# The Urination Index Biomarker

## Robust non-invasive detection of hyperglycemia in mouse models of metabolic dysregulation

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RRR
Replacement Reduction Refinement

**Danish 3R-Center Symposium**

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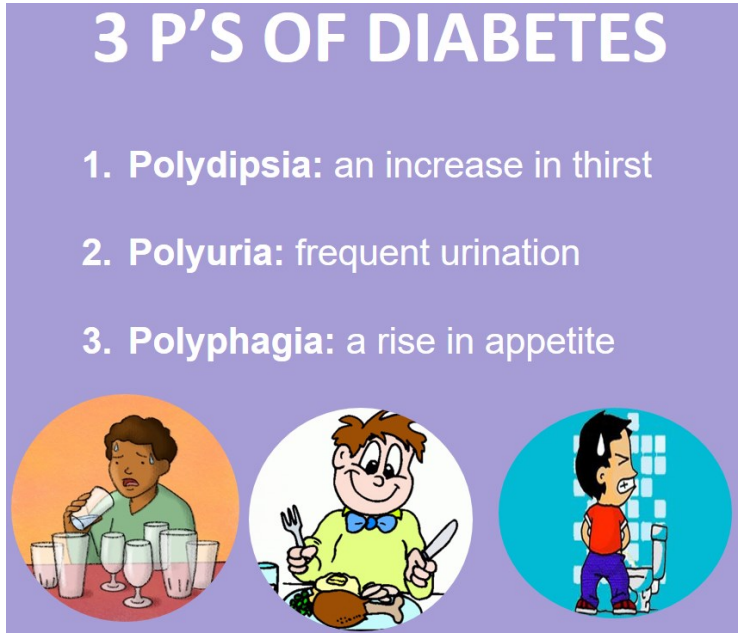
# Background - Glucose Homeostasis

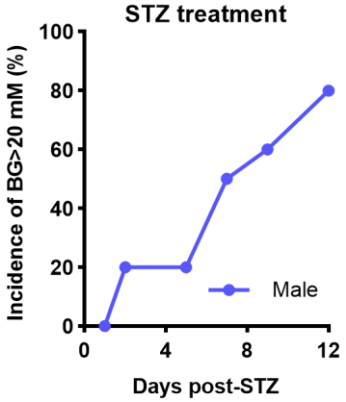
- Normal blood glucose range (euglycemia): 4-10 mM
- Hyperglycemia: >10 mM
- Type 1 Diabetes: Loss of pancreatic β-cells
- Type 2 Diabetes: Insulin resistance



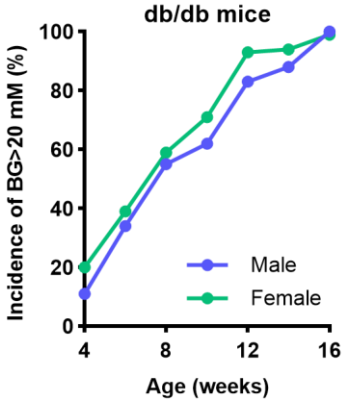

- **Polyuria:**
  - Main symptom of undiagnosed or poorly controlled diabetes
  - Caused by hyperglycemic saturation of renal glucose reabsorption
  - Excess glucose in urine draws more fluid (osmotic diuresis)
  - Leads to Polydipsia and severe risk of dehydration

# Background – Mouse Models of Diabetes

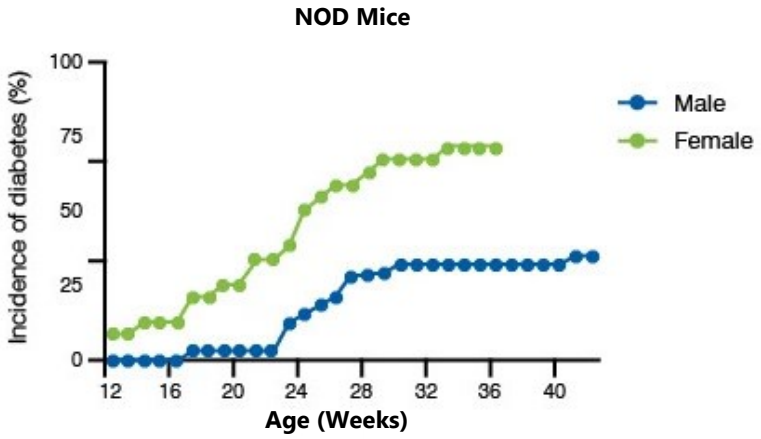
- **Obesity/Prediabetes**
  - Diet-induced obese (DIO) C57BL/6J or -N
    - Moderate obesity
    - Mild to moderate insulin resistance
- **Type 1 Diabetes**
  - NOD (Non-Obese Diabetic) mice:
    - Polygenic autoimmune type 1 diabetes
    - Spontaneous loss of pancreatic beta cells
  - Streptozotocin / Alloxan treatment:
    - Selective destruction of pancreatic beta cells
    - Rapid and severe hypoinsulinemia & hyperglycemia
- **Type 2 Diabetes**
  - Ob/ob mice:
    - Leptin deficiency → Polyphagia
    - Moderate to severe hyperglycemia & insulin resistance
  - Db/db mice:
    - Leptin receptor deficiency → Polyphagia
    - Severe hyperglycemia & insulin resistance
    - Diabetic complications (neuropathy, cardiomyopathy, infertility)



*CBMR unpublished data*



*JAX db/db phenotype data*

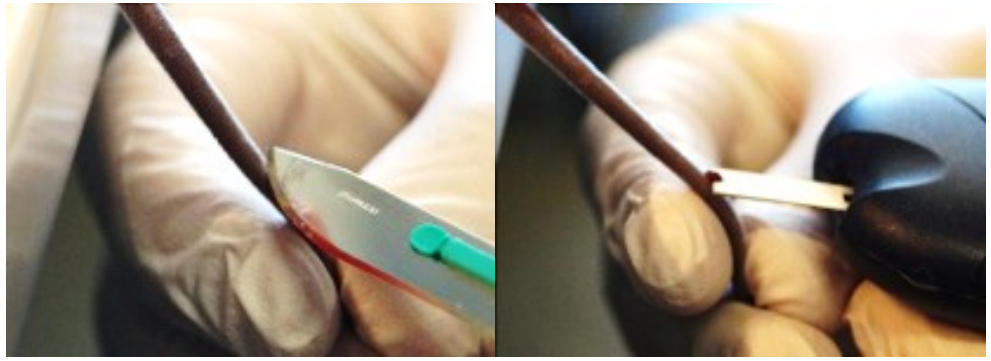


*Charles River NOD model data*

**Highly variable phenotype progression – both in time-course and magnitude**

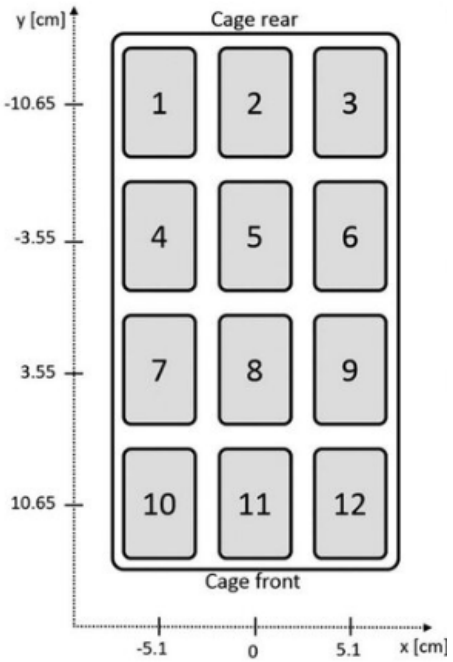
# Aim

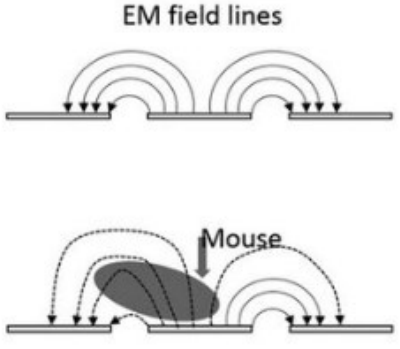
To investigate if tracking of bedding moisture in the home cage can be used to identify the onset of polyuria in mice as a diagnostic marker of hyperglycemia

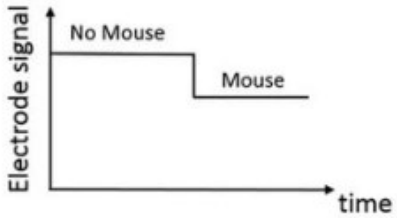


**Highly variable phenotype progression – both in time-course and magnitude**

# Background –Tecniplast DVC

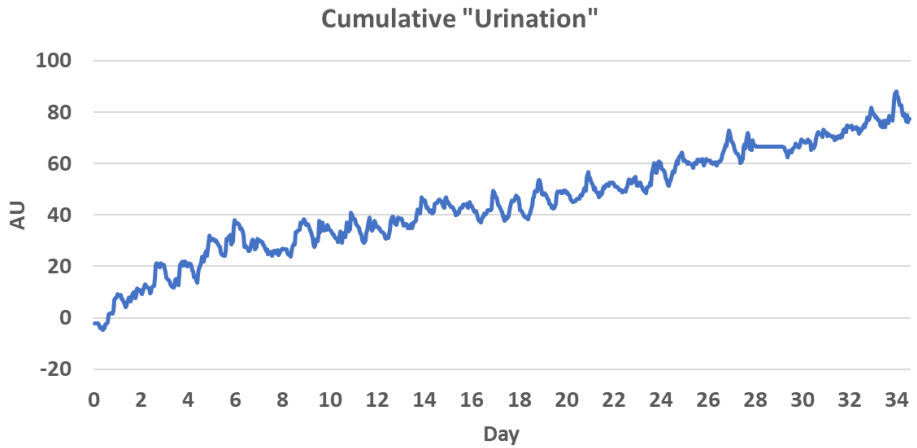


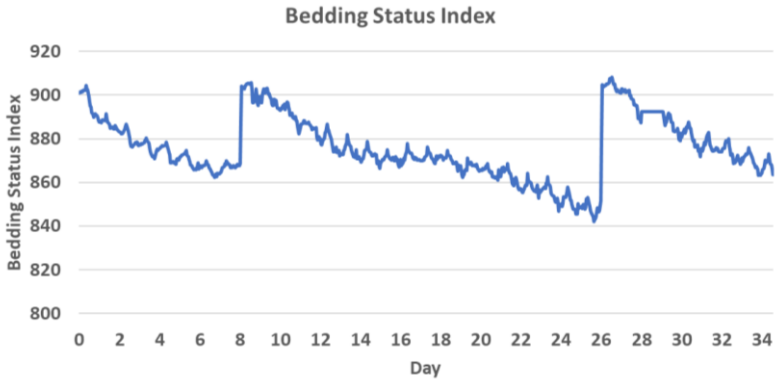



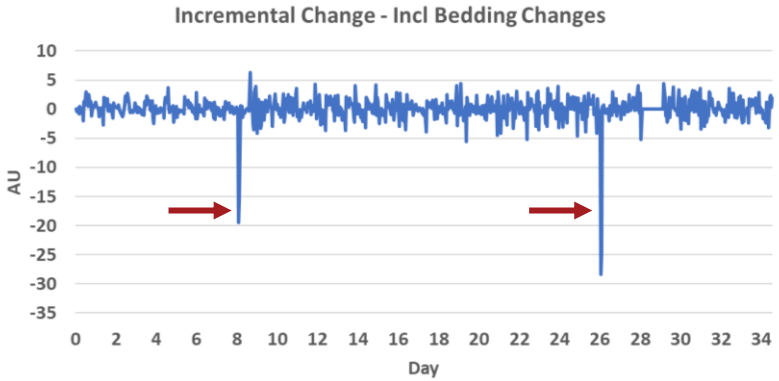



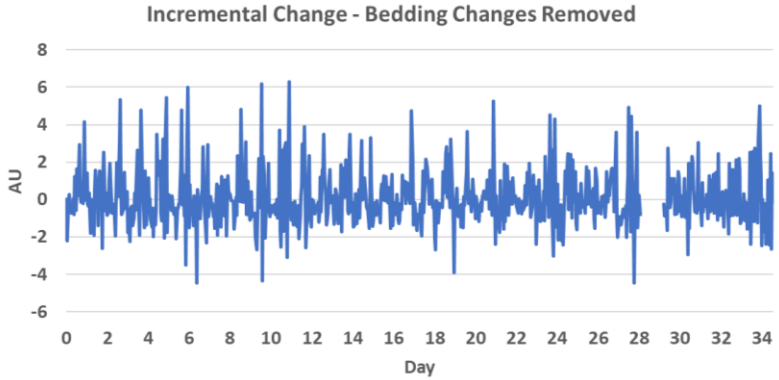
# Comparing Urination Rates

- **Estimating "Urination" with DVC**
  - Materials in the cage attenuates signal from DVC electrodes (bedding, nest, fluids, mouse, etc.)
  - Bedding status index = average signal strength from all electrodes
    - Declines in response to drinking, urination, mouse growth, water bottle leaks
    - Increases in response to evaporation, respiratory water loss, and bedding changes
  - Convert to incremental change and invert sign
  - Remove bedding changes
  - Calculate cumulative change



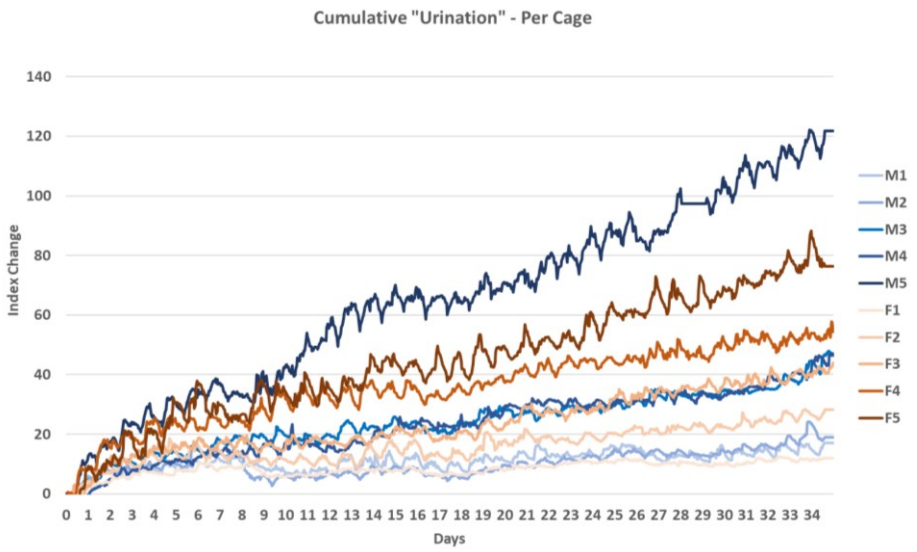



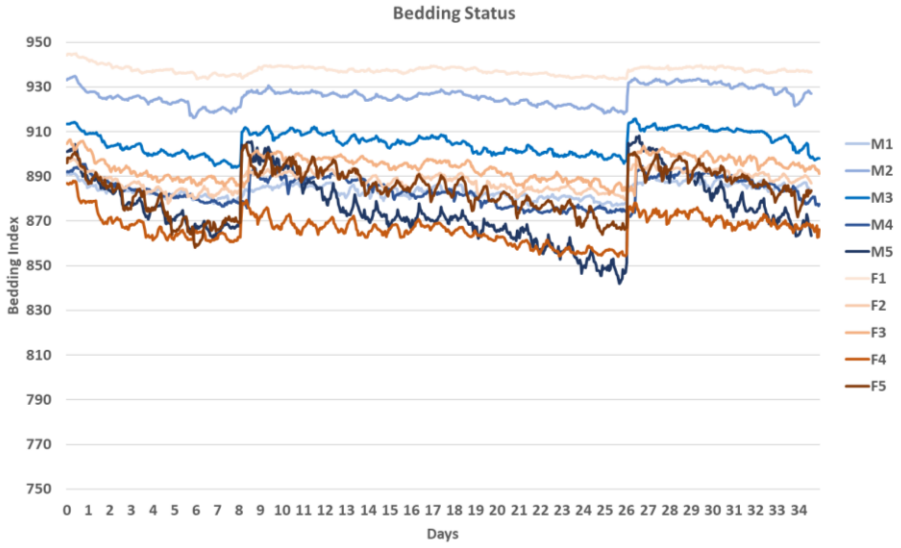



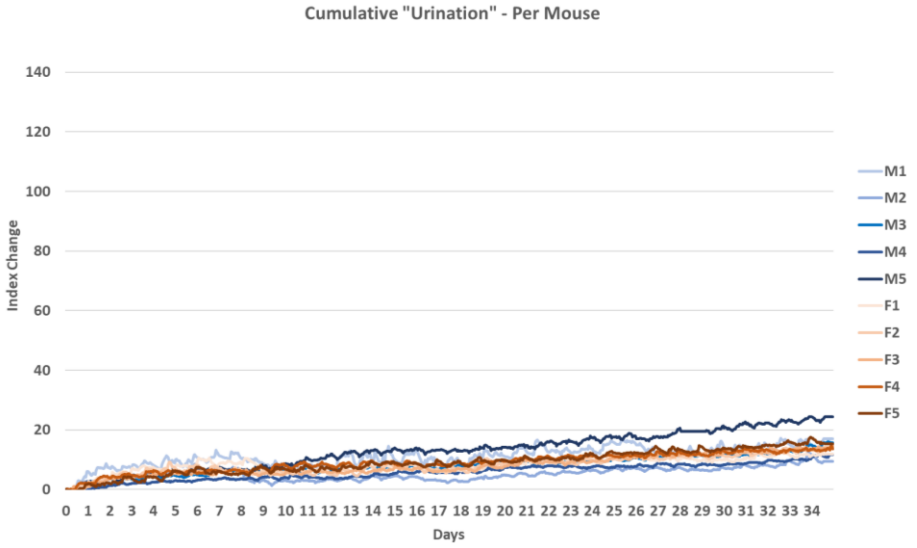



# Testing Linearity

- Proof-of-concept:
  - CD1 mice (Crl:CD1(ICR))
    - 1-5 mice per cage (housing density test)
    - 1 male and 1 female cage per group
    - ~30 weeks old

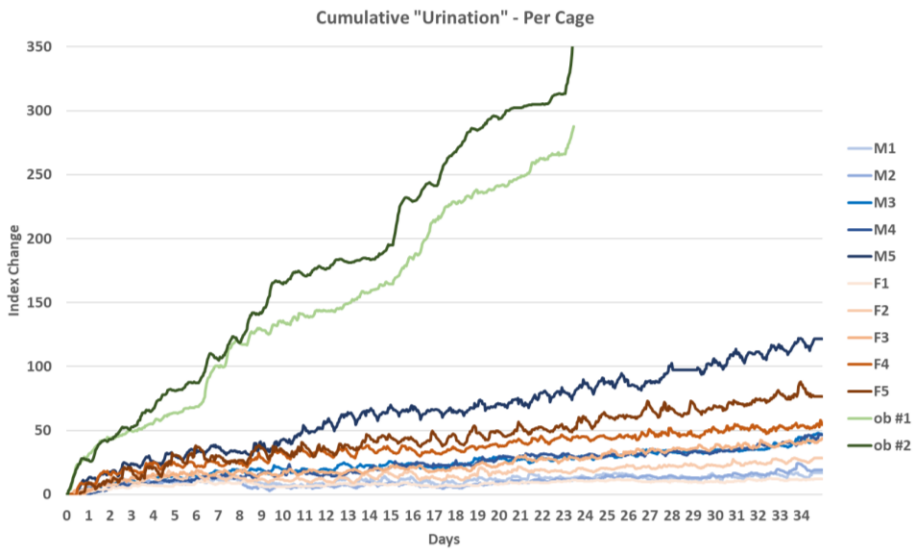


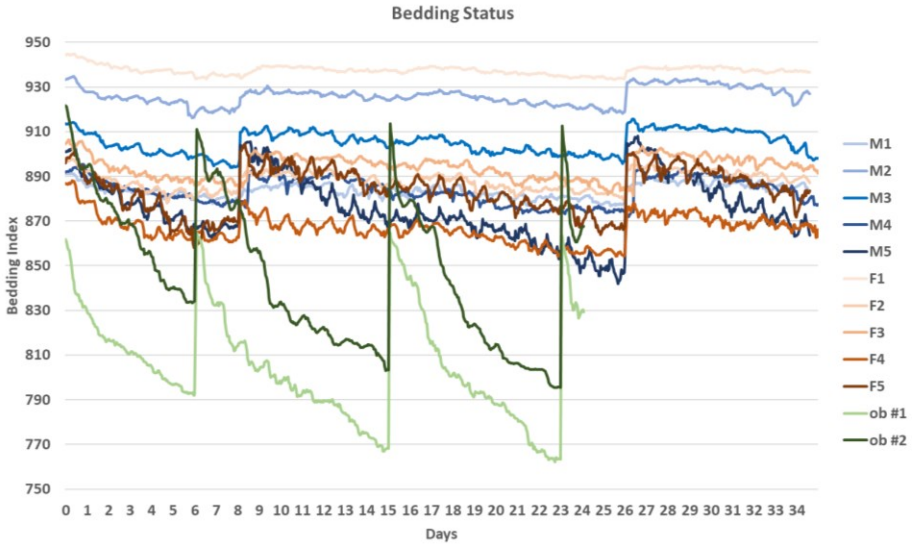



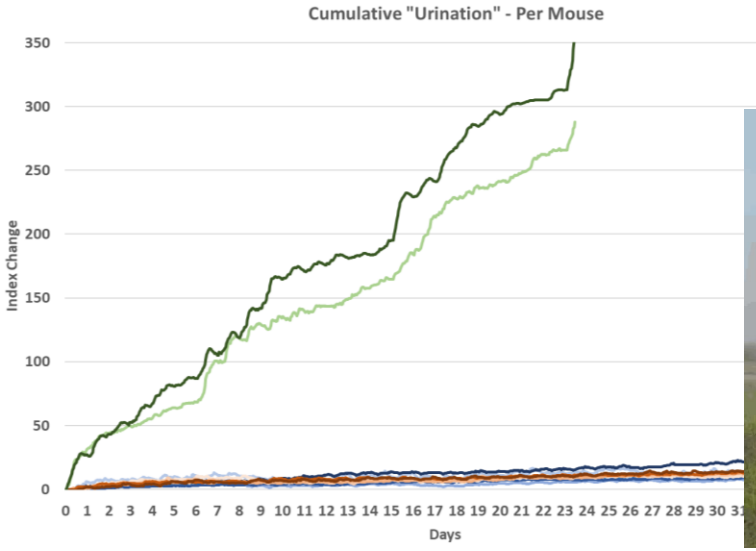



# Testing Linearity

- Proof-of-concept:
  - CD1 mice (Crl:CD1(ICR))
    - 1-5 mice per cage (housing density test)
    - 1 male and 1 female cage per group
    - ~30 weeks old
  - Ob/ob mice (B6.V-Lep$^{ob}$/JRj)
    - 2 male mice
    - ~25 weeks old
    - Individually housed, retired from study
    - Blood glucose consistently "Hi" (i.e. >33.3 mM)



# Pilot Studies

Type 2 Diabetes (University of Copenhagen)

Type 1 Diabetes (Charité, Berlin)

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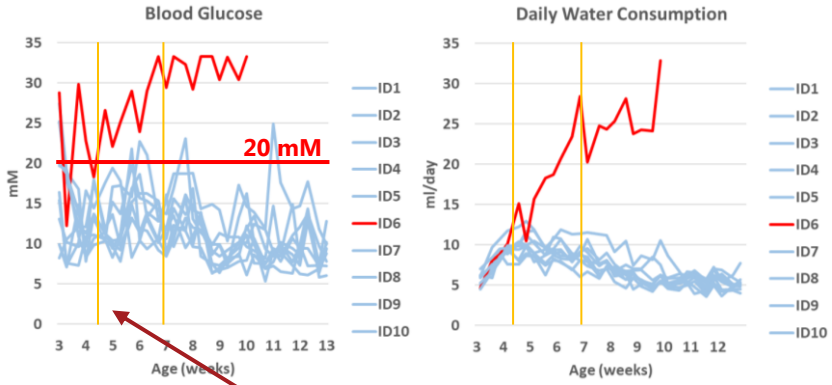
# Pilot Studies

- **Ob/Oh-Pee (Type 2 Diabetes):**
  - 10 male ob/ob mice (B6.V-Lepob/JRj), individually housed in DVC
  - 3 weeks old at study start
  - Blood glucose, body weight, and water consumption recorded 3x/week

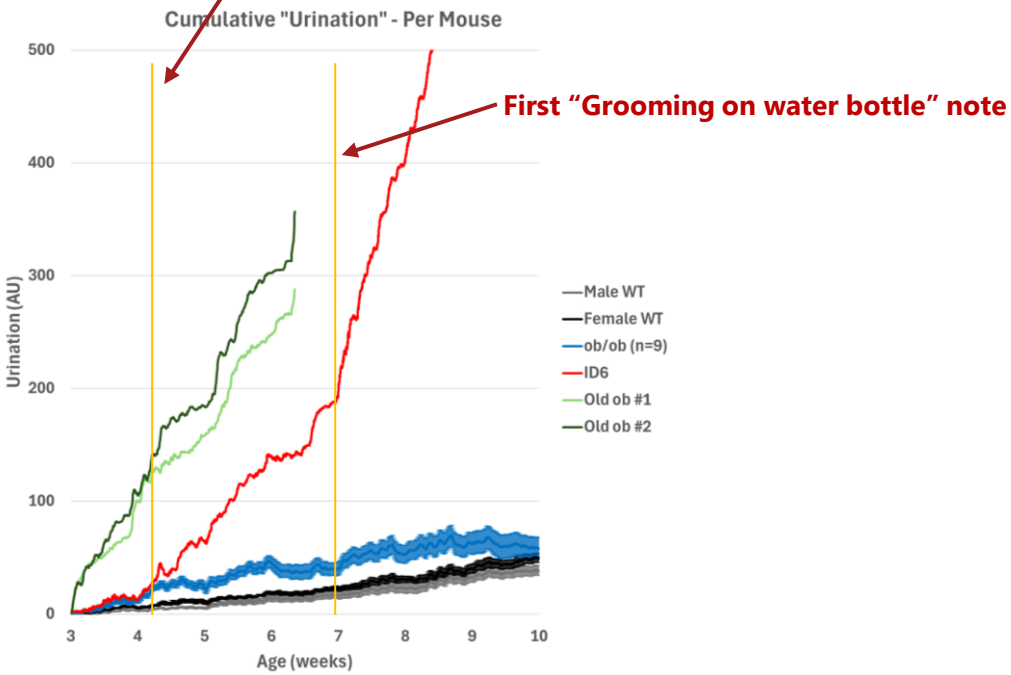
  - Reference groups:
    - 16 male & 16 female C57BL/6JRj mice, 4 per cage in DVC system
    - 3 weeks old at study start
    - DVC data only

# Pilot Studies

- **Ob/Oh-Pee (Type 2 Diabetes):**

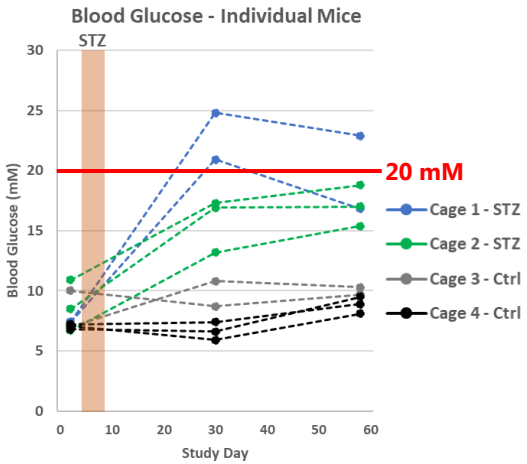


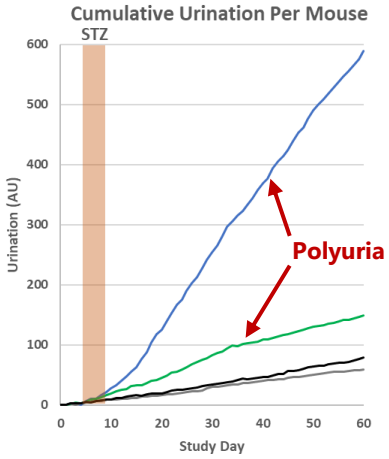

Sustained hyperglycemia from this point onwards

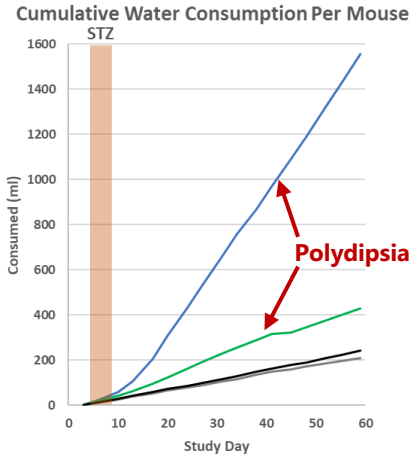


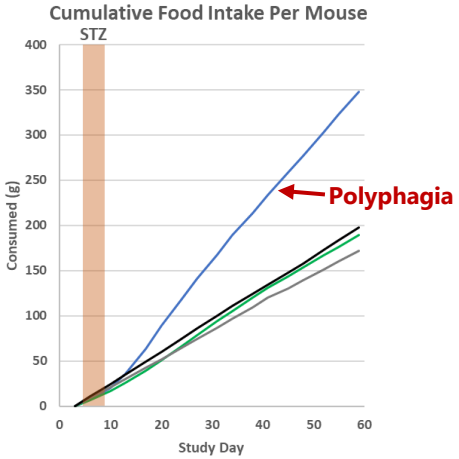

First "Grooming on water bottle" note

- **STZ-Pee (Type 1 Diabetes):**
  - 10 female C57BL/6J, housed in DVC (4 cages)
  - 10 weeks old at study start
  - Blood glucose: week 0, 4, and 8
  - Food (chow) and water consumption: Twice weekly
  - Grouping:
    - Cage 1: STZ (n=2)
    - Cage 2: STZ (n=3)
    - Cage 3: Control (n=2)
    - Cage 4: Control (n=3)
  - STZ treatment: 55 mg/kg daily for 5 consecutive days



# STZ-Pee 2.0

Type 1 Diabetes

Charité, Berlin

TS Nielsen Scientific Consult

# Type 1 Diabetes: STZ-Pee 2.0

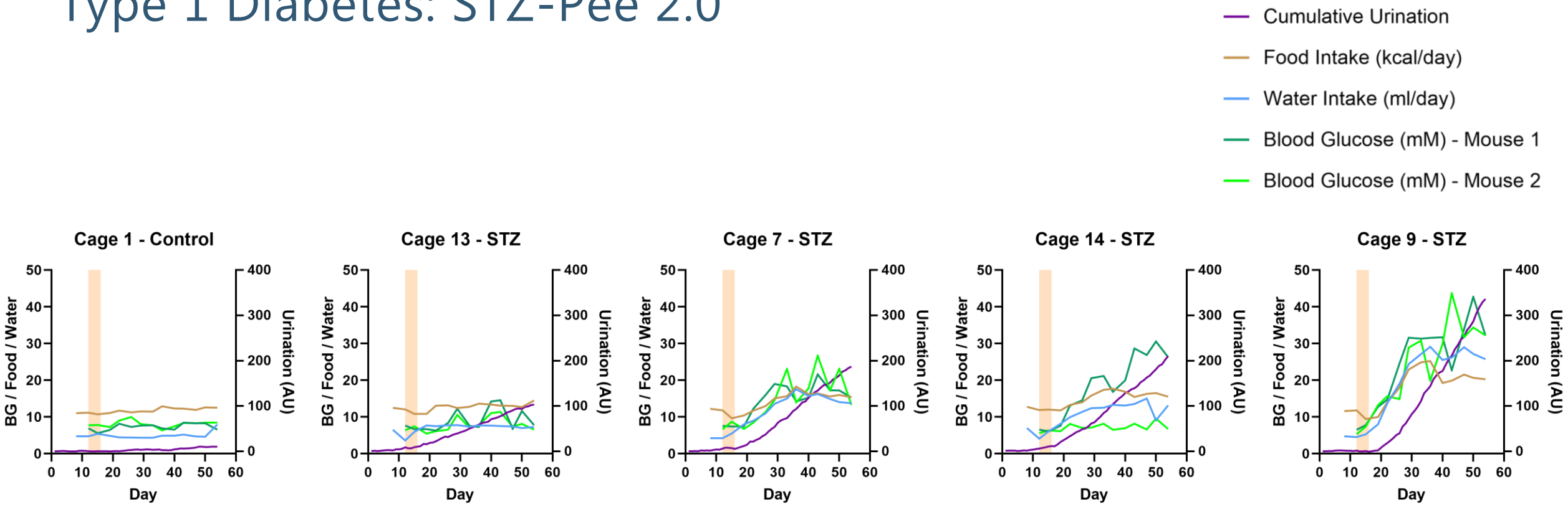
- Study Design:
  - **28 female C57BL/6J, pair-housed in DVC rack (14 cages)**
  - Age at study start: 7 weeks
  - Study duration: 10 weeks

  - Grouping:
    - Cage 1-6: **Control (n=6)**
    - Cage 7-14: **STZ (n=8)**

  - STZ treatment: 55 mg/kg daily for 5 consecutive days

  - Blood glucose, body weight, food, and water consumption recorded twice weekly

# Type 1 Diabetes: STZ-Pee 2.0



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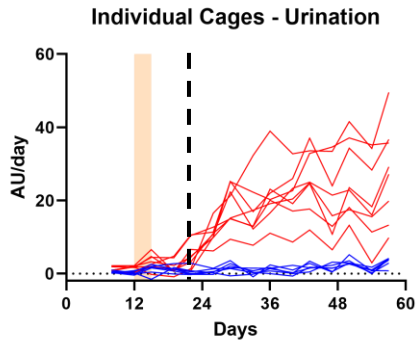
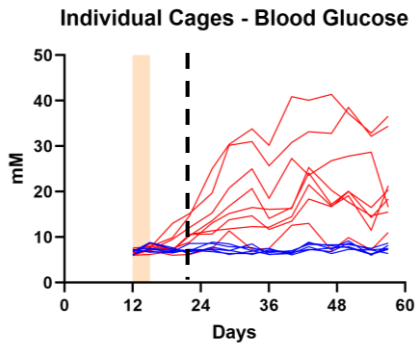
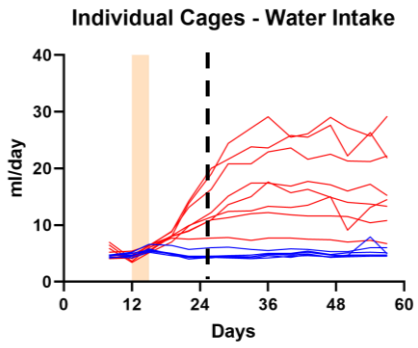
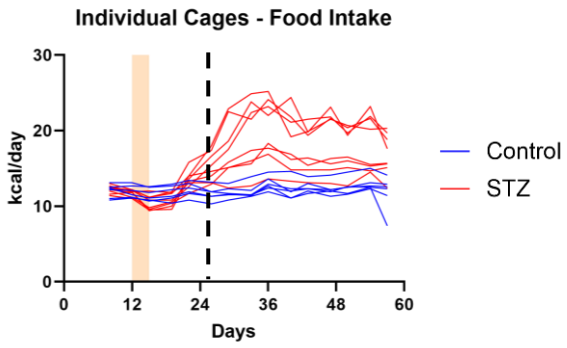
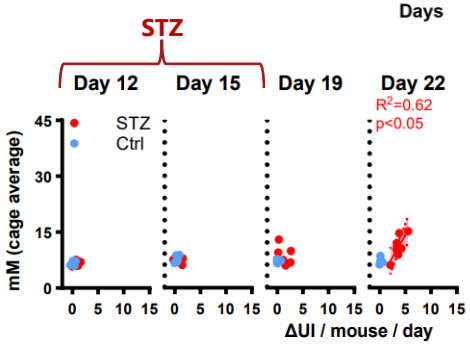


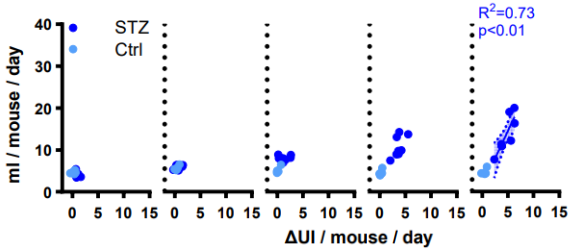




**Blood Glucose vs. UI:**




**Water Intake vs. UI:**

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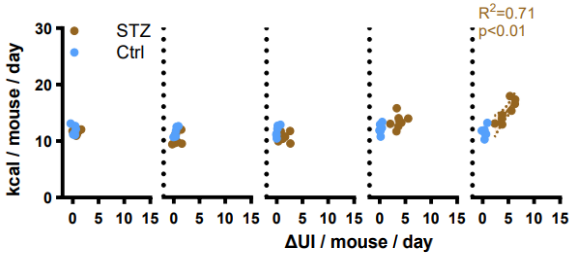
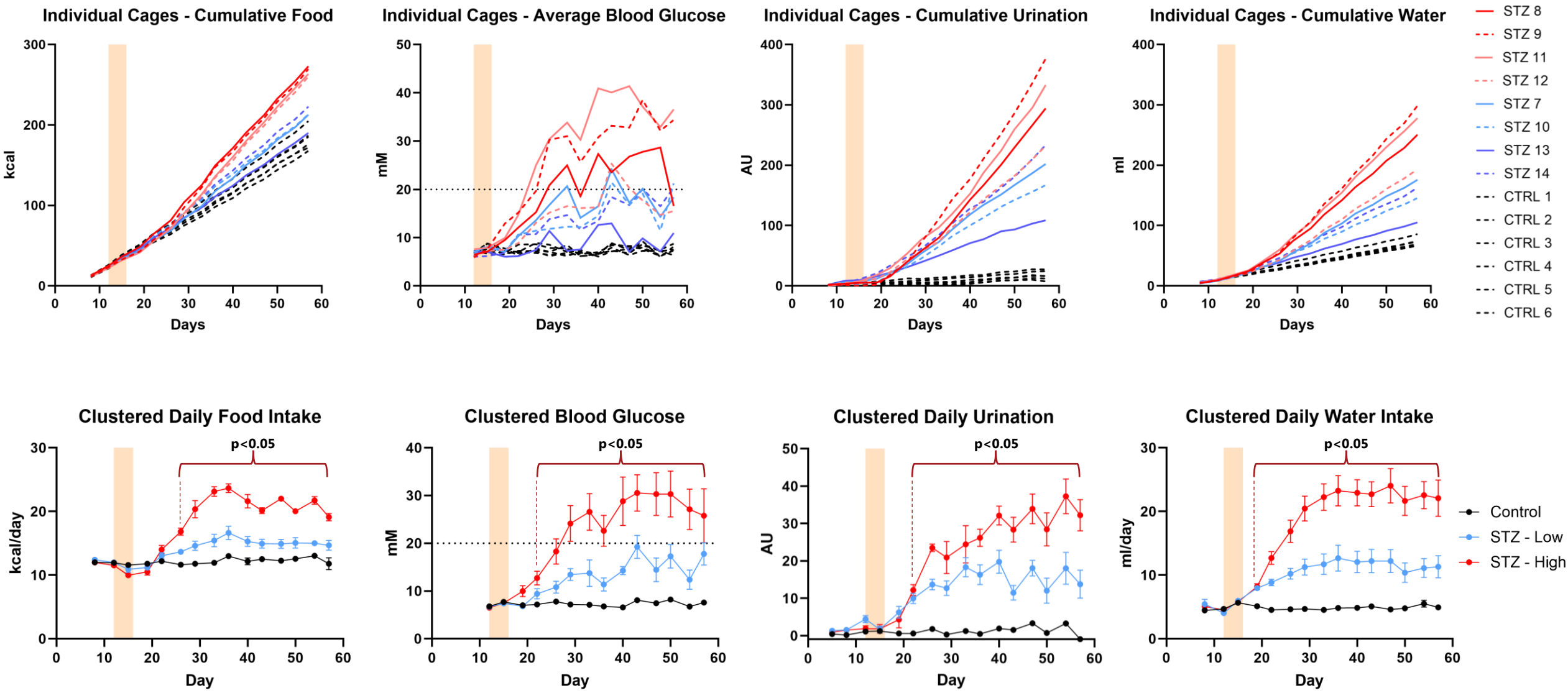


# Type 1 Diabetes: STZ-Pee 2.0



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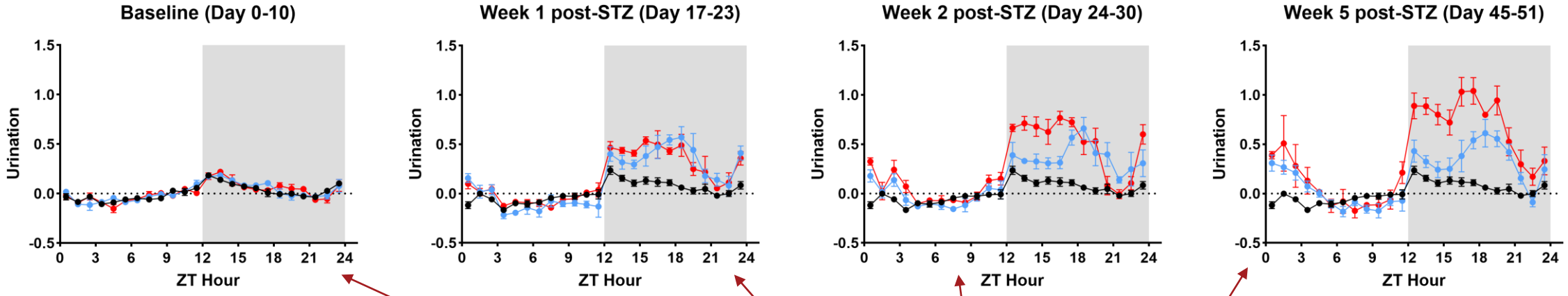


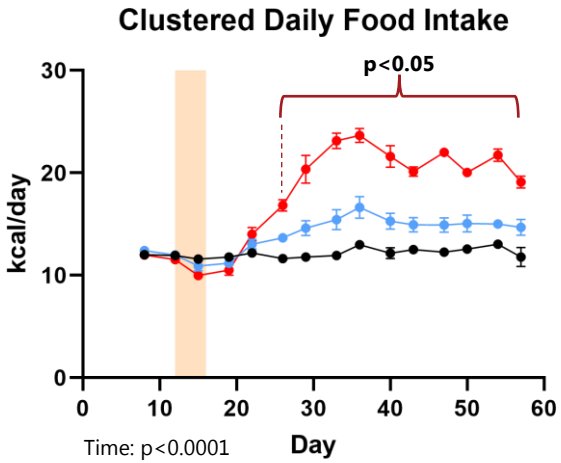


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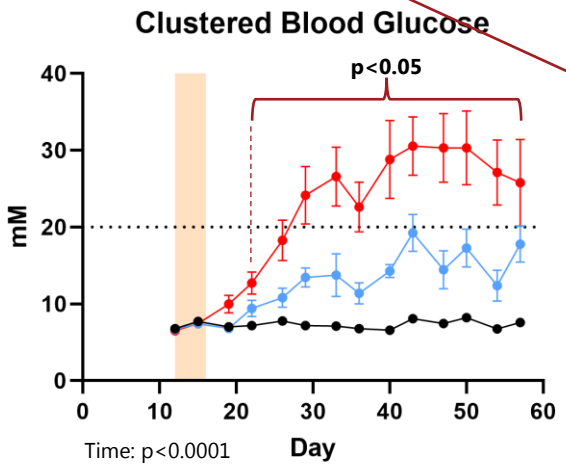
Group: p<0.0001
Time x Group: p<0.0001

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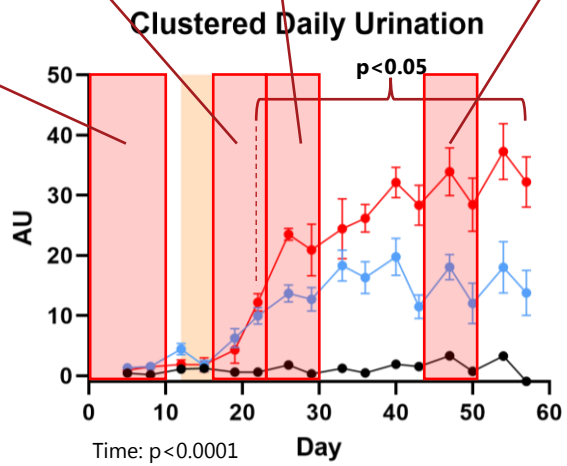


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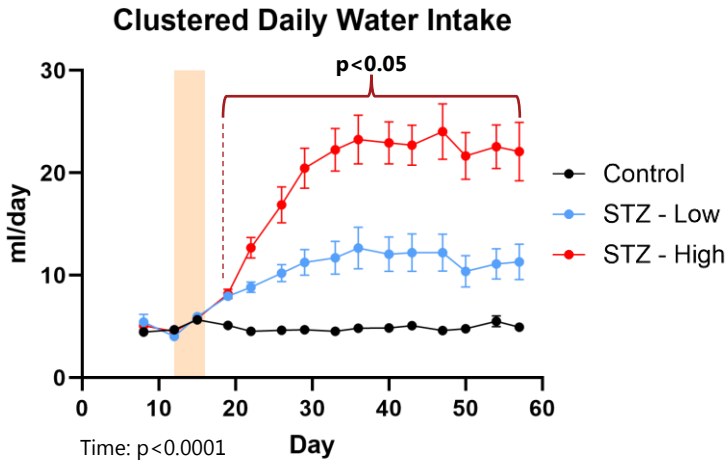


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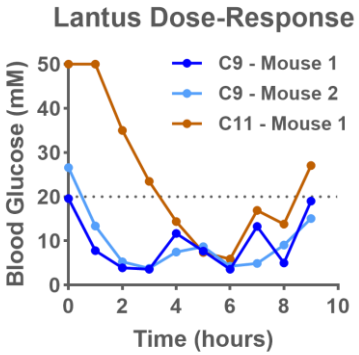
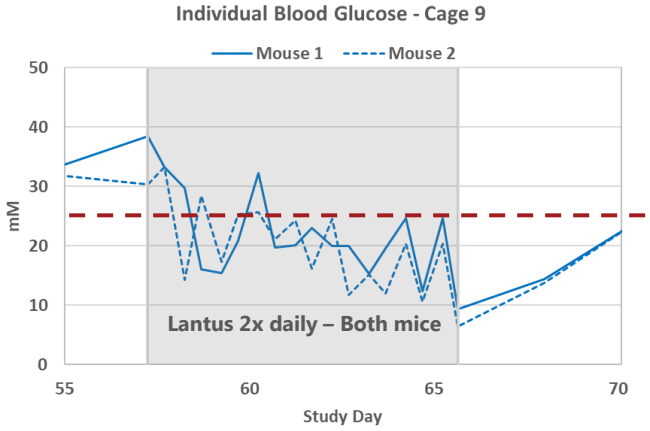
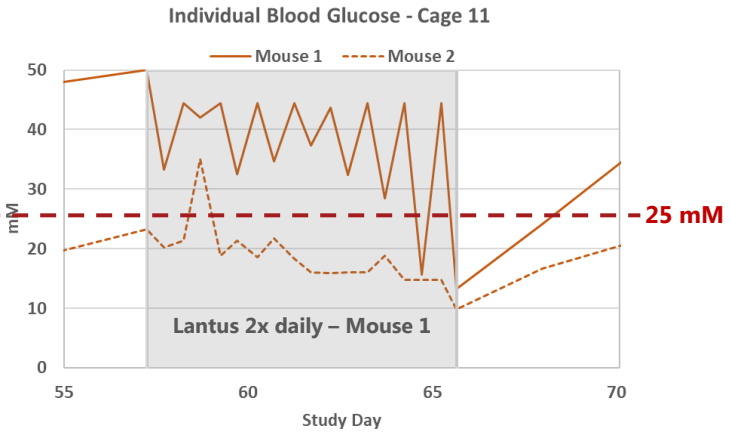
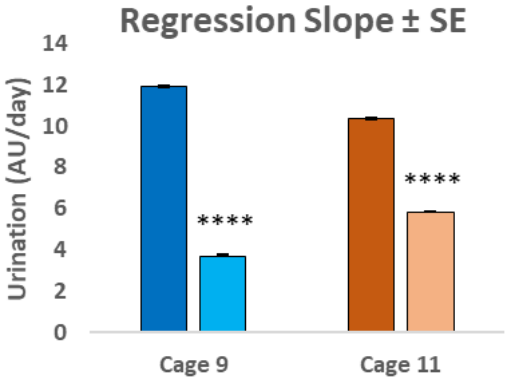
# Type 1 Diabetes: STZ-Pee 2.0

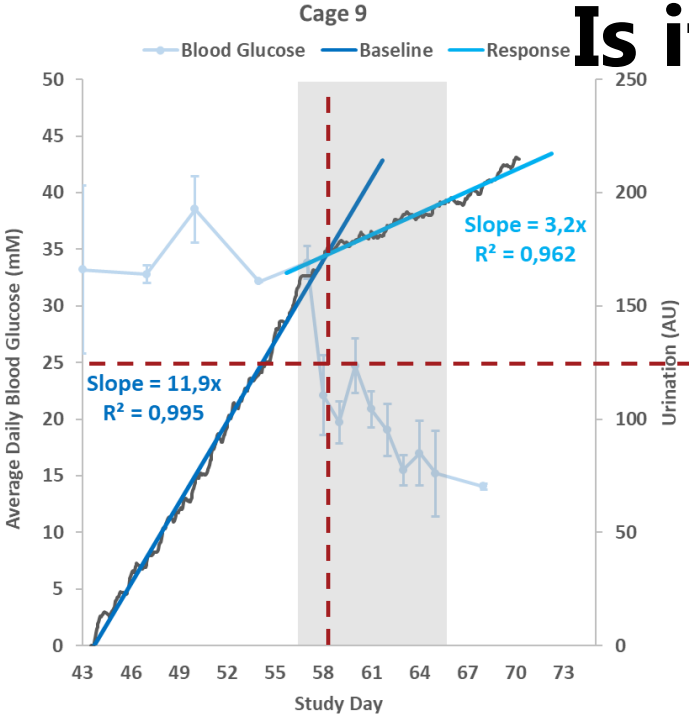


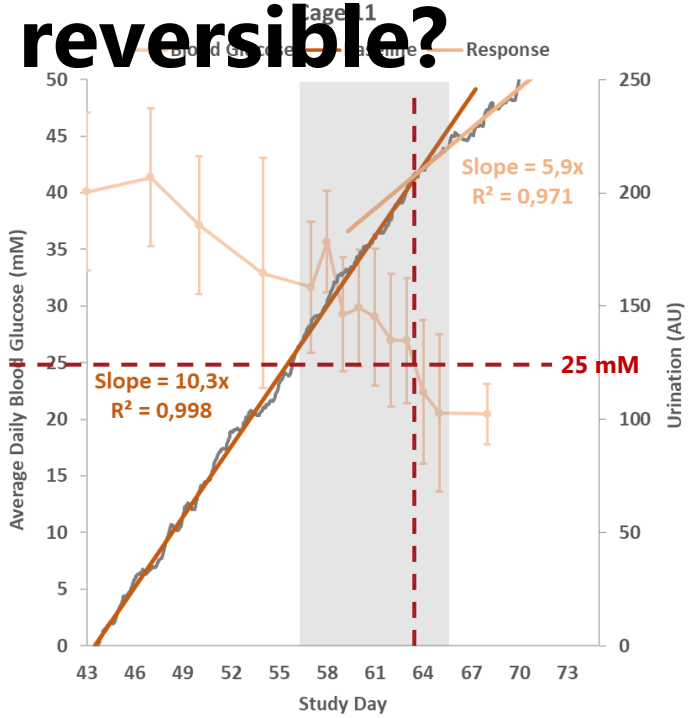



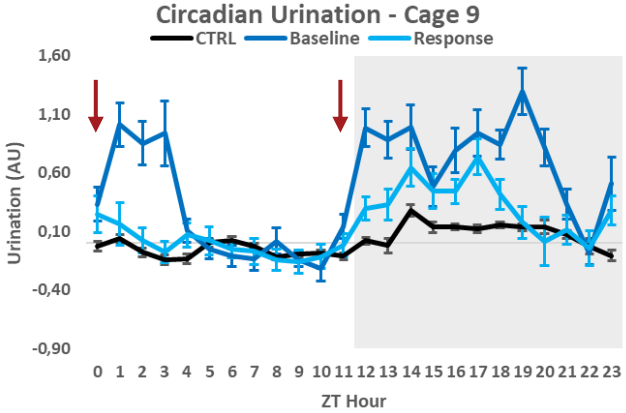



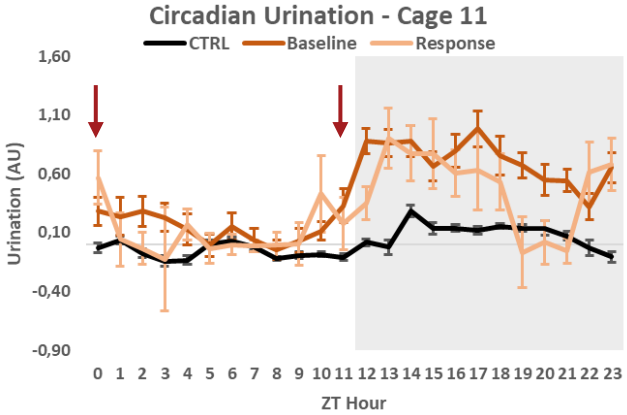




**Is it reversible?**



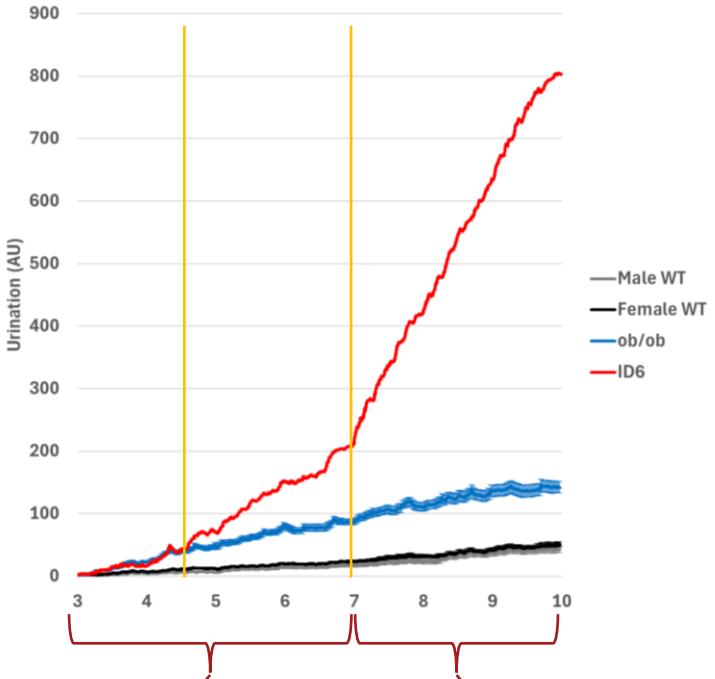
# Observer Effects

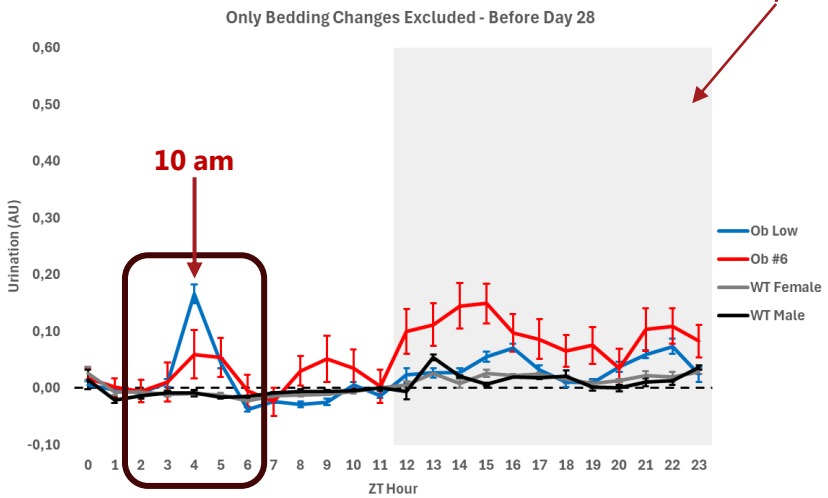
*”What we observe is not nature itself,*

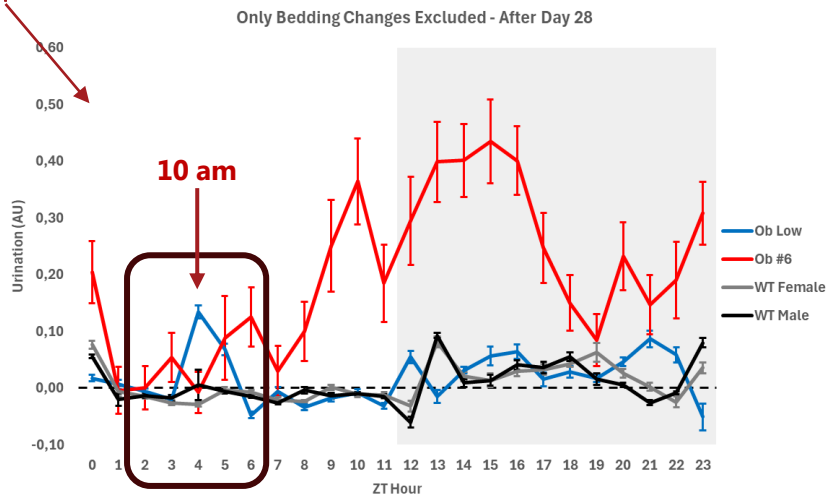
*but nature exposed to our method of questioning”*

*- Werner Heisenberg, 1958*

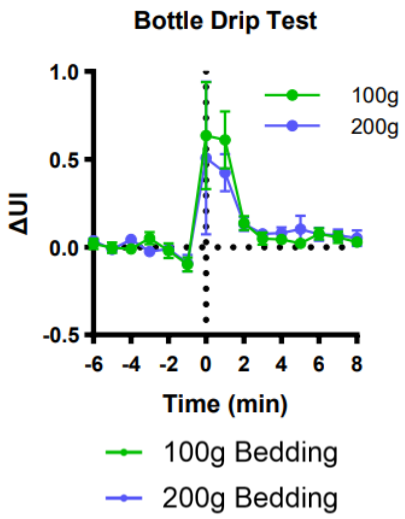
# Observer Effects - Ob/Oh-Pee Study

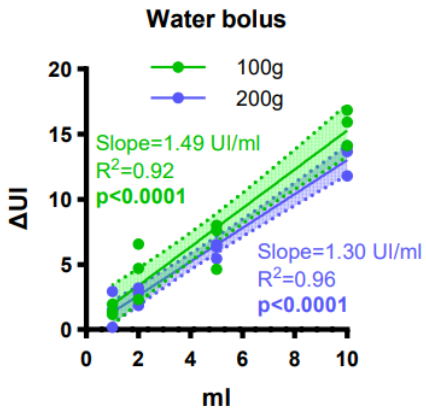


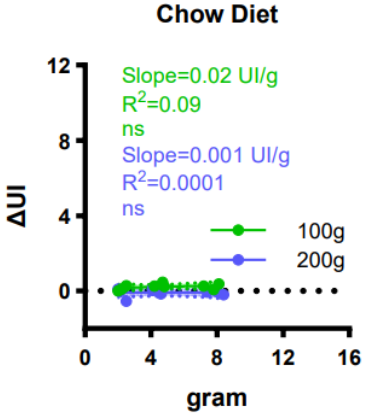



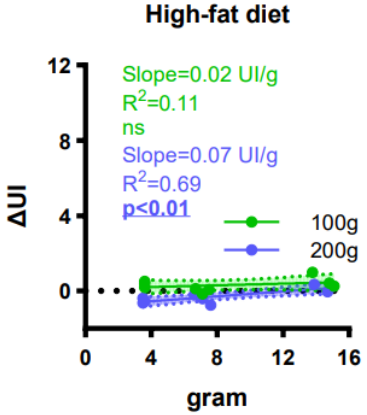



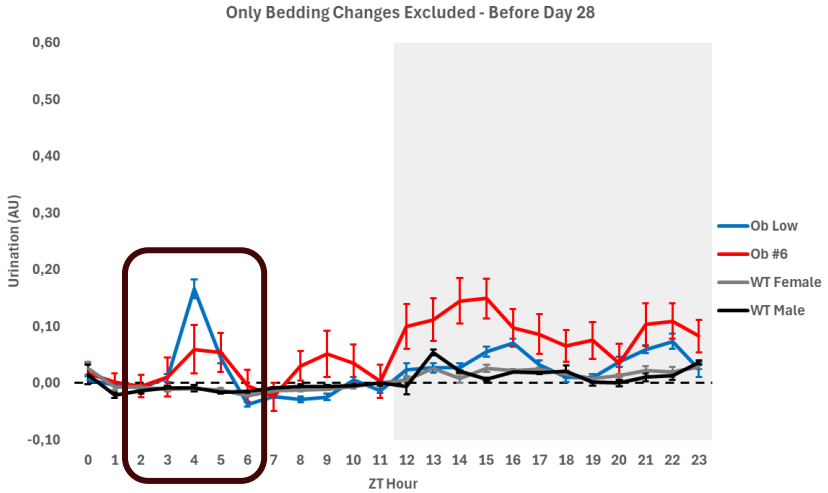
# Observer Effects - Ob/Oh-Pee Study

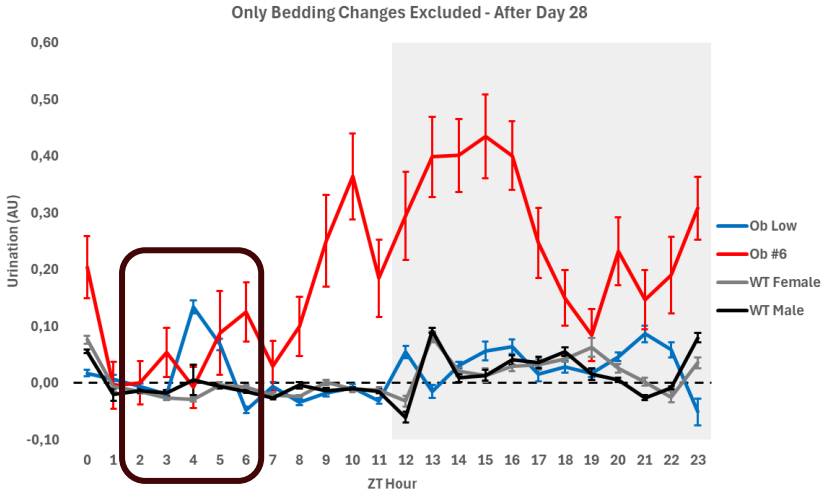


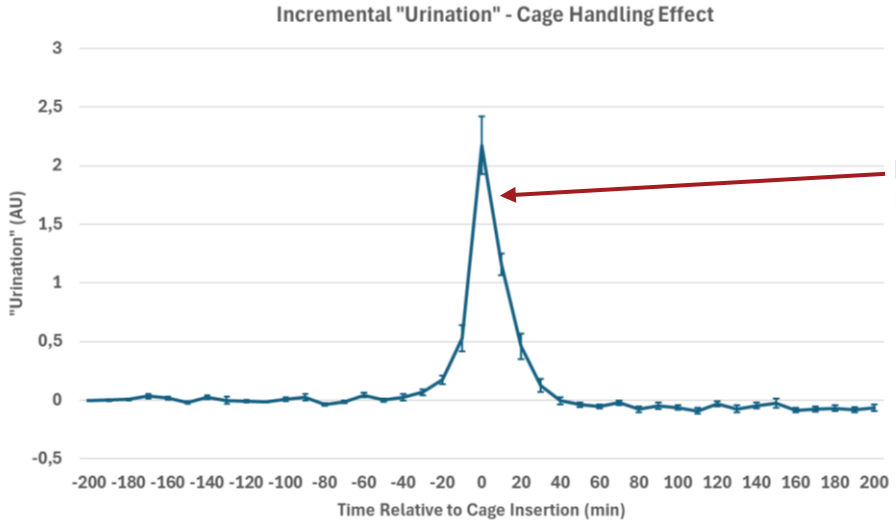



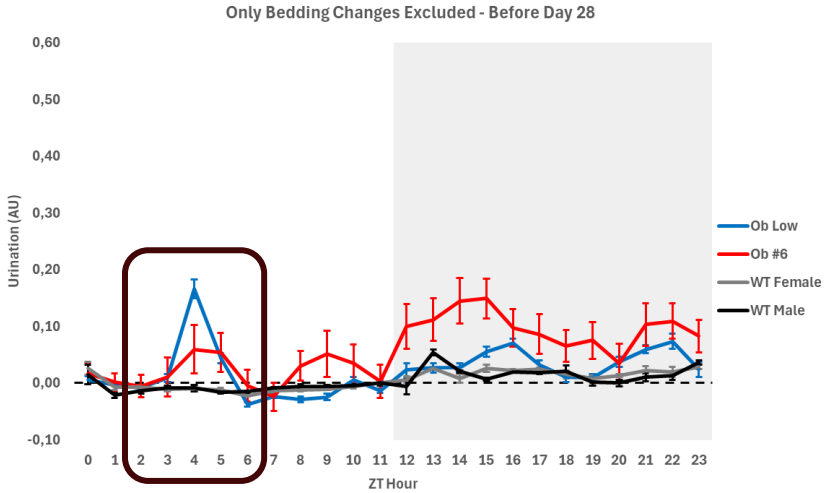



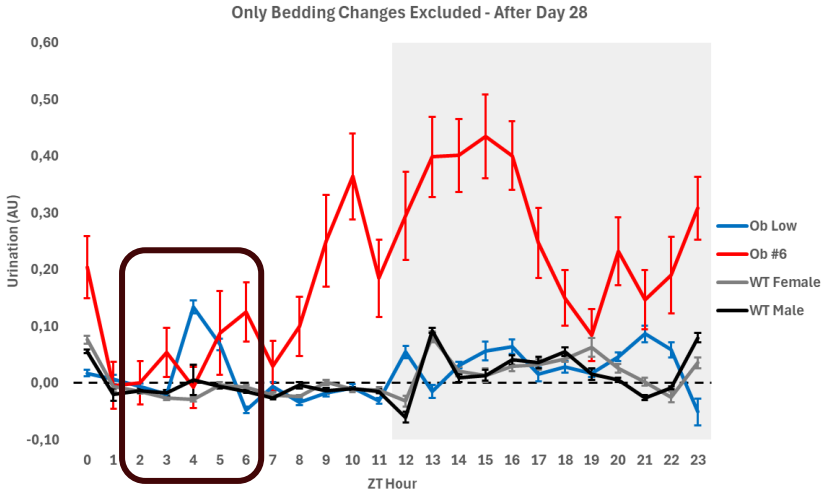



# Observer Effects - Ob/Oh-Pee Study

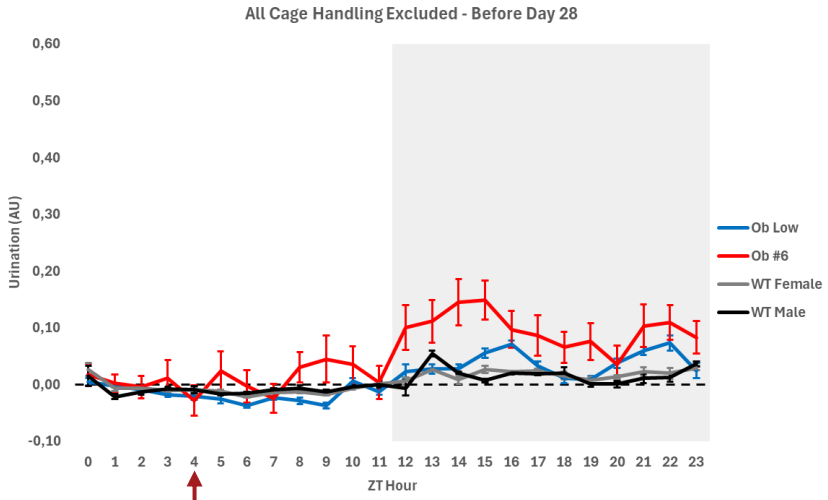


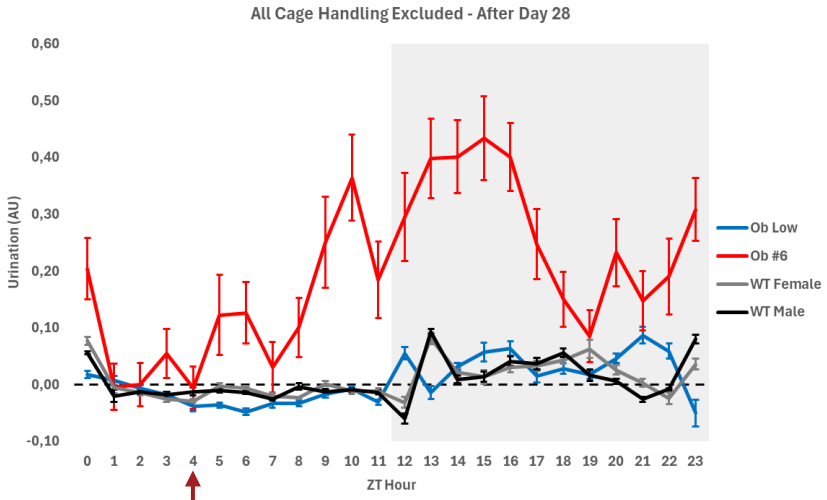

**Blood glucose measurements**
Data aligned to "Cage_Inserted" event

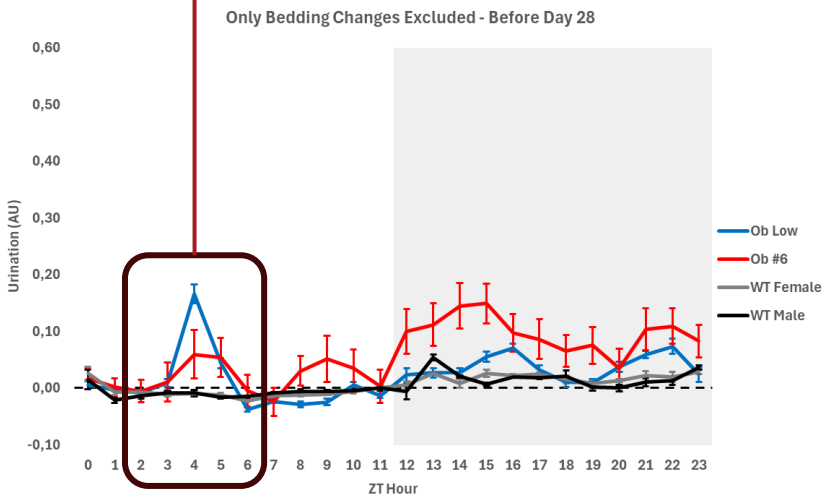


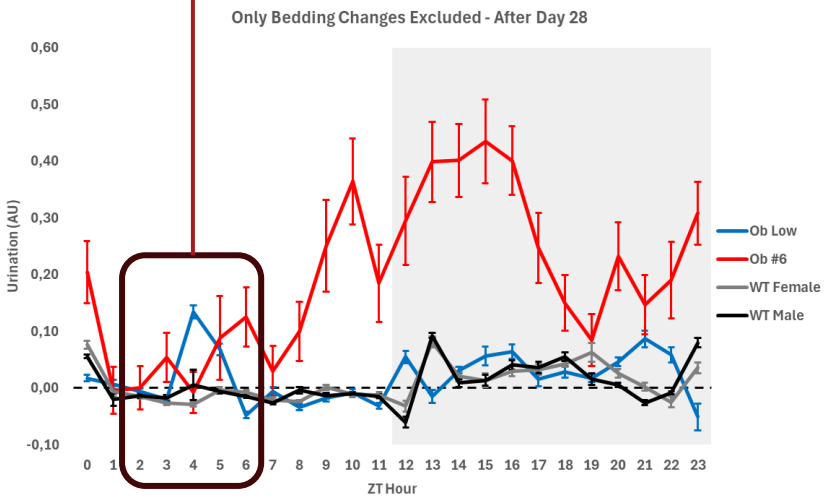



# Observer Effects - Ob/Oh-Pee Study

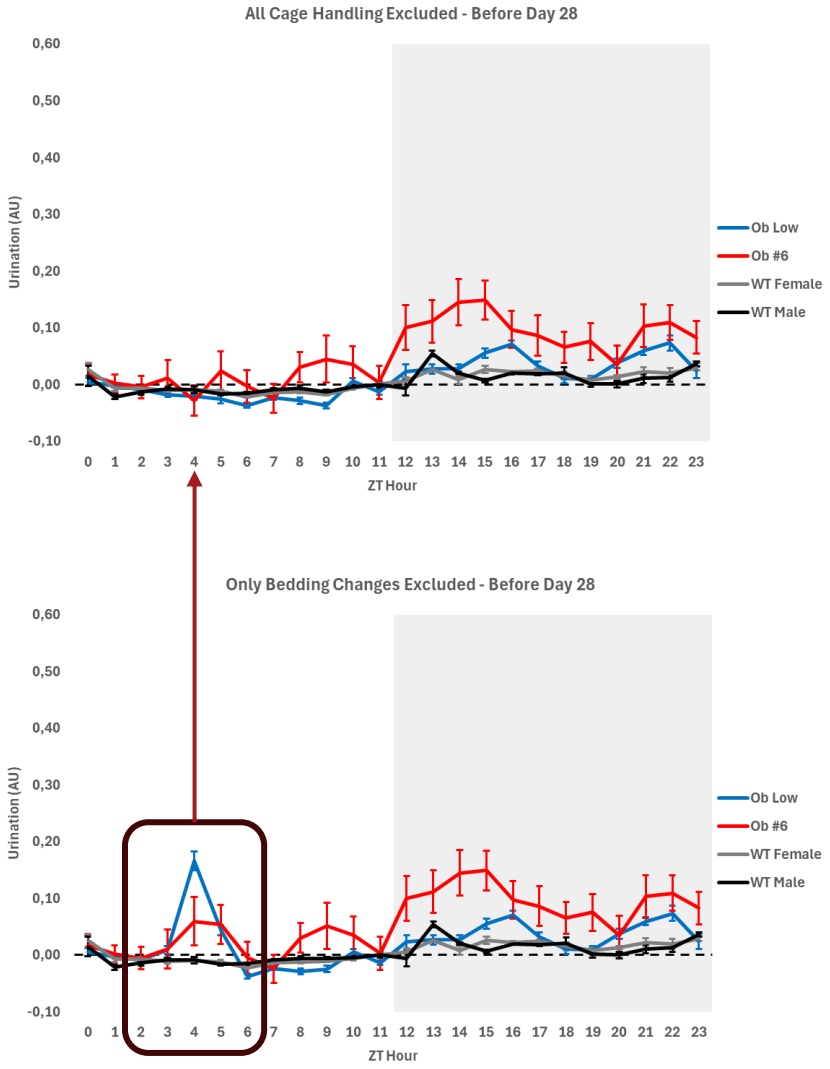


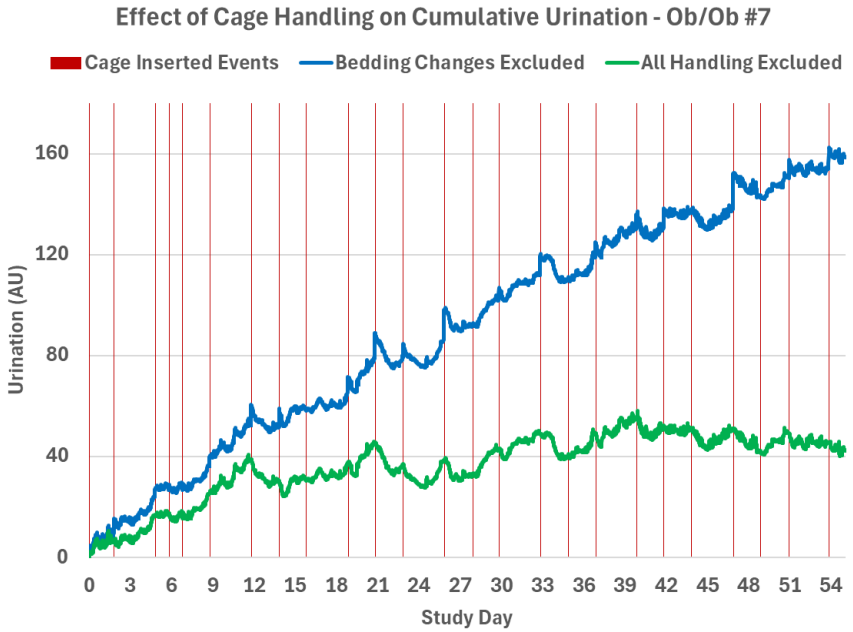



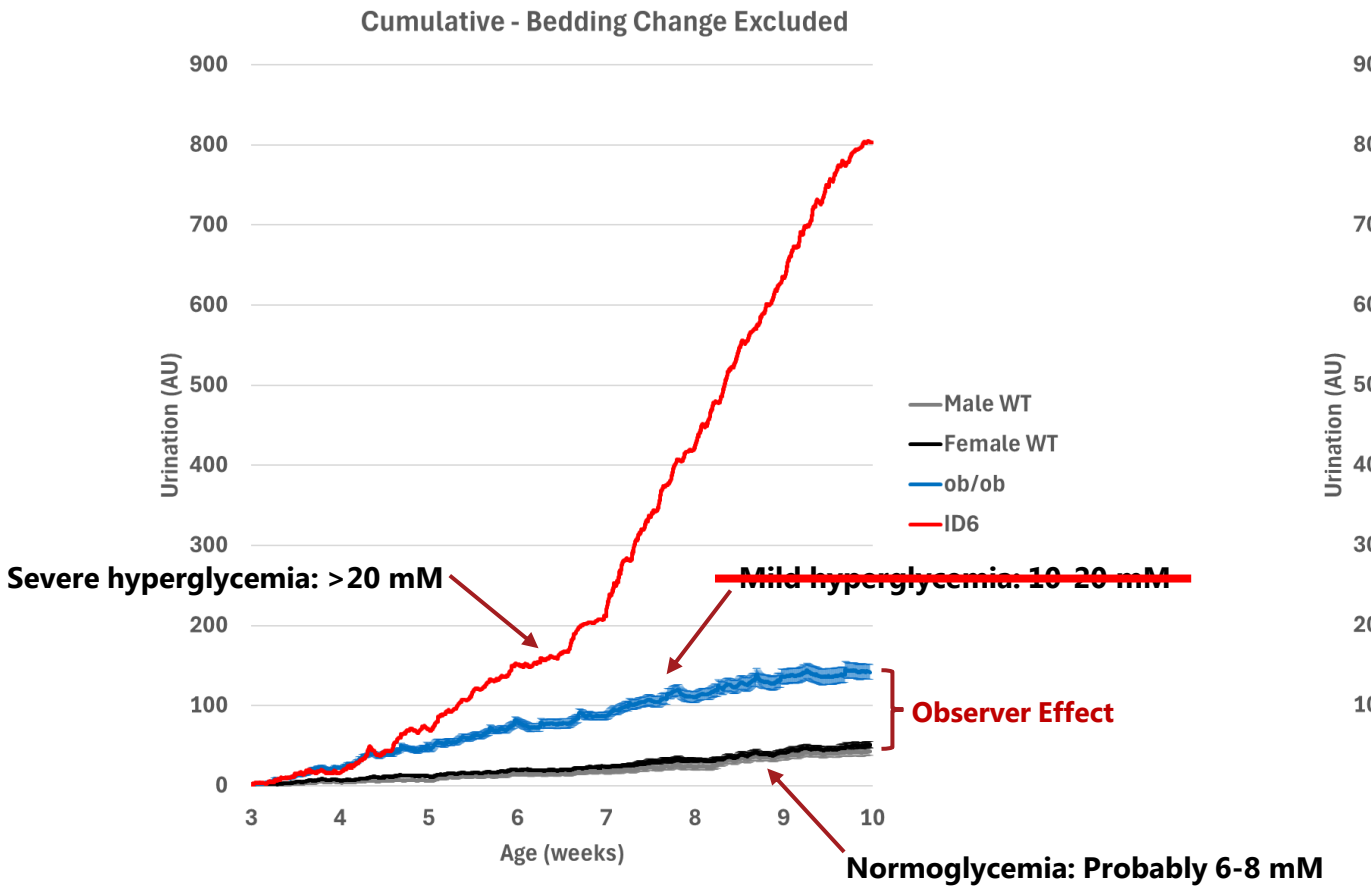



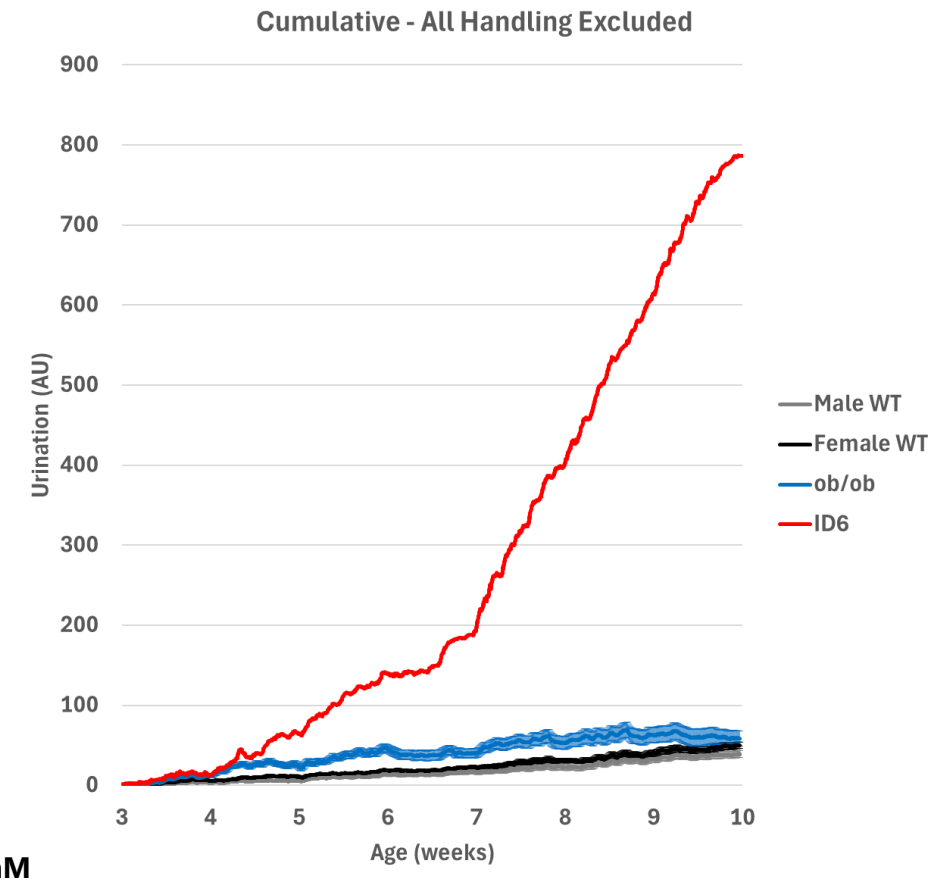
# Observer Effects - Ob/Oh-Pee Study



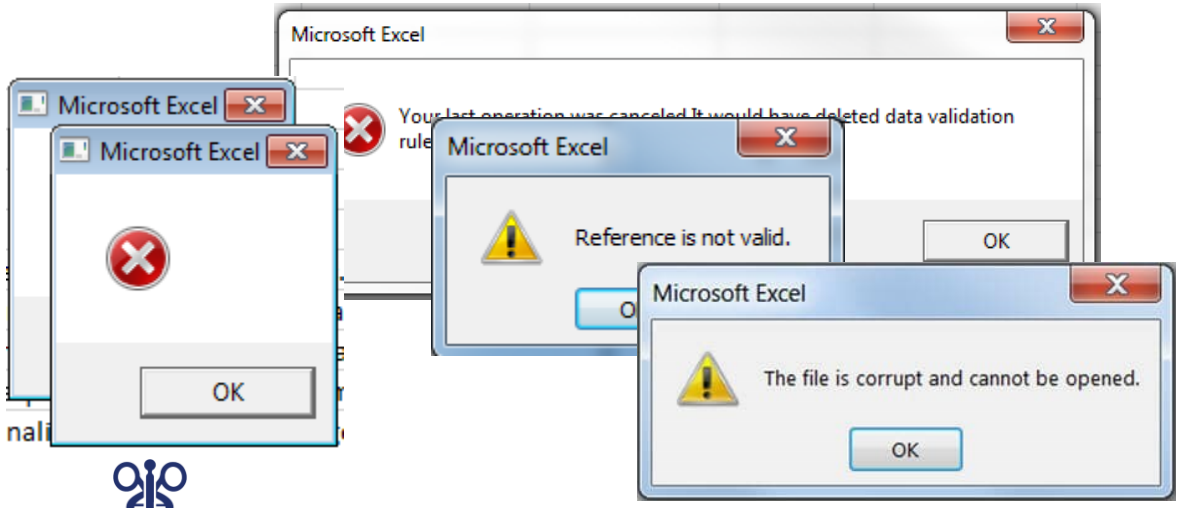



# Observer Effects - Ob/Oh-Pee Study



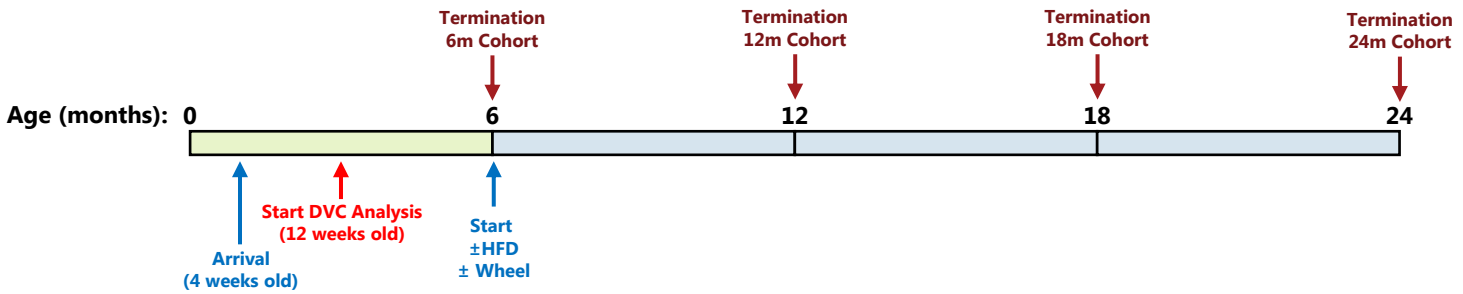



# Data Overload…




TS Nielsen Scientific Consult

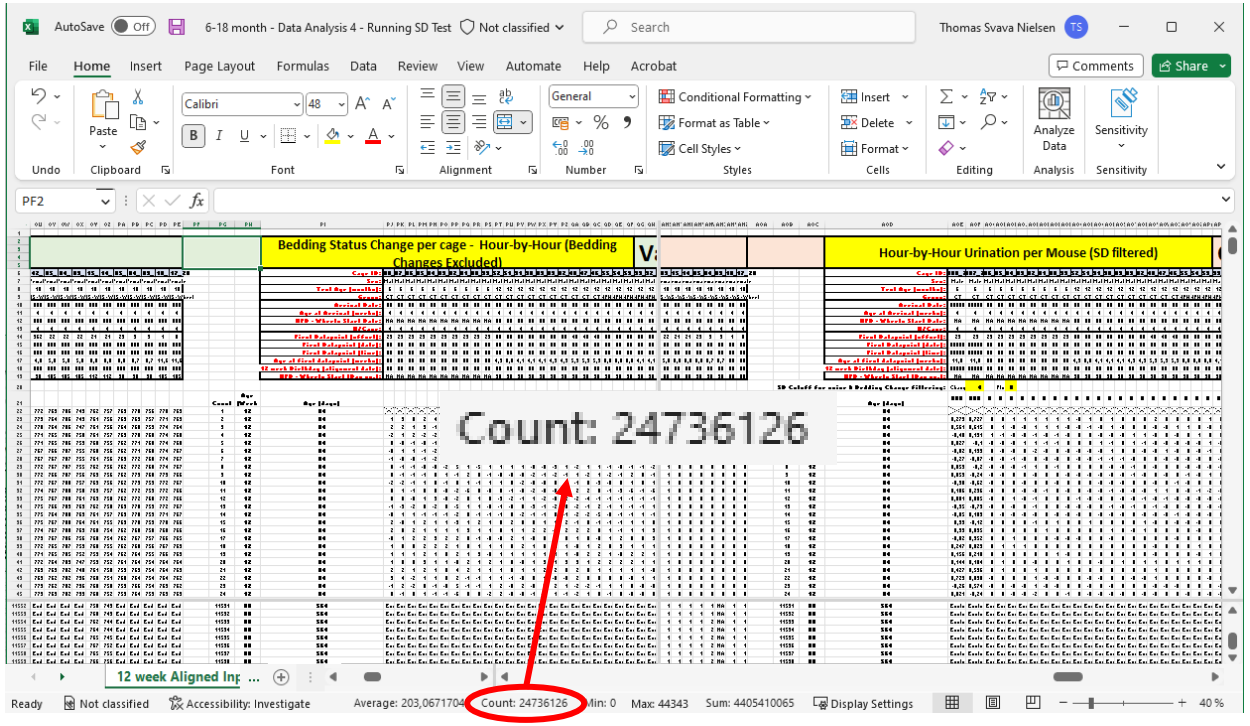
# Data Overload…

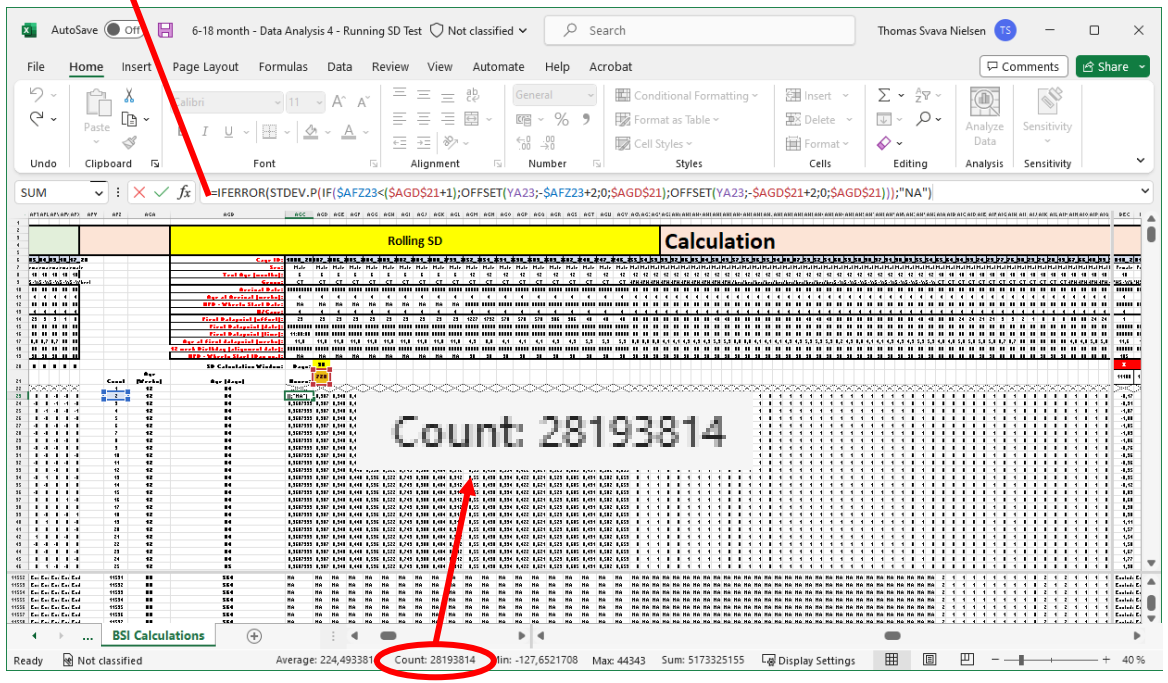



**INSPIRE Cohort (Angelo Parini, Université Toulouse):**

- 1456 mice (Swiss), housed in DVC racks (4 mice/cage)
- 364 cages
- 2 years
- 1 hour resolution

=IFERROR(STDEV.P(IF($AFZ23<($AGD$21+1);OFFSET(YA23;-$AFZ23+2;0;$AGD$21);OFFSET(YA23;-$AGD$21+2;0;$AGD$21)));"NA")



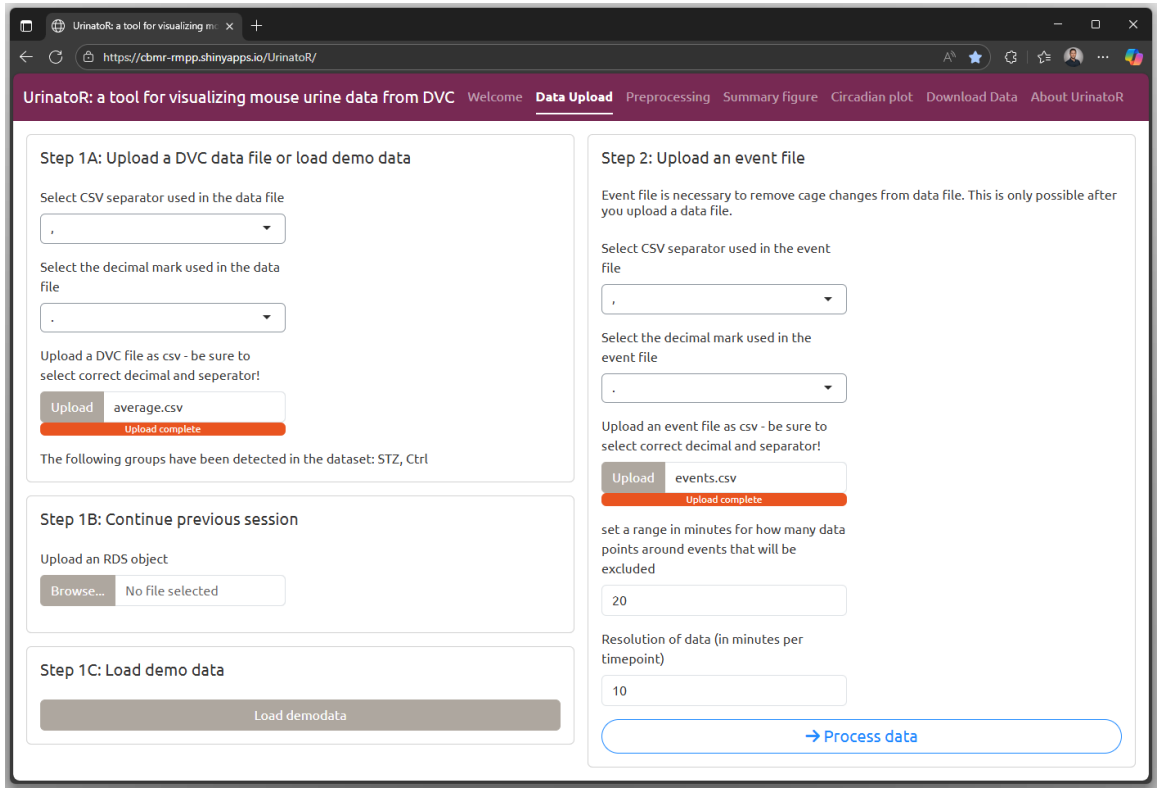

**52.929.940 cells with data…**

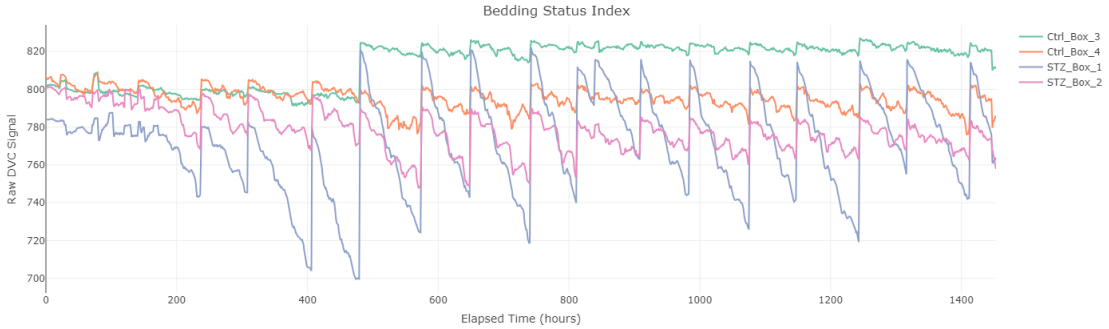
# Automating the Analysis

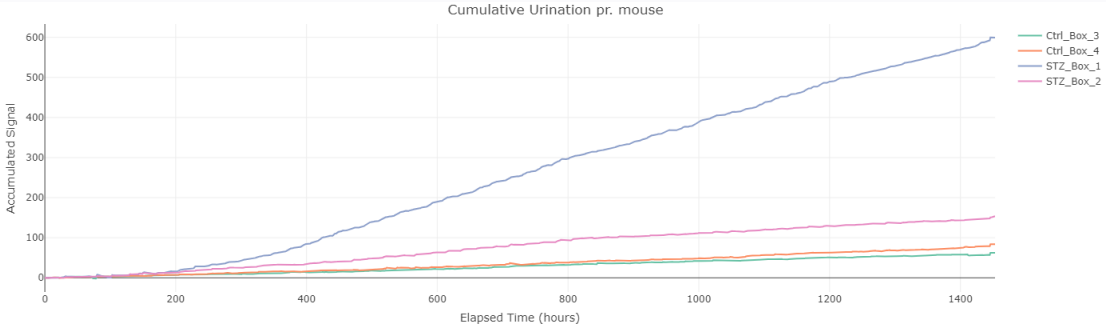
UrinatoR

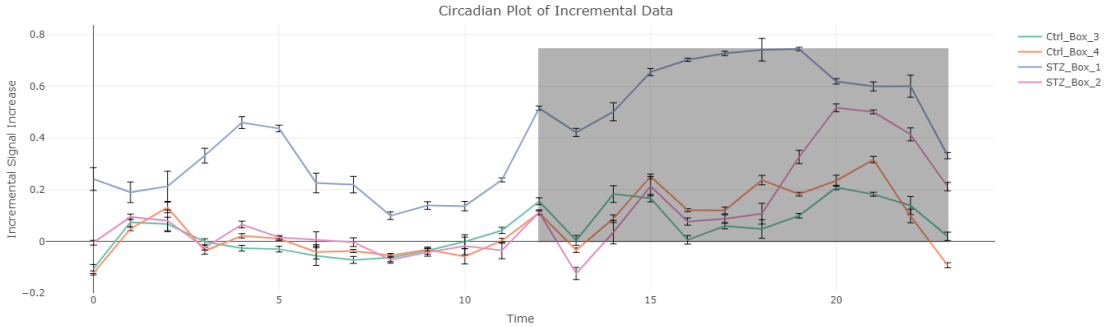
# UrinatoR

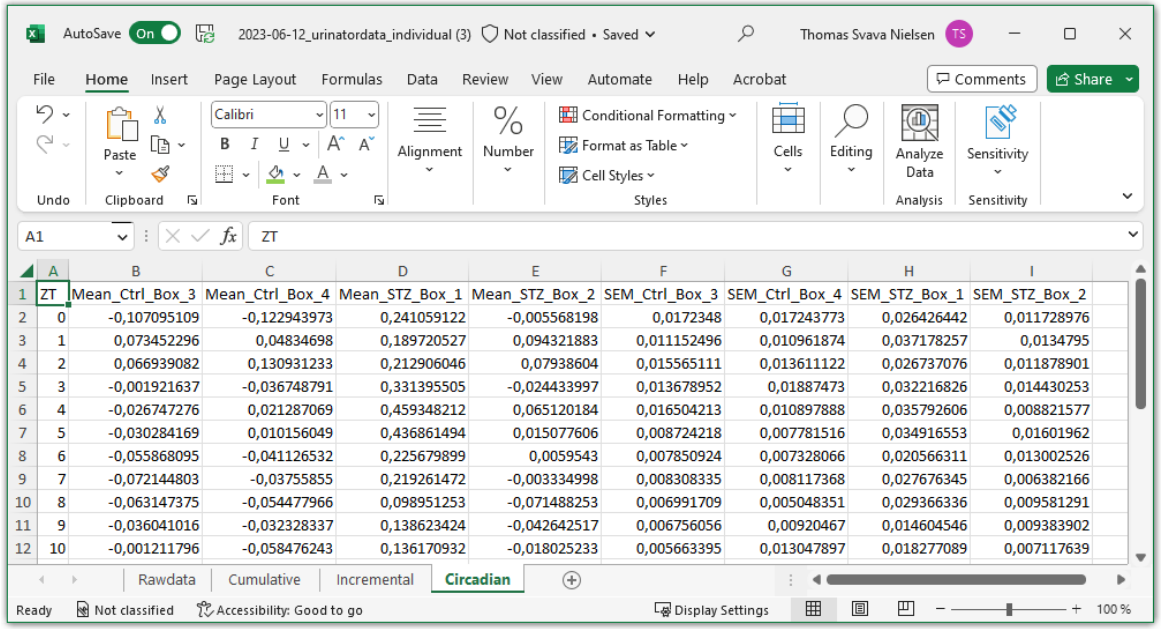
**UrinatoR How-To & Link to App and Pre-Print: [www.tsnscientific.com/urinator](www.tsnscientific.com/urinator)**



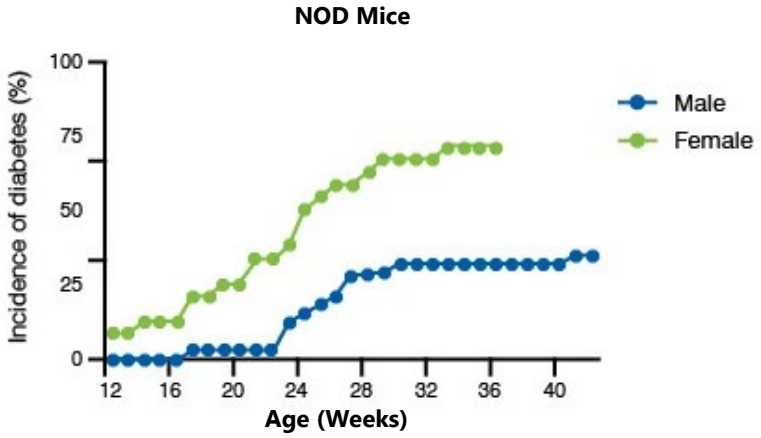




| ZT | Mean_Ctrl_Box_3 | Mean_Ctrl_Box_4 | Mean_STZ_Box_1 | Mean_STZ_Box_2 | SEM_Ctrl_Box_3 | SEM_Ctrl_Box_4 | SEM_STZ_Box_1 | SEM_STZ_Box_2 |
|---|---|---|---|---|---|---|---|---|
| 0 | -0,107095109 | -0,122943973 | 0,241059122 | -0,005568198 | 0,0172348 | 0,017243773 | 0,026426442 | 0,011728976 |
| 1 | 0,073452296 | 0,04834698 | 0,189720527 | 0,094321883 | 0,011152496 | 0,010961874 | 0,037178257 | 0,0134795 |
| 2 | 0,066939082 | 0,130931233 | 0,212906046 | 0,07938604 | 0,015565111 | 0,013611122 | 0,026737076 | 0,011878901 |
| 3 | -0,001921637 | -0,036748791 | 0,331395505 | -0,024433997 | 0,013678952 | 0,01887473 | 0,032216826 | 0,014430253 |
| 4 | -0,026747276 | 0,021287069 | 0,459348212 | 0,065120184 | 0,016504213 | 0,010897888 | 0,035792606 | 0,008821577 |
| 5 | -0,030284169 | 0,010156049 | 0,436861494 | 0,015077606 | 0,008724218 | 0,007781516 | 0,034916553 | 0,01601962 |
| 6 | -0,055868095 | -0,041126532 | 0,225679899 | 0,0059543 | 0,007850924 | 0,007328066 | 0,020566311 | 0,013002526 |
| 7 | -0,072144803 | -0,03755855 | 0,219261472 | -0,003334998 | 0,008308335 | 0,008117368 | 0,027676345 | 0,006382166 |
| 8 | -0,063147375 | -0,054477966 | 0,098951253 | -0,071488253 | 0,006991709 | 0,005048351 | 0,029366336 | 0,009581291 |
| 9 | -0,036041016 | -0,032328337 | 0,138623424 | -0,042642517 | 0,006756056 | 0,00920467 | 0,014604546 | 0,009383902 |
| 10 | -0,001211796 | -0,058476243 | 0,136170932 | -0,018025233 | 0,005663395 | 0,013047897 | 0,018277089 | 0,007117639 |

# Perspectives

## UrinatoR




- Elimination of screening blood/urine samples
- Remote monitoring of disease progression and severity
- Timing of interventions according to date of diabetes appearance
- Continuous assessment of intervention efficacy
- Detailed analysis of ambulatory and stress-free longitudinal and circadian changes in metabolism

- **Additionally, with integration in DVC server:**
- Continuous high-resolution monitoring of entire animal facility
- Automatic cage-level “Diabetes alerts” sent to researcher

DVC ANALYTICS
SCIENTIFIC PLATFORM

# Acknowledgements

**Rodent Metabolic Phenotyping Platform**

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Christian Oeing

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Inserm
La science pour la santé
From science to health
cnrs

**Tecniplast Digilab Team**

Stefano Gaburro

Giorgio Rosati

TECNIPLAST
innovation through passion
DVC
SCIENTIFIC PLATFORM

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- Home Cage Monitoring & Digital Biomarker Development
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www.TSNscientific.com thomas@TSNscientific.com +45 2685 6339

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  - Rent-a-surgeon: In-house preparation of operated animals for your project
  - Surgical training courses tailored to your needs
- **Development and training on advanced experimental models:**
  - Freely moving infusion/sampling models (acute or long-term)
  - Continuous home cage monitoring
  - Data analysis & digital biomarkers
- **Cutting-edge technologies and refinement tools:**

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VIGILITECH ADVANCED ANIMAL MONITORING
Kent Scientific
OLDEN LABS
Hundred

## Thank you for your attention!

**To pee, or not to pee, that is the question!**