

## HOT TOPIC

# Glucagon-Like Peptide 2\*

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### ABSTRACT

Glucagon-like peptide 2 (GLP-2) is a 33 amino acid peptide-encoded carboxyterminal to the sequence of GLP-1 in the proglucagon gene. Both GLP-1 and GLP-2 are secreted from gut endocrine cells and promote nutrient absorption through distinct mechanisms of action. GLP-2 regulates gastric motility, gastric acid secretion, intestinal hexose transport, and increases the barrier function of the gut epithelium. GLP-2 significantly enhances the surface area of the mucosal epithelium via stimulation of crypt cell proliferation and inhibition of apoptosis in the enterocyte and crypt compartments. The cytoprotective and reparative effects of GLP-2 are evident in rodent models of experimental intestinal injury. GLP-2 reduces mortality and de-

creases mucosal injury, cytokine expression, and bacterial septicemia in the setting of small and large bowel inflammation. GLP-2 also enhances nutrient absorption and gut adaptation in rodents or humans with short bowel syndrome. The actions of GLP-2 are transduced by the GLP-2 receptor, a G protein-coupled receptor expressed in gut endocrine cells of the stomach, small bowel, and colon. Activation of GLP-2 receptor signaling in heterologous cells promotes resistance to apoptotic injury *in vitro*. The cytoprotective, reparative, and energy-retentive properties of GLP-2 suggests that GLP-2 may potentially be useful for the treatment of human disorders characterized by injury and/or dysfunction of the intestinal mucosal epithelium. (*J Clin Endocrinol Metab* 86: 1759–1764, 2001)

THE GLUCAGON-LIKE PEPTIDES (GLPs) are produced in open type gut endocrine cells of the small and large intestine and play diverse roles in the regulation of energy homeostasis. Food ingestion sets in motion coordinated peptidergic responses that regulate nutrient transit through the gut, and nutrient absorption. Gut peptides also contribute to optimization of mucosal epithelial function for nutrient digestion and absorption. Following nutrient absorption into the blood stream, enteroendocrine-derived peptides such as GLP-1 facilitate nutrient disposal by regulating islet hormones and, indirectly, energy absorption via effects on liver, muscle, and adipose tissue. The aim of this review is to highlight recent advances in our understanding of the role played by GLP-2 in the regulation of intestinal epithelial biology and nutrient absorption.

The sequence of proglucagon contains a number of distinct peptides with pleiotropic actions, including glicentin, glucagon, oxyntomodulin, and two GLPs as well as two intervening or spacer peptides (Fig. 1). Whereas glucagon, GLP-1, and GLP-2 exert well-defined actions through known receptors, the biological actions of the remaining proglucagon-derived peptides remain less well characterized. Further-

more, no receptors have yet been isolated for glicentin, oxyntomodulin, or the intervening peptides (1–4).

Although the sequence of GLP-2 was not detected in anglerfish islet proglucagon complementary DNAs (cDNAs; Ref. 5), the GLP-2 sequence (Fig. 2) was detected in all isolated mammalian proglucagon cDNAs and genes (6–10) as a 33 amino acid peptide located carboxyterminal to GLP-1 and intervening peptide 2 (Figs. 1 and 2). Subsequent studies demonstrated that fish, chickens, and lizards generate GLP-2 in the gut as a result of tissue-specific alternative RNA splicing of proglucagon RNA transcripts (11, 12). In mammals, tissue-specific posttranslational processing liberates GLP-2 from proglucagon in the intestine and brain but not in pancreas, as a result of cell-specific expression of prohormone convertases in gut endocrine cells (13). Isolation and sequencing of GLP-2 from the porcine and human intestine confirmed that GLP-2 is a 33 amino acid peptide (Fig 2), corresponding to proglucagon 126–158, ending in a carboxy-terminal Asp residue (14, 15).

Initial studies of GLP-2 biological activity demonstrated increased adenylate cyclase activity in rat hypothalamic and pituitary membrane preparations following incubation with 50 pM GLP-2 (16). Subsequent analyses of GLP-2 activity in the gut either failed to demonstrate activity of GLP-2 or demonstrated GLP-2-mediated inhibition of serum-stimulated intestinal cell growth *in vitro* (17). Nevertheless, despite these negative findings, considerable experimental evidence correlated increased expression and secretion of intestinal proglucagon-derived peptides (PGDPs) with bowel injury and mucosal growth of the small intestine (18–22). Furthermore, two patients with glucagonomas exhibited marked small bowel villus hyperplasia that was reversed following surgical removal of the tumor (23, 24). Following observa-

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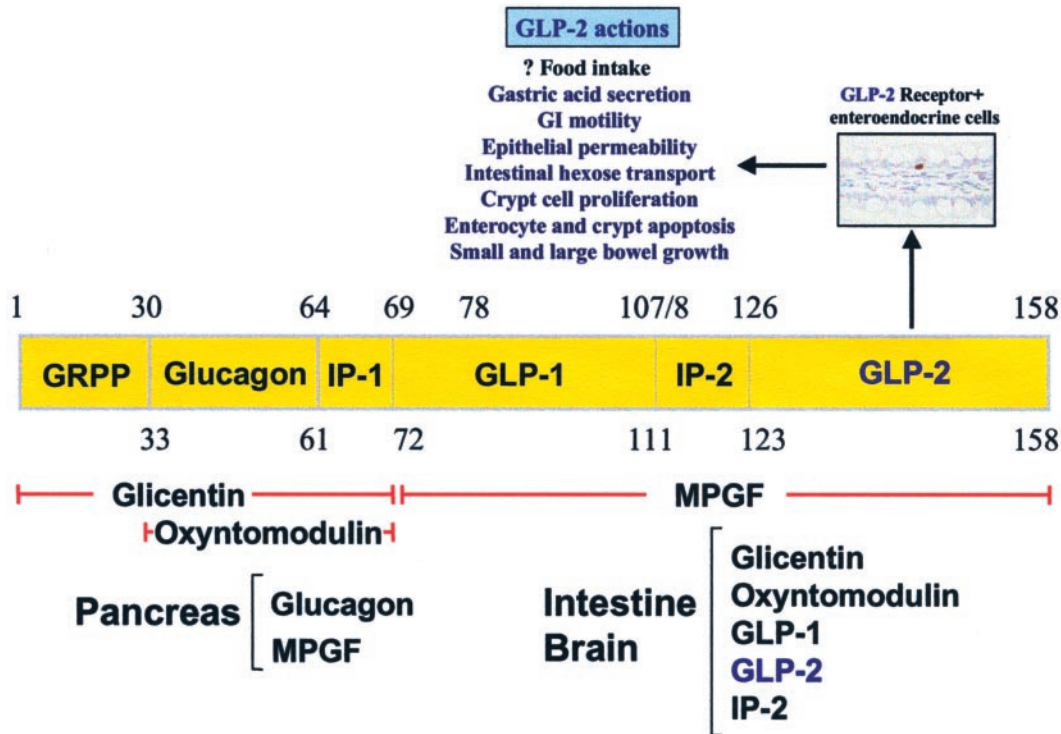


FIG. 1. Structure of mammalian proglucagon, the proglucagon-derived peptides, and the biological actions of GLP-2. The numbers *above* and *below* the proglucagon molecule correspond to the positions and length of specific PGDPs. MPGF, Major proglucagon fragment; IP, intervening peptide.

<b>Glucagon</b>	<b>HSQGT<b>T</b>TS<b>DY</b>SKY<b>L</b>DSRR<b>AQ</b>DFVQ<b>W</b>LMNT</b>
<b>GLP-1</b>	<b>HAEG<b>T</b>TS<b>DV</b>SSYLEG<b>QA</b>KE<b>F</b>IA<b>W</b>L<b>V</b>KGR<b>G</b></b>
<b>GLP-2</b>	<b>HADGS<b>F</b>SDE<b>M</b>NT<b>I</b>L<b>DN</b>LA<b>AR</b>DFIN<b>W</b>L<b>I</b>Q<b>T</b>K<b>I</b>T<b>D</b></b>

FIG. 2. Peptide sequences of human glucagon, GLP-1, and GLP-2. Amino acids common to all three peptides are shown in *bold*. Amino acids common to glucagon and GLP-1, GLP-1, and GLP-2, and glucagon and GLP-2 are shown in *green*, *blue*, and *red*, respectively.

tions that mice with sc glucagonomas developed massive small bowel hyperplasia, peptide injection studies identified GLP-2 as the PGDP with significant intestinotrophic activity (25).

#### GLP-2 synthesis, secretion, and degradation

A single proglucagon messenger RNA (mRNA) transcript, identical in sequence to proglucagon mRNAs in pancreas and brain (Fig. 1), is expressed in the mammalian small and large intestine (26–28). GLP-1 and GLP-2 are cosecreted from the gut (29) with the type and amount of nutrient ingestion representing a primary determinant of both intestinal proglucagon gene expression (30–33) and GLP-2 secretion in rodent, pig, and human studies (34–37). Fiber-enriched diets and fatty acids are potent stimulators of GLP-2 secretion in rodents and human subjects (36, 38).

Intestinal injury or resection is associated with increased levels of proglucagon mRNA transcripts in the intestinal remnant as a result of an increase in proglucagon RNA content in the remaining enteroendocrine cells (39–42). The kidney seems to be an important determinant of GLP-2 clearance as levels of immunoreactive GLP-2 are increased in human

patients with renal failure (43). Similarly, experimental nephrectomy results in delayed clearance and increased circulating levels of GLP-2 in rats (44, 45). The estimated elimination  $t_{1/2}$  of exogenously administered GLP-2 in human studies seems to be ~7.2 min (46), considerably longer than the  $t_{1/2}$  of GLP-1 in similar studies.

Analysis of rat and human plasma using a combination of high-performance liquid chromatography and site-specific GLP-2 antisera reveals the presence of two principal circulating molecular forms, GLP-2<sup>1–33</sup> and GLP-2<sup>3–33</sup> (15, 35, 46, 47). GLP-1, gastric inhibitory peptide, and GLP-2 all contain an alanine residue in position 2, rendering them ideal substrates for degradation by dipeptidyl peptidase IV (DP IV), a ubiquitous protease expressed in the gut and vascular endothelium (48, 49). Incubation of GLP-2 with DP IV *in vitro* results in cleavage to the bioinactive GLP-2<sup>3–33</sup> peptide, and inhibitors of DP IV prevent GLP-2 degradation both *in vitro* and *in vivo* (35, 46, 47). The importance of DP IV for the biological activity of GLP-2 is exemplified by studies in rats demonstrating considerably greater intestinotrophic activity of an exogenously administered GLP-2 analog resistant to DP IV-mediated inactivation (47). Similarly coadministration of a DP IV inhibitor potentiates the trophic activity of exogenous native GLP-2 in rats (50).

Increased circulating levels of GLP-2 are associated with the development of intestinal mucosal hyperplasia in rodents with experimental diabetes (38, 51). Administration of insulin to diabetic rats reduces the levels of circulating GLP-2 and reverses the small bowel mucosal hyperplasia (51). Human subjects with inflammatory bowel disease exhibit normal to increased levels of circulating bioactive GLP-2<sup>1–33</sup> (52), at-

tributable in part to a decrease in levels of circulating DP IV activity. Patients with Crohn's disease and marked involvement of the terminal ileum and patients with short bowel syndrome and lack of a colon in continuity with the remnant small bowel exhibit deficient levels of circulating GLP-2 and reduced meal-stimulated GLP-2 secretion, respectively (52, 53). In contrast, patients with ileal resection but a preserved colon exhibit increased fasting and meal-stimulated levels of GLP-2 and enhanced intestinal adaptation (54), providing a possible physiological explanation linking the presence of a colon with facilitated intestinal adaptation.

#### *Biological activities of GLP-2*

The gastrointestinal tract, from the stomach to the colon, is the principal target for GLP-2 action. GLP-2 inhibits stimulated gastric acid secretion in human subjects and reduces antral gastric motility in the pig (55, 56). Acute GLP-2 infusion rapidly increases intestinal hexose transport in rats, with significant increases in both hexose and SGLT-1 transport activity detectable within 60 min after initiation of iv GLP-2 infusion (57, 58). Administration of GLP-2 to normal mice produces significant increases in intestinal barrier function. Reduced epithelial permeability as measured by decreased ion and macromolecule transport, is detectable in Ussing chamber studies after a single injection of GLP-2 (59). Morphologically, intestinal epithelial cells appear narrower and longer, with increased numbers of longer microvilli detectable on the luminal surface of the enterocyte following several days of GLP-2 administration (59).

GLP-2 administered exogenously to mice and rats promotes expansion of the mucosal epithelium in the small and large bowel, with the most prominent trophic effects seen in the small bowel, specifically in the jejunum (25, 60). The trophic effects of GLP-2 are independent of the route of GLP-2 administration and are observed after iv, sc, or ip GLP-2 administration (57, 60–62). Although the optimal dosing and timing of GLP-2 administration for human clinical studies remains to be determined, GLP-2 is intestinotrophic in rodents even in daily or every other day administration regimens (60, 61). Whereas small changes in intestinal length have been detected after GLP-2 treatment (63), increased thickness of the intestinal mucosa, predominantly an increase in small bowel villus height and mucosal surface area, is invariably detected after several days of GLP-2 treatment (25, 47, 60, 61, 63). Enhanced thickness of the mucosal epithelium may be explained by GLP-2-stimulated increases in crypt cell proliferation, coupled with a decrease in the rate of enterocyte apoptosis (25, 61).

The GLP-2-treated bowel is functionally normal as assessed by analysis of macromolecule expression, and normal levels of mucosal enzymes are observed in the GLP-2-treated murine intestine (64). Absorption of carbohydrates, lipids, and proteins is normal to enhanced in GLP-2-treated mice (64). Similarly, GLP-2-treated rats exhibit enhanced absorption of glycine and galactose in association with increased mucosal DNA and protein content (65). Despite the suggestion that intracerebroventricular GLP-2 administration inhibits food intake (66), GLP-2 treatment of normal animals results in normal food intake, weight gain commensurate with

intestinal growth and an enhanced capacity for nutrient absorption (64).

The finding that enteral nutrients regulate GLP-2 secretion suggests a role for GLP-2 in mediating the trophic effect of nutrients on maintaining the normal thickness of the mucosal epithelium. Rats maintained on parenteral nutrition develop atrophy of the intestinal epithelium in both the small and large intestine, possibly as a result of reduced GLP-2 secretion. Intravenous coinfusion of GLP-2 and parenteral nutrition prevented the development of mucosal atrophy in the small but not the large bowel, illustrating the differential regional sensitivity of the gut to the trophic effects of GLP-2 (62, 67). Consistent with these findings, GLP-2 significantly improved the endogenous intestinal adaptive response to major small bowel resection in rats, with increased nutrient absorption and reduced intestinal permeability observed in the GLP-2-treated animals (68, 69).

The trophic and reparative effects of GLP-2 on the gut mucosa have also been observed in the setting of experimental intestinal injury. Following induction of indomethacin-induced intestinal inflammation, GLP-2 significantly reduced intestinal disease activity scores and cytokine expression, decreased bacterial sepsis, and reduced mortality in mice with enteritis (70). Remarkably, GLP-2 was most effective in ameliorating disease activity when administered as a pretreatment regimen before onset of indomethacin-induced enteritis (70). GLP-2 also increased mucosal DNA content and significantly reduced mortality in rats following vascular ischemia-reperfusion injury of the small intestine (71). The protective effects of GLP-2 in the gut have also been observed in the large bowel as mice with dextran sulfate colitis exhibit reduced parameters of disease activity, decreased intestinal interleukin expression, and significantly reduced weight loss after GLP-2 administration (72). Similarly, GLP-2 significantly reduced gross and microscopic mucosal damage and decreased cytokine expression in rats with antigen-induced inflammatory bowel disease (73).

The detection of GLP-2 receptor mRNA transcripts in the fetal and neonatal rat intestine (74) raises the possibility that GLP-2 may play a role in the development and maturation of the gastrointestinal tract. Daily administration of h[Gly2]-GLP-2 to neonatal rats enhanced stomach and small bowel weight and small bowel length (74). Furthermore, iv infusion of GLP-2 decreased proteolysis, reduced apoptosis, increased villus height, and was trophic to the gastrointestinal tract of immature pigs (75). Whether GLP-2 plays a role in growth and differentiation of the developing fetal gut remains unclear.

#### *Mechanisms underlying GLP-2 action: the GLP-2 receptor*

The actions of GLP-2 in the gut are mediated by a distinct GLP-2 receptor, a recently cloned member of the glucagon/GLP-1 G protein-coupled receptor superfamily (76). GLP-2R cDNAs isolated from intestinal and hypothalamic cDNA libraries are identical in sequence and encode a predicted receptor of 550 amino acids, exhibiting considerable amino acid identity with the glucagon and GLP-1 receptors. The GLP-2R gene was localized to human chromosome 17p13.3, a chromosomal region not yet linked to inheritance of fa-

mial intestinal diseases. Activation of GLP-2R signaling is coupled to an increase in cAMP with an EC<sub>50</sub> of ~0.58 nM GLP-2. In contrast, structurally related peptides such as glucagon, GLP-1, GIP, or exendin-4 do not activate the GLP-2R even at 10-nM concentrations (76). The intestinotrophic properties of GLP-2 derivatives in mice *in vivo* correlate well with the relative activation of GLP-2R signaling in transfected fibroblasts by these same peptides *in vitro* (76, 77). Analysis of the activity of alanine-substituted and both N- and C-terminally deleted GLP-2 molecules using the transfected GLP-2R expressed in fibroblasts identified a number of amino acid substitutions in the GLP-2 molecule that result in either diminished receptor binding and/or reduced receptor activation *in vitro* (77).

The GLP-2R is expressed in a highly tissue-specific manner, predominantly in the stomach, jejunum, ileum, and colon (76, 78). The results of Northern blotting, RNase protection, and RT-PCR experiments are consistent with the presence of a single GLP-2R transcript in the gastrointestinal tract and central nervous system of rodents and humans (78). The GLP-2R has been localized to subsets of enteroendocrine cells in the human gut. GLP-2R+ gut endocrine cells also exhibit immunopositivity for either GIP, serotonin, peptide YY, chromogranin, or GLP-1 (78). Although GLP-2 presumably exerts direct effects on enteroendocrine cells expressing the GLP-2R, it seems likely that many of the effects of GLP-2 on gastrointestinal target cells that do not express the GLP-2R are indirect, resulting in modulation of gastric motility, small bowel permeability, and both crypt cell proliferation and apoptosis. Hence, one model that explains GLP-2 action suggests that GLP-2 synthesized in and secreted from the small and large intestine exerts many of its actions in an autocrine, paracrine, or endocrine manner by stimulating the release of as yet unidentified mediators from GLP-2R+ gut endocrine cells. It seems likely that these GLP-2R+ enteroendocrine cells then release one or more factors that mediate the pleiotropic biological actions of GLP-2 in the gut (Fig. 1).

The observations that GLP-2 inhibits enterocyte and crypt compartment apoptosis following intestinal injury (70) prompted analysis of the mechanisms coupling GLP-2R signaling to reduced cell death. Remarkably, direct activation of GLP-2R signaling in transfected baby hamster kidney fibroblasts expressing the GLP-2 receptor (BHK-GLP-2R cells) confers resistance to cycloheximide-induced apoptosis (79). GLP-2 reduced activation of caspase-8, caspase-9, decreased cytochrome c release, and reduced caspase-3 cleavage, in a protein kinase A-independent manner. The antiapoptotic actions of GLP-2 are not diminished by inhibitors of the phosphatidylinositol-3 kinase or mitogen-activated protein kinase pathways (79). Furthermore, GLP-2 enhanced survival and decreased intestinal apoptosis in tumor-bearing mice treated with chemotherapy and reduced apoptosis and caspase activation in BHK-GLP-2R cells treated with irinotecan *in vitro* (80). These findings demonstrating a direct antiapoptotic effect of GLP-2 on cells expressing a GLP-2 receptor, taken together with the cytoprotective effects of GLP-2 *in vivo* on target cells that do not seem to express the GLP-2 receptor (70, 72, 80) suggest that GLP-2 inhibits cell death via both direct and indirect signaling pathways.

### Summary of current knowledge and unanswered questions

The available data demonstrate that GLP-2 regulates motility, nutrient absorption, epithelial permeability, cell proliferation, and apoptosis in the gastrointestinal tract. Whether one or more of these actions will prove to be essential for normal gut physiology in the absence of intestinal injury awaits the development of GLP-2 antagonists or rodent models of disrupted GLP-2 action. Similarly, the actions of GLP-2 have been principally delineated in rodents and the biological activities of GLP-2 in human subjects currently remain unclear. Nevertheless, the strong conservation of GLP-2 and GLP-2R sequences across species suggests that the physiological actions of GLP-2 in rodents and humans are likely to be comparable. In this regard, the results of a recent study of GLP-2 administration in human subjects with short bowel syndrome demonstrated enhanced energy absorption and increased crypt plus villus height in GLP-2-treated patients (81). Given the expression of the GLP-2 receptor in the central nervous system (76, 78), it seems likely that GLP-2, like GLP-1, also subserves one or more functions in the brain. Indeed, intracerebroventricular injection of GLP-2 in the rat reduces food intake (66), raising the possibility that GLP-2, perhaps like GLP-1, acts as a central satiety factor. The multiple actions of GLP-2 that include protection and restoration of the gut epithelium and enhancement of nutrient absorption will likely stimulate clinical testing of the therapeutic potential of this peptide in human diseases characterized by injury and/or dysfunction of the gut epithelium. Whether GLP-2 will ultimately prove therapeutically useful and safe for the treatment of human gastrointestinal diseases requires careful assessment in properly controlled clinical trials.

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