Cold exposure murine models

Bianca Hemmeryckx,Dries Bauters,H. Roger Lijnen

Abstract

Increasing energy expenditure by stimulating thermogenesis through activation of brown adipose tissue (BAT) and/or induction of browning of white adipose tissue (WAT) is considered a promising strategy to treat/prevent obesity and related metabolic diseases. Whereas WAT is adapted to store energy as triglycerides, BAT produces heat (non-shivering thermogenesis). In brown adipocytes, the uncoupling protein-1 (UCP-1) regulates conversion of energy into heat by uncoupling ATP production from mitochondrial respiration. Also in WAT adaptive UCP-1 positive adipocytes (brown in white: brite or beige) can arise, predominantly in subcutaneous (s) WAT. This browning of WAT is enhanced by exposure to cold.

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Guidelines

-Mouse needs to be out of cage, relaxed on a bench, a scalpel is used to cut of 1 mm of tail, the tail starts to bleed, glucose levels are measured on the second drop of blood.
-When the body temperature of the animals drop below 32°C, the experiment will be stopped (approved by the ethical committee of the KU Leuven).

Protocol

Step 1.
Determine the body weight of 8-weeks old male mice (C57BL/6J, C57BL/6N or gene-deficient mice with control littermates)

Step 2.
House the mice individually with access to standard chow (10.9 kJ/g) and drinking water ad libitum.

Step 3.
Expose the mice to cold temperatures (4-8°C) for 72 hours or 2 weeks by transferring the cages to a ventilated cooling unit (Friginox, France) with a 12-hour light/dark cycle (7AM/7PM).

Step 4.
Replace drinking water and change the cages with the standard chow bi-weekly. Check the health of the animals every day.

Step 5.

Record food intake, body weight and body temperature (rectal probe, TR-100, Fine Science Tools) upon changing of the cages.

Step 6.
After 72 hours or 2 weeks, fast animals for 6 hours (7:30 AM – 1:30 PM).

Step 7.

Determine blood glucose levels on a drop of blood taken from the nicked tail of a free-moving mouse using the Accu-Chek Aviva glucose meter (06988563016, Roche Diagnostics) and Accu-Chek Aviva glucose strips (06453970, Roche Diagnostics)

Step 8.

Sacrifice animals by intraperitoneal injection of 60 mg/kg sodium pentobarbital (Nembutal) or Dolethal.

-Nembutal (60 mg/ml): we make 10x dilution with saline.
-Dolethal (200 mg/ml): we make 30x dilution with saline.

REAGENTS
- Nembutal 60 mg/ml by Contributed by users
- Dolethal 200 mg/ml by Contributed by users

Step 9.
When animals are asleep, collect blood from the retro-orbital sinus on trisodium citrate and processed for plasma (http://dx.doi.org/10.17504/protocols.io.j9dcr26). Store plasma in aliquots at -80°C.

Step 10.

Immobilize the animals, cut open the chest and wash out the heart and the entire circulatory system with saline by putting a perfusion needle in the apex (left ventricle). Make a small cut in the right atrium to allow blood to flow out.

*Microflex Infusion set, orange: Ref. 240.05, Vygon Vet

Step 11.
Make a midline incision in the skin and make a incision at the height of the hind limbs.

Step 12.
Pull skin to the side so the inguinal subcutaneous white adipose tissue (sWAT) depots are visible. Remove the internal lymph nodes. Dissect out the sWAT depots.

Step 13.
Make a midline incision in the muscle layer to open the abdominal cavity.

Step 14.
Locate the intra-abdominal gonadal (GON) WAT depots and liver. Removed them.

Step 15.
Flip the mouse, locate the interscapular brown adipose tissue depot and remove it.

Step 16.
Weigh all dissected organs.

Step 17.
Make portions of each tissue for RNA, protein extraction or histology.

Step 18.
Snap-freeze the portions for RNA and protein extraction and store at -80°C.

Step 19.
Process the portions for histology (see separate protocol).

Step 20.

Clean dissection material and bench with Virkon S, detergent, water and 70% ethanol.

REAGENTS
Virkon™ S 5O tablets/bottle NC9821357 by Fisher Scientific

Step 21.
Dispose of the animal body according to internal institutional guidelines.