

# PhenoMaster: how to plan an indirect calorimetry experiment

SFB 1506 Seminar Series

Institute of Molecular Endocrinology and Physiology (IMEP)

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# Why use indirect calorimetry?

- Non-invasive whole-animal phenotyping of energy metabolism
- Links molecular pathways to integrated physiology in mice and rats
- Essential when body weight changes could reflect altered intake, activity, thermogenesis, or substrate selection
- Particularly useful in **aging studies** because age modifies basal metabolism, metabolic flexibility, activity, and thermoregulatory costs
- Respiratory exchange ratio, food and water intake, locomotor activity, and circadian patterns when integrated with metabolic cages
- Responses to fasting, feeding, pharmacology, thermogenic stimulation, or genetic manipulation



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- Respiratory exchange ratio, food and water intake, locomotor activity, and circadian patterns when integrated with metabolic cages
- Responses to fasting, feeding, pharmacology, thermogenic stimulation, or genetic manipulation
- Oxygen consumption ( $\text{VO}_2$ ) & carbon dioxide production ( $\text{VCO}_2$ )
- Energy expenditure derived from respiratory gas exchange as **respiratory exchange rate (RER)**
- **$\text{RER} = \text{VCO}_2 / \text{VO}_2$** 
  - RER: 0.7-0.8 low intensity exercise; fat as fuel
  - RER: 1 intense exercise; carbohydrates as fuel
  - RER: >1 high intensity exercise; anaerob



# Why use indirect calorimetry?

## INDIRECT CALORIMETRY IN LABORATORY ANIMALS

Measuring metabolism through gas exchange to assess energy expenditure



Non-invasive  
and continuous



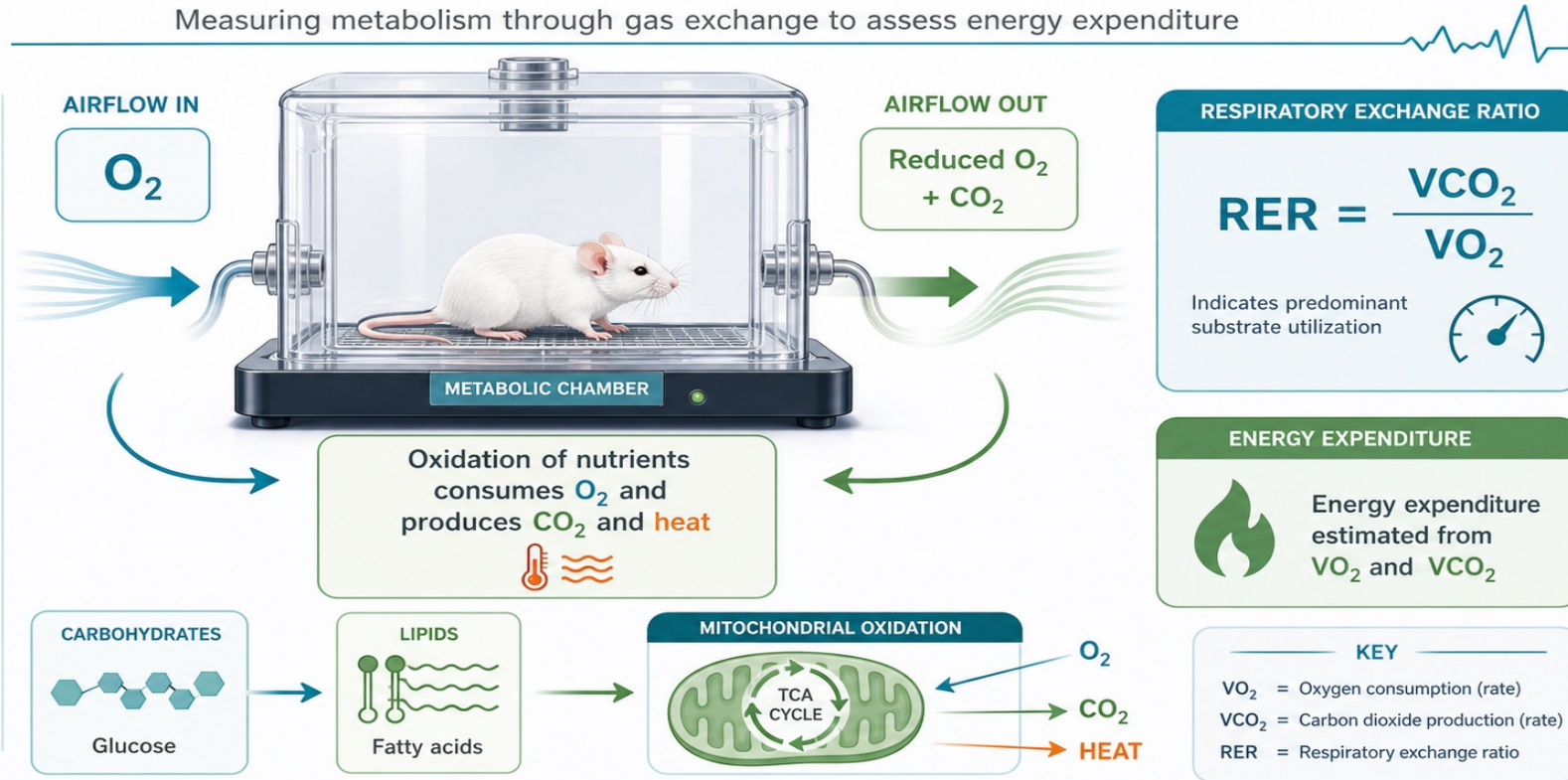
Physiological  
relevant



Real-time  
measurements



High sensitivity  
and precision



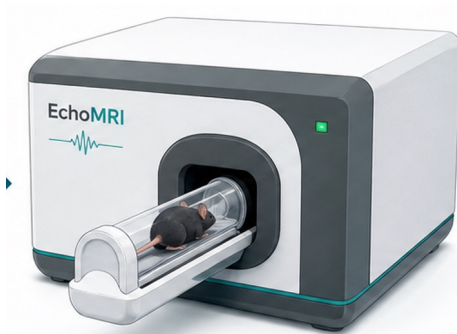
Perplexity.ai

Energy expenditure  $\approx$  Weir  
equation from  $VO_2$  and  $VCO_2$

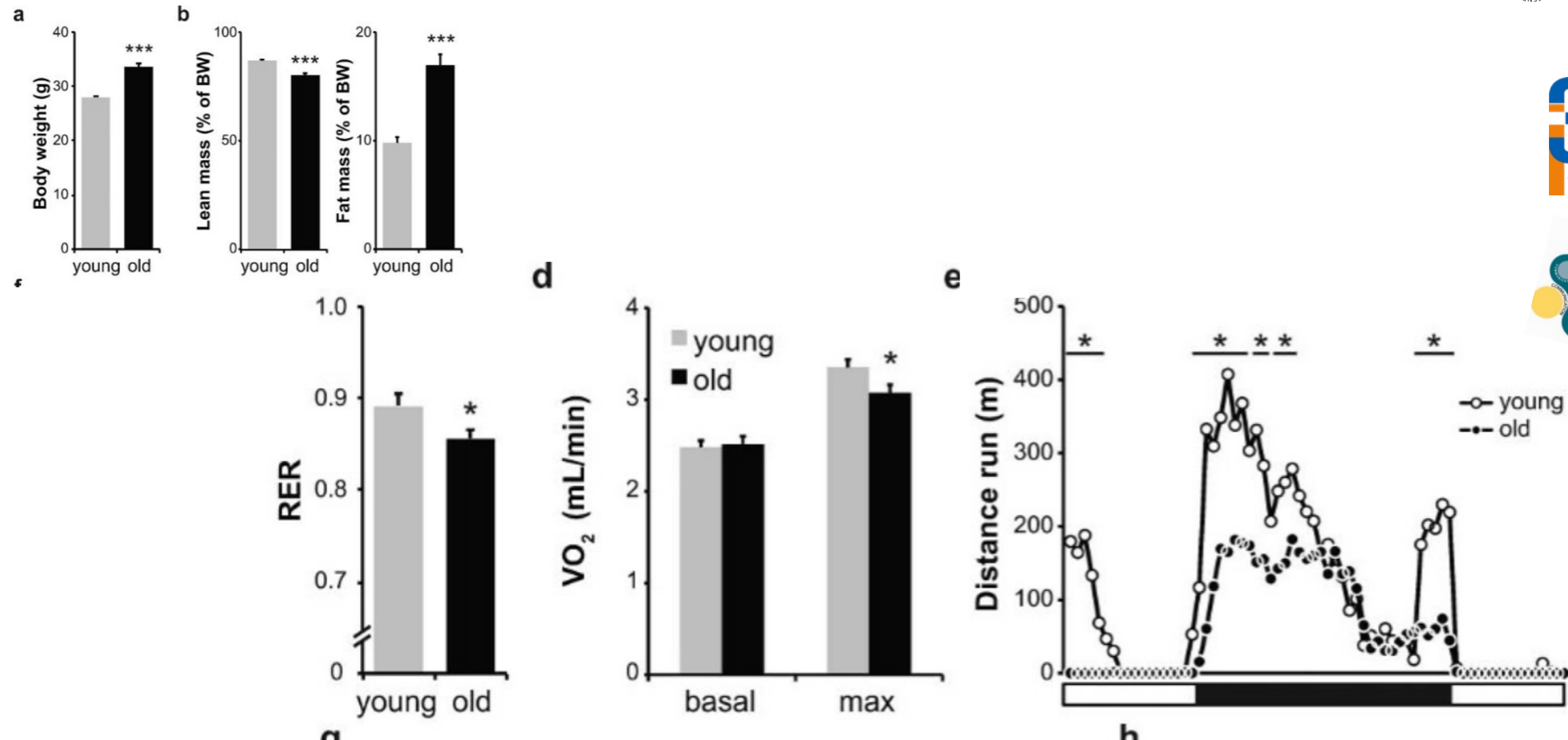
$$RER = VCO_2 / VO_2$$

13/07/2026

# Complementation with EchoMRI (purchased 2026, 1 min Determination of Fat and Lean Mass



# Why use indirect calorimetry?



Houtkooper RH, Argmann C, Houten SM, Cantó C, Jeninga EH, Andreux PA, Thomas C, Doenlen R, Schoonjans K & Auwerx J (2011) The metabolic footprint of aging in mice. *Sci Rep* 1: 134

# What is the PhenoMaster?

TSE Systems

Metabolic Analysis/ indirect Calorimetry

Behavioral Studies

Pysiological Research



# What is the PhenoMaster?

Light intensity & control

Cages

Temperature



Humidity

# What is the PhenoMaster?

## Light intensity & control

- adjustable intensity
- Control light & dark phase to mimic day-night rhythm
- Transition or direct switch
- Default: 40 % (ZBMF)
- Red light if scientists need to during the dark phase

## Temperature



## Cages

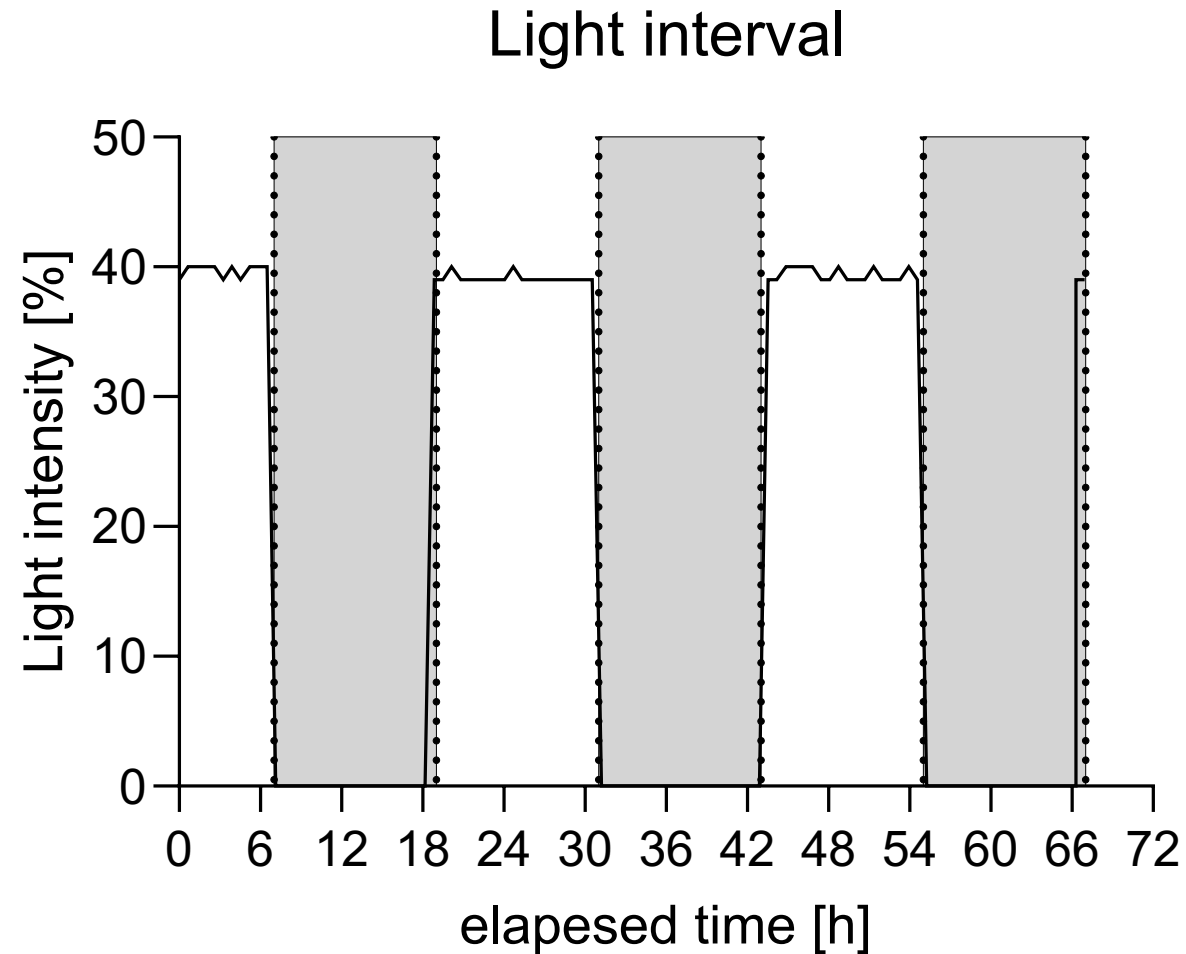
## Humidity

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# What is the PhenoMaster?

## Light intensity & control

## Cages



## Temperature

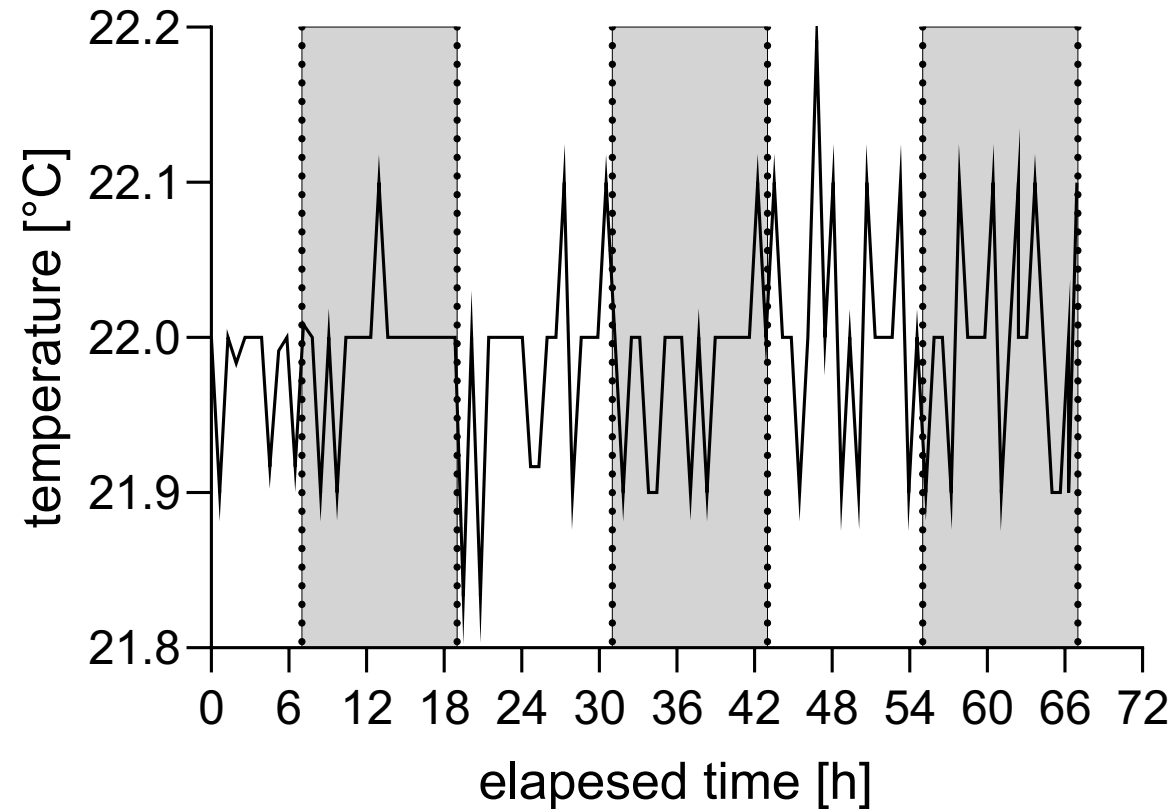
- Set from 4°C – 32 °C
- Default: 22 °C (ZBMF)
- Adjustable during a running experiment

## Humidity

# What is the PhenoMaster?

## Light intensity & control

## Temperature



## Temperature

- Set from 4°C – 32 °C
- Default: 22 °C (ZBMF)
- Adjustable during a running experiment

# What is the PhenoMaster?

## Light intensity & control



## Temperature

## Cages

- Up to 12 cages simultaneously
- Single-caged mice
- Measure intervals: 39 min/Cycle
- if only 1 mouse:  
continuous measurement mode

## Humidity

# What is the PhenoMaster?

Light intensity & control

Cages

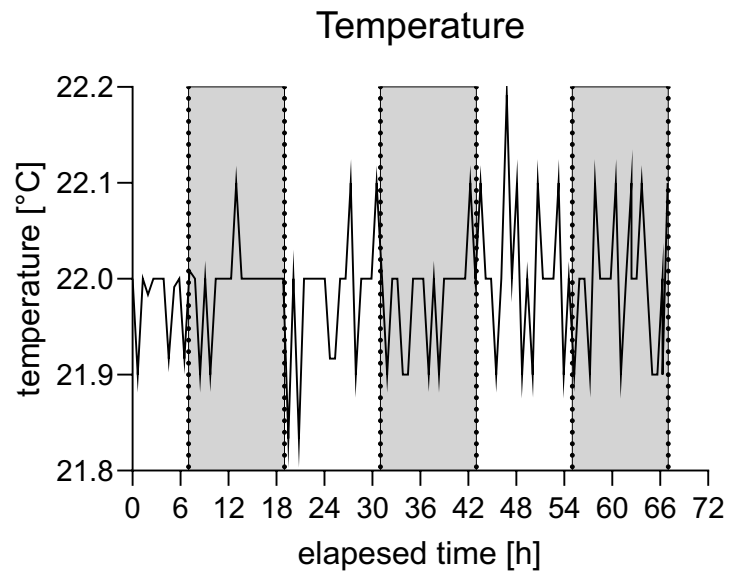
Temperature



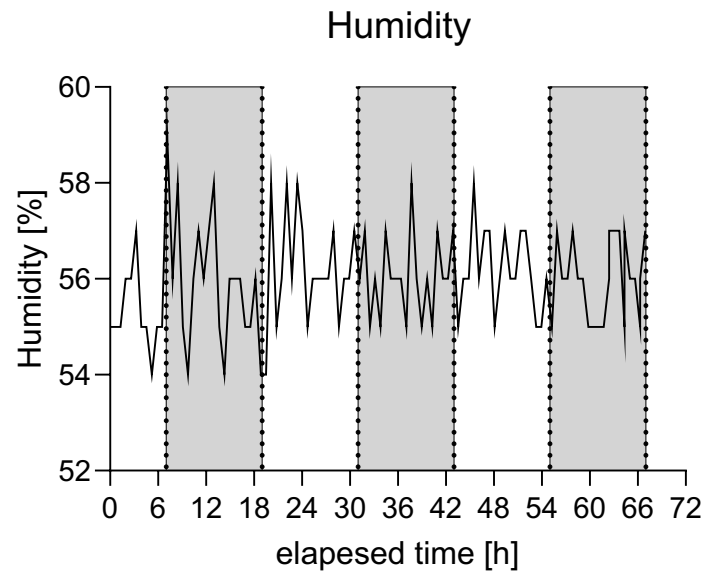
Humidity

- Adjustable
- VE water
- Default: 56% (ZBMF)

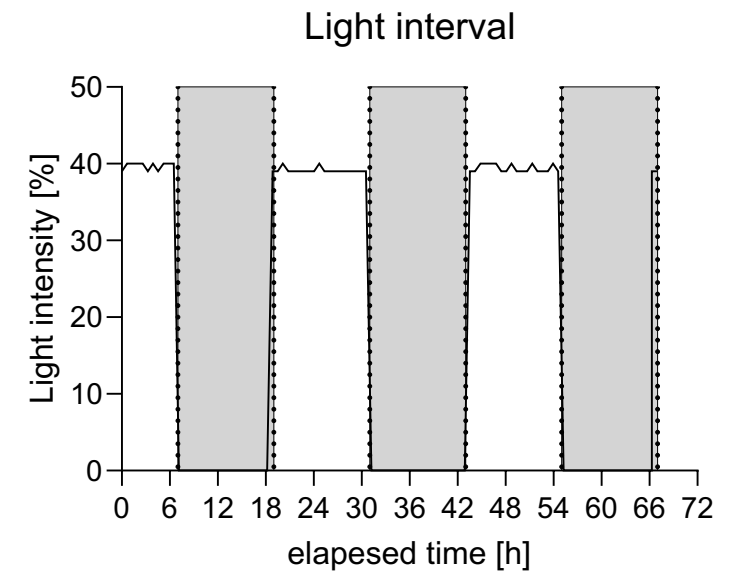
Set temp: 22°C



Set humidity: 56%



Set light intensitiy: 40%



# What is the PhenoMaster?

## Exercise

## Indirect Calorimetry

## Activity



## Weight sensors

## Enrichment

# What is the PhenoMaster?

## Exercise

- Time- controlled
- Speed, time, rpm

## Activity



## Enrichment

## Indirect Calorimetry

## Weight sensors

# What is the PhenoMaster?

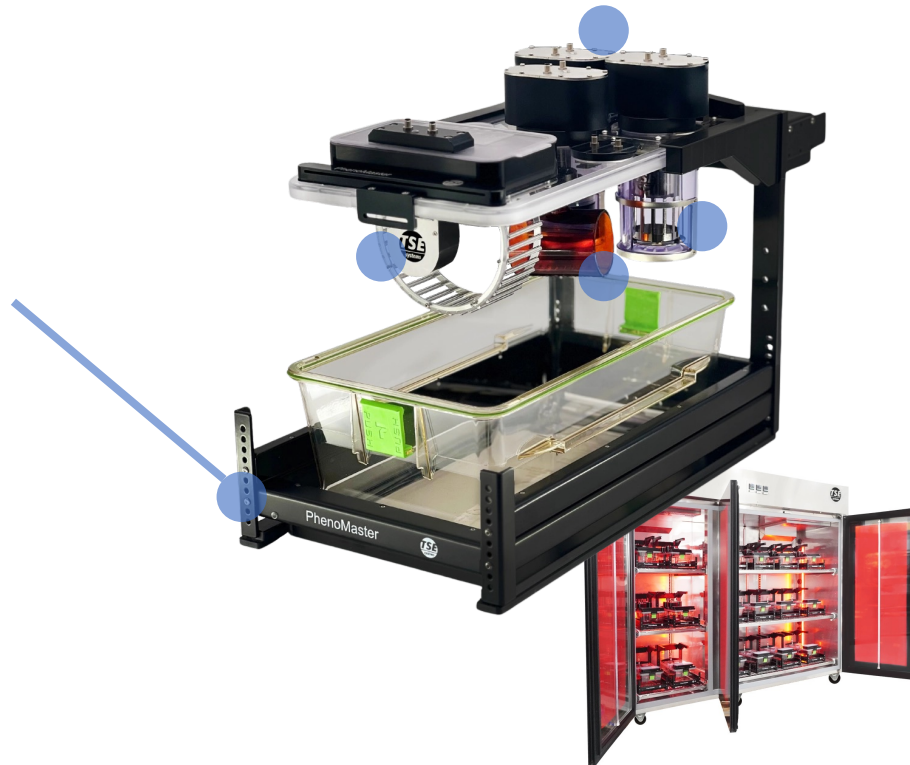
## Exercise

## Indirect Calorimetry

### Activity

- Infrared Activity Frame
- XY-T frame
- 100 Hz

### Enrichment



## Weight sensors

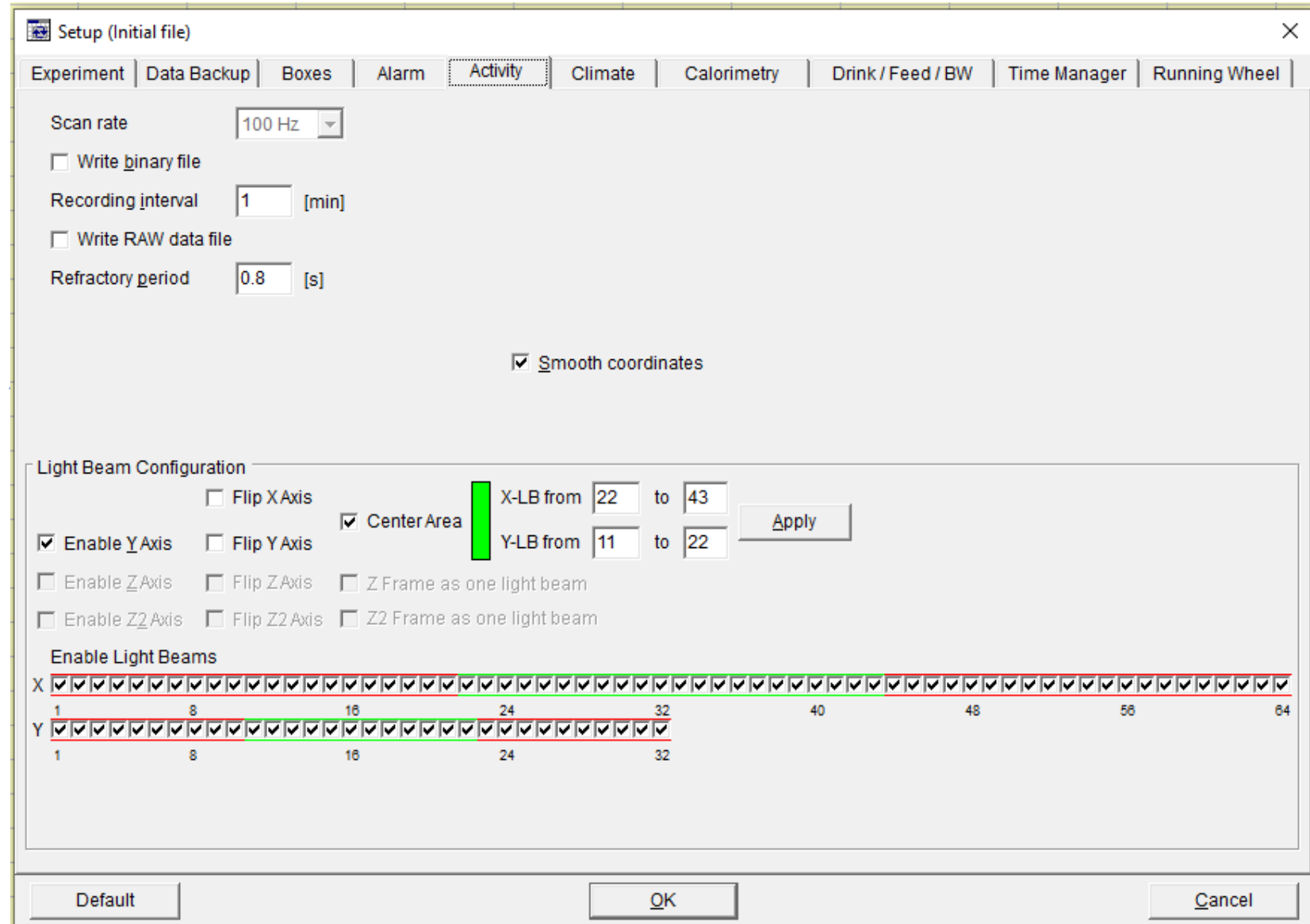
# What is the PhenoMaster?

## Exercise

### Activity

- Infrared Activity Frame
- XY-T frame
- 100 Hz

### Enrichment



The screenshot shows the 'Setup (Initial file)' window with the 'Activity' tab selected. The window contains various configuration options for the experiment.

**Activity Tab Settings:**

- Scan rate: 100 Hz
- ☐ Write binary file
- Recording interval: 1 [min]
- ☐ Write RAW data file
- Refractory period: 0.8 [s]
- ☒ Smooth coordinates

**Light Beam Configuration:**

- ☐ Flip X Axis
- ☒ Enable Y Axis
- ☐ Flip Y Axis
- ☒ Center Area
- X-LB from 22 to 43
- Y-LB from 11 to 22
- ☐ Enable Z Axis
- ☐ Flip Z Axis
- ☐ Z Frame as one light beam
- ☐ Enable Z2 Axis
- ☐ Flip Z2 Axis
- ☐ Z2 Frame as one light beam

**Enable Light Beams:**

X: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64

Y: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32

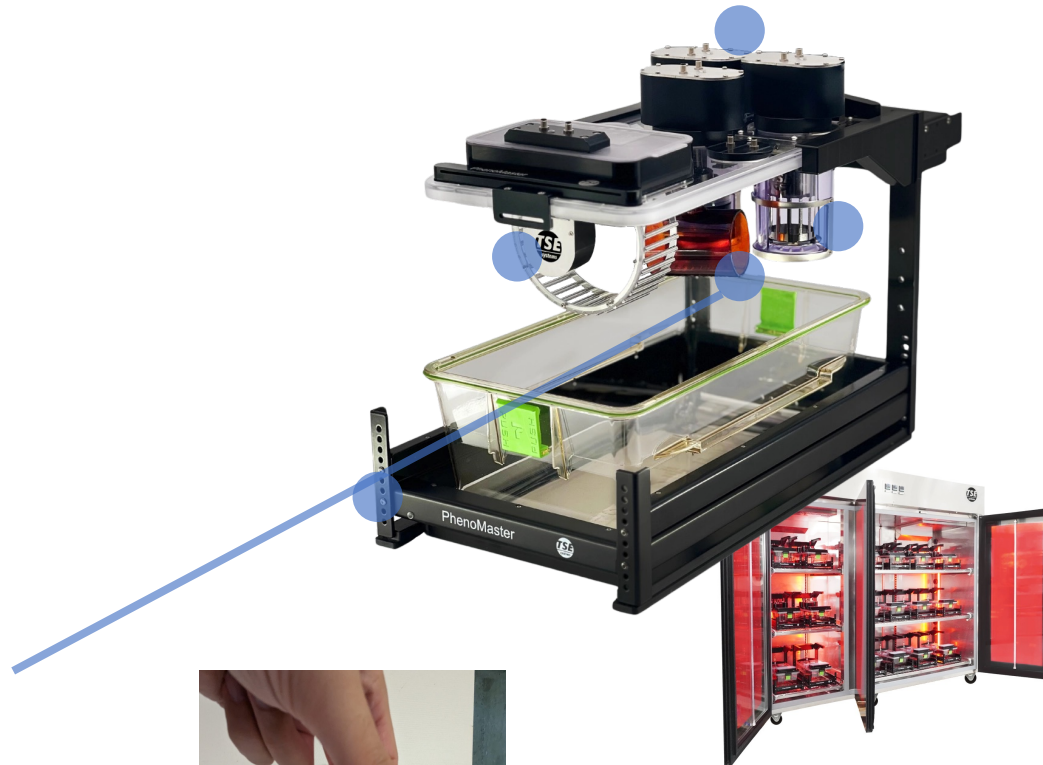
Buttons: Default, OK, Cancel

# What is the PhenoMaster?

## Exercise

## Indirect Calorimetry

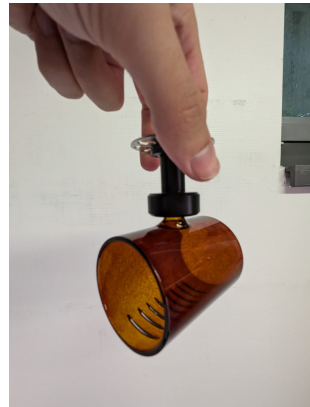
### Activity



### Weight sensors

### Enrichment

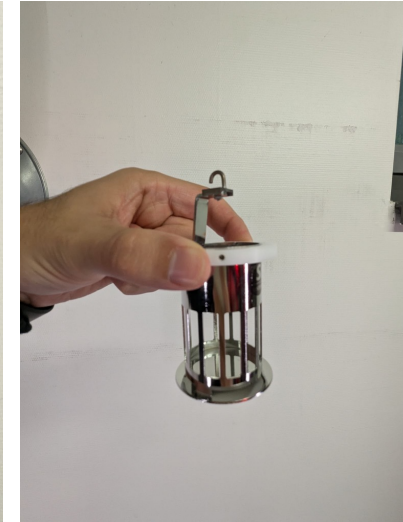
- Red tubular housing
- Exercise wheel



# What is the PhenoMaster?

## Exercise

### Activity



### Weight sensors

- Food & water
- records in real time
- Check amount without disturbing the mice

### Enrichment

# What is the PhenoMaster?

## Exercise

### Activity



### Indirect Calorimetry

- Oxygen consumption
- Carbon dioxide production
- Respiratory exchange ratio (RER)
- heat

### Weight sensors

### Enrichment

# What is the PhenoMaster?

## Exercise

### Activity

RER: Respiratory Exchange Ratio:  $VCO_2/VO_2$

RER: 0.7-0.8

RER: 1

as

RER: >1

low intensity exercise; fat as fuel

intense exercise; carbohydrates

fuel

high intensity exercise; anaerobic  
metabolism

Heat: burned calories calculated via Weir equation

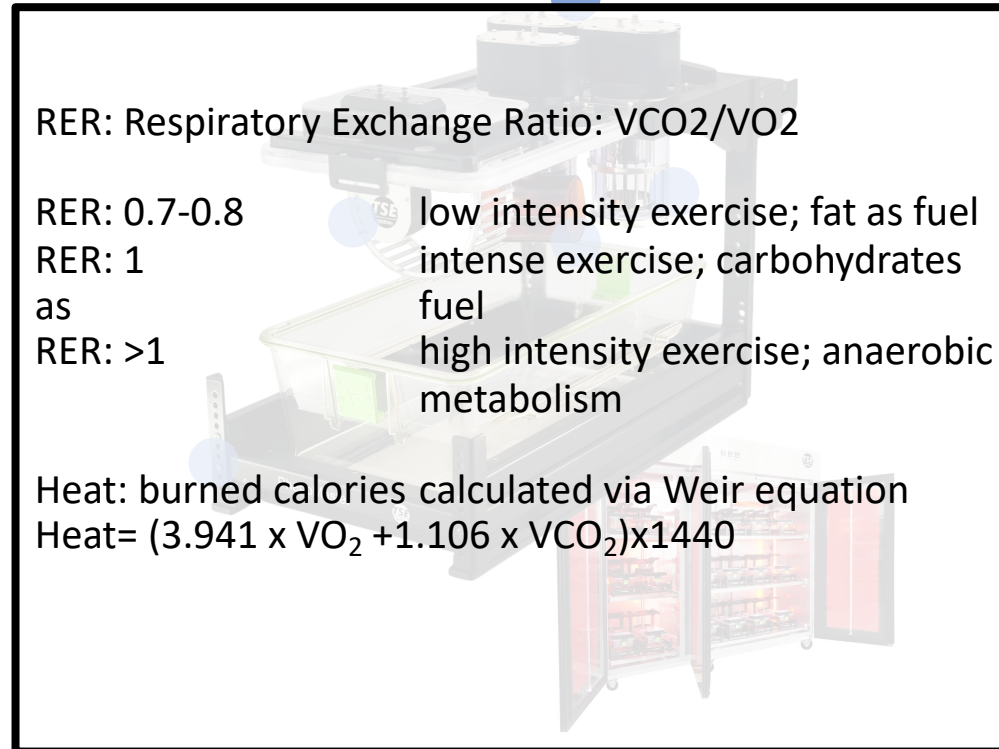
Heat=  $(3.941 \times VO_2 + 1.106 \times VCO_2) \times 1440$

### Indirect Calorimetry

- Oxygen consumption
- Carbon dioxide production
- Respiratory exchange ratio (RER)
- heat

### Weight sensors

### Enrichment



- In- house test run to conduct PhenoMaster procedure
- Already single-caged mice
- Ex- breeder

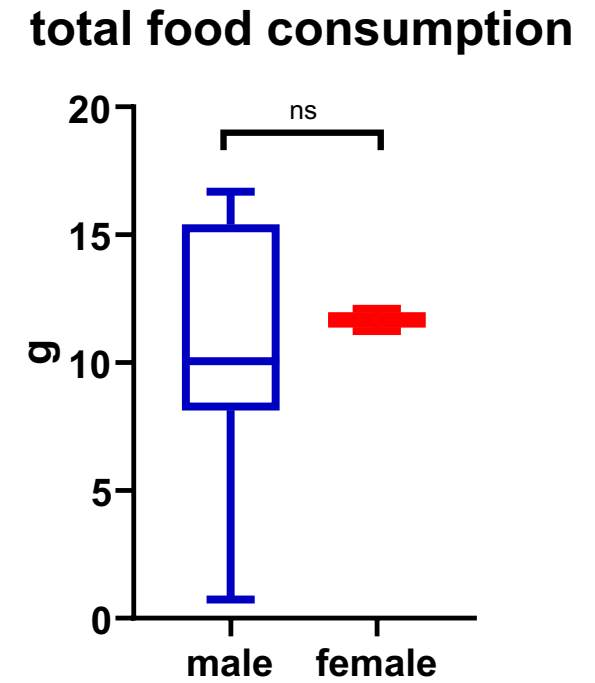
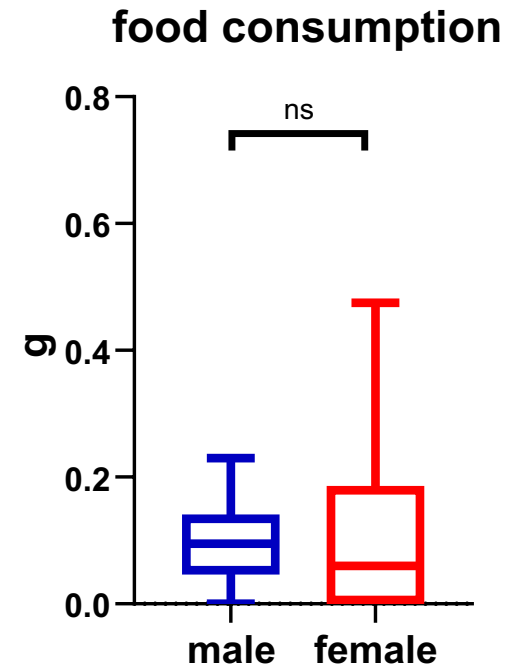
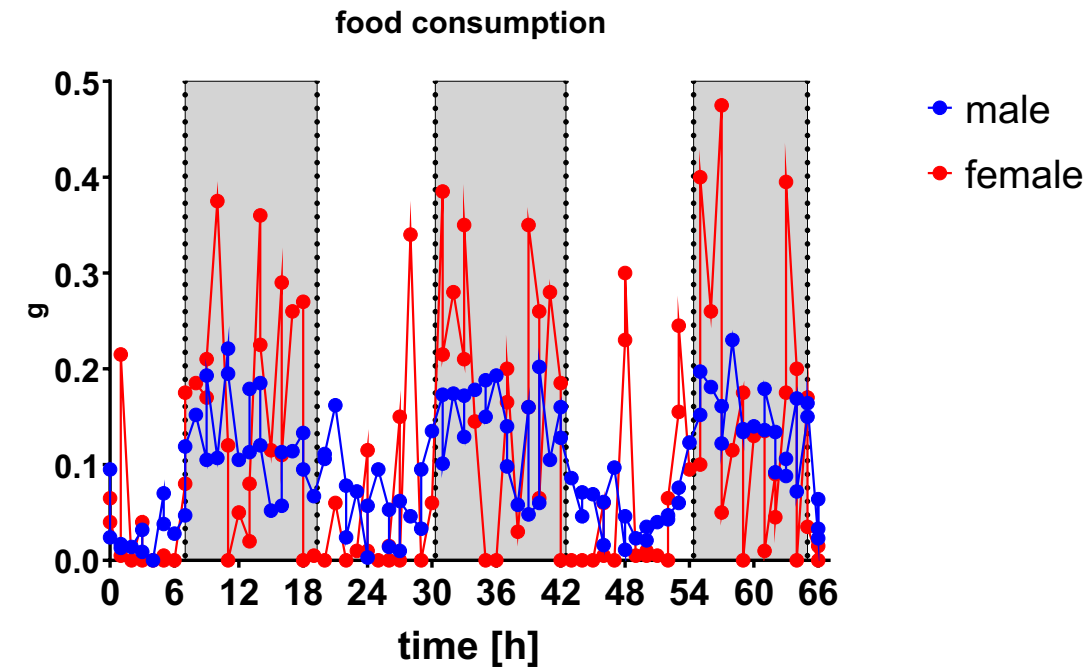


- In- house test run to conduct PhenoMaster procedure
- Already single-caged mice
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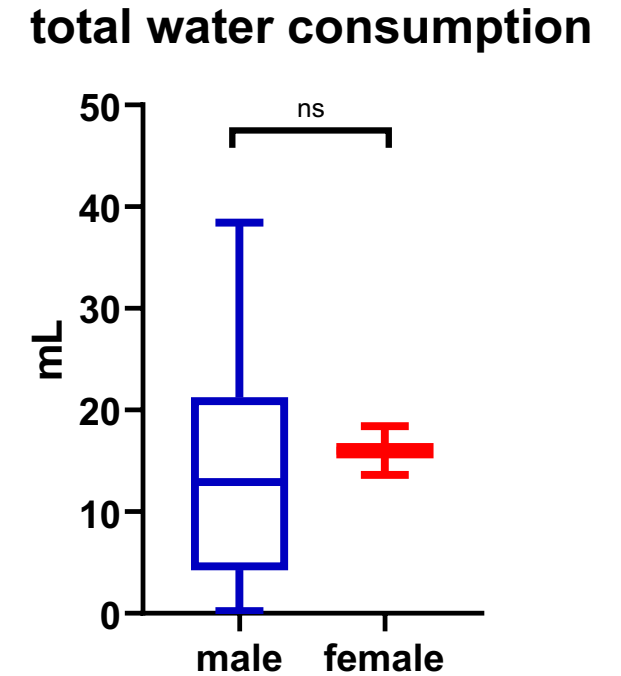
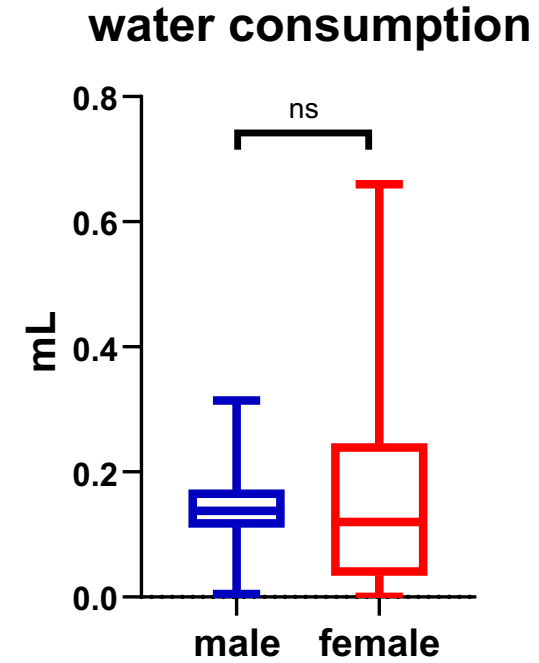
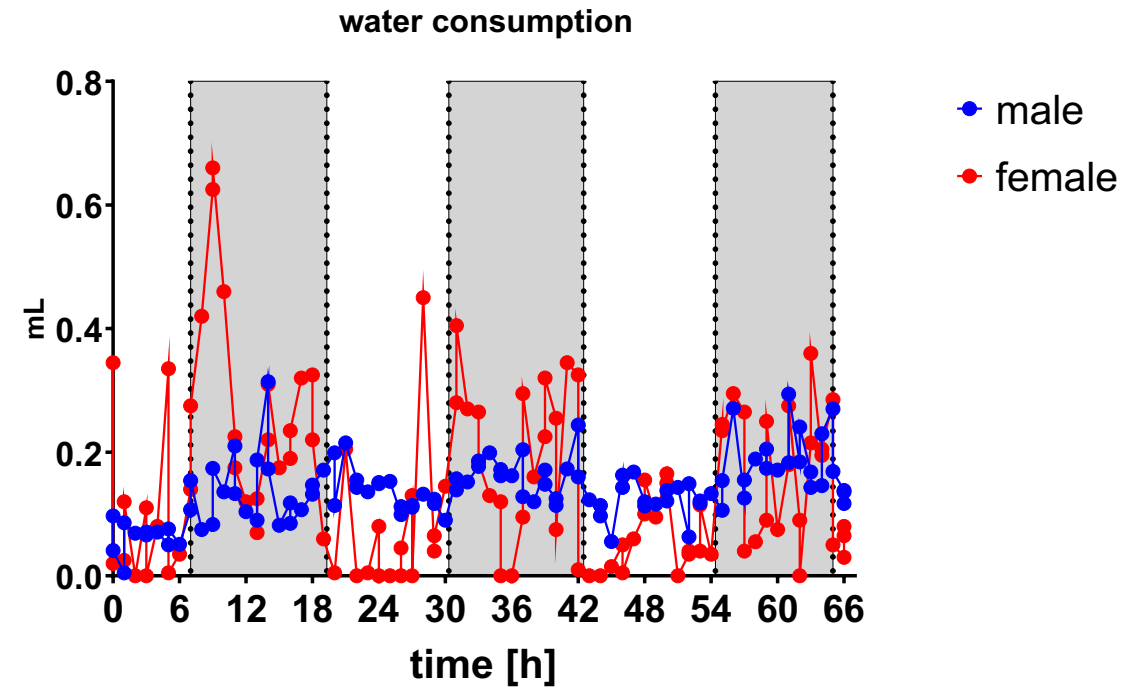
| ID   | Line                            | Background | Gender | Age       | Weight |
|------|---------------------------------|------------|--------|-----------|--------|
| 6806 | GRflox; LysMCre                 | B6         | F      | 108 weeks | 40,0 g |
| 9170 | CDK5flox;GT/Rosa<br>26-Cre ERT2 | B6         | F      | 13 weeks  | 21,5 g |
| 9037 | CDK5Flox;<br>Dmp1Cre            | B6         | M      | 20 weeks  | 25,0 g |
| 9068 | GRflox;<br>adiponectin-cre      | B6         | M      | 19 weeks  | 38,5 g |
| 9136 | GRflox;LysMCre                  | Bal        | M      | 15 weeks  | 27,5 g |
| 9137 | GRflox;LysMCre                  | Bal        | M      | 15 weeks  | 28,5 g |
| 8072 | GRflox;LysMCre                  | B6         | M      | 52 weeks  | 40,0 g |
| 8878 | R26flox<br>HSD11H6PD            | B6         | M      | 24 weeks  | 38,5 g |
| 9328 | GRflox;LysMCre                  | Bal        | M      | 11 weeks  | 32,5 g |
| 9017 | HSD11H6PD;<br>LysMCre           | B6         | M      | 20 weeks  | 30,0g  |
| 6791 | GRflox:LsyMCre                  | B6         | M      | 108 weeks | 35,0 g |
| 9221 | GRdel (B6)                      | B6         | M      | 16 weeks  | 30,5 g |



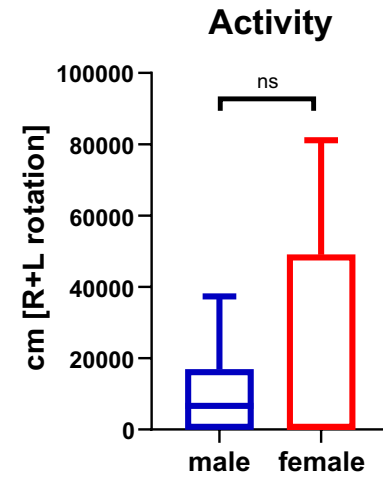
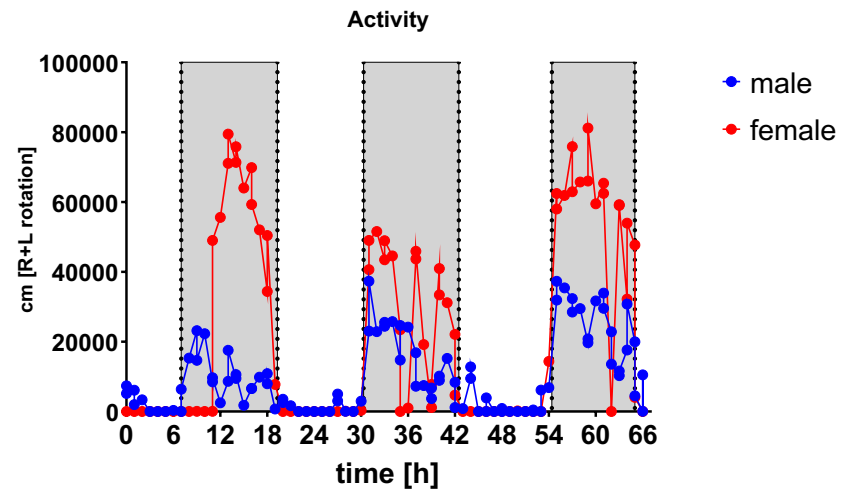
# Food consumption data



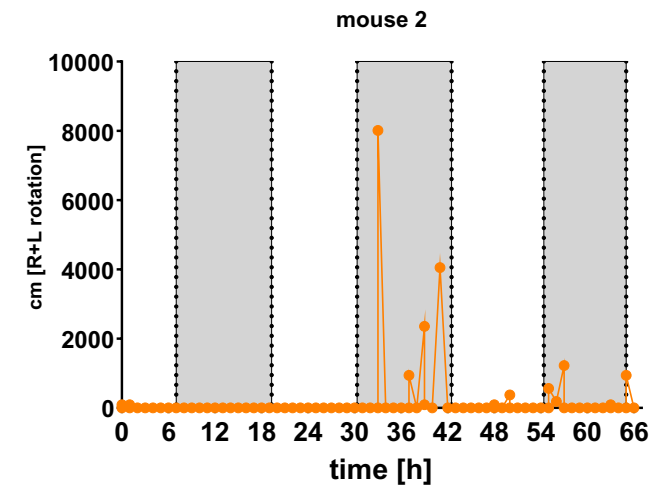
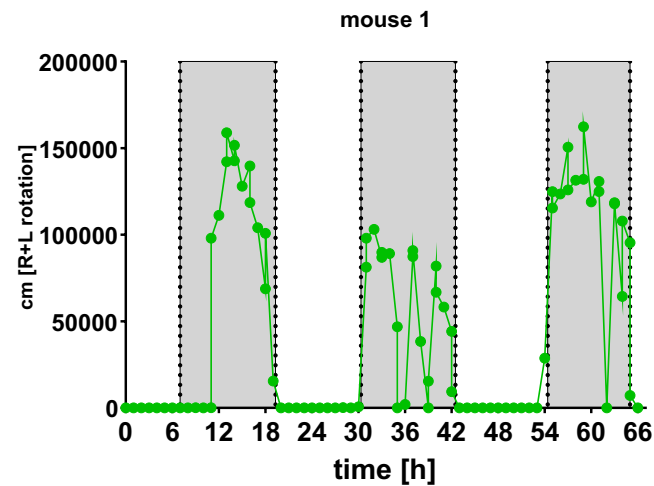
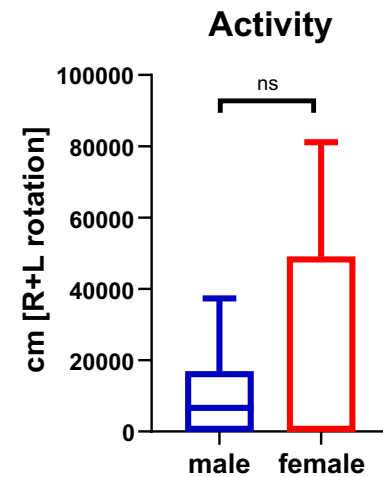
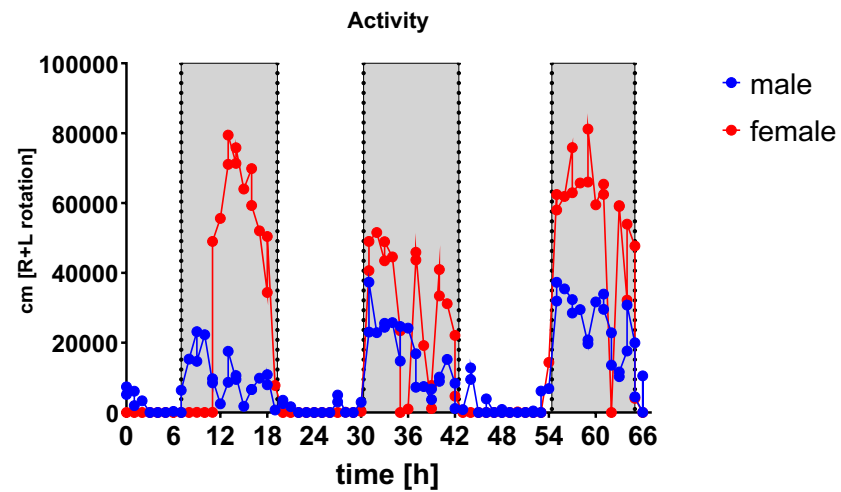
# water consumption data



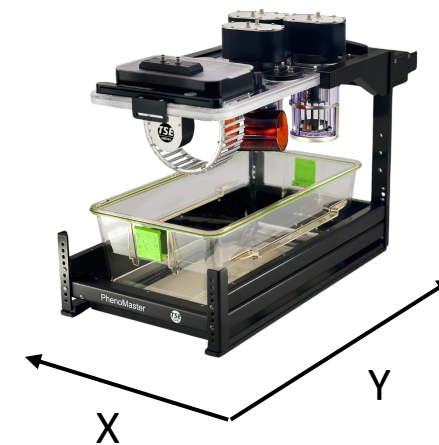
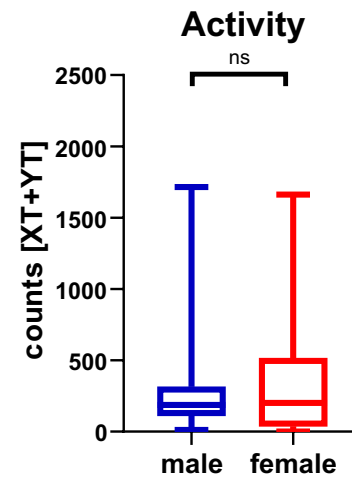
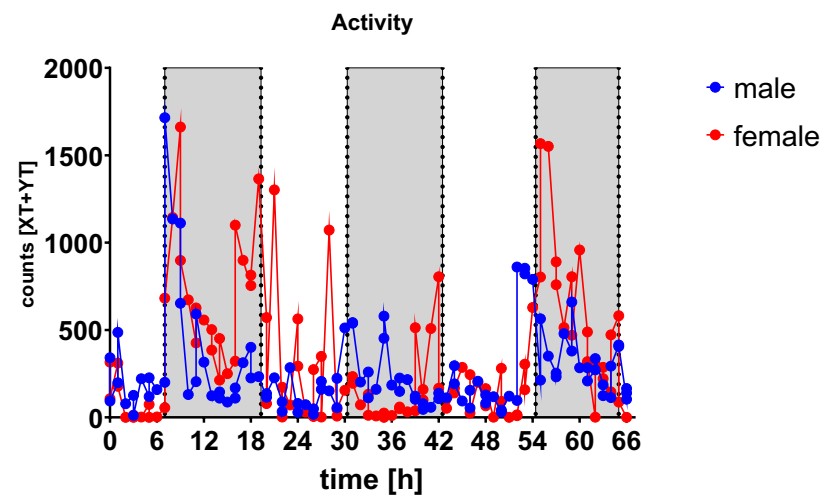
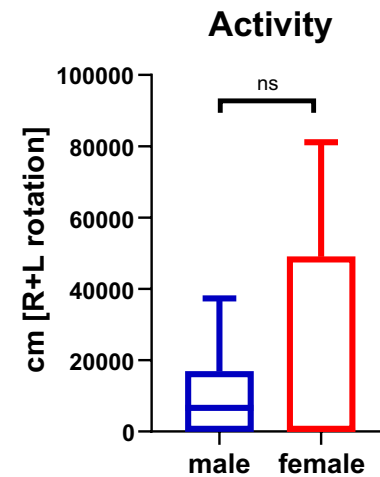
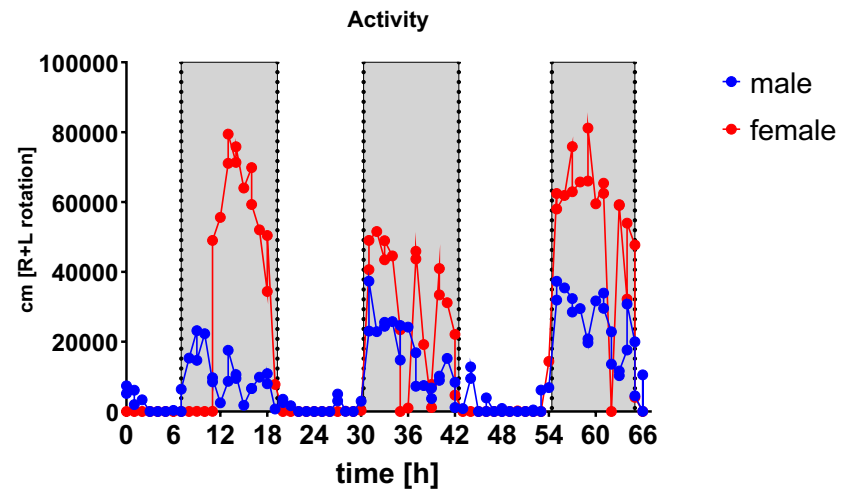
# Activity data

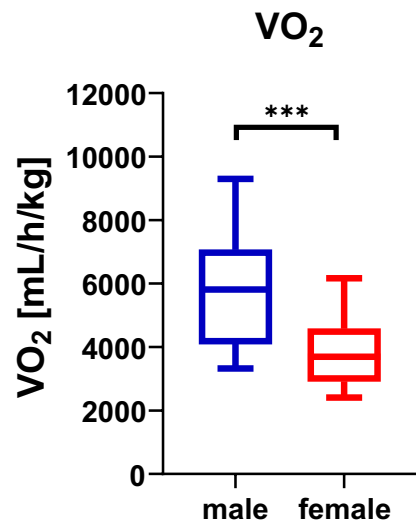
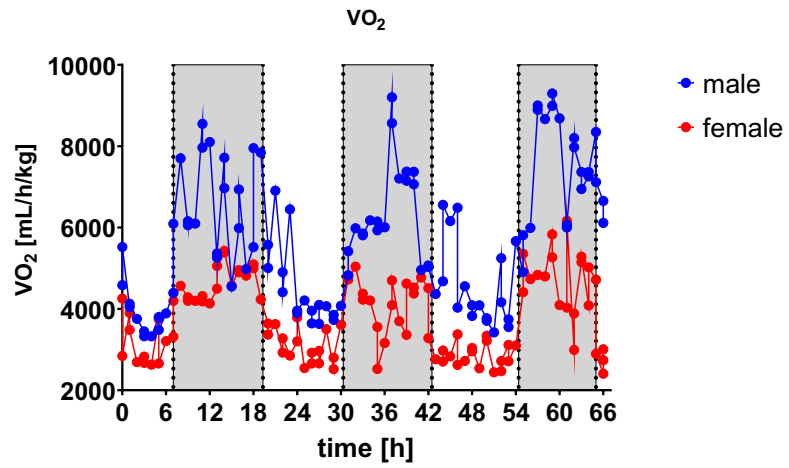


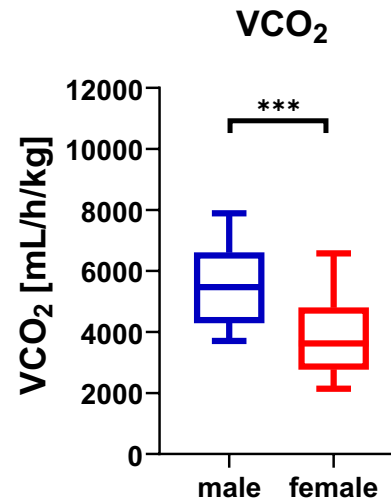
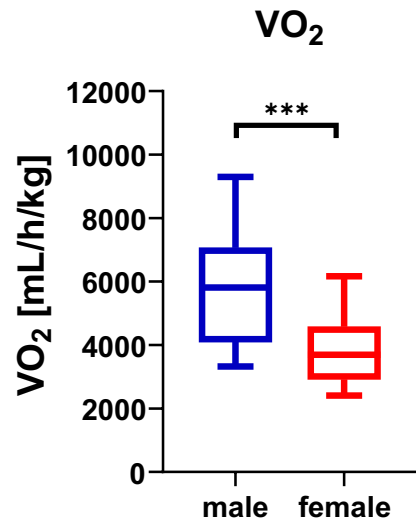
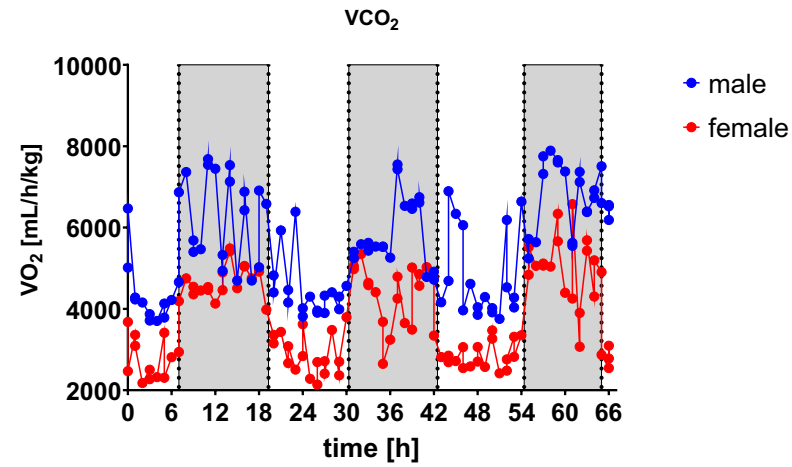
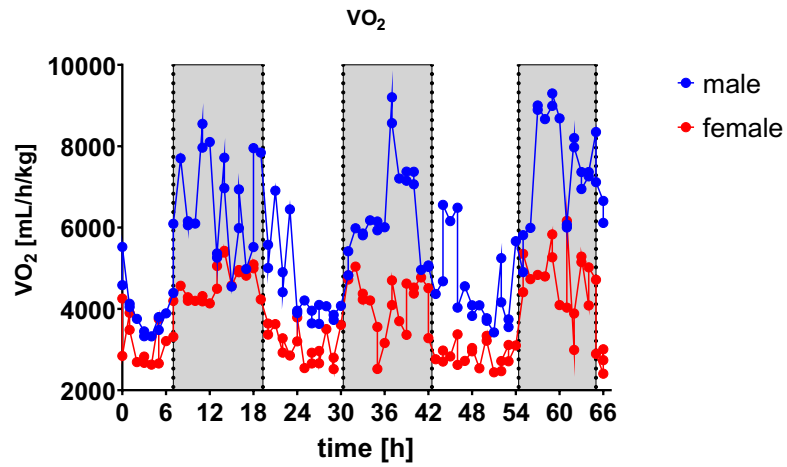
# Activity data

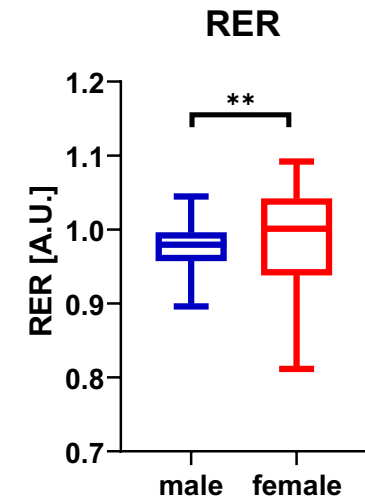
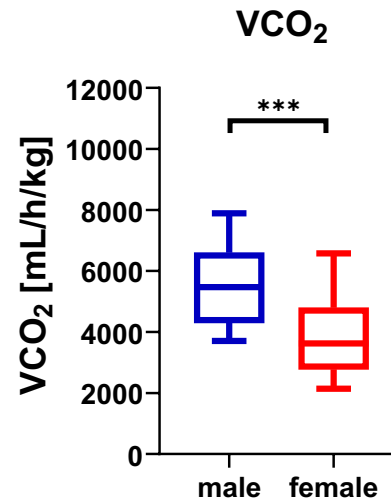
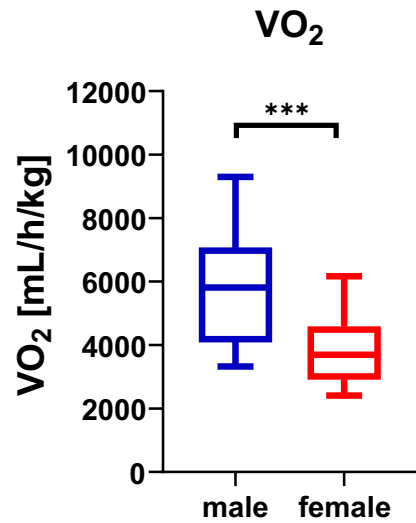
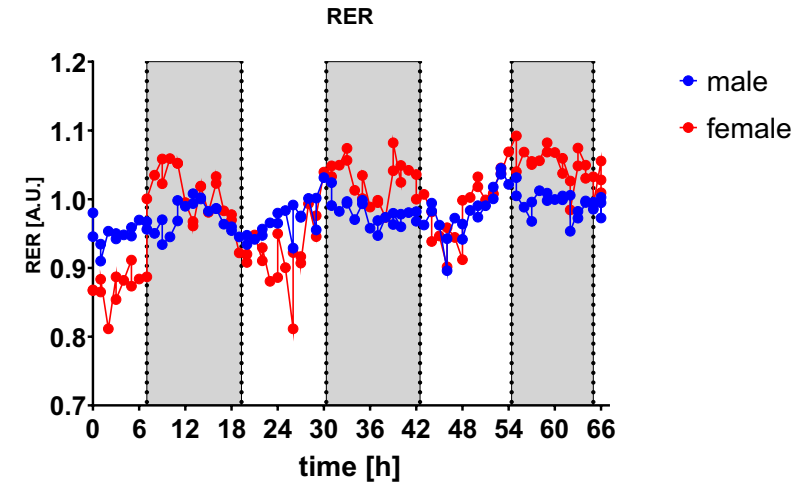
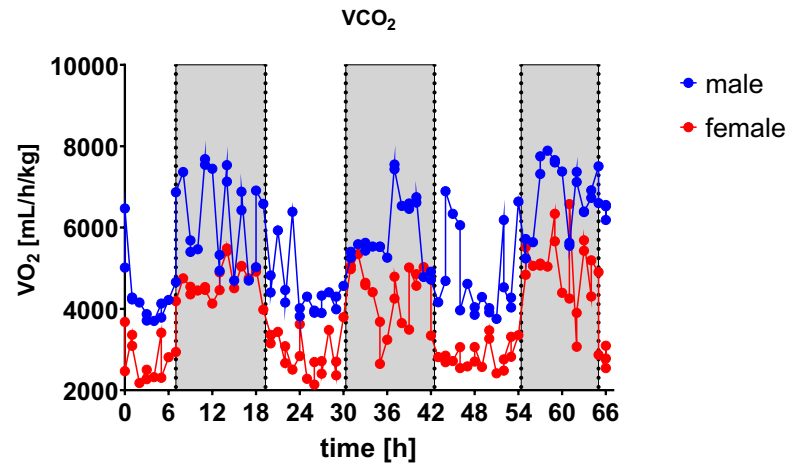
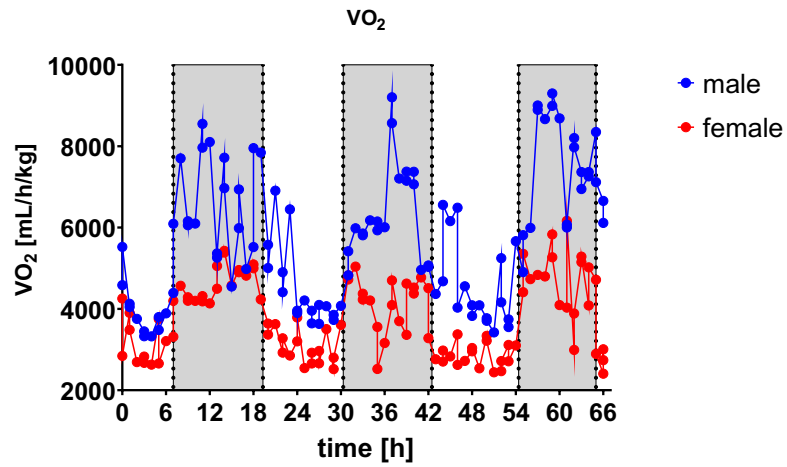


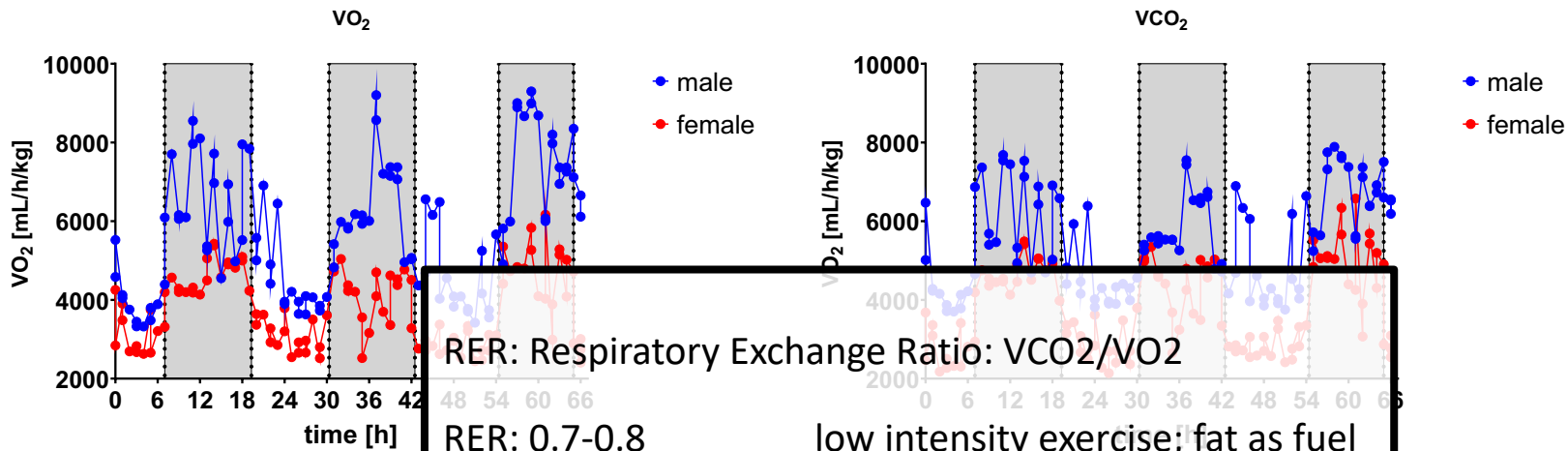
# Activity data











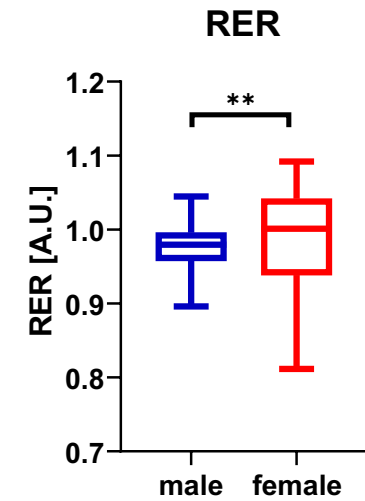
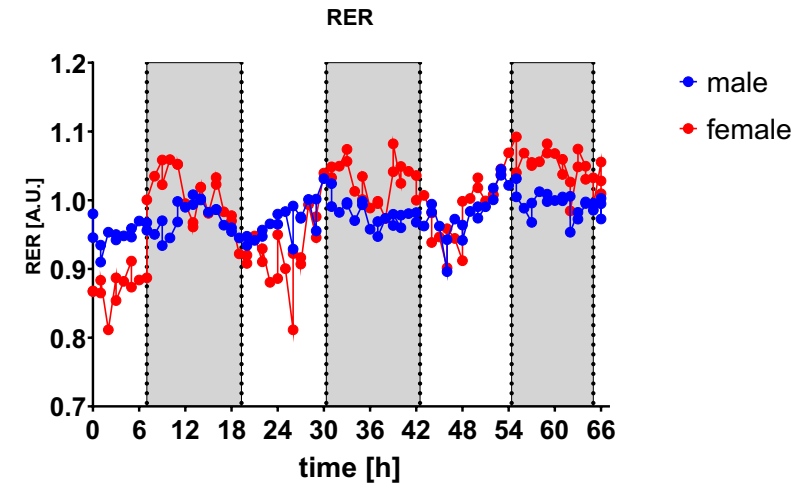
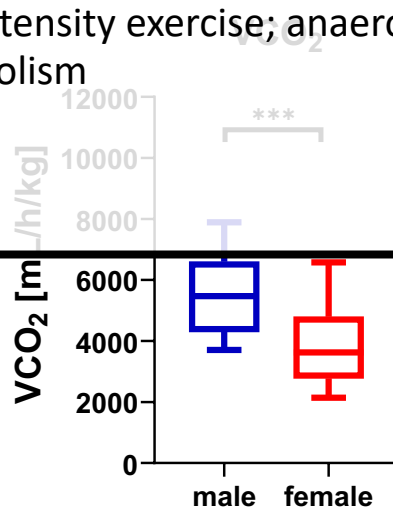
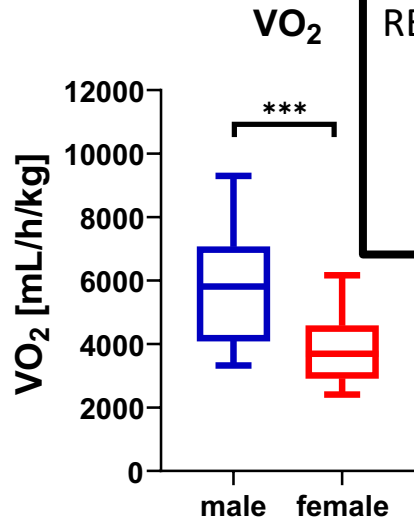
RER: Respiratory Exchange Ratio:  $VCO_2/VO_2$

RER: 0.7-0.8

RER: 1

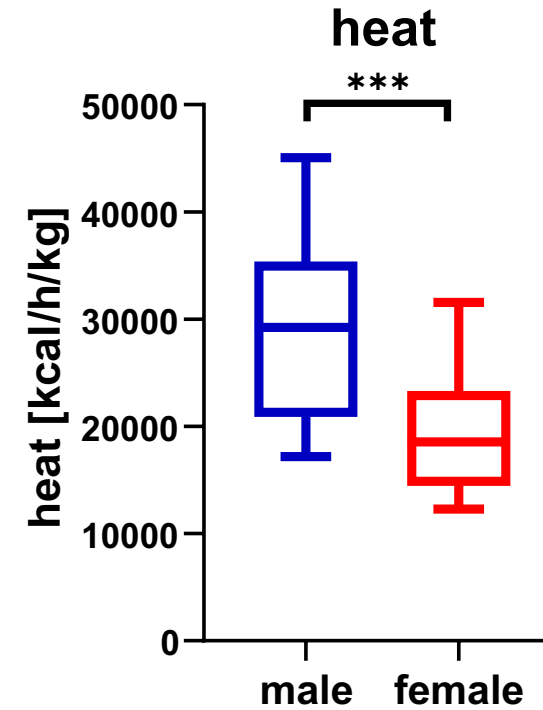
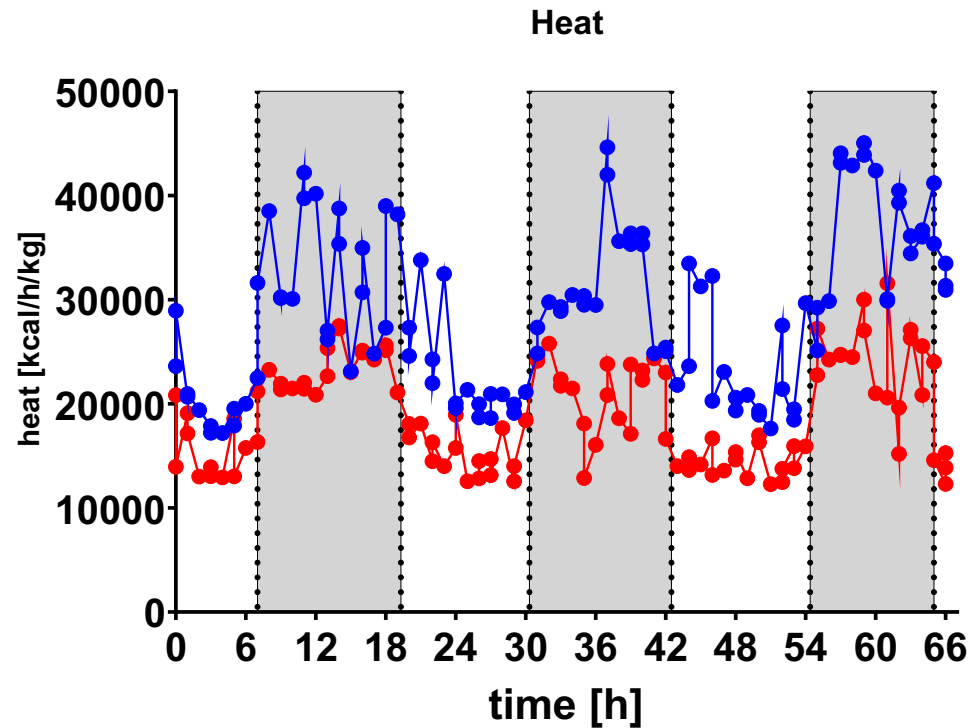
RER: >1

low intensity exercise; fat as fuel  
intense exercise; carbohydrates  
as fuel  
high intensity exercise; anaerobic  
metabolism



Heat: burned calories calculated via Weir equation  

$$\text{Heat} = (3.941 \times \text{VO}_2 + 1.106 \times \text{VCO}_2) \times 1440$$



## Food and water consumption

- No significant difference in the amount of consumed food or water
- Clear differences between light and dark phases
- Consumption in female mice was more dependant on switch between light and dark phases

## Activity

- No significant difference between activity between male and female mice
- Mice are more active during the dark phases
- Strong individual differences with wheel activity

## Indirect calorimetry

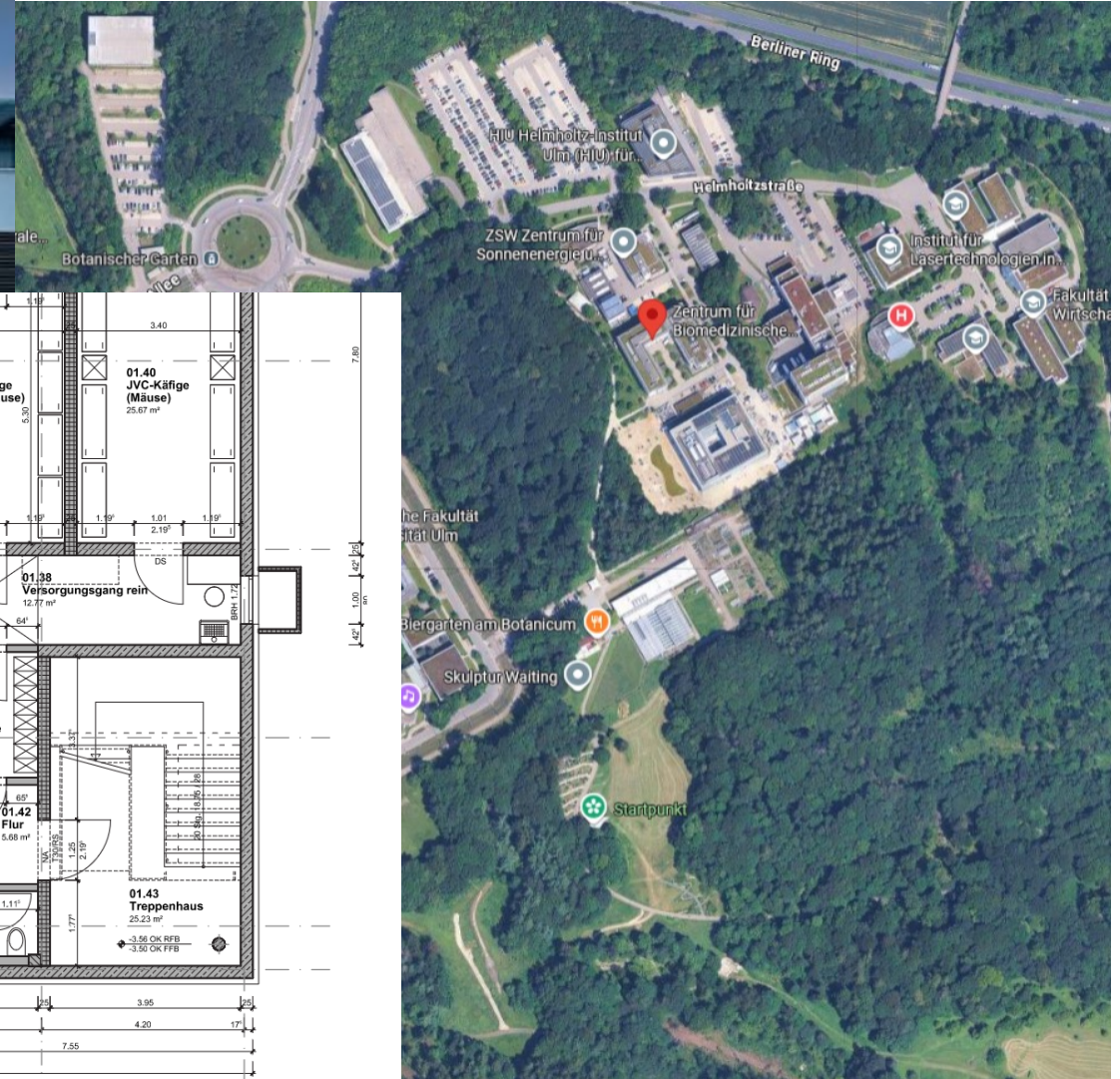
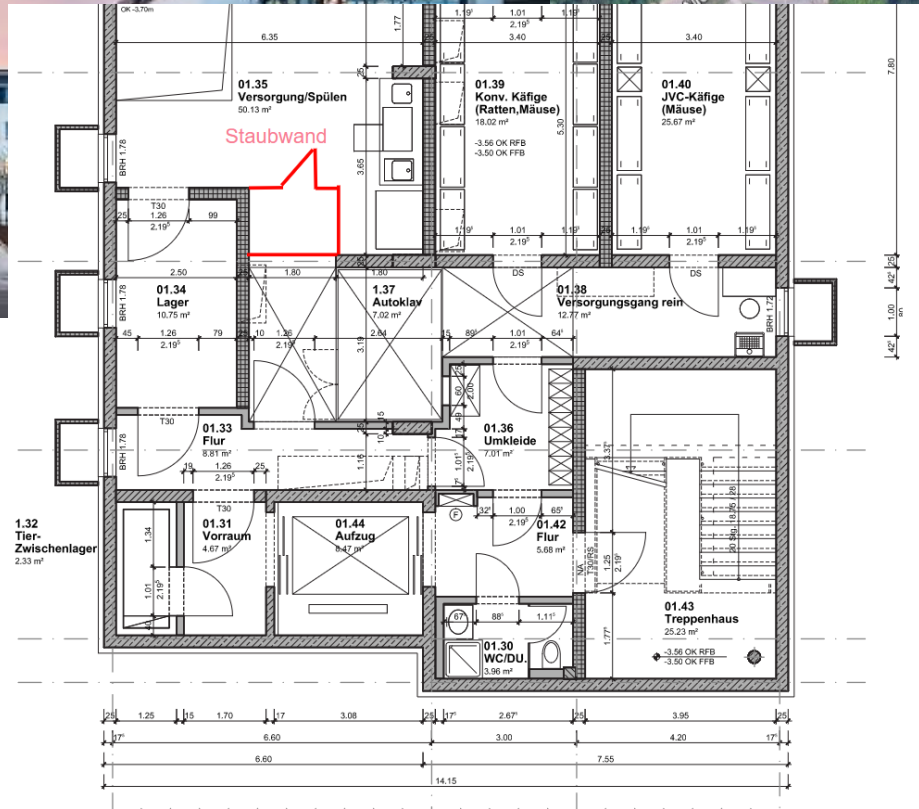
- Male and female mice produce energy mainly from carbohydrates (RER ~ 1)
- Male mice burn more calories compared to female mice



# Location



# Location



# Before the experiment starts



| i3RU | Researcher | TFZ |
|------|------------|-----|
|      | X          |     |
| (X)  | X          |     |
|      | X          | X   |
| X    |            |     |
| X    |            |     |

- Get in contact; planning the experiment
- Plan for 1 week: 2 days settling in; 3 days data acquisition
- Approved animal experimentation application/ Tierversuchsantrag
  - Provide example; text block example from TSE Systems
- Register needed cages at the animal care takers at the TFZ
  - At least 2 weeks prior
- Weight calibrations (once every 3 months)
- Gas calibrations (before every new experiment)

# Before the experiment starts



| i3RU | Researcher | TFZ |
|------|------------|-----|
|      | X          |     |
| (X)  | X          |     |
|      | X          | X   |
| X    |            |     |
| X    |            |     |

- Get in contact; planning the experiment
- Plan for 1 week: 2 days settling in; 3 days data acquisition

## Contacts

|                           |                                   |
|---------------------------|-----------------------------------|
| Prof. Dr. Jan Tuckermann: | jan.tuckermann@uni-ulm.de         |
| Denis Nalbantoglu:        | denis.nalbantoglu@uni-ulm.de      |
| Annika Herrmann-Janson:   | annika.herrmann-Janson@uni-ulm.de |

- Weight calibrations (once every 3 months)
- Gas calibrations (before every new experiment)

# Start of the experiment



| i3RU | Researcher | TFZ |
|------|------------|-----|
|      | X          |     |
| X    | X          |     |
| X    | X          |     |
| X    | X          |     |
| X    | (X)        |     |

- Weighing mice
  - Important for calorimetric calculations
- Transport of the mice into ZBMF/ room 1.34
  - Allowed mice: DRK, N26,M25, ZBMF
  - Return to regular housing: ZBMF; consultation with TFZ needed
- Transfer into new cages
- Transfer of water, food and bedding
- Set up and start of the PhenoMaster

# Running the experiment



| i3RU | Researcher | TFZ |
|------|------------|-----|
| X    |            |     |
| X    |            |     |
| (X)  | X          |     |

- Data acquisition check
- Check in on mice; twice a day
- Invasive interventions (injections, in between weighing,...)

# End of the experiment



| i3RU | Researcher | TFZ |
|------|------------|-----|
|      | X          |     |
|      | X          |     |
|      | X          | X   |
| (X)  | X          |     |
| X    |            | X   |

- Weighing the mice
- Sacrificing the mice or...
- ...Transfer back into regular housing
  - Mind the hygiene level of mice
  - Return to regular housing: ZBMF; consultation with TFZ needed
  - Contact: Annika Herrmann-Janson TFZ
- Exporting data files & analysis
- Clean up

# 3R Research and Projects

Let's improve the 3Rs in animal research!

<https://www.uni-ulm.de/nawi/3rc/>

SOP TSE Systems ZBMF, Institute of Molecular Endocrinology and Physiology and integrated 3R Center; Operator: Denis Nalbantoglu)

## Procedure Checklist

|                                                                                                                                                                                                                                                                                          | IMEP | Researcher | Animal caretaker ZBMF |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|------------|-----------------------|
| <b>Before the experiment starts</b>                                                                                                                                                                                                                                                      |      |            |                       |
| Contact IMEP via email to register for experiment (at least 3 weeks before you want to start an experiment in the Phenomaster) @ <a href="mailto:denis.nalbantoglu@uni-ulm.de">denis.nalbantoglu@uni-ulm.de</a> <a href="mailto:jan.tuckermann@uni-ulm.de">jan.tuckermann@uni-ulm.de</a> |      | X          |                       |
| Approved Tierversuchsantrag (Animal experimentation application)                                                                                                                                                                                                                         |      | X          |                       |
| Set specific date to start the experiment                                                                                                                                                                                                                                                | X    | X          | X                     |
| Plan exact experiment (conditions to be measured, are mice sacrificed afterwards? If mice are about to be returned, get approve from the TFZ. Usually, only mice from the ZBMF can return)                                                                                               | X    | X          |                       |
| Sign access list of room 1.34                                                                                                                                                                                                                                                            | X    | X          |                       |
| Fill out room protocols                                                                                                                                                                                                                                                                  | X    |            |                       |
| Perform weight calibrations of Phenomaster devices                                                                                                                                                                                                                                       | X    |            |                       |
| Perform Gas calibrations (a day prior to the experiment)                                                                                                                                                                                                                                 | X    |            |                       |
| Turn on Phenomaster to let it run warm (a day prior to the experiment)                                                                                                                                                                                                                   | X    |            |                       |
| Leitwarte/ Control pannel is informed about an upcoming Experiment in the Phenomaster                                                                                                                                                                                                    | X    |            |                       |
| <b>Transfer into the Phenomaster cages</b>                                                                                                                                                                                                                                               |      |            |                       |
| <i>Note: room 1.34 counts as a satellite room of the mouse housekeeping facility. Therefore, the same hygiene concept applies here as well. read more below.</i>                                                                                                                         |      |            |                       |
| All mice need to be weighed prior to the transfer to proper calculate indirect calorimetry                                                                                                                                                                                               | X    | X          |                       |
| Mice are provided ready to pick up in regular cages                                                                                                                                                                                                                                      |      | X          | X                     |
| Phenomaster cages are filled with bedding                                                                                                                                                                                                                                                |      | X          | X                     |
| Depending on the timescale of the experiment, extra food, water and bedding is provided                                                                                                                                                                                                  |      | X          | X                     |
| Mice are transported to the Phenomaster room 1.34                                                                                                                                                                                                                                        |      | X          |                       |
| Mice are transferred into Phenomaster cages                                                                                                                                                                                                                                              | X    | X          |                       |
| Food and Water from the mice cages are transferred into respective reservoirs in the Phenomaster cages                                                                                                                                                                                   | X    | X          |                       |

# Thank you for your Attention

## Contacts

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Institute of Molecular Endocrinology and Physiology

Die Mäuse werden nach der Eingewöhnungsphase in Einzelhaltung umgesetzt. Dies dient dazu, den Futterverbrauch pro Maus zu bestimmen, was in unterschiedlichen Gruppen nicht möglich ist. Einzelhaltung von männlichen Mäusen ist eine akzeptierte Form der Haltung bei erwachsenen Tieren (GV-SOLAS „On Single Housing of Mice for Scientific Purposes“ 2021). Einige Studien stellen heraus, dass die Einzelhaltung sogar die präferierte Form der Haltung für Männchen sein kann (Davies et al 2025).

Der Energieverbrauch der Mäuse soll mittels Metabolischer Käfige (TSE Phenomaster) bestimmt werden. Die metabolischen Käfige gleichen in Größe und Form den normalen Haltungskäfigen, und es besteht ebenfalls *ad libitum* Zugang zu Futter und Wasser. Die Mäuse werden in den ersten 48 Stunden an die Käfige gewöhnt und danach erfolgt eine kontinuierliche Messung für 72 Stunden. Die Mäuse können dabei ihrem normalen Tagesablauf nachgehen und müssen nicht gestört werden. Sie werden lediglich für die Injektionen aus dem Käfig genommen. Nach den 3 Tagen in den metabolischen Käfigen werden die Mäuse in die normale Haltung zurück überführt.

Bei metabolischen Käfigen wird der Sauerstoffverbrauch der Mäuse bestimmt, ebenso wie die CO<sub>2</sub> Abgabe. Daraus lässt sich der tatsächliche Energieverbrauch der Mäuse bestimmen. Ebenso können aussagen über Aktivität der Mäuse gemacht werden. Des Weiteren ist es möglich die Nahrungsaufnahme und die Wasseraufnahme automatisch zu erfassen.

