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Mouse intragastric infusion (*iG*) model

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Abstract

Direct intragastric delivery of a diet, nutrient or test substance can be achieved in rodents (mice and rats) on a long-term (2–3 months) basis using a chronically implanted gastrostomy catheter and a flow-through swivel system. This rodent intragastric infusion (*iG*) model has broad applications in research on food intake, gastrointestinal (GI) physiology, GI neuroendocrinology, drug metabolism and toxicity, obesity and liver disease. It achieves maximal control over the rate and pattern of delivery and it can be combined with normal *ad libitum* feeding of solid diet if so desired. It may be adopted to achieve infusion at other sites of the GI system to test the role of a bypassed GI segment in neuroendocrine physiology, and its use in genetic mouse models facilitates the genetic analysis of a central question under investigation.

INTRODUCTION

iG model for the study of food intake and GI physiology

Direct delivery of nutrients to stomach or intestine via an implanted catheter allows the testing of diverse and unique questions. These include the roles of a segment within the normal food intake route in neuroendocrine mechanisms of cephalic, GI and metabolic responses to nutrient intake^{1–7}. Mechanisms of inhibition of gastric emptying or postgastric satiety are studied by using *iG* or intraduodenal infusion of a liquid diet in mice that have been given receptor antagonists for intestinal or pancreatic endocrine peptides such as

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cholecystokinin or amylin^{8,9}. The use of the *iG* model facilitates the identification of rapid postoral mechanisms of flavor preference conditioning by sugar and fat as well as its potential interaction with an orosensory response^{3,5}. The maintenance of a long-term access (2–3 months) to the GI tract is often challenging. However, if it is achieved, it enables the study of the physiologic or metabolic adaptation to and pathophysiological consequences of experimental dietary conditioning. Such approaches are becoming crucial to studies on physiologic and metabolic complications associated with lifestyle-related diseases such as metabolic syndrome associated with obesity as described below.

Modeling liver disease using the *iG* model

One of the early objectives for creation of the rodent long-term *iG* model was the scientific desire to test whether maximal ethanol intake results in major alcoholic liver disease (ALD) in rodents that are naturally averse to ethanol and otherwise do not voluntarily consume adequate amounts of ethanol to damage the liver. Answers derived from the model for this critical question were both yes and no. With a maximal intake of ethanol reaching as high as 49% of caloric intake facilitated by forced *iG* ethanol administration, the rat develops severe hepatic steatosis and elevated alanine aminotransferase (ALT) levels even with a diet low in fat, which does not cause these changes in the models with *ad libitum* consumption of ethanol-containing liquid diet¹⁰. An increase in the dietary content of polyunsaturated fat causes further progression to steatohepatitis¹¹, and the addition of carbonyl iron¹² or enteral lipopolysaccharide administration¹³ potentiates liver fibrosis. Thus, although maximal ethanol intake indeed causes more serious liver injury than that observed in other *ad libitum* models, it alone is not sufficient for progression of ALD. The development of the mouse *iG* model has revolutionized the field because it allows genetic studies of ALD. The *iG* model using mice deficient in tumor necrosis factor (TNF) receptor I, CD14, Toll-like receptor 4, LBP, p47phox, ICAM-1 and so on revealed the importance of endotoxin-mediated activation of hepatic macrophages, their NADPH oxidase activation and generation of reactive oxygen species, subsequent TNF- α induction and proinflammatory response in the ALD pathogenesis (see ref. 14 for a review).

Application to obesity-associated research

Another important application of the mouse *iG* model is obesity-associated metabolic syndrome including nonalcoholic fatty liver disease (NAFLD), which is recognized as a global epidemic and the most common type of liver disease¹⁵. Although NAFLD and more pathologically advanced nonalcoholic steatohepatitis (NASH) are associated with obesity-associated insulin resistance (IR), the precise pathogenetic relationship between them historically could not be studied because of the lack of animal models that reproduce the natural history and course of the disease in humans. Genetic models such as mice deficient in leptin¹⁶ and leptin receptor¹⁷ are not clinically relevant and do not develop steatohepatitis. Mice with targeted ablation of peroxisome proliferator-activated receptor- α (*Ppara*)¹⁸, acyl-CoA oxidase (*Acox1*)¹⁹, methionine adenosyltransferase1A (*Mat1a*)²⁰ and phosphatase and tension homolog deleted on chromosome 10 (*Pten*)²¹ all develop deranged liver metabolism and consequent NAFLD or/and NASH, but these mutations are, again, rare in patients. Feeding rodents a diet deficient in choline and methionine results in NASH²², but the model does not result in the development of obesity or IR, and this extreme

malnutrition is not relevant to NAFLD and NASH in human patients. More natural nutrition models include feeding *ad libitum* a diet high in sucrose^{23,24}, fructose²³ or fat²⁴. However, these models produce mild obesity and NAFLD but not NASH. The most common cause of obesity in humans is hypercaloric alimentation. The *iG* model can be used to reproduce this condition by forced feeding. Indeed, *iG* feeding of male C57BL/6 mice with high-fat diet at a level up to 85% in excess of the standard intake spontaneously produces severe obesity, abdominal adiposity, hyperglycemia, hyperinsulinemia, hyperleptinemia, glucose tolerance, IR and NAFLD/NASH²⁵. A model such as this would allow studies on interrelationships among these metabolic and pathologic phenotypes in their pathogenesis.

Comparison with other methods and additional applications

As discussed above, the major difference between the *iG* model and other models is that the *iG* model achieves an absolute control over the intragastric delivery of nutrients or any liquid test substance. It allows a precise control over the feeding or administration rate (amount) and mode (continuous, intermittent or bolus). More importantly, it allows testing the role of a segment of the normal oroesophageal and GI route of nutrient intake by bypassing it via intragastric or intrainestinal access. Two limitations of the model are (i) that the diet or substance to be administered must be in a liquid form, and (ii) that it requires surgical expertise and swivel and infusion setup for chronic infusion.

Although this article describes technical details of the *iG* modeling in the mouse to help readers acquire essential methodological information, the same principle can be applied for the rat as described elsewhere²⁶. Dietary and infusion methods described pertain to continuous intragastric infusion of a liquid diet with a varying content of corn oil with or without ethanol administration as an example of the *iG* model application, but they can be modified according to different experimental needs.

Experimental design

Animals—Any strain, age or sex of mice can be used for the model. Genetically modified mice or mice subjected to nutritional or pharmacological treatments may differ in their response to dietary or ethanol administration. For the standard mouse *iG* model, we use 8-week-old male C57BL/6J mice obtained from the Jackson Laboratory. We allow at least 1 week of acclimatization after receiving mice before the *iG* surgery. Another week of a recovery period follows the surgery, and during this period a basic control diet is infused with or without regular chow.

Controls—Appropriate controls should be created for an experimental group, using identical strains, ages, sexes and *iG* feeding procedures. It is ideal to use the same littermates. For ethanol experiments, an isocaloric diet replacing calories from ethanol with dextrose solution can be used to pair feed. To examine the effects of *iG* overfeeding, mice fed at the standard caloric intake can be used as a control. In addition, if the amount of a nutrient is modified, the unmodified diet can be used for controls.

MATERIALS

REAGENTS

- Mice: any strain, age or sex can be used ! **CAUTION** Experiments involving mice must conform to governmental and institutional ethics regulations.

Gastrostomy catheter reagents

- Chloroform (Sigma, cat. no. C2432) ▲ **CRITICAL** Always use chloroform fresh from the bottle.

Anesthesia and pre-/postoperative reagents

- Ketamine HCl Injection, USP (Western Medical, cat. no. NDC-57319–542–02)
- Xylazine sterile solution (Western Medical, cat. no. NADA no. 139–236)
- Gentamicin sulfate veterinary ophthalmic ointment (Western Medical, cat. no. NDC17033–011–38)
- Buprenorphine (Buprenex) hydrochloride injection (Western Medical, cat. no. NDC 40042–010–01)
- Prodine (povidone-iodine) solution (Western Medical, cat. no. NDC57319–328–09)
- Lactated ringer's and 5% dextrose injection (Western Medical, cat. no. NDC0409–7929–09)
- Ethanol (70%, vol/vol; diluted from 100% solution)

Surgical reagents

- Sodium chloride irrigation (0.9%), USP (Western Medical, cat. no. NDC0409–7138–09)

Diet supplies

- Lactalbumin hydrolysate (Invitrogen, cat. no. 11800–042)
- Dextrose (Sigma, cat. no. G8270)
- Citric acid (Sigma, cat. no. C0759)
- Potassium phosphate (Sigma, cat. no. P5379)
- Calcium chloride (Sigma, cat. no. C8106)
- Sodium chloride (cat. no. S3014)
- Magnesium sulfate (Sigma, cat. no. M1880)
- Manganese sulfate (Sigma, cat. no. M7634)
- Potassium iodide (Sigma, cat. no. P207969)
- Ammonium molybdate (Sigma, cat. no. A7302)

- Cupric sulfate (Sigma, cat. no. C7631)
- Ferric ammonium citrate (cat. no. F5879)
- Zinc chloride (Sigma, cat. no. Z208086)
- Trace mineral mix (Dyets, cat. no. 210090) **▲ CRITICAL** A fresh batch should be aliquotted into small vials and stored in a refrigerator. Take out one vial for each use as needed.
- Vitamin mix AIN-76 (Dyets, cat. no. 300050) **▲ CRITICAL** Aliquots should be prepared in a manner similar to that mentioned above.
- Choline chloride (Dyets, cat. no. 400775) **▲ CRITICAL** Aliquots should be prepared in a manner similar to that mentioned above.
- Mazola corn oil or any other fat type (Local grocery store)
- Xanthan gum (Sigma, cat. no. G1253)
- Ethanol (100%, vol/vol; Fisher, cat. no. 04–355–451)

EQUIPMENT

Gastrostomy catheter materials

- Tygon tubing (inner diameter (i.d.) 0.508 mm × outer diameter (o.d.) 1.524 mm; i.d. 0.762 mm × o.d. 2.286 mm; Fisher, cat. nos. 1417015B, 1417015C)
- Silastic silicone tubing (i.d. 0.635 mm × o.d. 1.1938 mm, i.d. 0.762 mm × o.d. 1.651 mm; Fisher, cat. nos. 11–189–15B, 11–189–15C)
- Stainless steel wire T-340 V (Small Parts, cat. no. GWX-0110–30)
- Dacron felt (0.635 mm in thickness, PEI)
- RTV silicone rubber (Ellsworth, cat. no. 2118874)
- RTV catalyst no. 4 (Ellsworth, cat. no. 1103181)
- Precision swivel (22 G; Instech, cat. no. 375–22P)
- Intramedic Luer stub adaptor (23 G; VWR, cat. no. 63019–852)
- Intramedic Luer stub adaptor (20 G; VWR, cat. no. 63019–841)
- Syringe (1 ml; VWR, cat. no. BD309602)
- Y-shaped tubing connector (Small Parts, cat. no. STCY-18–10)
- Nylon tubing (Small Parts, cat. no. NSR-106B–C)

Surgical supplies

- 6/0 silk suture (Hospital Associates, cat. no. 1639G)
- Powdered sterile latex surgical gloves (Fisher, cat. no. 19–020–557B)
- Eye dressing surgical forceps (101.6 mm; VWR, cat. no. 21909–694)

- Nonsterile cotton gauze sponges (100 mm × 100 mm; Fisher, cat. no. MSD14–002–50)
- Surgical towels (457.2 mm × 838.2 mm)
- Sterilization pouch (190.5 mm × 330.2 mm; VWR, cat. no. 89140–802)
- Castroviejo needle holder (139.7 mm; VWR, cat. no. 10049–416)
- Jacobson Mosquito surgical forceps straight, curved (VWR, cat. nos. 219009–470, 21909–472)
- Miltex Jeweler-style forceps (Express Surgical (<http://www.expressurgical.com/>), cat. no. 17–305)
- Water heating pump (Fisher, cat. no. NC9803964)

Diet supplies

- Spectra mesh filter (70 µm; Spectrum lab, cat. no. 146–490)
- Sterile serological pipette (25 ml)
- Glass beakers (3 liters)
- Plastic beakers (4 liters)
- Graduated cylinders (2 liters)
- Graduated cylinders (1 liter)
- Stirrer and stirring magnetic bars
- VLP 200 mechanical vacuum pump (Ideal vacuum products, cat. no. VLP200)
- Plastic Buchner filtering funnel
- Pipetaid
- Commercial food blender (3 liters; Pacific Kitchen Equipment, cat. no. 38BL61)
- Glass filtering flask (2 liters)

Infusion setup supplies

- Syringe (10 ml; VWR, cat. no. BD309604)
- Shoe-box mouse cage with filter top lid
- Stainless steel rack for animal cages with steel rod for clamps
- Clamps
- Rubber stopper with a hole, cut to hold swivel
- Harvard Apparatus PHD 2000 Infusion pump with multirack kit (Harvard Apparatus, cat. no. PHD70–2003)
- Water-circulating heating pad

- Marker pen
- Silastic catheter tip

PROCEDURE

Catheter construction

- 1| Cut a Tygon tube (i.d. 0.508 mm × o.d. 1.524 mm; 'b' in Fig. 1) to a length of 350 mm.
- 2| Cut a Silastic tube (i.d. 0.64 mm × o.d. 1.19 mm; 'f' in Fig. 1a) to a length of 70 mm and immerse it in chloroform for a few minutes to expand the diameter.
! CAUTION This should be done in a fume hood.
- 3| Slide the Silastic tube over the end of the Tygon tube up to about 25 mm.
▲ CRITICAL STEP Hold the Silastic tube in place for 30 s to prevent it from slipping off of the Tygon tube.
- 4| Measure and mark (with a marker pen) a distance of 10 mm from the end of Tygon tube with the Silastic tube over it. Bend the tubing at this precise location and hold this bend by placing it into rubber tubing (i.d. 12 mm × o.d. 17 mm in a 10-mm length). Place the angled area supported by the rubber tubing into boiling water for about 3 s and remove the rubber tube from the angle point. This will create a 90–100° angle (Fig. 1c).
! CAUTION Be careful when handling boiling water and the hot plate.
▲ CRITICAL STEP Do not make too sharp of an angle, as it narrows the diameter of the Tygon tubing.
- 5| Attach a stainless steel wire (150 mm in length; 'd' in Fig. 1a) along the outer wall of the proximal Tygon tubing by securing it with short, chloroform-expanded Silastic tubing at four or five positions on the catheter ('c' in Fig. 1a).
- 6| Cut a piece of Dacron felt to a pear shape (8 mm width, 16 mm length) and pass the catheter through a hole made in the middle ('e' in Fig. 1a); next, glue it to the catheter angle with RTV silicone rubber resin and catalyst (Fig. 1c,d).
- 7| Cut a Dacron felt disc (6 mm in diameter), pass the Silastic catheter tip via a hole made in the middle and glue the disc to Silastic tubing at a location 33 mm distal to the tip of the Tygon tubing ('g' in Fig. 1a).
- 8| Place a 1-ml syringe with a 23-G Luer stub adaptor on the proximal end of the catheter (Fig. 1b).
- 9| Gas-sterilize the catheter in a sterilization pouch before its use.
■ PAUSE POINT The catheter can be presterilized and stored in a sealed pouch for 3–4 weeks.

Surgical procedure ● TIMING 40 min maximum

- 10| Anesthetize a mouse (body weight 24–27 g) by intraperitoneal injection of ketamine (80 mg kg⁻¹) and xylazine (10 mg kg⁻¹). Ketamine and xylazine should be premixed and diluted with saline.

▲ **CRITICAL STEP** The surgery procedure should be complete in ~40 min or less. Anesthesia achieved by this regimen should sufficiently cover this duration.
- 11| *Preparation of the mouse for aseptic surgery.* Clip the hair on the mid-abdomen and dorsal neck areas. Swab them with iodine solution and allow them to dry. Pull out the tongue and apply ointment on the mouse's eyes. Wipe the surgical areas with 70% (vol/vol) ethanol. Place the mouse on a surgical table that has been covered with a sterile drape and heated with a water-circulating heating pad (set at 37 °C) placed underneath the drape to prevent hypothermia. Use sterile gauze to cover areas that have not been prepared. Fill a catheter with saline using a 1-ml syringe.

▲ **CRITICAL STEP** Sterilized surgical instruments, towels, gowns, catheter, gloves and sutures must be used.
- 12| Place the mouse on its stomach. Make a skin incision (8–10 mm in length) on the dorsal midline from the base of the skull to the interscapular region (Fig. 2a). Use scissors to separate the skin from the underlying muscle tissue around the incision site and extending toward the left flank; this will create a subcutaneous tunnel.
- 13| Position the mouse on its back. Make a skin incision (8–10 mm) on the abdominal midline from the xiphoid cartilage extending to the midabdomen (Fig. 2b). Use scissors to separate the skin from the underlying muscle tissue. This separation process will move toward the left flank to complete a connection with the subcutaneous tunnel created from the neck.
- 14| Position the mouse on its right side. Insert a curved hemostat from the abdominal skin incision into the subcutaneous tunnel and out of the dorsal neck incision (Fig. 2c). By using the hemostat, gently grasp the catheter above the Dacron disc (Fig. 2d). Carefully pull the catheter tip through the subcutaneous tunnel out of the abdominal incision (Fig. 2e).

▲ **CRITICAL STEP** Do not grasp the Dacron disc. The integrity of the disc and its adhesion to the tubing may be compromised.
- 15| Make a midline incision on the linea alba to open the abdominal cavity (6–8 mm; Fig. 2f).
- 16| Insert a pointed curved hemostat into the abdomen, place the tip against the inner abdominal wall at the site ~20 mm toward the left flank from the incision, and then make a hole in the abdominal wall by pressuring the tip and penetrating the wall (use a scalpel tip to assist if needed). By using the hemostat, grasp and pull the catheter tip into the abdomen (Fig. 2g,h).

- 17| Use a curved forceps to find the forestomach by flipping the left liver lobe. Be careful not to damage the liver or any other major organs. The forestomach can be distinguished from the glandular portion of the stomach, as it is whiter in color. Gently grasp the forestomach and pull it out of the incision site (Fig. 2i).
- ! CAUTION** Be careful not to pinch and damage blood vessels on the forestomach. Place the stomach on gauze soaked with saline.
- 18| Use a jeweler's forceps to make a small puncture hole (a size of the diameter of Silastic tube) through the forestomach wall in the middle of the forestomach where there is minimal vasculature. Insert the catheter tip into forestomach. With curved forceps, hold the catheter in place (Fig. 2j). Use saline to prevent dehydration of the exposed stomach. Catheter tip placement and subsequent suturing are relatively easier in the morning when the mouse stomach is still full of diet consumed the previous night. As gastric emptying becomes more complete in the afternoon, gastric liquid content tends to spill out of the puncture site. Try to block and absorb this with gauze.
- 19| Use 6/0 silk or other nonabsorbable suture material to stitch the Dacron disc to the forestomach wall. Four to six stitches around the disc perimeter will secure its placement (Fig. 2k). After completing the suturing, gently infuse ~0.1 ml saline into the stomach using the saline-filled syringe attached to the proximal end of the catheter. Ensure that there is no leakage from the sutured disc.
- 20| Replace the whole stomach to the original resting position by pushing it into the abdomen while gently pulling the catheter from the dorsal neck side. The Dacron disc should be placed against the abdominal hole in the left flank.
- ! CAUTION** Ensure that the spleen and a splenic portion of pancreas are not trapped by the disc.
- 21| Close the peritoneal cavity using 6/0 silk or absorbable suture and a continuous suture pattern (Fig. 2l).
- ! CAUTION** Be careful not to puncture the internal organs, including abdominal fat.
- 22| Close the abdominal skin incision using 6/0 silk or other nonabsorbable suture and an interrupted suture pattern (Fig. 2m).
- 23| Position the mouse on its stomach. Position the anchoring Dacron along the dorsal neck muscle (Fig. 2n).
- 24| Use 6/0 silk to suture the Dacron onto the neck muscle by placing two stitches on either side (Fig. 2o).
- ! CAUTION** This suture is crucial to firmly anchor the catheter entry site. Ensure that you stitch a sufficiently deep layer of the muscle to achieve this.
- 25| Close the dorsal neck skin incision using 6/0 silk and a simple interrupted suture pattern (Fig. 2p).

- 26| For recovery, place the mouse in a shoe-box cage warmed by a heating pad. Connect the catheter to a swivel and connect a 1-ml syringe filled with saline to the swivel with Tygon tubing (i.d. 0.508 mm, 50 mm in length). Suspend the catheter by clamping the swivel above the cage. Inject Buprenex (0.025 mg kg⁻¹) as an analgesic subcutaneously when the mouse begins to recover from anesthesia. For hydration, warm lactated Ringer's solution can be administered subcutaneously (1 ml per mouse).
- 27| Cut the Nylon tubing to 50 mm in length and use it to cover the catheter above the neck. This will protect the catheter from being chewed by the mouse.

Animal housing and infusion setup ● TIMING 4–6 d (including recovery)

- 28| Leave the mouse to recover from anesthesia in a cage placed on a heating pad. Once the mouse has recovered from anesthesia, remove the cage from the heating pad and place it on a stainless steel rack (Fig. 3). Attach a rod with clamps to the rack to support the swivels above cages. Keep a 1-ml syringe connected to the swivel for 2 d after surgery until dietary infusion begins. During the 2-d postoperative period, provide water and regular chow.
- 29| On the second day after surgery, remove chow from the cage (from this stage onward, a basic liquid diet will be infused for a recovery period of 4–6 d, set up as described in the following steps).
- 30| Set the infusion rate on Harvard Apparatus PHD 2000 Infusion pump at 8 ml per 20 g body weight per 24 h for a 10-ml syringe with a diameter of 15 mm.

? TROUBLESHOOTING

- 31| Connect the 10-ml diet or dextrose/ethanol syringe to the 18-G Y-shaped tube connector ('c' in Fig. 3b) using a Tygon tube (i.d. 0.762 mm, 350 mm in length; 'd' in Fig. 3b). This Tygon tubing has a smaller diameter than the 18 G, but it will fit tightly into the Y-shaped tube connector.

? TROUBLESHOOTING

- 32| Use the Tygon tube (i.d. 0.508 mm; 50 mm in length) to connect the swivel and the Y-shaped connector ('b' in Fig. 3b).
- 33| Once all three ends of the Y-shaped connector are connected with appropriate tubing, fill the dead space of the lines with saline to ensure that there is no air.

? TROUBLESHOOTING

- 34| Connect the two ends of Tygon tubing to the diet and ethanol/dextrose syringes ('d' in Fig. 3b) via 20-G Intramedic Luer stub adaptors ('e' in Fig. 3b).
- 35| Infuse the basic diet (Table 1; Box 1) and dextrose (9.4% (wt/vol)) for a 4- to 6-d recovery period (Fig. 3c).

! CAUTION Pumps may generate heat, which can promote air bubble formation within diet syringes.

▲ CRITICAL STEP Disposable diet syringes may need to be changed, as this type of syringe plunger tends to lose lubrication and does not allow smooth infusion.

▲ CRITICAL STEP Transfer a frozen aliquot of diet from a -20°C freezer to a refrigerator 24 h before feeding in order to allow slow thawing. Thaw out just enough diet for each day so that freshly thawed diet is always fed.

▲ CRITICAL STEP The liquid diet described below contains no preservatives. For this reason, only freshly thawed diet should be used.

? TROUBLESHOOTING

Ethanol feeding ● TIMING ~2 min per mouse

- 36| For ethanol feeding experiments, prepare diluted ethanol solution using 95% (vol/vol) ethanol in advance to achieve incrementally increasing doses as shown in Table 2.

! CAUTION As 100% ethanol absorbs water from air, 95% (vol/vol) solution should be prepared immediately upon opening a new container of 100% ethanol.

- 37| Start infusing ethanol using a 10-ml syringe filled with an appropriate concentration of ethanol as detailed in Table 2 and the Harvard Apparatus infusion pump (Model PHD) set at an infusion rate of 8 ml per 20 g body weight per day.

- 38| As a control for ethanol feeding, start infusing isocaloric dextrose solution using the concentrations listed in Table 2.

Animal monitoring ● TIMING ~1 min per mouse

- 39| Monitor the condition of each mouse daily to assess the catheter, the catheter exit site at the dorsal neck, the general physical appearance and activity, and the presence of feces and urine in the cage. Check the mouse's body weight daily or weekly by disconnecting the infusion lines from the swivel and weighing the mouse with the catheter and the swivel. The weight of the latter can be subtracted to record the mouse's body weight.

? TROUBLESHOOTING

- 40| For ethanol infusion experiments, measure the degree of intoxication at least three times a day (morning, early and late afternoon). The dosages and schedule shown in Table 2 should achieve sufficient ethanol intake and blood alcohol levels (BALs) while minimizing the risk of overintoxication. However, during the third and fourth week of observations, one may observe individual variations in the response to ethanol dosing, thereby necessitating adjustments on an individual basis. The guideline described in Box 2 is helpful in the assessment of intoxication and implementation of individual adjustments.

- 41| Flush the catheter daily with saline when changing the diet. Cleaning of the catheter with a stainless steel wire may be performed especially when a diet with high viscosity is used. This will help prevent catheter clogging.

? TROUBLESHOOTING

? TROUBLESHOOTING

Troubleshooting advice can be found in Table 3.

● TIMING

Steps 10–27, surgical procedure: 40 min maximum per mouse

Steps 28–35, animal housing and infusion setup: 4–6 d (including recovery)

Steps 36–38, ethanol feeding: 2 min per mouse

Sample daily schedule (timing per mouse)

Steps 39–41, animal monitoring: 2 min maximum; catheter cleaning and flushing: 2 min maximum; recording the amount of diet and ethanol (or glucose) solution infused: 1 min maximum; filling a syringe with new diet and ethanol solution (or isocaloric dextrose solution): 2 min maximum

ANTICIPATED RESULTS

Anticipated results will obviously differ according to how the *iG* mouse model is used. As an example, we will discuss below anticipated results from the *iG* mouse model for 1–4 weeks of infusion of high-fat diet and ethanol. Table 4 summarizes key outcome parameters for liver damage: liver weight/body weight ratio as a measure of hepatomegaly; plasma ALT levels for a parameter of liver damage; and BALs. These parameters were determined at the end points. As shown, average BALs range from 300 to 350 mg dl⁻¹. With these levels of BAL, the animal's intoxication signs are usually rated from 0 to +1 according to the guidelines discussed above (see Box 2). Intoxication of +2 or +3 reflects a BAL of above 350 and 450 mg dl⁻¹, respectively. Thus, the degree of intoxication achieved in the *iG* model is substantially greater than that achieved in other models with *ad libitum* consumption of alcohol-containing diet or water (BALs in these models range from 100 to 200 mg dl⁻¹ at the utmost). Plasma ALT levels are quickly raised to the average values of 160 and 192 U l⁻¹ after 1 and 2 weeks of alcohol infusion, and they reach 260–270 U l⁻¹ at 3–4 weeks. These changes are closely associated with hepatomegaly, which is already evident at 1 week and becomes quite conspicuous at 4 weeks. The mortality rate of this model at 4 weeks is <10%.

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References

1. Tsukamoto H, Delgado G, Reidelberger RD, Largman C. Effects of cholecystokinin, food intake and cephalic stimuli on plasma levels of amylase, lipase, and immunoreactive cationic trypsinogen in rats. *Digestion*. 1986; 35:69–77. [PubMed: 2429883]
2. Colomb V, et al. Route of nutrient delivery affects insulin sensitivity and liver glucose transporter expression in rat. *Am. J. Physiol.* 1995; 269:E827–E833. [PubMed: 7491932]
3. Sclafani A, Glendinning JI. Sugar and fat conditioned flavor preferences in C57BL/6J and 129 mice: oral and postoral interactions. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2005; 289:R712–R720. [PubMed: 15845881]
4. de Araujo IE, et al. Food reward in the absence of taste receptor signaling. *Neuron*. 2008; 57:930–941. [PubMed: 18367093]
5. Schier LA, Davidson TL, Powley TL. Ongoing ingestive behavior is rapidly suppressed by a preabsorptive, intestinal ‘bitter taste’ cue. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2011; 301:R1557–R1568. [PubMed: 21865540]
6. Zukerman S, Ackroff K, Sclafani A. Rapid post-oral stimulation of intake and flavor conditioning by glucose and fat in the mouse. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2011; 301:R1635–R1647. [PubMed: 21975648]
7. Sclafani A, Glass DS, Margolskee RF, Glendinning JI. Gut T1R3 sweet taste receptors do not mediate sucrose-conditioned flavor preferences in mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2010; 299:R1643–R1650. [PubMed: 20926763]
8. Reidelberger RD, Heimann D, Kelsey L, Hulce M. Effects of peripheral CCK receptor blockade on feeding responses to duodenal nutrient infusions in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2003; 284:R389–R398. [PubMed: 12414437]
9. Reidelberger RD, et al. Amylin receptor blockade stimulates food intake in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2004; 287:R568–R574. [PubMed: 15130879]
10. Tsukamoto H, et al. Severe and progressive steatosis and focal necrosis in rat liver induced by continuous intragastric infusion of ethanol and low fat diet. *Hepatology*. 1985; 5:224–232. [PubMed: 3979954]
11. Tsukamoto H, Towner SJ, Ciofalo LM, French SW. Ethanol-induced liver fibrosis in rats fed high fat diet. *Hepatology*. 1986; 6:814–822. [PubMed: 3758935]
12. Tsukamoto H, et al. Induction of liver cirrhosis in rats fed alcohol and iron. *J. Clin. Invest.* 1995; 96:620–630. [PubMed: 7615836]
13. Mathurin P, et al. Exacerbation of alcoholic liver injury by enteral endotoxin in rats. *Hepatology*. 2000; 32:1008–1017. [PubMed: 11050051]
14. Tsukamoto H. Animal models of alcoholic liver injury. *Clin. Liver Dis.* 1998; 2:739–751.
15. Clark JM, Brancati FL, Diehl AM. Nonalcoholic fatty liver disease. *Gastroenterology*. 2002; 122:1649–1657. [PubMed: 12016429]
16. Pellemounter MA, et al. Effects of the obese gene product on body weight regulation in *ob/ob* mice. *Science*. 1995; 269:540–543. [PubMed: 7624776]
17. Chen H, et al. Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in *db/db* mice. *Cell*. 1996; 84:491–495. [PubMed: 8608603]
18. Costet P, et al. Peroxisome proliferator-activated receptor alpha-isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis. *J. Biol. Chem.* 1998; 273:29577–29585. [PubMed: 9792666]
19. Fan CY, et al. Steatohepatitis, spontaneous peroxisome proliferation and liver tumors in mice lacking peroxisomal fatty acyl-CoA oxidase Implications for peroxisome proliferator-activated receptor alpha natural ligand metabolism. *J. Biol. Chem.* 1998; 273:15639–15645. [PubMed: 9624157]
20. Lu SC, et al. Methionine adenosyltransferase 1A knockout mice are predisposed to liver injury and exhibit increased expression of genes involved in proliferation. *Proc. Natl. Acad. Sci. USA*. 2001; 98:5560–5565. [PubMed: 11320206]

21. Horie Y, et al. Hepatocyte-specific Pten deficiency results in steatohepatitis and hepatocellular carcinomas. *J. Clin. Invest.* 2004; 113:1774–1783. [PubMed: 15199412]
22. Yu S, et al. Adipocyte-specific gene expression and adipogenic steatosis in the mouse liver due to peroxisome proliferator-activated receptor 1 (PPAR 1) overexpression. *J. Biol. Chem.* 2003; 278:498–505. [PubMed: 12401792]
23. Poulosom R. Morphological changes of organs after sucrose or fructose feeding. *Prog. Biochem. Pharmacol.* 1986; 21:104–134. [PubMed: 3523499]
24. Surwit RS, et al. Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice. *Metabolism.* 1995; 44:645–651. [PubMed: 7752914]
25. Deng QG, et al. Steatohepatitis induced by intragastric overfeeding in mice. *Hepatology.* 2005; 42:905–914. [PubMed: 16175602]
26. Tsukamoto H, Mkrtchyan H, Dynnyk A. Intragastric ethanol infusion model in rodents. *Methods Mol. Biol.* 2008; 447:33–48. [PubMed: 18369909]

Diet preparation ● TIMING 3 d

A soluble and particulate-free liquid diet is required. For this reason, we use highly soluble lactalbumin hydrolysate and dextrose as protein and carbohydrate sources. As shown in Table 1, diets containing three different levels of polyunsaturated fat may be used. Corn oil can be replaced with a different type of fat as long as it does not solidify at room temperature. The procedure for preparing 6 liters of high-fat diet is described below. It can be scaled down to the volume of diet to be made or adjusted according to the content of fat in the diet.

1. Prepare ten mineral stock solutions (Table 1, minerals no. 1–10). Store them at 4°C. This process can be done ahead of time. Solutions must be brought to room temperature before use.

▲ CRITICAL STEP To completely dissolve potassium phosphate (mineral no. 1), it must be heated each time.

2. Dissolve 552 g of lactalbumin hydrolysate into 3 liters of Millipore water (or distilled and deionized water) in a 4-liter plastic beaker under constant stirring (solution A).
3. Add Millipore water in a 3-liter glass beaker and place on a stirrer/hot plate set to low heat. Dissolve dextrose (115.6 g for a high-fat diet) to 1,200 ml in water, and then dissolve 12 g of citric acid.
4. While stirring constantly, add mineral solutions no. 1–10 listed in Table 1.

▲ CRITICAL STEP The solutions should be added in the precise sequence shown in the table, one at a time, to avoid precipitation. Thereafter, dissolve the trace mineral mix.

5. Turn off the heat and let it cool down to body temperature, and then dissolve vitamin and choline chloride (solution B). Place it in a refrigerator.

■ PAUSE POINT Solutions A and B (Table 1) can be stored at 4°C overnight.

6. Take solution B out of the refrigerator and ensure that there is no precipitation at the bottom of the beaker. Warm the solution to room temperature under constant stirring.
7. Measure solution A and divide it into two equal volumes by using a 2-liter graduated cylinder.
8. Measure the volume of solution B and divide into two equal volumes by using a 1-liter graduated cylinder. As you measure solution B, filter it using a 70- μ m Spectra Nylon mesh.
9. Measure 193.2 g of Mazola corn oil (for high-fat diet) and 8.5 g of xanthan gum (for high-fat diet).
10. As a commercial food blender comes with a maximum volume of 3 liters, subtract half the volume of solution A, half the volume of solution B and the

volume of corn oil from 3 liters to determine how much water you need to add to blend a 3-liter diet.

11. Blend solution A, the calculated amount of water and corn oil together for 30 s.
12. Add approximately one-third of the xanthan gum and blend for 30 s, and then add another one-third of the xanthan gum and blend it for an additional 30 s.
13. Add solution B and the remaining third of the xanthan gum into the blender and blend for 1 min.
14. Vacuum-filter the diet using a 70- μ m Spectra Nylon mesh. Place the filtered diet in containers.
15. Repeat steps 11–14 for the remaining half to make a 6-liter diet.
16. Refrigerate the filtered diet overnight.
17. Aspirate and discard the top layer of foam and aliquot the diet into dated bottles for immediate freezing at -20°C .

■ **PAUSE POINT** Diet can be frozen for up to 1 month.

Intoxication degree and recovering procedure

Degree 0: Upon touching and pulling the tail, the animal swiftly tries to escape and assumes a defensive position toward you.

Degree +1: The animal is slightly slow to respond with slightly diminished motor coordination in the lower extremities. One may smell alcohol in the animal's cage. One may observe the animal without removing ethanol from the syringe; alternatively, remove 0.2–0.4 ml of ethanol if the intoxication is thought to be getting worse.

Degree +2: Motor coordination of the lower body is obviously impaired, but the animal can still move around in the cage. The animal may run from side to side instead of a straight direction. Remove 0.4–0.6 ml of ethanol only.

Degree +3: The animal moves only its head and forelegs while the rest of the body is immobilized. Remove 0.6 ml–0.8 ml of ethanol only or stop the pump for a short period until the animal begins to lift its own body weight and move around slowly.

Degree +4: Anesthetic stage accompanied by a loss of whole body motor coordination.

! CAUTION Stop the infusion pump for both diet and ethanol immediately, and remove the gastric content by aspiration via the catheter using a 1-ml syringe. Subcutaneously inject warm saline (~0.3 ml) every hour until the animal recovers to the +2 or +1 degree of intoxication. Thereafter, diet infusion can be resumed, followed by restarting ethanol infusion 1–2 h later.

▲ CRITICAL Check the animal's body temperature. Place the cage on a heating pad to prevent hypothermia. After recovery, frequent monitoring is needed as the animal may undergo withdrawal if its BAL drops rapidly in a short time period.

Degree –1: Withdrawal stage. The animal is jumpy, with paws digging into bedding, body shaking and/or a raised tail. The animal will be sensitive to noise, which may trigger a seizure. Slowly infuse 0.2–0.6 ml of ethanol solution directly through the catheter using a 1-ml syringe. This step may be repeated with 30- to 40-min intervals until physical signs of withdrawal disappear.

Figure 1. Catheter setup. **(a)** A mouse catheter diagram showing different components: a, swivel; b, Tygon tube; c, Silastic tube; d, stiffening wire; e, pear-shaped piece of Dacron; f, Silastic tube and g, Dacron disc. **(b)** An overall photo of the catheter. **(c)** Close-up side view of the catheter tip. **(d)** Close-up bottom view of the catheter tip.

Figure 2.

Surgical procedure for implantation of a gastric catheter. **(a)** Dorsal midline incision of the neck. **(b)** Ventral abdominal midline incision. The skin layer has been separated from the underlying abdominal muscle. **(c)** A curved hemostat is inserted through the ventral incision out of the neck incision via a subcutaneous tunnel. **(d)** The hemostat grasping the catheter above the Dacron (stomach) disc. **(e)** The catheter is pulled out of the ventral incision. **(f)** The peritoneum is opened. **(g)** A curved hemostat is inserted into the abdomen, placed against the inner wall of the abdomen and forced to penetrate the wall at a distance of 20 mm away from the midline in the left flank area. The hemostat grasping the catheter behind the Dacron (stomach) disc. **(h)** The catheter is pulled into the cavity and out of the midline incision. **(i)** The catheter tip is positioned near the area of insertion site of exposed forestomach. **(j)** A puncture hole is made in the middle of the forestomach, and the catheter tip is inserted into the forestomach. **(k)** The Dacron (stomach) disc is sutured to the forestomach wall by placing 4–6 stitches with 6/0 silk. **(l)** Closing of the abdominal incision with 6/0 silk. **(m)** The ventral skin incision is closed with 6/0 silk. **(n)** Dorsal Dacron is positioned along the cervical muscles within the skin incision. **(o)** Dacron is sutured to cervical muscles. **(p)** The skin incision is closed for the catheter exit site.

Figure 3.

Mice during the experiment. (a) An *iG* mouse inside the cage. (b) Diagram depicting how infusion lines are connected to a swivel: a, swivel; b, Silastic tube; c, Y-shaped tubing connector; d, Tygon tube; and e, 20-G Intramedic Luer stub adaptor. (c) Overview photo showing a setup for cages and infusion pumps on racks, as well as swivels supported by clamps and rods attached to the rack.

TABLE 1

Diet composition.

		For 6 liters		
		Low fat (8%)	Medium fat (25%)	High fat (35%)
A				
	Millipore water	3,000 ml	3,000 ml	3,000 ml
	Lactalbumin hydrolysate	552 g	552 g	552 g
B				
	Millipore water	780 ml	1,000 ml	1,200 ml
	Dextrose (D-glucose)	759.6 g	382 g	115.6 g
	Citric acid	12 g	12 g	12 g
Mineral	Stock solution			
(1)	Potassium phosphate 17.325% (wt/vol)	144 ml	144 ml	144 ml
(2)	Calcium chloride 43.39% (wt/vol)	72 ml	72 ml	72 ml
(3)	Sodium chloride 19.535% (wt/vol)	48 ml	48 ml	48 ml
(4)	Magnesium sulfate 8.888% (wt/vol)	24 ml	24 ml	24 ml
(5)	Manganese sulfate 0.605% (wt/vol)	24 ml	24 ml	24 ml
(6)	Potassium iodide 0.0215% (wt/vol)	24 ml	24 ml	24 ml
(7)	Ammonium molybdate 0.0125% (wt/vol)	24 ml	24 ml	24 ml
(8)	Cupric sulfate 1.22% (wt/vol)	24 ml	24 ml	24 ml
(9)	Ferric ammonium citrate 3.115% (wt/vol)	24 ml	24 ml	24 ml
(10)	Zinc chloride 0.255% (wt/vol)	24 ml	24 ml	24 ml
	Trace mineral mix	2.871 g	2.871 g	2.871 g
	Vitamin mix (AIN-76)	26.5 g	26.5 g	26.5 g
	Choline chloride	5.3 g	5.3 g	5.3 g
	Corn oil	82.8 g	260.4 g	386.4 g
	Xanthan gum	8.0 g	12.0 g	17.0 g

TABLE 2Ethanol and dextrose dosage schedule for mouse *iG* model.

Ethanol dose (g per kg ⁻¹ per day)	95% ethanol concentration (%, vol/vol)	Dextrose concentration (%, wt/vol)	Days/weeks
22.7	7.00	10.2	Days 1 and 2
24.3	7.49	10.9	Days 3 and 4
26	8.02	11.7	Days 5 and 6
27.5	8.48	12.4	Days 7 and 8
29.2	9.01	13.1	Days 9–14
30.9	9.54	13.9	Days 15–21
33	10.18	14.8	Days 22–28
35	10.8	15.7	Days 29–42

On the basis of an infusion rate of 8 ml per 20 g body weight per day. Ethanol concentration = ethanol dose (g kg⁻¹ per day)/400 (ml kg⁻¹ per day)/0.810 (density of 95% (vol/vol) ethanol at 25 °C) × 100 = ethanol dose × 0.3086. Dextrose concentration = ethanol dose (g kg⁻¹ per day) × 7.2 (Cal g⁻¹ for ethanol)/400 (ml kg⁻¹ per day)/4 (Cal g⁻¹ for dextrose) × 100 = ethanol dose × 0.45. Ethanol dose to be adjusted according to the tolerance of individual animals as assessed by the intoxication assessment guideline under animal monitoring.

TABLE 3

Troubleshooting table.

Step	Problem	Possible reason	Frequency in 4 week infusion	Solution
30,31,33,35	Air bubble formed in syringe	Heat from pump or air bubble in Tygon tubes	Common occurrence with heated pumps, but nearly 100% preventable	Fan can be placed on the rack to cool the pumps
35	Diarrhea	Old diet Poor quality of diet; contamination of diet, syringes or infusion lines	<3%	Prepare fresh diet Remake or use fresh diet; ensure diet syringes are changed frequently; change the infusion lines and swivel with a new set. These will eliminate the source of bacterial contamination. If diarrhea is associated with alcohol intoxication especially after heavy intoxication, reduce the dose of ethanol or remove 0.4–0.6 ml from an ethanol syringe every day until the condition improves. If diarrhea is severe, both ethanol and diet infusion may need to be stopped. To prevent dehydration, lactated Ringer's solution can be administered subcutaneously. Frequent monitoring is necessary. Clean the anal area with warm saline and apply antibiotic ointment
39	Chewed catheter	The protector tube may slide up and the animal will then be able to chew the catheter	<10% incidence	Chewed portion of the catheter can be replaced with a new Tygon tubing. A needle portion of 20-G Luer adaptor can be used for re-connection. Protector tube should be placed
	Infection	Skin does not heal well or is damaged for a variety of reasons	<3%	If the problem is minor, it can be corrected by surgical removal of the necrotic tissue and resuturing of fresh skin edges under anesthesia, accompanied by antibiotic application. It is crucial that an ethanol-infused mouse is anesthetized with a half of the regular dosage of anesthetics
39,41	Clogged catheter	Particulate matter in the diet	<5%	Check all dietary components, especially stock solutions, for minerals. Carefully remake the diet if necessary. Use a stainless steel wire to clean the catheter and flush it with saline. All the tubing system must be checked for smooth flow

TABLE 4

Liver parameter outcomes during 4 week *iG* feeding of alcohol and high-fat diet.

Weeks	Liver weight/body weight		ALT (U l ⁻¹)		BAL (mg dl ⁻¹)	
	Alcohol	Control	Alcohol	Control	Alcohol	Control
1	0.079 + 0.004	0.049 + 0.002	160.1 + 52.7	12.0 + 4.4	ND	ND
2	0.085 + 0.002	0.051 + 0.001	192.3 + 118.8	17.1 + 4.5	360.3 + 58.8	ND
3	ND	ND	276.5 + 112.6	11.4 + 11.8	ND	ND
4	0.102 + 0.005	0.043 + 0.001	260.1 + 39.0	25.4 + 5.3	309.8 + 25.5	ND

ND, not determined.

This table presents the ratio of Liver weight to body weight, ALT and BAL Levels at different time points of *iG* ethanol/high-fat diet infusion. Data shown are means ± s.e.m. *n* = 3–10 for each group.