Indirect Calorimetry IMPC_CAL_002

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.

Ontological description: MP:0005266 - abnormal metabolism.

Experimental Design

Minimum number of mutant animals: 7 mice for each sex.

Age of animals at test: 11-12 weeks.

Sexual dimorphism: In general, female mice have higher metabolism compared to males therefore statement is not entirely correct. 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:

1. Apply known volumes of CO_2 and O_2 to determine the sensitivity of the gas sensors and flow meters.
2. 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:

1. Set up a new experiment in accordance with the manual (or load a file from a previous experimental setting).
2. 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.
3. 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:

1. 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:

1. Ambulatory activity can be derived from the number beam splits during the session
2. Total activity can be derived from the number of fine movement (e.g. grooming behaviours) as well as ambulatory activity
3. An average of each of these parameters of activity is calculated hourly across the measurement period (between T-5 and T16).
4. 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.

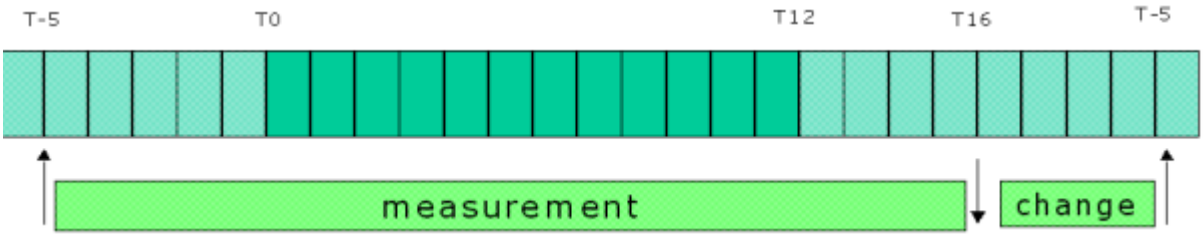

Figure 1. Daily workflow of calorimetric experimentation (Note: T0 designates start of dark cycle).

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

Parameters

	Version	Type	Increment	Option	Derived	Unit	Data Type
Body weight before experiment IMPC_CAL_001_001	1.3	simpleParameter				g	FLOAT
Body weight after experiment	1.3	simpleParameter				g	FLOAT

	Version	Type	Increment	Option	Derived	Unit	Data Type
IMPC_CAL_002_001							
Oxygen consumption IMPC_CAL_003_001	1.2	seriesParameter	datetime Minimum: 21			ml/h/animal	FLOAT
Carbon dioxide production IMPC_CAL_004_001	1.2	seriesParameter	datetime Minimum: 21			ml/h/animal	FLOAT
Heat production (metabolic rate) IMPC_CAL_005_001	1.2	seriesParameter	datetime Minimum: 21			kJ/h/animal	FLOAT
Ambulatory activity (no. of beam cuts) IMPC_CAL_006_001	1.2	seriesParameter	datetime Minimum: 21			count/hour	INT
Total activity (no. of fine movement + no. of beam cuts) IMPC_CAL_007_001	1.2	seriesParameter	datetime Minimum: 21			count/hour	INT
Total food intake IMPC_CAL_008_001	1.2	simpleParameter				g	FLOAT
Cumulative food intake IMPC_CAL_009_001	1.2	seriesParameter	datetime Minimum: 21			g	FLOAT
Respiratory Exchange Ratio IMPC_CAL_017_001	1.2	simpleParameter			IMPC_CAL_004_001 mean_of_increments IMPC_CAL_003_001 mean_of_increments /		FLOAT
Total water intake IMPC_CAL_021_001	1.1	simpleParameter				ml	FLOAT
Cumulative water intake IMPC_CAL_022_001	1.1	seriesParameter	datetime Minimum: 21			ml	FLOAT

Metadata

	Version	Type	Increment	Option	Derived	Unit	Data Type
Time of dark cycle start IMPC_CAL_010_002	2.0	procedureMetadata		pm8:00			TIME
Acclimation to respirometry	1.0	procedureMetadata		Yes			TEXT

	Version	Type	Increment	Option	Derived	Unit	Data Type
cages IMPC_CAL_012_001							
Duration of test IMPC_CAL_013_001	1.0	procedureMetadata		21		Hours	INT
Equipment ID IMPC_CAL_014_001	1.0	procedureMetadata					TEXT
Equipment manufacturer IMPC_CAL_015_001	1.1	procedureMetadata		O'hara Co. Ltd.			TEXT
Equipment model IMPC_CAL_016_001	1.2	procedureMetadata		FWI-3002 & IA-16M			TEXT
Experimenter ID IMPC_CAL_018_001	1.0	procedureMetadata					TEXT
Date equipment last calibrated IMPC_CAL_019_001	1.2	procedureMetadata					DATE
Time of dark cycle end IMPC_CAL_020_002	2.0	procedureMetadata		am8:00			TIME
Room temperature max IMPC_CAL_023_001	1.4	procedureMetadata		25		C	FLOAT
Room temperature min IMPC_CAL_024_001	1.2	procedureMetadata		21		C	FLOAT
Outer dimension of cage IMPC_CAL_027_001	1.0	procedureMetadata		29.3×14.4		cm	TEXT
Inner dimension of cage IMPC_CAL_028_001	1.0	procedureMetadata		25.5×11		cm	TEXT
Height from platform to lid assembly IMPC_CAL_029_001	1.0	procedureMetadata		14.7		cm	FLOAT
Available space for mouse IMPC_CAL_030_001	1.0	procedureMetadata		25.5×11×14.7		cm	TEXT
Infrared beam setting on X axis IMPC_CAL_031_001	1.0	procedureMetadata		17, 1.5, 3.0		cm	TEXT
Infrared beam setting on Y axis IMPC_CAL_032_001	1.0	procedureMetadata		7, 1.5, 3.0		cm	TEXT
Beam strip placement on exterior of chamber IMPC_CAL_034_001	1.0	procedureMetadata		Both			TEXT

	Version	Type	Increment	Option	Derived	Unit	Data Type
Lightbeam wavelength IMPC_CAL_035_001	1.0	procedureMetadata		940		nm	INT
Presence of bedding into the cage IMPC_CAL_038_001	1.0	procedureMetadata		Yes			TEXT
Igloo in cage IMPC_CAL_039_001	1.0	procedureMetadata		No			TEXT
Calibration method IMPC_CAL_040_001	1.0	procedureMetadata		Gas bottle method			TEXT