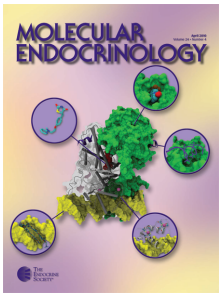
JCEM THE JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM

The Extrapancreatic Effects of Glucagon-Like Peptide-1 and Related Peptides

Rania Abu-Hamdah, Atoosa Rabiee, Graydon S. Meneilly, Richard P. Shannon, Dana K. Andersen and Dariush Elahi

J. Clin. Endocrinol. Metab. 2009 94:1843-1852 originally published online Mar 31, 2009; , doi: 10.1210/jc.2008-1296



THE ENDOCRINE SOCIETY®

The Extrapancreatic Effects of Glucagon-Like Peptide-1 and Related Peptides

Rania Abu-Hamdah, Atoosa Rabiee, Graydon S. Meneilly, Richard P. Shannon, Dana K. Andersen, and Dariush Elahi

Johns Hopkins University School of Medicine, Departments of Surgery (R.A.-H., A.R., D.K.A., D.E.) and Medicine (A.R., D.E.), Johns Hopkins Bayview Medical Center, Baltimore, Maryland 21224-2780; University of British Columbia (G.S.M.), Department of Medicine, Vancouver, British Columbia, Canada V5Z 1M9; and University of Pennsylvania School of Medicine (R.P.S.), Department of Medicine, Philadelphia, Pennsylvania 19104-2646

Context: Glucagon-like peptide-1 (GLP-1) 7-36 amide, an insulinotropic hormone released from the intestinal L cells in response to nutrient ingestion, has been extensively reviewed with respect to β-cell function. However GLP-1 receptors are abundant in many other tissues. Thus, the function of GLP-1 is not limited to the islet cells, and it has regulatory actions on many other organs.

Evidence Acquisition: A review of published, peer-reviewed medical literature (1987 to September 2008) on the extrapancreatic actions of GLP-1 was performed.

Evidence Synthesis: The extrapancreatic actions of GLP-1 include inhibition of gastric emptying and gastric acid secretion, thereby fulfilling the definition of GLP-1 as an enterogastrone. Other important extrapancreatic actions of GLP-1 include a regulatory role in hepatic glucose production, the inhibition of pancreatic exocrine secretion, cardioprotective and cardiotropic effects, the regulation of appetite and satiety, and stimulation of afferent sensory nerves. The primary metabolite of GLP-1, GLP-1 (9-36) amide, or GLP-1m, is the truncated product of degradation by dipeptidyl peptidase-4. GLP-1m has insulinomimetic effects on hepatic glucose production and cardiac function. Exendin-4 present in the salivary gland of the reptile, Gila monster (*Heloderma suspectum*), is a high-affinity agonist for the mammalian GLP-1 receptor. It is resistant to degradation by dipeptidyl peptidase-4, and therefore has a prolonged half-life.

Conclusion: GLP-1 and its metabolite have important extrapancreatic effects particularly with regard to the cardiovascular system and insulinomimetic effects with respect to glucose homeostasis. These effects may be particularly important in the obese state. GLP-1, GLP-1m, and exendin-4 therefore have potential therapeutic roles because of their diffuse extrapancreatic actions. **(*J Clin Endocrinol Metab* 94: 1843–1852, 2009)**

Glucagon-like peptide-1 (7-36) amide (GLP-1) is a 29-amino acid hormone secreted from the L cells of the small intestine by site-selective cleavage of the preproglucagon gene product (1). After the delineation of the DNA sequence of preproglucagon by Bell *et al.* (2) in hamster and man, the presence of two related peptides, GLP-1 and glucagon-like peptide-2, was proposed. Lopez *et al.* (3) isolated the same sequences in bovine preproglucagon cDNA. Habener's group then isolated the same sequence in the rat (4) and showed that GLP-1 was a potent insulinotropic agent (5) that qualified for the designation of an incretin (6, 7). GLP-1 is a mammalian brain-gut axis hormone that is also an endocrine paracrine hormone, an autonomic nervous system neurotransmitter (1, 8), and a natriuretic hormone (9).

GLP-1 is rapidly degraded to GLP-1 (9-36) amide, also referred to as GLP-1m, by the action of dipeptidyl peptidase-4 (DPP-4) (10, 11), which results in a circulating half-life for GLP-1 of 2 min (12) and is also cleared from the circulation by renal excretion (1). Peptides with alanine, proline, and hydroxyproline found in the N-terminal domain are cleaved by DPP-4, and GLP-1 is cleaved at the His 7, Aln 8 position leading to the

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2008-1296 Received June 16, 2008. Accepted March 20, 2009.
First Published Online March 31, 2009

Abbreviations: CNS, Central nervous system; DPP-4, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide-1; GLP-1R, GLP-1 receptor; GLUT, glucose transporter; IR, insulin receptor; PYY, peptide YY; T2DM, type 2 diabetes mellitus.

formation of the GLP-1m (13). Exendin-4, a GLP-1 receptor (GLP-1R) agonist, is present in the saliva of Gila monster (*Heloderma suspectum)*. It shares 53% of its amino acid sequence with the N-terminal region of mammalian GLP-1 (14). Exendin-4 has an extra nine amino acid residues at its C terminus. A major difference between the two agents is that the second amino acid of exendin-4 is glycine, which is resistant to DPP-4 cleavage. It has a circulating half-life of 30 min in man (15, 16).

GLP-1 and exendin-4 have been shown to be potently insulinotropic in both normal and diabetic subjects, and their roles as mediators of insulin release have therefore received much attention (12). Previous studies showed that exenatide (synthetic exendin-4) treatment resulted in lowering fasting and postprandial plasma glucose concentrations in patients with type 2 diabetes mellitus (T2DM) (17). GLP-1 is present and secreted from the L cells of several mammals including pig and rat (1, 18), and the peptide sequence of GLP-1 is identical in mouse, rat, and human (19, 20).

GLP-1 and exendin-4 have also been shown to have trophic effects on the β-cell (21). One mechanism responsible for the expansion of β-cell mass is inhibition of apoptosis shown for both GLP-1 and exendin-4 by Farilla *et al.* (22). The effect of GLP-1 on apoptosis appears to be mediated by the GLP-1R, and the expression of the GLP-1R in a nonpancreatic cell line renders these cells sensitive to the inhibition of programmed cell death by GLP-1 (23). It has also been shown that human islets treated with GLP-1 have a down-regulation of caspase 3 at the levels of mRNA of the active protein and an up-regulation of the anti-apoptotic protein Bcl-2 (22). A second mechanism responsible for the expansion of β-cell mass is enhanced cell proliferation. Mice treated with GLP-1 or exendin-4 show increased cell proliferation by bromodeoxyuridine labeling or by tritiated thymidine incorporation within the islets. The insulin-expressing cells are stimulated to proliferate by administration of GLP-1 *in vivo* (23). GLP-1 increases levels of β-cell cAMP and insulin gene transcription and stimulates glucose-dependent insulin release (24); however, unlike other depolarizing agents (such as the sulfonylureas), β-cell GLP-1R signaling is glucose dependent (24). GLP-1 also increases the gene expression and binding activity of transcription factor pancreatic and duodenal homeobox gene 1 most likely by a phosphatidylinositol-3-kinase-dependent pathway (25). Elimination of GLP-1R signaling in β-cells is associated with reduced intracellular cAMP and defective glucose-stimulated calcium influx (25).

The GLP-1R has been localized to the stomach, duodenum, exocrine pancreas, brainstem, thalamus, hypothalamus, hippocampus, heart, lung, and kidney, as well as the pancreatic islets (26). Furthermore, GLP-1 binding sites have been found in muscle cells, adipocytes, and the liver (27–30). The findings of GLP-1R outside of the islets provides strong evidence that GLP-1 has many extraislet effects and corroborates other studies (31–34) that show physiological effects of GLP-1 on a variety of extrapancreatic functions. This review summarizes the function of GLP-1 on different organs including the stomach, heart, liver, and the central nervous system (CNS).

GLP-1 Actions on Gastrointestinal Function

The inhibitory function of GLP-1 on gastric emptying and gastric acid secretion confirms the role of GLP-1 as an important enterogastrone, a hormone that inhibits proximal events of the gastrointestinal tract in a negative feedback manner (26). Nauck *et al.* (35) showed that iv administration of GLP-1 (7-36) amide and GLP-1 (7-37) has similar, profoundly inhibitory effects on the gastric emptying of a liquid mixed test meal in healthy, normoglycemic volunteers (Fig. 1) and that the effect of GLP-1 on gastric emptying is dose dependent and highly significant with physiological concentrations of approximately 25 pmol/liter. In eight healthy male volunteers, Schirra *et al.* (36) investigated the effect of different doses of GLP-1(7-36) amide (0.125 nmol/kg, 0.25 nmol/kg, or placebo) administered sc 5 min before the mixed meal. They quantified the pattern of gastric emptying of a mixed meal (300 kcal) as well as pancreatic secretion, antroduodenal motility, and the glycemic response and the release of insulin, C-peptide, and glucagon. The lag period or the time to reach maximal velocity of gastric emptying was dose-dependently prolonged in response to the sc infusion of GLP-1. Maximal emptying velocity, total emptying rate, and the exponential emptying rate were not changed, however (36). This study also showed that the sc infusion of GLP-1 resulted in a dose-dependent inhibition of duodenal and antral motility; that both doses of GLP-1 resulted in coordinated antroduodenal contractions; that GLP-1 initially reduced, then transiently stimulated the secretion of pancreatic enzymes; that both doses of GLP-1 resulted in a delaying postprandial insulin peak and enhanced total insulin release; and that the postprandial response of pancreatic polypeptide and glucagon was diminished. In another study, Schirra *et al.* (37) showed antro-pyloro-duodenal motility in humans and the actions of endogenously released GLP-1 on endocrine pancreas secretion. In their study of nine healthy volunteers, they used GLP-1R antagonist exendin (9-39) to test whether GLP-1 acts as an incretin and/or as an enterogastrone in humans. They showed that the endogenously released GLP-1 significantly enhanced postprandial insulin secre-

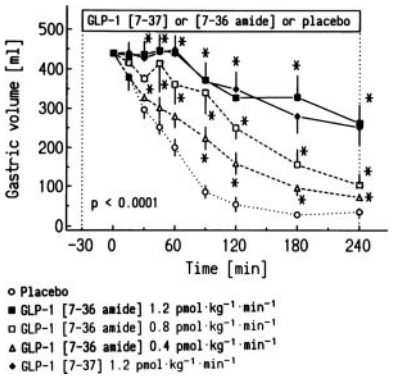


FIG. 1. The effect of GLP-1 on gastric emptying in man. Residual gastric volume after mixed liquid meal (8% amino acids plus 50 g sucrose in 400 ml) during iv infusion of GLP-1 (7-36) amide or (7-37) (means ± SE); symbols show different doses in nine healthy male volunteers. *P* values represent interaction of experiment (placebo/GLP-1) and time as calculated by repeated-measures ANOVA (RM-ANOVA). *, Significant differences ($P < 0.05$ by Student's *t* test) from experiments with placebo at individual time points. *Box*, Duration of infusion of GLP-1/placebo (35).

tion and suppressed the secretion of glucagon (37). During the fasting and postprandial state, antro-duodenal motility was inhibited by GLP-1, which qualifies GLP-1 as an enterogastrone. They also showed that the stimulation of pyloric motility that is induced by intestinal glucose was mediated by GLP-1. The presence of food in the gut causes the L cells of the intestine to release GLP-1 into the circulation, which not only stimulates the pancreas to produce insulin but also slows gastric emptying and may lead to a decrease in appetite (38–43).

The mechanisms by which GLP-1 inhibits gastric emptying appear to be complex and to involve communication with the central and peripheral nervous systems (44, 45). Gastric distension increases the expression of c-Fos in brainstem neurons that produce GLP-1 (46). In addition, administration of GLP-1 centrally resulted in reduction of food intake (47), which is accompanied with increased expression of the c-Fos in the brainstem of the rat (48, 49). The denervation of vagal afferent fibers abolishes the effects of GLP-1 on gastric emptying in the rat (50). The stimulation to the CNS is most likely responsible for the reduction in food intake, inhibition of gastric emptying, as well as inhibitory action on gastric motor function (47, 50). These actions are most likely mediated by increased action potential and calcium influx in neurons of the nodose ganglion (51).

Although small peptides such as GLP-1 and exendin-4 are capable of rapidly crossing the blood-brain barrier and directly accessing the CNS, higher molecular weight GLP-1R agonists, such as albumin-bound GLP-1, that do not cross the blood-brain barrier are still capable of inhibiting gastric emptying and food intake (52). These findings indicate the importance of ascending vagal afferents for GLP-1R-dependent control of gastrointestinal motility. Interestingly, studies by Meier *et al.* (53) showed that antagonizing the delaying effects of GLP-1 on gastric emptying by using a prokinetic agent such as erythromycin resulted in an augmentation of the insulin secretory response after meal ingestion. GLP-1Rs are also directly expressed in the stomach on gastric parietal cells, where GLP-1 may directly regulate gastric acid secretion (54). However, the effects of GLP-1 on gastric acid secretion were found to be absent in vagotomized human subjects (55). Hence, considerable evidence supports the importance of vagal innervation for GLP-1 regulation of gastric secretion and motility.

It should be noted that the effect of delayed gastric emptying has been generally demonstrated with physiological or supraphysiological exogenously administered GLP-1 (56, 57). Therefore, it remains unclear whether endogenously released GLP-1 has a significant effect on gastric emptying. Studies in healthy baboons have shown that with intragastric infusion of glucose and D-xylose (a marker for rate of emptying of glucose from stomach), plasma levels of D-xylose were similar when the effects of GLP-1 were blocked with exendin (9-36) amide or with a specific monoclonal antibody to GLP-1 (44, 58). Those findings suggested that gastric emptying is not increased when the effects of GLP-1 are blocked, at least in the baboon. The use of a DPP-4 inhibitor, which increases plasma levels of GLP-1, might be expected to delay gastric emptying, but Vella *et al.* (59) failed to observe any changes in the gastric emptying of a solid meal in patients with T2DM who were treated with such an inhibitor. Most recently, an iv-oral hyperglycemic clamp study in humans was reported during which 75 g glucose containing D-xylose was ingested. During the entire clamp, plasma glucose levels were held at a steady level despite the ingestion of glucose. Two studies were conducted, with blockade of GLP-1R in one. The rate of appearance of ingested D-xylose was not different between the two studies, indicating that endogenously released GLP-1 has at best only a modest effect on gastric emptying (60).

In some endocrine systems, negative feedback mechanisms regulate secretion of the hormone. The classic example is reproductive hormone regulation by the hypothalamus. Exogenous infusion of hormone may also exert negative feedback regulation of the endogenously released hormone. An example of this is the documented suppression of C-peptide levels when insulin is infused (61). In this context, we are not aware of any data that demonstrate regulation of endogenously released GLP-1 when it is infused exogenously.

GLP-1 and the Cardiovascular System

Early studies demonstrated the presence of transcripts for GLP-1Rs in the heart (62), but only recently has the cellular distribution of the receptors been localized. Ban *et al.* (63) identified GLP-1Rs via immunohistochemistry in cardiomyocytes and coronary and vascular endothelial cells as well as smooth muscle in mice.

Previous studies have shown that exogenous administration of GLP-1 exhibits both inotropic and chronotropic activity. The extent to which GLP-1 (7-36) exerts cardiovascular effects via increased inotropic and chronotropic actions appears to depend on the integrity of the autonomic nervous system. For example, Barragan *et al.* (64) showed that iv administration of GLP-1 dose-dependently increases arterial blood pressure and heart rate in anesthetized rats. Ahren (26) showed that GLP-1 increased both systolic and diastolic pressures under anesthesia and that those effects were not prevented by reserpine, propranolol, or phentolamine, suggesting a direct action of GLP-1. Yamamoto *et al.* (65) showed similar effects after peripheral and central administration of GLP-1 in anesthetized rats, observing dose-dependent increases in blood pressure and heart rate. Barragan *et al.* (66) showed that administration of the GLP-1R agonist, exendin-4, also increases blood pressure and heart rate in anesthetized rats. Notably, the pressor and chronotropic responses seen in rodents were not evident in normal conscious, chronically instrumented dogs over a dose range of 1–20 pmol $\cdot$ kg^{-1} $\cdot$ min^{-1} (67). Nikolaidis *et al.* (67) showed that myocardial function and cardiac output were improved after administration of GLP-1 in conscious, chronically instrumented canine models of cardiac injury or heart failure. GLP-1 increased cardiac output and reduced left ventricular end diastolic pressure in association with reduced systemic vascular resistance, and it improved myocardial insulin sensitivity and myocardial glucose uptake in dogs with rapid pacing-induced dilated cardiomyopathy (67). A clinical study showed that GLP-1 improves left ventricular ejection fraction and functional status in patients with congestive heart failure, without affecting heart rate or blood pressure, suggesting a mechanism other than direct inotropic or chronotropic effects (68).

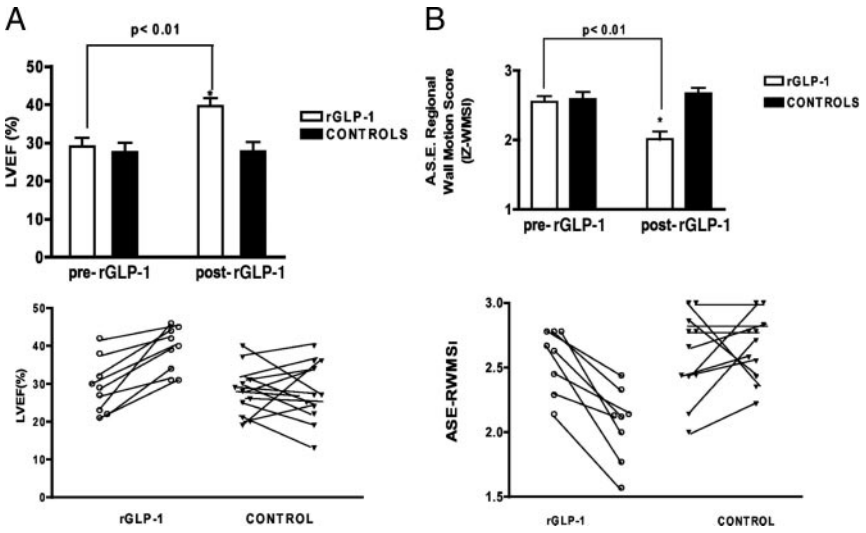


FIG. 2. The effect of GLP-1 on cardiac ejection fraction and wall motion in patients with acute myocardial infarction. A, Changes in left ventricular ejection fraction (LVEF) after 72 h of recombinant GLP-1 infusion *vs.* control subjects. *Lower panel* illustrates individual data. B, Changes in regional wall motion score at the per-infarct zone in recombinant GLP-1-treated patients *vs.* control subjects. *Lower panel* illustrates the individual data (34).

GLP-1 has been shown to reduce infarct size in the isolated perfused rat heart subjected to complete coronary artery occlusion (69, 70). When the cAMP inhibitor Rp-cAMP was present, the infarct-sparing actions of GLP-1 were abolished, implicating a cAMP-dependent mechanism. In these studies, GLP-1 was also associated with increased Akt expression, although these investigators did not measure myocardial glucose uptake. In contrast, Zhao *et al.* (71) demonstrated that GLP-1 (7-36) in normal rat hearts altered resting contractility and heart rate through a non-Akt-dependent mechanism. Specifically, these investigators showed that GLP-1, in contrast to insulin, had no effect on Akt phosphorylation or activation and did not result in increased glucose transporter (GLUT)-4 translocation, despite increased myocardial glucose uptake. Rather, GLP-1 (7-36) increased p38 MAPK activation, nitric oxide expression, and GLUT-1 translocation. Under circumstances of low flow ischemia, GLP-1 (7-36) mitigated myocardial stunning. Thus, the cellular signaling effects of GLP-1 (7-36) vary depending on the experimental circumstances (partial *vs.* complete coronary artery occlusion).

Remarkably, GLP-1 also exerts beneficial effects on cardiac function in human subjects after myocardial infarction and angioplasty. In one study, a 72-h infusion of GLP-1 in patients with acute myocardial infarction and ejection fractions less than 40% resulted in significantly improved left ventricular ejection fraction and improved regional and global wall motion scores, and it was associated with earlier hospital discharge (34) (Fig. 2). In a randomized study of the effects of GLP-1 on patients undergoing coronary artery bypass grafting, Sokos *et al.* (72) showed that the need for inotropic support and exogenous insulin was significantly reduced in patients who received GLP-1.

Whether the beneficial effects of GLP-1 on the injured heart are primarily directed via activation of cardiac GLP-1R signaling or indirectly via GLP-1R-dependent improvement in levels of glucose and insulin requires further investigation. The findings of a direct, cardioprotective effect in the isolated perfused rat and mouse heart argue strongly for the former (63, 71). However, it remains to be determined whether the cardioprotective effects are attributable to the increase in myocardial glucose uptake and glycolytic ATP or activation of distinct but related cellular pathways implicated in ischemic pre- or postconditioning.

The agonists of GLP-1R have been shown to have vascular and cardiac actions in humans as well as in rodents; these actions include the effects on cardiac output, blood pressure, contractility (65, 66, 68, 73), and cardioprotection (34, 67, 70, 74). Previous studies showed that GLP-1 is believed to exert its action through heptahelical G protein-coupled receptor (GLP-1R), which is functionally associated with adenylate cyclase through the stimulatory Gs (75, 76). Whether these mechanisms are operative and account for the putative beneficial effects of GLP-1 agonists remains to be determined conclusively. These studies will have important implications on the ultimate role of these agonists because chronic cAMP generation may be deleterious in clinical cardiovascular conditions. Moreover, the dose of the GLP-1 agonists that elicits a beneficial cardiovascular effect tends to be greater than the native peptide, raising important considerations of ligand-receptor interaction.

The demonstration that GLP-1 (9-36) amide, the principal metabolite of GLP-1, improves myocardial glucose uptake and ventricular contractility in dogs with pacing-induced dilated cardiomyopathy suggests that some of the cardiovascular effects of native GLP-1 may be mediated by a mechanism independent of the known GLP-1R (77). Ban *et al.* showed that GLP-1 (9-36) had favorable effects on postischemic contractile dysfunction in mice when administered after but not before occlusion. These investigators have suggested a two pathway schema for cardiovascular actions of GLP-1. The first depends on the GLP-1R action for inotropic, glucose uptake, ischemic preconditioning, and mild vasodilatory actions. The second pathway depends on the rapid degradation of GLP-1 to GLP-1m. Their data are compatible with the notion that although GLP-1m is not an inotrope, it has a small, significant cardioprotective effect in the setting of ischemic reperfusion injury. This is due to an increase of glucose uptake and vasodilation through a nitric oxide/cGMP-dependent pathway (63, 78, 79). These findings have important implications for the role of DPP-4 inhibition in the clinical utility of incretin biology.

Although the majority of experimental studies have used acute exposure to GLP-1 in assessing cardiovascular effects, a recent study by Poornima *et al.* (79) has examined the effects of 3 months of continuous infusion of GLP-1. These investigators demonstrated that chronic infusions of GLP-1 improved survival and preserved cardiac function in a rodent model of diabetes and hypertension that develops dilated cardiomyopathy and dies prematurely. These studies indicate that the salutary effects of

GLP-1 on cardiovascular performance are sustained after chronic exposure. Thus, the emerging cardiovascular profile of GLP-1 together with its effective antiglycemic actions portend significant clinical benefits in the treatment of T2DM where therapies that reduce macrovascular outcomes have been elusive.

GLP-1 Actions on the Liver

The hepatoportal region may be an important site of action of GLP-1 because there is a rapid degradation of GLP-1 in the plasma after its secretion into the mesenteric venous bed. During the postprandial phase, the concentration of GLP-1 increases in the mesenteric-portal venous system.

The effect of GLP-1 on hepatic glucose production has been reviewed by D'Alessio *et al.* (80). *In vitro* studies supporting GLP-1 effects on liver cells are most convincing from the laboratory of Valverde *et al.* (27) who showed that GLP-1 promotes glycogen accumulation in cultured rat liver cells. They observed that GLP-1 increases the activity of glycogen synthase-A, decreases the activity of glycogen phosphorylase-A, and promotes the incorporation of labeled glucose into glycogen in isolated rat hepatocytes. They also showed that these effects of GLP-1 are concentration dependent and increase with increasing levels of glucose. These effects of GLP-1 can also be reproduced with exendin and blocked by GLP-1R antagonist exendin (9-39) amide. The increased glycogen accumulation by GLP-1 or insulin is significantly reduced when glucagon is added to the media, which is also accompanied by a significant reduction in cAMP (27, 81, 82).

An *in vivo* dog study by Dardevet *et al.* (32) suggested the presence of GLP-1 sensors or receptors in the hepatoportal region. They showed that the insulin-independent effect of GLP-1 on hepatic glucose uptake is consistent with the presence of specific GLP-1Rs that could activate kinases and/or factors involved in glycogen synthesis and glucose uptake.

In a study to evaluate the beneficial therapeutic effects of exendin on hepatic steatosis in ob/ob mice (16), it was shown that GLP-1 and exendin-4 both have the potential for a direct lipid-lowering effect on hepatocytes, making either peptide a potential candidate for the treatment of nonalcoholic fatty liver disease. The presence of GLP-1Rs in isolated rat hepatocytes was shown in this study by an immunoblot analysis. However, in contrast to the study of Valverde *et al.* (27), GLP-1 and exendin-4 resulted in a marked increase in cAMP production; when the hepatocytes were pretreated with the GLP-1R antagonist exendin (9-39), the activity of cAMP was significantly reduced to below basal levels.

In addition to its role in hepatic glucose uptake and glycogen formation, GLP-1 may also mediate the regulation of hepatic glucose output by insulin. The hepatic insulin receptor (IR) and the hepatocyte membrane-bound GLUT2 have been shown to be internalized into the endosomal compartment after feeding or insulin administration (83), and the internalization of hepatocyte GLUT2 appears to mediate the suppression of hepatic glucose output by insulin (84). The endocytosis of the hepatic IR and GLUT2 appears coupled, suggesting an IR-GLUT2 complex on the hepatocyte plasma membrane (85). In states of apancreatic diabetes, such as chronic pancreatitis, the internalization of the IR-GLUT2 complex is impaired, and hepatic glucose production becomes unresponsive to suppression by insulin (86). Treatment with GLP-1 in rats with chronic pancreatitis was found to reverse this impairment (87), suggesting a role for GLP-1 in the regulation of IR and GLUT2 endocytosis, and therefore hepatic glucose production.

Our group showed that an infusion of GLP-1 in obese volunteers resulted in an increase in glucose uptake that was not the result of increased endogenous insulin secretion (88). These results were similar to what Dardevet *et al.* (32) found, *i.e.* that pharmacological doses of GLP-1 resulted in increased glucose utilization, independent of changes in insulin. Such human studies and others in pigs and dogs suggest an important extrapancreatic effect of the principal metabolite of GLP-1, GLP-1 (9-36) amide, or GLP-1m. GLP-1m was previously found to lack insulinotropic activity and has therefore been considered to be biologically inactive (89). It has been shown that infusion of GLP-1 (7-36) amide results in high levels of GLP-1m because of its cleavage by DPP-4 in plasma (11, 90). Indeed, we and others have shown that when steady-state levels were achieved during infusion of full-length peptide, approximately 80% of the circulating plasma levels of peptide were in the form of GLP-1m (88, 90). Therefore we hypothesized that the insulinomimetic action of GLP-1 might be due to GLP-1m formation, and we undertook glucose clamp studies in lean and obese subjects with the aim of elucidating the effects of GLP-1m. Glucose turnover was measured during two 2-h euglycemic clamp studies in which saline or GLP-1m was infused from 0 to 60 min. Half of the volunteers underwent a third clamp in which the known GLP-1R was blocked with the infusion of the GLP-1 (7-36) antagonist, exendin (9-39) amide, starting 60 min before infusion of GLP-1m. In lean subjects, no glucose infusion was necessary to sustain euglycemia during saline or GLP-1m infusion. However, in obese subjects glucose infusion was necessary during GLP-1m infusion because of a marked (>50%) inhibition of hepatic glucose production. Plasma insulin levels remained constant in lean subjects but rose significantly in obese subjects after termination of the peptide infusion. During GLP-1R blockade, infusion of glucose was immediately required, on starting GLP-1m infusions, in all subjects because of a more dramatic reduction in hepatic glucose production and a delayed and modest insulinotropic response (33). Thus, GLP-1m inhibits hepatic glucose production and is a weak insulinotropic agent (Fig. 3). These properties are especially apparent and pronounced in obese subjects and only become apparent in lean subjects during GLP-1R blockade. These previously unrecognized antidiabetogenic actions of GLP-1m, which is always generated when GLP-1 (7-36) is administered or secreted, suggest a role for GLP-1m as a therapeutic agent in controlling blood glucose (33).

The observation noted above that GLP-1m improved myocardial glucose uptake and ventricular contractility in dogs with dilated cardiomyopathy equally as GLP-1 (7-36) suggests that a putative GLP-1m receptor may be present in cardiac tissue (77). Increased functional recovery and cardiomyocyte viability after ischemic reperfusion injury in isolated hearts from wild-type mice were also observed in mice lacking a functional GLP-1R

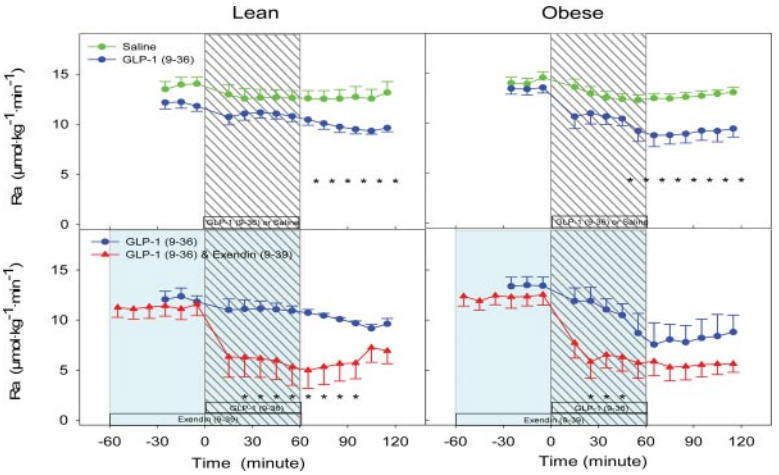


FIG. 3. The effect of GLP-1m on hepatic glucose production in man. Rates of appearance of glucose (Ra, *top panel*) in 12 lean (*left*) and 12 obese (*right*) volunteers who received GLP-1 (9-36) amide (GLP-1m) or saline from 0 to 60 min. Rates of appearance of glucose (*bottom panel*) in seven lean (*left*) and six obese (*right*) volunteers who received GLP-1m from 0 to 60 min. Exendin (9-39) amide was infused from −60 to 60 min (mean ± SE). *, Significant difference between the two studies at indicated times (33).

(Glp1r$^{-/-}$) (63), which further supports this possibility. Furthermore, in the latter study, a reduction of ischemic change was observed during reperfusion when GLP-1m was administered both in wild-type and Glp1r$^{-/-}$ mice. This was accompanied with increased cGMP release, vasodilation, and coronary flow. Taken together, the data from animal and human studies strongly demonstrate that in both the cardiac tissue and the liver, the action of GLP-1m is not mediated through activation of the known GLP-1R, which suggests that a yet unidentified GLP-1m receptor is present in these tissues.

Despite these reports of an insulin-independent effect of GLP-1 (or GLP-1m) on the liver, and despite other *in vitro* studies that demonstrate effects that are attributable to a presumed hepatic GLP-1R, it is acknowledged that there is controversy with respect to GLP-1 (or GLP-1m) effects in the liver, muscle, and adipose tissue (31), and the presence and/or species differentiation of an identifiable receptor in these tissues (91, 92). GLP-1m has been administered in humans by other investigators (89, 93) and in general did not show any effect. However, the design of these studies did not allow for evaluation of effects of GLP-1m because it was infused along with GLP-1, or with DPP-4 inhibitors, and tracers were not used to determine site-specific glucose kinetics. Our observation that GLP-1m infusion results in the suppression of hepatic glucose production (33) strongly suggests a role of GLP-1m on hepatic glucose production. In the absence of measurements of hepatic glucose dynamics, these effects would appear to result in enhanced insulin sensitivity after GLP-1 infusion.

GLP-1 and the CNS

GLP-1 is synthesized in the caudal part of the nucleus of the solitary tract (94), and its receptors are widespread throughout the brain, particularly in the paraventricular nucleus (48, 95, 96). The presence of GLP-1 and the GLP-1R in the CNS indicate that GLP-1 also acts centrally in addition to its actions on the peripheral system. It has been shown previously that injection of GLP-1 intracisternally causes a delay of liquid gastric emptying (50). Nakade *et al.* (97) showed that the peripheral sympathetic nervous system and the central corticotropin-releasing factor receptors are involved in the central GLP-1-mediated delay of solid gastric emptying in rats.

Nishizawa *et al.* (98) showed that administration of GLP-1 into the portal vein increases the firing frequency in the vagal afferents in rats. This suggests that the release of GLP-1 from the gut is rapidly signaled to the brain through this afferent pathway (98). This may represent the functional basis for neurally mediated inhibition of gastric emptying, gastric acid secretion, and exocrine pancreatic secretion by GLP-1, effects that have been shown to require intact sensory and efferent parasympathetic nerves (26).

Intracerebroventricular administration of GLP-1R agonists inhibits food intake in rodents (96, 99), and GLP-1Rs have been localized to hypothalamic nuclei, which are important for the regulation of satiety. Repeated intracerebroventricular administration of GLP-1 in rats produces weight loss (Fig. 4), whereas intracerebroventricular administration of the GLP-1R antagonist exendin (9-39) for 3 d produced weight gain, and exendin (9-39) administered together with the central orexigenic agent neuropeptide Y resulted in an increased food intake and weight gain compared with that observed with neuropeptide Y alone (100). It should be noted that the L cells corelease GLP-1 and peptide YY (PYY), and immunohistological studies have shown that these peptides are colocalized and coreleased from these cells. PYY (3-36), the major circulating form of PYY, has been shown to be a potent orexigenic agent in rats and man (101, 102). Evidence therefore supports the corelease of GLP-1 and PYY as having important roles as mediators of satiety. It has been shown that GLP-1 may regulate the hypothalamic pituitary axis via effects on LH, TSH, CRH, oxytocin, and vasopressin secretion (103, 104). The available evidence suggests that taste and/or food aversion induced by GLP-1 is mediated by different CNS pathways (47, 99, 105).

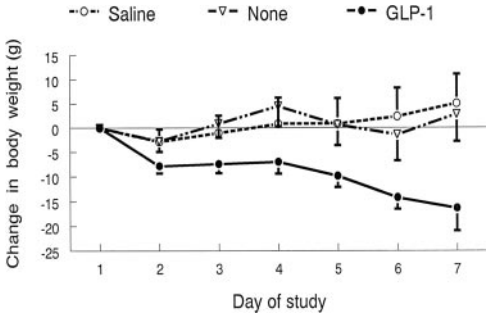


FIG. 4. The effect of intracerebroventricular (icv) GLP-1 on body weight in the rat. Body weight after daily icv injection of GLP-1 or saline. The *solid circles* represent animals given 3 nmol GLP-1, and *open circles* represent control animals that received saline (100).

GLP-1 and PYY are secreted not only from L cells in the small intestine (from duodenum to ileum, with the greatest concentration in the ileum) but also from mammalian taste cells. Egan and colleagues (106) have shown that human duodenal L cells and taste cells of the tongue express the sweet taste receptor G protein gustducin, which is probably involved in the regulation of GLP-1 release. These investigators have shown that in many L cells, GLP-1, gustducin, and PYY are colocalized. They also have shown that GLP-1 is produced in two subsets of mammalian taste cells (type 2 and type 3) and that GLP-1Rs are present on adjacent intragemmal afferent nerve fibers (107). It is possible that GLP-1 (and PYY) activate the CNS events resulting in an anorexigenic effect, before stimulating islet hormones (108). Chronic peripheral administrations of GLP-1R agonists (Exendin, Liraglutide) have been consistently associated with reductions in food intake and weight loss in rats and humans (109–112). However, in a study of continuous sc administration of GLP-1 for 6 wk at a rate of 4.8 pmol $\cdot$ kg^{-1} $\cdot$ min^{-1}, only 1.9 kg of weight loss was documented (113). Furthermore, in a 12-wk continuous sc administration study of a lower dose of GLP-1 (1.5 pmol $\cdot$ kg^{-1} $\cdot$ min^{-1}), there was no weight loss (114). Therefore, it is possible that, in humans, reduction of appetite by GLP-1 is manifested only acutely and does not persist long term. Alternatively, to demonstrate weight loss with exogenous GLP-1 administration, much larger doses are required.

Cabou *et al.* (115) showed that brain GLP-1R signaling simultaneously controls heart rate, femoral arterial blood flow, and glucose utilization in an awake free-moving mouse, and that brain GLP-1 signaling regulates reactive nitric oxide and reactive oxygen species that are likely important for the coordinated regulation of metabolic and cardiovascular function. An increase in vagus nerve activity was associated with brain to periphery signaling, implying that the action of GLP-1R signaling for control of nitric oxide and reactive oxygen species is also glucose dependent (115). Previous studies showed that GLP-1 was able to relax the femoral artery tone in a dose-response manner in rats (116) and that it is associated with vasodilatation induced by acetylcholine (117). On the other hand, when GLP-1 or its analogs is infused systemically in humans, it does not induce hyper- or hypotension as has been seen in animals (68, 118).

Conclusions

Our understanding of the extrapancreatic effects of the incretin hormones has expanded exponentially over the past two decades, and it is clear that GLP-1, exendin-4, and GLP-1m all have actions beyond the pancreatic islets (Fig. 5). The roles of these peptides on peripheral organs such as the gastrointestinal tract, CNS, and heart appear well established. The roles of GLP-1 and GLP-1m in the liver remain to be clarified and await consensus on the localization of receptors for GLP-1 and GLP-1m on the hepatocyte. Although many of the extrapancreatic effects of GLP-1 appear to be insulinomimetic, it is possible that some mechanisms of action by GLP-1 and/or GLP-1m are independent of insulin-regulated pathways. That GLP-1 has a broad range of effects in nutrient metabolism and energy balance is now clear.

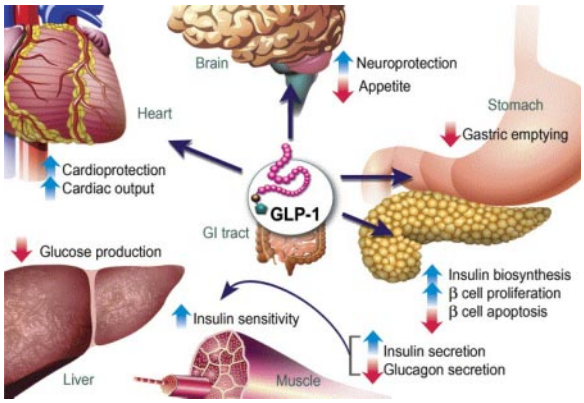


FIG. 5. GLP-1 actions on peripheral tissues. GLP-1 acts directly on the endocrine pancreas, heart, stomach, and brain, whereas actions on liver and muscle are direct and/or indirect (45).

It is also clear that an exciting spectrum of possible therapeutic applications is rapidly emerging.

Acknowledgments

The authors acknowledge with thanks the assistance of Ms. Melissa Scudder in the preparation of the manuscript. We gratefully acknowledge the support of the Alan McGavin Medicine Endowment of the University of British Columbia.

Address all correspondence and requests for reprints to: Dariush Elahi, Ph.D., Departments of Surgery and Medicine, Johns Hopkins Bayview Medical Center, 4940 Eastern Avenue, Baltimore, Maryland 21224-2780. E-mail: delahi1@jhmi.edu.

This work has been supported in part by National Institutes of Health Grants AG 00599 and AG 623175.

Disclosure Summary: The authors have nothing to disclose.

References

1. **Frezza EE, Wachtel MS, Chiriva-Internati M** 2007 The multiple faces of glucagon-like peptide-1–obesity, appetite, and stress: what is next? A review. Dig Dis Sci 52:643–649
2. **Bell GI, Santerre RF, Mullenbach GT** 1983 Hamster preproglucagon contains the sequence of glucagon and two related peptides. Nature 302:716–718
3. **Lopez LC, Frazier ML, Su CJ, Kumar A, Saunders GF** 1983 Mammalian pancreatic preproglucagon contains three glucagon-related peptides. Proc Natl Acad Sci USA 80:5485–5489
4. **Heinrich G, Gros P, Lund PK, Bentley RC, Habener JF** 1984 Pre-proglucagon messenger ribonucleic acid: nucleotide and encoded amino acid sequences of the rat pancreatic complementary deoxyribonucleic acid. Endocrinology 115:2176–2181
5. **Mojsov S, Weir GC, Habener JF** 1987 Insulinotropin: glucagon-like peptide I (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. J Clin Invest 79:616–619
6. **Lund PK** 2005 The discovery of glucagon-like peptide 1. Regul Pept 128:93–96
7. **Habener JF** 1993 The incretin notion and its relevance to diabetes. Endocrinol Metab Clin North Am 22:775–794
8. **Perry T, Lahiri DK, Chen D, Zhou J, Shaw KT, Egan JM, Greig NH** 2002 A novel neurotrophic property of glucagon-like peptide 1: a promoter of nerve growth factor-mediated differentiation in PC12 cells. J Pharmacol Exp Ther 300:958–966
9. **Gutzwiller JP, Tschopp S, Bock A, Zehnder CE, Huber AR, Kreyenbuehl M, Gutmann H, Drewe J, Henzen C, Goeke B, Beglinger C** 2004 Glucagon-like peptide 1 induces natriuresis in healthy subjects and in insulin-resistant obese men. J Clin Endocrinol Metab 89:3055–3061

10. **Deacon CF, Johnsen AH, Holst JJ** 1995 Degradation of glucagon-like peptide-1 by human plasma *in vitro* yields an N-terminally truncated peptide that is a major endogenous metabolite *in vivo*. J Clin Endocrinol Metab 80:952–957
11. **Kieffer TJ, McIntosh CH, Pederson RA** 1995 Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 *in vitro* and *in vivo* by dipeptidyl peptidase IV. Endocrinology 136:3585–3596
12. **Drucker DJ, Nauck MA** 2006 The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet 368:1696–1705
13. **Green BD, Gault VA, O'harte FP, Flatt PR** 2004 Structurally modified analogues of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) as future antidiabetic agents. Curr Pharm Des 10: 3651–3662
14. **Eng J, Kleinman WA, Singh L, Singh G, Raufman JP** 1992 Isolation and characterization of exendin-4, an exendin-3 analogue, from *Heloderma suspectum* venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas. J Biol Chem 267:7402–7405
15. **Edwards CM, Stanley SA, Davis R, Brynes AE, Frost GS, Seal LJ, Ghatei MA, Bloom SR** 2001 Exendin-4 reduces fasting and postprandial glucose and decreases energy intake in healthy volunteers. Am J Physiol Endocrinol Metab 281:E155–E161
16. **Ding X, Saxena NK, Lin S, Gupta NA, Gupta N, Anania FA** 2006 Exendin-4, a glucagon-like protein-1 (GLP-1) receptor agonist, reverses hepatic steatosis in ob/ob mice. Hepatology 43:173–181
17. **Kolterman OG, Buse JB, Fineman MS, Gaines E, Heintz S, Bicsak TA, Taylor K, Kim D, Aisporna M, Wang Y, Baron AD** 2003 Synthetic exendin-4 (exenatide) significantly reduces postprandial and fasting plasma glucose in subjects with type 2 diabetes. J Clin Endocrinol Metab 88:3082–3089
18. **Eissele R, Göke R, Willemer S, Harthus HP, Vermeer H, Arnold R, Göke B** 1992 Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. Eur J Clin Invest 22:283–291
19. **Irwin DM** 2001 Molecular evolution of proglucagon. Regul Pept 98:1–12
20. **Estall JL, Drucker DJ** 2006 Glucagon and glucagon-like peptide receptors as drug targets. Curr Pharm Des 12:1731–1750
21. **Wang Y, Perfetti R, Greig NH, Holloway HW, DeOre KA, Montrose-Rafizadeh C, Elahi D, Egan JM** 1997 Glucagon-like peptide-1 can reverse the age-related decline in glucose tolerance in rats. J Clin Invest 99:2883–2889
22. **Farilla L, Bulotta A, Hirshberg B, Li Calzi S, Khoury N, Noushmehr H, Bertolotto C, Di Mario U, Harlan DM, Perfetti R** 2003 Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. Endocrinology 144:5149–5158
23. **List JF, Habener JF** 2004 Glucagon-like peptide 1 agonists and the development and growth of pancreatic β-cells. Am J Physiol Endocrinol Metab 286: E875–E881
24. **Drucker DJ** 2001 Minireview: the glucagon-like peptides. Endocrinology 142:521–527
25. **Buteau J, Roduit R, Susini S, Prentki M** 1999 Glucagon-like peptide-1 promotes DNA synthesis, activates phosphatidylinositol 3-kinase and increases transcription factor pancreatic and duodenal homeobox gene 1 (PDX-1) DNA binding activity in β (INS-1)-cells. Diabetologia 42:856–864
26. **Ahrén B** 2004 GLP-1 and extra-islet effects. Horm Metab Res 36:842–845
27. **Valverde I, Morales M, Clemente F, López-Delgado MI, Delgado E, Perea A, Villanueva-Peñacarrillo ML** 1994 Glucagon-like peptide 1: a potent glycogenic hormone. FEBS Lett 349:313–316
28. **Villanueva-Peñacarrillo ML, Alcántara AI, Clemente F, Delgado E, Valverde I** 1994 Potent glycogenic effect of GLP-1(7-36)amide in rat skeletal muscle. Diabetologia 37:1163–1166
29. **Galera C, Clemente F, Alcantara A, Trapote MA, Perea A, Lopez-Delgado MI, Villanueva-Penacarrillo ML, Valverde I** 1996 Inositolphosphoglycans and diacyglycerol are possible mediators in the glycogenic effect of GLP-1(7-36)amide in BC3H-1 myocytes. Cell Biochem Funct 14:43–48
30. **Wheeler MB, Lu M, Dillon JS, Leng XH, Chen C, Boyd 3rd AE** 1993 Functional expression of the rat glucagon-like peptide-I receptor, evidence for coupling to both adenylyl cyclase and phospholipase-C. Endocrinology 133: 57–62
31. **Holst JJ** 2007 The physiology of glucagon-like peptide 1. Physiol Rev 87: 1409–1439
32. **Dardevet D, Moore MC, Neal D, DiCostanzo CA, Snead W, Cherrington AD** 2004 Insulin-independent effects of GLP-1 on canine liver glucose metabolism: duration of infusion and involvement of hepatoportal region. Am J Physiol Endocrinol Metab 287:E75–E81
33. **Elahi D, Egan JM, Shannon RP, Meneilly GS, Khatri A, Habener JF, Andersen DK** 2008 GLP-1 (9-36) amide, cleavage product of GLP-1 (7-36) amide, is a glucoregulatory peptide. Obesity 16:1501–1509
34. **Nikolaidis LA, Mankad S, Sokos GG, Miske G, Shah A, Elahi D, Shannon RP** 2004 Effects of glucagon-like peptide-1 in patients with acute myocardial infarction and left ventricular dysfunction after successful reperfusion. Circulation 109:962–965
35. **Nauck MA, Niedereichholz U, Ettler R, Holst JJ, Orskov C, Ritzel R, Schmiegel WH** 1997 Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. Am J Physiol 273:E981–E988
36. **Schirra J, Kuwert P, Wank U, Leicht P, Arnold R, Göke B, Katschinski M** 1997 Differential effects of subcutaneous GLP-1 on gastric emptying, antroduodenal motility, and pancreatic function in men. Proc Assoc Am Physicians 109:84–97
37. **Schirra J, Nicolaus M, Roggel R, Katschinski M, Storr M, Woerle HJ, Göke B** 2006 Endogenous glucagon-like peptide 1 controls endocrine pancreatic secretion and antro-pyloro-duodenal motility in humans. Gut 55:243–251
38. **Näslund E, Barkeling B, King N, Gutniak M, Blundell JE, Holst JJ, Rössner S, Hellström PM** 1999 Energy intake and appetite are suppressed by glucagon-like peptide-1 (GLP-1) in obese men. Int J Obes Relat Metab Disord 23:304–311
39. **Flint A, Raben A, Ersbøll AK, Holst JJ, Astrup A** 2001 The effect of physiological levels of glucagon-like peptide-1 on appetite, gastric emptying, energy and substrate metabolism in obesity. Int J Obes Relat Metab Disord 25:781–792
40. **Meier S, Hücking K, Ritzel R, Holst JJ, Schmiegel WH, Nauck MA** 2003 Absence of a memory effect for the insulinotropic action of glucagon-like peptide 1 (GLP-1) in healthy volunteers. Horm Metab Res 35:551–556
41. **Silvestre RA, Rodríguez-Gallardo J, Egido EM, Marco J** 2003 Interrelationship among insulin, glucagon and somatostatin secretory responses to exendin-4 in the perfused rat pancreas. Eur J Pharmacol 469:195–200
42. **Ling Z, Wu D, Zambre Y, Flamez D, Drucker DJ, Pipeleers DG, Schuit FC** 2001 Glucagon-like peptide 1 receptor signaling influences topography of islet cells in mice. Virchows Arch 438:382–387
43. **Nagai K, Tsuchiya K, Ezaki T, Tsuchiya M, Ohgawara H** 2004 Effect of GLP-1 (glucagon-like peptide 1:7-36 amide) on porcine pancreatic endocrine cell proliferation and insulin secretion. Pancreas 28:138–145
44. **D'Alessio D** 2008 Intestinal hormones and regulation of satiety: the case for CCK, GLP-1, PYY, and Apo A-IV. JPEN J Parenter Enteral Nutr 32:567–568
45. **Drucker DJ** 2006 The biology of incretin hormones. Cell Metab 3:153–165
46. **Vrang N, Phifer CB, Corkern MM, Berthoud HR** 2003 Gastric distension induces c-Fos in medullary GLP-1/2-containing neurons. Am J Physiol Regul Integr Comp Physiol 285:R470–R478
47. **Kinzig KP, D'Alessio DA, Seeley RJ** 2002 The diverse roles of specific GLP-1Rs in the control of food intake and the response to visceral illness. J Neurosci 22:10470–10476
48. **Larsen PJ, Tang-Christensen M, Jessop DS** 1997 Central administration of glucagon-like peptide-1 activates hypothalamic neuroendocrine neurons in the rat. Endocrinology 138:4445–4455
49. **Dakin CL, Small CJ, Batterham RL, Neary NM, Cohen MA, Patterson M, Ghatei MA, Bloom SR** 2004 Peripheral oxyntomodulin reduces food intake and body weight gain in rats. Endocrinology 145:2687–2695
50. **Imeryüz N, Yeðen BC, Bozkurt A, Coþkun T, Villanueva-Peñacarrillo ML, Ulusoy NB** 1997 Glucagon-like peptide-1 inhibits gastric emptying via vagal afferent-mediated central mechanisms. Am J Physiol 273:G920–G927
51. **Kakei M, Yada T, Nakagawa A, Nakabayashi H** 2002 Glucagon-like peptide-1 evokes action potentials and increases cytosolic Ca2+ in rat nodose ganglion neurons. Auton Neurosci 102:39–44
52. **Baggio LL, Huang Q, Brown TJ, Drucker DJ** 2004 A recombinant human glucagon-like peptide (GLP)-1-albumin protein (albugon) mimics peptidergic activation of GLP-1R-dependent pathways coupled with satiety, gastrointestinal motility, and glucose homeostasis. Diabetes 53:2492–2500
53. **Meier JJ, Kemmeries G, Holst JJ, Nauck MA** 2005 Erythromycin antagonizes the deceleration of gastric emptying by glucagon-like peptide 1 and unmasks its insulinotropic effect in healthy subjects. Diabetes 54:2212–2218
54. **Schmidtler J, Dehne K, Allescher HD, Schusdziarra V, Classen M, Holst JJ, Polack A, Schepp W** 1994 Rat parietal cell receptors for GLP-1-(7-36) amide: Northern blot, cross-linking, and radioligand binding. Am J Physiol 267: G423–G432
55. **Wettergren A, Wøjdemann M, Meisner S, Stadil F, Holst JJ** 1997 The inhibitory effect of glucagon-like peptide-1 (GLP-1) 7-36 amide on gastric acid secretion in humans depends on an intact vagal innervation. Gut 40:597–601
56. **Delgado-Aros S, Kim DY, Burton DD, Thomforde GM, Stephens D, Brinkmann BH, Vella A, Camilleri M** 2002 Effect of GLP-1 on gastric volume, emptying, maximum volume ingested, and postprandial symptoms in humans. Am J Physiol Gastrointest Liver Physiol 282:G424–G431
57. **Schirra J, Katschinski M, Weidmann C, Schäfer T, Wank U, Arnold R, Göke**

B 1996 Gastric emptying and release of incretin hormones after glucose ingestion in humans. J Clin Invest 97:92–103
58. **D'Alessio DA, Vogel R, Prigeon R, Laschansky E, Koerker D, Eng J, Ensinck JW** 1996 Elimination of the action of glucagon-like peptide 1 causes an impairment of glucose tolerance after nutrient ingestion by healthy baboons. J Clin Invest 97:133–138
59. **Vella A, Bock G, Giesler PD, Burton DB, Serra DB, Saylan ML, Dunning BE, Foley JE, Rizza RA, Camilleri M** 2007 Effects of dipeptidyl peptidase-4 inhibition on gastrointestinal function, meal appearance, and glucose metabolism in type 2 diabetes. Diabetes 56:1475–1480
60. **Salehi M, Vahl TP, D'Alessio DA** 2008 Regulation of islet hormone release and gastric emptying by endogenous GLP-1 following glucose ingestion. J Clin Endocrinol Metab 93:4909–4916
61. **Elahi D, Nagulesparan M, Hershcopf RJ, Muller DC, Tobin JD, Blix PM, Rubenstein AH, Unger RH, Andres R** 1982 Feedback inhibition of insulin secretion by insulin: relation to the hyperinsulinemia of obesity. N Engl J Med 306:1196–1202
62. **Bullock BP, Heller RS, Habener JF** 1996 Tissue distribution of messenger ribonucleic acid encoding the rat glucagon-like peptide-1 receptor. Endocrinology 137:2968–2978
63. **Ban K, Noyan-Ashraf MH, Hoefer J, Bolz SS, Drucker DJ, Husain M** 2008 Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. Circulation 117:2340–2350
64. **Barragán JM, Rodríguez RE, Blázquez E** 1994 Changes in arterial blood pressure and heart rate induced by glucagon-like peptide-1-(7-36) amide in rats. Am J Physiol 266:E459–E466
65. **Yamamoto H, Lee CE, Marcus JN, Williams TD, Overton JM, Lopez ME, Hollenberg AN, Baggio L, Saper CB, Drucker DJ, Elmquist JK** 2002 Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons. J Clin Invest 110:43–52
66. **Barragán JM, Eng J, Rodríguez R, Blázquez E** 1999 Neural contribution to the effect of glucagon-like peptide-1-(7-36) amide on arterial blood pressure in rats. Am J Physiol 277:E784–E791
67. **Nikolaidis LA, Elahi D, Hentosz T, Doverspike A, Huerbin R, Zourelias L, Stolarski C, Shen YT, Shannon RP** 2004 Recombinant glucagon-like peptide-1 increases myocardial glucose uptake and improves left ventricular performance in conscious dogs with pacing-induced dilated cardiomyopathy. Circulation 110:955–961
68. **Sokos GG, Nikolaidis LA, Mankad S, Elahi D, Shannon RP** 2006 Glucagon-like peptide-1 infusion improves left ventricular ejection fraction and functional status in patients with chronic heart failure. J Card Fail 12:694–699
69. **Bose AK, Mocanu MM, Carr RD, Yellon DM** 2005 Glucagon like peptide-1 is protective against myocardial ischemia/reperfusion injury when given either as a preconditioning mimetic or at reperfusion in an isolated rat heart model. Cardiovasc Drugs Ther 19:9–11
70. **Nikolaidis LA, Doverspike A, Hentosz T, Zourelias L, Shen YT, Elahi D, Shannon RP** 2005 Glucagon-like peptide-1 limits myocardial stunning following brief coronary occlusion and reperfusion in conscious canines. J Pharmacol Exp Ther 312:303–308
71. **Zhao T, Parikh P, Bhashyam S, Bolukoglu H, Poornima I, Shen YT, Shannon RP** 2006 Direct effects of glucagon-like peptide-1 on myocardial contractility and glucose uptake in normal and postischemic isolated rat hearts. J Pharmacol Exp Ther 317:1106–1113
72. **Sokos GG, Bolukoglu H, German J, Hentosz T, Magovern Jr GJ, Maher TD, Dean DA, Bailey SH, Marrone G, Benckart DH, Elahi D, Shannon RP** 2007 Effect of glucagon-like peptide-1 (GLP-1) on glycemic control and left ventricular function in patients undergoing coronary artery bypass grafting. Am J Cardiol 100:824–829
73. **Golpon HA, Puechner A, Welte T, Wichert PV, Feddersen CO** 2001 Vasorelaxant effect of glucagon-like peptide-(7-36)amide and amylin on the pulmonary circulation of the rat. Regul Pept 102:81–86
74. **Bose AK, Mocanu MM, Carr RD, Brand CL, Yellon DM** 2005 Glucagon-like peptide 1 can directly protect the heart against ischemia/reperfusion injury. Diabetes 54:146–151
75. **Mayo KE, Miller LJ, Bataille D, Dalle S, Göke B, Thorens B, Drucker DJ** 2003 International Union of Pharmacology. XXXV. The glucagon receptor family. Pharmacol Rev 55:167–194
76. **Thorens B** 1992 Expression cloning of the pancreatic β cell receptor for the gluco-incretin hormone glucagon-like peptide 1. Proc Natl Acad Sci USA 89:8641–8645
77. **Nikolaidis LA, Elahi D, Shen YT, Shannon RP** 2005 Active metabolite of GLP-1 mediates myocardial glucose uptake and improves left ventricular performance in conscious dogs with dilated cardiomyopathy. Am J Physiol Heart Circ Physiol 289:H2401–H2408
78. **Brunner F, Maier R, Andrew P, Wölkart G, Zechner R, Mayer B** 2003 Attenuation of myocardial ischemia/reperfusion injury in mice with myocyte-specific overexpression of endothelial nitric oxide synthase. Cardiovasc Res 57:55–62
79. **Poornima IB, Bhashyam S, Parikh P, Bolukoglu H, Shannon R** 2008 Chronic glucagon-like peptide-1 infusion sustains left ventricular function and prolongs survival in spontaneously hypertensive, heart failure prone rats. Circ Heart Fail 1:153–160
80. **D'Alessio D, Vahl T, Prigeon R** 2004 Effects of glucagon-like peptide 1 on the hepatic glucose metabolism. Horm Metab Res 36:837–841
81. **Alcántara AI, Morales M, Delgado E, López-Delgado MI, Clemente F, Luque MA, Malaisse WJ, Valverde I, Villanueva-Peñacarrillo ML** 1997 Exendin-4 agonist and exendin (9-39) amide antagonist of the GLP-1 (7-36) amide effects in liver and muscle. Arch Biochem Biophys 341:1–7
82. **López-Delgado MI, Morales M, Villanueva-Peñacarrillo ML, Malaisse WJ, Valverde I** 1998 Effects of glucagon-like peptide 1 on the kinetics of glycogen synthase A in hepatocytes from normal and diabetic rats. Endocrinology 139:2811–2817
83. **Nathan JD, Zdankiewicz PD, Wang J, Spector SA, Aspelund G, Jena BP, Seymour NE, Geibel JP, Andersen DK** 2001 Impaired hepatocyte glucose transport protein (GLUT2) internalization in chronic pancreatitis. Pancreas 22:172–178
84. **Andersen DK, Ruiz CL, Burant CF** 1994 Insulin regulation of hepatic glucose transporter protein is impaired in chronic pancreatitis. Ann Surg 219:679–686; discussion, 686–677
85. **Eisenberg ML, Maker AV, Slezak LA, Nathan JD, Sritharan KC, Jena BP, Geibel JP, Andersen DK** 2005 Insulin receptor (IR) and glucose transporter 2 (GLUT2) proteins form a complex on the rat hepatocyte membrane. Cell Physiol Biochem 15:51–58
86. **Brunicardi FC, Chaiken RL, Ryan AS, Seymour NE, Hoffmann JA, Lebovitz HE, Chance RE, Gingerich RL, Andersen DK, Elahi D** 1996 Pancreatic polypeptide administration improves abnormal glucose metabolism in patients with chronic pancreatitis. J Clin Endocrinol Metab 81:3566–3572
87. **Andersen DK** 2007 Mechanisms and emerging treatments of the metabolic complications of chronic pancreatitis. Pancreas 35:1–15
88. **Egan JM, Meneilly GS, Habener JF, Elahi D** 2002 Glucagon-like peptide-1 augments insulin-mediated glucose uptake in the obese state. J Clin Endocrinol Metab 87:3768–3773
89. **Vahl TP, Paty BW, Fuller BD, Prigeon RL, D'Alessio DA** 2003 Effects of GLP-1-(7-36)NH2, GLP-1-(7-37), and GLP-1-(9-36)NH2 on intravenous glucose tolerance and glucose-induced insulin secretion in healthy humans. J Clin Endocrinol Metab 88:1772–1779
90. **Deacon CF, Nauck MA, Toft-Nielsen M, Pridal L, Willms B, Holst JJ** 1995 Both subcutaneously and intravenously administered glucagon-like peptide I are rapidly degraded from the NH2-terminus in type II diabetic patients and in healthy subjects. Diabetes 44:1126–1131
91. **Campos RV, Lee YC, Drucker DJ** 1994 Divergent tissue-specific and developmental expression of receptors for glucagon and glucagon-like peptide-1 in the mouse. Endocrinology 134:2156–2164
92. **Sandhu H, Wiesenthal SR, MacDonald PE, McCall RH, Tchipashvili V, Rashid S, Satkunarajah M, Irwin DM, Shi ZQ, Brubaker PL, Wheeler MB, Vranic M, Efendic S, Giacca A** 1999 Glucagon-like peptide 1 increases insulin sensitivity in depancreatized dogs. Diabetes 48:1045–1053
93. **Zander M, Madsbad S, Deacon CF, Holst JJ** 2006 The metabolite generated by dipeptidyl-peptidase 4 metabolism of glucagon-like peptide-1 has no influence on plasma glucose levels in patients with type 2 diabetes. Diabetologia 49:369–374
94. **Jin SL, Han VK, Simmons JG, Towle AC, Lauder JM, Lund PK** 1988 Distribution of glucagonlike peptide I (GLP-I), glucagon, and glicentin in the rat brain: an immunocytochemical study. J Comp Neurol 271:519–532
95. **Van Dijk G, Thiele TE, Donahey JC, Campfield LA, Smith FJ, Burn P, Bernstein IL, Woods SC, Seeley RJ** 1996 Central infusions of leptin and GLP-1-(7-36) amide differentially stimulate c-FLI in the rat brain. Am J Physiol 271: R1096–R1100
96. **Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JP, Smith DM, Ghatei MA, Herbert J, Bloom SR** 1996 A role for glucagon-like peptide-1 in the central regulation of feeding. Nature 379:69–72
97. **Nakade Y, Tsukamoto K, Pappas TN, Takahashi T** 2006 Central glucagon like peptide-1 delays solid gastric emptying via central CRF and peripheral sympathetic pathway in rats. Brain Res 1111:117–121
98. **Nishizawa M, Nakabayashi H, Kawai K, Ito T, Kawakami S, Nakagawa A, Niijima A, Uchida K** 2000 The hepatic vagal reception of intraportal GLP-1 is via receptor different from the pancreatic GLP-1R. J Auton Nerv Syst 80:14–21

99. **Tang-Christensen M, Larsen PJ, Göke R, Fink-Jensen A, Jessop DS, Møller M, Sheikh SP** 1996 Central administration of GLP-1-(7-36) amide inhibits food and water intake in rats. Am J Physiol 271:R848–R856
100. **Meeran K, O'Shea D, Edwards CM, Turton MD, Heath MM, Gunn I, Abusnana S, Rossi M, Small CJ, Goldstone AP, Taylor GM, Sunter D, Steere J, Choi SJ, Ghatei MA, Bloom SR** 1999 Repeated intracerebroventricular administration of glucagon-like peptide-1-(7-36) amide or exendin-(9-39) alters body weight in the rat. Endocrinology 140:244–250
101. **Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, Wren AM, Brynes AE, Low MJ, Ghatei MA, Cone RD, Bloom SR** 2002 Gut hormone PYY(3-36) physiologically inhibits food intake. Nature 418:650–654
102. **Batterham RL, Cohen MA, Ellis SM, Le Roux CW, Withers DJ, Frost GS, Ghatei MA, Bloom SR** 2003 Inhibition of food intake in obese subjects by peptide YY3-36. N Engl J Med 349:941–948
103. **Beak SA, Small CJ, Ilovaiskaia I, Hurley JD, Ghatei MA, Bloom SR, Smith DM** 1996 Glucagon-like peptide-1 (GLP-1) releases thyrotropin (TSH): characterization of binding sites for GLP-1 on α-TSH cells. Endocrinology 137:4130–4138
104. **Beak SA, Heath MM, Small CJ, Morgan DG, Ghatei MA, Taylor AD, Buckingham JC, Bloom SR, Smith DM** 1998 Glucagon-like peptide-1 stimulates luteinizing hormone-releasing hormone secretion in a rodent hypothalamic neuronal cell line. J Clin Invest 101:1334–1341
105. **Seeley RJ, Blake K, Rushing PA, Benoit S, Eng J, Woods SC, D'Alessio D** 2000 The role of CNS glucagon-like peptide-1 (7-36) amide receptors in mediating the visceral illness effects of lithium chloride. J Neurosci 20:1616–1621
106. **Jang HJ, Kokrashvili Z, Theodorakis MJ, Carlson OD, Kim BJ, Zhou J, Kim HH, Xu X, Chan SL, Juhaszova M, Bernier M, Mosinger B, Margolskee RF, Egan JM** 2007 Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. Proc Natl Acad Sci USA 104:15069–15074
107. **Shin YK, Martin B, Golden E, Dotson CD, Maudsley S, Kim W, Jang HJ, Mattson MP, Drucker DJ, Egan JM, Munger SD** 2008 Modulation of taste sensitivity by GLP-1 signaling. J Neurochem 106:455–463
108. **Egan JM, Margolskee RF** 2008 Taste cells of the gut and gastrointestinal chemosensation. Mol Interv 8:78–81
109. **Szayna M, Doyle ME, Betkey JA, Holloway HW, Spencer RG, Greig NH, Egan JM** 2000 Exendin-4 decelerates food intake, weight gain, and fat deposition in Zucker rats. Endocrinology 141:1936–1941
110. **Young AA, Gedulin BR, Bhavsar S, Bodkin N, Jodka C, Hansen B, Denaro M** 1999 Glucose-lowering and insulin-sensitizing actions of exendin-4: studies in obese diabetic (ob/ob, db/db) mice, diabetic fatty Zucker rats, and diabetic rhesus monkeys (*Macaca mulatta*). Diabetes 48:1026–1034
111. **Schnabel CA, Wintle M, Kolterman O** 2006 Metabolic effects of the incretin mimetic exenatide in the treatment of type 2 diabetes. Vasc Health Risk Manag 2:69–77
112. **Garber A, Henry R, Ratner R, Garcia-Hernandez PA, Rodriguez-Pattzi H, Olvera-Alvarez I, Hale PM, Zdravkovic M, Bode B** 2009 Liraglutide versus glimepiride monotherapy for type 2 diabetes (LEAD-3 Mono): a randomised, 52-week, phase III, double-blind, parallel-treatment trial. Lancet 373:473–481
113. **Zander M, Madsbad S, Madsen JL, Holst JJ** 2002 Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and β-cell function in type 2 diabetes: a parallel-group study. Lancet 359:824–830
114. **Meneilly GS, Greig N, Tildesley H, Habener JF, Egan JM, Elahi D** 2003 Effects of 3 months of continuous subcutaneous administration of glucagon-like peptide 1 in elderly patients with type 2 diabetes. Diabetes Care 26:2835–2841
115. **Cabou C, Campistron G, Marsollier N, Leloup C, Cruciani-Guglielmacci C, Pénicaud L, Drucker DJ, Magnan C, Burcelin R** 2008 Brain glucagon-like peptide-1 regulates arterial blood flow, heart rate, and insulin sensitivity. Diabetes 57:2577–2587
116. **Nyström T, Gonon AT, Sjöholm A, Pernow J** 2005 Glucagon-like peptide-1 relaxes rat conduit arteries via an endothelium-independent mechanism. Regul Pept 125:173–177
117. **Basu A, Charkoudian N, Schrage W, Rizza RA, Basu R, Joyner MJ** 2007 Beneficial effects of GLP-1 on endothelial function in humans: dampening by glyburide but not by glimepiride. Am J Physiol Endocrinol Metab 293:E1289–E1295
118. **Saraceni C, Broderick TL** 2007 Effects of glucagon-like peptide-1 and long-acting analogues on cardiovascular and metabolic function. Drugs R D 8:145–153