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Methods for studying rodent intestinal lipoprotein production and metabolism

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Abstract

Lipid absorption begins with the digestion of dietary triacylglycerol and ultimately results in the secretion of triacylglycerol in chylomicrons into the lymphatics. Additionally, the intestine also secretes numerous proteins and peptides involved in lipid and lipoprotein metabolism in response to food. Ultimately, chylomicrons and these proteins, peptides, and hormones are found in lymph. The lymph fistula rat model has traditionally been used to study this intestinal absorption of nutrients, especially lipids, but recently, this model has also been used for studying the secretion of hormones by the small intestine. The protocols described in this article include the lymph fistula rat and mouse model, as well as in vivo chylomicron metabolism studies. These experimental models are helpful for the study of metabolic phenotypes, the characterization of intestinal lipid absorption and transport, and determining peripheral metabolism of intestinally derived lipoproteins.

Keywords

lipids; lipoproteins; chylomicrons; lymph; mouse; lymph fistula model

INTRODUCTION

Utilization of the lymph fistula model

Of the *in vivo* models used to study the digestion, uptake, and lymphatic transport of lipids, the lymph fistula rat model has been one of the most commonly used. This particular approach involves the steady infusion of lipid by intragastric or intraduodenal cannula. After an equilibration period of about 5 – 6 hours, lymphatic output of the infused lipid reaches a steady state. Therefore, it is possible to compare the output and composition of lipids within the lymph with the pre-infusion levels to measure absorption. By infusing triacylglycerol with radioactively labeled fatty acids, it is possible to determine the lymphatic transport of those fatty acids in chylomicrons. At the end of the infusion period, a measurement (the percent recovery) of the lipid retained in the lumen, mucosa, and lymph, provides an estimate of the uptake, mucosal retention, and transport of that lipid into chylomicrons (Bennett and Simmonds, 1962; Simmonds et al., 1968).

The lymph fistula model has a number of advantages: **1)** The animals are studied in their conscious state and are therefore devoid of the effects of anesthesia (which inhibits chylomicron production and lymph flow); **2)** This model also allows for the continuous sampling of lymph during the entire course of an experiment; **3)** There is less dilution of any

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secreted mediators or peptides, since lymph flow (2-3 mL/h during fasting and increased to 4-5 mL/h during feeding) is much slower than the portal blood flow (14-20 mL/min for an adult rat). For example, when we compare lymphatic GLP-1 concentration with portal plasma GLP-1 concentrations following the ingestion of an Ensure® (Abbott Laboratories, North Chicago, IL) meal, there is a nearly 10-fold higher GLP-1 concentration in lymph versus portal plasma; **4)** There are significantly less degrading enzymes, such as dipeptidyl peptidase-IV (DPP-IV) and aminopeptidase, present in lymph than in plasma. Consequently, there is less degradation of the peptides secreted by the gut epithelium; **5)** Chylomicron particles are better preserved in the lymph compartment than in the plasma component and therefore the composition of lymph chylomicrons are more likely to mimic the composition of newly secreted chylomicrons in the periphery; **6)** The lymph fistula rat model allows an investigator to quantitate and characterize the lipoproteins secreted by the gut following the ingestion of a lipid meal. The lipoproteins in lymph are mostly in their nascent state since they have not been exposed to extensive modification by the enzymes in the plasma as well as the action of lipoprotein lipase. While the composition of chylomicrons has been studied extensively over the years by numerous investigators, the composition and the factors regulating the secretion of high density lipoproteins by the gut have not been extensively studied since the original studies of Glickman and his colleagues (Bearnot et al., 1982); and finally **7)** The lymph fistula rat model also allows an investigator to obtain labeled chylomicrons for metabolic studies.

> The procedures within this manuscript require substantial experience in small rodent surgery. Instructions for fundamental surgical procedures are beyond the scope of this manuscript. It is the responsibility of investigator to ensure that technicians/researchers are properly trained to ensure that animal suffering and waste is minimized and that all procedures are carried out in accordance with animal regulatory board guidelines.

BASIC PROTOCOL 1 – LYMPH FISTULA IN THE RAT

Introduction

The original lymph fistula procedure was first described by Bollman et al. (Bollman et al., 1948). The procedure involves a midline abdominal incision and the retraction of the liver upward and towards the right; the intestines and stomach are gently displaced to the left of the animal.

Following an overnight recovery, lymph fistula rats can be used for a mixed meal challenge, for the collection of chylomicrons, and/or to measure the output of intestinal hormones into lymph. This experimental design allows investigators to collect lymph for measuring the lymph flow rate, output of TG and cholesterol (or other infused lipids), chylomicron composition, and hormone concentrations. The collection regimen can be modified according to the need of the investigators.

Materials

Adult male rats, 280-250g

Sterile surgical equipment (fine forceps, iris scissors, drapes, lighting, heating pad)

Liposyn® III (Hospira Inc. Lake Forest, Illinois, USA) or olive oil (food grade)

Inhaled anesthetic (recommended: Isofluorane, e.g. from Butler Schein, Dublin OH) and necessary equipment to administer anesthetic

PVC cannula; highly recommended: OD 0.80 × ID 0.5 mm (111031); Critchley Electrical Products Pty. Ltd, Auburn, NSW, Australia)

Cyanoacrylate adhesive (Krazy Glue, Columbus, OH)

Peristaltic pump; low flow (1-10 mL)

Sterile normal saline with 5% glucose Duodenal or stomach infusion tube (recommended: VWR cat#60985-708, .040 ID × .085 OD × .023 wall)

Mixed meal bolus (such as Ensure, Abbott Nutrition, Columbus, OH; 3.125 kcal/animal – 0.075 g fat (21.6%), 0.5 g carbohydrate (64.0%), 0.1125 g protein (14.4%)

0.9% sterile saline

Temperature-controlled recovery housing

Conical vials suitable for lymph collection

Surgery for Fistula Induction in the Rat—

1. Fast rats overnight
2. Anesthetize animals

   In terms of anesthesia, the inhalation type (isoflurane) is by far the best since the animals recover from the anesthesia quickly and also ensures good lymph flow while reducing the chance of clotting.
3. The duodenum is stretched transversely towards the left to expose the superior mesenteric artery and the intestinal lymph duct.

   To help with the visualization of the lymph duct, one can feed a small amount (3 ml) of Liposyn® III or 0.5 ml of olive oil by gavage before the surgery.

   The type of cannula is absolutely critical in ensuring successfully cannulation of the duct. We have found that the best type of cannula is polyvinylchloride tubing (since the tubing has enough stiffness to facilitate the cannulation process, but is not so stiff as to pierce the lymph duct during cannulation).
4. Using ophthalmic iris scissors, make a small incision in the lymph duct; one should immediately observe the leakage of intestinal lymph from the duct into the peritoneal cavity.
5. Holding the tip of the cannula with a pair of fine forceps, one can insert the tip of the lymph cannula into the duct.

   It is important not to push the cannula too far into the duct because this may prevent the drainage of the lymph when the retracted organs are returned to the original position. Instead of using suture to securely tie around the duct and the cannula, we found that it is best to simply secure the cannula with a drop of cyanoacrylate glue. This procedure is simple and works extremely well.
6. If the surgery is successful, one should see the constant drainage of lymph promoted by gravity.
7. An intraduodenal cannula is installed by inserting a silicone feeding tube, advanced 1 cm beyond the pylorus into the duodenum, which is secured by a purse-string ligature in the stomach and sealed by a drop of cyanoacrylate glue to prevent leakage.

8. The lymph cannula and the intraduodenal feeding tube are exteriorized through the right flank.

9. After surgery, the animals were allowed to recover overnight in restraint cages situated in a warm chamber (28°C) prior to the start of the experiments (see Critical Parameters and Troubleshooting in Commentary). Animals also receive a constant 5% glucose in saline infusion overnight to help with fluid loss through the draining lymph.

   It is advisable to inject about 2 – 3 ml of the saline into the gut prior to suturing the incisions to increase the lymph flow and thus reduce the chance of clotting after surgery.

Mixed Meal Challenge and Lymph Collection—Following an overnight recovery, lymph fistula rats can be used for a mixed meal challenge, for the collection of chylomicrons, or to measure the output of intestinal hormones into lymph.

10 After animals have recovered overnight, fasting lymph is collected for one hour in eppendorf tubes on ice, to establish fasting lymph output and hormone secretion.

11 Rats are then given a 3 ml mixed meal bolus via their duodenal cannula.

12 Thirty minutes following the nutrient bolus, a 0.9% saline infusion is provided at 3 ml/h for the remainder of the study period.

13 Lymph samples are continuously collected in conical vials on ice every ten minutes for the first hour (fasting lymph) and then every hour thereafter following the mixed meal bolus.

   In addition to the numerous advantages of this model described in the introduction, an additional advantage of the method is that a second cannula can be placed in the hepatic portal vein of the same animal. This offers a second site in which to sample nutrients, hormones, and lipids that are absorbed via the portal vein as well as through the lymphatics.

   Real-time comparisons between the appearances of these molecules in the portal circulation versus the lymphatics are at the cutting edge of research into the intestinal absorption and secretion of lipids, incretin hormones, and lipophilic toxins (Jandacek et al., 2010; Jandacek et al., 2009; Kohan et al., 2011b; Yoder et al., 2010).

BASIC PROTOCOL 2 – LYMPH FISTULA IN THE MOUSE

With the availability of various knockout and transgenic models, the lymph fistula mouse model is obviously a powerful model in answering a number of interesting questions which otherwise would be difficult to answer in the *in vivo* setting. For instance, utilizing the Apobec1 knockout mice, Lo et al. (Lo et al., 2008) demonstrated that the gastrointestinal tract of the KO mice secreted fewer apo B molecules into lymph (compared with WT), during both fasting and active lipid absorption, suggesting that the KO intestine produces fewer TG-rich lipoproteins (including chylomicrons and VLDL) than WT animals. This interesting study led to the finding that apo B48 is the preferred protein of the intestine for efficient chylomicron formation and lipid absorption.

Despite its usefulness, the technical difficulties involved in this model have precluded this technique from being widely used by different laboratories. With experience, we have

achieved success rates ranging from 50 – 60%. One interesting observation, after studying at least a few hundred mice, is the fact that the mouse is very different from the rat in its lymph flow response to fat feeding. Unlike rats, the lymph flow rate of mice does not increase significantly with fat absorption. This is different from the scenario in both rats and humans of which lymph flow rates increased markedly with active fat absorption. The physiological importance of this difference is not known.

The basic surgical protocol is similar between rats and mice, with a few exceptions noted below.

Materials

Adult C57BL/6 mice (30g or larger)

Anesthetic (Ketamine/Xylazine)

PVC cannula (ID 0.20 × OD 0.50 mm (111008); Critchley Electrical Products Pty. Ltd, Auburn, NSW, Australia)

Cyanoacrylate adhesive (Krazy Glue, Columbus, OH)

Peristaltic pump; very low flow

Silicone feeding tube for intraduodenal cannula (0.5 mm ID, 0.8 mm OD; Tyco Electronics, Castle Hill, Australia)

Temperature-controlled restraint cages for overnight recovery

Sterile 0.9% saline w/0.5% glucose

Sterile 0.9% saline

Liposyn® III (Hospira Inc. Lake Forest, Illinois, USA)

Conical vials suitable for lymph collection

Mouse Intestinal Lymph Fistula Surgery

1. Adult male C57BL/6 mice (approximately 30 g or larger) are fasted for 24-h prior to surgery, but retain free access to water.
2. Mice are anesthetized with Ketamine/xylazine (100/10 mg/kg) then the main mesenteric lymphatic duct is cannulated with polyvinyl chloride tubing
3. The lymph cannula is secured with a drop of cyanoacrylate glue (Krazy Glue, Columbus, Ohio, USA).
4. An intraduodenal cannula is installed by inserting a silicone feeding tube, advanced 1 cm beyond the pylorus into the duodenum, which is secured by a purse-string ligature in the stomach and sealed by a drop of cyanoacrylate glue to prevent leakage.
5. The lymph cannula and the intraduodenal feeding tube are exteriorized through the right flank.
6. After surgery, the animals were allowed to recover overnight in restraint cages situated in a warm chamber (28°C) prior to the start of the experiments.
7. Immediately post-surgery, mice receive a continuous intraduodenal infusion of 5% glucose-0.9% saline solution at 0.3 ml/h for 6–7 h, and then received a continuous infusion of 0.9% saline at 0.3 ml/h overnight.
8. Allow mice to recover overnight.

Lipid Infusion and Lymph Collection—Following an overnight recovery, lymph fistula mice can be used for a mixed meal challenge, for the collection of chylomicrons, or to measure the output of intestinal hormones into lymph.

9 After mice have recovered overnight, fasting lymph is collected for one hour to establish fasting lymph output and hormone secretion.

10 Mice are then given a 0.3 ml lipid bolus, such as Liposyn® III, via their duodenal cannula.

11 Thirty minutes following the nutrient bolus, a 0.9% saline infusion is provided at 0.3 ml/h for the remainder of the study period.

12 Lymph samples are continuously collected in conical vials on ice every ten minutes for the first hour (fasting lymph) and then every hour thereafter following the mixed meal bolus.

Unlike the rat (with a lymph flow of approximately 1.5-3 ml/hour), an investigator can expect to collect approximately 100 – 300 μl of lymph per hour from mice post-infusion.

BASIC PROTOCOL 3 – *IN VIVO* CHYLOMICRON METABOLISM ASSAY (applies to both mouse and rat)

Since chylomicrons are the primary postprandial plasma lipoprotein, understanding their metabolism is critical. Nascent chylomicrons are secreted into the intestinal lymphatic vessels and enter the bloodstream via the thoracic lymph duct (Kohan et al., 2010). Once they enter the periphery, the chylomicron triacylglycerol is rapidly hydrolyzed by lipoprotein lipase on the surface of capillary endothelial cells. This clearance of chylomicron triacylglycerol produces a smaller denser particle known as a chylomicron remnant. This particle is cleared from circulation by the liver through the low density lipoprotein receptor-related protein-1 (LRP1), also known as the chylomicron remnant receptor (Martins et al., 1996; Redgrave et al., 1996).

Using either the lymph fistula rat or mouse to provide donor chylomicrons is a key aspect to the in vivo measurement of chylomicron metabolism, since the donor rat/mouse lymph is a source of chylomicrons that has not been exposed to LPL. A measurement of the rate of clearance of chylomicrons from the blood can yield quite a lot of information about chylomicron particle size, interactions with lipoprotein lipase, and the effects of various apolipoproteins. The lymph fistula mouse model is particularly useful because chylomicrons can be isolated from genetic knock-outs, which may be deficient in specific chylomicron binding proteins and can then be assessed for impaired metabolism.

For this procedure, usually doubly labeled triacylglycerol or cholesterol are infused into the duodenum of the “donor” lymph fistula rats, followed by a collection of those “donor chylomicrons” from the secreted lymph. Due to the doubly labeled lipid infusion, an investigator is able to obtain chylomicrons with labeled triacylglycerol and cholesterol.

While Redgrave and colleagues (Mortimer et al., 1992; Mortimer et al., 1988; Mortimer et al., 1995; Redgrave et al., 1993) have used artificial chylomicrons for metabolic studies, there is still concern that the artificial chylomicrons may not be metabolized the same way as native chylomicrons made by the gut.

Materials

Lymph fistula animals (either rats or mice; Basic Protocols 1 steps 1-8 or Basic Protocol 2 steps 1-8, respectively)

5.0 mCi ^{3}H-labeled glycerol Trioleate (PerkinElmer)

1.0 mCi ^{14}C-cholesterol (PerkinElmer)

Liposyn® III (Hospira Inc. Lake Forest, Illinois, USA)

Conical tubes suitable for lymph collection

Refrigerated centrifuge with Beckman 50.3 Ti rotor (Beckman Instruments, Palo Alto, CA)

Liquid scintillation counter with dual window (for ^{3}H and ^{14}C) capability

Triglyceride assay kit (recommended: Randox TG kits, Randox Laboratories, Crumlin, UK)

Cholesterol assay kit (recommended: Infinity cholesterol kits, Thermo Electron, Noble Park, Victoria, Australia)

Insulin syringe (1 mL)

Recipient rodents of the same breed, age, and sex as selected lymph fistula animals

Generation of Doubly-Labeled “Donor” Chylomicrons—

1. To generate doubly-labeled chylomicrons for the chylomicron metabolism assay, lymph fistula mice receive a bolus infusion of lipid emulsion containing 5.0 mCi ^{3}H-labeled glycerol trioleate, 1.0 mCi ^{14}C-cholesterol, in 0.3ml of Liposyn® III the morning following the placement of lymphatic and duodenal cannula.
2. Lymph is then collected on ice for 6 h post-infusion.
3. As noted above, one can expect to collect approximately 100 – 300 μl of lymph per hour from mice, so lymph may need to be pooled from multiple animals (or from multiple time points) to obtain a large enough sample for metabolic studies.

   It should be noted that peak cholesterol output into lymph occurs later than TG output. If chylomicrons rich in labeled cholesterol are needed, lymph collection could be extended by several hours to obtain lymph with higher counts of the labeled cholesterol.

Isolation of Chylomicrons—

4 To isolate the doubly labeled chylomicrons, pooled lymph is centrifuged at 25K at 10° C for 60 min using a Beckman 50.3 Ti rotor.

   Isolation of the top creamy portion will yield a heterogeneous sample of lipid-rich intestinal lipoproteins, and may include some intestinal VLDL particles. For a more stringent isolation of only chylomicron-sized particles, lymph can be subjected to density centrifugation (Redgrave et al., 1975).

5 Both the ^{3}H-tryglyceride and ^{14}C-cholesterol content can be assessed by liquid scintillation counting, and total triglyceride and cholesterol content of the chylomicrons are assessed by standard chemical assay (although the fractionated lymph will need to be diluted).

It should be noted that S.B Clark (Gantz et al., 1990) has demonstrated that in some instances, the spinning the chylomicrons at low temperatures can cause deformation of the particles. Thus, if one is working with chylomicrons that contain a lot of saturated fatty acids, it may be better to spin the chylomicrons at a higher temperature.

We have successfully measured chylomicron clearance using both isolated chylomicrons and whole lymph (that has not been spun) and found that centrifugation does not change the clearance of the chylomicrons in our experimental system.

Chylomicron Clearance Assay (requires new, recipient animals)—We have used 3000 μg of chylomicron triglyceride (in phosphate buffered saline in a total volume of 100 μl) as our dose of lipid, since this amount corresponds to less than 1% of the daily ingested lipid and is not likely a pharmacological dose.

6 Load 100 μl of the “donor” chylomicrons in an insulin syringe and record the weight.

7 Inject into the jugular vein of male “recipient” mice that have been fasted for 5 h.

8 Set the syringe aside and take blood samples at 2, 4, 6, 8, 10, and 20 min post-injection.

Be sure to record the final weight of the syringe to accurately reflect the injected dose.

9 Radioactivity in the plasma can be measured by liquid scintillation counting, and the percent dose remaining in plasma at each time point can be calculated based on a theoretical total plasma volume of 4% total body weight and the known dpm of the 3000 μg dose.

Commentary

Background

The dietary intake of fat has received considerable attention over the last few decades since diets high in fat have been linked to high blood lipids and an increased risk of cardiovascular disease (Austin, 1991; Kannel et al., 1964; Levy and Kannel, 1988). Dietary fat constitutes a significant source of calories in the Western diet. The major dietary lipid is triacylglycerol, comprised of various fatty acids esterified to a glycerol backbone. Triacylglycerol molecules are hydrophobic, and thus must undergo considerable digestion to be absorbed by the intestine and secreted into the circulation via the intestinal lymphatics.

The digestion of dietary fat begins in the stomach, where both lingual and gastric acid lipases hydrolyze triacylglycerol to form mainly diacylglycerol and free fatty acids. Gastric lipase plays a particularly important role in fat digestion in neonates since the pancreatic lipase system is immature at this stage of development (Abrams et al., 1988; Grand et al., 1976; Liao et al., 1984).

However, the bulk of triglyceride digestion occurs in the upper part of the intestine as a result of the activity of pancreatic lipase. Elegant work by Mattson and colleagues showed that pancreatic lipase acts mainly on the sn-1 and sn-3 positions of the triacylglycerol molecule to release 2-monoacylglycerol (2-MG) and free fatty acids (Borgstroem, 1964; Mattson and Volpenhein, 1964). The monoacylglycerol and fatty acids are then solubilized by bile salts into mixed micelles. The mixed micelles, located at the surface of the

enterocyte, facilitate the uptake of monoacylglycerol and fatty acids by passive diffusion (Borgstrom, 1985; Hofmann and Borgstroem, 1964; Hofmann and Borgstrom, 1962; Woollett et al., 2006). They are then re-synthesized into triacylglycerol by the enzymes of the monoacylglycerol pathway (Johnston, 1976; Kuksis, 1986; Lehner and Kuksis, 1995; Rao and Johnston, 1966).

Inside the enterocyte, triacylglycerol is then incorporated into a lipid droplet coated with cholesterol, phospholipid, and apolipoproteins. The particle is further processed in the Golgi apparatus to form a mature chylomicron particle, which is released by the enterocyte into the intercellular space (ICS) by exocytosis (Luchoomun and Hussain, 1999; Mahley et al., 1971; Sabesin and Frase, 1977).

Chylomicron transport in lymph—Once chylomicrons have been secreted from the enterocytes into the ICS, they must cross the lamina propria to enter the lymphatics. This transport is hampered by the basement membrane lining the base of the enterocytes. After a lipid-rich meal, during active fat absorption, a large number of chylomicrons can be found within the ICS between enterocytes. Therefore, the ICS becomes greatly distended, which may loosen the tight junctions between enterocytes. It is known that the integrity of the tight junction is compromised by chronic consumption of high fat diet, potentially related to fat absorption and the trafficking of chylomicrons (Kohan et al., 2010).

Active fat absorption not only stimulates the formation and secretion of chylomicrons into lymph, it also stimulates the rate of lymph flow, protein flux, and lymphocyte trafficking (Barrowman, 1978; Ji et al., 2011). Fat absorption also stimulates other cells in the intestinal mucosa including macrophages, polymorphonuclear leukocytes, and enteroendocrine cells to release cytokines and hormones (Miura et al., 1998).

Postprandially, the small intestine produces predominantly chylomicrons (Tso et al., 1984); during the fasted state very low density lipoproteins (VLDL) are also produced by the small intestine (Ockner et al., 1969). Currently, the separation of intestinal CM and VLDL is based on operational criteria. Lipoproteins that have a Svedberg flotation (S_f) rate exceeding 400 are classified as CMs; those with a S_f rate of 20-400 are defined as VLDL (Lindgren, 1972; Redgrave et al., 1975). There have been considerable advances in the last few years regarding the intracellular packaging and secretion of chylomicrons (Mansbach and Siddiqi, 2010; Siddiqi et al., 2010).

Critical parameters and Troubleshooting

For Basic Protocol 1-Post-operative care—Attention to detail is crucial for the success of the lymph fistula rat studies and the postoperative comfort of animals.

We used stainless steel cages with stainless steel floors to prevent any damage to the paws and limbs resulting from bars or the gauze type of flooring. A recessed circular area of floor with perforations is installed in the rear of the restraining cage to allow for urine to escape or for the collection through a detachable plastic funnel clipped to the outside of the cage.

Although the animal is restrained, a considerable amount of to and fro movement of the hind legs is permitted without twisting of the hindquarters. The twisting of the forequarters is prevented by passing a coarse suture thread (4-0) during the lymph fistula surgery through the loose skin over the shoulder and knotted loosely around the top bar of the cage.

The bars are removable apart from several structural supports and are slipped into place when the unconscious rat is laid on the floor of the cage after the operation. The bars are

held in place by sliding a guard plate over the back plate of the cage. Any feces excreted are gently removed with forceps.

It is imperative to keep the animals warm since they are unable to nestle together to get warm in cages in the vivarium. We recommend keeping the animals at about 28° C.

To compensate for fluid and electrolyte loss due to lymphatic drainage, we infuse a 5% glucose-saline solution into the duodenum (at 3 ml/h) for 6-7 h post-surgery. This is followed by an overnight infusion of saline at 3 ml/h prior to the experimental infusion. Postoperatively, Buprenex is administered at a dose of 0.1 mg/kg for pain relief.

With a skilled surgical staff and excellent postoperative care, we have routinely achieved success rates of 80 – 90 %. The most important attribute for successful lymph fistula studies is patience.

Additional considerations-Basic Protocol 1—In the paper first describing the lymph fistula rat surgery, they include the cannulation of the thoracic duct as well as the intestinal lymph duct (Bollman et al., 1948). Although cannulation of the thoracic duct offers a more complete collection of the lymph as well as lipoproteins (including chylomicrons) produced by the gastrointestinal tract, a tremendous disadvantage is that it is surgically a more difficult procedure than intestinal lymph cannulation. Additionally, the thoracic duct lymph originates not only from the gastrointestinal tract but also from other organs including the kidney. In contrast, the lymph collected from the intestinal lymph duct is derived exclusively from the gastrointestinal tract. It is important to note that there can be leakage of proteins and small lipoproteins from the plasma into the lymph, and in addition, a few percent of the gastrointestinal lymph can be lost through minor lymphatic drainages of the gastrointestinal tract that are not collected by the major intestinal lymph duct. This occurs in animals with either an intestinal or thoracic cannula, and is well illustrated by the appearance of radioactive apolipoproteins synthesized by the lymph that appeared in the mesenteric lymph of the same animals (Wu and Windmueller, 1978; Wu and Windmueller, 1979).

Additional considerations: Basic protocol 3, chylomicron clearance assay—The isolation of double-labeled chylomicrons can also be used to generate chylomicron remnants, by incubating purified chylomicrons with post-heparin plasma as a source of lipoprotein lipase (Kohan et al., 2011a). Remnants prepared using this method acquire apoC-II, which activates hepatic lipoprotein lipase, and apoE, for clearance by the liver, from HDL present in the post-heparin plasma (Mortimer et al., 1995; Vigne and Havel, 1981). Chylomicron remnants can then be used to study specific transport processes in isolated cells in culture (such as adipocytes and hepatocytes).

Anticipated Results

For the lymph fistula rat you can expect to collect between 3 – 4 ml of lymph per hour during active fat absorption. By analyzing chemically the TG, cholesterol, and phospholipid outputs, one gets an accurate output of the amount of different lipids transported by the enterocytes of the gut as TG-rich lipoproteins (mostly chylomicrons but also contain a small amount of very low density lipoproteins, also referred to as small chylomicrons by some investigators). By infusing different amounts and composition of lipids in the emulsion, one can determine how each of these variables affects the lymphatic transport of chylomicrons. Another utility of the lymph fistula rat model is it allows the investigator to harvest and characterize the nascent chylomicrons secreted by the gut prior to their metabolism in the periphery. Of course by labeling the chylomicrons with either radioactive lipids or stable isotopic lipids, one can inject these labeled chylomicrons into naïve recipient animals to look at chylomicron metabolism.

From a lymph fistula mouse, you can expect to collect approximately 150ul of lymph per hour for approximately 6 hours. If you measure TG and/or cholesterol mass in each hour of lymph collected, you can easily generate a rate of lipid secretion into lymph. If you are pooling the lymph (for the chylomicron metabolism protocol), you can expect to collect approximately 1-1.5ml of lymph per lymph fistula mouse. For the chylomicron metabolism protocol, by sampling plasma for 2-20 min post-injection, you will be able to generate a decay curve describing the loss of dpm (both ^{3}H-TG and ^{14}C-cholesterol if used) from the plasma. A difference in the metabolism of chylomicrons may point to changes in lipoprotein lipase (LPL) activity, hepatic uptake, or peripheral tissue uptake.

Time Considerations

Day 1 - lymph and duodenal cannulation. Rodents recover overnight with a continuous saline/glucose infusion.

Day 2 - bolus dose of lipid administered intraduodenally, followed by the collection of lymph from the lymphatic cannula over the next 6 hours. The lymph is collected in eppendorf tubes on ice, in 1-hour aliquots. Alternatively, lymph can be collected in 1 tube (a 15 ml centrifuge tube) on ice, if you are planning to harvest total chylomicrons.

Day 3 - TG and cholesterol assays on aliquots of lymph, or if you are preparing for the chylomicron metabolism assay, you would isolate the chylomicron fraction followed by scintillation counting and TG assay.

Day 4 (for chylomicron metabolism assay only) – prepare the recipient rodents, and inject in the jugular vein the appropriate amount of donor chylomicrons. Isolate blood from the tail over a 20 minute period post-chylomicron injection. Assay blood for radiolabel of interest (either [^{3}H]-TG or [^{14}C]-cholesterol).

CONCLUSIONS

As described above, both lipids (and lipophilic compounds) and hormones are transported in the intestinal lymph after being absorbed by the gastrointestinal system. Therefore, direct sampling of the lymph through lymph cannulation has been remarkably important in studying the physiology of intestinal nutrient absorption. We have used the lymph fistula rat model extensively in our fat absorption studies and interested readers will find the following papers interesting and informative (Kohan et al., 2012; Lo et al., 2008; Nauli et al., 2006).

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ABBREVIATIONS

CM	chylomicron
HDL	high density lipoprotein
LDL	low density lipoprotein
VLDL	very low density lipoprotein
PL	phospholipids
TG	triglyceride

TRLs	triglyceride rich lipoproteins
ICS	intercellular space

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