFHCD group.

Groups	**Males** (n=29)		**Females** (n=28)	
	n	mean ± SD	n	mean ± SD
Tuft size (μm²)				
Control	7	10294 ± 1308	6	8585 ± 757
HFHCD	6	9351 ± 1269	8	7968 ± 632
HFHCD+liraglutide	8	9145 ± 562	4	7978 ± 85.3
HFHCD+metformin	8	9428 ± 391	7	8164 ± 573
Glomerular podocin (% area)				
Control	7	30.4 ± 3.0	6	30.9 ± 3.9
HFHCD	6	28.3 ± 3.9	8	27.2 ± 3.6
HFHCD+liraglutide	8	28.4 ± 3.9	5	33.1 ± 4.9
HFHCD+metformin	8	25.9 ± 1.7	7	29.5 ± 3.2
Glomerular nestin (% area)				
Control	7	9.1 ± 1.7	6	11.1 ± 2.1*
HFHCD	6	8.0 ± 1.8	8	7.2 ± 1.8
HFHCD+liraglutide	8	7.2 ± 3.2	5	6.9 ± 1.9
HFHCD+metformin	8	8.2 ± 2.1	7	11.0 ± 2.1**
Glomerular desmin (% area)				
Control	7	5.5 ± 2.9	6	3.7 ± 1.0
HFHCD	6	4.3 ± 2.8	8	3.5 ± 1.8
HFHCD+liraglutide	8	7.0 ± 4.2	5	3.9 ± 2.2
HFHCD+metformin	8	6.0 ± 3.0	7	4.2 ± 3.0
Glomerular CD44 (% no of glom.)				
Control	7	23.3 ± 3.3*	6	18.7 ± 8.0
HFHCD	6	40.0 ± 10.1	8	19.0 ± 9.6
HFHCD+liraglutide	8	40.5 ± 15.1	5	17.8 ± 7.3
HFHCD+metformin	8	38.3 ± 11.4	7	11.9 ± 5.6
Glomerular collagen IV (% area)				
Control	7	15.6 ± 3.6	6	16.3 ± 2.0
HFHCD	6	10.2 ± 5.0	8	15.2 ± 5.6
HFHCD+liraglutide	8	13.6 ± 1.8	5	18.3 ± 3.8
HFHCD+metformin	8	13.0 ± 3.7	7	15.3 ± 5.8

Supplementary Table 2. Gender differences in HFHCD-induced renal injury- Cortex and mRNA.

aSMA = alpha smooth muscle actin, HFHCD = high-fat-high-carbohydrate diet, n = number, SD = standard deviation, TI = tubulointerstitial * p<0.05, ** p<0.01 versus HFHCD group.

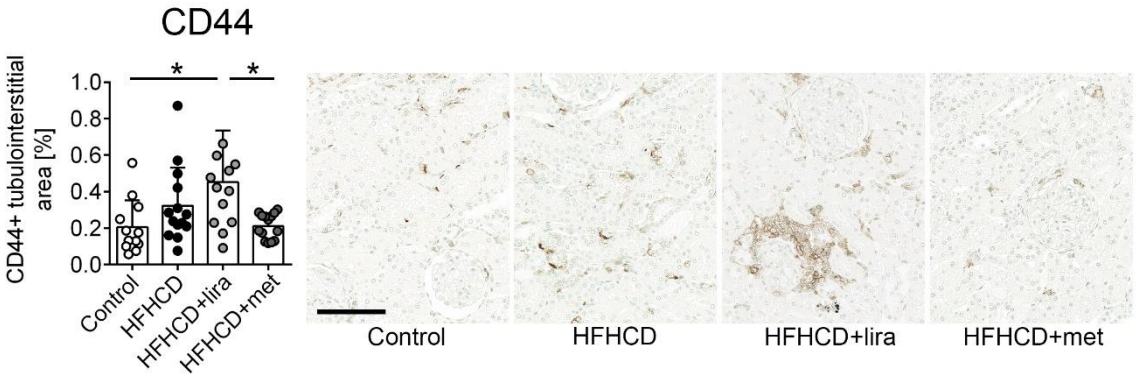
Groups	**Males** (n=29)		**Females** (n=28)	
	n	mean ± SD	n	mean ± SD
Cortex lipocalin 2 (no of tubules)				
Control	7	1.8 ± 0.6	6	0.6 ± 0.4
HFHCD	5	2.0± 0.3	8	1.0 ± 0.5
HFHCD+liraglutide	8	1.4 ± 0.8	5	1.1 ± 0.5
HFHCD+metformin	7	2.2 ± 0.7	6	0.8 ± 0.4
Cortex TI injury (% area)				
Control	7	25.5 ± 20.1	6	14.4 ± 9.8
HFHCD	6	29.9 ± 21.3	8	10.6 ± 8.4
HFHCD+liraglutide	8	37.4 ± 15.4	5	22.5 ± 16.1
HFHCD+metformin	8	27.2 ± 21.3	7	18.7 ± 15.8
Cortex CD68+ cells (no of cells)				
Control	7	20.0 ± 7.4	6	11.0 ± 3.6
HFHCD	6	32.5 ± 18.9	8	9.7 ± 4.2
HFHCD+liraglutide	8	33.8 ± 10.0	5	16.0 ± 4.9
HFHCD+metformin	8	28.2 ± 13.2	7	11.8 ± 5.3
Cortex CD44 (% area)				
Control	6	0.1 ± 0.1	6	0.3 ± 0.2
HFHCD	6	0.5 ± 0.2	8	0.2 ± 0.1
HFHCD+liraglutide	8	0.5 ± 0.3	5	0.4 ± 0.2
HFHCD+metformin	7	0.3 ± 0.0	7	0.2 ± 0.1
Cortex collagen I (% area)				
Control	7	4.4 ± 1.2	6	4.3 ± 1.3
HFHCD	6	5.2 ± 1.5	8	4.0 ± 1.3
HFHCD+liraglutide	8	5.8 ± 1.6	5	4.2 ± 1.0
HFHCD+metformin	8	5.0 ± 1.7	7	3.6 ± 0.8
Cortex collagen IV (% area)				
Control	7	20.7 ± 2.7	6	20.3 ± 2.1
HFHCD	5	19.0 ± 2.1	8	19.9 ± 4.5
HFHCD+liraglutide	8	23.8 ± 2.2**	5	22.6 ± 3.0
HFHCD+metformin	8	20.7 ± 3.1	7	18.9 ± 5.0
Cortex aSMA (%area)				
Control	7	0.8 ± 0.4	6	0.9 ± 0.2
HFHCD	6	0.8 ± 0.2	8	0.8 ± 0.3
HFHCD+liraglutide	8	1.0 ± 0.3	5	1.3 ± 0.5*
HFHCD+metformin	8	0.5 ± 0.2	7	0.8 ± 0.2
***Ccl2* mRNA expression (CCL2)**				
Control	7	1.0 ± 0.6	6	1.1 ± 0.6
HFHCD	6	1.1 ± 1.0	8	1.1 ± 0.8
HFHCD+liraglutide	8	1.0 ± 0.6	5	0.5 ± 0.2
HFHCD+metformin	8	0.8 ± 0.3	7	0.8 ± 0.2
***Il1b* mRNA expression (IL1-β)**				
Control	7	1.0 ± 0.7	6	1.0 ± 1.3
HFHCD	6	1.5 ± 1.0	8	0.8 ± 0.4
HFHCD+liraglutide	8	2.2 ± 1.5	5	0.7 ± 0.2
HFHCD+metformin	8	0.9 ± 0.7	7	0.6 ± 0.4



Supplementary Figure 1. Ultrastructural changes in podocytes after HFHCD in female rats.

(A-C) Representative pictures from glomeruli of the control group. The glomerular structure was inconspicuous: only a few mesangial cells, open capillaries, fenestrated thin endothelium, thin basement membranes, podocytes with fine foot processes (arrow). (D-F) In the HFHCD group mesangial cells expanded in some glomeruli. Podocytes showed signs of stress: frequently, microvilli protruded into the urinary space (arrowheads) and very rare foot process effacement (arrow). (G-I) Both treatment groups, liraglutide and metformin, showed similar signs of podocyte stress. Microvilli formation (arrowheads) was observed in several glomeruli.

HFHCD = high-fat-high-carbohydrate diet; C = capillary, E = endothelial cell, GBM = glomerular basement membrane, M = mesangial cell, P = podocyte, US = urinary space, arrow = podocyte foot process, arrowhead = microvilli; scale bars = 5000, 1000 and 500 nm, respectively.



Supplementary Figure 2. Tubulointerstitial CD44 expression.

CD44 positive area of kidney cortex was quantified in immunohistochemical stainings. The HFHCD+liraglutide treated rats showed significantly higher expression compared to the control and HFHCD+metformin group.

HFHCD = high-fat-high-carbohydrate diet, lira = liraglutide, met = metformin; shown are individual animals and mean ± SD; * $p<0.05$.