REVIEW

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Regulation of intestinal lipid and lipoprotein metabolism by the proglucagon-derived peptides glucagon like peptide 1 and glucagon like peptide 2

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Purpose of review
The intestine is highly efficient at absorbing and packaging dietary lipids onto the structural protein apoB48 for distribution throughout the body. Here, we summarize recent advances into understanding the physiological and pharmacological actions of the proglucagon-derived peptides: glucagon like peptide 1 (GLP-1) and glucagon like peptide 2 (GLP-2) on intestinal lipoprotein secretion.

Recent findings
Several recent studies have elucidated mechanisms underlying the paradoxical effects of GLP-1 and GLP-2 on intestinal production of triglyceride-rich lipoproteins (TRLs). Both gut-derived peptides are secreted on an equimolar basis in response to the same nutrient stimulus. Despite neither receptor demonstrating clear localization to enterocytes, a single injection of a GLP-1R agonist rapidly decreases delivery of intestinally packaged fatty acids into the plasma, while conversely GLP-2 receptor (GLP-2R) activation acutely increases TRL concentrations in plasma.

Summary
The regulation of TRL secretion is dependent on the coordination of many processes: fatty acid availability uptake, assembly onto the apoB48 polypeptide backbone, secretion and reuptake, which the hormonal, neural, inflammatory and metabolic milieu can all strongly influence. Understanding of how GLP-1 and GLP-2 receptor agonists control TRL production has clinical importance given that GLP1R agonists were recently demonstrated not only to provide glycemic control but also to prevent major adverse cardiovascular events in patients with T2DM and the success of GLP-2R agonists in treating short bowel disease.

Keywords
glucagon like peptide 1, glucagon like peptide 2, intestine, lipoprotein, postprandial

INTRODUCTION

Patients with type 2 diabetes mellitus (T2DM) are at an increased risk of death from cardiovascular disease (CVD) [1,2]. The particulars of the disturbed metabolic milieu responsible for the increase in CVD risk are complex. Intensive glucose lowering has been definitively linked to improved microvascular health; however, a clear benefit on macrovascular vessel health has been elusive [3–5]. The atherogenic, dyslipidemia in T2DM is characterized by an overproduction and/or delayed catabolism of triglyceride-rich particles (TRLs), including apolipoprotein (apoB)48-containing chylomicrons and apoB100-containing VLDLs, cholesterol-rich remnant particles, small dense LDLs and a reduction in circulating HDLs [6]. LDL-cholesterol lowering with statins is a mainstay in the management of dyslipidemia in patients with T2DM [7]. However, even when cholesterol targets are met, the risk of CVD is still significant. Prospective clinical trials have demonstrated that hypertriglyceridemia is an

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Curr Opin Lipidol 2018, 29:95–103

DOI:10.1097/MOL.0000000000000495

KEY POINTS

- Increased concentrations of intestinally derived apolipoproteinB48 and elevations in postprandial triglycerides are risk factors for the development of cardiovascular disease (CVD).
- Treatment of patients with type 2 diabetes mellitus at a high risk of CVD with GLP-1R agonists reduces major adverse cardiovascular events.
- Treatment of patients with short bowel disease with the GLP-2R agonist, teduglutide, reduces dependence on parental nutrition.
- Given the paradoxical effects of the cosecreted gut hormones GLP-1 and GLP-2 on intestinal lipoprotein production deciphering the dominant receptor mediated mechanisms, which regulate triglyceride-rich lipoprotein secretion in response to GLP-1R and GLP-2R activation require further elucidation.

additional independent risk factor for the development of CVD [8,9] and the extent of coronary atherosclerosis in patients with T2DM is positively correlated with plasma concentrations of TRL [10]. Nonfasting triglyceride concentrations are correlated with cholesterol-rich remnants and when elevated are associated with an increased risk of myocardial infarction (MI) and death [9]. Results from the Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE IT) have demonstrated that the addition of ezetimibe to statin treatment offered further benefit in the reduction of major adverse cardiovascular events (MACEs) [11]. Also, recent studies have demonstrated that apoB48 levels better predict increased adiposity than traditional lipid risk factors [12▪], suggesting it may also be a harbinger of a disturbed metabolic milieu. Therefore, therapies that target both the atherogenic dyslipidemia and hyperglycemia observed in patients with T2DM are of significant interest for potential CVD risk reduction.

Glucagon like peptide -1 (GLP-1) is a gut-derived peptide hormone, which is an exquisite regulator of glucose-dependent insulin secretion. GLP-1, together with the incretin hormone glucose-dependent insulinotropic polypeptide (GIP), is responsible for up to 70% of nutrient-stimulated insulin secretion and therefore indirectly facilitates postprandial glucose clearance [13]. Activation of the β-cell GLP-1 receptor (GLP-1R) has well described effects, including potentiation of glucose stimulated insulin secretion, increased synthesis of insulin, prevention of β-cell apoptosis and stimulation of somatostatin contributing to a reduction in glucagon secretion [14–16]. However, levels of GLP-1 in circulation are very low, owing to degradation by the serine protease dipeptidyl peptidase 4 (DPP4) [17]. Sustained activation of the GLP-1 receptor (GLP-1R) can be achieved through long-acting agonists resistant to cleavage by DPP4 or through compounds which inhibit DPP4 activity [18]. In addition to the sodium glucose cotransporter 2 (SGLT2) inhibitor empagliflozin, two GLP-1R agonists, liraglutide and semaglutide, have demonstrated a decrease in MACE in patients with T2DM [19,20▪▪,21▪▪]. Therefore, in addition to its significant effects on the endocrine pancreas, the less prominent but potentially equally important postprandial lipid-lowering aspects of GLP-1 physiology may improve the postprandial dyslipidemia associated with T2DM and reduce the risk for CVD [22]. However, understanding the physiological role of GLP-1 on intestinal lipoprotein secretion and how it may be disturbed in T2DM is complicated due to the cosecretion on an equimolar basis with glucagon like peptide 2 (GLP-2). In contrast to the reduction in postprandial TRL observed with induction of GLP-1 signalling, GLP-2 receptor (GLP-2R) activation has been demonstrated to promote lipid absorption [23]. In this review, we focus on new developments in both the physiological and pharmacological regulation of TRL metabolism in response to treatment with GLP-1 and GLP-2.

REGULATION OF INTESTINAL-DERIVED TRIGLYCERIDE-RICH LIPOPROTEIN SECRETION

Unlike the liver, which can control its intake of lipid by regulating receptor-mediated endocytosis, the intestine must act in a dynamic fashion at the extremes of nutrient exposure [24,25]. Luminal fat content is a key signal for TRL production but represents only one of the many hormone and nutrition signals, which are integrated to control the process [26]. Within the intestinal lumen, fats are hydrolyzed and associate with bile acids to be taken up by both passive and active transport [27]. Upon entry, the fatty acids (FAs) can be re-esterified and incorporated from the endoplasmic reticulum (ER) into cytosolic lipids droplets or loaded onto the apoB48 polypeptide, the obligate scaffold for the assembly of dietary lipids through the actions of microsomal triglyceride transfer protein (MTP). These triglyceride -rich particles undergo maturation and association with other and secreted from the enterocyte into lymphatic lacteal. Intestinal lymphatics within the villi are the main route of entry for dietary lipid into the bloodstream. Chylomicrons are too large to cross endothelial barriers and have been described to enter the lacteal through both paracellular transport in endothelial vesicles

and transcytosis through open junctions [28]. The transport process is initiated minutes after a meal and continues for hours. The lymphatics drain from all sections of the small bowel to mesenteric and celiac nodes and onto intestinal lymph trunks and the venous circulation through the thoracic duct [29]. This routing bypasses the liver, making absorbed nutrients immediately available to all tissues.

SECRETION OF PREPROGLUCAGON PEPTIDES

Preproglucagon (*Gcg*)+ enteroendocrine cells compose approximately 1% of the intestinal epithelium [30] and are dispersed throughout the duodenum but more enriched in the ileum in rats and pigs and distal colon in mice [31]. Single cell sequencing has determined that Gcg+ cells can be classified into three overlapping but distinct hormone-secreting populations [32▪▪]. Activation of a number of nutrient receptors residing on the luminal side of the L-cell stimulate release of stored peptide hormones in secretory vesicles at the basolateral membrane in close proximity to capillaries and nerve endings [33,34]. In healthy participants, fasting plasma concentrations of GLP-1 are within the 5–10 pmol/l range, which elevates to approximately 50 pmol/l after ingestion of a mixed meal in blood [35,36]. Interestingly, recent work has suggested that FA sensing by L-cells may also occur basolaterally in a process involving postlipoprotein lipase (LPL)-mediated hydrolysis of formed chylomicrons [37▪▪]. These data support previous studies whereby disruption of chylomicron synthesis by treatment with a surfactant reduced GLP-1 secretion [38].

PRECLINICAL STUDIES EVALUATING PHYSIOLOGICAL AND PHARMACOLOGICAL CONCENTRATIONS OF GLUCAGON LIKE PEPTIDE-1 ON INTESTINAL LIPOPROTEIN METABOLISM

Despite being pharmacologically exploited for its potent effects on the islet, GLP-1 is best described as an intestinal signal peptide that works as an elegant messenger during the postprandial period to fine-tune endocrine responses to nutrients. GLP-1 has been characterized to inhibit gastric acid secretion [39], reduce gastric emptying [40,41], slow gastric motility [42], reduce lymph flow [43], relax the gastric fundus [44], inhibit antro-pyloroduodenal motility [45] and act as an 'ileal break' [46]. Both elevations in endogenous GLP-1 and pharmacological concentrations reached with GLP-1R agonists have demonstrated significant reductions in postprandial lipoprotein secretion [47,48]. Alternatively, *Glp1r*$^{-/-}$ mice demonstrate an increase in triglyceride-rich, apoB48-containing lipoproteins postfat load when compared with littermate controls. Similarly, administration of the GLP-1R antagonist exendin 9–39 (50 nmol/kg) to hamsters increased apoB48 mass in the plasma TRL fraction thus supporting a role for endogenous or physiological GLP-1 signaling in regulating triglyceride secretion [47].

GLP-1R expression has been reported in mouse and human bowel [49–51] and treatment of intestinal segments with exendin-4 reduce apoB48 secretion to the media [47]. Flow cytometry analysis of the absorptive layer of the intestine has demonstrated that the GLP-1R is not expressed in Villin+ enterocytes but instead is localized to intraepithelial lymphocytes [52]. Consistent with its effects throughout the alimentary canal, the GLP-1R has also been localized in mice using in-situ hybridization to gastric parietal cells in the glandular stomach, Brunners glands and the nerve plexus throughout the small and large bowel [53▪▪].

In the absence of a direct signalling mechanism in enterocytes to regulate postprandial lipid handling, a number of secondary pathways have been investigated. Acute treatment of mice with exendin-4 (24 nmol/kg) decreases the rate of appearance of triglyceride and apoB48 into plasma [47], suggesting while the chronic GLP-1R-mediated anorectic effects or weight loss may contribute clinically to improved lipid profiles, they are not the exclusive driver of reduced lipoprotein secretion (Fig. 1a). Also, the lowering of triglyceride and apoB48 production in response to exendin-4 persisted even when oil was given 1 h prior to treatment to allow FA to exit the stomach and enter the small bowel, therefore eliminating inhibition of antral contractility and reduced gastric emptying as the main mechanism for decreasing TRL-production (Fig. 1a). As GLP-1R agonists increase meal-stimulated insulin secretion, a significant regulator of postprandial lipoprotein secretion [54], analysis of plasma insulin levels and the relative effects of these concentrations on intestinal lipoprotein secretion determined that the reduction in the number of TRL cannot solely be explained by increased insulin secretion [47] (Fig. 1a).

The intestine has been proposed to play a role in control of lymph flow [28]. Initial lymphatics lack the ability to generate tone due to an absence of smooth muscle cells and therefore are dependent on the peristaltic motion of the intestinal wall to propel flow. During the transition from a fasted to fed state, lymph flow in the intestine is normally increased, particularly in response to ingested fat [55]. The concentrations of GLP-1 in lymph have been reported to significantly exceed those in plasma

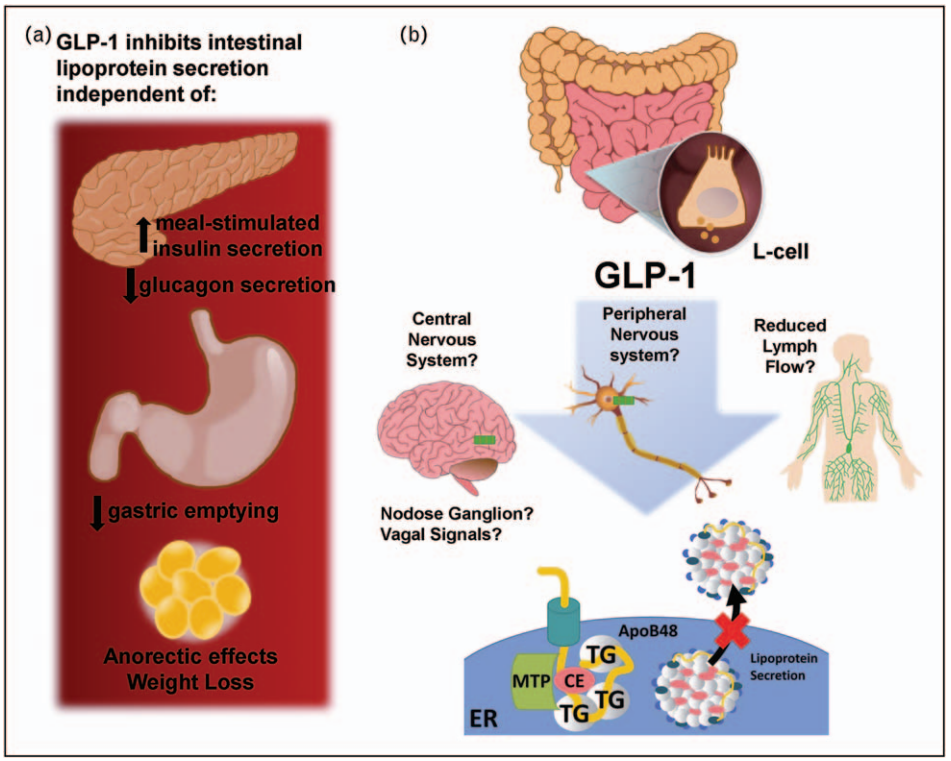

FIGURE 1. (a) GLP-1R mediated decrease in the production rate of intestinally derived, triglyceride-rich lipoproteins is independent of increased insulin secretion and reduced glucagon secretion, reduced gastric emptying and weight loss or anorectic effects. (b) Potential mechanisms in need of validation for GLP-1R mediated reductions in TRL-TG and TRL- apoB48 synthesis and secretion including signalling through the peripheral gut:brain axis, lymph flow and the central nervous system.

[56] and injection of recombinant GLP-1 (20 pmol/kg/min) into lipid-infused rats demonstrated a 50% decrease in the rate of lymph flow [43]. Studies in cannulated rats that received an intraduodenal infusion with ^{3}H-triolein demonstrated a significant decrease in the recovery of ^{3}H-triolein in the lymph suggesting a decrease in entry of lipid into the lymphatics with GLP-1 treatment (20 pmol/kg/min) [43]. An increase in label was found within the mucosa layer and not the lumen suggesting uptake and esterification of FA into triglyceride within enterocytes was not disturbed [43]. In accordance with this, evaluation of feces from *Glp1r*$^{-/-}$ mice or mice treated with the GLP-1R agonist taspoglutide demonstrated no significant increase in lipid content [57,58]. Exendin-4 treatment has been reported to result in a modest (~20%) reduction in MTP activity but did not affect MTP protein levels. These data were associated with reduced Oil red O staining in Villi of the jejunum 2 h after oil gavage [59]. Therefore, it is clear GLP-1 has significant effects on lipid handling within the intestine but interpreting the pathways involved requires further study. GLP-1R activation also has a described role in both the small and large intestine to mediate mucosal expansion [49]; however, what role secondary mediators such as fibroblast growth factor 7, (*Fgf7*) have in lipid handling and lipoprotein secretion remain unclear (Fig. 1b).

Studies in rats have demonstrated that intestinal transit is sensitive to treatment with native GLP-1; however, concentrations of GLP-1 greatly exceeding physiological concentrations do not affect the contractility of gastrointestinal muscle strips suggesting an indirect and potential neuronal effect to reduce intestinal transit time [60]. GLP-1R are distributed throughout the brain and peripherally in the nodose ganglion [61,62]. Injection of exendin-4 into the ventricles of the brain reduces intestinal lipoprotein secretion in hamsters. However, intracerebroventricular (i.c.v.) injections of exendin 9–39 did not prevent the inhibition of TRL production by exendin-4 suggesting a potential combination of peripheral and neuronal pathways [59]. The plasticity observed in lipoprotein regulation is consistent with recent studies evaluating the GLP-1R+ circuits within the brain that control glucose and body weight. Deletion of the *Glp1r* in the hypothalamus, proopiomelanocortin neurons,or paraventricular nucleus did not individually disrupt GLP-1R feeding and glucose responses, although clearly each population can contribute [63▪▪].

CLINICAL STUDIES EVALUATING GLUCAGON LIKE PEPTIDE-1 RECEPTOR AGONISTS AND INTESTINAL LIPOPROTEIN METABOLISM

Collectively, placebo-controlled clinical trials with GLP-1R agonists as a monotherapy have illustrated a consistent 0.2–0.3 mmol reduction in triglyceride concentrations [22]. Importantly, many of the mechanistic insights provided from preclinical studies have been reproduced in clinical settings. Kinetic studies in healthy men in which a primed constant infusion of deuterated leucine was administered under pancreatic clamp conditions in the fed state have demonstrated that insulin acutely suppresses apoB48 production rates in the postprandial state (47–62%) [54]. In-vivo support for GLP-1R activation to reduce TRL production independent of increased insulin secretion and reduced gastric emptying came from similar kinetic study protocols undertaken in which healthy participants were administrated a fixed high fat, mixed macronutrient liquid meal through a nasoduodenal tube. Under these experimental conditions, acute treatment with the GLP-1R agonist, exenatide, was still able to significantly reduce postprandial apoB48 production [64].

Patients with impaired glucose tolerance or recent onset T2DM injected with exenatide [10 μg, subcutaneous (sc)] demonstrated a marked decrease in serum concentrations of triglyceride, remnant-triglyceride and apoC3 [65]. A null mutation (R19X) discovered in *APOC3*, in which carriers express half the amount of protein, is associated with lower fasting and postprandial triglyceride (1–6 h) postfat load [66] and apoC3-enriched TRLS have a slower rate of triglyceride hydrolysis particularly when LPL was bound to GPIHBP1 [67▪▪]. Therefore, chronic changes in apoC3 do have the potential to influence lipoprotein secretion. However, kinetic studies have not to date demonstrated a reduction in fractional catabolic rate of TRL-particles and analysis of patients treated for 1 year with exenatide (10 μg twice daily) versus insulin glargine have demonstrated significant reductions in triglyceride, free fatty acids (FFA), HDL-cholesterol, VLDL-cholesterol, and apoB48; however, no differences were noted in apoB100, apoA1, apoA2 or importantly apoC3 [67▪▪].

PRECLINICAL STUDIES EVALUATING THE PHYSIOLOGICAL AND PHARMACOLOGICAL EFFECTS OF GLUCAGON LIKE PEPTIDE-2 ON INTESTINAL LIPOPROTEIN SECRETION

Initial studies characterizing the effects of treatment of female CD-1 mice with synthetic, rat GLP-2 (2.5 μg sc, twice daily for 10 days) demonstrated an increase in intestinal lipid absorption as GLP-2 enhanced plasma triolein levels [68]. Consistent with this acute, intravenous treatment of chow-fed Syrian golden hamsters (GLP-2, 20 pmol/kg body weight/min) after an oral fat load increased plasma levels of radiolabelled triolein and apoB48 [69]. Studies in *Glp2r*$^{-/-}$ mice and experiments utilizing GLP-2 (3–33), an antagonist of the GLP-2R [70], which can also exert weak agonist activity [71], have demonstrated that endogenous GLP-2R activation regulates mucosal expansion in response to refeeding; however, a direct effect on lipoprotein action has not been elucidated. Interestingly, high fat diet-fed CD-1 mice treated with GLP-2(3–33) had no change in lipid content in faeces, suggesting that inhibition of endogenous GLP-2 action does not disturb lipid absorption [72▪].

Like GLP-1, GLP-2 signals through a G-protein coupled receptor, which has been localized within the gastrointestinal tract and found to be enriched within the jejunum [73,74▪]. GLP-2Rs have also been associated in intestinal compartments rich in enteric neurons [75]. Consistent with these studies, recent in-situ hybridization analysis in mice has determined that GLP-2R expression is negligible in absorptive epithelial cells and abundant in the nerve plexus throughout the gut [53▪▪]. In addition, GLP-2Rs were localized to the muscle layers of the nonglandular stomach, lamina propria of the mucosal layer, in the circular and longitudinal muscles of the duodenum [53▪▪].

Fast performance liquid chromatography analysis of plasma lipoproteins isolated after a fat load demonstrated an enrichment in the chylomicron remnant/VLDL range in hamsters administered GLP-2 (1–33) (0.25 mg/kg) and also in wildtype mice treated with the GLP-2 analogue (hGly^{2}-GLP-2, 0.25 mg/kg) compared with controls [76]. Mechanistically, the increase in TRL was demonstrated to be due to a three-fold increase in intestinal lipoprotein production. In support of increased production, radiolabelling experiments in which primary hamster jejunal segments were treated with GLP-2 demonstrated an increase in newly synthesized apoB48 into the media and these data were not due to an overall increase in protein synthesis or increased enterocyte survival [76]. Treatment of hamsters with GLP-2 accelerated the rate of incorporation of gavaged radiolabelled triolein into plasma TG. Consistent with increased FA uptake, acute GLP-2 treatment increased levels of glycosylated CD36 in hamster-derived enterocytes and increased localization to the subapical domain of enterocyte villi. Further experiments in mice lacking CD36 (*Cd36*$^{-/-}$), demonstrated that treatment with hGly^{2}-GLP-2 (0.25 mg/kg) failed to increase luminal

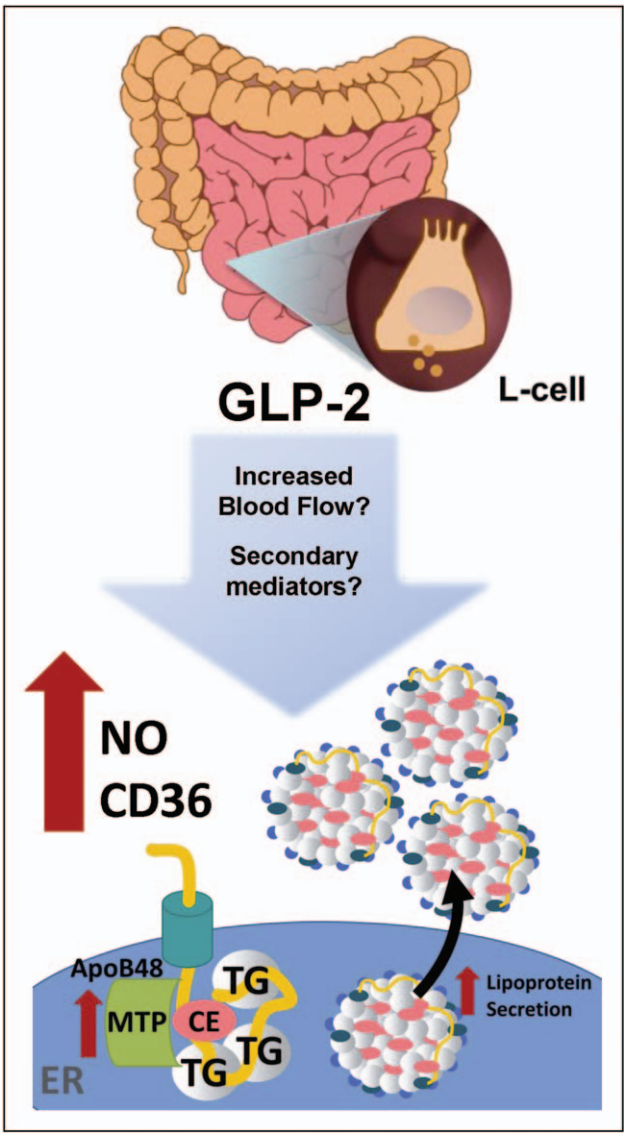

FIGURE 2. Emerging evidence suggests that GLP-2R activation increases the secretion of preformed chylomicron particles. Potential mechanisms in need of validation for GLP-2R mediated increases in TRL-TG and TRL-apoB48 synthesis and secretion including increased blood flow and secondary mediators.

triolein uptake and increase plasma TRL-TG and apoB48 [76]. It is clear that CD36 facilitates the increase in FA uptake in response to GLP-2R signalling; however, what signalling pathway GLP-2 exploits to increase glycosylation and activity of the CD36 transmembrane receptor remains undetermined (Fig. 2).

In 12-day-old piglets fed by parental nutrition, human GLP-2 (500 pmol/kg/h) increased portal blood flow, which was inhibited by NG-Nitro-L-arginine methyl ester (L-NAME, 50 μmol/kg/h) suggesting a nitric oxide dependent mechanism [77]. Nitric oxide is the product of nitric oxide synthase (NOS), which is freely permeable and can easily diffuse through biological membranes [78]. Therefore, as nitric oxide is a known mediator of GLP-2 action on increasing mesenteric blood flow, its role in lipoprotein secretion was further investigated. Consistent with a role in the regulation of lipoprotein secretion by GLP-2, genetic elimination of eNOS resulted in fewer TRLs and triglyceride accumulation in the jejunum, which was unable to be rescued by intraperitoneal (i.p.) injection of Gly^{2}-GLP-2 [79]. In addition, a significant increase in MTP activity was described in hamsters 90 min after GLP-2 (0.25 mg/kg) treatment and this effect was completely abrogated by treatment with L-NAME [79] (Fig. 2).

Not surprisingly similar to GLP-1, significant concentrations of GLP-2 appear within intestinal lymph in response to oral administration of a range of FAs [80]; however, the significance of elevating endogenous concentrations remains to be determined. Also, many secondary mediators have been identified as targets of GLP-2 action, including the growth factors: keratinocyte growth factor, insulin-like growth factors, epidermal growth factor and related ligands in the ErbB family [23]; however, it remains to be determined what role if any these secondary mediators have in GLP-2 mediated effects on lipoprotein secretion.

CLINICAL STUDIES EVALUATING GLUCAGON LIKE PEPTIDE-2 ACTION ON INTESTINAL LIPOPROTEIN SECRETION

GLP-2 (2 pmol/kg/min) treatment of healthy male participants fed a low-calorie content meal significantly increased postprandial plasma triglyceride and FFA concentrations [81]. Administration of a single, pharmacological dose of native GLP-2 (1500 μg subcutaneously) during a high-fat, macronutrient-fixed formula administered in a nasoduodenal tube resulted in a rapid increase in TRL-apoB48 and TRL-TG with peak concentrations of plasma triglyceride and apoB48 being reached within 1 h after administration. Further studies using retinyl palmitate to label lipid incorporation in triglyceride-rich lipoproteins 7 h prior to treatment with GLP-2 clearly demonstrated an acute increase in retinyl palmitate labelled TRL triglyceride and apoB-48 concentrations suggesting GLP-2 mediates the release of preformed chylomicron particles [82]. Studies in healthy, obese males regarding the endogenous contribution of GLP-1 and GLP-2 demonstrate that significant variation occurs in the postprandial TRL fraction and variation in GLP-1 and GLP-2 concentrations did not account for this

disparity [83]. However, similar to the kinetic studies discussed above, GLP-2 levels significantly correlated with the rapid elevation in area under the curve for both TRL-TG concentrations and apoB48 [83].

CONCLUSION

GLP-1 and GLP-2 are secreted in equimolar concentrations in response to nutrients; clearly, they both have significant effects on lipoprotein secretion, however, deciphering the precise mechanisms has been difficult. Both have their biological activity regulated through cleavage by the serine protease DPP4, GLP-1 has a half-life of 1–2 min [84], while GLP-2 cleavage is much slower at 7 min [85]. Many factors challenge our interpretation of how GLP-1 and GLP-2 regulate TRL secretion, including controlling for fluctuations in pancreatic hormones, gastric emptying, intestinal motility, lymphatic and splanchnic blood flow, exchangeable apolipoproteins and the differences in metabolic milieu and inflammation, which arise from studying healthy participants versus diabetic patients. In hamsters, a physiological infusion of GLP-1 and GLP-2 results in an initial increase in lipid absorption and increased plasma concentrations of TRL-apoB48, which after prolonged coinfusion leads to decreased levels of TRL-apoB48 in plasma. Interestingly, under conditions of insulin resistance, hamsters displayed an increased postprandial lipid response consistent with the dominance of a GLP-2 response [69]. As GLP-1R agonists become more widely prescribed to prevent cardiovascular death in patients with T2DM and GLP-2R agonists are tested in a variety of intestinal disorders understanding both the physiological actions and pharmacological properties takes on a new importance.

Acknowledgements

I would like to thank Natasha Trzaskalski for her help with the figures.

Financial support and sponsorship

Work in the author's laboratory is funded through an NSERC Discovery Grant RGPIN-2017-05996.

Conflicts of interest

Erin Mulvihill has received honoraria from Merck in the past 12 months.

This study has not been published in its current form or a substantially similar form and is not under consideration by another publication.

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