Effect of ezetimibe on incretin secretion in response to the intestinal absorption of a mixed meal

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Yang L, Li X, Ji Y, Kohan AB, Wang DQ, Howles PN, Hui DY, Lai J, Tso P. Effect of ezetimibe on incretin secretion in response to the intestinal absorption of a mixed meal. Am J Physiol Gastrointest Liver Physiol 299: G1003–G1011, 2010. First published July 22, 2010; doi:10.1152/ajpgi.00294.2010.—Ezetimibe is a potent inhibitor of cholesterol absorption by enterocytes. Although ezetimibe minimally affects the absorption of triglyceride, it is unknown whether ezetimibe affects the secretion of the incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). It has been shown that ezetimibe-treated mice are protected from diet-induced insulin resistance. Since GIP and GLP-1 promote the actions of insulin, we hypothesized that ezetimibe may affect the secretion of GIP and GLP-1 by enteroendocrine cells into lymph in response to the intestinal absorption of a mixed meal (Ensure). To test this hypothesis, we used the lymph fistula rat model to determine GIP and GLP-1 concentrations in lymph during the 2 h after the infusion of Ensure. Ezetimibe significantly reduced lymphatic cholesterol output during fasting, without coincident decreases in glucose, protein, and triglyceride outputs. However, ezetimibe did not influence cholesterol output after infusion of Ensure. Interestingly, ezetimibe significantly reduced the secretion of both GIP and GLP-1 into lymph after the infusion of Ensure. Therefore, the inhibitory effect of ezetimibe on GIP and GLP-1 secretion by enteroendocrine cells occurs outside of the effects of glucose, protein, or triglyceride secretion by the intestine.

While the cholesterol biosynthetic and metabolic pathways are quite well defined, the mechanisms controlling dietary and biliary cholesterol absorption by the intestine are still poorly understood. It is known, however, that the azetidinone drug ezetimibe is a potent inhibitor of the absorption of both dietary and biliary cholesterol in rodents and humans (13, 36, 44). This inhibition of cholesterol absorption by ezetimibe involves the intestinal sterol transport protein Niemann-Pick C1 Like 1 (NPC1L1) (2, 15, 21).

Ezetimibe treatment significantly reduces low-density lipoprotein cholesterol levels in rodents, rhesus monkeys, and humans (14, 34, 42, 43, 47), without affecting the absorption of triglyceride, vitamins A and D, or bile acids by the intestine (11, 45). However, several studies have reported that ezetimibe treatment results in a modest decrease in plasma triglyceride concentrations (3, 18, 29), most likely due to a specific reduction of saturated fat absorption (26). Ezetimibe inhibits intestinal cholesterol absorption up to 92–96% in a dose-dependent manner (45, 47, 49). However, it is not known whether ezetimibe has pleiotropic effects on the other functions of the intestine.

In addition to contributing to whole-body cholesterol homeostasis through the uptake and absorption of dietary and endogenous (from bile and also from enterocytes shed by the small intestine) cholesterol, the small intestine also secretes incretin hormones in response to ingested nutrients. The incretin hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), are produced by enteroendocrine K cells (10) and L cells (30) of the intestine, respectively, and are released into the circulation in response to ingestion of macronutrients. Mixed meals or individual nutrients (including carbohydrate, fatty acids, and essential amino acids) stimulate the release of GIP and GLP-1 (16, 23, 33, 40). Our recent studies have shown that the intraduodenal infusion of both lipid and glucose (independently or together) induces the release of GIP and GLP-1 by the enteroendocrine cells into intestinal lymph (27, 28). Furthermore, secretion of GIP and GLP-1 into the lymph increases dose dependently in response to an increase in dietary fat (50).

Both GIP and GLP-1 enhance postprandial insulin secretion in a glucose concentration-dependent manner (19, 25). In addition to their insulinoressive effects, GIP has a direct insulin-like action on lipid metabolism by stimulating de novo lipogenesis in both adipose tissue and liver and increasing lipoprotein lipase activity (24, 31, 51).

In addition to its effects on cholesterol absorption, ezetimibe treatment also confers protection against diet-induced insulin...
resistance (26). Since GIP and GLP-1 are insulinotrophic, we hypothesized that ezetimibe may have an additional effect on the secretion of these incretin hormones in response to the intestinal absorption of a mixed meal (Ensure). Whether ezetimibe has any effects on the nutrient-induced GIP and GIP secretion is unknown and is the major aim of this study. Using the lymph fistula rat model, we investigated the effect of ezetimibe administration on GIP and GLP-1 secretion in response to the intraduodenal infusion of a mixed meal, Ensure.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats weighing 280–320 g (Harlan, Indianapolis, IN) were individually housed in a temperature-controlled (21 ± 1°C) vivarium on a 12:12-h light-dark cycle (lights on at 0600). Standard chow (LM-485 Mouse/Rat Sterilizable Diet, Harlan Laboratories) and water were provided ad libitum (except where noted). All animal procedures were approved by the University of Cincinnati Institutional Animal Care and Use Committee and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Lymph fistula surgery. All surgery and experiments were conducted 1–2 wk after rats were received. Rats were fasted for 24-h prior to surgery but retained free access to water. Rats were anesthetized with isoflurane anesthesia, then the superior mesenteric lymphatic duct was cannulated with polyvinyl chloride tubing (0.5 mm ID, 0.8 mm OD; Tyco Electronics, Castle Hill, Australia) according to the method of Bollman et al. (7) with slight modifications. The lymph cannula was secured with cyanoacrylate glue (Krazy Glue, Columbus, OH). Intraduodenal cannulation was performed by inserting a silicone feeding tube (1.02 mm ID, 2.16 mm OD; VWR International, West Chester, PA) ~2 cm beyond the pylorus into the duodenum via a fundal incision of the stomach. The tube was secured by a purse-string ligature in the stomach and sealed by a drop of cyanoacrylate glue to prevent leakage. The lymph cannula and the intraduodenal feeding tube were exteriorized through the right flank. After surgery, the animals were placed in Bollman restraint cages (5) and allowed to recover overnight; the animals were kept in a temperature-regulated chamber (28°C) to prevent hypothermia and received a continuous intraduodenal infusion of 5% glucose-saline solution (145 mM NaCl, 4 mM KCl, and 0.28 M glucose) at 3 ml/h for 6–7 h. Rats then received continuous infusion of saline (0.15 M NaCl) at 3 ml/h overnight prior to Ensure infusion to compensate for fluid and electrolyte loss due to lymphatic drainage.

Treatments and lymph collection. After overnight recovery, fasting lymph was collected on ice for 30 min prior to the start of the intraduodenal infusions. Rats received either an intraduodenal infusion of 1 ml of saline (0.15 M NaCl) or a 1 ml bolus of ezetimibe (0.67 mg/kg body wt in 0.15 M NaCl) (time = 0 min). The continuous infusion of saline at 3 ml/h was continued for 120 min during which lymph was collected on ice at 30-min intervals. All rats then received a 3-ml bolus dose of Ensure (4.43 kcal) through the intraduodenal feeding tube (time = 0 min). At 30 min post-Ensure infusion (time = 30 min), the saline infusion was resumed at 3 ml/h, and lymph was collected on ice at 10–30 min intervals for 120 min post-Ensure infusion. Each lymph sample was treated with 10% per volume antiproteolytic cocktail (0.25 M EDTA, 0.80 mg/ml aprotinin). At the end of the lymph-collection period rats were euthanized.

Materials. Ezetimibe (trade name Zetia) was purchased from Sequoia Research Products (Pangbourne, UK). Ezetimibe was prepared by dissolving 10 mg in 1 ml 100% ethanol, and then diluting the dose to 0.67 mg/kg body wt in 0.15 M NaCl. Ensure (Abbott Laboratories, North Chicago, IL) is a mixed-nutrient liquid containing 14 kcal% protein, 21 kcal% fat, and 64 kcal% carbohydrate with a caloric content of ~1.48 kcal/ml.

Measurement of glucose, protein, triglyceride, and cholesterol in lymph. Glucose concentrations in lymph were determined by a modification of the glucose oxidase-peroxidase method described by Trinder (41) using a commercially available kit (Genzyme Diagnostics, Charlottetown, P.E.I., Canada). Protein concentration was measured by the Bradford method (9) using Bio-Rad protein assay reagents (Bio-Rad Laboratories, Hercules, CA). Lymphatic triglyceride concentrations were determined using a commercially available kit (Randox Laboratories, Crumlin, Northern Ireland, UK). Total cholesterol concentration was measured using commercially available enzymatic cholesterol esterase/oxidase/peroxidase kit, as described previously (1, 20) (Thermo Fisher Scientific, Middletown, VA).

Measurement of lymph concentration of incretin hormones of rats. Lymphatic GIP and GLP-1 levels were measured by enzyme-linked immunosorbent assays (ELISA). GIP was measured using the rat mouse GIP (total) ELISA kit (Millipore, St. Charles, MO). The GIP ELISA measures both the active GIP-(1–42) and inactive form of GIP-(3–42). Lymphatic GLP-1 levels were measured by Glucagon-Like Peptide-1 (Active) ELISA Kit (Millipore). The GLP-1 ELISA measures bio logically active GLP-1(7–37) and GLP-1(7–36) NH2 and does not cross-react with glucagon, GLP-2, and inactive GLP-1(9–37) and GLP-1(9–37) NH2.

Statistical analysis. All values are expressed as means ± SE. Statistical significance between groups was assessed by two-way ANOVA. If significant differences were found, pairwise comparisons were done using a t-test at each time point. P < 0.05 were considered statistically significant. All statistical analyses were carried out by the statistics program GraphPad Prism, version 5 (GraphPad Software, La Jolla, CA).

RESULTS

Ezetimibe has no effect on the total output of lymph stimulated by a mixed meal. Individual nutrients as well as a mixed meal stimulate lymph flow (27, 28). The intraduodenal infusion of Ensure approximates the effect of the ingestion of a mixed meal on both lymph flow and the secretion of nutrients into lymph (12, 28). The lymph flow rates in rats treated with ezetimibe vs. saline are shown in Fig. 1A. In the fasting state, the average rate of lymph flow in saline-treated rats was 3.28 ± 0.25 ml/h and was not significantly different from rats treated with ezetimibe, which had an average lymph flow rate of 2.76 ± 0.26 ml/h. In saline-treated rats, lymph flow increased to a peak rate of 5.70 ± 1.01 ml/h at 40 min after Ensure infusion, whereas the lymph flow rate in ezetimibe-treated rats increased to a peak of 8.21 ± 0.57 ml/h at 40 min post-Ensure infusion. For both groups, lymph flow rates gradually declined to basal levels by 120 min post-Ensure infusion. Although the peak lymph flow rate in ezetimibe-treated rats was higher (8.21 ± 0.57 vs. 5.72 ± 1.01 ml/h), there was no significant difference in the cumulative outputs of lymph in the 2 h prior to and 2 h post-Ensure infusion between two groups (Fig. 1B). This finding is consistent with a previous report that the ezetimibe analog SCH48461 has no effect on lymph volume outputs (46).

Fasting cholesterol levels in lymph are decreased in ezetimibe-treated rats. We wished to determine whether ezetimibe would inhibit fasting cholesterol absorption as well as cholesterol absorption post-Ensure infusion. As shown in Fig. 2A, lymphatic cholesterol concentration was examined. For the saline-treated control rats, lymphatic cholesterol concentration increased dramatically within 10 min of Ensure infusion, peaking at 40 min at 23.44 ± 3.63 mg/dl. Lymphatic cholesterol concentration began to decrease by 50 min postinfusion, reaching the lowest concentration of 5.85 ± 0.58 mg/dl by 90 min.
For ezetimibe-treated rats, fasting lymphatic cholesterol concentration was lower than that in saline-treated rats 2 h after ezetimibe treatment (8.38 ± 0.75 mg/dl vs. 12.09 ± 1.22 mg/dl). After Ensure infusion in ezetimibe-treated rats, cholesterol concentration increased within 20 min and reached its peak level of 16.90 ± 1.04 mg/dl at 30 min. Compared with the saline-treated rats, ezetimibe treatment significantly reduced the elevation of lymphatic cholesterol concentration at 10, 20, and 30 min (P < 0.05). Ezetimibe concentration then decreased to 4.07 ± 0.79 mg/dl by 90 min.

Fig. 1. Ezetimibe has no effect on the total output of lymph after an intraduodenal bolus of Ensure. A: lymph was collected from rats treated with either saline (□) (n = 11) or ezetimibe (●) (n = 13) for 2 h prior to and post-Ensure infusion as described in MATERIALS AND METHODS. Lymph flow rate was defined as the volume of lymph collected per hour. B: cumulative lymph flow was measured in the 2 h prior to and 2 h post-Ensure infusion. Values are expressed as means ± SE. *P < 0.05, **P < 0.01 between ezetimibe- and saline-treated rats at the same time point.

postinfusion. For ezetimibe-treated rats, fasting lymphatic cholesterol concentration was lower than that in saline-treated rats 2 h after ezetimibe treatment (8.38 ± 0.75 mg/dl vs. 12.09 ± 1.22 mg/dl). After Ensure infusion in ezetimibe-treated rats, cholesterol concentration increased within 20 min and reached its peak level of 16.90 ± 1.04 mg/dl at 30 min. Cholesterol concentration then decreased to 4.07 ± 0.79 mg/dl by 90 min. Compared with the saline-treated rats, ezetimibe treatment significantly reduced the elevation of lymphatic cholesterol concentration at 10, 20, and 30 min (P < 0.05). Ezetimibe concentration then decreased to 4.07 ± 0.79 mg/dl by 90 min. Compared with the saline-treated rats, ezetimibe treatment significantly reduced the elevation of lymphatic cholesterol concentration at 10, 20, and 30 min (P < 0.05). Ezetimibe

Fig. 2. Ezetimibe treatment decreases fasting cholesterol levels in lymph. Lymph was collected from rats treated with either saline (□) (n = 11) or ezetimibe (●) (n = 13) as described in Fig. 1. A: lymphatic cholesterol concentration was determined by measuring the total amount of cholesterol per volume lymph. B: lymphatic output of cholesterol was measured as total amount of cholesterol per hour. C: cumulative cholesterol output was measured for the 2 h prior to and 2 h post-Ensure infusion. Values are expressed as means ± SE. *P < 0.05, ***P < 0.001 vs. saline control.
Ezetimibe affects lymphatic incretin secretion

Ezetimibe does not affect the total output of glucose, protein, or triglyceride into the lymph induced by Ensure. We also determined the effect of ezetimibe on lymphatic glucose, protein, and triglyceride outputs. We determined the lymphatic output of glucose, protein, and triglyceride by measuring their concentrations in lymph over time. As shown in Fig. 3A, there was only one time point in which glucose output was different between the two groups of rats at 30 min post-Ensure infusion, with glucose levels in the lymph of ezetimibe-treated rats being higher than the saline controls (17.79 ± 3.11 mg/h vs. 11.76 ± 3.62 mg/h) (P < 0.05). As shown in Fig. 3B, the ezetimibe-treated rats had greater peak protein output in lymph at 30 min post-Ensure infusion vs. controls (67.88 ± 14.37 vs. 36.73 ± 13.78 mg/h) (P < 0.01). Similarly, in Fig. 3C, ezetimibe treatment significantly raises triglyceride output at only two time points, 22.37 ± 3.15 vs. 13.41 ± 3.81 mg/h at 30 min (P < 0.01) and 34.21 ± 1.91 vs. 23.54 ± 3.21 mg/h at 40 min (P < 0.001).

Because we saw some significant differences in the output of glucose, protein, and triglyceride into the lymph of ezetimibe-treated rats at specific time points, we also wanted to know whether ezetimibe treatment would affect the total output of these dietary constituents from the intestine into lymph taking into account the flow rate. As shown in Fig. 3, D-F, the cumulative output glucose, protein, and triglyceride was calculated. In the fasted state ezetimibe treatment has no effect on the total output of glucose, protein, or triglyceride into lymph. As expected, the infusion of Ensure significantly increases the cumulative lymphatic output of these dietary constituents in the 2 h postinfusion (relative to the fasting period), but there is no significant difference in total output of glucose, protein, or triglyceride between the ezetimibe- and saline-treated groups in the 2 h postinfusion.

Ezetimibe reduces the secretion of lymphatic GIP in response to Ensure. Using the lymph fistula rat model, we have previously shown that the incretin hormones are secreted from enteroendocrine cells in response to glucose and triglyceride in the intestine (28). Mixed meals such as Ensure also stimulate the release of GIP and GLP-1 (12, 28). Therefore, we wondered whether ezetimibe also affects the secretion of these hormones in the absence of an effect on glucose, protein, and triglyceride output. To determine whether ezetimibe would influence the response of GIP secretion to the intraduodenal infusion of Ensure, lymphatic GIP concentrations were measured (Fig. 4A). The concentration of lymphatic GIP in rats treated with saline increased as early as 20 min after the infusion of Ensure, with a peak concentration of 993.72 ± 153.85 pM at 40 min. Therefore, GIP secretion was increased by 28-fold over its basal values. GIP concentrations in lymph returned to basal levels by 120 min. In contrast, following the administration of ezetimibe, the peak concentration of lymphatic GIP was only 638.61 ± 63.78 pM at 40 min, or 19-fold above baseline. Compared with the saline control rats, the lymphatic GIP concentration in ezetimibe-treated rats decreased significantly at 40 min by 35.7% (P < 0.05), at 50 min by 50.7% (P < 0.001), and at 60 min by 56.3% (P < 0.01) after infusion of Ensure.

Again, taking into account the increased lymph flow in ezetimibe-treated rats at these time points, we measured lymphatic GIP output prior to and post-Ensure infusion. Lymphatic output of GIP from the intestine followed a similar pattern as GIP concentration (Fig. 4B). Infusion of Ensure induced a significant increase in lymphatic GIP output, which was significantly blunted in rats treated with ezetimibe at 50 min (P < 0.05) and 60 min (P < 0.01) postinfusion. As shown in Fig. 4C, the cumulative output of GIP into lymph was calculated. In the fasted state, GIP output was not affected by ezetimibe treatment. However, in the fed state (2 h post-Ensure infusion) there was a significant decrease in the amount of GIP secreted from the intestine (23.5%, P < 0.05) in rats treated with ezetimibe. Therefore, ezetimibe treatment significantly diminishes the secretion of GIP into lymph in response to a mixed meal, irrespective of its lack of effect on lymphatic triglyceride and glucose.

Ezetimibe reduces the secretion of lymphatic GLP-1 in response to Ensure. To test whether ezetimibe treatment influences the secretion of the other incretin hormone, GLP-1, lymphatic GLP-1 concentrations were measured in the 2 h prior to and post-Ensure infusion (Fig. 5A). In rats treated with saline, lymphatic GLP-1 concentrations increased at 10 min post-Ensure infusion, peaking at 700.95 ± 131.37 pM by 40 min (a 55-fold increase over basal levels). GLP-1 secretion returned to basal levels 120 min after the infusion of Ensure. In ezetimibe-treated rats, however, lymphatic GLP-1 concentrations only reached a peak of 439.72 ± 48.25 pM at 30 min (40-fold over fasting levels). Ezetimibe-treated rats displayed a marked decrease in the amount of time peak levels of GLP-1 persisted in the lymph, with a rapid decline in GLP-1 concentrations back to baseline by 60–120 min after the infusion of Ensure. Compared with the saline-treated rats, ezetimibe treatment significantly decreased the elevation in lymphatic GLP-1 concentration stimulated by Ensure by 48.7% (P < 0.0001) at
Fig. 3. Total lymphatic glucose, protein, and triglyceride outputs are not affected by ezetimibe treatment. Lymphatic glucose (A), protein (B), and triglyceride (C) output was measured in lymph collected from rats treated with either saline (□) (n = 11) or ezetimibe (■) (n = 13) as in Fig. 1. Cumulative output of glucose (D), protein (E), and triglyceride (F) was measured for the 2 h prior to and 2 h post-Ensure infusion. Values are means ± SE. *P < 0.05, **P < 0.01, ***P < 0.001 vs. saline control.
Fig. 4. Ezetimibe treatment reduced lymphatic glucose-dependent insulino-tropic polypeptide (GIP) secretion. Lymph was collected from rats treated with either saline (□) \( n = 11 \) or ezetimibe (●) \( n = 13 \) as in Fig. 1. Lymphatic GIP concentration (A) and output (B) were measured. C: cumulative GIP output was measured for the 2 h prior to and 2 h post-Ensure infusion. Values are means ± SE. *\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \) vs. saline control.

Fig. 5. Ezetimibe treatment reduced lymphatic glucagon-like peptide-1 (GLP-1) secretion. Lymph was collected from rats treated with either saline (□) \( n = 11 \) or ezetimibe (●) \( n = 13 \) as in Fig. 1. Lymphatic GLP-1 concentration (A) and output (B) were measured. C: cumulative GLP-1 output was measured for the 2 h prior to and 2 h post-Ensure infusion. Values are means ± SE. *\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \) vs. saline control.
40 min, 74% \((P < 0.001)\) at 50 min, and 75.4% \((P < 0.01)\) at 60 min following the infusion of Ensure.

Again, taking into account the difference in lymph flow rates during this time period, we also calculated the lymphatic GLP-1 output (Fig. 5B). These data follow the GLP-1 concentration data in Fig. 5A, with lymphatic output of GLP-1 significantly increased after Ensure infusion in the saline-treated rats, whereas ezetimibe-treatment significantly blunted this effect 68.3% at 50 min \((P < 0.001)\) and by 79.7% at 60 min \((P < 0.01)\) post-Ensure infusion.

In the 2 h prior to Ensure infusion, again there was no effect of ezetimibe treatment on the cumulative output of GLP-1 (Fig. 5C). However, there is a significant decrease in GLP-1 output in the 2 h post-Ensure infusion (with ezetimibe-treated rats having 26.1% less GLP-1 than their saline-treated counterparts). From these data, we conclude that ezetimibe treatment significantly diminishes the secretion of GLP-1 into lymph in response to a mixed meal, even though there is no corresponding effect on lymphatic triglyceride and glucose outputs.

**DISCUSSION**

It is well recognized that ezetimibe is a potent and selective agent that inhibits the intestinal absorption of cholesterol; however, it is unknown whether ezetimibe also has an effect on the secretion of the intestinal incretin hormones GIP and GLP-1. The purpose of the present study was to investigate the potential role of ezetimibe on the lymphatic secretion of GIP and GLP-1 in response to a mixed meal, Ensure. To our knowledge, this is the first report investigating the effect of ezetimibe on incretin secretion by the intestine. We used the lymph fistula rat model to determine the amount of intestinally-secreted GLP-1 and GIP in lymph.

We have recently validated in a number of studies (12, 27, 28) that the lymph fistula model is an excellent model for studying the secretion of incretins stimulated by macronutrients. Although GIP and GLP-1 are typically measured in plasma, their concentrations are quite low due to portal dilution (portal blood flow is 14–18 ml/min while lymph flow is 2–3 ml/h) and their rapid degradation by dipeptidyl-peptidase IV (DPP-IV). In lymph however, the activity of DPP-IV is 12-fold lower than in plasma during fasting, leading to elevated concentrations of GIP and GLP-1 (27). We were able to measure total GIP in lymph with a commercially available ELISA kit since the active GIP kit is not commercially available yet. With regard to GLP-1 we were able to measure the active form by ELISA. However, it has been shown that in lymph, the amount of active GLP-1 closely reflects the amount of total GLP-1 due to the relative lack of degradation by DPP-IV (27). Therefore, the lymph fluid compartment gives us a unique opportunity to sensitively detect changes in incretin hormone secretion relative to plasma. Also, it should be emphasized that the measurement of incretin levels in lymph has an added advantage of providing us with insights into the concentration of the incretins that the enteric neurons and dendritic cells of the lamina propria are exposed to.

Using the lymph fistula rat model, we measured the absorption and lymphatic transport of cholesterol both 2 h prior to and post the mixed meal (Ensure) and the effects of ezetimibe on this process. We found that ezetimibe treatment does not have an effect on total lymph flow (Fig. 1), which corroborates previous reports (46). Lymphatic cholesterol concentration and output increased following infusion of Ensure. The effect of ezetimibe was most striking in the fasted state, where ezetimibe treatment caused a significant decrease in lymphatic cholesterol output (Fig. 2, B and C). Since Ensure is very low in cholesterol (0.02 mg/ml) and also because cholesterol uptake by the small intestine was inhibited by ezetimibe (13, 36, 44), the elevated cholesterol concentrations in lymph post-Ensure infusion are likely derived from the biliary source because of gallbladder contraction induced by Ensure infusion and the intracellular cholesterol pool of the intestine (17, 48). More importantly, in the fasted state, the main source of cholesterol in the intestine comes from biliary cholesterol pool (4); therefore, we expected that ezetimibe decreased cholesterol output into lymph in the fasting animals (Fig. 2C) since it inhibits the uptake of cholesterol by the intestine.

We also measured the lymphatic outputs of glucose, protein, and triglyceride in lymph in response to the infusion of Ensure and the effects of ezetimibe on this process. We found no significant differences between the saline and ezetimibe-treated rats in the cumulative outputs of glucose, protein, and triglyceride in the lymph either in the 2 h prior to or post-Ensure infusion.

Our previous studies have shown that intraduodenal infusion of Ensure (4.43 kcal in 3 ml) significantly increases lymphatic GIP and GLP-1 outputs and their concentrations in lymph relative to fasting levels (12, 28). In the present study, we have shown a 49- and 78-fold increase in lymphatic GIP and GLP-1 outputs, respectively, after the intraduodenal infusion of Ensure (Figs. 4B and 5B). However, treatment of rats with ezetimibe markedly decreased the lymphatic outputs of GIP and GLP-1 despite no change in the output of glucose, protein, and triglyceride from the intestine into the lymph (Figs. 4 and 5). Furthermore, the cumulative GIP and GLP-1 outputs in ezetimibe-treated rats significantly decreased in the 2 h post-Ensure infusion.

Our data suggest that, regardless of the intestinal secretion of glucose, protein, and triglyceride, ezetimibe treatment has a distinct effect on the release of GLP-1 and GIP hormones into the lymph. Since these incretin hormones are important in the maintenance of whole-body glucose homeostasis, this acute effect of ezetimibe treatment is of potential clinical interest. The exact biological mechanism by which acute ezetimibe administration is acting to decrease incretin hormone secretion in response a mixed meal is unknown; whether this effect is a direct effect upon the incretin-secreting L and K cells of the intestine or whether it may be an indirect effect due to the inhibition of NPC1L1 is curious. We know that chronic treatment of mice fed a western diet with ezetimibe actually increases insulin sensitivity (26), suggestive of increased GIP and GLP-1 secretion in that situation, but actual measurement of secretion of incretins was not done. Further studies will be required to elucidate whether acute treatment with ezetimibe reduces the secretion of incretins induced by lipid only or whether it also applies to other nutrients.

In addition, our data are very interesting since they suggest that GIP and GLP-1 secretion are not always coupled to the outputs of glucose, protein, and triglyceride into the lymph. Because the concentrations of these nutrients in lymph are not affected to any significant extent by the ezetimibe treatment, lymphatic GIP and GLP-1 outputs and concentrations are
greatly affected by ezetimibe treatment. This is especially interesting given that glucose and triglyceride are the primary physiological stimuli for intestinal GIP and GLP-1 release.

In summary, we have demonstrated that ezetimibe significantly inhibits GIP and GLP-1 secretion in the intestine induced by Ensure. This effect appears to be unrelated to lymphatic glucose, protein, or triglyceride concentrations. Our study also confirms previous reports of the inhibition of intestinal cholesterol absorption by ezetimibe. Taking into account that the output of cholesterol into lymph during active absorption (stimulated by Ensure infusion) may also be derived from intracellular sources and that this process is probably not inhibited by ezetimibe. We believe the effect of ezetimibe on incretin secretion induced by macronutrients is potentially important and warrants additional studies.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

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