Diet-induced obesity murine model

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Abstract

Obesity can be induced by exposing mice to a western-type diet high in sugar and fat for 15 weeks. Control animals receive a standard fat diet for 15 weeks.

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Guidelines

-HFD is stored at +4°C
-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.

Protocol

Step 1.

Randomize 8-weeks old male mice (C57BL/6J, C57BL/6N or gene-deficient mice with control littermates) to two groups based on body weight.

Step 2.

Give one group 7g standard chow (SFD, 10.9 kJ/g) each day/mouse, and the other group 7g western high-fat diet (HFD, 22 kJ/g; kcal from fat 42%, 43% from carbohydrates and 15% from protein) for 15 weeks. As food intake averages 4g/day, the animals are fed ad libitum. Provide drinking water ad libitum.

REAGENTS
ssniff® EF R/M acc. TD88137 mod E15721-34 by Contributed by users

Step 3.

House the animals in a temperature (20-23°C) and humidity (30-40%)-controlled room with a 12-hour light/dark cycle (7AM/7PM). Give 1-2 small wooden sticks to every cage containing mice on a HFD in order to allow correct wearing off of their teeth.

Step 4.

Replace drinking water and change the cages every week. Check the health of the animals every day.

Step 5.
Replace each diet weekly, record food intake and body weight weekly.

Step 6.

After 15 weeks of diet administration, 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 meter and Accu-Chek Aviva glucose strips.

REAGENTS
- Accu-Chek Aviva glucose strips 06453970 by Contributed by users

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.

REAGENTS
- Microflex Infusion set, orange 240.05 by Contributed by users

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 Vircon 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.