Gastroenterology 2014;147:1275–1284

Glucagon-Like Peptide-2 Regulates Release of Chylomicrons From the Intestine

Satya Dash,[1,2,*] Changting Xiao,[1,2,*] Cecilia Morgantini,[1,2] Philip W. Connelly,[2,3] Bruce W. Patterson,[4] and Gary F. Lewis[1,2]

[1]Department of Medicine, Department of Physiology, Banting and Best Diabetes Centre, [2]Division of Endocrinology, Department of Medicine, University of Toronto, Toronto, Ontario, Canada; [3]Keenan Research Centre for Biomedical Science of St. Michael's Hospital, Toronto, Ontario, Canada; [4]Center for Human Nutrition, Department of Internal Medicine, Division of Geriatrics and Nutritional Science, Washington University School of Medicine, St. Louis, Missouri

BACKGROUND & AIMS: The intestine efficiently incorporates and rapidly secretes dietary fat as chylomicrons (lipoprotein particles comprising triglycerides, phospholipids, cholesterol, and proteins) that contain the apolipoprotein isoform apoB-48. The gut can store lipids for many hours after their ingestion, and release them in chylomicrons in response to oral glucose, sham feeding, or unidentified stimuli. The gut hormone glucagon-like peptide-2 (GLP-2) facilitates intestinal absorption of lipids, but its role in chylomicron secretion in human beings is unknown. **METHODS:** We performed a randomized, single-blind, cross-over study, with 2 study visits 4 weeks apart, to assess the effects of GLP-2 administration on triglyceride-rich lipoprotein (TRL) apoB-48 in 6 healthy men compared with placebo. Subjects underwent constant intraduodenal feeding, with a pancreatic clamp and primed constant infusion of deuterated leucine. In a separate randomized, single-blind, cross-over validation study, 6 additional healthy men ingested a high-fat meal containing retinyl palmitate and were given either GLP-2 or placebo 7 hours later with measurement of TRL triglyceride, TRL retinyl palmitate, and TRL apoB-48 levels. **RESULTS:** GLP-2 administration resulted in a rapid (within 30 minutes) and transient increase in the concentration of TRL apoB-48, compared with placebo ($P = .03$). Mathematic modeling of stable isotope enrichment and the mass of the TRL apoB-48 suggested that the increase resulted from the release of stored, presynthesized apoB-48 from the gut. In the validation study, administration of GLP-2 at 7 hours after the meal, in the absence of additional food intake, robustly increased levels of TRL triglycerides ($P = .007$), TRL retinyl palmitate ($P = .002$), and TRL apoB-48 ($P = .04$) compared with placebo. **CONCLUSIONS:** Administration of GLP-2 to men causes the release of chylomicrons that comprise previously synthesized and stored apoB-48 and lipids. This transiently increases TRL apoB-48 levels compared with placebo. Clinical trials number at www.clinicaltrials.gov: NCT 01958775.

Keywords: Enterocyte; Plasma Lipid; Fatty Acid; Human Trial.

Dietary triglycerides (TGs) are digested in the intestine by lipase enzymes, yielding monoacylglycerol and fatty acids, which are absorbed from the lumen of the gut, re-esterified to TGs, and assembled into apolipoprotein (apo)B-48–containing chylomicron particles within the enterocyte.[1,2] Chylomicrons are secreted from the basolateral surface of enterocytes into the lamina propria and the lymphatic system[3] before reaching the circulation.[2] TG digestion and absorption is extremely efficient and rapid, with more than 95% of ingested TGs absorbed by the intestine.[2] Despite the efficiency of TG absorption and chylomicron production and release, the gut has the capacity to store TGs from a meal. The stored TGs subsequently can be released rapidly as chylomicrons in response to factors such as glucose,[3] sham feeding,[4] and further mixed meal ingestion.[5]

The gut hormone glucagon-like peptide-2 (GLP-2) is encoded by the proglucagon gene and secreted by L cells in response to nutrient ingestion.[6] Studies in the Syrian Golden hamster have shown that GLP-2 acutely enhances chylomicron secretion.[7,8] In addition, GLP-2 enhances nutrient absorption, gut barrier function, intestinal blood flow, crypt cell proliferation, and reduces inflammation.[6] As a result, GLP-2 is of proven therapeutic benefit in patients with malabsorption secondary to short-bowel syndrome.[9,10] The GLP-2 analogue teduglutide is now approved in a number of countries for treatment of this condition.[6,11] Only 1 study has previously examined the effects of GLP-2 on plasma lipids in human beings.[12] Intravenous infusion of native GLP-2 was shown to increase postprandial plasma TG and free fatty acid (FFA) concentrations.[12] The effect of GLP-2 on apoB-100 (indicative of VLDL) and apoB-48 (indicative of intestinal chylomicrons) metabolism is not known.

We examined the acute effects of a single, subcutaneous dose of GLP-2 on intestinal and hepatic lipoprotein production in healthy human beings, during constant high-fat, mixed-macronutrient formula infusion, through a nasoduodenal tube and under conditions of a pancreatic clamp as previously described[13] (study A). Because hypothesis-generating non–steady-state solutions suggested

*Authors share co-first authorship.

Abbreviations used in this paper: ANOVA, analysis of variance; apoB, apolipoprotein B; $AUC_{0–3}$, area under the concentration curve in the first 3 hours after treatment; BMD, bone mineral density; DPP-IV, dipeptidyl peptidase IV; FCR, fractional catabolic rate; FFA, free fatty acid; GLP-2, glucagon-like peptide-2; $iAUC_{0–3}$, incremental area under the curve in the first 3 hours after treatment; PR, production rate; S_f, Svedberg floatation rate; TG, triglyceride; TRL, triglyceride-rich lipoprotein.

0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2014.08.037

that GLP-2 increases intestinal triglyceride-rich lipoprotein (TRL) (chylomicron) concentration by releasing previously synthesized apoB-48 rather than stimulating secretion of newly synthesized particles, we performed an additional study to test this hypothesis (study B). Volunteers were given a liquid meal enriched with retinyl palmitate, thus labeling the lipid absorbed from that meal.[14] In the absence of further food ingestion, a single dose of GLP-2 administered 7 hours after the high-fat meal increased TRL TG, apoB-48, and retinyl palmitate levels, suggesting that GLP-2 releases chylomicrons comprising previously synthesized and stored apoB-48 and lipid from the gut in human beings.

Materials and Methods

Six healthy normoglycemic and normolipidemic volunteers, on no medication, participated in study A, and a further 6 volunteers participated in study B. Both studies were randomized, single-blind, cross-over studies with 2 visits separated by 4 weeks (GLP-2 was administered during one visit and placebo was administered during the other). Baseline demographics and biochemistry are shown in Table 1. For study A, participants underwent lipoprotein kinetic studies with constant intraduodenal feeding and administration of a pancreatic clamp and L-[5,5,5-$^2H_3$]-leucine (d3-leucine) (Figure 1*A*).[13] The primary outcome was the change in TRL apoB-48 concentration. Further details about mathematic modeling and biochemical assays are available online in the Supplementary Materials and Methods section. For study B, participants had a liquid meal along with retinyl palmitate to label chylomicrons produced from the meal (Figure 5*A*). Seven hours later, in the absence of food intake, GLP-2 or placebo was administered and the increment in TRL TG, apoB-48, and retinyl palmitate concentration was assessed (primary outcome). Further details are available in the Supplementary Information section. All authors had access to the study data and reviewed and approved the study manuscript.

Study Oversight

Both studies were performed according to the principles of the Declaration of Helsinki and were approved by the institutional research ethics board (University Health Network Research Ethics Board, Toronto, Ontario, Canada). All participants provided their written informed consent.

Table 1. Baseline Demographics and Biochemistry

Characteristic	Study A (n = 6), mean ± SEM	Study B (n = 6), mean ± SEM
Age, *y*	40.0 ± 5	48.8 ± 3.3
Body weight, *kg*	74.8 ± 5.1	81.5 ± 4.6
Body mass index, *kg/m²*	24.4 ± 1.2	25.4 ± 0.7
Fasting plasma glucose level, *mmol/L*	5.1 ± 0.1	4.8 ± 0.2
Fasting plasma TG level, *mmol/L*	0.9 ± 0.1	0.6 ± 0.04

Results

Study A: Determination of Intestinal and Hepatic TRL Particle Secretion in Response to GLP-2

GLP-2 administration increases plasma GLP-2 concentration. Administration of 1500 μg of GLP-2 subcutaneously significantly increased plasma total GLP-2 concentration compared with placebo (Supplementary Figure 1*A*). GLP-2 concentration decreased at 4 hours to approximately half of the level at 2 hours.

GLP-2 transiently increases plasma TG concentration. GLP-2 treatment transiently increased plasma TG concentration with a significant increment in plasma TG in the first 3 hours after administration by analysis of variance (ANOVA) of the increment ($P = .001$). In addition, both the peak concentration at 1 hour (placebo, 0.92 ± 0.13 vs GLP-2, 1.23 ± 0.20 mmol/L; $P = .03$) and the area under the concentration curve in the first 3 hours (AUC_{0-3}) after administration (placebo, 2.7 ± 0.4 vs GLP-2, 3.1 ± 0.4 mmol/L/h; $P = .03$) were significantly higher with GLP-2 compared with placebo (Figure 2*A*). There was an increase in TRL TG with GLP-2 treatment by ANOVA ($P = .04$). The peak

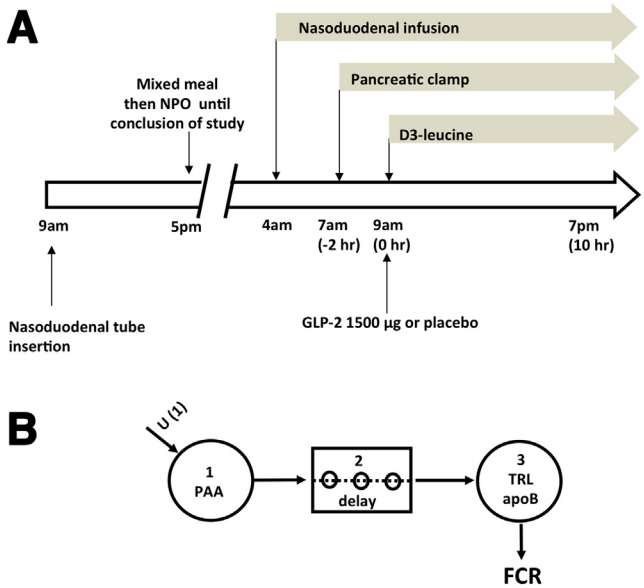

Figure 1. (*A*) Outline of lipoprotein kinetic study (study A). Volunteers had a nasoduodenal tube inserted the day before the study. After an overnight fast, a liquid mixed macronutrient formula was infused through the nasoduodenal tube for 15 hours from 4 AM on the day of the study. A pancreatic clamp (with infusion of somatostatin, insulin, glucagon, and growth hormone) was started at 7 AM. TRL kinetics were studied with a primed, constant infusion of deuterated leucine (d3-leucine) for 10 hours starting at 9 AM. At 9 AM volunteers received a subcutaneous dose of either GLP-2 (1500 ug) or placebo. NPO, Nil per oral except water. (*B*) Multicompartmental model for analysis of TRL apoB-100 kinetics and TRL apoB-48 placebo treatment. Infused d3-leucine enters the plasma amino acid pool (PAA) (compartment 1). After a delay (compartment 2), it is incorporated into TRL apoB (compartment 3). Enrichment time-course curves were analyzed with the multicompartmental model to derive FCR.