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Meal-contingent intestinal lymph sampling from awake, unrestrained rats

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Arnold M, Dai Y, Tso P, Langhans W. Meal-contingent intestinal lymph sampling from awake, unrestrained rats. Am J Physiol Regul Integr Comp Physiol 302: R1365–R1371, 2012. First published April 18, 2012; doi:10.1152/ajpregu.00497.2011.—Standard procedures for intestinal lymph collection involve continuous, quantitative drainage of the lymph fluid in anesthetized or restrained animals that are often euthanized within 48 h. We here describe a novel technique for the nonocclusive cannulation of the major intestinal lymph duct in rats that allows for repetitive in vivo sampling of intestinal lymph from unrestrained, awake, and ad libitum-fed animals. The distinctive feature of this novel technique is that a 5- to 7-mm long piece of Vialon tubing (OD/ID: 0.8/0.7 mm) with a small hole in its wall is first implanted into the major intestinal lymph duct for stabilization. The tapered tip (OD: ≈0.1 mm) of the catheter is then inserted into the hole of the tubing and fixed in place with a polyamid suture and a drop of tissue glue. In our hands, catheters implanted this way remain patent for up to 6 wk after surgery. In an initial experiment we collected lymph from six adult rats before (0) and 15, 30, 45, 60, 75, 90, 120, and 180 min (120 μL each) after the onset of isocaloric (12.5 kcal) low-fat (LF) or high-fat (HF) test meals and measured active glucagon-like peptide-1 (GLP-1). Intestinal lymphatic GLP-1 concentration increased \( P < 0.05 \) from ≈4 pmol/l (0 min) to a peak of 33 ± 6 (means ± SE) or 22 ± 4 pmol/l at 15 (HF) or 30 min (LF) after meal onset and gradually returned to baseline levels by 180 min. With this new technique fewer animals are required to generate physiologically relevant data for various aspects of gastrointestinal physiology that involve the lymphatic system. Furthermore, the advantage of this system is that the animal can act as its own control when the effect of different experimental protocols is tested.

lymphatic system; gastrointestinal physiology; fat absorption; intestinal peptides; glucagon-like peptide-1

The intestinal lymphatic system drains the interstitial space of the gastrointestinal (GI) tract of fluid, proteins, and lipids that are not absorbed into blood capillaries. During dietary fat absorption, chylomicrons are released from the enterocytes into the intercellular space, cross the basement membrane to reach the lamina propria of the mucosa, and are finally collected into the lymphatics (11). Lipophilic substances and orally administered lipophilic drugs are transported in association with lymph lipoproteins and therefore follow the same path from the small intestine to the systemic circulation (4, 21). Enteroendocrine cells release GI peptides into the lamina propria. These peptides are also collected in intestinal lymph, where some of them are found in higher concentrations than in the hepatic portal blood (15) because of the low concentration of dipeptidyl peptidase-IV (DPP-IV) and the low flow rate of intestinal lymph compared with the hepatic portal vein blood (11). The high intestinal lymph concentrations of GI peptides, such as glucagon-like peptide-1 (GLP-1) or cholecystokinin, are functionally important for their putative paracrine effects on afferent nerves terminating in the lamina propria of the GI mucosa (see Ref. 13). In addition, evidence accumulates indicating that enterocyte fatty acid oxidation may constitute a mechanism for dietary fat sensing in the control of eating (14), releasing into the interstitial fluid of the lamina propria a chemical mediator that triggers an eating-modulatory afferent nerve signal (12, 14). Finally, because the intestine with its large surface area is an important entry point for pathogenic microorganisms, effective intestinal immune responses are crucial for survival (18). These immune responses include, for instance, the production of immunoglobulins and cytokines by mucosal immune cells (18) and the migration of dendritic cells from the intestine to the mesenteric lymph nodes (17). Even eating triggers an intestinal immune response (8, 18, 19) that can be detected systemically (8), but it is still not fully understood how the gut-associated lymphoid tissue differentiates usually harmless food antigens from potentially dangerous microbial antigens. One promising approach to specifically investigate important aspects of all these phenomena is to collect intestinal lymph under physiologically relevant conditions and to measure the concentrations of the pertinent molecules (lipids, peptides, or small, nonpeptidergic mediators) or cells in intestinal lymph, which closely resembles the composition of the interstitial fluid in the lamina propria of the intestinal mucosa (16, 20). Consequently, this is what the neurons and dendritic cells in the lamina propria are exposed to.

Several animal models for continuous intestinal lymph fluid collection have been described (see Ref. 6). The commonly employed procedures, however, use restrained and/or anesthetized animals and usually continuous drainage of all intestinal lymph fluid. As a result, these techniques are nonsurvival procedures, i.e., the animals are euthanized after a comparatively short period of time. The most sophisticated method described so far uses conscious, minimally restrained rats, but the animals are housed in metabolic cages, consume exclusively liquid diet (dextrose in Ringer-lactate), and the lymph is collected quantitatively and continuously (6, 9). With continuous and complete drainage of the lymph, however, the rats

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lose substantial amounts of fluid, electrolytes, and proteins that have to be replaced by intragastric or intravenous infusion of rehydration solutions. The experiments with such animals therefore rarely last longer than 48 h. Moreover, infusion of rehydration solutions in intestinal lymph duct-cannulated rats may also affect the rate of appearance of intestinal lymph lipoproteins and the lymph flow. Thus Tso et al. (26) demonstrated significant increases in lymph flow and chylomicon transport after enteral rehydration in rats due to expansion of the interstitial matrix and enhanced interstitial fluid formation. The expansion of the interstitial matrix will facilitate the diffusion of the chylomicrons into the lacteals while the increase in interstitial fluid pressure facilitates the entry of lymphatic fluid from the lamina propria into the central lacteals. Techniques for intestinal lymph collection in laboratory animals that use either anesthetized or fully restrained (Bollman cages) animals also are not ideal for a critical in vivo evaluation of the role of substances that are released into mesenteric lymph in response to eating. We here describe a novel surgical technique for the nonocclusive cannulation of the major intestinal lymph duct in rats. This method allows for repetitive in vivo sampling of intestinal lymph fluid from unrestrained, awake, and ad libitum-fed animals, i.e., under fully physiological conditions. We present data from an experiment in which intestinal lymph was sampled in relation to isocaloric high-fat (HF) and low-fat (LF) meals, and the extent in which intestinal lymph duct-cannulated rats were switched to individual housing but kept in the same cage model.

All experimental procedures were approved by the institution’s official Animal Care Committee, i.e., by the Canton of Zurich Veterinary Office.

**Lymph duct catheters.** The mesenteric lymph duct catheter consisted of a 23-cm piece of polyurethane tubing (Art. MRE-025; 0.3 × 0.64 mm; Microrenathane, Braintree Scientific, Braintree, MA) (see Fig. 1). One end of this tubing was tapered to about 0.1 mm OD by pulling in 145°C vegetable oil, the other (proximal) end was fitted with a sampling port made from a 23-cm piece of 26-gauge (0.45 × 25 mm) surgical stainless-steel tubing (Sterican; B. Braun, Melsungen, Germany). The stainless-steel tubing was polished at both ends and bent into a V shape. The connection between the polyurethane and stainless-steel tubing was shielded with a 1.5-cm piece of silicon tubing (ID 0.508 mm, OD 0.914 mm; Gore WL, Newark, DE). To ensure adhesion to skin and fascia after surgery, the sampling port of the catheter was fixed (3/0 Supramid, SERAG Wiessner, Naila, Germany) to a 1.5 × 2 cm oval piece of polypropylene surgical mesh (Marlex; Bard Implants, Billerica, MA). To stabilize the lymph duct, another 5- to 7-mm piece of Vialon tubing (0.9 × 25 mm REF 381223 or 0.7 × 19 mm REF 381212, BD Insyte IV Catheters; Becton Dickinson, Madrid, Spain) with a small hole in its wall was first implanted into the major intestinal lymph duct. Before implantation all catheter parts were sterilized in a disinfectant solution (KodanTintur forte farblos; Schluer and Mayr, Norderstedt, Germany) for 30 min, and then thoroughly rinsed with sterile 0.9% saline, and implanted using sterile techniques.

**Lymph fluid sampling tubes.** Three-millimeter long pieces of silicone tubing (0.65 × 1.19 mm, Gore WL) were fixed to one end of two 2-cm pieces of polyurethane tubing (Art. MRE-025; 0.3 × 0.64 mm; Microrenathane, Braintree Scientific) (see Fig. 2). The silicone tubing ends of the two pieces of polyurethane tubing were then fitted into the ends of a 10-cm polyethylene tube (PE-200, 1.4 × 1.9 mm; Smith Medical International). A mixing wire (Best Medizin und Kunststofftechnik) was also placed inside the polyethylene tube. The sampling tubes were then sealed by wrapping the connections into very thin layers of parafilm. A 1.5-cm piece of 26-gauge (0.45 × 25 mm) surgical stainless-steel tubing (Sterican; B. Braun, Melsungen, Germany) to a 1.5 × 2 cm oval piece of polypropylene surgical mesh (Marlex; Bard Implants, Billerica, MA). To stabilize the lymph duct, another 5- to 7-mm piece of Vialon tubing (0.9 × 25 mm REF 381223 or 0.7 × 19 mm REF 381212, BD Insyte IV Catheters; Becton Dickinson, Madrid, Spain) with a small hole in its wall was first implanted into the major intestinal lymph duct. Before implantation all catheter parts were sterilized in a disinfectant solution (KodanTintur forte farblos; Schluer and Mayr, Norderstedt, Germany) for 30 min, and then thoroughly rinsed with sterile 0.9% saline, and implanted using sterile techniques.

**Animals and housing.** Adult (300–360 g body wt on arrival) male Long-Evans rats (bred from founders from Charles River, Sulzfeld, Germany) were housed in groups (n = 5, each) for the first 5 days in type IV (595 × 380 × 200 mm, 1 × w × h) cages with sawdust bedding. The colony room was kept at a 12:12 h light-dark cycle (lights off 13:00 h) with an ambient temperature of 20–22°C. Starting around 12 days before surgery the animals were kept in pairs of two and transferred to cages with grated stainless steel floors, which were furnished with “sleeping tubes” made of hard plastic. The rats had ad libitum access to tap water and were kept on a HF diet (RD Western Diet, Research Diet, Nr. D10022601M, with 49% kcal derived from fat), except when noted otherwise (see Surgical procedures and Meal-contingent intestinal lymph sampling). For 10 days before surgery they were also adapted to receiving every day a HF diet test meal (2.4 g noncaloric mesh mixed with corn oil, 0.1% saccharin, 100% kcal from fat) in a plastic food cup. One day before surgery, the rats were switched to individual housing but kept in the same cage model.

All experimental procedures were approved by the institution’s official Animal Care Committee, i.e., by the Canton of Zurich Veterinary Office.

**Fig. 1. Lymph duct catheter.** See text for further details.
was polished at both ends and fitted into one of the two pieces of polyurethane tubing.

**Surgical procedures.** Rats received subcutaneous injections of 4 mg/kg trimethoprim-20 mg/kg sulfadoxin (Borgal 24%, Intervet, Shering-Plough Animal Health, Kenilworth, NJ) for infection prophylaxis a few hours before surgery. They also received 0.05 mg/kg sc atropine (Sintetica, Mendrisio, Switzerland) 15 min before surgery. The HF test meal (2.4 g of the noncaloric mesh with corn oil, 100% energy from fat; see **Animals and housing**) was given 1–2 h before surgery to stimulate lymph flow. The whitish-opaque lymph fluid inside ensured easy identification of the mesenteric lymph duct (Figs. 3 and 4). Noncaloric mesh and oil was used as a scape goat instead of the regular HF diet to avoid formation of a conditioned avoidance against one of the potential test diets. Anesthesia was induced by intraperitoneal injection (1.0 ml/kg) of 70 mg/kg ketamine (Ketasol-100; Dr. E. Gräub, Bern, Switzerland) and 6.0 mg/kg xylazine (Rompun; Bayer, Leverkusen, Germany) and 0.8 mg/kg acepromazine (Fatro SA, Ozzano Emilia, Italy). Additional intraperitoneal doses of 0.1 ml ketamine were administered every 20–40 min, if necessary.

The sampling port of the catheter with the surgical mesh (Fig. 1) was led subcutaneously from 2-cm midline incisions 3 cm distal to the scapulas to a puncture wound between the scapulas where the sampling port was exteriorized. The distal end of the catheter was led subcutaneously from the neck to a 5-cm rostral-caudal midline skin incision, and the skin was separated by blunt dissection from the underlying muscle. The skin in the neck was closed with resorbable 5–0 Vicryl suture (Ethicon, Norderstedt, Germany). Then a 5-cm laparotomy was made beneath the skin incision. Approximately 1 cm lateral to the midline laparotomy, a stainless steel cannula (20 G) was poked through the musculature, and the tip of the catheter was carefully inserted into the cannula and pulled into the abdominal cavity. The small and large intestines were exteriorized, placed on the animals left side, and covered with gauze that was kept moist with warm saline. The liver was retracted toward the diaphragm and kept out of the way with a piece of wet gauze. The superior mesenteric lymph duct runs parallel to the mesenteric artery and extends laterally from the right kidney to the small intestine, at which point it terminates in an intestinal lymph node. The inferior mesenteric and hepatic communicating lymph ducts, when present, were ligated (10–0 Polyamid suture, Ethilon, Ethicon) to increase lymph flow in the superior mesenteric duct and to prevent a potential contamination of the intestinal lymph by lymph of hepatic origin (Fig. 4). The superior mesenteric lymph duct was then freed from surrounding connective tissue. A loop of 10-0 polyamid thread was placed around the previously prepared Vialon tubing. A small incision with a 24-gauge steel cannula (0.51 mm OD) was made into the anterior wall of the mesenteric lymph duct, and the Vialon tubing was placed into the

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**Fig. 2.** Lymph fluid sampling tube. See text for further details.

**Fig. 3.** In situ view of the anatomical picture with the major and inferior intestinal lymph ducts visible beneath the superior mesenteric artery. The black circle identifies the area of interest.
lymph duct so that the hole in its wall was positioned beneath the insertion site. The catheter was filled with heparinized saline (100 IU/ml), and the thin, tapered tip was inserted 2–3 mm into the hole in the wall of the Vialon tubing, with the tip of the catheter pointing upstream, and fastened with the polyamid thread that was placed around the Vialon TM tubing (Fig. 4). The insertion site of the anterior lymph duct wall was closed around the inserted catheter with a suture of polyamid thread, and a drop of tissue adhesive (Histoacryl, B. Braun) was applied to seal and secure the insertion site. The intestines were rinsed with warm Ringer-lactate solution (B. Braun) and replaced into the abdominal cavity. Abdominal skin and muscle were closed with resorbable sutures (muscle: 3–0, skin: 5–0 Vicryl, Ethicon).

Immediately after the surgery was completed, the catheters were once again checked for patency and filled with hypertonic, heparinized glycercol (100 U/ml in 50% glycercol). Thereafter, 5 mg/kg carprofen sc (Rimadyl E. Gräub, Bern, Switzerland) was injected for analgesia. Additional injections of 4 mg/kg trimethoprim-20 mg/kg sulfadoxin sc (Borgal 24%, Intervet, Shering-Plough Animal Health, Kenilworth, NJ) and 5 mg/kg carprofen sc (Rimadyl, E. Gräub) were given on the subsequent 2 days. The catheters were flushed daily with 100 IU/ml heparinized sterile PBS (pH 7.2, GIBCO, Aukland, New Zealand). Just before sampling, the lymph fluid sampling tubes (see above) were filled with 13 µl of a mixture of EDTA (40 mg/ml, Titriplex 3, Sigma, St. Louis, MO) and DPP-IV inhibitor (200 µl/ml, catalog no. DPP4, Millipore, Billerica, MA) to yield a final concentration of 3.9 mg EDTA and 20 µl DPP-IV inhibitor per ml lymph. After 5.5 h food deprivation (4.5 h before dark onset, 1 h into dark phase), a baseline intestinal lymph sample (120 µl) was taken as follows: the animal was taken to an adjacent room, placed on the experimenters lap and allowed to move freely during the whole procedure. The catheter was connected to a 1-ml syringe filled with water, with a blunt 26-gauge cannula attached to a polyurethane tubing and with a small air bubble at the distal end. About 20 µl was sampled within 1 min and discarded. Thereafter, the lymph sampling tube (see above) was connected, and 120 µl lymph was slowly sampled at a rate of about 20 µl/min. This was done manually by a skilled experimenter who managed to maintain a reasonably stable withdrawal rate over several minutes. Preliminary attempts to sample intestinal lymph with slow acting pumps were less satisfactory because the pumps were unable to adequately react to temporary resistances in lymph withdrawal by modulating the suction. During the whole procedure the sampled lymph was constantly cooled with a cooling pad and mixed with a magnet several times. The samples were transferred to a microfuge tube, frozen immediately in dry ice, and stored (−70°C) for later analysis of active GLP-1. The catheters were then filled with 20 µl of sterile PBS, and the animals were put back into their home cage. About 30 min later animals were offered an isocaloric (12 kcal) HF or LF test meal. All rats ate the test meal within 7 min. Additional intestinal lymph samples (120 µl, each) were taken at 15, 30, 45, 60, 90, 120, and 180 min after meal onset.

Analysis of active GLP-1. The samples were defrosted on wet ice and ethanol extracted as follows: 100 µl lymph was transferred into a 600-µl microfuge tube that contained 380 µl of ice-cold 96% ethanol, vortexed for 1 min, and placed in an ice bath for 30 min. The tubes were then inverted again and centrifuged at 10,000 rpm and 4°C for 10 min. Immediately afterwards, 350 µl supernatant were pipetted into glass tubes (7 mm × 60 mm) and dried in the vacuum oven at least three times before the experiment. A few hours before the lymph sampling, the catheters were flushed to remove the glycercol and filled with 100 IU/ml heparinized sterile PBS (pH 7.2, GIBCO, Aukland, New Zealand). Just before sampling, the lymph fluid sampling tubes (see above) were filled with 13 µl of a mixture of EDTA (40 mg/ml, Titriplex 3, Sigma, St. Louis, MO) and DPP-IV inhibitor (200 µl/ml, catalog no. DPP4, Millipore, Billerica, MA) to yield a final concentration of 3.9 mg EDTA and 20 µl DPP-IV inhibitor per ml lymph. After 5.5 h food deprivation (4.5 h before dark onset, 1 h into dark phase), a baseline intestinal lymph sample (120 µl) was taken as follows: the animal was taken to an adjacent room, placed on the experimenters lap and allowed to move freely during the whole procedure. The catheter was connected to a 1-ml syringe filled with water, with a blunt 26-gauge cannula attached to a polyurethane tubing and with a small air bubble at the distal end. About 20 µl was sampled within 1 min and discarded. Thereafter, the lymph sampling tube (see above) was connected, and 120 µl lymph was slowly sampled at a rate of about 20 µl/min. This was done manually by a skilled experimenter who managed to maintain a reasonably stable withdrawal rate over several minutes. Preliminary attempts to sample intestinal lymph with slow acting pumps were less satisfactory because the pumps were unable to adequately react to temporary resistances in lymph withdrawal by modulating the suction. During the whole procedure the sampled lymph was constantly cooled with a cooling pad and mixed with a magnet several times. The samples were transferred to a microfuge tube, frozen immediately in dry ice, and stored (−70°C) for later analysis of active GLP-1. The catheters were then filled with 20 µl of sterile PBS, and the animals were put back into their home cage. About 30 min later animals were offered an isocaloric (12 kcal) HF or LF test meal. All rats ate the test meal within 7 min. Additional intestinal lymph samples (120 µl, each) were taken at 15, 30, 45, 60, 90, 120, and 180 min after meal onset.

Analysis of active GLP-1. The samples were defrosted on wet ice and ethanol extracted as follows: 100 µl lymph was transferred into a 600-µl microfuge tube that contained 380 µl of ice-cold 96% ethanol, vortexed for 1 min, and placed in an ice bath for 30 min. The tubes were then inverted again and centrifuged at 10,000 rpm and 4°C for 10 min. Immediately afterwards, 350 µl supernatant were pipetted into glass tubes (7 mm × 60 mm) and dried in the vacuum oven at
30°C for at least 3 h. The samples were placed in an ice bath and reconstituted with 73 μl assay buffer from the ELISA test kit for active GLP-1 (Art. EGLP-35K, Millipore, MO). The samples were run in two separate assays, single measurements of the samples and the assay performed according to the manufacturers recommendation with a slight modification: 50 μl sample volume and 50 μl assay buffer was used instead of 100 μl. The within and between assay coefficients of variation for this ELISA (determined in our lab) are 8.6 and 13.9%, respectively.

RESULTS

Surgery and catheter patency. Ten of 12 rats (83%) survived the surgery and 6 rats entered experiments after 16–22 days of recovery and 2 other preliminary experiments. Two animals were euthanized during surgery because their anatomical idiosyncrasies prevented successful catheter implantation. One animal was euthanized on postsurgical day 4 because its weight loss and general clinical condition required us to terminate the experiment based on the discontinuation criteria demanded by the Veterinary Office. The body weight of the remaining 9 animals declined immediately after surgery by 5.3 ± 0.7% (means ± SE) but returned to the presurgical level between postoperative days 4 and 17. Catheters remained patent for 26 ± 6 days (means ± SE, range: 5–61 days).

Intestinal lymph GLP-1. Intestinal lymphatic GLP-1 concentration increased (P < 0.05) from ≈4 pmol/l (0 min) to a peak of 33 ± 6 (means ± SE) or 22 ± 4 pmol/l at 15 (HF) or 30 min (LF) after meal onset and gradually returned to baseline levels by 180 min (Fig. 5). The 15-min values and the area under the curve were significantly different between the HF and LF test meal.

DISCUSSION

Here we describe a novel and unique technique for the cannulation of the major intestinal lymph duct in rats. The high success rate of the surgery that is achievable by a skilled experimental surgeon, the option to obtain multiple, repetitive lymph samples under truly physiological conditions, as well as the long duration of catheter patency and, hence, usable time of the animals for longitudinal studies make this procedure a valuable addition to the experimental surgical repertoire that is available for studies of GI physiology.

Given the importance of the intestinal lymphatic system for GI physiology and pathophysiology (e.g., 4, 8, 9, 11, 15, 18, 21), the advantages and potential benefits of the new technique are obvious: the procedure allows for repetitive, within-subjects lymph sampling for in vivo measurements of several target parameters. This approach will particularly be applicable to determine how the chronic treatment, e.g., chronic feeding of a HF diet affects the secretion of incretins when challenged by the same meal over time. Because each animal can act as its own control, fewer animals are needed to generate more physiologically relevant data for various aspects of GI physiology. Mesenteric lymph flow in unrestrained rats was reported to be 1–5 ml/h (6, 9), depending on whether the animals were anesthetized or not. After a meal this value presumably further increases (6). This suggests that our sampling rates in postabsorptive, freely moving animals should not profoundly disturb the intestinal lymph fluid dynamics, but further studies are necessary to critically examine this point. The exact flow rate of mesenteric lymph under our experimental conditions is unknown, and one limitation of our model is that so far we cannot measure it under these conditions. While this is a drawback for all measurements that aim at quantifying the amount of any substrate absorbed from the small intestine into lymph, it is presumably not that important for an estimate of the concentration of a GI peptide or another chemical mediator that the effector cells at the site of action are exposed to in the gut wall. For instance, to evaluate the possible paracrine effect of a GI peptide on afferent nerves (3) the peptide’s concentration in intestinal lymph is important because it is the best available measure of its concentration in the interstitial fluid of the lamina propria (16, 20), whereas lymph flow is less important in this context.

A general limitation of the rat model of lymph sampling for translational purposes is that bile flow in the rat is continuous and independent of food intake, whereas in humans and many other species food or lipids are necessary to stimulate digestive processes that affect lymph formation and flow. Therefore, the pre- and postprandial intestinal environment in rats does not fully match the situation in humans. On the other hand, various other limitations of the commonly used procedures, such as the effects of infusing rehydration solutions or of course also lipids into the small intestine do not apply to our model. Also, the effects of anesthesia, which is often used for lymph collection experiments (see Ref. 6), are difficult to estimate. Some data suggest that the primary disadvantage of anesthesia may not be the anesthesia, per se, but rather the inability of anesthetized animals to effectively disperse and process lipid-based delivery systems (6). It is our experience that anesthetized animals transport lipid by the gut significantly slower than the conscious animals.

To validate our novel intestinal lymph sampling technique for studies of GI peptide functions in relation to eating, we measured intestinal lymphatic levels of active GLP-1 in response to isocaloric HF or LF meals in a within-subjects design. Accumulating evidence indicates that endogenous GLP-1 influences eating by acting in a paracrine fashion on vagal afferents terminating in the wall of the GI tract (2, 11, 15, 23, 24). The findings obtained with our model show that within...
the time frame of a normal meal, active GLP-1 increased in intestinal lymph and therefore at the site of GLP-1’s supposedly paracrine action in the wall of the small intestine. This fulfills one of the important criteria for the physiological relevance of a peptide as satiation signal summarized by Geary (7). Presumably because of the low flow rate of intestinal lymph coupled with low concentration of degrading enzymes such as DPP-IV, the GLP-1 concentration in intestinal lymph appears to be elevated much longer than in the hepatic portal vein under similar conditions (2). This finding was replicated in the same animals using the same technique several days later (data not shown), which indicates that the obtained measurements are reliable and reproducible. The longer presence of GLP-1 at the site of action may be functionally relevant for a possible paracrine effect of endogenous GLP-1. We could not directly compare intestinal lymph with hepatic portal vein concentrations of active GLP-1 because the rats in the present study were not equipped with a hepatic portal vein catheter, but when we measured hepatic portal vein active GLP-1 concentration in rats in response to a 3-g standard chow meal in another study, we found only a moderate and transient increase from about 5 to 16 pg/ml (~1.5–4.9 pmol/l) (2), which seemed to be smaller than what we report here for intestinal lymph. Together with the present data this is consistent with a report by Kohan et al. who observed a substantially higher concentration of GLP-1 in intestinal lymph than in hepatic portal vein plasma in response to intragastric administration of Ensure in immobilized rats (11). To revisit this question, we will certainly combine the technique described here with a hepatic portal vein catheter in one of the next studies.

Interestingly, intestinal lymph GLP-1 concentration was higher in response to the HF than in response to the LF meal. Both, carbohydrates and fats are usually considered to be potent stimuli of GLP-1 release from endocrine cells (10). The stronger effect of fat was therefore surprising because a possible enhancement of intestinal lymph flow after the HF compared with the HC meal should have decreased rather than increased the concentration of active GLP-1 in intestinal lymph. On the other hand, we also found a substantial increase in hepatic portal vein active GLP-1 concentration in response to a HF meal in rats under slightly different conditions (25). We can only speculate about the reasons for the difference between the HF and LF diets: Our previous findings indicate that pharmacological inhibition of diacylglycerol acyltransferase-1 (DGAT-1), i.e., interference with intestinal triacylglycerol resynthesis, further enhances the dietary lipid-induced increase in intestinal lymphatic GLP-1 level (25), suggesting that intestinal fat handling somehow stimulates GLP-1 release. Further studies are necessary to identify the exact mechanism. Comparing intragastric infusions of different macronutrients with respect to their effects on intestinal lymphatic levels of total GLP-1 in anesthetized and immobilized animals, Lu et al. (15) reported that dextrin and intralipid each elicited similar increases in peak levels of GLP-1 concentration, whereas the combination of dextrin and intralipid increased intestinal lymph levels of total GLP-1 in a synergistic fashion. In our study in which the rats consumed real meals, carbohydrates were of course present in the small intestine during consumption of the HF meal and may have accounted for a stronger synergistic effect on GLP-1 release in this condition than the low levels of fat in the LF meal were able to generate.

**Perspectives and Significance**

The intestinal lymphatic system plays a major role in gut immune mechanisms, lipophilic drug delivery, nutrient absorption, and GI peptide function. The novel technique for the cannulation of the major mesenteric lymph duct described here provides a valuable tool for such studies. The dramatic obesity-curbing and antidiabetic effects of bariatric surgery that appears to be in part due to an enhanced stimulation of GI peptide release (e.g., Ref. 22) has revitalized interest in the effects of endogenous peptides. As many of these effects appear to be paracrine, the intestinal lymphatic concentrations of GI peptides are functionally relevant. Yet another interesting aspect is that intestinal immune reactions may contribute to HF diet-induced changes in vagal afferent function and, hence, to the often observed overeating of HF diets (5). The here-described novel technique for the cannulation of the major mesenteric lymph duct in rats provides a promising new tool for a critical examination of such issues. It allows for repetitive in vivo sampling of intestinal lymph fluid from unrestrained, awake, and ad libitum-fed animals and, hence, avoids several limitations of the commonly employed procedures for intestinal lymph collection in laboratory rodents related to the continuous, quantitative sampling of intestinal lymph from restrained or anesthetized animals. Moreover, preliminary trials revealed that this technique can also be applied after 12 h food deprivation. We therefore believe that this technique, alone and in combination with hepatic portal vein catheterization and other sophisticated techniques, has great potential for investigations into many GI functions involving the lymphatic system.

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**DISCLOSURES**

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**AUTHOR CONTRIBUTIONS**

Author contributions: M.A. and W.L. conception and design of research; M.A. and Y.D. performed experiments; M.A. and Y.D. analyzed data; M.A., Y.D., P.T., and W.L. approved final version of manuscript; P.T. and W.L. edited and revised manuscript.

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