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

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BACKGROUND & AIMS: Excessive postprandial lipemia is a prevalent condition that results from intestinal oversecretion 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/very 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.[1] 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.[2] In addition to augmenting the risk of atherosclerosis, postprandial hypertriglyceridemia is also an important facet of the metabolic dyslipidemia observed in insulin resistance,[3] and there is evidence to suggest that intestinal cholesterol absorption is up-regulated in type 1 diabetes.[4]

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).[5] The intestine is capable of secreting small, dense, lipid-poor apoB48-containing lipoproteins,[6] 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[7] and that insulin resistance is associated with intestinal apoB48 overproduction[8,9] sug-

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 stimulation of downstream mediators such as keratinocyte growth factor[13] and insulin-like growth factor-1.[16]

GLP-2 also exerts rapid actions converging on stimulation of nutrient absorption in the gut. GLP-2 enhances hexose transport through up-regulation of sodium-dependent glucose transporter-1[17] and glucose transporter 2[18] in the brush border membrane. Intriguingly, long-chain fatty acids stimulate secretion of proglucagon-derived peptides from the gut,[19] and acute GLP-2 administration enhances lipid absorption in normal human subjects.[20] Nevertheless, the mechanism(s) through which GLP-2 modulates intestinal lipid secretion and/or metabolism remain poorly understood. In this study, we utilized both Syrian Golden hamster and mouse models to characterize the effect of GLP-2 on intestinal lipid metabolism. Because hamsters secrete apoB48 exclusively from the intestine, this facilitates delineation of intestinal contributions to circulating lipoprotein levels. We present new evidence implicating GLP-2 as a potent stimulator of apoB48-containing TRL secretion from the intestine and describe potential mechanisms via which GLP-2 augments the TG pool for secretion.

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.[21] 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 isoflourane 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) (Tyloxapol; 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 anaesthesia, 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 hGly[2]-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 μmol/L microspin polysulphone filter (Alltech, Mandel 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 CaCl$_2$, 100 μmol/L DTPA, 0.02% NaN$_3$, 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-^{3}H(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 heparinized 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-^{3}H(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.[18] Enterocytes were isolated as previously described[8] 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 $\pm$ 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% $\pm$ 0.75% $\cdot$ min^{-1} and 2.57% $\pm$ 0.9% $\cdot$ 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 [^{35}S]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 [^{35}S]-labelled apoB48 (Supplementary Figure 2C). This increase in apoB48 synthesis was specific because GLP-2 did not significantly affect ATP-

Figure 1. In vivo effects of GLP-2 on apoB48-containing TRL secretion. (*A*) Plasma collected from hamsters challenged with an oral gavage of olive oil coupled with an intravenous bolus of Triton is shown. The *time points* represent the minutes after the initial fat load. (*B*) Time-dependent increase of apoB48 mass in the plasma TRL fraction from chow-fed hamsters given an oral olive oil gavage and Triton infusion, as determined by immunoblotting. ApoB48 mass was normalized to baseline fasting levels determined immediately prior to the oil gavage (time 0) and before GLP-2 injection (time, 20 minutes). Representative apoB48 blots are shown above the graphical representation. N = 7 per group, *P < .05. (*C*) Representative FPLC lipoprotein profile of plasma from fat-loaded hamsters not given an intravenous bolus of Triton WR-1339. Profiles are representative of 3 FPLC runs per group. (*D*) TG and (*E*) cholesterol concentrations of the plasma TRL fraction, 120 minutes following fat load and without Triton infusion. N = 4 per group, *P < .05.

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-^{3}H(N)]triolein, but

without Triton WR-1339 injection. By 60 minutes after the gavage, there was a 2-fold increase in ^{3}H 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 ^{3}H radioactivity at both 60 (Figure 2B) and 90 minutes (Figure 2C). No difference in total intestinal ^{3}H counts was detected (data not shown).

The expression of specific protein transporters implicated in intestinal fatty acid absorption was examined by
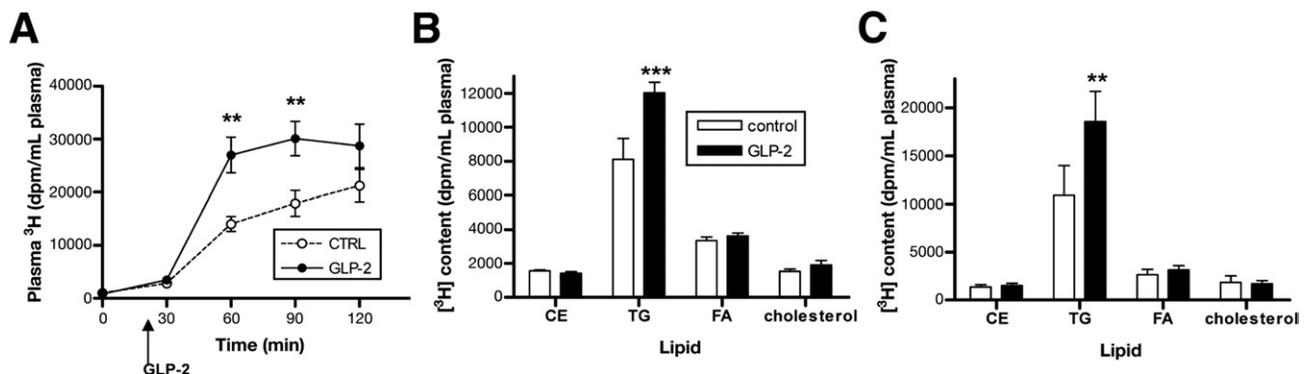
Figure 2. GLP-2 accelerates absorption of luminal fatty acids in hamsters. (*A*) Rate of appearance of radioactivity in plasma following an oral gavage of 3 μCi [9,10-^{3}H(N)]triolein in 200 μL olive oil without an intravenous bolus of Triton WR-1339. N = 7 per group, **P < .01. Thin layer chromatography was performed on the hexane-soluble phase of plasma to characterize the incorporation of the ^{3}H label into secreted lipids at (*B*) 60 minutes and (*C*) 90 minutes. N = 4 per group, **P < .01, ***P < .001.
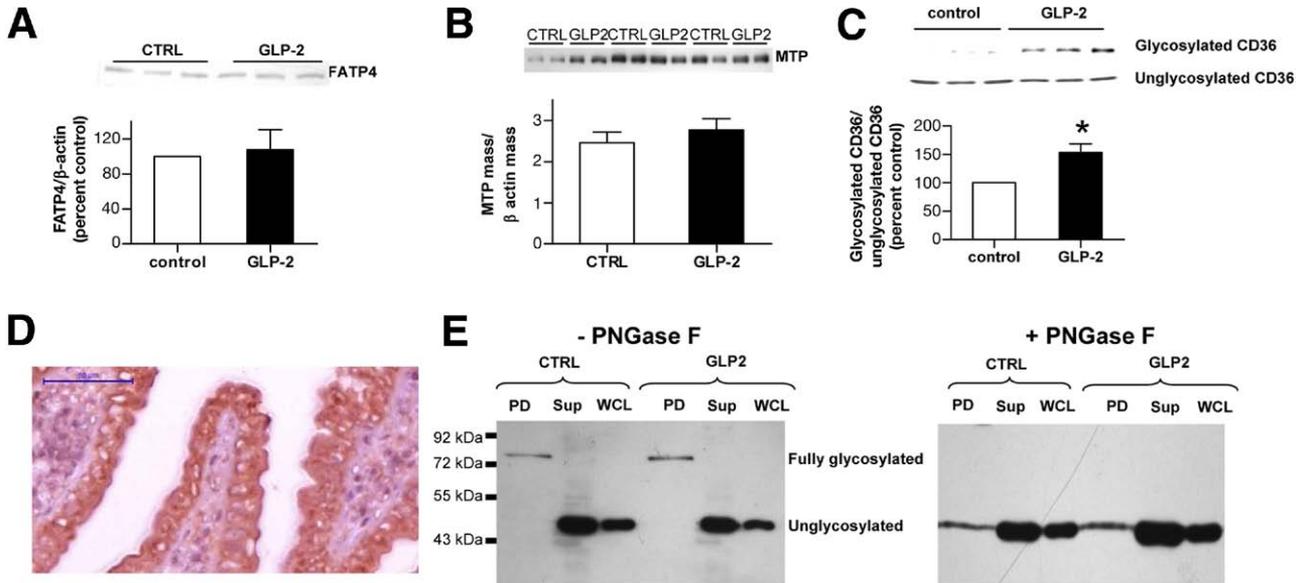
Figure 3. GLP-2 enhances intestinal CD36 action in hamsters. (*A*) FATP4 expression in hamster enterocytes. FATP4 immunoblot is shown above graphical representation. FATP4 expression was normalized to cellular levels of β-actin. N = 4 per group. (*B*) Immunoblot analysis of MTP protein in enterocytes from hamsters treated with GLP-2 in vivo in comparison with control, normalized to β-actin. Immunoblots of individual animals in duplicate shown above. N = 3 per group. (*C*) Ratio of fully glycosylated CD36 to unglycosylated CD36 as determined by immunoblotting. N = 4 per group, *$P < .05$. (*D*) Immunohistochemistry of hamster intestinal villi CD36 expression with an anti-CD36 antibody, visualized with diaminobenzidine (DAB) deposition (*brown*) and counterstained with hematoxylin and eosin. (*E*) Intestinal apical membrane proteins were biotinylated in situ, subjected to streptavidin pull down, and probed with anti-CD36 antibody by immunblotting. The biotinylated proteins were also treated with PNGase F for 120 minutes. *PD*, biotinylated intestinal apical proteins pulled down with streptavidin; *Sup*, supernatant after streptavidin pull down; *WCL*, whole cell lysate previous to addition of streptavidin-agarose beads. Molecular weight markers are indicated on the *left*.

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
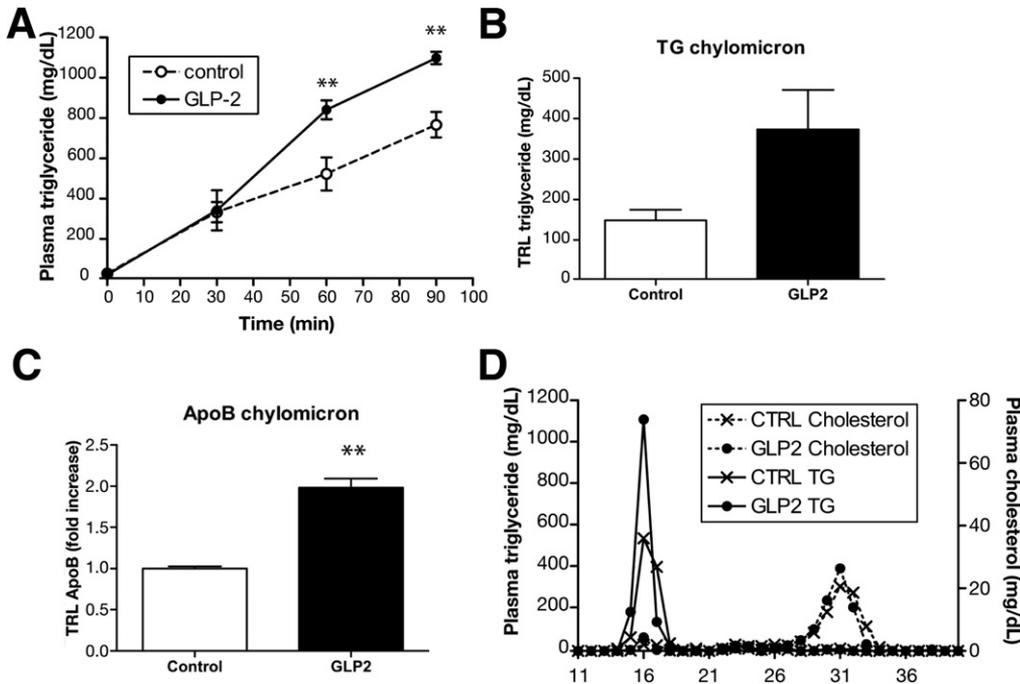
Figure 4. Stimulatory effects of GLP-2 on intestinal lipid absorption and apoB48-chylomicron production in wild-type mice. (*A*) Plasma TG accumulation after a fat load (200 μL olive oil) and IV Triton WR-1339 injection in wild-type mice fasted for 5 hours with or without Gly^2-GLP-2 as described in Materials and Methods section. N = 4–6, **$P < .01$. (*B*) TG and (*C*) apoB48 accumulation in TRL fraction purified 90 minutes after triton WR-1339 injection as described in Materials and Methods section. N = 4–6 per group, **$P < .01$. (*D*) Lipoprotein profile of plasma pooled from 6 mice per group 90 minutes after the fat load.

is required for GLP-2-stimulated intestinal lipid absorption, we assessed GLP-2 action in $Cd36^{-/-}$ mice. Exogenous GLP-2 had no effect on levels of plasma TG after an oral fat load in $Cd36^{-/-}$ mice (Figure 5*A*). The time-dependent increase in plasma apoB48 following Triton WR-1339 injection indicate that $Cd36^{-/-}$ mice are indeed competent in secreting postprandial apoB48-containing lipoproteins. Consistent with the lack of GLP-2 action on TG absorption in $Cd36^{-/-}$ mice, no significant differences were noticed among TG (Figure 5*B*), cholesterol (Figure 5*C*), and apoB48 mass (Figure 5*D*) 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^2-GLP-2 failed to augment luminal [^{3}H]triolein uptake in $Cd36^{-/-}$ mice (Figure 5*E*).

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 $Cd36^{-/-}$ mice (Figure 5*F*), consistent with observations of Nassir et al.[22] There was however abundant oil red O staining in the distal jejunum of the $Cd36^{-/-}$ 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 $Cd36^{-/-}$ mice (Figure 5*F*).

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 [^{3}H]triolein uptake into the circulation,[23] 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,[19] 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.[20] 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 [^{3}H]triolein experiment. Because GLP-2 has not been shown to affect pancreatic
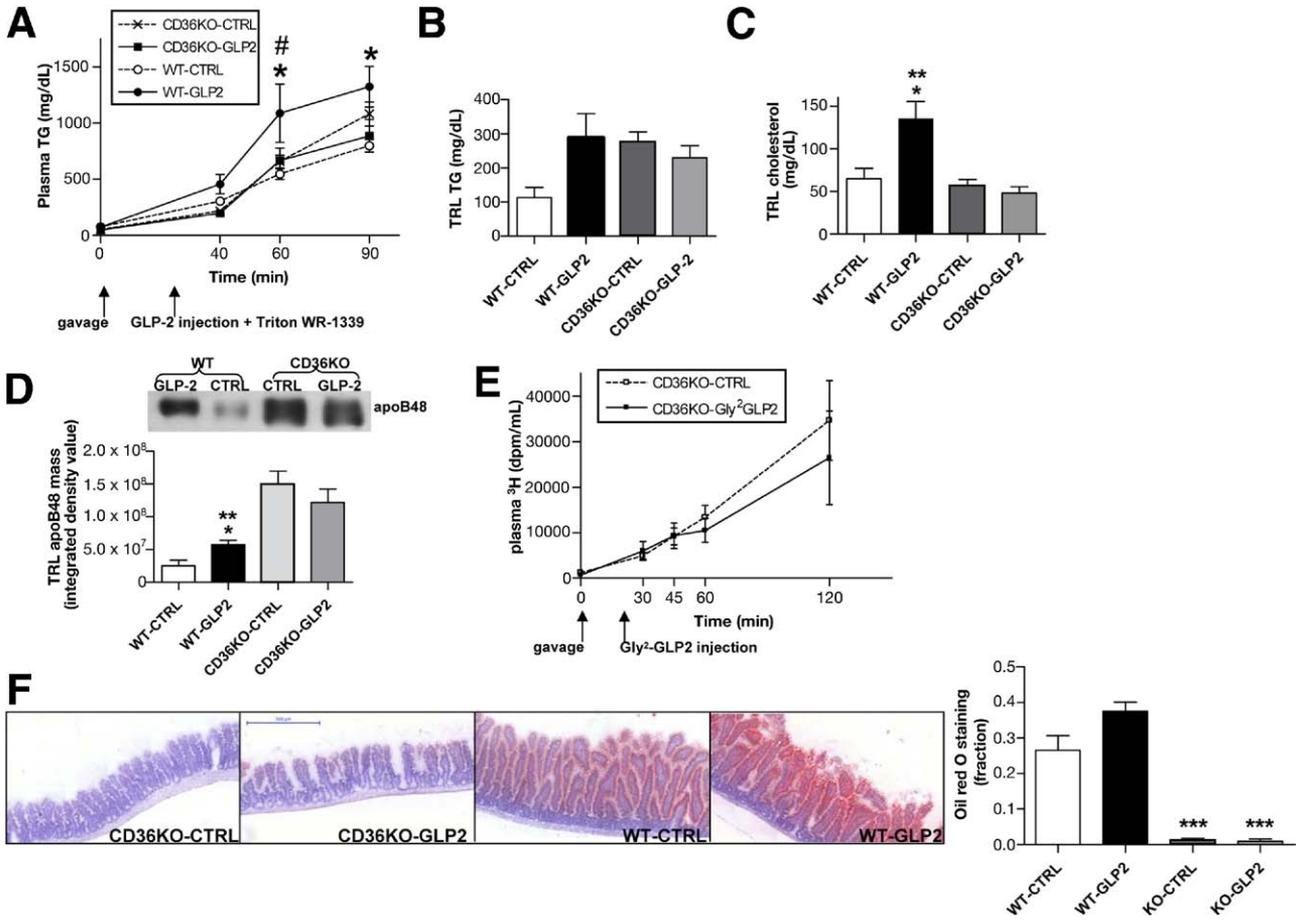
Figure 5. Lack of GLP-2 mediated stimulation of intestinal lipoprotein secretion in *Cd36*^{−/−} mice. *Cd36*^{−/−} mice bred on the C57BL/6 background were challenged with an oral fat load, injected intraperitoneally with hGLP-2[(1–33)], and given an intravenous bolus of Triton WR-1339 to inhibit lipoprotein catabolism. (*A*) Plasma TG following an oral fat load. N = 3 or 4 per group, *$P < .05$, wild-type GLP-2 vs wild-type control, #$P < .05$, wild-type GLP-2 vs *Cd36*^{−/−} control. (*B*) TG mass, (*C*) cholesterol mass, and (*D*) apoB48 mass in the TRL fraction; n = 3 or 4 per group; *$P < .05$, wild-type GLP-2 vs wild-type control; **$P < .01$, wild-type GLP-2 vs *Cd36*^{−/−} groups. (*E*) Plasma tritium levels in response to an oral gavage of 3 μCi [9,10-[3]H(N)]triolein in 200 μL olive oil, Gly[2]-GLP-2 injection, and no Triton WR-1339; n = 5 per group. (*F*) Representative oil red O stains of the proximal intestine with quantification of average area stained by oil red O, based on 10 separate fields of view per animal. N = 3 per group, ***$P < .001$, *Cd36*^{−/−} mice vs wild-type mice.

exocrine function,[24] 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,[25] 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.[26]

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[27] and appears to contribute significantly to intestinal cholesterol absorption.[28] Recent studies in CD36-deficient mice also established this scavenger receptor as an intestinal long-chain fatty acid transporter.[22] 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.[29] 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.[30] 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.[17]

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 VLDL[31] because of impaired clearance of TG-rich VLDL-sized[32] and chylomicron-sized[33] particles. Impairment in lipoprotein lipase-independent clearance pathways could also explain the elevated TRL TG and apoB48 observed in $Cd36^{-/-}$ mice.[33] Enteroendocrine-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 Gly^2-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 inflection 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 gastric[36] and intestinal[37] 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.

References

1. Boquist S, Ruotolo G, Tang R, et al. Alimentary lipemia, postprandial triglyceride-rich lipoproteins, and common carotid intima-media thickness in healthy, middle-aged men. Circulation 1999; 100:723–728.
2. Nakano T, Nakajima K, Niimi M, et al. Detection of apolipoproteins B-48 and B-100 carrying particles in lipoprotein fractions extracted from human aortic atherosclerotic plaques in sudden cardiac death cases. Clin Chim Acta 2008;390:38–43.
3. Taskinen MR. Diabetic dyslipidaemia: from basic research to clinical practice. Diabetologia 2003;46:733–749.
4. Gylling H, Tuominen JA, Koivisto VA, et al. Cholesterol metabolism in type 1 diabetes. Diabetes 2004;53:2217–2222.
5. Hussain MM. A proposed model for the assembly of chylomicrons. Atherosclerosis 2000;148:1–15.
6. Guo Q, Avramoglu RK, Adeli K. Intestinal assembly and secretion of highly dense/lipid-poor apolipoprotein B48-containing lipoprotein particles in the fasting state: evidence for induction by insulin resistance and exogenous fatty acids. Metabolism 2005;54: 689–697.
7. Federico LM, Naples M, Taylor D, et al. Intestinal insulin resistance and aberrant production of apolipoprotein B48 lipoproteins in an animal model of insulin resistance and metabolic dyslipidemia: evidence for activation of protein tyrosine phosphatase-1B, extracellular signal-related kinase, and sterol regulatory element-binding protein-1c in the fructose-fed hamster intestine. Diabetes 2006;55:1316–1326.
8. Haidari M, Leung N, Mahbub F, et al. Fasting and postprandial overproduction of intestinally derived lipoproteins in an animal model of insulin resistance. Evidence that chronic fructose feeding in the hamster is accompanied by enhanced intestinal de novo lipogenesis and ApoB48-containing lipoprotein overproduction. J Biol Chem 2002;277:31646–31655.
9. Zoltowska M, Ziv E, Delvin E, et al. Cellular aspects of intestinal lipoprotein assembly in *Psammomys obesus*: a model of insulin resistance and type 2 diabetes. Diabetes 2003;52:2539–2545.
10. Estall JL, Drucker DJ. Glucagon-like peptide-2. Annu Rev Nutr 2006;26:391–411.
11. Yusta B, Huang L, Munroe D, et al. Enteroendocrine localization of GLP-2 receptor expression in humans and rodents. Gastroenterology 2000;119:744–755.
12. Guan X, Karpen HE, Stephens J, et al. GLP-2 receptor localizes to enteric neurons and endocrine cells expressing vasoactive pep-

tides and mediates increased blood flow. Gastroenterology 2006;130:150–164.

13. Orskov C, Hartmann B, Poulsen SS, et al. GLP-2 stimulates colonic growth via KGF, released by subepithelial myofibroblasts with GLP-2 receptors. Regul Pept 2005;124:105–112.

14. Tang-Christensen M, Larsen PJ, Thulesen J, et al. The proglucagon-derived peptide, glucagon-like peptide-2, is a neurotransmitter involved in the regulation of food intake. Nat Med 2000;6:802–807.

15. Koehler JA, Yusta B, Drucker DJ. The HeLa cell glucagon-like peptide-2 receptor is coupled to regulation of apoptosis and ERK1/2 activation through divergent signaling pathways. Mol Endocrinol 2005;19:459–473.

16. Dube PE, Forse CL, Bahrami J, et al. The essential role of insulin-like growth factor-1 in the intestinal tropic effects of glucagon-like peptide-2 in mice. Gastroenterology 2006;131:589–605.

17. Cheeseman CI. Up-regulation of SGLT-1 transport activity in rat jejunum induced by GLP-2 infusion in vivo. Am J Physiol 1997;273:R1965–R1971.

18. Au A, Gupta A, Schembri P, et al. Rapid insertion of GLUT2 into the rat jejunal brush-border membrane promoted by glucagon-like peptide 2. Biochem J 2002;367:247–254.

19. Rocca AS, LaGreca J, Kalitsky J, et al. Monounsaturated fatty acid diets improve glycemic tolerance through increased secretion of glucagon-like peptide-1. Endocrinology 2001;142:1148–1155.

20. Meier JJ, Nauck MA, Pott A, et al. Glucagon-like peptide 2 stimulates glucagon secretion, enhances lipid absorption, and inhibits gastric acid secretion in humans. Gastroenterology 2006;130:44–54.

21. Qin B, Qiu W, Avramoglu RK, et al. Tumor necrosis factor-α induces intestinal insulin resistance and stimulates the overproduction of intestinal apolipoprotein B48-containing lipoproteins. Diabetes 2007;56:450–461.

22. Nassir F, Wilson B, Han X, et al. CD36 is important for fatty acid and cholesterol uptake by the proximal but not distal intestine. J Biol Chem 2007;282:19493–19501.

23. Brubaker PL, Izzo A, Hill M, et al. Intestinal function in mice with small bowel growth induced by glucagon-like peptide-2. Am J Physiol 1997;272:E1050–E1058.

24. Pedersen NB, Hjollund KR, Johnsen AH, et al. Porcine glucagon-like peptide-2: structure, signaling, metabolism and effects. Regul Pept 2008;146:310–320.

25. Stahl A, Hirsch DJ, Gimeno RE, et al. Identification of the major intestinal fatty acid transport protein. Mol Cell 1999;4:299–308.

26. Milger K, Herrmann T, Becker C, et al. Cellular uptake of fatty acids driven by the ER-localized acyl-CoA synthetase FATP4. J Cell Sci 2006;119:4678–4688.

27. Drover VA, Ajmal M, Nassir F, et al. CD36 deficiency impairs intestinal lipid secretion and clearance of chylomicrons from the blood. J Clin Invest 2005;115:1290–1297.

28. Nauli AM, Nassir F, Zheng S, et al. CD36 is important for chylomicron formation and secretion and may mediate cholesterol uptake in the proximal intestine. Gastroenterology 2006;131:1197–1207.

29. Eyre NS, Cleland LG, Tandon NN, et al. Importance of the carboxyl terminus of FAT/CD36 for plasma membrane localization and function in long-chain fatty acid uptake. J Lipid Res 2007;48:528–542.

30. Luiken JJ, Koonen DP, Willems J, et al. Insulin stimulates long-chain fatty acid utilization by rat cardiac myocytes through cellular redistribution of FAT/CD36. Diabetes 2002;51:3113–3119.

31. Febbraio M, Abumrad NA, Hajjar DP, et al. A null mutation in murine CD36 reveals an important role in fatty acid and lipoprotein metabolism. J Biol Chem 1999;274:19055–19062.

32. Goudriaan JR, den Boer MA, Rensen PC, et al. CD36 deficiency in mice impairs lipoprotein lipase-mediated triglyceride clearance. J Lipid Res 2005;46:2175–2181.

33. Densupsoontorn N, Carpentier YA, Racine R, et al. CD36 and proteoglycan-mediated pathways for (n-3) fatty acid enriched triglyceride-rich particle blood clearance in mouse models in vivo and in peritoneal macrophages in vitro. J Nutr 2008;138:257–261.

34. Pohl J, Ring A, Korkmaz U, et al. FAT/CD36-mediated long-chain fatty acid uptake in adipocytes requires plasma membrane rafts. Mol Biol Cell 2005;16:24–31.

35. Levy E, Stan S, Delvin E, et al. Localization of microsomal triglyceride transfer protein in the Golgi: possible role in the assembly of chylomicrons. J Biol Chem 2002;277:16470–16477.

36. Amato A, Baldassano S, Serio R, et al. Glucagon-like peptide-2 relaxes mouse stomach through vasoactive intestinal peptide release. Am J Physiol Gastrointest Liver Physiol 2009;296:G678–G684.

37. Bozkurt A, Naslund E, Holst JJ, et al. GLP-1 and GLP-2 act in concert to inhibit fasted, but not fed, small bowel motility in the rat. Regul Pept 2002;107:129–135.

38. Fischer KD, Dhanvantari S, Drucker DJ, et al. Intestinal growth is associated with elevated levels of glucagon-like peptide 2 in diabetic rats. Am J Physiol 1997;273:E815–E820.

39. Gleeson A, Anderton K, Owens D, et al. The role of microsomal triglyceride transfer protein and dietary cholesterol in chylomicron production in diabetes. Diabetologia 1999;42:944–948.

BASIC–
ALIMENTARY TRACT

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 microsome luminal contents.[1] 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 hGly[2]-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)[(1–33)]. The fragments were then radioactively labeled with 225 μCi of [35S]methionine for 30 minutes. After washing with PBS, the fragments were chased in 40 mmol/L methionine-enriched DMEM for 30, 60, and 90 minutes, following which, tissues were homogenized with a Polytron homogenizer, and media were collected. Immunoprecipitation and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed as previously described.[2] ABCG5 was immunoprecipitated with a rabbit anti-ABCG5 antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Following SDS-PAGE, the gel was dried and exposed to a phosphor imaging screen (Molecular Diagnostics). The screen was then read with the Storm 840 Phosphorimager (Amersham, Piscataway, NJ).

Results

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 $\times$ 10^5 $\pm$ 2.06 $\times$ 10^5 dpm/mg protein vs 3.83 $\times$ 10^5 $\pm$ 0.85 $\times$ 10^5 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% $\pm$ 0.048% vs 0.136% $\pm$ 0.005%, GLP-2 vs control, $P < .05$; at 90 minutes, 0.640% $\pm$ 0.027% vs 0.352% $\pm$ 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% $\pm$ 0.05% vs 0.643% $\pm$ 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

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

1. Nassir F, Wilson B, Han X, et al. CD36 is important for fatty acid and cholesterol uptake by the proximal but not distal intestine. J Biol Chem 2007;282:19493–19501.
2. Haidari M, Leung N, Mahbub F, et al. Fasting and postprandial overproduction of intestinally derived lipoproteins in an animal model of insulin resistance. Evidence that chronic fructose feeding in the hamster is accompanied by enhanced intestinal de novo lipogenesis and ApoB48-containing lipoprotein overproduction. J Biol Chem 2002;277:31646–31655.
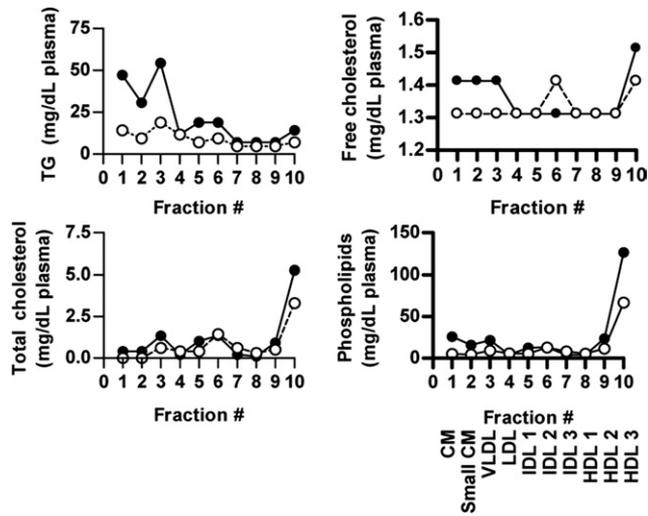
Supplementary Figure 1. Plasma lipoprotein profiling by salt-density sedimentation in hamsters. Hamsters were given an oral gavage of olive oil and followed by injection with GLP-2 or PBS 20 minutes later, and no Triton WR-1339 was used in this experiment. Profiling was performed on plasma collected 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.
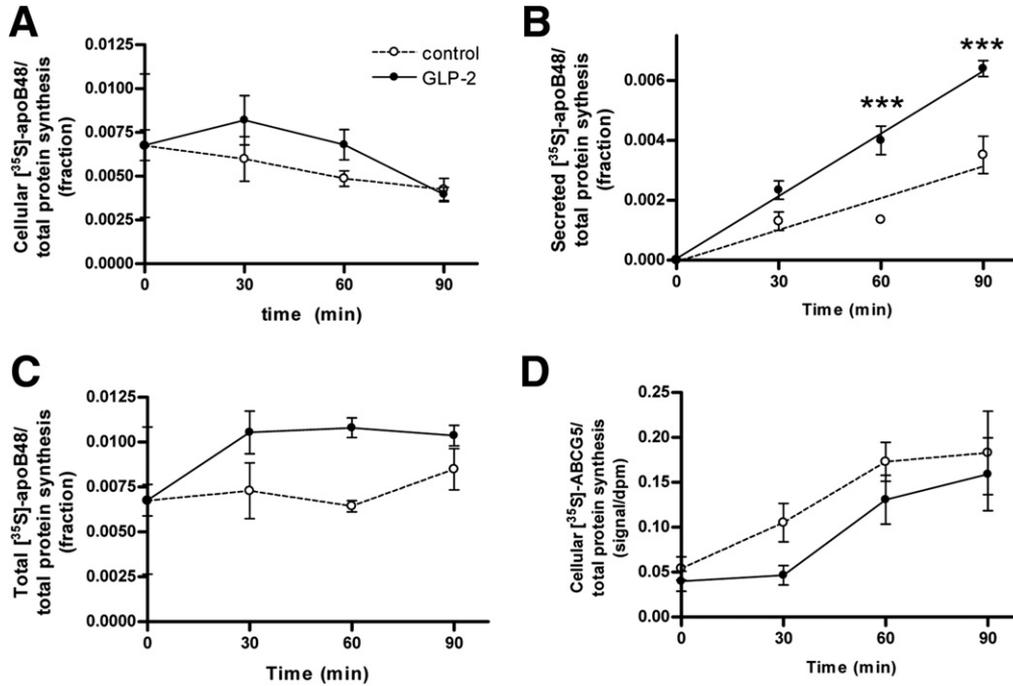
Supplementary Figure 2. Ex vivo effects of GLP-2 on apoB48-containing lipoprotein secretion. [^{35}S]-Labeled apoB48 was measured during the cold methionine-enriched chase after a 30-minute pulse in jejunal fragments freshly isolated from chow-fed hamsters and treated with or without GLP-2 ex vivo and normalized to total protein synthesis. (*A*) Intracellular levels of ^{35}S-apoB48. (*B*) Secreted ^{35}S-apoB48. (*C*) Total levels of ^{35}S-apoB48. N = 3 per group; *$P <$ .05, ***$P <$ .001. (*D*) Cellular ^{35}S-ABCG5. N = 4 per group.
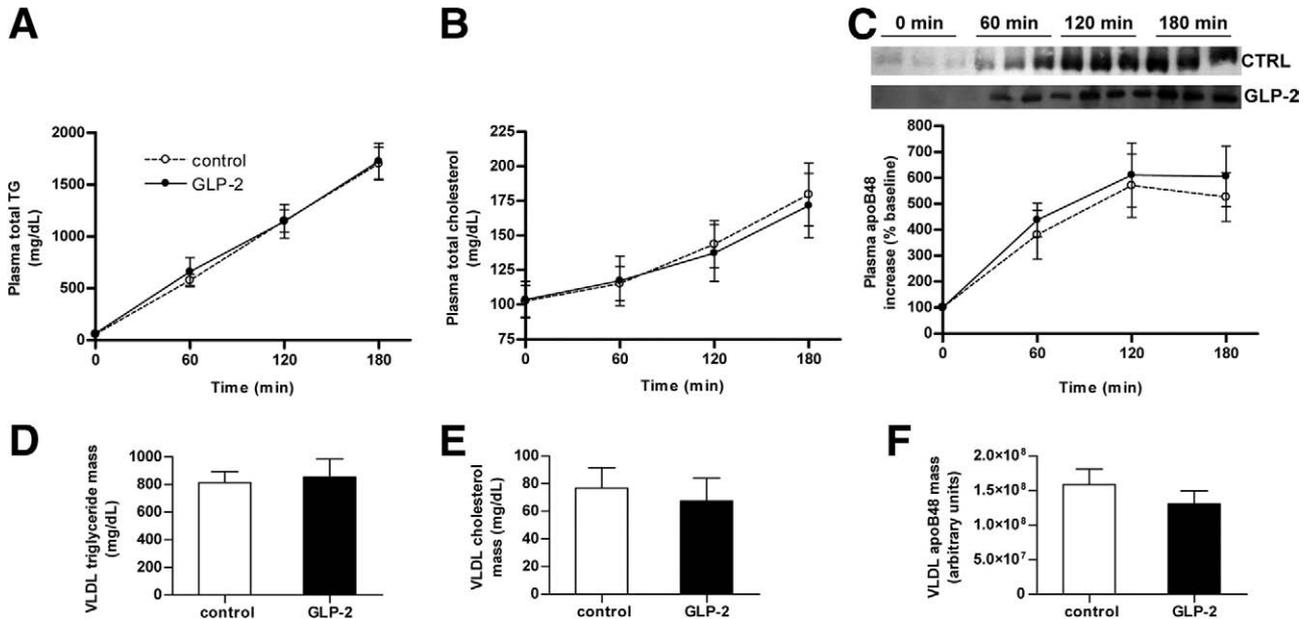
Supplementary Figure 3. In vivo hepatic lipoprotein secretion in fasting C57BL/6 mice. Fasted C57BL/6 mice were administered an intravenous bolus of Triton WR-1339 and a single intraperitonal injection of Gly^2-GLP-2 or PBS control. No fat load was given. Blood was sampled, and the 180-minute sample 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.