Glucagon-Like Peptide-2 Increases Intestinal Lipid Absorption and Chylomicron Production via CD36

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See editorial on page 775.

BACKGROUND & AIMS: Excessive postprandial lipemia is a prevalent condition that results from intestinal over-secretion of apolipoprotein B48 (apoB48)-containing lipoproteins. Glucagon-like peptide-2 (GLP-2) is a gastrointestinal-derived intestinotropic hormone that links nutrient absorption to intestinal structure and function. We investigated the effects of GLP-2 on intestinal lipid absorption and lipoprotein production. METHODS: Intestinal lipid absorption and chylomicron production were quantified in hamsters, wild-type mice, and Cd36−/− mice infused with exogenous GLP-2. Newly synthesized apoB48 was metabolically labelled in primary hamster jejunal fragments. Fatty acid absorption was measured, and putative fatty acid transporters were assessed by immunoblotting. RESULTS: Human GLP-2 increased secretion of the triglyceride (TG)-rich lipoprotein (TRL)-apoB48 following oral administration of olive oil to hamsters; TRL and cholesterol mass each increased 3-fold. Fast protein liquid chromatography profiling indicated that GLP-2 stimulated secretion of chylomicron/low-density lipoprotein-sized particles. Moreover, GLP-2 directly stimulated apoB48 secretion in jejunal fragments cultured ex vivo, increased expression of fully glycosylated cluster of differentiation 36/fatty acid translocase (CD36), and induced intestinal absorption of [3H]triolein. The ability of GLP-2 to increase intestinal lipoprotein production was lost in Cd36−/− mice. CONCLUSIONS: GLP-2 stimulates intestinal apoB48-containing lipoprotein secretion, possibly through increased lipid uptake, via a pathway that requires CD36. These findings suggest that GLP-2 represents a nutrient-dependent signal that regulates intestinal lipid absorption and the assembly and secretion of TRLs from intestinal enterocytes.

Postprandial lipemia, particularly excessive accumulation of intestinally derived lipoproteins, is gaining recognition as a cardiovascular risk factor because the magnitude of postprandial lipemia correlates with intima media thickness. Apolipoprotein B48 (apoB48) serves as the main structural apolipoprotein of the buoyant, triglyceride (TG)-rich chylomicrons (CM) that deliver dietary lipids to tissues. Interestingly, CM remnants and apoB48 particles have been detected in atherosclerotic plaques. In addition to augmenting the risk of atherosclerosis, postprandial hypertriglyceridemia is also an important facet of the metabolic dyslipidemia observed in insulin resistance, and there is evidence to suggest that intestinal cholesterol absorption is up-regulated in type 1 diabetes.

Despite increasing awareness that intestinally derived lipoproteins may contribute to the pathophysiology of atherosclerosis, our understanding of the cellular pathways involved in CM production and secretion remains limited. CM assembly is proposed to occur in a 3-step manner: (1) assembly of the primordial lipoprotein during the translocation of apoB48 into the endoplasmic reticulum, (2) accumulation of TG-rich lipid droplets in the lumen of smooth endoplasmic reticulum, and (3) core expansion achieved through additional lipidation of the primordial particle as it proceeds through the secretory pathway to generate a large TG-rich lipoprotein (TRL). As the nascent apoB48 polypeptide traverses the secretory system, its lipidation is achieved through the action of microsomal triglyceride transfer protein (MTP). The intestine is capable of secreting small, dense, lipid-poor apoB48-containing lipoproteins, but the secretion of TRL is dependent on TG availability, which can be derived from the diet or intracellular de novo lipogenesis.

The observations that insulin can acutely inhibit intestinal apoB48 secretion and that insulin resistance is associated with intestinal apoB48 overproduction suggest...
gest that intestinal lipid metabolism is sensitive to endocrine signals. Glucagon-like peptide-2 (GLP-2), a 33-amino acid peptide cosecreted with GLP-1 from enteroendocrine L cells in response to carbohydrate and fat ingestion, is an attractive candidate for such an endocrine signal. GLP-2 is highly intestinotrophic and prevents the intestinal mucosal hypoplasia observed with parenteral nutrition.10 GLP-2 exerts its actions through the GLP-2 receptor (GLP-2R), a G-protein-coupled receptor that exhibits a highly restricted specific pattern of expression in enteroendocrine cells,11 enteric neurons,12 subepithelial myofibroblasts,13 and the central nervous system.14 GLP-2R activation results in cyclic adenosine monophosphate accumulation and protein kinase A activation.15 Notably, GLP-2R messenger RNA or protein has not been detected in absorptive enterocytes. Thus, GLP-2’s proliferative and cytoprotective actions on the intestinal epithelium must occur indirectly, likely involving GLP-2R-dependent stimulatory actions on the intestinal epithelium.

Materials and Methods

Antibodies and Chemicals

Rabbit polyclonal anti-human cluster of differentiation 36/fatty acid translocase (CD36) antibody (Cayman Chemical, Ann Arbor, MI), rabbit polyclonal anti-human FATP4 (kind gift from Dr Paul A. Watkins, Johns Hopkins University), and rabbit anti-MTP antibody (kind gift of Dr André Theriault, University of Hawaii) were used for immunoblots of protein from isolated hamster enterocytes.

Animals

Male Syrian Golden hamsters (Mesocricetus auratus) from 130 to 150 g (Charles River, Montreal, Quebec, Canada) were housed individually in a 12:12-hour light/dark cycle. Animals were fed ad libitum with a standard chow diet. Male Cd36−/− mice bred on the C57BL/6 background from 12 to 15 weeks of age were obtained from Dr Maria Febbraio, Cleveland Clinic via Dr Kevin Kain, University of Toronto. Following at least a 1-week acclimatization period, animals underwent the in vivo protocols described below. All procedures were approved by the Hospital for Sick Children and Toronto General Hospital Animal Care Committees. C57BL/6 male mice 8–12 weeks old (Charles River, Montreal, Quebec, Canada) were maintained on standard rodent chow under a normal 12:12-hour light/dark cycle.

Determination of TRL apoB48 Secretion In Vivo in Hamsters

The in vivo protocol for Syrian Golden hamsters was performed as previously described.23 Briefly, the right jugular vein was cannulated with a silastic catheter (VWR), filled with heparinized saline (40 IU/mL in 0.9% NaCl), and exteriorized at back of neck under isoflurane anesthesia. Animals were allowed to recover overnight, and henceforth, experiments were performed on conscious hamsters. Following a 16-hour overnight fast, a 400 µL blood sample was collected into heparinized tubes (Microtainer PST tubes with lithium heparin; BD, Franklin Lakes, NJ) from the jugular catheter as the baseline reading and succeeded by an oral gavage of 200 µL olive oil. Twenty minutes following the gavage, the hamster was administered human GLP-2(1–33) (0.25 mg/kg) (Bachem Bioscience Inc, King of Prussia, PA) by intraperitoneal injection and followed immediately by an intravenous bolus of Triton WR-1339 (20% wt/vol in saline, 0.5 g/kg) (Tyloxapal; Sigma-Aldrich Co, St. Louis, MO). Four hundred microliters of blood was sampled at 30-minute intervals until 120 minutes postgavage, at which point the intestine was excised under isoflurane anesthesia, and the hamster was killed.

Determination of TRL apoB48 Secretion In Vivo in Mice

Analysis of intestinal TG and ApoB48 secretion in mice was performed following a similar procedure. After a 5-hour fast, mice were administered 200 µL olive oil by gavage, and, 20 minutes later, Triton WR-1339 (15% in saline, 0.5 g/kg) with or without the GLP-2 analogue hGly2−GLP-2 (0.25 mg/kg body weight) was injected intravenously (IV). Fifty-microliter blood samples were collected via the tail vein prior to (time 0) and 30 and 60 minutes after IV injection for determination of plasma triglyceride and apoB48. Ninety minutes after IV injection, mice were killed, and blood was collected by cardiac puncture.

Isolation of TRL

The plasma layer was separated by centrifuging the blood for 15 minutes at 4°C at 5000 rpm. The plasma was supplemented with sodium azide and a cocktail of
protease inhibitors (Roche Diagnostics, Mannheim, Germany), and 150 μL was layered under 4 mL of 1.006 g/mL potassium bromide solution. The layered plasma was centrifuged at 10°C for 70 minutes at 35,000 rpm using an SW55Ti rotor (Beckmann Coulter, Mississauga, Ontario, Canada). The top 300 μL was collected as the TRL fraction.

**Fast Protein Liquid Chromatography of Plasma Lipoproteins**

Blood was collected by cardiac puncture 2 hours following a gavage of 200 μL olive oil. Two hundred microliters of plasma was filtered through a 0.45 μm filter and microspin polysulphone filter (Alltech, Manfold Scientific, Lachine, PQ) and separated according to size through a HR 10/300 GL Superose 6 column (Pharmacia, Uppsala, Sweden). A solution of 10 mmol/L Tris, 150 mmol/L NaCl, 2 mmol/L CaCl₂, 100 μmol/L DTPA, 0.02% NaN₃, pH 7.4, was pumped through at a flow rate of 0.5 mL/min, and 0.5 mL fractions were collected. TG and cholesterol concentrations of the fractions were ascertained with 1-step colorimetric assays (Roche Diagnostics).

**Monitoring Triolein Absorption In Vivo**

Hamsters with a jugular catheter were gavaged with 3 μCi [9,10-³H(N)] triolein mixed in with 200 μL olive oil and then injected intraperitoneally with 0.25 mg/kg GLP-2 20 minutes later. Two hundred microliters of blood was sampled from the jugular catheter into heparinised tubes at half-hour intervals. The activity of tritium in 20 μL of plasma was determined by scintillation counting in triplicate. After an overnight fast, mice were administered 3 μCi [9,10-³H(N)] triolein mixed in with 200 μL Intralipid (10%), and 50 μL blood samples were collected via the tail vein.

**In Situ Apical Membrane Protein Biotinylation**

To specifically label enterocyte brush border membrane proteins with biotin, intact proximal jejunum was filled with a solution of 1.5 mg/mL sulfo-NHS-SS-Biotin (Pierce, Rockford, IL) as described. To enterocytes were isolated as previously described and lysed in 1% Triton X-100, 150 mmol/L NaCl, 50 mmol/L EDTA, 50 mmol/L Tris, pH 7.5. Biotinylated proteins were pulled down by incubating 0.75 mg of total protein with 50 μL streptavidin agarose beads (Pierce) at 4°C overnight. The beads were washed, and the biotin was cleaved from the protein by boiling the sample in 8% SDS containing β-mercaptoethanol and dithiothreitol.

**Statistical Analysis**

Two-way repeated measures ANOVA with the Bonferroni post hoc analysis was performed on time course experiments. For enterocyte expression of proteins and lipid synthesis assays, a paired t test was used. Results are expressed as mean ± SEM.

**Results**

**GLP-2 Acutely Increases Circulating Levels of apoB48-Containing TRL in Hamsters**

Hamsters were administered human GLP-2(1–33) (0.25 mg/kg) by intraperitoneal injection and challenged with a fat load, and the apoB48 mass in the TRL fraction of the plasma was monitored by immunoblotting. An intravenous bolus of Triton WR-1339 was also introduced to inhibit lipoprotein catabolism, thus maintaining the buoyancy of newly secreted CM. As shown in the photograph in Figure 1A, treatment with GLP-2 resulted in a marked lipemia that was visibly noticeable, especially by 120 minutes postgavage. Quantification of the apoB48 mass by immunoblotting demonstrated that GLP-2 increased the mass of circulating apoB48-containing TRL by 60 minutes (Figure 1B). The slopes were 4.36% ± 0.75% · min⁻¹ and 2.57% ± 0.9% · min⁻¹ for GLP-2- vs vehicle-treated hamsters, respectively.

We next assessed whether GLP-2 produces changes in intestinal lipoproteins by both fast protein liquid chromatography (FPLC) analysis and salt-density sedimentation of plasma from hamsters challenged with a fat load but not given a bolus of Triton WR-1339. FPLC separates lipoproteins according to size via gel filtration. One hundred twenty minutes after the gavage, levels of TG and cholesterol were higher in the very low-density lipoprotein (VLDL)/CM remnant-sized fractions in plasma from GLP-2-treated hamsters (Figure 1C). No appreciable differences were observed in the low-density lipoprotein (LDL) and high-density lipoprotein (HDL) fractions, suggesting that GLP-2's stimulatory effect on circulating lipoprotein levels is limited largely to the TRL. Complete profiling of plasma lipoproteins by sedimentation yielded similar observations, with higher TG and free cholesterol concentrations in the large CM, small CM, and large VLDL fractions isolated from GLP-2-treated hamster plasma (Supplementary Figure S1). The concentration of TG in the TRL fraction of GLP-2-treated hamsters was 3-fold higher than in control hamsters (Figure 1D). TRL cholesterol was also 3-fold higher in GLP-2-treated hamsters (Figure 1E). Metabolic labelling of primary hamster jejunal segments with [³⁵S]methionine was performed to see whether these in vivo observations could be recapitulated ex vivo. To rule out the possibility that the effect of GLP-2 on specific protein synthesis may be a result of GLP-2-enhanced enterocyte survival, ApoB48 and ABCG5 synthesis data were normalized to total protein synthesis (trichloroacetic acid precipitable counts). Addition of GLP-2 to the incubation media did not affect cellular levels of apoB48 (Supplementary Figure 2A) but resulted in significantly enhanced secretion of newly synthesized apoB48 (Supplementary Figure 2B) that is reflected in significantly greater total levels of [³⁵S]-labelled apoB48 (Supplementary Figure 2C). This increase in apoB48 synthesis was specific because GLP-2 did not significantly affect ATP-
binding cassette transporter G5 (ABCG5) synthesis (Supplementary Figure 2D).

**GLP-2 Accelerates Absorption of Luminal Fatty Acids**

To understand the mechanism by which GLP-2 induces apoB48-lipoprotein secretion, we next examined fatty acid absorption. To indirectly measure intestinal fatty acid transport, hamsters were challenged with a fat load that contained 3 μCi of [9,10-3H(N)]triolein, but without Triton WR-1339 injection. By 60 minutes after the gavage, there was a 2-fold increase in 3H radioactivity in the plasma of GLP-2-treated hamsters (Figure 2A). The majority of the tritium label was incorporated into secreted TG, which accounted for the GLP-2-induced increase in plasma 3H radioactivity at both 60 (Figure 2B) and 90 minutes (Figure 2C). No difference in total intestinal 3H counts was detected (data not shown).

The expression of specific protein transporters implicated in intestinal fatty acid absorption was examined by
Western blot analyses. No difference was noted in the protein levels of fatty acid transporter 4 (FATP4) (Figure 3A); similarly, intestinal MTP protein expression was found not to change with acute GLP-2 treatment (Figure 3B). However, GLP-2 significantly increased the expression of glycosylated CD36 (Figure 3C). Immunohistochemical visualization of CD36 suggests that this protein is expressed on hamster intestinal villi tips and appears to be present on the apical membrane and intracellularly (Figure 3D), poised for luminal fatty acid uptake. To verify that high-molecular-weight CD36 is the isoform that is expressed on the enterocyte apical membrane, a cell membrane-impermeable biotinylating agent (sulfo-NHS-SS-biotin) was introduced into the intestinal lumens of both control and GLP-2-treated hamsters in situ to specifically label apically expressed proteins of the epithelium. As shown in the “PD” lane of Figure 3E, streptavidin affinity pull down of biotinylated proteins revealed only a higher molecular weight protein slightly larger than 88 kilodaltons in enterocytes from both control and GLP-2-treated hamsters. However, this isoform of CD36 was not prominent in the streptavidin pull down of supernatant and whole cell lysate samples under both conditions. Such glycosylation behavior of CD36 was observed in 3 animals per treatment group. Treatment with PNGase F to remove all N-linked oligosaccharides yielded only the lower molecular weight band across all lanes. Thus, a potential mechanism by which GLP-2 may promote fat absorption is through increasing apical CD36 by means of advanced glycosylation.

**Acute GLP-2 Treatment Increases apoB48-Containing TRL Production in Mice**

To determine whether GLP-2 exerts similar actions on intestinal lipid absorption and lipoprotein production in mice, experiments were performed in wild-type mice with hGly2-GLP-2, a human dipeptidylpeptidase-4-resistant GLP-2 analogue. hGly2-GLP-2 rapidly increased the accumulation of plasma TG in the presence of Triton WR-1339 (Figure 4A) in a linear manner ($R^2 = 0.9918$ for control mice and $0.9966$ for GLP-2-treated mice), as well as the levels of TG and apoB48 in TRL fraction (Figure 4B and C) in wild-type mice after an oral gavage of olive oil in the presence of Triton WR-1339. Furthermore, FPLC profiling showed that exogenous GLP-2 increased TG levels in the VLDL/CM remnant fractions when lipoprotein clearance was not inhibited (Figure 4D). These experiments demonstrate that the stimulatory effects of GLP-2 on intestinal lipid absorption and CM secretion are conserved in different species.

**GLP-2-Stimulated Fat Absorption Requires CD36**

The immunoblots shown in Figure 3 suggested that GLP-2 rapidly promotes intestinal fat uptake by regulating the trafficking of CD36. To determine whether CD36...
is required for GLP-2-stimulated intestinal lipid absorption, we assessed GLP-2 action in C<sup>36</sup>-/- mice. Exogenous GLP-2 had no effect on levels of plasma TG after an oral fat load in C<sup>36</sup>-/- mice (Figure 5A). The time-dependent increase in plasma apoB48 following Triton WR-1339 injection indicate that C<sup>36</sup>-/- mice are indeed competent in secreting postprandial apoB48-containing lipoproteins. Consistent with the lack of GLP-2 action on TG absorption in C<sup>36</sup>-/- mice, no significant differences were noticed among TG (Figure 5B), cholesterol (Figure 5C), and apoB48 mass (Figure 5D) measurements on the TRL fraction, even though there were significant increases in TRL-apoB48 in their littermate controls. Hence, the actions of GLP-2 on the stimulation of CM secretion require CD36. Additionally, Gly<sup>2</sup>-GLP-2 failed to augment luminal [<sup>3</sup>H]triolein uptake in C<sup>36</sup>-/- mice (Figure 5E).

As quantified by scoring 10 different histologic sections of the small intestine, there was negligible oil red O staining in sections of proximal intestine from C<sup>36</sup>-/- mice (Figure 5F), consistent with observations of Nassir et al. There was however abundant oil red O staining in the distal jejunum of the CD<sup>36</sup>-/- mice (data not shown), which could explain why these mice were competent in TG secretion despite the lack of neutral lipid detected in their proximal intestine. Finally, we observed increased lipid accumulation in the enterocytes of GLP-2-treated wild-type but not C<sup>36</sup>-/- mice (Figure 5F).

**Discussion**

There is growing evidence that, in addition to its intestinotrophic effects, GLP-2 may act as an endocrine signal controlling intestinal nutrient absorption. Although we previously demonstrated that chronic GLP-2 administration enhanced [<sup>3</sup>H]triolein uptake into the circulation, there are limited data available as to the role of GLP-2 in regulating acute intestinal absorption, packaging, and secretion of chylomicron lipids. Long-chain fatty acids have been shown to regulate proglucagon-derived peptide secretion from ileal enteroendocrine L cells, suggesting that these cells may act as important postprandial sensors for the presence of luminal fatty acids and secrete factors such as GLP-1/GLP-2 to regulate intestinal lipid absorption and/or metabolism. Data presented in this manuscript establish a role for GLP-2 in the regulation of intestinal lipid and lipoprotein metabolism. GLP-2 was found to acutely and rapidly bring about changes in the intestine that ultimately result in an exaggerated postprandial lipemia. The most pronounced changes in plasma lipids were noticed in the TRL fraction of circulating lipoproteins, both in apoB48 mass and lipid content, which indicates that GLP-2 promotes the lipidation of nascent apob48 polypeptides. These data provide a mechanistic explanation for the rise in postprandial TG observed in human subjects infused with GLP-2. Because no change was noted in MTP mass of GLP-2-treated enterocytes, GLP-2 likely acts to stimulate chylomicron assembly and secretion by providing more lipid substrate for lipidation of the growing CM particle.

Increased GLP-2-stimulated packaging and secretion of diet-derived fatty acids as CMs was observed, as indicated by results from the [<sup>3</sup>H]triolein experiment. Because GLP-2 has not been shown to affect pancreatic
exocrine function, changes in pancreatic lipase levels to accelerate the hydrolysis of luminal TG is likely not a contributing factor to GLP-2-enhanced fat absorption. Evidence is amassing that long-chain fatty acid transport across the intestinal brush border membrane is protein mediated. The role of FATP4 was considered, but no difference in enterocyte FATP4 protein expression was noted between control and GLP-2-treated hamsters. Although FATP4 has been proposed as an important mediator of intestinal fatty absorption, recent evidence suggests that FATP4’s role lies within its acyl CoA-synthetase activity at the endoplasmic reticulum rather than as a solute carrier at the brush border membrane.

Our data suggest that GLP-2-accelerated fat absorption can be attributed at least in part to increased expression of fully glycosylated CD36. The highly glycosylated CD36 appears to be the isoform exclusively expressed on the enterocyte apical membrane as determined by in situ biotinylation. CD36/fatty acid translocase is essential for chylomicron secretion and appears to contribute significantly to intestinal cholesterol absorption. Recent studies in CD36-deficient mice also established this scavenger receptor as an intestinal long-chain fatty acid transporter. It has been suggested that the carbohydrate moieties of CD36 are important for its subcellular localization. Deletions in the C-terminus of CD36 downregulates its expression on the cell surface, and this was accompanied by reduced glycosylation. The effect of GLP-2 on CD36 was rapid, detected within 40 minutes of peptide injection. Interestingly, CD36 translocation to the cell surface from intracellular membranes is a process sensitive to metabolic signals in cardiac myocytes.

GLP-2 has previously been documented to influence the trafficking of another nutrient transporter, sodium-dependent glucose transporter-1, from intracellular stores to the brush border membrane of enterocytes.
Because Western blot analysis of enterocytes suggests that GLP-2 modifies CD36 glycosylation, studies in Cd36−/− mice were performed to confirm the transporter’s role. In contrast to the stimulatory effects of GLP-2 observed in wild-type mice, the actions of GLP-2 on intestinal lipid and lipoprotein secretion were absent in Cd36−/− mice. The rate of TG, cholesterol, and apoB48 secretion in GLP-2 and control-treated Cd36−/− mice were all superimposable. TRL TG and apoB48 were higher in Cd36−/− mice compared with wild type (Figure 5), but this may be attributable to this mouse model's elevated fasting VLDL31 because of impaired clearance of TG-rich VLDL-sized32 and chylomicron-sized33 particles. Impairment in lipoprotein lipase-independent clearance pathways could also explain the elevated TRL TG and apoB48 observed in Cd36−/− mice.33 Enteropancreatic-regulated post-translational modification of CD36 in the intestine may contribute to mechanisms controlling lipid absorption. However, the pathways regulating CD36 movement to the apical membrane in enterocytes have yet to be elucidated, and the intracellular compartments in which CD36 resides are still unidentified. Interestingly, a large portion of the enterocyte’s CD36 is not found at the plasma membrane, as shown by the strong signal of the 43- to 55-kilodalton-sized band in the supernatant and whole cell lysate lanes of Figure 3E. CD36 has also been localized to the Golgi apparatus in adipocytes,34 like MTP,35 so it may play a role in further lipidation of the apoB48-containing particle in that compartment. Cd36−/− mice have been reported to accumulate neutral lipids in enterocytes during a high fat load,27 hinting at the importance of intracellular movement of CD36 to target fatty acids for assembly and secretion as CMs.

Figure 1B suggests that there is an inflection point in increased TRL-apoB48 secretion in GLP-2-treated hamsters at 60 minutes, after which the difference between the 2 groups diminished, unlike the observations made in wild-type mice in Figure 4. Because dipeptidylpeptidase-4-sensitive native GLP-2 was administered to the hamsters, whereas dipeptidylpeptidase-4-resistant Gly2-GLP-2 was used in mice in Figure 4, it can be inferred that sustained GLP-2 action is necessary to maintain up-regulated CM secretion. In addition, given that GLP-2 resulted in an infection in TG secretion between 40 minutes and 60 minutes in both wild-type and Cd36−/− mice (Figure 5A), and that the results presented in Supplementary Figure S3A refute differential hepatic TG secretion, it suggests that these kinetics of lipoprotein secretion may be a CD36-independent actions of GLP-2 that can modulate fat absorption in vivo, perhaps because of alterations in gastric36 and intestinal37 motility.

In conclusion, our data demonstrate that GLP-2 promotes assembly and secretion of intestinally derived apoB48-containing TRL through accelerated dietary fat absorption and increased lipidation and secretion of apoB48-containing CM particles. This suggests that GLP-2 may facilitate the efficient absorption of dietary fats. The potent stimulatory role of GLP-2 raises the intriguing possibility of a link between GLP-2 function and postprandial dyslipidemia observed in conditions such as type 1 diabetes. Elevated levels of GLP-2 have been reported and implicated in mediating the intestinal hyperplasia in the streptozotocin-induced diabetic rat model.38 This same animal model also exhibits excessive postprandial lipemia.39 GLP-2 may thus provide a possible endocrine explanation for the intestinal apoB48-containing lipoprotein oversecretion observed in pathologic conditions.

Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of Gastroenterology at www.gastrojournal.org, and at doi: 10.1053/j.gastro.2009.05.051.

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Received September 17, 2008. Accepted May 20, 2009.

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Conflicts of interest
The authors disclose the following: Daniel J. Drucker (Dr Drucker is a party to a licensing agreement among the University of Toronto, the University Health Network, and NPS Pharmaceuticals Inc in regard to the clinical development of GLP-2). The remaining authors disclose no conflicts.

Funding
Supported by CIHR grant MOP-53093 (to K.A.) and CIHR grant MOP-14799 (to D.J.D.). J.H. is supported by the NSERC CGS M, Restracomp, and CIHR CGS D fellowships.
Supplementary Materials and Methods

Density Ultracentrifugation of Plasma Lipoproteins

One hundred fifty microliters of plasma obtained from hamsters 40 minutes after fat loading was subjected to density ultracentrifugation over a KBr gradient as previously described for microsomal luminal contents. Concentrations of triglyceride (TG) and total cholesterol (Roche Diagnostics, Mannheim, Germany) and free cholesterol and phospholipids (Wako Diagnostics, Osaka, Japan) were assayed in each fraction using enzymatic-based colorimetric assays.

Determination of Hepatic Lipoprotein Secretion In Vivo

C57BL/6 mice were fasted for 5 hours. An IV bolus of Triton WR-1339 was introduced by tail vein injection (15% in saline, 0.5 g/kg), followed by an intraperitoneal injection of hGly2-GLP-2 (0.25 mg/kg body weight) or phosphate-buffered saline (PBS). Fifty-microliter blood samples were collected from the tail vein prior to (0 minutes) and each hour for 3 hours following the intravenous (IV) injection. At 3 hours, mice were killed, and blood was collected by cardiac puncture.

Metabolic Labelling of Primary Jejunal Fragments Ex Vivo

Hamsters were given an oral gavage of 200 μL olive oil. Sixty minutes later, the jejunum was excised under isoflurane anesthesia, and the hamster was killed. The jejunum was flushed with ice-cold PBS, pH 7.4, and sliced longitudinally to reveal the mucosa; 0.5-mm-long fragments of jejunum were divided randomly among wells, with the mucosal side facing up. The fragments were prepulsed for 45 minutes in Dulbecco’s modified Eagle medium (DMEM) lacking methionine and cysteine and supplemented with 1% L-glutamine, 10% fetal bovine serum, 1% penicillin/streptomycin, and with/without the addition of 100 nmol/L human glucagon-like peptide-2 (GLP-2) (GLP-2) because insignificant increases in protein synthesis were detected (6.47 × 10⁵ ± 2.06 × 10⁵ dpm/mg protein vs 3.83 × 10⁵ ± 0.85 × 10⁵ dpm/mg protein, GLP-2 vs control), consistent with other studies. Significantly greater amounts of [35S]-apoB48 were immunoprecipitated from the media of jejunal fragments treated with GLP-2 after 60 and 90 minutes (Supplementary Figure 2B), suggesting direct GLP-2 stimulation of apoB48 secretion (at 60 minutes, 0.400% ± 0.048% vs 0.136% ± 0.005%, GLP-2 vs control, P < .05; at 90 minutes, 0.640% ± 0.027% vs 0.352% ± 0.062%, GLP-2 vs control, P < .001). The increase in apoB48 secretion was associated with greater amounts of total [35S]-apoB48 at 60 minutes (1.08% ± 0.05% vs 0.643% ± 0.031%, GLP-2 vs control) (Supplementary Figure 2C). To ascertain whether the GLP-2-stimulated increase in apoB48 synthesis was specific, we monitored the synthesis of another protein, adenosine triphosphate (ATP)-binding cassette transporter G5 (ABCG5). As shown in Supplementary Figure 2D, GLP-2 treatment did not increase the amount of cellular newly synthesized ABCG5.

Effect of GLP-2 on Hepatic Secretion of Triglyceride-Rich Lipoprotein

Because the liver is a major contributor to circulating apoB48 levels in C57BL/6J mice and the fast protein liquid chromatography fractionation was not sufficient to resolve CM remnants from VLDL particles, hepatic lipoprotein secretion was assessed in fasting mice. When Triton WR-1339 was administered to

Lipoprotein Profile Analysis

Complete profiling of plasma lipoproteins by sedimentation yielded similar observations, with higher TG and free cholesterol concentrations in the large chylomicron (CM), small CM, and large very low-density lipoprotein (VLDL) fractions isolated from GLP-2 treated hamster plasma (Supplementary Figure 1).

Direct Ex Vivo Stimulation of apoB48 Secretion by GLP-2

To delineate the mechanisms underlying the effects of GLP-2 on intestinal lipid absorption, we analyzed the direct actions of GLP-2 on fragments of hamster jejunum by labelling newly synthesized apoB48 with [35S]-methionine with a 30-minute pulse. ApoB48 was immunoprecipitated at the end of the pulse (0 minutes) and every 30 minutes thereafter during chase with unlabelled methionine. As shown in Supplementary Figure 2A, no significant differences in intracellular [35S]-labelled apoB48 were observed between control and GLP-2-treated intestinal fragments. Values were expressed as a fraction of total [35S]methionine incorporation to account for any global changes in protein metabolism (Supplementary Figure 2A) because insignificant increases in protein synthesis were detected (6.47 × 10⁵ ± 2.06 × 10⁵ dpm/mg protein vs 3.83 × 10⁵ ± 0.85 × 10⁵ dpm/mg protein, GLP-2 vs control), consistent with other studies. Significantly greater amounts of [35S]-apoB48 were immunoprecipitated from the media of jejunal fragments treated with GLP-2 after 60 and 90 minutes (Supplementary Figure 2B), suggesting direct GLP-2 stimulation of apoB48 secretion (at 60 minutes, 0.400% ± 0.048% vs 0.136% ± 0.005%, GLP-2 vs control, P < .05; at 90 minutes, 0.640% ± 0.027% vs 0.352% ± 0.062%, GLP-2 vs control, P < .001). The increase in apoB48 secretion was associated with greater amounts of total [35S]-apoB48 at 60 minutes (1.08% ± 0.05% vs 0.643% ± 0.031%, GLP-2 vs control) (Supplementary Figure 2C). To ascertain whether the GLP-2-stimulated increase in apoB48 synthesis was specific, we monitored the synthesis of another protein, adenosine triphosphate (ATP)-binding cassette transporter G5 (ABCG5). As shown in Supplementary Figure 2D, GLP-2 treatment did not increase the amount of cellular newly synthesized ABCG5.
fasting C57BL/6 mice in the absence of a fat load, control and GLP-2-treated mice had identical rates of hepatic TG (Supplementary Figure 3A), cholesterol (Supplementary Figure 3B), and apoB48 (Supplementary Figure 3C) output. TRL levels of TG (Supplementary Figure 3D), cholesterol (Supplementary Figure 3E), and apoB48 (Supplementary Figure 3F) were also very similar, thereby excluding a role for the liver in GLP-2-induced postprandial hyperlipidemia.

References


Supplementary Figure 1. Plasma lipoprotein profiling by salt-density sedimentation in hamsters. Hamsters were given an oral gavage of olive oil and followed by injection with GLP-2 or PBS 20 minutes later, and no Triton WR-1339 was used in this experiment. Profiling was performed on plasma collected 20 minutes after peptide injection. Representative density profile depicted TG (upper left), total cholesterol (lower left), free cholesterol (upper right), and phospholipids (lower right) content of plasma lipoproteins separated by ultracentrifugation on a discontinuous KBr gradient. Fractions are arranged as numbers 1 to 10 in order of increasing density.
Supplementary Figure 2. Ex vivo effects of GLP-2 on apoB48-containing lipoprotein secretion. [35S]-Labeled apoB48 was measured during the cold methionine-enriched chase after a 30-minute pulse in jejunal fragments freshly isolated from chow-fed hamsters and treated with or without GLP-2 ex vivo and normalized to total protein synthesis. (A) Intracellular levels of 35S-apoB48. (B) Secreted 35S-apoB48. (C) Total levels of 35S-apoB48. N = 3 per group; *P < .05, ***P < .001. (D) Cellular 35S-ABCG5. N = 4 per group.

Supplementary Figure 3. In vivo hepatic lipoprotein secretion in fasting C57BL/6 mice. Fasted C57BL/6 mice were administered an intravenous bolus of Triton WR-1339 and a single intraperitoneal injection of Gly²-GLP-2 or PBS control. No fat load was given. Blood was sampled, and the 180-minute sample was spun at 35,000 rpm for 18 hours to collect total VLDL. Plasma total (A) TG, (B) cholesterol, and (C) apoB48 accumulation over 3 hours. VLDL (D) TG, (E) cholesterol, and (F) apoB48 at 180 minutes. N = 6–8 per group, P > .05 for all parameters.