Diabetic Complications Consortium

Application Title: Impact of Diabetes on Renal Transporters in Females and Males

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1. Project Accomplishments:

Aim 1.

- **Addressed most all the objectives in Specific Aim 1** with n=4 rats/group (replicate in additional rats for rigor and reproducibility).
- PhD student working on this project presented finding "so far" to the annual USC/Keck/CHLA Diabetes and Obesity Research Symposium and won one of the three poster awards.
- **Submitted an NIH grant with the aim to do mathematical modelling of the female vs male diabetic kidney.**
- Summary of results detailed below.

Aim 2. Established the protocol for detecting transporters in urinary exosomes (EV). Results below. Set up a collaboration with the Ecelbarger lab (Georgetown) to measure miRNAs in the ZSF1 rat urinary exosomes.

2. **Specific Aim 1.** Determine the impact of T2D in both males (M) and females (F) in two accepted and widely studied models of DN: 1) obese (vs. lean) ZSF1 rats, 2) db/db (vs. db/m) C57BL/6J mice. Provide transporter profiles (abundance, covalent modification, distribution) of renal Na^+, K^+, HCO_3^-, glucose, and organic acid transporters, claudins, water and K^+ channels and associated regulators. Assess physiologic and hemodynamic impact on excretion and plasma levels. Seminar style" summary of data and text presented.

1. Morphometrics and Electrolyte handling:

- Females gained less weight than males overall; fractional weight gain greater in OM than OF
- Pronounced perirenal fat in both OM and OF which is implicated in compromised renal function (J Hall, 2019 Nature Rev Neph)
- Obese animals had greater food and water intake, thus, greater NaCl intake, urine volume (UV), UNaV and UKV
- Fat weight was not estimated
- Three- fold hypertrophy of liver in both sexes
- Tibia length not impacted by obesity – **normalize data to tibia length not body weight**

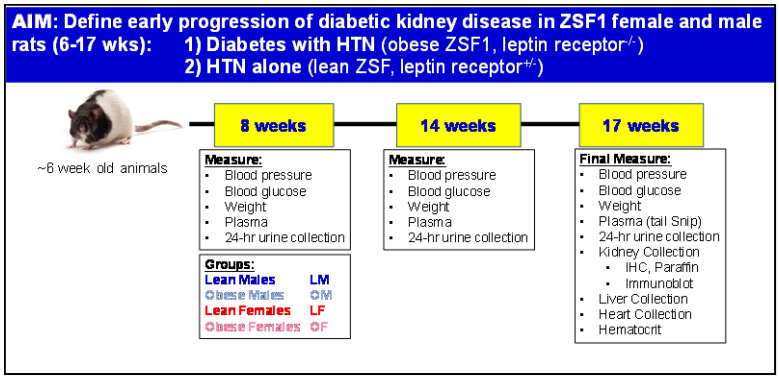


Table 1. 14-week body weight, food and water intake, Na^+ and K^+ excretion

Parameters	Lean Males - LM (n=4)	Obese Males - OM (n=4)	Lean Females - LF (n=4)	Obese Females - OF (n=4)
Body weight (BW, g)	396 ± 4	564 ± 11[a]	233 ± 7[a]	409 ± 9[bc]
Food Intake (g/day)	23 ± 2	37 ± 2[a]	19 ± 1	26 ± 2[b]
Water Intake (ml/day)	28 ± 2	54 ± 4[a]	28 ± 3	28 ± 3[c]
24hr Urine Volume (UV, ml)	13 ± 2	42 ± 7[a]	14 ± 1	16 ± 3[c]
Urinary Sodium Excretion (UNaV, mmol/24 hrs)	2.0 ± 0.2	4.1 ± 0.3[a]	2.0 ± 0.2	2.2 ± 0.1[c]
Urinary Potassium Excretion (UKV, mmol/24 hrs)	4.2 ± 0.5	8.4 ± 0.6[a]	4.0 ± 0.3	4.5 ± 0.2[c]

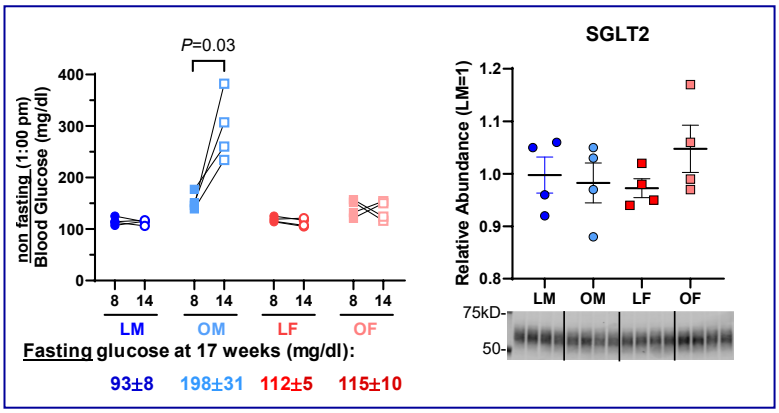
Table 2. 17-week organ weights and tibia lengths

Parameters	Lean Males - LM (n=4)	Obese Males - OM (n=4)	Lean Females - LF (n=4)	Obese Females - OF (n=4)
Heart (g)	1.25 ± 0.06	1.49 ± 0.05[a]	0.86 ± 0.01[a]	1.21 ± 0.01[bc]
Kidney (g)	1.66 ± 0.04	2.01 ± 0.08[a]	1.02 ± 0.03[a]	1.43 ± 0.06[bc]
Liver (g)	11.7 ± 0.9	32.0 ± 1[a]	6.9 ± 0.3[a]	16.3 ± 0.6[bc]
Tibia (cm)	4.03 ± 0.09	4.08 ± 0.09	3.63 ± 0.05[a]	3.73 ± 0.1[c]

[a]p<0.05 compared to LM [b]p<0.05 compared to LF [c]p<0.05 compared to OM

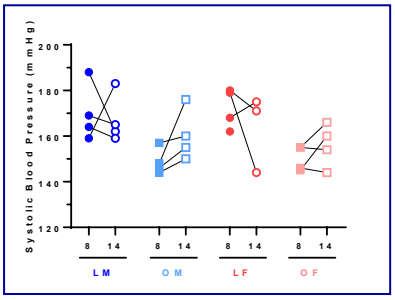
2. Glucose Handling

- Obese males exhibited pronounced hyperglycemia that increased with age
- Obese females exhibited less hyperglycemia which did not rise between 8-14 wks
- 17 wk fasted glucose showed same pattern
- Lean animals had normal blood glucose
- Renal proximal tubule Na–glucose cotransporter (SGLT2) is not different in the ZSF1 at 17 weeks.
- *WORK IN PROGRESS: Measure urine glucose and SGLT1*



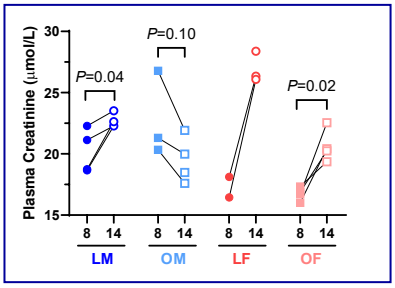
3. Blood pressure

- Since ZSF1 are offspring of SHR, both lean and obese animals exhibit hypertension
- At 8 wks lean males and females exhibit higher blood pressure than obese male and females. No further increase between 8 and 14 wks.
- Obese males blood pressure tended to increase between 8 and 14 wks (*P*=0.13)
- Obese females blood pressure was variable between 8-14 wks



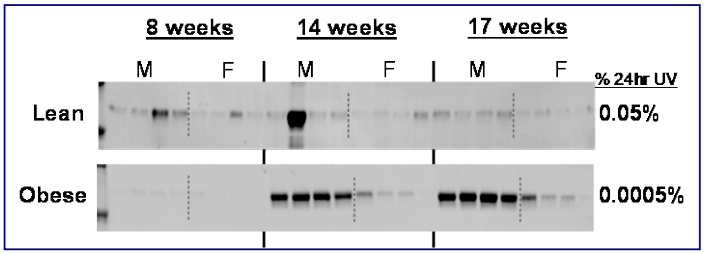
4. Estimated Glomerular Filtration Rate (GFR)

- Plasma creatinine tends to fall in obese males, evidence for **hyperfiltration** driven by hyperglycemia in early diabetes
- Plasma creatinine increases in other groups; perhaps a function of muscle growth (or low n's)
- *WORK IN PROGRESS: measure GFR directly with sinistrin clearance.*



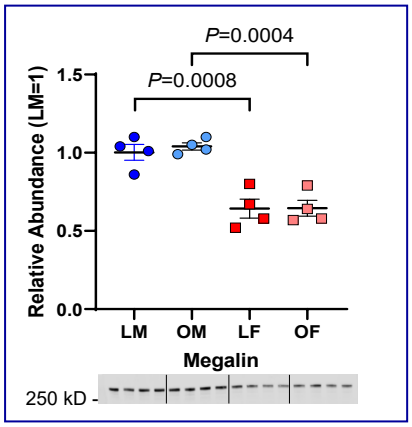
5a. Albuminuria, a measure of renal injury

- Obese males and females have greater albuminuria than lean littermates *despite similar hypertension. Note that 100X more urine assayed for LM, LF than OM, OF.*
- Obese females have less albuminuria than males but it increases over time
- *Urine albumin correlates with hyperglycemia… causal link?*
- *WORK IN PROGRESS: measure PT and glomerular intracellular albumin (which can be toxic)*



5b. Megalin and cubilin are involved in reabsorbing filtered albumin

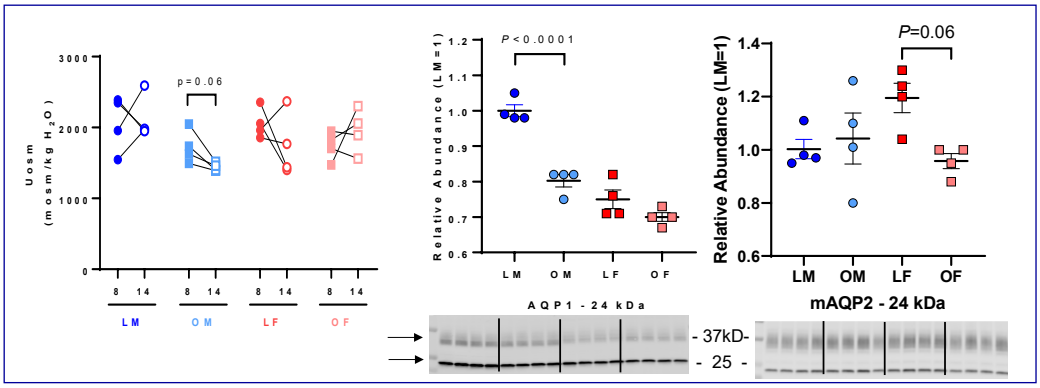
- Megalin is more abundant in males than females and not different between obese and lean.
  WORK IN PROGRESS: measure cubilin
- **Albuminuria not likely due to reduced capacity to reabsorb albumin, rather, to increased filtration.**



6. Urine concentrating ability - *Polyuria is typical in diabetes*

- Obese M consume more water and excrete more urine than obese F, lean M and lean F

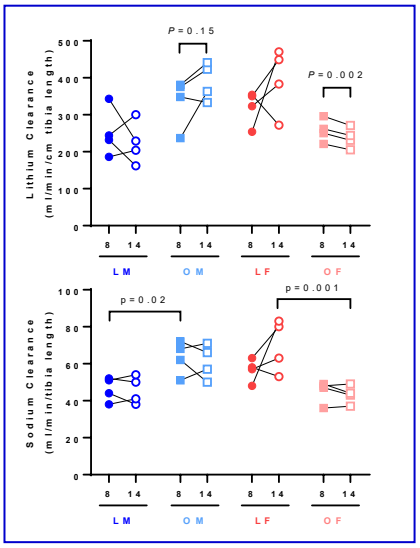
- Urine osmolality, a measure of concentrating ability, falls in obese males between 8-14 wks
- *WORK IN PROGRESS: measure plasma osmolality for evidence of hypernatremia.*



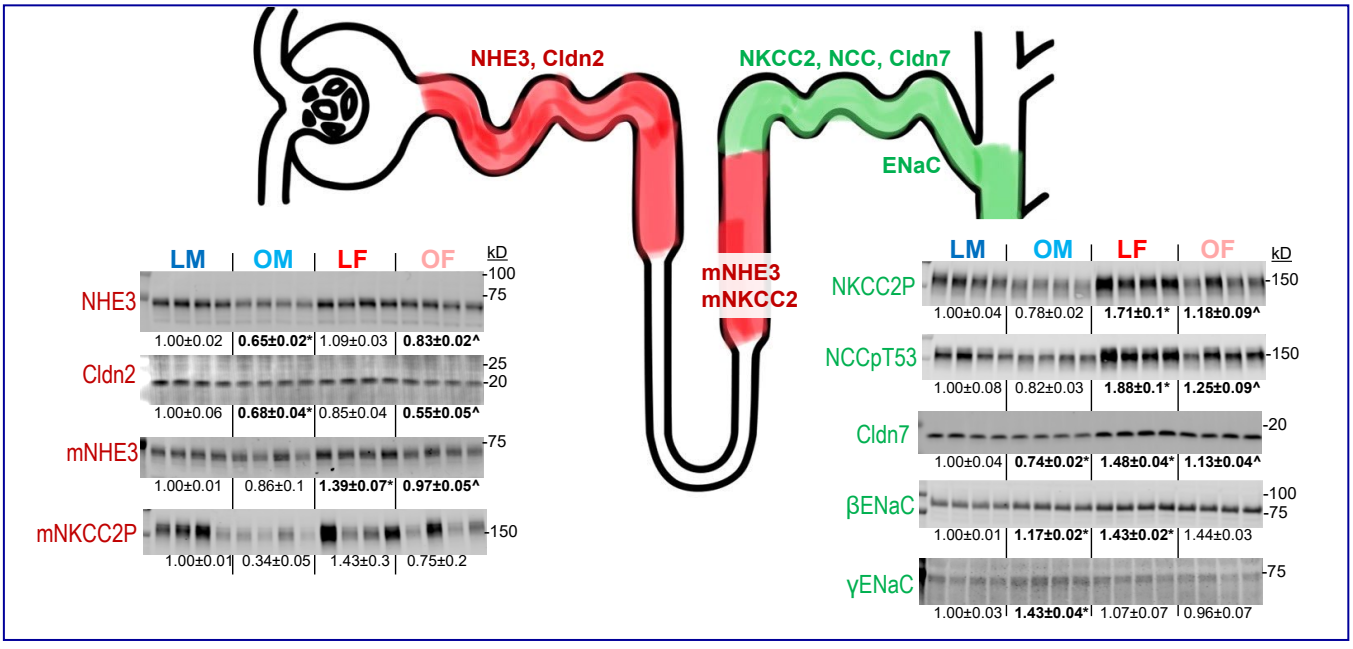
- As previously reported by us, females have less abundant proximal water channels (AQP1) than males, also evident in ZSF1 rats. Less AQP1 abundance in obese males than lean males
- The collecting duct AQP2, critical to urinary concentration tends to be lower in obese vs. lean F

7. Renal sodium handling

Lithium Clearance (CLi^+) is a measure of volume flow from the proximal nephron;
Sodium Clearance (CNa^+) is a measure of sodium handling along the entire nephron



- Tendency for higher CLi^+ in obese males may be secondary to hyperglycemia, hyperfiltration and reduced NHE3 (see below)
- Lower CLi^+ in obese females may be secondary to *early* hyperglycemia increasing Na^+ reabsorbed by proximal tubule SGLT2
- Higher overall CNa^+ in obese males than lean males is consistent with increased Na^+ intake driving lower fractional reabsorption
- Lower overall CNa^+ in obese females than lean females is may, again, be secondary to early hyperglycemia increasing Na^+ reabsorbed by SGLT2
- Sexual dimorphisms in Na^+ transporters established by us in SD rats are evident in ZSF1 rats, e.g. lower Cldn2, higher NKCC2, NCC, Cldn7
- Obese M,F exhibit lower Na^+ transporters' abundance and phosphorylation than lean M,F



Aim 1. Summary, Conclusions and Future directions.

Males exhibit more rapid diabetic kidney disease progression than females of the same age between 8-17 weeks of age: Greater weight gain, hyperglycemia, liver hypertrophy, fall in plasma creatinine (hyperfiltration), albuminuria, and suppressed urine concentrating ability

- **Role of food intake:** Males consume 42% more food (and NaCl) than females of the same age which may → liver hypertrophy, hyperglycemia, increase GFR and challenge kidneys

Hypertension alone can provoke renal injury and albuminuria. Albuminuria is *exacerbated* in obese M and F

- **Role of hyperglycemia:** Hyperglycemia in OM>>> OF>> LM, LF similar to the rank order of albuminuria implicating an effect of diabetes/hyperglycemia (independent of BP) on early renal damage, and supporting a "female advantage"

Obese animals have lower abundance of key renal Na^+ transporters, independent of sexual dimorphisms.

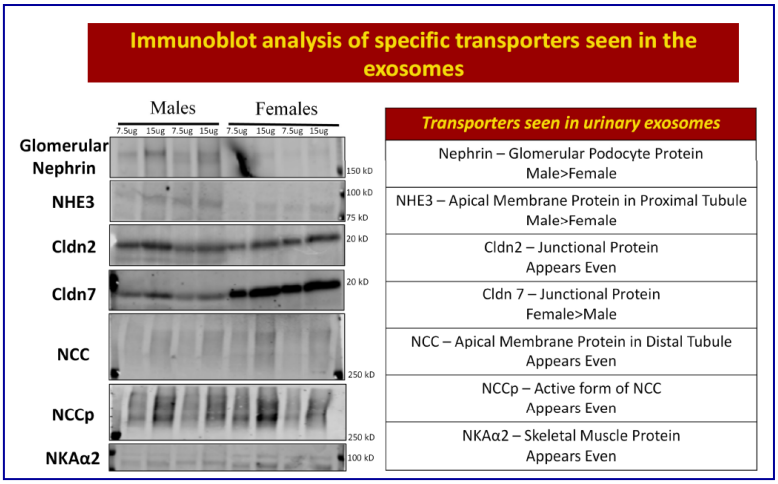
- **Lower Na^+ transporters should be "protective;"** differences may reflect higher NaCl intake or impact of perirenal fat.

***Overall, the female advantage in these diabetic rats appears to be a function of lower calories and/or lower salt intake*.**

- Presumably, obese females eventually develop similar hyperglycemia, tissue hypertrophy and albuminuria as seen in males as their obesity worsens.
- To test the hypothesis that greater salt intake or greater calorie intake accounts for the difference in progression between males and females (independent of sex), males can be pair fed similar salt or calories as female rats.

Aim 2.

We set up protocol to isolate urinary microvesicles (EV) and detected transporters in the EVs. Samples have been sent to collaborator at Georgetown to determine sexual dimorphisms in miRNAs in OM and OF.



3. **Publications:** None yet. We will submit abstracts to ASN Kidney Week 2020 and write a paper after we do another set of rats for rigor and reproducibility.