Indirect Calorimetry NINGLA_CAL_003

Purpose

Indirect calorimetry provides detailed information on the energy metabolism of mutant mice. Energy expenditure is evaluated through indirect calorimetry by measuring oxygen consumption with an open flow respirometric system. CO_2 and O_2 sensors measure the difference in CO_2 and O_2 concentrations in air volumes flowing through control or animal cages. The amount of oxygen consumed over a given period of time can thus be calculated, as far as the air flow through the cage is known. Data are expressed as ml O_2 $h^{-1}$$animal^{-1}$. The system also monitors CO_2 production, therefore, the respiratory exchange ratio (RER) and heat production can be calculated. An activity and food and water intake monitoring system can also be integrated into the set up in order to investigate circadian pattern and behaviour.

Experimental Design

Minimum number of animals :

7

M or

7

F

Age at test:

Week 54

Sexual dimorphism: In general, when considering body mass, female and male mice have similar rates of oxygen consumption. However, genotype x sex interaction are rare therefore testing only males is acceptable.

It is essential that all phenotyping experimentation is conducted at the same time of day because physiological and biochemical parameters e.g. metabolic rate, body temperature and activity are subject to temporal rhythms. In the indirect calorimetry module standard measurements begin five hours before lights-off (lights off = T0) and are finished at T16 i.e. four hours after lights-on the next morning. Optional: Mice can be given one day of acclimation before the trial, and the trial can be continued for more than 21 hours.

Equipment

1. Calorimetric system equipped with respirometer, feeder and water bottles

2. Ambulatory activity monitor (dependent on system specifications)
3. Food and water intake monitor
4. Computer with apparatus software installed

Procedure

1. Optionally mice are allowed to acclimatise to the phenotyping room, to the calorimetry cage, food hoppers and drinking bottles 24 hours before testing.
2. Prepare and calibrate the calorimetric apparatus to confirm the accuracy of the gas sensors and flow meters. Specifically prior to each experiment:

a. Apply known volumes of CO_2 and O_2 to determine the sensitivity of the gas sensors and flow meters.
b. Run a complete calibration protocol according to the manufacturer's recommendations.

3. Provide each calorimetry cage with sufficient food and water for a period of ~24 (or 48) hours.
4. Weigh the mouse.
5. Place the mouse into a calorimetry cage with food and water available *ad libitum*.
6. Label the chamber with the corresponding subject identification and close it ensuring there is adequate air flow.
7. Initiate the calorimetric system for measurement:

a. Set up a new experiment in accordance with the manual (or load a file from a previous experimental setting).
b. Start recording measurements five hours before lights off for a total duration of 21 hours at minimum. Optional: 24 hours acclimation can be applied and the recording may continue for 48 hours.
c. The latency of CO_2 and O_2 activity transmitted and recorded is dependent on the number of chambers in use but will be logged periodically.

8. Generating a data report:

a. Upload all data from the experimentation including:

- Gas analysis VO2 and VCO2 (ml/h/animal)
- Heat production (kJ/h/animal)
- Periodicity of measurements taken throughout experimentation (Figure 1)
- Animal and the corresponding chamber that was used
- The respiratory exchange ratio (RER) can be calculated using the VCO2/VO2 ratio.

9. Activity parameters recorded will depend on the specification of calorimetric system used:

a. Ambulatory activity can be derived from the number beam splits during the session
b. Total activity can be derived from the number of fine movement (e.g. grooming behaviours) as well as ambulatory activity
c. An average of each of these parameters of activity is calculated hourly across the measurement period (between T-5 and T16).

d. Water and food intake (cumulative, hourly or total food and water intake, between T-5 and T16, will be computable depending on the calorimetric system used).

10. Remove each mouse from its chamber in turn at the end of the experimental session and record its weight. Return to their home cage.
11. Monitor the animals carefully to observe any abnormal behaviour(s). Ensure that food and water are available *ad libitum.*
12. Wash and wipe clean the chambers with warm water and dilute alcohol or appropriate disinfectant respectively.

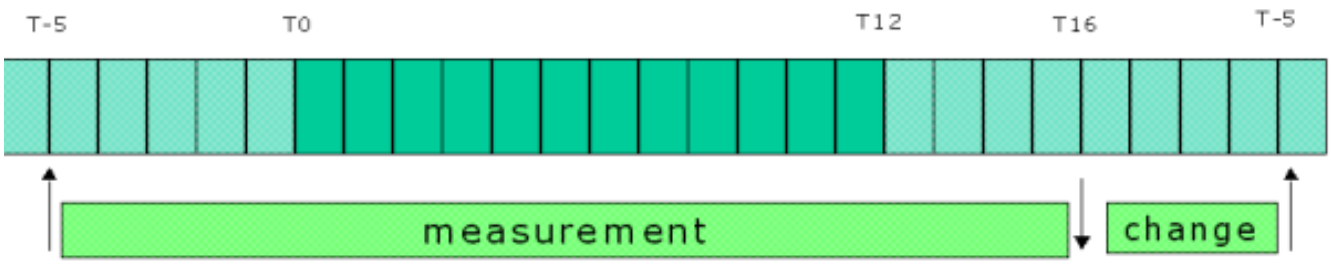


Figure 1. Daily workflow of calorimetric experimentation (Note: T0 designates start of dark cycle).

Notes

The system requires periodic calibration of the gas sensors and flow meters to ensure precise measurements. The calibration procedure consists of the application of a gas of known composition and adjusting control knobs in the front of the oxygen and carbon dioxide sensors to obtain readings that reflect the contents of the calibration gas. It is recommended that the system be calibrated prior to the start of each experiment. The analyzers should not be shut down if not urgently required for maintenance. If this has to be done a warm up time of at least 90 minutes is required for the gas sensors for calibration (refer to manufacturer's manual). Calibrations and shut downs should be recorded in the laboratory journal.

Calorimetry test is to be performed before the ECG/ECHO test to avoid effects of hair removal on the calorimetry results.

The information about the date of the experiment, that is the date when the measurement is performed, is an important parameter which is to be submitted in the Experiment xml file (dateOfExperiment="2013-02-28").

Data QC

1. Respiratory Exchange Rate (RER) is between 0.7-1.00
2. Mice show normal feeding and drinking behaviour
3. Mice show stable weight before and after calorimetry
4. Correct calibration of gases according to manufacturer's manual

MetaData and examples

Metadata	Example
Time of dark cycle start	The starting time of the dark cycle. E.g. 7 pm
Time of dark cycle end	The ending time of the dark cycle. E.g. 7 am
Room temperature	The range of min. and max. temperatures of the calorimetry chamber. If the calorimetry machine doesn't register the chamber temperature, the room temperature in which the machine resides can be reported instead. E.g. 20.0-24.0 (C°). If the temperature is constant throughout the experiment, a single temperature can be submitted. E.g. 20.0 (C°). Do not submit the subtraction between the 2 values!
Acclimation to calorimetry cages	E.g. Yes/No.
Duration of test	Duration of the test without including the acclimation period. Can be a minimum of 21 hours or more, when acclimation is done. E.g. 21 (hours).
Equipment ID	ID of the machine used when more than 1 is used having same model and manufacturer. E.g. machine 1, machine 2, machine Minnie, machine Mickey Mouse, etc.
Equipment manufacturer	Manufacturer of the equipment. E.g. TSE Systems GmbH.
Equipment model	Model of the equipment. E.g. Labmaster CaloSys.
Experimenter ID	An ID of any format to be used coherently both inside the same procedure and for all procedures. E.g. Harw_001, or 1/2/3.
Date equipment last calibrated	Most recent date in which the equipment (or any part of) used in the procedure was subject to a calibration event.

Outer dimension of cage	(L, cm) x (W, cm) x (H, cm). E.g. 21.4 x 11.5 x 13.3
Inner dimension of cage	(L, cm) x (W, cm) x (H, cm). E. g. 21.4 x 11.5 x 13.3
Height from platform** to lid assembly	cm. E.g. 8.6
Available space for mouse	(L, cm) x (W, cm) x (H, cm). E.g. 20.3 x 10.4 x 8.6
Infrared beam setting on X axis	Orizontal activity: locomotion. N beams with L cm spacing, H cm above platform**. E.g. 16, 1.3, 3.0
Infrared beam setting on Y axis	Orizontal activity: locomotion. N beams with L cm spacing, H cm above platform**. E.g. 16, 1.3, 3.0
Infrared beam setting on Z* axis	Vertical activity: rearing/jumping. N beams with L cm spacing, H cm above platform**. E.g. 16, 1.3, 6.8
Beam strip placement on exterior of chamber****	Left/right, front/back, both
Lightbeam wavelength	nm, E.g. 950
Scanning rate	Hz, E.g. 100
Refractory period	s, E.g. 0.8
Presence of bedding into the cage	Yes/No
Igloo in cage	Yes/No

*Z has same direction as X only is higher with respect to the platform

** the floor where the mouse stand

*** This value is the "Height from platform** to lid assembly"

Parameters and Metadata

Date equipment last calibrated NINGLA_CAL_019_001 | v1.2

procedureMetadata

Req. Analysis: false **Req. Upload:** false **Is Annotated:** false

---

Acclimation to respirometry cages NINGLA_CAL_012_001 | v1.0

procedureMetadata

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

Options: No, Yes,

Inner dimension of cage NINGLA_CAL_028_001 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** false **Is Annotated:** false

Unit Measured: cm

Body weight before experiment NINGLA_CAL_001_001 | v1.3

simpleParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

Unit Measured: g

Presence of bedding into the cage NINGLA_CAL_038_001 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** false **Is Annotated:** false

Options: No, Yes,

Chamber temperature max NINGLA_CAL_026_001 | v1.1

procedureMetadata

Req. Analysis: false **Req. Upload:** false **Is Annotated:** false

Unit Measured: C

Duration of test NINGLA_CAL_013_001 | v1.0

procedureMetadata

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

Unit Measured: Hours

Options: 23, 22, 48, 21,

Room temperature max NINGLA_CAL_023_001 | v1.4

procedureMetadata

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

Unit Measured: C

Total activity (no. of fine movement + no. of beam cuts) NINGLA_CAL_007_001 | v1.2

seriesParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** true

Unit Measured: count/hour

Increments: Minimum 21

Beam strip placement on exterior of chamber NINGLA_CAL_034_001 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** false **Is Annotated:** false

Options: Left/right, Both, Front/back,

Available space for mouse NINGLA_CAL_030_001 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** false **Is Annotated:** false

Unit Measured: cm

Acclimation period NINGLA_CAL_041_001 | v1.0

procedureMetadata

Req. Analysis: false **Req. Upload:** false **Is Annotated:** false

Unit Measured: Hours

Equipment manufacturer NINGLA_CAL_015_001 | v1.1

procedureMetadata

Req. Analysis: true **Req. Upload:** true **Is Annotated:** false

Options: TSE Systems GmbH, O'hara Co. Ltd., Columbus Instruments, Sable Systems,

---

Experimenter ID NINGLA_CAL_018_001 | v1.0

procedureMetadata

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

---

Cumulative food intake NINGLA_CAL_009_001 | v1.2

seriesParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** true

Unit Measured: g

Increments: Minimum 21

Equipment model NINGLA_CAL_016_001 | v1.2

procedureMetadata

Req. Analysis: true **Req. Upload:** true **Is Annotated:** false

Options: PhenoMaster/LabMaster CaloSys, (Dri)+Fed, Act.X,Y 16mice,
PhenoMaster/Labmaster CaloSys, (Dri)+Fed, Act.X,Y 12Mice, FWI-3002 & IA-16M,
Oxymax FAST, SM-MARS 8 channel Metabolic system,
PhenoMaster/Labmaster CaloSys, (Dri)+Fed, Act.X,Y 20Mice, Oxymax/CLAMS,
SAMPLEMAX,
PhenoMaster/Labmaster CaloSys, (Dri)+Fed, Act.X,Y 12Mice - Gas system 994620 series,
PhenoMaster/Labmaster CaloSys, (Dri)+Fed, Act.X,Y 32Mice,
PhenoMaster/Labmaster CaloSys – TSE Systems GmbH,

Lightbeam wavelength NINGLA_CAL_035_001 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** false **Is Annotated:** false

Unit Measured: nm

Outer dimension of cage NINGLA_CAL_027_001 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** false **Is Annotated:** false

Unit Measured: cm

Respiratory Exchange Ratio series NINGLA_CAL_042_001 | v1.0

seriesParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** false

Increments: Minimum 21

Total food intake NINGLA_CAL_008_001 | v1.2

simpleParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** true

Unit Measured: g

Time of dark cycle start NINGLA_CAL_010_003 | v3.0

procedureMetadata

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

---

Cumulative water intake NINGLA_CAL_022_001 | v1.1

seriesParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** true

Unit Measured: ml

Increments: Minimum 21

Time of dark cycle end NINGLA_CAL_020_003 | v3.1

procedureMetadata

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

---

Scanning rate NINGLA_CAL_036_001 | v1.1

procedureMetadata

Req. Analysis: true **Req. Upload:** false **Is Annotated:** false

Unit Measured: Hz

Igloo in cage NINGLA_CAL_039_001 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** false **Is Annotated:** false

Options: Yes, No,

Respiratory Exchange Ratio NINGLA_CAL_017_001 | v1.2

simpleParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** true

Derivation:
div(meanOfIncrements('NINGLA_CAL_004_001',21), meanOfIncrements ('NINGLA_CAL_003_001',21))

Equipment ID NINGLA_CAL_014_001 | v1.0

procedureMetadata

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

---

Chamber temperature min NINGLA_CAL_025_001 | v1.1

procedureMetadata

Req. Analysis: false **Req. Upload:** false **Is Annotated:** false

Unit Measured: C

Carbon dioxide production NINGLA_CAL_004_001 | v1.2

seriesParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Unit Measured: ml/h/animal

Increments: Minimum 21

Calibration method NINGLA_CAL_040_001 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** false **Is Annotated:** false

Options: Reference range method, Gas bottle method,

Oxygen consumption NINGLA_CAL_003_001 | v1.2

seriesParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Unit Measured: ml/h/animal

Increments: Minimum 21

Room temperature min NINGLA_CAL_024_001 | v1.2

procedureMetadata

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

Unit Measured: C

Infrared beam setting on Z axis NINGLA_CAL_033_001 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** false **Is Annotated:** false

Unit Measured: cm

Body weight after experiment NINGLA_CAL_002_001 | v1.3

simpleParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

Unit Measured: g

Heat production (metabolic rate) NINGLA_CAL_005_001 | v1.2

seriesParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Unit Measured: kJ/h/animal

Increments: Minimum 21

Height from platform to lid assembly NINGLA_CAL_029_001 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** false **Is Annotated:** false

Unit Measured: cm

Ambulatory activity (no. of beam cuts) NINGLA_CAL_006_001 | v1.2

seriesParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** true

Unit Measured: count/hour

Increments: Minimum 21

Infrared beam setting on X axis NINGLA_CAL_031_001 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** false **Is Annotated:** false

Unit Measured: cm

Total water intake NINGLA_CAL_021_001 | v1.1

simpleParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** true

Unit Measured: ml

Refractory period NINGLA_CAL_037_001 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** false **Is Annotated:** false

Unit Measured: s

Infrared beam setting on Y axis NINGLA_CAL_032_001 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** false **Is Annotated:** false

Unit Measured: cm
