Experimental Diabetes Research

Glucagon-Like Peptide-1 and Diabetes 2012

Guest Editors: Matteo Monami, Giovanni Di Pasquale, Anne Rowzee, Carlo Maria Rotella, and Edoardo Mannucci

Glucagon-Like Peptide-1 and Diabetes 2012

Experimental Diabetes Research

Glucagon-Like Peptide-1 and Diabetes 2012

Guest Editors: Matteo Monami, Giovanni Di Pasquale, Anne Rowzee, Carlo Maria Rotella, and Edoardo Mannucci

Editorial Board

Contents

Glucagon-Like Peptide-1 and Diabetes 2012, Matteo Monami, Giovanni Di Pasquale, Anne Rowzee, Carlo Maria Rotella, and Edoardo Mannucci
Volume 2012, Article ID 768760, 1 page

Bone: Incretin Hormones Perceiver or Receiver?, Ilaria Dicembrini, Edoardo Mannucci, and Carlo Maria Rotella
Volume 2012, Article ID 519784, 5 pages

Effects of Glucagon-Like Peptide-1 Receptor Agonists on Body Weight: A Meta-Analysis, Matteo Monami, Ilaria Dicembrini, Niccolò Marchionni, Carlo M. Rotella, and Edoardo Mannucci
Volume 2012, Article ID 672658, 8 pages

Physiology and Emerging Biochemistry of the Glucagon-Like Peptide-1 Receptor, Francis S. Willard and Kyle W. Sloop
Volume 2012, Article ID 470851, 12 pages

Small Molecule Drug Discovery at the Glucagon-Like Peptide-1 Receptor, Francis S. Willard, Ana B. Bueno, and Kyle W. Sloop
Volume 2012, Article ID 709893, 9 pages

Cardiovascular Benefits of GLP-1-BasedTherapies in Patients with Diabetes Mellitus Type 2: Effects on Endothelial and Vascular Dysfunction beyond Glycemic Control, Thomas Forst, Matthias M. Weber, and Andreas Pfützner
Volume 2012, Article ID 635472, 9 pages

Hindawi Publishing Corporation
Experimental Diabetes Research
Volume 2012, Article ID 768760, 1 page
doi:10.1155/2012/768760

Editorial

Glucagon-Like Peptide-1 and Diabetes 2012

Matteo Monami,[1] Giovanni Di Pasquale,[2] Anna Rowzee,[2] Carlo Maria Rotella,[3] and Edoardo Mannucci[4]

[1] *Section of Geriatric Cardiology and Medicine, Careggi Teaching Hospital, Via delle Oblate 4, 50141 Florence, Italy*
[2] *National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, USA*
[3] *Obesity Agency, Careggi Teaching Hospital, Florence, Italy*
[4] *Diabetes Agency, Careggi Teaching Hospital, Florence, Italy*

Correspondence should be addressed to Matteo Monami, mmonami@libero.it

Received 21 November 2012; Accepted 21 November 2012

The present special issue was aimed at elucidating the role and the effects of glucagon-like peptide-1 (GLP-1) in type 2 diabetes. GLP-1 is a gastrointestinal hormone, mainly secreted after meals, capable of increasing glucose-stimulated insulin release and inhibits food intake. The physiological activity of this hormone has been demonstrated to be impaired in obese subjects and in patients with type 2 diabetes in comparison with healthy subjects.

Available data suggest that GLP-1 plays a relevant role in the regulation of postprandial glucose metabolism in physiologic conditions. Several new drugs act through the GLP-1 signaling system to stimulate insulin release and regulate blood glucose levels in patients with diabetes. Therefore, a special issue exploring the physiological properties of GLP-1 and the possible applications in several clinical settings is particularly warranted.

This special issue is composed of 5 articles: two mechanistic studies and three systematic reviews and meta-analyses, exploring GLP-1-induced signaling mechanisms and molecular identification and cloning of the GLP-1 receptor. The reviews and meta-analyses are focused on the promising beneficial extraglycaemic effects of the incretin-based therapy, including those on lipid profile and cardiovascular risk.

Matteo Monami
Giovanni Di Pasquale
Anna Rowzee
Carlo Maria Rotella
Edoardo Mannucci

Hindawi Publishing Corporation
Experimental Diabetes Research
Volume 2012, Article ID 519784, 5 pages
doi:10.1155/2012/519784

Review Article

Bone: Incretin Hormones Perceiver or Receiver?

Ilaria Dicembrini,[1] Edoardo Mannucci,[2] and Carlo Maria Rotella[1]

[1] *Section of Endocrinology, Department of Clinical Pathophysiology, Careggi Teaching Hospital, University of Florence and Obesity Agency, 50127 Florence, Italy*
[2] *Careggi Teaching Hospital, Diabetes Agency, 50127 Florence, Italy*

Correspondence should be addressed to Carlo Maria Rotella, c.rotella@dfc.unifi.it

Received 6 March 2012; Accepted 9 May 2012

Academic Editor: Giovanni Di Pasquale

Novel incretin-based drugs, such as glucagon-like peptide-1 receptor agonists (GLP-1 RA) and dipeptidyl peptidase-4 inhibitors (DPP-4i), have been last introduced in the pharmacological treatment of type 2 diabetes. In the last few years, the interest on the relationship of gut hormones with bone metabolism in diabetes has been increasing. The aim of present paper is to examine *in vitro* and *in vivo* evidence on the connections between incretin hormones and bone metabolism. We also discuss results of clinical trials and metaanalysis, explore the effects of incretin drugs *in vitro* on osteogenic cells and osteoclasts, and speculate on the possibility of different effects of GLP-1 RA and DPP-4i on the risk of bone fractures risk in humans. Although existing preliminary evidence suggests a protective effect on the bone, at least for DPP-4i, further controlled, long-term studies with measurement of bone markers, bone density, and clinical fractures rates are needed to substantiate and confirm those findings.

1. Introduction

Glucose, protein, and fat and mixed meal ingestion is associated with a significant reduction in markers of bone resorption, detectable by twenty minutes after feeding [1]. Bone formation is also influenced, but it seems to be less responsive to nutrients than resorption [2]. Biochemical assessment of bone turnover demonstrates that food intake is the major cause of the reduced bone turnover during daytime, which is followed by a nocturnal increase [3]. In addition, the observation that parenteral feeding is related to bone mass reduction [4] suggests a functional link between gut and bone metabolism through hormones responding to nutrients absorption, such as, incretins. The concept of incretins has been introduced to define gastrointestinal hormones released after meal ingestion, which modulate glucose homeostasis, mainly through both glucose-induced enhancement of insulin secretion and inhibition of glucagon release, such as glucagon-like peptide-1 (GLP-1). Beneficial extraglycemic actions on body weight, blood pressure, dyslipidemia, cardiac and endothelial function are further reported. Novel drugs based on the incretin system, such as, glucagon-like peptide-1 receptor agonists (GLP-1 RA) and dipeptidyl peptidase-4 inhibitors (DPP-4i), have been approved for the therapy of type 2 diabetes [5]. In the last few years, the interest on the relationship of gut hormones with bone formation and turnover in diabetes has been increasing, with preliminary data suggesting the possibility of positive effects of GLP-1 RA and DPP-4i on bone health. The aim of present paper is to examine in vitro and in vivo evidences on the connections between incretin hormones and bone metabolism. We also discuss results of clinical trials and meta-analysis, thus explore investigating the in vitro effects of incretin drugs in vitro on osteoblasts and osteoclasts, and speculate on the cells and presenting the possibility of different effects of GLP-1 RA and DPP-4i effects on the risk of bone fractures risk in humans clinical studies.

2. The Gut-Brain-Bone Axis and Diabetes

The regulation of bone turnover in response to feeding is complex with probable involvement of several mediators. The most important mediators identified are intestinal (GLP-1, GLP-2, Glucose-dependent Insulinotropic Peptide or GIP, and Peptide YY) and pancreatic beta cell (insulin,

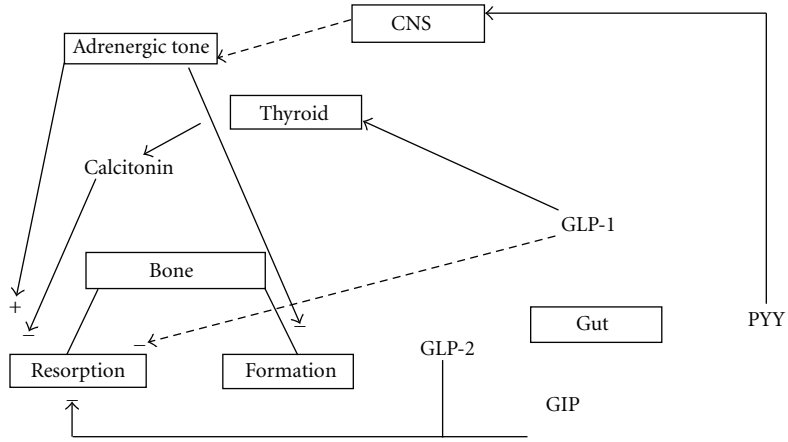


Figure 1: Gut mediators of the acute bone turnover in response to feeding. GLP-1: glucagon-like peptide-1; GLP-2: glucagon-like peptide-2; GIP: glucose-dependent insulinotropic peptide; PYY: peptide YY; CNS: central Nervous System. Broken lines represent putative pathways.

amylin, preptin, and pancreatic polypeptide) hormones [6]. Pancreatic peptides have direct actions on bone cells, while Peptide YY probably acts through arcuate nucleus in the central nervous system, thus regulating adrenergic tone and bone metabolism (Figure 1).

Diabetes is related to an increased risk of bone fractures [7]. A systematic review performed on 16 eligible studies indicates a significant increased risk of hip fracture both in type 2 diabetic women (overall relative risk (RR) 2.1; 95% confidence interval (CI): 1.3, 2.2) and men (overall RR 2.8; 95% CI: 2.6, 15.1) [8]. The observed increase in fracture risk is likely to be related to impaired bone quality rather than to bone mineral density. The related mechanisms, due at least in part to hyperglycemia, neuropathy, and higher incidence of hypovitaminosis D, are not yet fully understood [9]. However, disease progression is associated with low bone turnover, suggesting potential influences of antidiabetic agents on bone density and fracture rates. The increased incidence of bone fractures in patients with diabetes could also be due, at least part, to the effect of glucose- lowering therapies. It has been observed that long-term treatment with thiazolidinediones (TZDs) is associated with an increased risk of fracture in women with type 2 diabetes compared with other antidiabetic agents [10, 11]. The effect of TZD on bone fractures could be due to a specific inhibition of osteoblast differentiation and activity [12]. Furthermore, most available studies report a higher incidence of bone fractures in insulin-treated patients, in comparison with noninsulin-treated type 2 diabetic individuals [13], even after adjusting for concomitant antidiabetic medications [14]. The underlying mechanism is not completely understood, however, the contribution of an increased risk of falls induced by hypoglycemia cannot be excluded [15]. Moreover, a modifiable nutritional factor, such as, vitamin D deficiency, is also believed to play a role. Recent epidemiological evidence supports an increasing prevalence of hypovitaminosis D, inversely related to BMI, in all subset populations including children and adolescents [16]. Low 25-OH vitamin D levels are associated with higher probability of future diagnosis of type 2 diabetes, and in patients with established diabetes, with an increased incidence and progression of macro- and microvascular complications [17]. Cross-sectional studies confirmed an association between vitamin D status and risk of falls [18], but evidence from randomized clinical trials is required.

3. GLP-1 and Bone: Mechanisms of Action

GLP-1 is secreted by intestinal endocrine L cells, mainly after nutrient intake and rapidly inactivated by DPP-4 produced by endothelial cells. GLP-1 stimulates insulin secretion and inhibits glucagon secretion both in a glucose-dependent manner, thus ameliorating glucose homeostasis. A wide range of extrapancreatic actions on body weight, lipid profile and cardiovascular system has been recently described [5]. Mixed meal [19] and oral-glucose-load-induced [20] GLP-1 response have been reported to be reduced in Type 2 diabetes in comparison with healthy subjects; considering the possible involvement of GLP-1 in bone metabolism, the impairment of the GLP-1 axis could theoretically contribute to the increased risk of fractures in type 2 diabetes.

The actions of GLP-1 are predominantly mediated by a G protein-coupled receptor (GLP-1 R) expressed in the pancreas, stomach, intestine, kidney, lung, vascular system, heart, and brain. GLP-1 R activation stimulates adenylate cyclase, with formation of cyclic adenosine monophosphate (cAMP) and subsequent phosphorylation of protein kinase A [21]. In rodents, GLP-1 R has been detected on parafollicular thyroid C cells and GLP-1-mediated activation leads to C-cell proliferation and to calcitonin release, which could contribute to decrease bone resorption [22]. Moreover, genetic disruption of GLP-1 R in Glp-1 $r^{-/-}$ knockout mice resulted in decreased cortical bone mass, and increased osteoclasts number. The bone resorption increase appeared to be sensitive to an acute calcitonin administration, thus promoting a calcitonin-dependent pathway in the GLP-1 mediated control of bone metabolism [23]. However, important differences on the expression levels of GLP-1 R between rodents and human have been described. In rodents

(both mice and rats) C cells are relatively abundant, and calcitonin represents an important regulatory hormone in calcium homeostasis. In humans, conversely, C cells are significantly less represented and the physiological role of the hormone, except for some circumstances, such as, pregnancy and lactation, is uncertain [24]. Knudsen et al. showed a lack of functional response to GLP-1 in terms of cAMP production and calcitonin release in human TT thyroid C-cell line compared to rat C-cell lines MTC 6–23 and CA-77. The clinical relevance of these findings was confirmed by large clinical trials performed in type 2 diabetic patients treated with GLP-1 R agonists [22].

The possibility that GLP-1 might directly act on bone cells has also been investigated. The G protein-coupled GLP-1 R is expressed on human osteoblastic precursor cells [25] but not on mature osteoblasts [26]. The osteoblast activity modulation by GLP-1 seems to be related to different development stage. In human bone marrow stromal cells, GLP-1 promotes cellular proliferation and cytoprotection, preventing differentiation into adipocytes [27]. It has been recently demonstrated that GLP-1 can functionally interact with osteoblastic cells through a receptor, different from the GLP-1 R previously described. In liver and muscle [28], the effects of GLP-1 on glucose homeostasis are not related to a cAMP stimulation but to a rapid hydrolysis of glycosylphosphatidylinositoli (GIPs), generating inositolphosphoglycans (IPGs) and to a phosphatidylinositol-3 kinase (PI3K) and mitogen activated protein kinase (MAPK) activities. In a well-characterized later stage of osteoblastic cell line, such as MC3T3-EI, GLP-1 has shown to promote the immediate hydrolysis of GPIs, and this effect is consistent with the specific binding to a functional receptor independent of the cAMP-linked GLP-1 R. These data support the effect of IPGs as a second messenger and a GLP-1-induced stimulation upon PI3K and the existence of MAPK activities in osteoblastic cells [29] but required confirmation in vivo, particularly in humans.

In streptozotocin-induced diabetic and fructose-stimulated insulin-resistant rats, an insulin- and PTH-independent bone anabolic effect of GLP-1 has been recently shown, following 3-day continuous infusion on the trabecular bone structure [30]. In both these experimental models, GLP-1 and Exendin-4 (a natural GLP-1 RA) increased osteoprotegerin/receptor activated of NF-$\kappa\beta$ ligand (OPG/RANKL) ratio, interacting with the Wnt pathway in osteoblasts to decrease bone remodeling. In particular, analysis of bone structure by microcomputer tomography supported a trend toward a small-size increase of BMD in the appendicular skeleton [31]. Similar results were reported in high-fat diet fed rats, following the same administration scheme [32]. These studies suggest a GLP-1-induced inhibition of bone resorption by osteoclasts, through direct effects on osteoblasts both in animal models of type 2 diabetes and metabolic syndrome, thus promoting a further careful evaluation of bone effects in ongoing Phase III clinical trials investigating the efficacy of a long-acting GLP-1 R analog, such as, liraglutide, in the treatment of obesity.

In response to feeding, as previously reported, different gut mediators are cosecreted. GIP, an incretin peptide, such as, GLP-1, is released from enteroendocrine K cells and functional GIP receptors are detected on osteoblasts-like cells, thus regulating their proliferation and activity. However, GIP receptors are in vitro downregulated by continuous exposure to GIP, thus requiring a pulsatile hormone release to stimulate osteoblasts [26]. Transgenic mice overexpressing GIP show increased bone mass and reduced bone loss with aging [33]. At the same time, GLP-2 and peptide YY are cosecreted with GLP-1 from L cells after feeding. GLP-2 receptors are expressed on osteoclasts, and a related decrease on bone resorption has been shown in vitro [26]. Peptide YY knock-out mice showed a significant decreased bone mass and a further increase of bone loss after ovariectomy [34].

4. Incretins and Bone: Clinical Evidence in Humans

Long-term exposure of type 2 diabetic patients to exenatide, an incretin mimetic agent, was not significantly associated to an increased bone fracture risk, despite the progressive weight loss: at 82 weeks an average weight reduction of 4.4 kg was reported, with a mean of 11.9 kg (−11.4% of baseline body weight) in highest weight loss quartile [35]. Several previously reported studies have shown that a 5–10% weight loss is associated to a significant decrease in bone mass and to an increase of bone resorption, especially in obese postmenopausal women [36]. Moreover, bone mineral density and markers of calcium homeostasis (serum alkaline phosphatase, calcium and phosphate) were not affected by 44 week treatment with exenatide in comparison to insulin glargine, a long-acting insulin, in type 2 diabetic subjects [37].

In a recent small double blind randomized clinical trial enrolling drug naïve type 2 diabetic patients, one-year treatment with DPP-4i (vildagliptin 100 mg daily) was not significantly related to significant change both in markers of bone resorption and calcium homeostasis in comparison to placebo [38].

A recent meta-analysis was performed including 28 clinical trials with a duration of at least 24 weeks, enrolling 11,880 and 9,175 patients for DPP-4i and comparators, respectively. Following a treatment of 35 weeks mean duration, 63 bone fractures were reported as serious adverse events. Despite short duration of trials, absence of discrimination between sex and pre-/postmenopausal state and evaluation of only severe bone fractures, DPP-4i, compared with placebo or other treatments, were associated with a reduced risk of fractures (Mantel-Haenszel odds ratio [MH-OR] 0.60, 95% CI 0.37–0.99, $P = 0.045$), even after the exclusion of comparisons with thiazolidinediones or sulfonylureas (MH-OR 0.56, 0.33–0.93, $P = 0.026$) [39].

On the other hand, GLP-2 injection in postmenopausal women resulted in a significant reduction of bone turnover in a dose-dependent manner [40]. The decrease of bone resorption by GLP-2 required an intact gastrointestinal tract, where GLP-2 receptors have been located in the myenteric plexus. The lack of GLP-2 response in jejunostomy patients

[41] supported the afferent nerve fibres involvement in the regulation of bone metabolism by GLP-2.

5. Conclusions

The mechanisms through which feeding regulates bone turnover is complex and probably involved several mediators. Gastrointestinal peptides, such as, GLP-1, GIP, GLP-2 and peptide YY have been shown to favour bone formation over resorption. In the last few years, growing experimental evidences reported positive effects of novel incretin-based antidiabetic drugs on bone health. Clinical data on bone fractures risk profile during GLP-1 RA and DPP-4i therapies could vary with respect to their concomitant different (positive and neutral, resp.) effect on body weight. A positive action of GLP-1 RA on bone homeostasis could be overshadowed by weight loss-induced bone mass decrease, thus determining neutrality of GLP-1 RA treatment on bone fracture risk profile in human clinical trials. Moreover, despite stimulation of GLP-1 R through specific agonists, inhibition of incretin-hormone degrading enzyme DDP-4 enhances postprandial availability of different gut mediators of acute bone metabolism, such as, GLP-1, GIP, GLP-2, and peptide YY. Additional beneficial effects on bone health could be achieved by DPP-4i, in comparison to GLP-1 RA, through an overall involvement of the gut-brain-bone axis [6].

Taken together, this evidence could further explain potential different effects of GLP-1 RA and DPP-4i on bone fracture incidence and calcium homeostasis in human clinical studies. Further controlled, long-term studies with measurement of bone markers, bone density, and clinical fractures rates will be required to demonstrate conclusive efficacy along with underlying mechanisms responsible for incretin-related bone protection both in diabetic and not diabetic obese population. Pending further evidence, it is mandatory to promote the mainstay of osteoporosis prevention in type 2 diabetes: physically active, healthy lifestyle, and optimization of glucose control with low hypoglycemic risk, along with vitamin D repletion in deficient patients [42].

References

[1] D. B. Henriksen, P. Alexandersen, N. H. Bjarnason et al., "Role of gastrointestinal hormones in postprandial reduction of bone resorption," *Journal of Bone and Mineral Research*, vol. 18, no. 12, pp. 2180–2189, 2003.

[2] J. A. Clowes, R. A. Hannon, T. S. Yap, N. R. Hoyle, A. Blumsohn, and R. Eastell, "Effect of feeding on bone turnover markers and its impact on biological variability of measurements," *Bone*, vol. 30, no. 6, pp. 886–890, 2002.

[3] N. H. Bjarnason, E. E. G. Henriksen, P. Alexandersen, S. Christgau, D. B. Henriksen, and C. Christiansen, "Mechanism of circadian variation in bone resorption," *Bone*, vol. 30, no. 1, pp. 307–313, 2002.

[4] J. A. Clowes, S. Khosla, and R. Eastell, "Potential role of pancreatic and enteric hormones in regulating bone turnover," *Journal of Bone and Mineral Research*, vol. 20, no. 9, pp. 1497–1506, 2005.

[5] I. Dicembrini, L. Pala, and C. M. Rotella, "From theory to clinical practice in the use of GLP-1 receptor agonists and DPP-4 inhibitors therapy," *Exp Diabetes Res*, vol. 2011, Article ID 898913, 8 pages, 2011.

[6] J. S. Walsh and D. B. Henriksen, "Feeding and bone," *Archives of Biochemistry and Biophysics*, vol. 503, no. 1, pp. 11–19, 2010.

[7] N. B. Khazai, G. R. Beck, and G. E. Umpierrez, "Diabetes and fractures: an overshadowed association," *Current Opinion in Endocrinology, Diabetes and Obesity*, vol. 16, no. 6, pp. 435–445, 2009.

[8] M. Janghorbani, R. M. Van Dam, W. C. Willett, and F. B. Hu, "Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture," *American Journal of Epidemiology*, vol. 166, no. 5, pp. 495–505, 2007.

[9] T. Takiishi, C. Gysemans, R. Bouillon, and C. Mathieu, "Vitamin D and diabetes," *Endocrinology and Metabolism Clinics of North America*, vol. 39, no. 2, pp. 419–446, 2010.

[10] A. Grey, M. Bolland, G. Gamble et al., "The peroxisome proliferator-activated receptor-γ agonist rosiglitazone decreases bone formation and bone mineral density in healthy postmenopausal women: a randomized, controlled trial," *Journal of Clinical Endocrinology and Metabolism*, vol. 92, no. 4, pp. 1305–1310, 2007.

[11] S. E. Kahn, B. Zinman, J. M. Lachin et al., "Rosiglitazone-associated fractures in type 2 diabetes: an analysis from ADOPT," *Diabetes Care*, vol. 31, no. 5, pp. 845–851, 2008.

[12] S. Benvenuti, I. Cellai, P. Luciani et al., "Rosiglitazone stimulates adipogenesis and decreases osteoblastogenesis in human mesenchymal stem cells," *Journal of Endocrinological Investigation*, vol. 30, no. 9, pp. RC26–RC30, 2007.

[13] K. K. Nicodemus and A. R. Folsom, "Type 1 and type 2 diabetes and incident hip fractures in postmenopausal women," *Diabetes Care*, vol. 24, no. 7, pp. 1192–1197, 2001.

[14] M. Monami, B. Cresci, A. Colombini et al., "Bone fractures and hypoglycemic treatment in type 2 diabetic patients: a case-control study," *Diabetes Care*, vol. 31, no. 2, pp. 199–203, 2008.

[15] D. E. Bonds, J. C. Larson, A. V. Schwartz et al., "Risk of fracture in women with type 2 diabetes:the women's health initiative observational study," *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 9, pp. 3404–3410, 2006.

[16] V. Ganji, X. Zhang, and V. Tangpricha, "Serum 25-hydroxyvitamin D concentrations and prevalence estimates of hypovitaminosis D in the U.S. population based on assay-adjusted data," *Journal of Nutrition*, vol. 142, no. 3, pp. 498–507, 2012.

[17] C. Mathieu and B. J. Van Der Schueren, "Vitamin D deficiency is not good for you," *Diabetes Care*, vol. 34, no. 5, pp. 1245–1246, 2011.

[18] M. Chung, E. M. Balk, M. Brendel et al., "Vitamin D and calcium: a systematic review of health outcomes," *Evidence Report/Technology Assessment*, no. 183, pp. 1–420, 2009.

[19] T. Vilsbøll, T. Krarup, C. F. Deacon, S. Madsbad, and J. J. Holst, "Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients," *Diabetes*, vol. 50, no. 3, pp. 609–613, 2001.

[20] E. Mannucci, A. Ognibene, F. Cremasco et al., "Glucagon-like peptide (GLP)-1 and leptin concentrations in obese patients with Type 2 diabetes mellitus," *Diabetic Medicine*, vol. 17, no. 10, pp. 713–719, 2000.

[21] J. J. Holst, "The physiology of glucagon-like peptide 1," *Physiological Reviews*, vol. 87, no. 4, pp. 1409–1439, 2007.

[22] L. B. Knudsen, L. W. Madsen, S. Andersen et al., "Glucagon-like peptide-1 receptor agonists activate rodent thyroid C-cells causing calcitonin release and C-cell proliferation," *Endocrinology*, vol. 151, no. 4, pp. 1473–1486, 2010.

[23] C. Yamada, Y. Yamada, K. Tsukiyama et al., "The murine glucagon-like peptide-1 receptor is essential for control of bone resorption," *Endocrinology*, vol. 149, no. 2, pp. 574–579, 2008.

[24] P. F. Hirsch and H. Baruch, "Is calcitonin an important physiological substance?" *Endocrine*, vol. 21, no. 3, pp. 201–208, 2003.

[25] E. L. Pacheco-Pantoja, L. R. Ranganath, J. A. Gallagher, P. J. M. Wilson, and W. D. Fraser, "Receptors and effects of gut hormones in three osteoblastic cell lines," *BMC Physiology*, vol. 11, article 12, 2011.

[26] R. J. Bollag, Q. Zhong, P. Phillips et al., "Osteoblast-derived cells express functional glucose-dependent insulinotropic peptide receptors," *Endocrinology*, vol. 141, no. 3, pp. 1228–1235, 2000.

[27] C. Sanz, P. Vázquez, C. Blázquez, P. A. Barrio, M. D. M. Alvarez, and E. Blázquez, "Signaling and biological effects of glucagon-like peptide 1 on the differentiation of mesenchymal stem cells from human bone marrow," *American Journal of Physiology*, vol. 298, no. 3, pp. E634–E643, 2010.

[28] M. L. Villanueva-Peñacarrillo, E. Delgado, M. A. Trapote et al., "Glucagon-like peptide-1 binding to rat hepatic membranes," *Journal of Endocrinology*, vol. 146, no. 1, pp. 183–189, 1995.

[29] B. Nuche-Berenguer, S. Portal-Núñez, P. Moreno et al., "Presence of a functional receptor for GLP-1 in osteoblastic cells, independent of the cAMP-linked GLP-1 receptor," *Journal of Cellular Physiology*, vol. 225, no. 2, pp. 585–592, 2010.

[30] B. Nuche-Berenguer, P. Moreno, P. Esbrit et al., "Effect of GLP-1 treatment on bone turnover in normal, type 2 diabetic, and insulin-resistant states," *Calcified Tissue International*, vol. 84, no. 6, pp. 453–461, 2009.

[31] B. Nuche-Berenguer, P. Moreno, S. Portal-Nuñez, S. Dapía, P. Esbrit, and M. L. Villanueva-Peñacarrillo, "Exendin-4 exerts osteogenic actions in insulin-resistant and type 2 diabetic states," *Regulatory Peptides*, vol. 159, no. 1–3, pp. 61–66, 2010.

[32] B. Nuche-Berenguer, D. Lozano, I. Gutiérrez-Rojas et al., "GLP-1 and exendin-4 can reverse hyperlipidic-related osteopenia," *Journal of Endocrinology*, vol. 209, no. 2, pp. 203–210, 2011.

[33] K. H. Ding, X. M. Shi, Q. Zhong et al., "Impact of glucose-dependent insulinotropic peptide on age-induced bone loss," *Journal of Bone and Mineral Research*, vol. 23, no. 4, pp. 536–543, 2008.

[34] K. E. Wortley, K. Garcia, H. Okamoto et al., "Peptide YY regulates bone turnover in rodents," *Gastroenterology*, vol. 133, no. 5, pp. 1534–1543, 2007.

[35] L. Blonde, E. J. Klein, J. Han et al., "Interim analysis of the effects of exenatide treatment on A1C, weight and cardiovascular risk factors over 82 weeks in 314 overweight patients with type 2 diabetes," *Diabetes, Obesity and Metabolism*, vol. 8, no. 4, pp. 436–447, 2006.

[36] C. S. Riedt, M. Cifuentes, T. Stahl, H. A. Chowdhury, Y. Schlussel, and S. A. Shapses, "Overweight postmenopausal women lose bone with moderate weight reduction and 1 g/day calcium intake," *Journal of Bone and Mineral Research*, vol. 20, no. 3, pp. 455–463, 2005.

[37] M. C. Bunck, B. Eliasson, A. Cornér et al., "Exenatide treatment did not affect bone mineral density despite body weight reduction in patients with type 2 diabetes," *Diabetes, Obesity and Metabolism*, vol. 13, no. 4, pp. 374–377, 2011.

[38] M. C. Bunck, M. Poelma, E. M. Eekhoff et al., "Vildagliptin effects on postprandial markers of bone resorption and calcium homeostasis in recently diagnosed, well-controlled, type 2 diabetes patients," *Journal of Diabetes*, vol. 4, no. 2, pp. 181–185, 2012.

[39] M. Monami, I. Dicembrini, A. Antenore, and E. Mannucci, "Dipeptidyl peptidase-4 inhibitors and bone fractures: a meta-analysis of randomized clinical trials," *Diabetes Care*, vol. 34, no. 11, pp. 2474–2476, 2011.

[40] D. B. Henriksen, P. Alexandersen, B. Hartmann et al., "Four-month treatment with GLP-2 significantly increases hip BMD. A randomized, placebo-controlled, dose-ranging study in postmenopausal women with low BMD," *Bone*, vol. 45, no. 5, pp. 833–842, 2009.

[41] I. B. Gottschalck, P. B. Jeppesen, B. Hartmann, J. J. Holst, and D. B. Henriksen, "Effects of treatment with glucagon-like peptide-2 on bone resorption in colectomized patients with distal ileostomy or jejunostomy and short-bowel syndrome," *Scandinavian Journal of Gastroenterology*, vol. 43, no. 11, pp. 1304–1310, 2008.

[42] G. Bardini, I. Dicembrini, M. Serio, and C. M. Rotella, "Vitamin D therapy in adults with diabetes mellitus," *Nature Reviews Endocrinology*, vol. 7, no. 1, p. c1, 2011.

Hindawi Publishing Corporation
Experimental Diabetes Research
Volume 2012, Article ID 672658, 8 pages
doi:10.1155/2012/672658

Research Article

Effects of Glucagon-Like Peptide-1 Receptor Agonists on Body Weight: A Meta-Analysis

Matteo Monami,[1] Ilaria Dicembrini,[2] Niccolò Marchionni,[1] Carlo M. Rotella,[2] and Edoardo Mannucci[3]

[1] *Geriatric Cardiology, Careggi Teaching Hospital and University of Florence, 50141 Florence, Italy*
[2] *Obesity Agency, Careggi Teaching Hospital and University of Florence, 50141 Florence, Italy*
[3] *Diabetes Agency, Careggi Teaching Hospital and University of Florence, 50141 Florence, Italy*

Correspondence should be addressed to Edoardo Mannucci, edoardo.mannucci@unifi.it

Received 22 January 2012; Accepted 19 March 2012

Academic Editor: Giovanni Di Pasquale

Glucagon-Like Peptide-1 receptor agonists (GLP-1RAs), approved as glucose-lowering drugs for the treatment of type 2 diabetes, have also been shown to reduce body weight. An extensive Medline, Cochrane database, and Embase search for "exenatide," "liraglutide," "albiglutide," "semaglutide," and "lixisenatide" was performed, collecting all randomized clinical trials on humans up to December 15, 2011, with a duration of at least 24 weeks, comparing GLP-1 receptor agonists with either placebo or active drugs. Twenty two (7,859 patients) and 7 (2,416 patients) trials with available results on body weight at 6 and 12 months, respectively, were included. When compared with placebo, GLP-1RAs determine a reduction of BMI at 6 months of -1.0 $[-1.3; -0.6]$ kg/m^2. Considering the average BMI at baseline (32.4 kg/m^2) these data means a weight reduction of about 3% at 6 months. This result could seem modest from a clinical standpoint; however, it could be affected by many factors contributing to an underestimation of the effect of GLP-1RA on body weight, such as non adequate doses, inclusion criteria, efficacy of GLP-1RA on reducing glycosuria, and association to non-pharmacological interventions not specifically aimed to weight reduction.

1. Introduction

Most drugs developed for the therapy of obesity have failed to show a sufficient efficacy and safety for long-term treatment. In particular, agents which stimulate energy expenditure (e.g., thyroid hormones, sympathoadrenergic drugs, or sibutramine) do not have an adequate cardiovascular safety, whereas centrally acting anorexants either are ineffective in the long term (e.g., serotonin reuptake inhibitors) or show neuropsychiatric adverse effects (e.g., amphetamine derivatives or cannabinoid receptor antagonists) [1]. As a result, orlistat, which inhibits lipid absorption, is the only available drug for obesity in many countries. Even for drugs which do not show relevant problems of long-term safety, such as orlistat, the unsatisfactory tolerability profile limits clinical use.

Glucagon-like peptide-1 (GLP-1) is a gastrointestinal hormone, produced mainly in the postprandial phase, which stimulates insulin secretion and inhibits glucagon release in a dose-dependent fashion [2]. Due to this properties, the hormone reduces hyperglycemia without inducing hypoglycemia in patients with type 2 diabetes [3]. The rapid inactivation of GLP-1 in vivo and the consequent short half-life (a few minutes after subcutaneous administration) prevents its therapeutic use. Long-acting GLP-1 receptor agonists, which can be administered via subcutaneous injection once or twice a day or once a week, have been developed as glucose-lowering drugs for the treatment of type 2 diabetes [4], but they have also been shown to reduce body weight [5, 6]. The effects of GLP-1 and its agonists on body weight appears to be due to a reduction in food intake, mainly determined by a direct central (hypothalamic) effect of the hormone [7]. The stimulation of GLP-1 receptor also retards gastric emptying; this latter effect is again due, at least partly, to a central action, mediated via the autonomous nervous system [8]. One of the side effect of GLP-1 receptor agonists, nausea (sometimes associated with vomiting), could contribute to the weight

reducing effect; however, weight loss has also been observed when analyzing separately patients who do not report nausea [8].

In fact, some drugs of this class (i.e., liraglutide and long-acting exenatide) are currently under development for the treatment of obesity [9–12]. A phase II, 20-week trial enrolling patients without diabetes showed that liraglutide has a higher efficacy than orlistat in promoting weight loss [13]. Another longer-term (52 weeks) trial with same molecule, the results of which have not been published in full but partly disclosed [14], confirms that liraglutide is an interesting option for the treatment of obesity. Another molecule of the same class, exenatide, has been reported to induce a significant weight loss in a 24-week placebo-controlled trial [15]. Most of what is known on the effect of GLP-1 receptor agonists on body weight comes from clinical trials performed on patients with type 2 diabetes, with glucose control as the principal endpoint. Currently ongoing trials enrolling subjects with obesity and without diabetes will provide, in due time, further information. In the meanwhile, a systematic evaluation of data collected in studies on type 2 diabetes can provide a more defined picture of what we can realistically expect from GLP-1 receptor agonists as weight-reducing agents.

A recent meta-analysis has shown a weight loss of approximately 3% at endpoint in available published trials, with a duration ranging from 20 to 52 weeks [6]. This analysis does not provide information on the time-course of weight loss with GLP-1 receptor agonists. Furthermore, no distinction is made between placebo- and active comparator-controlled trials, with some of the comparators (i.e., insulin, thiazolidinediones, and sulfonylureas) possibly inducing weight gain. Aim of the present meta-analysis is to assess the effects of GLP-1 receptor agonists on body weight at 6 and 12 months of treatment, separating placebo-controlled trials from comparisons with active drugs. Furthermore, a meta-regression analysis will be performed to explore predictors of weight change during treatment.

2. Methods

The meta-analysis was reported following the PRISMA checklist [16].

2.1. Data Sources, Searches, and Extraction. An extensive Medline, Cochrane database, and Embase search for all articles in English using the keywords "exenatide", "liraglutide", "albiglutide", "semaglutide", and "lixisenatide" was performed collecting all randomized clinical trials on humans up to December 15, 2011. Completed but still unpublished trials were identified through a search of http://www.clinicaltrials.gov/ website. FDA (http://www.fda.gov/) and European Medicines Agency (EMA, http://www.ema.europa.eu/) reviews of approved drugs, as well as published infor-mation provided to FDA in response to queries during the approval process, were also searched for retrieval of unpublished trials. Results of those trials were retrieved, if available, on http://www.novonordisk-trials.com/ or http://www.clinicaltrials.org/. For unpublished and published trials which were not exhaustively disclosed, an attempt was made (through e-mail) to contact principal investigators in order to retrieve missing data. For all published trials, results reported in papers were used as the primary source of information, when available.

The identification of relevant abstracts, the selection of studies based on the criteria described previously, and the subsequent data extraction were performed independently by two of the authors (E. Mannucci, M. Monami), and conflicts these resolved by the third investigator (N. Maschionni).

2.2. Study Selection. A meta-analysis was performed including all randomized clinical trials, with a duration of at least 24 weeks, comparing full therapeutic doses Glucagon-like Peptide-1 (GLP-1) receptor agonists (i.e., at least 1.8 mg/day liraglutide, 20 μg/day for exenatide b.i.d., 2 mg/day for exenatide once weekly) and with placebo or other active drugs. Trials with a shorter duration were excluded, due to the fact that they could not yield relevant information on body weight reduction. No review protocol was published elsewhere. Trials without any information on body mass index (BMI) at 6 or 12 months were also excluded.

2.3. Quality Assessment. The quality of trials was assessed using some of the parameters proposed by Jadad et al. [17]. The score was not used as a criterion for the selection of trials, whereas some items were used only for descriptive purposes.

2.4. Data Synthesis and Analysis. The principal outcome was the effect of full therapeutic doses of GLP-1 receptor agonists, compared with other hypoglycemic agents or placebo, on BMI at 6 months and 12 months (when available). Between-group differences in endpoint BMI were assessed as a measure of treatment effect, without considering differences from baseline. Secondary outcomes included glycated hemoglobin (HbA1c) at 6 and 12 months. Separate analyses were performed for trials with different GLP-1 receptor agonists and with different comparators, whenever possible. Furthermore, separate analyses were performed for trials with different principal endpoints. Metaregression analysis was performed on placebo-controlled trials, in order to identify possible predictors of weight loss.

Heterogeneity was calculated using the I^2 statistics. Weighted mean differences were calculated for BMI and HbA1c at 6 and 12 months, and a random effects model was used for the meta-analysis. Publication/disclosure bias was estimated separately for placebo-controlled trials and studies versus active comparators, using Kendall's tau without continuity correction, and one-sided P, were calculated, together with the fail-safe N, and Funnel plot analysis. All those analyses were performed using Comprehensive Meta-analysis Version 2, Biostat, (Englewood, NJ, USA).

3. Results

The trial flow summary is reported in Figure 1. Trials with available results on body mass index at 6 months were 21

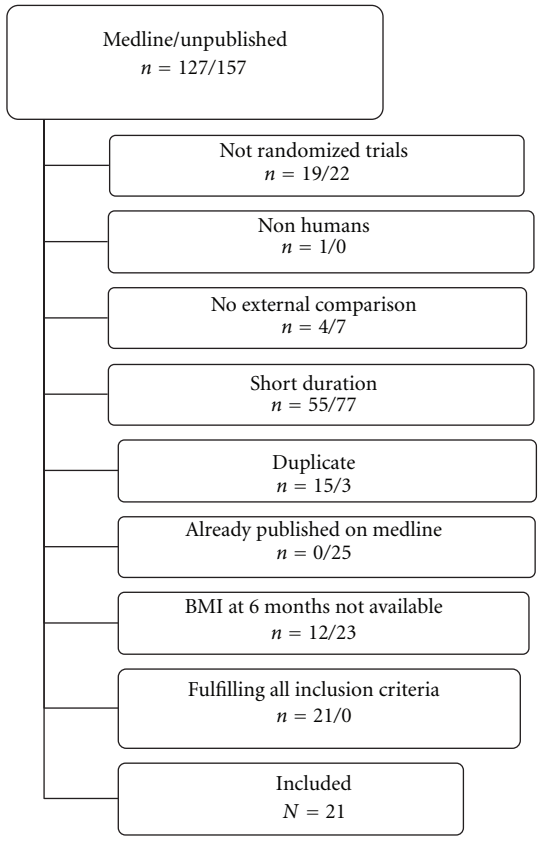


Figure 1: Trial flow summary.

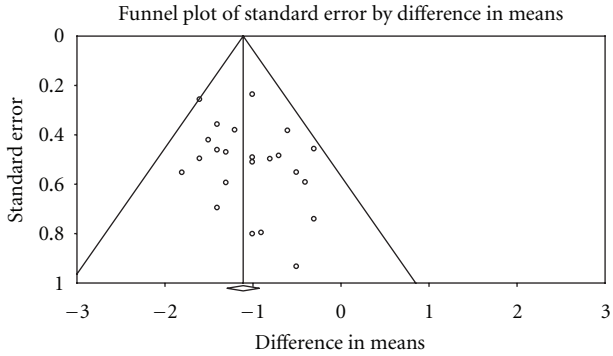


Figure 2: Funnel plot for bias/disclosure publication.

(19 of which in patients with diabetes), whereas those with data at one year were 7 (6 of which on diabetes); the characteristics of those studies are summarized in Table 1. Funnel plot analysis on 6-month trials on diabetes (Figure 2) did not reveal any major publication/disclosure bias for BMI, as confirmed by Kendall's tau ($t = 0.14$, $P = 0.36$) and fail-safe N (number of missing studies that would bring $P > 0.05$: 733). I^2 for BMI at 6 months was 83.6 ($P < 0.001$).

3.1. Results at 6 Months. Only two trials [15, 18] in subjects with obesity not associated with diabetes reported outcomes on body weight of exenatide at 6 months (with a significant ($P = 0.002$) BMI reduction of 1.6 (0.6–2.5) kg/m^2 in comparison with placebo). One further trial, which enrolled patients with type 2 diabetes, had been designed for the assessment of weight reduction with exenatide as the principal outcome [19], with similar results.

In the 19 trials performed in patients with diabetes, GLP-1 receptor agonists were associated with a significantly lower BMI at 6 months in comparison with placebo and with any active glucose-lowering agent, with the exception of the only 2 available head-to-head comparisons with thiazolidinediones. No differences in the weight-reducing effects were observed between exenatide and liraglutide (Figure 3(a)). A subgroup analysis of placebo-controlled trials was performed on the basis of the minimum BMI chosen as inclusion criterion; in trials excluding ($N = 4$) or including ($N = 5$) nonoverweight (BMI < 25 kg/m^2), the difference in 6-month BMI between active treatment and control groups was -1.0 [-1.6; -0.4] and -0.8 [-1.3; -0.3] kg/m^2, respectively (both $P < 0.001$).

For 18 out of 19 of those trials, the principal endpoint was HbA1c, which was significantly reduced by GLP-1 receptor agonists in comparison with placebo, DPP4 inhibitors, and thiazolidinediones, whereas differences with respect to sulfonylureas and insulin were not statistically significant (Figure 3(b)).

Metaregression analysis was performed on all placebo-controlled trials, including those on nondiabetic individuals, irrespective of the principal endpoint of the study. In the 11 available trials, mean baseline BMI, age, and duration of diabetes (in the 9 trials on patients with diabetes) were not significantly correlated with treatment effect on BMI.

3.2. Results at 12 Months. Results on BMI at 12 months were available in 7 trials, 6 of which were performed in patients with diabetes. The only one trial [14] enrolling subjects without diabetes, which had weight loss as its principal endpoint, liraglutide, induced a significant reduction of weight in comparison with placebo (-1.2 [-2.3; -0.1] kg/m^2 in 1-year BMI; $P = 0.04$). The results of the other 6 trials, all with active comparators, are summarized in Table 2. In these studies, a further reduction of body weight was observed after the first six months of treatment. Similar results were obtained when the only trial which did not report 6-month BMI [14] was excluded from the analysis (data not shown).

4. Discussion

The few available trials designed with weight loss as the principal endpoint and enrolling nondiabetic patients with obesity have shown that GLP-1 receptor agonists have a potential use as drugs for the treatment of overweight [14, 15]. Similar results were obtained in a trial on overweight patients with polycystic ovary syndrome, in which restoration of menstrual cycles was the principal endpoint [20]. The much wider evidence collected in subjects with type 2 diabetes confirms this effect, as previously reported [6, 18]. This action is consistent across trials, and it cannot be attributed to selective reporting as shown by Funnel

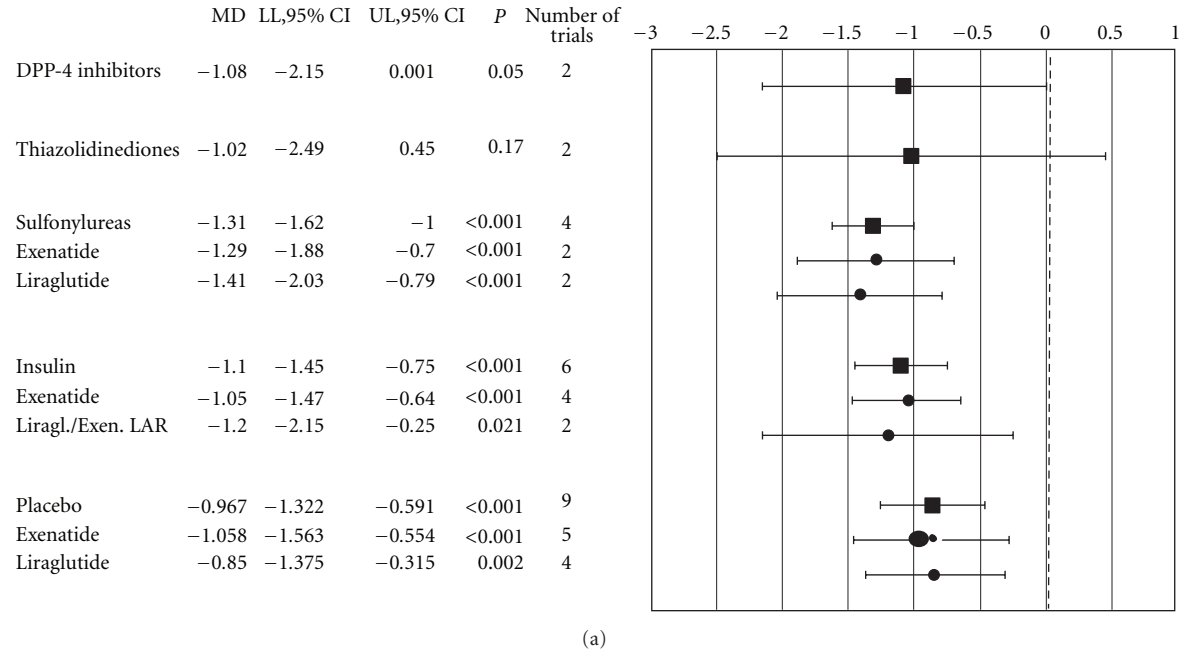
Table 1

Study (Reference)	Number of patients (ID/C)	Comparator	Trial duration (wks)	Age (ys)	Duration of DM (ys)	BMI Baseline (Kg/m^2)	BMI at 6-month (Kg/m^2)	HbA1c baseline (%)	HbA1c at 6-month (%, ID/C)
Liraglutide									
Marre et al. [21]	234/114	Placebo	26	56	7.0	30.0	29.9/30.3	8.5	7.5/8.7
Nauck et al. [22]	242/121	Placebo	26	57	8.0	31.3	30.1/31.1	8.3	7.3/8.5
Zinman et al. [23]	355/175	Placebo	26	55	9.0	33.5	33.0/33.7	8.5	7.0/7.9
Russell-Jones et al. [24]	230/114	Placebo	26	57	9.0	30.9	29.9/31.2	8.3	7.0/8.1
Nauck et al. [22]#	242/242	Glimepiride	26	57	8.0	31.1	30.1/31.6	8.3	7.3/7.4
Garber et al. [25]	498/248	Glimepiride	52	53	5.0	33.0	32.3/33.6	8.3	7.1/7.8
Marre et al. [21]#	234/232	Rosiglitazone	26	56	7.0	29.8	29.9/30.2	8.4	7.5/8.1
Pratley et al. [26]	225/219	Sitagliptin	52	55	6.0	32.8	29.9/31.5	8.4	6.9/7.3
Russell-Jones et al. [27]#	230/232	Glargine	26	57	9.0	30.3	29.9/30.9	8.3	7.0/7.2
Exenatide LAR									
Diamant et al. [27]	233/233	Glargine	26	58	8.0	32.0	31.1/32.5	8.3	6.8/7.0
Bergenstal et al. [28]	160/165	Pioglitazone	26	52	6.0	32.0	31.2/33.0	8.5	7.2/7.4
Bergenstal et al. [28]#	160/166	Sitagliptin	26	52	6.0	32.0	31.2/31.7	8.5	7.2/7.7
Exenatide									
Obesity									
Rosenstock et al. [15]	73/79	Placebo	24	46	0.0	39.5	37.8/38.8	5.6	5.6/5.7
Elkind-Hirsch et al. [20]	20/20	Placebo	24	29	0.0	40.4	39.1/40.8	NR	NR/NR
Type 2 diabetes									
Moretto et al. [29]	77/78	Placebo	24	54	2.0	31.5	30.4/31.4	7.8	6.9/7.6
Buse et al. [30]	129/123	Placebo	30	55	6.3	33.3	32.4/33.8	8.6	7.8/8.7
Buse et al. [31]	138/123	Placebo	30	59	12.0	33.5	33.2/33.5	8.4	6.6/7.5
Kendall et al. [19]	241/247	Placebo	30	55	9.0	34.0	32.9/33.7	8.5	7.7/8.6
DeFronzo et al. [32]	113/113	Placebo	30	53	5.8	34.0	33.0/33.9	8.2	7.4/8.3
Derosa et al. [33]	61/62	Glibenclamide	52	56	NR	28.6	27.3/28.9	8.8	8.1/8.0
Derosa et al. [34]	57/54	Glimepiride	52	55	NR	28.4	27.5/28.5	8.7	7.9/8.1
Gallwitz et al. [35]	248/246	BiAsp	26	57	5.0	33.1	32.0/33.2	7.9	6.9/6.8
Nauck et al. [36]	253/248	BiAsp	52	59	9.9	30.4	29.9/30.5	8.6	7.5/7.6
Heine et al. [37]	282/267	Glargine	26	59	9.5	31.3	30.6/32.0	8.2	7.2/7.1
Bunck et al. [38]	36/33	Glargine	52	58	5.0	30.5	29.9/30.4	7.5	6.7/6.8

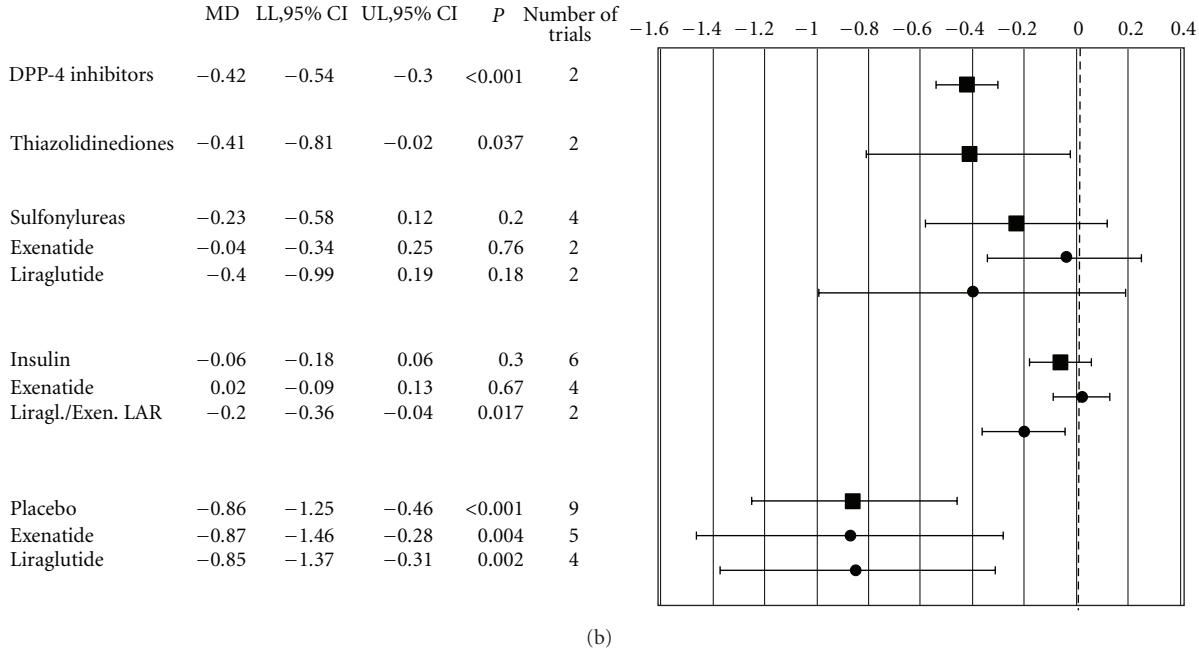
#Studies with multiple comparators; NR: not reported; ID: interventional drug; C: comparator; DM: diabetes mellitus; wks: weeks.

Table 2: Weighted mean differences in 6- and 12-month BMI between GLP-1 receptor agonists and different active comparators.

	Number of trials	6-month BMI	*P*	12-month BMI	*P*
Overall	6	−1.2 [−1.5; −0.8]	<0.001	−1.9 [−3.0; −0.8]	<0.001
DPP-4 inhibitors	1	−1.6 [−2.6; −0.8]	<0.001	−1.7 [−2.7; −0.7]	0.001
Sulphonylureas	3	−1.4 [−2.4; −0.7]	0.001	−2.3 [−4.2; −0.5]	0.012
Insulin	2	−0.7 [−1.4; 0.0]	0.048	−1.5 [−2.1; −0.8]	<0.001



(a)



(b)

Figure 3: Weighted mean differences in 6-month BMI (a) and HbA1c (b) between GLP-1 receptor agonists and different active comparators or placebo, in trials performed in type 2 diabetic patients. MD: weighted mean differences; LL: lower limits; UL: upper limits.

plot analysis and Kendall's tau calculation. GLP-1 receptor agonists have a beneficial effect on body weight not only in comparisons with drugs that induce weight gain (such as insulin or sulfonylureas) but also with respect to placebo. The only exception is represented by direct comparisons with thiazolidinediones: in this case, despite a mean difference in 6-month BMI similar to that observed for other active comparators, the statistical significance is not reached, due to the small number of available trials.

In order to evaluate the weight reducing effect of GLP-1 receptor agonists, the most interesting results are those obtained in placebo-controlled trials, which allow to discriminate the beneficial action of these drugs from the adverse effects on body weight of other glucose-lowering agents. In these studies, the mean weight loss at 6 months is 1.0 kg/m^2; considering that the average BMI at baseline is about 33.9 kg/m^2, this means that the actual ponderal reduction is in the 3% range. The estimated weight loss seems to be larger than that reported in a previous meta-analysis [6]; this result could be due to the exclusion of patients treated with submaximal doses of GLP-1 receptor agonists. This result could seem modest from a clinical standpoint; however, several factors should be considered. In all trials on patients with diabetes except one the principal endpoint was the improvement in HbA1c, and not weight loss. This means that patients were selected on the basis of unsatisfactory glucose control, and not for their overweight; the minimum BMI for inclusion was not specified in some studies, and ranged from 25 to 45 kg/m^2 in the others, meaning that, in all trials, part of the patients enrolled were not actually obese. Notably, those trials that excluded normal-weight subjects showed a greater effect of GLP-1 receptor agonists on weight loss. Furthermore, patients with diabetes could have greater difficulties in losing weight than similarly overweight subjects with normal glucose tolerance. In those who had elevated HbA1c at baseline, the reduction of glycosuria determined by drug treatment could have been an obstacle to weight loss. Finally, the nonpharmacological interventions associated to drugs in trials for glycemic control in type 2 diabetes are not specifically aimed at weight reduction. All these factors could have contributed to an underestimation of the effect of GLP-1 receptor agonists on body weight. It should also be recognized that weight loss in clinical trials could be quite different from that obtained in real-life conditions. The selection of patients with greater compliance and the more accurate follow-up produces a greater weight loss from baseline in randomized clinical trials. On the other hand, for the same reasons, as long as the between-group differences are assessed, as in the present study, the lifestyle/dietary intervention associated with drug treatment in randomized trials can partly mask the actual effect of the drug.

It should also be considered that treatment with GLP-1 receptor agonists could have some further beneficial effects on other metabolic alterations of obese patients (e.g., insulin resistance, risk of diabetes, blod pressure, etc.), beyond weight loss. The assessment of those effects was not among the aims of the present meta-analysis.

The effect of GLP-1 and its receptor agonists on food intake and body weight is dose dependent [13]. For this reason, it is possible that doses needed for the treatment of obesity are higher than those indicated for type 2 diabetes. For example, liraglutide 3.0 mg/day induces a greater weight loss than 1.8 mg/day, whereas no additional effect on blood glucose is expected over 1.8 mg/day [13]. Obviously, at least some of the adverse effects of these drugs (e.g., nausea and vomiting) are also dose-dependent and they could be amplified by the increase in daily doses. In the case that recommended doses for obesity exceed in a relevant manner those for diabetes, the safety profile of GLP-1 receptor agonists, which is satisfactory when they are used in the treatment of type 2 diabetes, should be verified on a sufficiently wide amount of data.

Some interesting information can be obtained from the analysis of data collected in trials on type 2 diabetes with a 1-year follow-up; in fact, the effect of GLP-1 receptor agonists at 1 year seems to be larger than that observed, in the same trials, after 6 months of treatment. The number of studies is limited, and none of them includes a comparison with placebo; in fact, a longer-term treatment without any active drug would be unethical in patients with unsatisfactory control of diabetes. Active comparisons can be misleading, as the comparators often induce weight gain (e.g., insulin, sulfonylureas, thiazolidinediones); this means that the increased difference between GLP-1 receptor agonists and control groups at 1 year could be partly due to weight gain induced by comparators. Despite these limitations, the possibility that the maximum effect of GLP-1 receptor agonists on body weight is reached after 6 months should be considered and taken into account in the design of future clinical trials.

Conflict of Interests

M. Monami has received speaking fees from Astra Zeneca, Bristol Myers Squibb, Eli-Lilly, Merck, Novo Nordisk, and Takeda. I. Dicembrini has received speaking fees from Bayer, Eli-Lilly, Novo Nordisk, and Takeda. C. M. Rotella has received consultancy fees from Eli Lilly, research grants from Eli Lilly, and speaking fees from Eli Lilly, Novo Nordisk, and Sanofi-Aventis. E. Mannucci has received consultancy fees from Merck and Novartis, speaking fees from Astra Zeneca, Bristol Myers Squibb, Merck, and Novartis, and research grants from Merck, Novartis, and Takeda. This work was performed as part of the institutional activity of the authors, without financial support from any third party.

References

[1] M. L. Vetter, L. F. Faulconbridge, V. L. Webb, and T. A. Wadden, "Behavioral and pharmacologic therapies for obesity," *Nature Reviews Endocrinology*, vol. 6, no. 10, pp. 578–588, 2010.

[2] M. E. Doyle and J. M. Egan, "Mechanisms of action of glucagon-like peptide 1 in the pancreas," *Pharmacology and Therapeutics*, vol. 113, no. 3, pp. 546–593, 2007.

[3] B. Ahrén, "GLP-1 for type 2 diabetes," *Experimental Cell Research*, vol. 317, no. 9, pp. 1239–1245, 2011.

[4] A. J. Garber, "Long-acting glucagon-like peptide 1 receptor agonists: a review of their efficacy and tolerability," *Diabetes Care*, vol. 34, pp. S279–S284, 2011.

[5] B. Gallwitz, "Glucagon-like peptide-1 analogues for Type 2 diabetes mellitus: current and emerging agents," *Drugs*, vol. 71, no. 13, pp. 1675–1688, 2011.

[6] T. Vilsbø, M Christensen, A. E. Junker, F. K. Knop, and L. L. Gluud, "Effects of glucagon-like peptide-1 receptor agonists on weight loss: systematic review and meta-analyses of randomised controlled trials," *British Medical Journal*. In press.

[7] M. R. Hayes, B. C. De Jonghe, and S. E. Kanoski, "Role of the glucagon-like-peptide-1 receptor in the control of energy balance," *Physiology and Behavior*, vol. 100, no. 5, pp. 503–510, 2010.

[8] S. E. Kanoski, L. E. Rupprecht, S. M. Fortin, B. C. De Jonghe, and M. R. Hayes, "The role of nausea in food intake and body weight suppression by peripheral GLP-1 receptor agonists, exendin-4 and liraglutide," *Neuropharmacology*, vol. 62, no. 5-6, pp. 1916–1927, 2012.

[9] "Effect of Liraglutide on Body Weight in Non-diabetic Obese Subjects or Overweight Subjects With Co-morbidities: SCALE—Obesity and Pre-diabetes," http://www.clinicaltrials.gov/ct2/show/NCT01272219/.

[10] "Effect of Liraglutide on Body Weight in Overweight or Obese Subjects With Type 2 Diabetes: SCALE—Diabetes," http://www.clinicaltrials.gov/ct2/show/NCT01272232/.

[11] "The Effects of Exenatide (Byetta(Trademark)) on Energy Expenditure and Weight Loss in Nondiabetic Obese Subjects," http://www.clinicaltrials.gov/ct2/show/NCT00856609/.

[12] "The Effect of Exenatide on Weight and Hunger in Obese, Healthy Women," http://www.clinicaltrials.gov/ct2/show/NCT00456885/.

[13] A. Astrup, S. Rössner, L. Van Gaal et al., "Effects of liraglutide in the treatment of obesity: a randomised, double-blind, placebo-controlled study," *The Lancet*, vol. 374, no. 9701, pp. 1606–1616, 2009.

[14] "Comparison of Liraglutide Versus Placebo in Weight Loss Maintenance in Obese Subjects: SCALE Maintenance," http://www.clinicaltrials.gov/ct2/show/NCT00781937?term=NCT00781937/.

[15] J. Rosenstock, L. J. Klaff, S. Schwartz et al., "Effects of exenatide and lifestyle modification on body weight and glucose tolerance in obese subjects with and without pre-diabetes," *Diabetes Care*, vol. 33, no. 6, pp. 1173–1175, 2010.

[16] D. Moher, A. Liberati, J. Tetzlaff et al., "Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement," *Annals of Internal Medicine*, vol. 151, no. 4, pp. 264–269, 2009.

[17] A. R. Jadad, R. A. Moore, D. Carroll et al., "Assessing the quality of reports of randomized clinical trials: is blinding necessary?" *Controlled Clinical Trials*, vol. 17, no. 1, pp. 1–12, 1996.

[18] K. Elkind-Hirsch, O. Marrioneaux, M. Bhushan, D. Vernor, and R. Bhushan, "Comparison of single and combined treatment with exenatide and metformin on menstrual cyclicity in overweight women with polycystic ovary syndrome," *Journal of Clinical Endocrinology and Metabolism*, vol. 93, no. 7, pp. 2670–2678, 2008.

[19] D. M. Kendall, M. C. Riddle, J. Rosenstock et al., "Effects of exenatide (exendin-4) on glycemic control over 30 weeks in patients with type 2 diabetes treated with metformin and a sulfonylurea," *Diabetes Care*, vol. 28, no. 5, pp. 1083–1091, 2005.

[20] M. Monami, N. Marchionni, and E. Mannucci, "Glucagon-like peptide-1 receptor agonists in type 2 diabetes: a meta-analysis of randomized clinical trials," *European Journal of Endocrinology*, vol. 160, no. 6, pp. 909–917, 2009.

[21] M. Marre, J. Shaw, M. Brändle et al., "Liraglutide, a once-daily human GLP-1 analogue, added to a sulphonylurea over 26 weeks produces greater improvements in glycaemic and weight control compared with adding rosiglitazone or placebo in subjects with Type 2 diabetes (LEAD-1 SU)," *Diabetic Medicine*, vol. 26, no. 3, pp. 268–278, 2009.

[22] M. Nauck, A. Frid, K. Hermansen et al., "Efficacy and safety comparison of liraglutide, glimepiride, and placebo, all in combination with metformin, in type 2 diabetes: the LEAD (liraglutide effect and action in diabetes)-2 study," *Diabetes Care*, vol. 32, no. 1, pp. 84–90, 2009.

[23] B. Zinman, J. Gerich, J. B. Buse et al., "Efficacy and safety of the human glucagon-like peptide-1 analog liraglutide in combination with metformin and thiazolidinedione in patients with type 2 diabetes (LEAD-4 Met+TZD)," *Diabetes Care*, vol. 32, no. 7, pp. 1224–1230, 2009.

[24] D. Russell-Jones, A. Vaag, O. Schmitz et al., "Liraglutide Effect and Action in Diabetes 5 (LEAD-5) met+SU Study Group. Liraglutide vs insulin glargine and placebo in combination with metformin and sulfonylurea therapy in type 2 diabetes mellitus (LEAD-5 met+SU): a randomised controlled trial," *Diabetologia*, vol. 52, no. 10, pp. 2046–2055, 2009.

[25] A. Garber, R. Henry, R. Ratner et al., "Liraglutide versus glimepiride monotherapy for type 2 diabetes (LEAD-3 Mono): a randomised, 52-week, phase III, double-blind, parallel-treatment trial," *The Lancet*, vol. 373, no. 9662, pp. 473–481, 2009.

[26] R. Pratley, M. Nauck, T. Bailey et al., "1860-LIRA-DPP-4 Study Group. One year of liraglutide treatment offers sustained and more effective glycaemic control and weight reduction compared with sitagliptin, both in combination with metformin, in patients with type 2 diabetes: a randomised, parallel-group, open-label trial," *International Journal of Clinical Practice*, vol. 65, no. 4, pp. 397–407, 2011.

[27] M. Diamant, L. Van Gaal, S. Stranks et al., "Once weekly exenatide compared with insulin glargine titrated to target in patients with type 2 diabetes (DURATION-3): an open-label randomised trial," *The Lancet*, vol. 375, no. 9733, pp. 2234–2243, 2010.

[28] R. M. Bergenstal, C. Wysham, L. MacConell et al., "Efficacy and safety of exenatide once weekly versus sitagliptin or pioglitazone as an adjunct to metformin for treatment of type 2 diabetes (DURATION-2): a randomised trial," *The Lancet*, vol. 376, no. 9739, pp. 431–439, 2010.

[29] T. J. Moretto, D. R. Milton, T. D. Ridge et al., "Efficacy and tolerability of exenatide monotherapy over 24 weeks in antidiabetic drug-naive patients with type 2 diabetes: a randomized, double-blind, placebo-controlled, parallel-group study," *Clinical Therapeutics*, vol. 30, no. 8, pp. 1448–1460, 2008.

[30] J. B. Buse, R. R. Henry, J. Han, D. D. Kim, M. S. Fineman, and A. D. Baron, "Exenatide-113 Clinical Study Group. Effects of exenatide (exendin-4) on glycemic control over 30 weeks in sulfonylurea-treated patients with type 2 diabetes," *Diabetes Care*, vol. 27, no. 11, pp. 2628–2635, 2004.

[31] J. B. Buse, R. M. Bergenstal, L. C. Glass et al., "Use of twice-daily exenatide in Basal insulin-treated patients with type 2 diabetes: a randomized, controlled trial," *Annals of Internal Medicine*, vol. 154, no. 2, pp. 103–112, 2011.

[32] R. A. DeFronzo, R. E. Ratner, J. Han, D. D. Kim, M. S. Fineman, and A. D. Baron, "Effects of exenatide (exendin-4)

on glycemic control and weight over 30 weeks in metformin-treated patients with type 2," *Diabetes Care*, vol. 28, no. 5, pp. 1092–1100, 2005.

[33] G. Derosa, P. Maffioli, S. A. T. Salvadeo et al., "Exenatide versus glibenclamide in patients with diabetes," *Diabetes Technology and Therapeutics*, vol. 12, no. 3, pp. 233–240, 2010.

[34] G. Derosa, P. Putignano, A. C. Bossi et al., "Exenatide or glimepiride added to metformin on metabolic control and on insulin resistance in type 2 diabetic patients," *European Journal of Pharmacology*, vol. 666, no. 1-3, pp. 251–256, 2011.

[35] B. Gallwitz, M. Böhmer, T. Segiet et al., "Exenatide twice daily versus premixed insulin aspart 70/30 in metformin-treated patients with type 2 diabetes: a randomized 26-week study on glycemic control and hypoglycemia," *Diabetes Care*, vol. 34, no. 3, pp. 604–606, 2011.

[36] M. A. Nauck, S. Duran, D. Kim et al., "A comparison of twice-daily exenatide and biphasic insulin aspart in patients with type 2 diabetes who were suboptimally controlled with sulfonylurea and metformin: a non-inferiority study," *Diabetologia*, vol. 50, no. 2, pp. 259–267, 2007.

[37] R. J. Heine, L. F. Van Gaal, D. Johns, M. J. Mihm, M. H. Widel, and R. G. Brodows, "Exenatide versus insulin glargine in patients with suboptimally controlled type 2 diabetes: a randomized trial," *Annals of Internal Medicine*, vol. 143, no. 8, pp. 559–569, 2005.

[38] M. C. Bunck, M. Diamant, A. Cornér et al., "One-year treatment with exenatide improves β-cell function, compared with insulin glargine, in metformin-treated type 2 diabetic patients: a randomized, controlled trial," *Diabetes Care*, vol. 32, no. 5, pp. 762–768, 2009.

Hindawi Publishing Corporation
Experimental Diabetes Research
Volume 2012, Article ID 470851, 12 pages
doi:10.1155/2012/470851

Review Article

Physiology and Emerging Biochemistry of the Glucagon-Like Peptide-1 Receptor

Francis S. Willard[1] and Kyle W. Sloop[2]

[1] *Translational Science and Technologies, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285, USA*
[2] *Endocrine Discovery, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285, USA*

Correspondence should be addressed to Kyle W. Sloop, sloop_kyle_w@lilly.com

Received 31 December 2011; Accepted 25 January 2012

Academic Editor: Matteo Monami

The glucagon-like peptide-1 (GLP-1) receptor is one of the best validated therapeutic targets for the treatment of type 2 diabetes mellitus (T2DM). Over several years, the accumulation of basic, translational, and clinical research helped define the physiologic roles of GLP-1 and its receptor in regulating glucose homeostasis and energy metabolism. These efforts provided much of the foundation for pharmaceutical development of the GLP-1 receptor peptide agonists, exenatide and liraglutide, as novel medicines for patients suffering from T2DM. Now, much attention is focused on better understanding the molecular mechanisms involved in ligand induced signaling of the GLP-1 receptor. For example, advancements in biophysical and structural biology techniques are being applied in attempts to more precisely determine ligand binding and receptor occupancy characteristics at the atomic level. These efforts should better inform three-dimensional modeling of the GLP-1 receptor that will help inspire more rational approaches to identify and optimize small molecule agonists or allosteric modulators targeting the GLP-1 receptor. This article reviews GLP-1 receptor physiology with an emphasis on GLP-1 induced signaling mechanisms in order to highlight new molecular strategies that help determine desired pharmacologic characteristics for guiding development of future nonpeptide GLP-1 receptor activators.

1. Introduction

The glucoregulatory role of the gut is demonstrated by studies showing insulin secretion is profoundly more robust following glucose ingestion compared to the insulinotropic response achieved by parenteral administration of intravenously infused glucose [1–5]. This physiologic phenomenon, coined the "incretin effect," is primarily mediated by two enteric factors known as the incretins: glucagon-like peptide-1 (7-37)/(7-36)-amide (GLP-1) and glucose dependent insulinotropic polypeptide (1-42) (GIP) [6–13]. In addition to glucose, the presence of other macronutrients in a mixed meal, such as lipids and amino acids, in the intestinal lumen stimulate a similar incretin response [14, 15]. When moving through the intestine, nutrients interact directly with sensory receptors and integral membrane channel and transporter proteins localized on the microvillus-rich apical membrane surface of open-type endocrine cells. These cells are embedded in the mucosal lining throughout various regions of the intestinal tract and release the incretins upon nutrient stimulation. In L-cells, located throughout the intestine but predominantly found in the ileum of the distal small intestine and colon [16, 17], GLP-1 is produced by posttranslational cleavage of the 160-amino acid proglucaon precursor protein [18, 19], a process requiring prohormone convertase-1/3 [20–22]. GIP is the single peptide derived from proteolytic processing of a 153-amino acid precursor protein [23] expressed in endocrine K-cells located mainly in the duodenum and proximal jejunum of the upper small intestine [24].

1.1. Physiologic Action of GLP-1. Upon release into the circulation, GLP-1 and GIP facilitate glucose disposal by directly acting on pancreatic islets to enhance postprandial insulin secretion [6–13]. This process is mediated by two class B1 (secretin-like family) seven transmembrane spanning,

heterotrimeric G-protein coupled receptors (GPCRs) that signal in response to binding and occupancy by GLP-1 [25, 26] and GIP [27], respectively. Both of these GPCRs predominantly couple to the $G\alpha_s$ subunit which activates adenylyl cyclases to increase intracellular cyclic 3′5′AMP (cAMP). Genetic deletion of both receptors in mice leads to glucose intolerance and defects in glucose stimulated insulin secretion [28]. In addition to ligand stimulated cAMP generation, β-arrestin interaction [29, 30] and signaling pathways that mobilize intracellular calcium are important effectors of incretin action [31, 32].

In humans, the incretin effect is often reduced in patients suffering from type 2 diabetes mellitus (T2DM) [33]. To combat this, initial strategies to develop incretin based "replacement" therapies largely focused on GLP-1 receptor analogs because studies suggested diabetic patients are resistant to GIP treatment [34, 35], while GLP-1 infusion elicits a strong insulin secretory response and can normalize hyperglycemia [36–39]. In contrast to GIP, GLP-1 also induces several additional antidiabetic effects, including inhibition of glucagon secretion [6, 8] and gastric emptying [40–42] (which both help improve postprandial glucose control) and a decrease in appetite and food intake [43–47]. These latter effects are mediated by the GLP-1 receptor expressed in extrapancreatic tissues, most notably those of the gastrointestinal tract and central nervous system.

While infusion regimens demonstrate remarkable antidiabetic pharmacology, elimination metabolism and pharmacokinetic characteristics of native GLP-1 present major hurdles to developing it as an effective pharmaceutical agent. One significant challenge in pursing GLP-1 based molecules is that GLP-1 is rapidly inactivated by dipeptidyl peptidase 4 (DPP4), a plasma membrane bound enzyme that is positioned with its active site orientated towards the extracellular space. This ubiquitously expressed "ectopeptidase" cleaves the N-terminal dipeptide, His^7-Ala^8, to inactivate GLP-1 [48, 49]. Removal of these residues dramatically reduces the binding affinity of the peptide for the GLP-1 receptor, thus abolishing its ability to effectively activate receptor signaling [50]. DPP4 is highly expressed on the surface of endothelial cells lining blood vessels; consequently, GLP-1 is immediately vulnerable to inactivation following release into the circulation [51]. Upon cleavage, the inactive GLP-1 metabolite is eliminated by the kidney [52]. As a result of rapid postsecretory proteolysis and renal elimination, the biological half-life of GLP-1 is estimated to be between 1 to 2 minutes [53, 54]. These characteristics limit the pharmaceutical potential of native GLP-1.

1.2. GLP-1 Receptor Peptide Agonists. Several efforts pursued novel GLP-1 analogs with improved metabolic properties. A common approach was to introduce N-terminally substituted modifications to reduce DPP4 sensitivity [54–56]. To date, attempts solely focused on amino acid substitutions of native GLP-1 to identify longer acting molecules likely have been hampered by other issues such as renal clearance and secondary degradation by other endopeptidases. However, two alternate approaches proved successful in advancing more stable, degradation resistant GLP-1 receptor agonists. Both exenatide and liraglutide are approved for marketing by several government regulatory agencies for the treatment of T2DM.

Exenatide is a 39-amino acid peptide GLP-1 receptor agonist that is fully efficacious in cellular assays and competitive with native GLP-1 in receptor binding studies [26, 57–59]. It is the synthetic version of exendin-4 which was among several bioactive peptides containing an N-terminal histidine identified in crude venom preparations extracted from perimandibular salivary glands of Helodermatidae lizards [60, 61]. Exendin-4 was isolated from the poisonous venom of the Gila monster, *Heloderma suspectum* [60], a lizard indigenous to the southwest United States in the Gila River area of New Mexico and Arizona [62]. In addition to mimicking the physiologic glucoregulatory actions of native GLP-1, exendin-4 is a poor DPP4 substrate [63] and is cleared from the body primarily by glomerular filtration in the kidney [64, 65]. Consequently, exenatide has a longer duration of action compared to GLP-1 [66–68] and an estimated biological half-life of approximately 4 hours [64, 69]. In April of 2005, under the brand name *Byetta*, exenatide became the first enteroendocrine based therapeutic approved by the United States Food and Drug Administration (FDA) for the treatment of T2DM.

The second GLP-1 receptor agonist approved to treat T2DM is liraglutide (NN2211). For this molecule, a "fatty acid derivatization" strategy was used to prolong the *in vivo* action of GLP-1. This approach attaches a fatty acid moiety to GLP-1 in order to facilitate GLP-1 binding to serum albumin. Liraglutide is acylated on Lys^{26} with a covalently attached palmitoyl (C16:0) chain [70]. As this modification enables binding to albumin, GLP-1 is then sterically protected from DPP4 degradation [70]. The plasma half-life of liraglutide is estimated to be between 11 and 15 hours [71, 72]. Under the brand name *Victoza*, liraglutide received marketing approval by the FDA in January of 2010 for the treatment of T2DM. *Byetta* and *Victoza* are both commonly prescribed medicines.

2. GLP-1 Receptor Signal Transduction and Second Messenger Pathways

As the best characterized *in vivo* action of GLP-1 is an acute insulinotropic effect mediated by the GLP-1 receptor in pancreatic β-cells, the signal transduction coupling mechanisms of this receptor primarily have been analyzed using *ex vivo* preparations of pancreatic islets, transformed pancreatic β-cell lines, and recombinant GLP-1 receptor expressing systems, Accordingly, critical evaluation of GLP-1 receptor signal transduction in extrapancreatic tissues can be made by inference only. Use of the various peptide GLP-1 receptor agonists to define the *in vitro* pharmacologic properties of the receptor should define key assay systems to enable optimizing small molecule GLP-1 receptor activators.

2.1. GLP-1 Receptor Activation. Both GLP-1 and exendin-4 are α-helical peptides that interact with the GLP-1 receptor by binding multiple extracellular contact points to induce

receptor signaling [73–75]. Similar to the "two-step" mechanism proposed for other class B1 GPCRs [76] (Figure 1), the GLP-1 receptor utilizes an N-terminal extracellular domain as an "affinity trap" to recognize and bind peptide ligands [77, 78]. The N-terminal domain of the GLP-1 receptor is conserved among class B1 GPCRs forming an α-β-$\beta\alpha$ protein fold that has structural homology to the sushi/CCP/SCR protein folds [79, 80] (Figure 2). This structure, referred to as an "ectodomain" (ECD), is a trilayer fold composed of an N-terminal α-helix, a middle section of two antiparallel β strands, and a final lobe composed of two additional antiparallel β sheets and a short α-helical region ($\beta\alpha$) (Figure 2). The overall structure of the ECD is stabilized by three pairs of disulfide bonds formed between six conserved cysteine residues that lock the three layers of the ECD together [81] (Figure 2). X-ray crystal structures of exendin-4 and GLP-1 bound to the ECD confirm the "affinity trap" hypothesis showing the C-terminal α-helical region of GLP-1 or exendin-4 is positioned within a binding cleft of the N-terminal ECD [74, 75] (Figure 3). Both GLP-1 and exendin-4 are amphipathic in nature, and this defines their structurally conserved interaction mechanism with the ECD. The hydrophobic faces of GLP-1 and exendin-4 make the majority of interactions with the ECD and likely are the key contributors to ECD/ligand affinity with only a minor contribution of binding energy provided by the hydrophilic regions of GLP-1 receptor agonist peptides [74, 75] (Figure 3).

The second step of the class B1 GPCR activation model predicts the ECD docks the bound peptide in a position that promotes direct contact of N-terminal residues of the ligand with the central activation pocket of the receptor, a region consisting of three interconnecting extracellular loops often referred to as the helical bundle or "J" (juxtamembrane) domain. GLP-1 (or exendin-4) binding to this core region induces a conformational rearrangement of the membrane-spanning α-helices, eliciting a shift of the intracellular receptor loops to stimulate intracellular signal transduction (Figure 1).

Structural information regarding GLP-1 receptor peptide ligands is available primarily from NMR studies. The data are consistent with other class B1 GPCR ligands [83] in that GLP-1(7-36) and exendin-4 peptides are likely α-helical in structure with disordered N-termini, although the artificial environment in which these studies are conducted must be used to qualify any interpretation of the experimental data [84, 85]. Structural studies of class B1 GPCR ECDs have been very informative regarding the molecular mechanisms of peptide ligand selectivity and have generated new hypotheses regarding class B1 GPCR activation mechanisms. However, the field awaits definitive structural data to explain how peptide agonists activate their cognate class B1 GPCRs.

2.2. G-Protein Coupling. The GLP-1 receptor primarily couples to the $G\alpha_s$ heterotrimeric G-protein. Upon ligand binding, the resulting conformational change activates intrinsic guanine nucleotide exchange factor activity of the receptor to catalyze release of bound GDP from the $G\alpha_s$. The $G\alpha_s$ then rapidly binds GTP which leads to dissociation of $G\alpha_s$ and $G\beta\gamma$, consequently activating downstream effector pathways. Activated $G\alpha_s$ allosterically stimulates membrane-associated adenylyl cyclases to catalyze conversion of ATP to cAMP, which acts as an intracellular second messenger mediating GLP-1 signaling.

Elevation of cAMP in the pancreatic β-cell is a critical event in the process of glucose dependent insulin secretion and is likely the key mechanism by which GLP-1 and exendin-4 act on β-cells to potentiate insulin secretion [25, 26, 86]. However, early reports highlighted the ability of the GLP-1 receptor to couple to alternative signaling pathways, including phospholipase C (PLC) and the mobilization of intracellular Ca^{2+} [87, 88], consistent with the known effects of GLP-1 to stimulate Ca^{2+} mobilization in β-cells [89]. Further, multiple reports indicate GLP-1 receptor couples to Ca^{2+} mobilization when heterologously expressed [90, 91]. In these systems, it is generally assumed Ca^{2+} mobilization is a $G\alpha_q$ mediated process. In support of this, studies utilizing the azidoanilide-GTP cross-linking method show the GLP-1 receptor can cause activation of the $G\alpha_q$- and $G\alpha_i$-families of G-proteins in GLP-1 receptor expressing CHO cells [88]. Conversely, recent experiments using membrane GTPγS binding assays demonstrate GLP-1 receptor activation does not induce measurable activation of $G\alpha_q$ or $G\alpha_i$ despite the presence of substantial PLC independent Ca^{2+} mobilization in GLP-1 receptor expressing HEK cells [91]. *In vivo*, pancreatic β-cell specific dual inactivation of $G\alpha_q$ and $G\alpha_{11}$ does not affect GLP-1 potentiation of glucose stimulated insulin secretion [92], whereas insulinotropic action through known $G\alpha_{q/11}$ coupled GPCRs, GPR40 and the M_3 muscarinic receptor, is ablated. While this study elegantly demonstrates that $G\alpha_{q/11}$ signal transduction is not required for GLP-1 receptor mediated insulin secretion (using perifused islets), it is problematic that a single dose of 100 μM GLP-1 (a concentration greater than 5 orders of magnitude above the K_d and peak circulating levels of active GLP-1) was used in the studies [49, 92].

While a role for $G\alpha_{q/11}$ signal transduction in β-cell GLP-1 receptor action is generally excluded, a PLC and Ca^{2+} mobilization pathway may be operant. Experiments using mouse β-cells indicate the elevation of cAMP by GLP-1 receptor signaling results in activated EPAC2 that stimulates PLC [31] and Ca^{2+} channel recruitment [93] to facilitate calcium induced calcium release, a process integral for robust insulin secretion. These data provide a potential mechanism whereby sole activation of the $G\alpha_s$ pathway induces cAMP- and PLC/Ca^{2+} dependent responses in β-cells. In light of the contrasting data, it is apparent that the phenotype of GLP-1 receptor signaling may differ according to the cellular context in which the receptor is expressed, a phenomenon now widely recognized but not well understood within the GPCR field [94]. Accordingly, the definitive *in vivo* G-protein coupling profile of the GLP-1 receptor is unclear, although $G\alpha_s$ induced cAMP accumulation is certainly integral to the biological response of GLP-1 receptor activation. It is of interest that other class B1 GPCRs demonstrate physiologically relevant coupling to multiple G-proteins; the parathyroid hormone receptor is

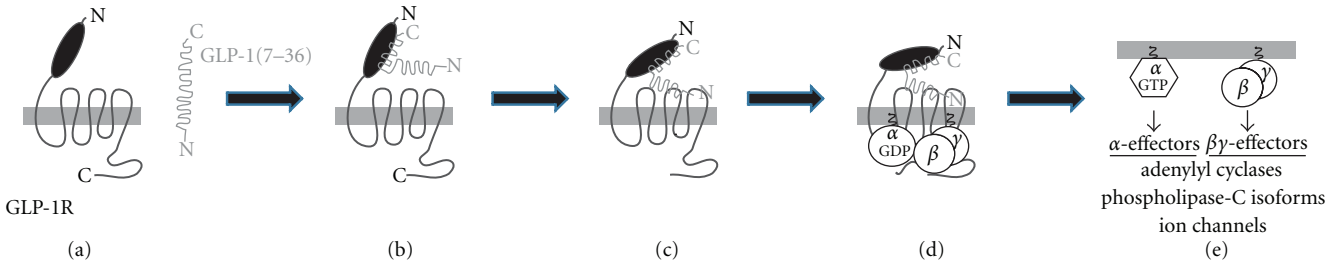


Figure 1: The activation mechanism of the GLP-1 receptor. Biochemical and structural studies have led to a model of class B1 GPCR activation by peptide hormones referred to as the "two-step" mechanism [82]. (a) In the unliganded state, the GLP-1 receptor (GLP-1R) is in a predominately inactive conformation. The natural ligand GLP-1(7-36)-NH_2 is freely diffusible in solution and likely has substantial intrinsic α-helical structure. (b) An initial binding event between the globular ectodomain at the N-terminal of the GLP-1R and the C-terminal of the GLP-1(7-36)-NH_2 peptide occurs. This "low affinity" interaction acts as a tether or "affinity trap" to localize GLP-1 at the GLP-1R. (c) The weak affinity of the N-terminus of GLP-1(7-36)-NH_2 is then able to productively engage with transmembrane domain and loop residues of the receptor to induce a high affinity interaction and likely a conformational change in the GLP-1R. (d) Coincident with agonist binding, the G-protein bound conformation of the GLP-1R is stabilized. This represents the classic high affinity agonist bound state. (e) The high affinity agonist bound state is transient in an intact system as the GLP-1R stimulates guanine nucleotide exchange on the α-subunit of the G-protein heterotrimer, leading to G-protein dissociation and independent or synergistic activation of effector proteins by liberated Gα·GTP and G$\beta\gamma$.

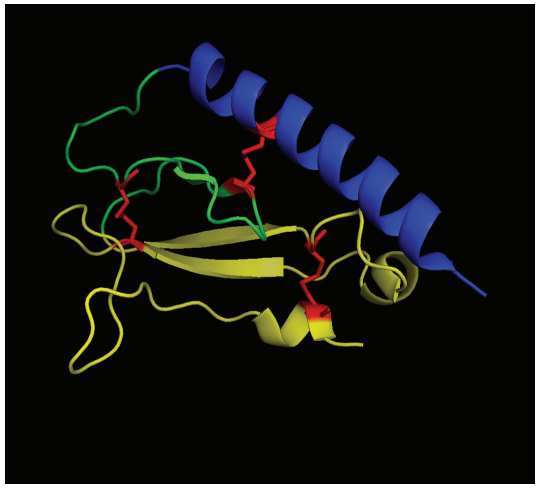


Figure 2: Structure of the GLP-1 receptor ectodomain. The overall structure of the GLP-1 receptor ectodomain is depicted (PDB ID: 3IOL) [74]. The tripartite α-β-$\beta\alpha$ structure is annotated using color; from N-terminal to C-terminal α (blue), β (green), $\beta\alpha$ (yellow). The three conserved disulfide bonds that stabilize the tertiary structure of the ectodomain are colored in red.

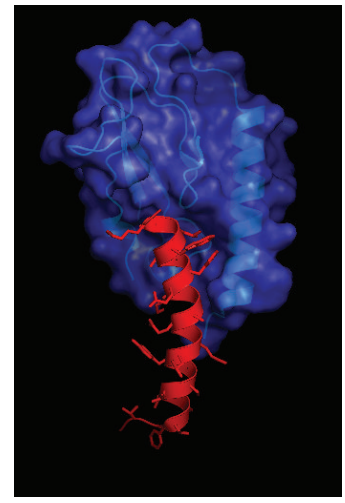


Figure 3: Structural determinants of ligand binding to the GLP-1 receptor ectodomain. (a) Structure of the GLP-1 receptor ectodomain bound to GLP-1. Blue ribbon and space filling model is GLP-1 receptor and the red ribbon and stick model is GLP-1. Data are derived from PDB ID: 3OL.

the best characterized example [95]. The use of genetically modified mice, RNA interference, or novel pharmacological tools such as the $G\alpha_{q/11}$ inhibitor YM-254890 [96] may serve to answer whether the GLP-1 receptor functionally couples to G-proteins other than $G\alpha_s$ in the endogenous context, particularly in extrapancreatic cell types.

2.3. β-Arrestin Coupling. Despite numerous studies characterizing β-arrestin interactions with GPCRs, only a limited number of reports investigate interactions between incretin receptors and arrestin proteins. Bioluminescence resonance energy transfer studies demonstrate both β-arrestin-1 and -2 interact with the GLP-1 receptor in an agonist dependent manner [97]. Classically, GPCR recruitment of GPCR kinases (GRKs) and β-arrestins is characterized as inducing desensitization of G-protein mediated signal transduction [98]; β-arrestin binding blocks G-protein mediated signaling and facilitates receptor internalization. However, emerging data suggest receptor activated β-arrestins can stimulate signaling pathways independently of G-protein activation [99]. Thus, β-arrestin signaling has physiologic consequences distinct from G-protein coupled signaling [99]. It is, therefore, of great interest to understand the functional outcome of β-arrestin regulation of the GLP-1 receptor.

In INS-1E insulinoma β-cells, siRNA knockdown of β-arrestin-1 reduces GLP-1 induced insulin secretion [30]. These experiments implicate β-arrestin-1 in GLP-1 receptor activity, but the mechanism responsible for the diminution of GLP-1 action is not explicitly characterized. An alternate explanation for lower GLP-1 stimulated insulin secretion could be reduced tonic inhibition from anti-insulinotropic $G\alpha_i$-coupled GPCRs resulting from β-arrestin-1 removal. Further, in this study, insulin secretion induced by glucose alone is severely attenuated by β-arrestin-1 knockdown making it difficult to establish direct causality between β-arrestin-1 knockdown and GLP-1 receptor dependent signaling.

In studies using MIN6 insulinoma β-cells, GLP-1 receptor stimulation is shown to induce a biphasic activation of ERK [100]. This effect is comprised of an initial cAMP-dependent transient activation of ERK and a prolonged β-arrestin-1 dependent activation of ERK [100]. β-arrestin-1 dependent ERK activity promotes Bad phosphorylation and consequently mediates prosurvival effects of GLP-1 receptor agonists on high glucose induced apoptosis. Because many biochemical mechanisms in MIN6 cells are operant in mouse islets, this report elegantly delineates separable pathways for GLP-1 receptor induced insulin secretion ($G\alpha_s$-cAMP axis) versus antiapoptotic signaling (β-arrestin-1 $\rightarrow$ p90RSK $\rightarrow$ Bad axis). It should be noted, though, that this study also contains experimental caveats; for example, the authors are unable to cause glucotoxic apoptosis in primary islet cultures and thus fail to validate the efficacy of GLP-1 anti-apoptotic signaling in their islet system.

In vivo analysis of insulin secretion in β-arrestin-1 knockout mice indicates glucose stimulated insulin secretion is reduced by over 80% [30, 100]. Accordingly, it is problematic to ascribe any physiologic alterations to GLP-1 receptor agonists in β-arrestin-1 knockout mice as being directly due to the GLP-1 receptor, given that β-arrestins are likely crucial signal regulatory proteins for hundreds of GPCRs. This is exemplified by the recent observation that β-arrestin-2 knockout mice are insulin resistant [101]. A key point to contextualize these studies is that although numerous reports in rodents show positive effects of GLP-1 or exendin-4 on pancreatic β-cell replication, β-cell mass, and function in preclinical models [102, 103], less data are available regarding GLP-1 agonist modulation of β-cell apoptosis or neogenesis in humans (discussed in [104]). One report does, however, demonstrate GLP-1 mediated attenuation of apoptosis and enhanced insulin responsiveness in an *ex vivo* human islet preparation [105]. Similarly, recent evidence demonstrates that GLP-1 receptor agonism induces β-cell replication in human islet grafts [106].

3. Structural Evaluation of the GLP-1 Receptor

Understanding the molecular mechanisms whereby peptide ligands induce GLP-1 receptor signaling should enhance new efforts to optimize small molecule activators of the GLP-1 receptor. Various approaches are being explored that may ultimately inform more rationale design strategies for small molecule GLP-1 receptor agonists. A comprehensive review of currently disclosed low molecular weight GLP-1 receptor activators is presented in this issue of *Experimental Diabetes Research*; see Willard et al. for review. Importantly, new strategies to exploit potential small molecule binding to the GLP-1 receptor are advancing as a result of progress in GPCR molecular and structural biology. Extensive site-directed mutagenesis studies have been carried out on the GLP-1 receptor as have structure activity studies on peptide GLP-1 ligands. It is beyond the scope of this review to cover these studies, however, we recognize that such efforts have been a valuable starting point in efforts to understand GLP-1 receptor biochemistry [107, 108].

3.1. Intramolecular Endogenous Peptide Agonists. Dong and colleagues proposed a novel activation mechanism for class B1 GPCRs [109] whereby peptide ligand interaction with the receptor induces a conformational change that exposes an intramolecular "endogenous agonist" epitope within the ECD of a GPCR. The "endogenous agonist" motif is proposed to act at the transmembrane domains to facilitate receptor activation. This hypothesis is largely derived from mutagenesis, peptide cross-linking, and molecular modeling studies using the secretin receptor [109]. In support of the concept, synthetic peptides derived from the ECD of the secretin receptor are shown to be low potency, high efficacy agonists of the secretin receptor. The minimized pharmacophore for the secretin receptor is a tri-peptide Trp^{70}-Asp^{71}-Asn^{72} [109]. Translation of this finding to the GLP-1 receptor identified an analogous peptide, Asn^{63}-Arg^{64}-Thr^{65}-Phe^{66}-Asp^{67} (NRTFD), as a low potency but fully efficacious GLP-1 receptor agonist [110]. Although these short peptides have low affinity and poor receptor selectivity, making them unlikely starting points for lead optimization and drug development, the molecules identify a potential binding site for compound action on the GLP-1 receptor. Structural elucidation of these features should be enlightening. The GLP-1 receptor "endogenous agonist" peptide maps to the β1 strand of the GLP-1 receptor ECD crystal structure [74]. However, analyses of these structural data suggest Asn^{63} is solvent exposed, but the majority of the peptide is not; these results make it difficult at this point to clearly understand the molecular mechanism proposed for the "endogenous agonist" peptides without further information around the structure or dynamics of the GLP-1 receptor. A subsequent report used an elegant cross-linking approach coupled with radiochromatography (see Section 3.3) to identify the site of action of the NRFTD peptide as being extracellular loop 3 in close proximity to transmembrane domain 6 [111]. The mechanism of action of the NRFTD peptide may be analogous to that of the "pepducins" [112]. Pepducins are short peptides derived from the intracellular loops of GPCRs that act allosterically to modulate receptor signaling [113].

3.2. Crystal Structure of the GLP-1 Receptor ECD. Receptor binding and functional studies show both GLP-1 (K_d = 0.3 nM) and exendin-4 (K_d = 0.1 nM) bind with high affinity and are full agonists at the GLP-1 receptor [26,

57]. In addition, competition binding studies suggest these peptides use the same ligand binding site within the receptor [114]. However, in experiments exploring the initial ligand-receptor binding event, data show exendin-4 binds the isolated soluble form of the GLP-1 receptor ECD with much greater affinity (13 nM) compared to GLP-1 (800 nM) [114, 115]. It was hypothesized that this phenomenon occurs because exendin-4 contains nine additional amino acids at its C-terminus that enable further binding contacts with the ECD [77, 115]. Importantly, the crystal structures of the ECD in complex with either bound GLP-1 or exendin-$4_{(9\text{-}39)}$ show these peptides share a very similar mode of binding, and there is no interaction between the last seven residues of the nine amino acid C-terminal extension of exendin-4 with the ECD [74, 75] (Figure 3). Alternatively, biophysical studies suggest the higher propensity of exendin-4 to form helical conformations in solution compared to GLP-1 results in its enhanced binding affinity [73, 114]. However, this area is still in need of further exploration because other reports using a membrane-tethered form of the ECD demonstrate GLP-1 (IC_{50} = 160 nM) and exendin-4 (IC_{50} = 20 nM) bind the receptor with closer affinity [116]. Recent studies highlight this incongruity as exendin-4 (K_d = 0.9 μM) and GLP-1 (K_d = 1 μM) have equivalent affinities for the ECD, as measured by surface plasmon resonance [117]. While studies using purified forms of the ECD are informative, these latter results may highlight the relatively artificial nature of approaches using the soluble form of the ECD. Therefore, experimental methodologies allowing structural characterization of the full length, intact GLP-1 receptor are needed.

3.3. GLP-1 Receptor Photoaffinity Labeling. In the absence of a high resolution crystal structure of the full length GLP-1 receptor, photoaffinity labeling has been used as an alternate approach to identify potentially important ligand-receptor interactions. An inherent advantage of this technique is that whole cells expressing the native receptor or membrane preparations enriched with a receptor of interest are used so the receptor is folded and presented in its proper structural orientation [118–120]. These studies typically use a radio-labeled version of the natural ligand engineered to contain a photoreactive moiety such as *p*-benzoyl-l-phenylalanine (Bpa). Ultraviolet photolysis of a probe in complex with its receptor covalently labels residues of the receptor that are in close spatial approximations with important structural regions of the ligand. The labeled amino acids within the targeted receptor can then be identified using manual cycles of Edman degradation sequencing of isolated receptor fragments [121]. For the GLP-1 receptor, this technique has established spatial approximations between several ligand-receptor residues that likely occur in the "agonist occupied" receptor conformation. In studies intended to further assess the initial binding event, C-terminal residues, Ala^{24} and Gly^{35}, of GLP-1 are shown to dock in close proximity near Glu^{133} and Glu^{125} of the ECD, respectively [122]. These data are in line with the 2.1 Å resolution crystal structure of the ECD-GLP-1 complex showing GLP-1 binding occurs via a continuous C-terminal α-helix formed by the sequence spanning Thr^{13} and Val^{33} with residues between Ala^{24} and Val^{33} directly interacting with the ECD [74]. Overall, these data are consistent with the initial binding event proposed by the "two-step" model. Somewhat surprisingly, results from studies testing GLP-1 probes with Bpa incorporated at positions 12 and 16 show docking of this region also near residues contained within the ECD. These data predict Phe^{12} and Val^{16} are positioned near Tyr^{145} and Leu^{141}, respectively, of the ECD, sites located in the distal region of the ECD immediately upstream of the first transmembrane segment of the receptor [123, 124].

Importantly, photoaffinity cross-linking studies have also established potential contacts between the extracellular loops of the receptor with residues of GLP-1. This work is helping provide better insights into the orientation of the ligand bound N-terminal region of GLP-1 with the receptor core. For studies aimed at identifying structural elements involved with the "second step" of GLP-1 binding and receptor activation, an N-terminus labeled photo-labile GLP-1 probe was generated. Because changes to the N-terminal His^7 are not well tolerated [125], a probe with Bpa N-terminally attached to His^7 (at a new position 6) was used to better understand spatial approximations between the most N-terminal residues of GLP-1 and the receptor. These data show the N-terminus positions near Tyr^{205} in the first extracellular loop of the receptor [123]. Similarly, using a mid-region position 20 probe, Trp^{297} within the second extracellular loop of the receptor positions within close proximity to Leu^{20} of GLP-1 [124]. Taken together, X-ray structural data of the ECD in complex with the C-terminus of GLP-1 and the cumulative results from photoaffinity labeling studies provide experimentally derived information with which to generate a more accurate molecular model of the ligand binding pocket for the GLP-1 receptor [124].

3.4. Emerging Biochemical Technologies. Although progress is continuing, integrated strategies pairing classic receptor pharmacology with newer biophysical and structural biology techniques are needed to progressively refine a model for GLP-1 receptor activation. New techniques aimed at understanding ligand dependent conformational changes in the GLP-1 receptor should aide small molecule discovery pursuits. One emerging approach is the application of solution phase peptide amide hydrogen/deuterium exchange (HDX) coupled with mass spectrometry (MS) for the study of GPCRs. In contrast to photoaffinity labeling, HDX-MS does not require generating probes that require incorporation of a bulky moiety, such as the hydrophobic Bpa, and must retain the binding and activation properties of the natural ligand. Alternatively, HDX-MS is based on the principle that for proteins in solution, amide bond hydrogen atoms are exchangeable, and differences in the rate of exchange are indicative of local accessibility and thus can reflect the conformational status of a protein [126, 127]. Deuterium incorporation into the peptide backbone increases protein mass, and upon protease cleavage, the location and degree of hydrogen/deuterium exchange can be

mapped via MS analysis. Although technically difficult to apply to membrane proteins, methods have advanced that better enable purification procedures for isolating GPCRs using detergents that help maintain native structure, protein solubility, and functional activity. From these advances, HDX-MS has now been used to study ligand induced conformational changes of the β2 adrenergic receptor [128, 129]. These biochemical studies demonstrate that distinct receptor conformations are elicited by ligands with different intrinsic efficacies. Further, an elegant parallel MS approach has utilized covalent derivatization of cysteine and lysine residues with stable isotope labeled reactive functionalities to assess dynamic conformational changes elicited by β2 adrenergic receptor ligands [130]. These studies highlight the diversity of receptor conformations induced by ligands with apparently similar functional capacities. Together, this work provides further experimental evidence for the existence of multiple ligand specific conformations of GPCRs.

3.5. Recent Advances in GPCR Crystallography. The first GPCR crystal structure determined was that of Rhodopsin in 2000 [131]. While informative about the general principles of GPCR structure, and having utility as a homology model template for closely related class A GPCRs, this information has not significantly impacted drug discovery activities for class B1 GPCRs. However, recent advances in GPCR biochemistry and macromolecular crystallography have accelerated the pace of structure determination for this important target class [132]. Since 2007, multiple new class A GPCR structures have been determined, including adrenergic, adenosine, chemokine, dopamine, and histamine receptors [132]. In many cases, structures of multiple ligands with cognate GPCRs are solved. Moreover, the recent determination of a G-protein bound complex of an activated GPCR represents a landmark achievement of GPCR crystallography [133]. Several new technological advances have been developed to facilitate these pursuits, including novel detergents [134], creative receptor-fusion proteins [135], camelid nanobodies [136], and lipidic cubic phase crystallization [137]. Consequently, we anticipate that significant effort may now turn toward determining the crystal structures of class B1 GPCRs.

4. Conclusions

It is clear the GLP-1/GLP-1 receptor axis is a key physiologic regulator of glucose metabolism, and diabetic patients treated with degradation resistant GLP-1 receptor peptide agonists, exenatide and liraglutide, experience improved glucose homeostasis. Therefore, efforts to identify orally active small molecule GLP-1 receptor agonists are justifiably being pursued. While several scaffolds are now reported (see Willard et al. in this issue of *Experimental Diabetes Research*), high-throughput screening campaigns and other discovery approaches have largely failed to identify quality chemical starting points that have been successfully optimized into therapeutic agents. The lack of apparent success is likely due to the inherent difficulty of lower molecular weight, nonpeptide molecules to mimic the complex nature of peptide ligand binding to the GLP-1 receptor ECD and transmembrane regions needed to induce intrinsic structural changes in the receptor to elicit signal transduction.

Fortunately, the availability of peptide ligands for the GLP-1 receptor enables very detailed assessment of GLP-1 receptor signaling pathways, and newer biophysical techniques are helping interrogate the integral mechanisms involved in receptor activation. Further, advances in structural biology methodologies for GPCRs are now rapidly occurring, and application of these techniques to class B1 GPCRs will be groundbreaking. Assimilation of structural information for the full length, intact GLP-1 receptor and improved assay systems with which to monitor the GLP-1 receptor in different conformational states will likely be critical to advancing nascent efforts to identify GLP-1 receptor small molecule ligands. Once novel compounds emerge, it will be important to optimize molecules using testing schemes that incorporate signal transduction mechanisms of GLP-1 physiology, especially GLP-1 receptor stimulation of cAMP production to enhance glucose dependent insulin secretion.

Author Contributions

F. S. Willard and K. W. Sloop contributed equally to this paper.

Conflict of Interest

Both authors are employees of Eli Lilly and Company and may own company stock or possess stock options.

References

[1] M. A. Nauck, E. Homberger, and E. G. Siegel, "Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses," *The Journal of Clinical Endocrinology & Metabolism*, vol. 63, no. 2, pp. 492–498, 1986.

[2] H. Elrick, L. Stimmler, C. J. Hlad, and Y. ARAI, "Plasma insulin response to oral and intravenous glucose administration," *The Journal of Clinical Endocrinology & Metabolism*, vol. 24, pp. 1076–1082, 1964.

[3] M. J. Perley and D. M. Kipnis, "Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic sujbjects," *Journal of Clinical Investigation*, vol. 46, no. 12, pp. 1954–1962, 1967.

[4] N. Mcintyre, C. D. Holdsworth, and D. S. Turner, "New interpretation of oral glucose tolerance," *The Lancet*, vol. 2, no. 7349, pp. 20–21, 1964.

[5] R. H. Unger and A. M. Eisentraut, "Entero-insular axis," *Archives of Internal Medicine*, vol. 123, no. 3, pp. 261–266, 1969.

[6] C. Orskov, J. J. Holst, and O. V. Nielsen, "Effect of truncated glucagon-like peptide-1 [Proglucagon-(78–107) amide] on endocrine secretion from pig pancreas, antrum, and nonantral stomach," *Endocrinology*, vol. 123, no. 4, pp. 2009–2013, 1988.

[7] J. J. Holst, C. Orskov, O. V. Nielsen, and T. W. Schwartz, "Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut," *FEBS Letters*, vol. 211, no. 2, pp. 169–174, 1987.

[8] B. Kreymann, G. Williams, M. A. Ghatei, and S. R. Bloom, "Glucagon-like peptide-1 7–36: a physiological incretin in man," *The Lancet*, vol. 2, no. 8571, pp. 1300–1304, 1987.

[9] R. M. Elliott, L. M. Morgan, J. A. Tredger, S. Deacon, J. Wright, and V. Marks, "Glucagon-like peptide-1(7–36)amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24−h secretion patterns," *Journal of Endocrinology*, vol. 138, no. 1, pp. 159–166, 1993.

[10] K. B. Lauritsen, A. J. Moody, K. C. Christensen, and S. Lindkaer Jensen, "Gastric inhibitory polypeptide (GIP) and insulin release after small-bowel resection in man," *Scandinavian Journal of Gastroenterology*, vol. 15, no. 7, pp. 833–840, 1980.

[11] S. Mojsov, G. C. Weir, and J. F. Habener, "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," *Journal of Clinical Investigation*, vol. 79, no. 2, pp. 616–619, 1987.

[12] J. C. Brown, "Gastric inhibitory polypeptide," *Monographs on Endocrinology*, vol. 24, pp. 1–88, 1982.

[13] J. Dupre, S. A. Ross, D. Watson, and J. C. Brown, "Stimulation of insulin secretion by gastric inhibitory polypeptide in man," *The Journal of Clinical Endocrinology & Metabolism*, vol. 37, no. 5, pp. 826–828, 1973.

[14] J. M. Falko, S. E. Crockett, S. Cataland, and E. L. Mazzaferri, "Gastric inhibitory polypeptide (GIP) stimulated by fat ingestion in man," *The Journal of Clinical Endocrinology & Metabolism*, vol. 41, no. 2, pp. 260–265, 1975.

[15] F. B. Thomas, E. L. Mazzaferri, and S. E. Crockett, "Stimulation of secretion of gastric inhibitory polypeptide and insulin by intraduodenal amino acid perfusion," *Gastroenterology*, vol. 70, no. 4, pp. 523–527, 1976.

[16] T. Kauth and J. Metz, "Immunohistochemical localization of glucagon-like peptide 1. Use of poly- and monoclonal antibodies," *Histochemistry*, vol. 86, no. 5, pp. 509–515, 1987.

[17] R. Eissele, R. Goke, S. Willemer et al., "Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man," *European Journal of Clinical Investigation*, vol. 22, no. 4, pp. 283–291, 1992.

[18] G. I. Bell, R. Sanchez Pescador, P. J. Laybourn, and R. C. Najarian, "Exon duplication and divergence in the human preproglucagon gene," *Nature*, vol. 304, no. 5924, pp. 368–371, 1983.

[19] J. J. Holst, "Gut glucagon, enteroglucagon, gut glucagonlike immunoreactivity, glicentin—current status," *Gastroenterology*, vol. 84, no. 6, pp. 1602–1613, 1983.

[20] Y. Rouille, S. Kantengwa, J. C. Irminger, and P. A. Halban, "Role of the prohormone convertase PC3 in the processing of proglucagon to glucagon-like peptide 1," *The Journal of Biological Chemistry*, vol. 272, no. 52, pp. 32810–32816, 1997.

[21] X. Zhu, A. Zhou, A. Dey et al., "Disruption of PC1/3 expression in mice causes dwarfism and multiple neuroendocrine peptide processing defects," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 16, pp. 10293–10298, 2002.

[22] M. E. Rothenberg, C. D. Eilertson, K. Klein et al., "Processing of mouse proglucagon by recombinant prohormone convertase 1 and immunopurified prohormone convertase 2 *in vitro*," *The Journal of Biological Chemistry*, vol. 270, no. 17, pp. 10136–10146, 1995.

[23] J. Takeda, Y. Seino, K. Tanaka et al., "Sequence of an intestinal cDNA encoding human gastric inhibitory polypeptide precursor," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 84, no. 20, pp. 7005–7008, 1987.

[24] A. M. Buchan, J. M. Polak, and C. Capella, "Electronimmunocytochemical evidence for the K cell localization of gastric inhibitory polypeptide (GIP) in man," *Histochemistry*, vol. 56, no. 1, pp. 37–44, 1978.

[25] B. Thorens, "Expression cloning of the pancreatic beta cell receptor for the gluco- incretin hormone glucagon-like peptide 1," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 89, no. 18, pp. 8641–8645, 1992.

[26] B. Thorens, A. Porret, L. Buhler, S. P. Deng, P. Morel, and C. Widmann, "Cloning and functional expression of the human islet GLP-1 receptor: demonstration that exendin-4 is an agonist and exendin-(9–39) an antagonist of the receptor," *Diabetes*, vol. 42, no. 11, pp. 1678–1682, 1993.

[27] T. B. Usdin, E. Mezey, D. C. Button, M. J. Brownstein, and T. I. Bonner, "Gastric inhibitory polypeptide receptor, a member of the secretin- vasoactive intestinal peptide receptor family, is widely distributed in peripheral organs and the brain," *Endocrinology*, vol. 133, no. 6, pp. 2861–2870, 1993.

[28] F. Preitner, M. Ibberson, I. Franklin et al., "Gluco-incretins control insulin secretion at multiple levels as revealed in mice lacking GLP-1 and GIP receptors," *Journal of Clinical Investigation*, vol. 113, no. 4, pp. 635–645, 2004.

[29] R. Jorgensen, L. Martini, T. W. Schwartz, and C. E. Elling, "Characterization of glucagon-like peptide-1 receptor beta-arrestin 2 interaction: a high-affinity receptor phenotype," *Molecular Endocrinology*, vol. 19, no. 3, pp. 812–823, 2005.

[30] N. Sonoda, T. Imamura, T. Yoshizaki, J. L. Babendure, J. C. Lu, and J. M. Olefsky, "Beta-Arrestin-1 mediates glucagon-like peptide-1 signaling to insulin secretion in cultured pancreatic beta cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 18, pp. 6614–6619, 2008.

[31] I. Dzhura, O. G. Chepurny, G. G. Kelley et al., "Epac2-dependent mobilization of intracellular Ca^{2+} by glucagon-like peptide-1 receptor agonist exendin-4 is disrupted in beta-cells of phospholipase C-beta knockout mice," *Journal of Physiology*, vol. 588, part 24, pp. 4871–4889, 2010.

[32] M. S. Islam, "Calcium signaling in the islets," *Advances in Experimental Medicine and Biology*, vol. 654, pp. 235–259, 2010.

[33] M. Nauck, F. Stockmann, R. Ebert, and W. Creutzfeldt, "Reduced incretin effect in Type 2 (non-insulin-dependent) diabetes," *Diabetologia*, vol. 29, no. 1, pp. 46–52, 1986.

[34] I. R. Jones, D. R. Owens, A. J. Moody, S. D. Luzio, T. Morris, and T. M. Hayes, "The effects of glucose-dependent insulinotropic polypeptide infused at physiological concentrations in normal subjects and Type 2 (non-insulin-dependent) diabetic patients on glucose tolerance and B-cell secretion," *Diabetologia*, vol. 30, no. 9, pp. 707–712, 1987.

[35] T. Krarup, N. Saurbrey, and A. J. Moody, "Effect of porcine gastric inhibitory polypeptide on beta-cell function in type I and type II diabetes mellitus," *Metabolism*, vol. 36, no. 7, pp. 677–682, 1987.

[36] M. A. Heimesaat, C. Orskov, J. J. Holst, R. Ebert, and W. Creutzfeldt, "Preserved incretin activity of glucagon-like peptide 1 [7–36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus," *Journal of Clinical Investigation*, vol. 91, no. 1, pp. 301–307, 1993.

[37] D. M. Nathan, E. Schreiber, H. Fogel, S. Mojsov, and J. F. Habener, "Insulinotropic action of glucagonlike peptide-I-(7–37) in diabetic and nondiabetic subjects," *Diabetes Care*, vol. 15, no. 2, pp. 270–276, 1992.

[38] M. Gutniak, C. Orskov, J. J. Holst, B. Ahren, and S. Efendic, "Antidiabetogenic effect of glucagon-like peptide-1 (7–36)amide in normal subjects and patients with diabetes mellitus," *The New England Journal of Medicine*, vol. 326, no. 20, pp. 1316–1322, 1992.

[39] M. A. Nauck, N. Kleine, C. Orskov, J. J. Holst, B. Willms, and W. Creutzfeldt, "Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7–36 amide) in Type 2 (non-insulin-dependent) diabetic patients," *Diabetologia*, vol. 36, no. 8, pp. 741–744, 1993.

[40] M. A. Nauck et al., "Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans," *American Journal of Physiology*, vol. 273, no. 5, part 1, pp. E981–E988, 1997.

[41] B. Willms, J. Werner, J. J. Holst, C. Ørskov, W. Creutzfeldt, and M. A. Nauck, " Gastric emptying, glucose responses, and insulin secretion after a liquid test meal: effects of exogenous glucagon-like peptide-1 (GLP-1)-(7–36) amide in type 2 (noninsulin-dependent) diabetic patients," *The Journal of Clinical Endocrinology & Metabolism*, vol. 81, no. 1, pp. 327–332, 1996.

[42] J. M. Wishart, M. Horowitz, H. A. Morris, K. L. Jones, and M. A. Nauck, "Relation between gastric emptying of glucose and plasma concentrations of glucagon-like peptide-1," *Peptides*, vol. 19, no. 6, pp. 1049–1053, 1998.

[43] K. Meeran, D. O'Shea, C. M. B. Edwards et al., "Repeated intracerebroventricular administration of glucagon-like peptide-1-(7–36) amide or exendin-(9–39) alters body weight in the rat," *Endocrinology*, vol. 140, no. 1, pp. 244–250, 1999.

[44] A. Flint, A. Raben, A. Astrup, and J. J. Holst, "Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans," *Journal of Clinical Investigation*, vol. 101, no. 3, pp. 515–520, 1998.

[45] J. P. Gutzwiller, J. Drewe, B. Göke et al., "Glucagon-like peptide-1 promotes satiety and reduces food intake in patients with diabetes mellitus type 2," *American Journal of Physiology*, vol. 276, no. 5, part 2, pp. R1541–R1544, 1999.

[46] J. P. Gutzwiller, B. Göke, J. Drewe et al., "Glucagon-like peptide-1: a potent regulator of food intake in humans," *Gut*, vol. 44, no. 1, pp. 81–86, 1999.

[47] M. D. Turton, D. O'Shea, I. Gunn et al., "A role for glucagon-like peptide-1 in the central regulation of feeding," *Nature*, vol. 379, no. 6560, pp. 69–72, 1996.

[48] R. Mentlein, B. Gallwitz, and W. E. Schmidt, "Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7–36)amide, peptide histidine methionine and is responsible for their degradation in human serum," *European Journal of Biochemistry*, vol. 214, no. 3, pp. 829–835, 1993.

[49] C. F. Deacon, A. H. Johnsen, and J. J. Holst, "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*," *The Journal of Clinical Endocrinology & Metabolism*, vol. 80, no. 3, pp. 952–957, 1995.

[50] L. B. Knudsen and L. Pridal, "Glucagon-like peptide-1-(9–36) amide is a major metabolite of glucagon-like peptide-1-(7–36) amide after *in vivo* administration to dogs, and it acts as an antagonist on the pancreatic receptor," *European Journal of Pharmacology*, vol. 318, no. 2-3, pp. 429–435, 1996.

[51] L. Hansen, C. F. Deacon, C. Ørskov, and J. J. Holst, "Glucagon-like peptide-1-(7–36)amide is transformed to glucagon-like peptide-1-(9–36)amide by dipeptidyl peptidase IV in the capillaries supplying the L cells of the porcine intestine," *Endocrinology*, vol. 140, no. 11, pp. 5356–5363, 1999.

[52] C. F. Deacon, L. Pridal, L. Klarskov, M. Olesen, and J. J. Holst, "Glucagon-like peptide 1 undergoes differential tissue-specific metabolism in the anesthetized pig," *American Journal of Physiology*, vol. 271, no. 3, part 1, pp. E458–E464, 1996.

[53] T. Vilsboll, H. Agersø, T. Krarup, and J. J. Holst, "Similar elimination rates of glucagon-like peptide-1 in obese type 2 diabetic patients and healthy subjects," *The Journal of Clinical Endocrinology & Metabolism*, vol. 88, no. 1, pp. 220–224, 2003.

[54] E. G. Siegel, B. Gallwitz, G. Scharf et al., "Biological activity of GLP-1-analogues with N-terminal modifications," *Regulatory Peptides*, vol. 79, no. 2-3, pp. 93–102, 1999.

[55] U. Ritzel, U. Leonhardt, M. Ottleben et al., "A synthetic glucagon-like peptide-1 analog with improved plasma stability," *Journal of Endocrinology*, vol. 159, no. 1, pp. 93–102, 1998.

[56] C. F. Deacon, L. B. Knudsen, K. Madsen, F. C. Wiberg, O. Jacobsen, and J. J. Holst, "Dipeptidyl peptidase IV resistant analogues of glucagon-like peptide-1 which have extended metabolic stability and improved biological activity," *Diabetologia*, vol. 41, no. 3, pp. 271–278, 1998.

[57] R. Goke, H. C. Fehmann, T. Linn et al., "Exendin-4 is a high potency agonist and truncated exendin-(9–39)-amide an antagonist at the glucagon-like peptide 1-(7–36)-amide receptor of insulin- secreting beta-cells," *The Journal of Biological Chemistry*, vol. 268, no. 26, pp. 19650–19655, 1993.

[58] H. C. Fehmann, J. Jiang, J. Schweinfurth, M. B. Wheeler, A. E. Boyd, and B. Goke, "Stable expression of the rat GLP-I receptor in CHO cells: activation and binding characteristics utilizing GLP-I(7–36)-amide, oxyntomodulin, exendin- 4, and exendin(9–39)," *Peptides*, vol. 15, no. 3, pp. 453–456, 1994.

[59] H. C. Fehmann, J. Jiang, J. Schweinfurth et al., "Ligand-specificity of the rat GLP-I receptor recombinantly expressed in Chinese hamster ovary (CHO-) cells," *Zeitschrift fur Gastroenterologie*, vol. 32, no. 4, pp. 203–207, 1994.

[60] J. Eng, W. A. Kleinman, L. Singh, G. Singh, and J. P. Raufman, "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," *The Journal of Biological Chemistry*, vol. 267, no. 11, pp. 7402–7405, 1992.

[61] J. Eng, P. C. Andrews, W. A. Kleinman, L. Singh, and J. P. Raufman, "Purification and structure of exendin-3, a new pancreatic secretagogue isolated from Heloderma horridum venom," *The Journal of Biological Chemistry*, vol. 265, no. 33, pp. 20259–20262, 1990.

[62] F. E. Russell and C. M. Bogert, "Gila monster: its biology, venom and bite—a review," *Toxicon*, vol. 19, no. 3, pp. 341–359, 1981.

[63] A. Thum, K. Hupe-Sodmann, R. Göke, K. Voigt, B. Göke, and G. P. McGregor, "Endoproteolysis by isolated membrane peptidases reveal metabolic stability of glucagon-like peptide-1 analogs, exendins-3 and -4," *Experimental and Clinical Endocrinology and Diabetes*, vol. 110, no. 3, pp. 113–118, 2002.

[64] C. M. Edwards, S. A. Stanley, R. Davis et al., "Exendin-4 reduces fasting and postprandial glucose and decreases

energy intake in healthy volunteers," *American Journal of Physiology*, vol. 281, no. 1, pp. E155–E161, 2001.

[65] L. Simonsen, J. J. Holst, and C. F. Deacon, "Exendin-4, but not glucagon-like peptide-1, is cleared exclusively by glomerular filtration in anaesthetised pigs," *Diabetologia*, vol. 49, no. 4, pp. 706–712, 2006.

[66] N. H. Greig, H. W. Holloway, K. A. De Ore et al., "Once daily injection of exendin-4 to diabetic mice achieves long-term beneficial effects on blood glucose concentrations," *Diabetologia*, vol. 42, no. 1, pp. 45–50, 1999.

[67] M. Szayna, M. E. Doyle, J. A. Betkey et al., "Exendin-4 decelerates food intake, weight gain, and fat deposition in Zucker rats," *Endocrinology*, vol. 141, no. 6, pp. 1936–1941, 2000.

[68] A. A. Young, B. R. Gedulin, S. Bhavsar et al., "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*, vol. 48, no. 5, pp. 1026–1034, 1999.

[69] O. G. Kolterman, D. D. Kim, L. Shen et al., "Pharmacokinetics, pharmacodynamics, and safety of exenatide in patients with type 2 diabetes mellitus," *American Journal of Health-System Pharmacy*, vol. 62, no. 2, pp. 173–181, 2005.

[70] L. B. Knudsen, P. F. Nielsen, P. O. Huusfeldt et al., "Potent derivatives of glucagon-like peptide-1 with pharmacokinetic properties suitable for once daily administration," *Journal of Medicinal Chemistry*, vol. 43, no. 9, pp. 1664–1669, 2000.

[71] H. Agerso, L. B. Jensen, B. Elbrønd, P. Rolan, and M. Zdravkovic, "The pharmacokinetics, pharmacodynamics, safety and tolerability of NN2211, a new long-acting GLP-1 derivative, in healthy men," *Diabetologia*, vol. 45, no. 2, pp. 195–202, 2002.

[72] B. Elbrond, G. Jakobsen, S. Larsen et al., "Pharmacokinetics, pharmacodynamics, safety, and tolerability of a single-dose of NN2211, a long-acting glucagon-like peptide 1 derivative, in healthy male subjects," *Diabetes Care*, vol. 25, no. 8, pp. 1398–1404, 2002.

[73] J. W. Neidigh, R. M. Fesinmeyer, K. S. Prickett, and N. H. Andersen, "Exendin-4 and glucagon-like-peptide-1: NMR structural comparisons in the solution and micelle-associated states," *Biochemistry*, vol. 40, no. 44, pp. 13188–13200, 2001.

[74] C. R. Underwood, P. Garibay, L. B. Knudsen et al., "Crystal structure of glucagon-like peptide-1 in complex with the extracellular domain of the glucagon-like peptide-1 receptor," *The Journal of Biological Chemistry*, vol. 285, no. 1, pp. 723–730, 2010.

[75] S. Runge, H. Thøgersen, K. Madsen, J. Lau, and R. Rudolph, "Crystal structure of the ligand-bound glucagon-like peptide-1 receptor extracellular domain," *The Journal of Biological Chemistry*, vol. 283, no. 17, pp. 11340–11347, 2008.

[76] M. Castro, V. O. Nikolaev, D. Palm, M. J. Lohse, and J. P. Vilardaga, "Turn-on switch in parathyroid hormone receptor by a two-step parathyroid hormone binding mechanism," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 44, pp. 16084–16089, 2005.

[77] S. Al-Sabah and D. Donnelly, "A model for receptor-peptide binding at the glucagon-like peptide-1 (GLP-1) receptor through the analysis of truncated ligands and receptors," *British Journal of Pharmacology*, vol. 140, no. 2, pp. 339–346, 2003.

[78] S. Runge, B. S. Wulff, K. Madsen, H. Bräuner-Osborne, and L. B. Knudsen, "Different domains of the glucagon and glucagon-like peptide-1 receptors provide the critical determinants of ligand selectivity," *British Journal of Pharmacology*, vol. 138, no. 5, pp. 787–794, 2003.

[79] M. H. Perrin, C. R. R. Grace, R. Riek, and W. W. Vale, "The three-dimensional structure of the n-terminal domain of corticotropin-releasing factor receptors sushi domains and the B1 family of G protein-coupled receptors," *Annals of the New York Academy of Sciences*, vol. 1070, pp. 105–119, 2006.

[80] A. A. Pioszak and H. E. Xu, "Molecular recognition of parathyroid hormone by its G protein-coupled receptor," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 13, pp. 5034–5039, 2008.

[81] A. Bazarsuren, U. Grauschopf, M. Wozny et al., "*In vitro* folding, functional characterization, and disulfide pattern of the extracellular domain of human GLP-1 receptor," *Biophysical Chemistry*, vol. 96, no. 2-3, pp. 305–318, 2002.

[82] S. R. Hoare, "Mechanisms of peptide and nonpeptide ligand binding to Class B G-protein-coupled receptors," *Drug Discovery Today*, vol. 10, no. 6, pp. 417–427, 2005.

[83] L. Jin, S. L. Briggs, S. Chandrasekhar et al., "Crystal structure of human parathyroid hormone 1-34 at 0.9−A resolution," *The Journal of Biological Chemistry*, vol. 275, no. 35, pp. 27238–27244, 2000.

[84] K. Thornton and D. G. Gorenstein, "Structure of glucagon-like peptide(7–36) amide in a dodecylphosphocholine micelle as determined by 2D NMR," *Biochemistry*, vol. 33, no. 12, pp. 3532–3539, 1994.

[85] N. H. Andersen, Y. Brodsky, J. W. Neidigh, and K. S. Prickett, "Medium-dependence of the secondary structure of exendin-4 and glucagon-like-peptide-1," *Bioorganic and Medicinal Chemistry*, vol. 10, no. 1, pp. 79–85, 2002.

[86] C. A. Leech, O. G. Chepurny, and G. G. Holz, "Epac2-Dependent Rap1 Activation and the Control of Islet Insulin Secretion by Glucagon-Like Peptide-1," *Vitamins and Hormones*, vol. 84, no. C, pp. 279–302, 2010.

[87] M. B. Wheeler, M. Lu, J. S. Dillon, X. H. Leng, C. Chen, and A. E. Boyd, "Functional expression of the rat glucagon-like peptide-I receptor, evidence for coupling to both adenylyl cyclase and phospholipase-C," *Endocrinology*, vol. 133, no. 1, pp. 57–62, 1993.

[88] C. Montrose-Rafizadeh, P. Avdonin, M. J. Garant et al., "Pancreatic glucagon-like peptide-1 receptor couples to multiple G proteins and activates mitogen-activated protein kinase pathways in Chinese hamster ovary cells," *Endocrinology*, vol. 140, no. 3, pp. 1132–1140, 1999.

[89] C. A. Cullinan, E. J. Brady, R. Saperstein, and M. D. Leibowitz, "Glucose-dependent alterations of intracellular free calcium by glucagon-like peptide-1((7–36amide)) in individual ob/ob mouse beta-cells," *Cell Calcium*, vol. 15, no. 5, pp. 391–400, 1994.

[90] C. Koole, D. Wootten, J. Simms et al., "Allosteric ligands of the glucagon-like peptide 1 receptor (GLP-1R) differentially modulate endogenous and exogenous peptide responses in a pathway-selective manner: implications for drug screening," *Molecular Pharmacology*, vol. 78, no. 3, pp. 456–465, 2010.

[91] K. Coopman, Y. Huang, N. Johnston, S. J. Bradley, G. F. Wilkinson, and G. B. Willars, "Comparative effects of the endogenous agonist glucagon-like peptide-1 (GLP-1)-(7–36) amide and the small-molecule ago-allosteric agent "compound 2" at the GLP-1 receptor," *Journal of Pharmacology and Experimental Therapeutics*, vol. 334, no. 3, pp. 795–808, 2010.

[92] A. Sassmann, B. Gier, H. J. Gröne, G. Drews, S. Offermanns, and N. Wettschureck, "The Gq/G11-mediated signaling pathway is critical for autocrine potentiation of insulin

secretion in mice," *Journal of Clinical Investigation*, vol. 120, no. 6, pp. 2184–2193, 2010.

[93] T. Yada, K. Itoh, and M. Nakata, "Glucagon-like peptide-1-(7–36)amide and a rise in cyclic adenosine 3′,5′- monophosphate increase cytosolic free Ca^{2+} in rat pancreatic beta-cells by enhancing Ca^{2+} activity," *Endocrinology*, vol. 133, no. 4, pp. 1685–1692, 1993.

[94] T. Kenakin, "Efficacy in drug receptor theory: outdated concept or under-valued tool?" *Trends in Pharmacological Sciences*, vol. 20, no. 10, pp. 400–405, 1999.

[95] J. Guo, M. Liu, D. Yang et al., "Phospholipase C signaling via the parathyroid hormone (PTH)/PTH-related peptide receptor is essential for normal bone responses to PTH," *Endocrinology*, vol. 151, no. 8, pp. 3502–3513, 2010.

[96] J. Takasaki, T. Saito, M. Taniguchi et al., "A novel Galphaq/11-selective inhibitor," *The Journal of Biological Chemistry*, vol. 279, no. 46, pp. 47438–47445, 2004.

[97] R. Jorgensen, V. Kubale, M. Vrecl, T. W. Schwartz, and C. E. Elling, "Oxyntomodulin differentially affects glucagon-like peptide-1 receptor beta-arrestin recruitment and signaling through Galpha(s)," *Journal of Pharmacology and Experimental Therapeutics*, vol. 322, no. 1, pp. 148–154, 2007.

[98] N. J. Freedman and R. J. Lefkowitz, "Desensitization of G protein-coupled receptors," *Recent Progress in Hormone Research*, vol. 51, pp. 319–351, 1996, discussion 352-353.

[99] S. Rajagopal, K. Rajagopal, and R. J. Lefkowitz, "Teaching old receptors new tricks: biasing seven-transmembrane receptors," *Nature Reviews Drug Discovery*, vol. 9, no. 5, pp. 373–386, 2010.

[100] J. Quoyer, C. Longuet, C. Broca et al., "GLP-1 mediates antiapoptotic effect by phosphorylating bad through a beta-arrestin 1-mediated ERK1/2 activation in pancreatic beta-cells," *The Journal of Biological Chemistry*, vol. 285, no. 3, pp. 1989–2002, 2010.

[101] B. Luan, J. Zhao, H. Wu et al., "Deficiency of a beta-arrestin-2 signal complex contributes to insulin resistance," *Nature*, vol. 457, no. 7233, pp. 1146–1149, 2009.

[102] Y. Li, T. Hansotia, B. Yusta, F. Ris, P. A. Halban, and D. J. Drueker, "Glucagon-like peptide-1 receptor signaling modulates beta cell apoptosis," *The Journal of Biological Chemistry*, vol. 278, no. 1, pp. 471–478, 2003.

[103] Q. Wang and P. Brubaker, "Glucagon-like peptide-1 treatment delays the onset of diabetes in 8 week-old db/db mice," *Diabetologia*, vol. 45, no. 9, pp. 1263–1273, 2002.

[104] R. Burcelin and S. Dejager, "GLP-1: what is known, new and controversial in 2010?" *Diabetes and Metabolism*, vol. 36, no. 6, part 1, pp. 503–509, 2010.

[105] L. Farilla, A. Bulotta, B. Hirshberg et al., "Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets," *Endocrinology*, vol. 144, no. 12, pp. 5149–5158, 2003.

[106] L. Tian, J. Gao, G. Weng et al., "Comparison of exendin-4 on beta-cell replication in mouse and human islet grafts," *Transplant International*, vol. 24, no. 8, pp. 856–864, 2011.

[107] M. E. Doyle and J. M. Egan, "Mechanisms of action of glucagon-like peptide 1 in the pancreas," *Pharmacology and Therapeutics*, vol. 113, no. 3, pp. 546–593, 2007.

[108] D. Donnelly, "The structure and function of the glucagon-like peptide-1 receptor and its ligands," *British Journal of Pharmacology*. In press.

[109] M. Dong, D. I. Pinon, Y. W. Asmann, and L. J. Miller, "Possible endogenous agonist mechanism for the activation of secretin family G protein-coupled receptors," *Molecular Pharmacology*, vol. 70, no. 1, pp. 206–213, 2006.

[110] M. Dong, F. Gao, D. I. Pinon, and L. J. Miller, "Insights into the structural basis of endogenous agonist activation of family B G protein-coupled receptors," *Molecular Endocrinology*, vol. 22, no. 6, pp. 1489–1499, 2008.

[111] M. Dong, D. I. Pinon, and L. J. Miller, "Site of action of a pentapeptide agonist at the glucagon-like peptide-1 receptor. Insight into a small molecule agonist-binding pocket," *Bioorganic and Medicinal Chemistry Letters*, vol. 22, no. 1, pp. 638–641, 2012.

[112] L. Covic, A. L. Gresser, J. Talavera, S. Swift, and A. Kuliopulos, "Activation and inhibition of G protein-coupled receptors by cell-penetrating membrane-tethered peptides," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 2, pp. 643–648, 2002.

[113] J. M. Janz, Y. Ren, R. Looby et al., "Direct interaction between an allosteric agonist pepducin and the chemokine receptor CXCR4," *Journal of the American Chemical Society*, vol. 133, no. 40, pp. 15878–15881, 2011.

[114] S. Runge, S. Schimmer, J. Oschmann et al., "Differential structural properties of GLP-1 and exendin-4 determine their relative affinity for the GLP-1 receptor N-terminal extracellular domain," *Biochemistry*, vol. 46, no. 19, pp. 5830–5840, 2007.

[115] R. L. de Maturana, A. Willshaw, A. Kuntzsch, R. Rudolph, and D. Donnelly, "The isolated N-terminal domain of the glucagon-like peptide-1 (GLP-1) receptor binds exendin peptides with much higher affinity than GLP-1," *The Journal of Biological Chemistry*, vol. 278, no. 12, pp. 10195–10200, 2003.

[116] R. J. Mann, N. E. Nasr, J. K. Sinfield, E. Paci, and D. Donnelly, "The major determinant of exendin-4/glucagon-like peptide 1 differential affinity at the rat glucagon-like peptide 1 receptor N-terminal domain is a hydrogen bond from SER-32 of exendin-4: research paper," *British Journal of Pharmacology*, vol. 160, no. 8, pp. 1973–1984, 2010.

[117] K. Schroder-Tittmann, E. Bosse-Doenecke, S. Reedtz-Runge et al., "Recombinant expression, *in vitro* refolding, and biophysical characterization of the human glucagon-like peptide-1 receptor," *Biochemistry*, vol. 49, no. 36, pp. 7956–7965, 2010.

[118] A. Bisello, A. E. Adams, D. F. Mierke et al., "Parathyroid hormone-receptor interactions identified directly by photocross-linking and molecular modeling studies," *The Journal of Biological Chemistry*, vol. 273, no. 35, pp. 22498–22505, 1998.

[119] M. Dong, Z. Li, D. I. Pinon, T. P. Lybrand, and L. J. Miller, "Spatial approximation between the amino terminus of a peptide agonist and the top of the sixth transmembrane segment of the secretin receptor," *The Journal of Biological Chemistry*, vol. 279, no. 4, pp. 2894–2903, 2004.

[120] M. Dong, D. I. Pinon, R. F. Cox, and L. J. Miller, "Molecular approximation between a residue in the amino-terminal region of calcitonin and the third extracellular loop of the class B G protein-coupled calcitonin receptor," *The Journal of Biological Chemistry*, vol. 279, no. 30, pp. 31177–31182, 2004.

[121] Z. Ji, E. M. Hadac, R. M. Henne, S. A. Patel, T. P. Lybrand, and L. J. Miller, "Direct identification of a distinct site of interaction between the carboxyl-terminal residue of cholecystokinin and the type A cholecystokinin receptor using photoaffinity labeling," *The Journal of Biological Chemistry*, vol. 272, no. 39, pp. 24393–24401, 1997.

[122] Q. Chen, D. I. Pinon, L. J. Miller, and M. Dong, "Molecular basis of glucagon-like peptide 1 docking to its intact receptor

studied with carboxyl-terminal photolabile probes," *The Journal of Biological Chemistry*, vol. 284, no. 49, pp. 34135–34144, 2009.

[123] Q. Chen, D. I. Pinon, L. J. Miller, and M. Dong, "Spatial approximations between residues 6 and 12 in the amino-terminal region of glucagon-like peptide 1 and its receptor: a region critical for biological activity," *The Journal of Biological Chemistry*, vol. 285, no. 32, pp. 24508–24518, 2010.

[124] L. J. Miller, Q. Chen, P. C.-H. Lam et al., "Refinement of glucagon-like peptide 1 docking to its intact receptor using mid-region photolabile probes and molecular modeling," *The Journal of Biological Chemistry*, vol. 286, no. 18, pp. 15895–15907, 2011.

[125] K. Adelhorst, B. B. Hedegaard, L. B. Knudsen, and O. Kirk, "Structure-activity studies of glucagon-like peptide-1," *The Journal of Biological Chemistry*, vol. 269, no. 9, pp. 6275–6278, 1994.

[126] S. W. Englander, T. R. Sosnick, J. J. Englander, and L. Mayne, "Mechanisms and uses of hydrogen exchange," *Current Opinion in Structural Biology*, vol. 6, no. 1, pp. 18–23, 1996.

[127] V. Katta and B. T. Chait, "Conformational changes in proteins probed by hydrogen-exchange electrospray-ionization mass spectrometry," *Rapid Communications in Mass Spectrometry*, vol. 5, no. 4, pp. 214–217, 1991.

[128] X. Zhang, E. Y. T. Chien, M. J. Chalmers et al., "Dynamics of the beta2-adrenergic G-protein coupled receptor revealed by hydrogen-deuterium exchange," *Analytical Chemistry*, vol. 82, no. 3, pp. 1100–1108, 2010.

[129] G. M. West, E. Y. T. Chien, V. Katritch et al., "Ligand-dependent perturbation of the conformational ensemble for the GPCR beta(2) adrenergic receptor revealed by HDX," *Structure*, vol. 19, no. 10, pp. 1424–1432, 2011.

[130] A. W. Kahsai, K. Xiao, S. Rajagopal et al., "Multiple ligand-specific conformations of the beta(2)-adrenergic receptor," *Nature Chemical Biology*, vol. 7, no. 10, pp. 692–700, 2011.

[131] K. Palczewski, T. Kumasaka, T. Hori et al., "Crystal structure of rhodopsin: a G protein-coupled receptor," *Science*, vol. 289, no. 5480, pp. 739–745, 2000.

[132] J. A. Salon, D. T. Lodowski, and K. Palczewski, "The significance of G protein-coupled receptor crystallography for drug discovery," *Pharmacological Reviews*, vol. 63, no. 4, pp. 901–937, 2011.

[133] S. G. Rasmussen, B. T. Devree, Y. Zou et al., "Crystal structure of the beta(2) adrenergic receptor-Gs protein complex," *Nature*, vol. 477, no. 7366, pp. 549–555, 2011.

[134] P. S. Chae, S. G. F. Rasmussen, R. R. Rana et al., "Maltose-neopentyl glycol (MNG) amphiphiles for solubilization, stabilization and crystallization of membrane proteins," *Nature Methods*, vol. 7, no. 12, pp. 1003–1008, 2010.

[135] D. M. Rosenbaum, V. Cherezov, M. A. Hanson et al., "GPCR engineering yields high-resolution structural insights into beta2-adrenergic receptor function," *Science*, vol. 318, no. 5854, pp. 1266–1273, 2007.

[136] S. G. Rasmussen, H.-J. Choi, J. J. Fung et al., "Structure of a nanobody-stabilized active state of the $\beta 2$ adrenoceptor," *Nature*, vol. 469, no. 7329, pp. 175–180, 2011.

[137] M. Caffrey, "Crystallizing membrane proteins for structure-function studies using lipidic mesophases," *Biochemical Society Transactions*, vol. 39, no. 3, pp. 725–732, 2011.

Hindawi Publishing Corporation
Experimental Diabetes Research
Volume 2012, Article ID 709893, 9 pages
doi:10.1155/2012/709893

Review Article

Small Molecule Drug Discovery at the Glucagon-Like Peptide-1 Receptor

Francis S. Willard,[1] Ana B. Bueno,[2] and Kyle W. Sloop[3]

[1] *Translational Science and Technologies, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285, USA*
[2] *Centro de Investigación Lilly, Eli Lilly and Company, 28108 Alcobendas, Madrid, Spain*
[3] *Endocrine Discovery, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285, USA*

Correspondence should be addressed to Kyle W. Sloop, sloop_kyle_w@lilly.com

Received 31 December 2011; Accepted 24 January 2012

Academic Editor: Matteo Monami

The therapeutic success of peptide glucagon-like peptide-1 (GLP-1) receptor agonists for the treatment of type 2 diabetes mellitus has inspired discovery efforts aimed at developing orally available small molecule GLP-1 receptor agonists. Although the GLP-1 receptor is a member of the structurally complex class B1 family of GPCRs, in recent years, a diverse array of orthosteric and allosteric nonpeptide ligands has been reported. These compounds include antagonists, agonists, and positive allosteric modulators with intrinsic efficacy. In this paper, a comprehensive review of currently disclosed small molecule GLP-1 receptor ligands is presented. In addition, examples of "ligand bias" and "probe dependency" for the GLP-1 receptor are discussed; these emerging concepts may influence further optimization of known molecules or persuade designs of expanded screening strategies to identify novel chemical starting points for GLP-1 receptor drug discovery.

1. Introduction

The glucagon-like peptide-1 (GLP-1) receptor is a member of the peptide hormone binding class B1 (secretin-like receptors) family of seven transmembrane spanning, heterotrimeric G-protein coupled receptors (GPCRs). The best characterized physiologic role of the GLP-1 receptor is to help regulate insulin secretion from pancreatic β cells [1]. GLP-1 binding to the receptor activates $G\alpha_s$, stimulating membrane-associated adenylyl cyclases and cyclic 3′5′AMP (cAMP) production which enhances glucose dependent insulin secretion. The GLP-1 receptor peptide agonists, exenatide (exendin-4) and liraglutide, are widely approved medicines for the treatment of type 2 diabetes mellitus (T2DM) [2].

Identifying and developing small molecular weight organic compounds that mimic the orthosteric binding and receptor activation properties of GLP-1 peptide agonists is difficult. Class A GPCRs, for which many therapeutic small molecules have been developed [3], are structurally distinct from class B1 GPCRs. Class B1 receptors contain a larger independently folded globular ectodomain (ECD) at their N-termini. Peptide ligand binding to the ECD to initiate signaling of class B1 GPCRs is mechanistically different compared to class A receptors whose ligands primarily make contact with residues located within the membrane spanning α-helical regions [4]. For class B1 receptors, peptide ligands make numerous contacts with the ECD and extracellular loops of the transmembrane bundle [4]. For class A receptors, medicinal chemistry efforts have successfully exploited the endogenous ligand binding sites within transmembrane domains [3]. Recent reports solving X-ray crystal structures of class A GPCRs demonstrate the molecular interactions used for ligand binding [5–10].

While basic research efforts to better understand the intricate mechanisms regulating GLP-1 receptor function are being aided by advancements in GPCR molecular and structural biology (see review by Willard and Sloop in this issue of *Experimental Diabetes Research*), the field awaits determination of a high resolution crystal structure of a class B1 GPCR for use as a template to facilitate rational drug design for these difficult targets. In recent years, though, there have

been an increasing number of reports showing discovery of structurally diverse small molecule ligands for the GLP-1 receptor. While the molecular details of compound-receptor binding are largely not determined, evidence supporting interaction of ligands with the GLP-1 receptor is provided in many cases. While several of these molecules only have utility as research tools, some may represent pharmacophores to be further optimized for clinical evaluation. Importantly, although not thought to be utilized endogenously to regulate GLP-1 receptor signaling, there does appear to be evidence generated for some scaffolds indicating the presence of an allosteric pocket(s) in the GLP-1 receptor [11–13], possibly located within the transmembrane domains. As a therapeutic strategy, small molecules targeting this pocket may be optimized to enhance binding and signaling of endogenous GLP-1 receptor peptides.

The development of orally available modulators of the GLP-1 receptor for therapeutic evaluation not only requires identification of specific nonpeptide ligands but also necessitates optimizing molecules to possess appropriate physicochemical properties. This is the medicinal chemistry concept of "drug-like" compounds, that is, molecules possessing functional groups and/or having properties consistent with the majority of known drugs [14]. Careful analyses of orally active, marketed drugs have resulted in several proposed rules for guiding optimization of key physical properties of compounds. Examples of these include the pioneering "rule of five" from Lipinski [15] (Rule of five: MW $<$ 500 Da; log P $<$ 5; number of hydrogen bond donors (HBD) $<$ 5; number of hydrogen bond acceptors (HBA) $<$ 10. Compounds that violate two or more of these rules have very low probability of being developed as an oral drug.) and properties identified by Veber et al. [16] (Polar surface area (PSA) $\leq$ 140; number of rotatable bonds $\leq$10. Compounds that meet these two criteria have high probability of achieving good oral bioavailability.). These guidelines are commonly used in medicinal chemistry strategies, and therefore, the drug-like profile of several GLP-1 receptor ligands is evaluated herein.

Below are descriptions of the best characterized small molecule GLP-1 receptor ligands. Although clinical development of any of these compounds is uncertain, the data suggest small molecules can be identified that target the GLP-1 receptor. In addition to descriptions of published GLP-1 receptor agonists, other chemotypes, including antagonists and molecules only reported in the patent literature that lack thorough biological characterization, are presented. The comprehensive dissemination of knowledge for small molecule ligands of this receptor may inspire advances in chemical and biological approaches for the GLP-1 receptor.

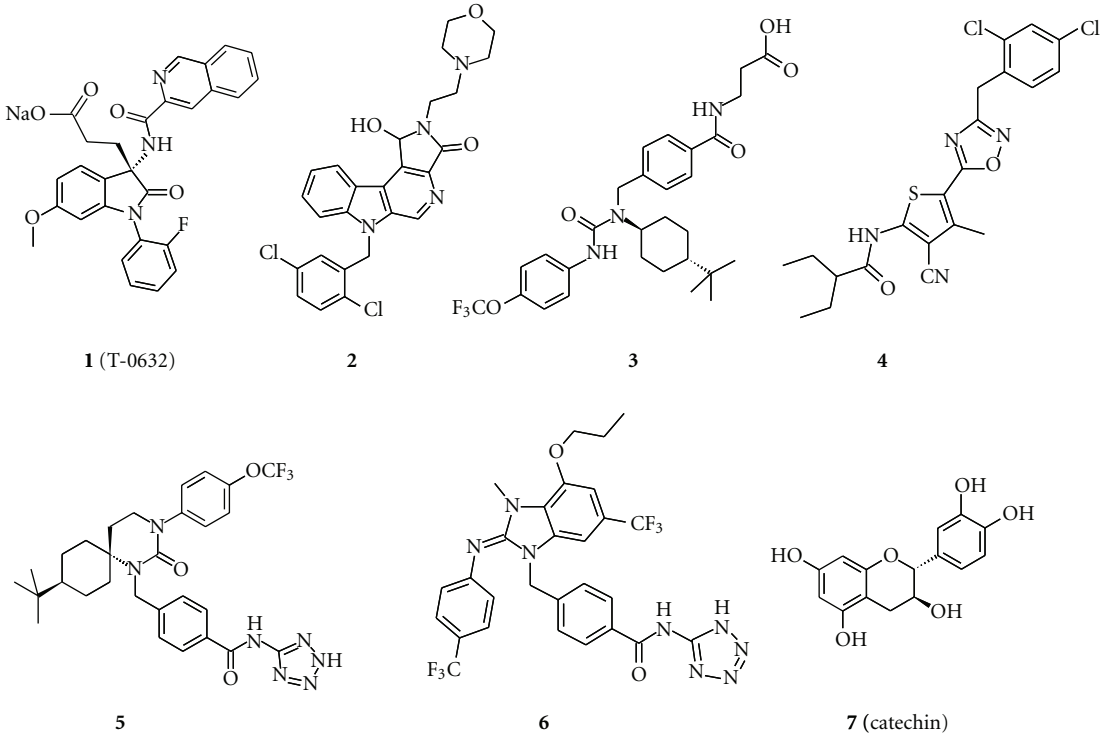
2. Low Molecular Weight GLP-1 Receptor Antagonists

2.1. PNU-126814. A small molecule GLP-1 receptor antagonist, PNU-126814, was disclosed in an abstract [17]. This compound is described to have submicromolar binding affinity for the GLP-1 receptor and inhibit GLP-1 induced cAMP modulation and insulin secretion in RINmF5 insulinoma cells. Unfortunately, the chemical structure of this compound has not been disclosed.

2.2. T-0632. The first small molecule modulator of the GLP-1 receptor to be described in the public domain with both annotated biology and chemistry is the antagonist T-0632. Beinborn et al. discovered that the cholecystokinin receptor 1 antagonist, T-0632 (Figure 1 (**1**) sodium (*S*)-3-[1-(2-fluorophenyl)-2,3-dihydro-3-[(3-isoquinolinyl-carbonyl) amino]-6-methoxy-2-oxo-1*H*-indole]propanoate), is a noncompetitive antagonist of the human GLP-1 receptor with low micromolar potency [18]. The binding site of this molecule is hypothesized to be in the ECD of the receptor as Trp^{33}, within the ECD, was identified as a critical determinant for compound action. Thus, the Trp^{33}Ser mutation in the human receptor results in a ~100-fold decrease in the binding affinity of the antagonist. Trp^{33} is within 10 Å of the peptide binding cleft in the crystal structures of GLP-1 and exendin-4 complexed with the GLP-1 receptor ECD [19, 20]. Trp^{33} does not make direct contact with the peptide ligands exendin-4 or GLP-1, but the data suggest that a small molecule binding event in this region of the protein could account for inhibitory activity. T-0632 can be considered an allosteric modulator of the GLP-1 receptor. Additionally, there is some evidence indicating the compound behaves as an inverse agonist in a constitutively active GLP-1 receptor system [21]. Although this compound could be a good tool for *in vivo* studies considering its physicochemical properties (it passes all of the Lipinski and Veber rules), the weak affinity of T-0632 for the GLP-1 receptor combined with its subnanomolar CCK1 antagonist activity renders it largely inadequate as a research tool to study the GLP-1 receptor.

2.3. 9-Benzylpyrido[3,4-b]Indoles. Agouron, Inc. described a family of molecules, exemplified by **2** (Figure 1 (**2**) 6-(2,5-dichlorobenzyl)-1-hydroxy-2-(2-morpholin-4-ylethyl)-1,6-dihydropyrrolo[3′,4′ : 5,6]pyrido[3,4-b]indol-3(2*H*)-one), as GLP-1 receptor antagonists [22]. Unfortunately, the patent describing these molecules does not include specific functional data, so the pharmacology of this chemotype of putative GLP-1 receptor antagonists remains to be elucidated. However, it is apparent that selective class B1 GPCR antagonists with well-defined pharmacology have utility in understanding biological systems and their sensitivity to therapeutic intervention [23].

2.4. Nonselective Glucagon Receptor Antagonists. A variety of weak GLP-1 receptor antagonists have been identified during investigations of glucagon receptor antagonists as potential therapeutic agents for T2DM. This finding suggests these two receptors may share a similar binding pocket. Figure 1 depicts representative molecules that bind both the GLP-1 and glucagon receptors. Molecules **3** to **6** (Figure 1 (**3**) *trans*-3-[[4-[[(4-*tert*-butylcyclohexyl)-(ptolylcarbamoyl) amino]methyl]benzoyl]amino]propanoic acid [24]; (**4**) *N*-[3-cyano-5-[3-[(2,4-dichlorophenyl)-methyl]-1,2,4-oxadiazol-5-yl]-4-methyl-2-thienyl]-2-ethyl-butan amide [25]; (**5**) *trans*-4-[[9-*tert*-butyl-2-oxo-3-(*p*-tolyl)-1,3-diazaspiro [5.5]



1 (T-0632) **2** **3** **4**

5 **6** **7** (catechin)

Figure 1: Chemical structures of GLP-1 receptor antagonists. Representative depictions of (**1**) T-0632, (**2**) 9-benzylpyrido[3,4-b]indole, (**3-6**) nonselective glucagon receptor antagonists, and (**7**) catechin.

undecan-1-yl]methyl]-*N*-(2*H*-tetrazol-5-yl)benzamide [26]; (**6**) 4-[[(2Z)-3,6-dimethyl-4-propoxy-2-(*p*-tolylimino)benzimidazol1yl]methyl]-*N*-(1*H*-tetrazol-5-yl)benzamide [27]) bind the GLP-1 receptor with affinities in the micromolar range.

A common characteristic of these compounds is high lipophilicity (calculated to be >5 for all four compounds) and molecular weight (around 500 Da). Unfortunately, it is not known whether such a high number of hydrophobes is required for GLP-1 receptor binding; focused optimization of these series against the GLP-1 receptor has not been reported. Importantly, the lipophilic nature of these molecules does not preclude achieving oral exposure as demonstrated by compound **6** in two animal species [27]. While all of these compounds are more potent glucagon receptor antagonists, and little is known about their respective structure activity relationships (SAR) against the GLP-1 receptor, some of the molecules could be attractive starting points for identifying potent and selective GLP-1 receptor ligands.

2.5. Catechin. The polyphenolic natural product, catechin (**7**) (Figure 1 (**7**) *trans*-2-(3,4-dihydroxyphenyl)chromane-3,5,7-triol), has been shown to be a functionally selective, negative allosteric modulator of the GLP-1 receptor [28]. This compound is further discussed in Section 4.

3. Low Molecular Weight GLP-1 Receptor Agonists

3.1. Quinoxalines. Teng, Knudsen et al. at Novo Nordisk disclosed a series of quinoxalines exemplified by **8** (Figure 2, (**8**) 2-[6,7-dichloro-3-(trifluoromethyl)quinoxalin-2-yl]sulfanyl-5-methyl-1,3,4-thiadiazole) (usually referred to in literature as "Compound 1") and **9** (Figure 2, (**9**) N-*tert*-butyl-6,7-dichloro-3-methylsulfonyl-quinoxalin-2-amine) (usually referred to in literature as "Compound 2") [12, 29]. Initial screening using a competitive binding assay did not provide useful hits from ~500,000 compounds. A change in strategy to perform screening using a functional assay led to the identification of the quinoxaline scaffold from ~250,000 compounds. Compound **9** is a full agonist in GLP-1 receptor dependent cAMP accumulation experiments and shows specificity for the GLP-1 receptor versus other class B1 GPCRs. Compound **9** is characterized as an ago-allosteric modulator of the GLP-1 receptor; it displays intrinsic activity and also enhances binding of GLP-1 to the GLP-1 receptor [12]. Moreover, compound **9** action is not blocked by exendin-$4_{(9-39)}$, further supporting an allosteric mechanism of action of this molecule. Additional studies clearly demonstrate that compound **9** increases the binding affinity of both GLP-1 and oxyntomodulin for the GLP-1 receptor [11]. Together, these data are important because the results indicate the compound interacts with a site independent of the orthosteric binding pocket, suggesting the existence of an exploitable allosteric site for small molecules.

The definitive experiment to show compound **9** is a *bona fide* GLP-1 receptor ligand is the demonstration that it significantly potentiates glucose dependent insulin secretion in wild type mouse islets but not in islets from GLP-1 receptor knockout mice [12]. A subsequent report shows intraperitoneal administration of **9** is insulinotropic

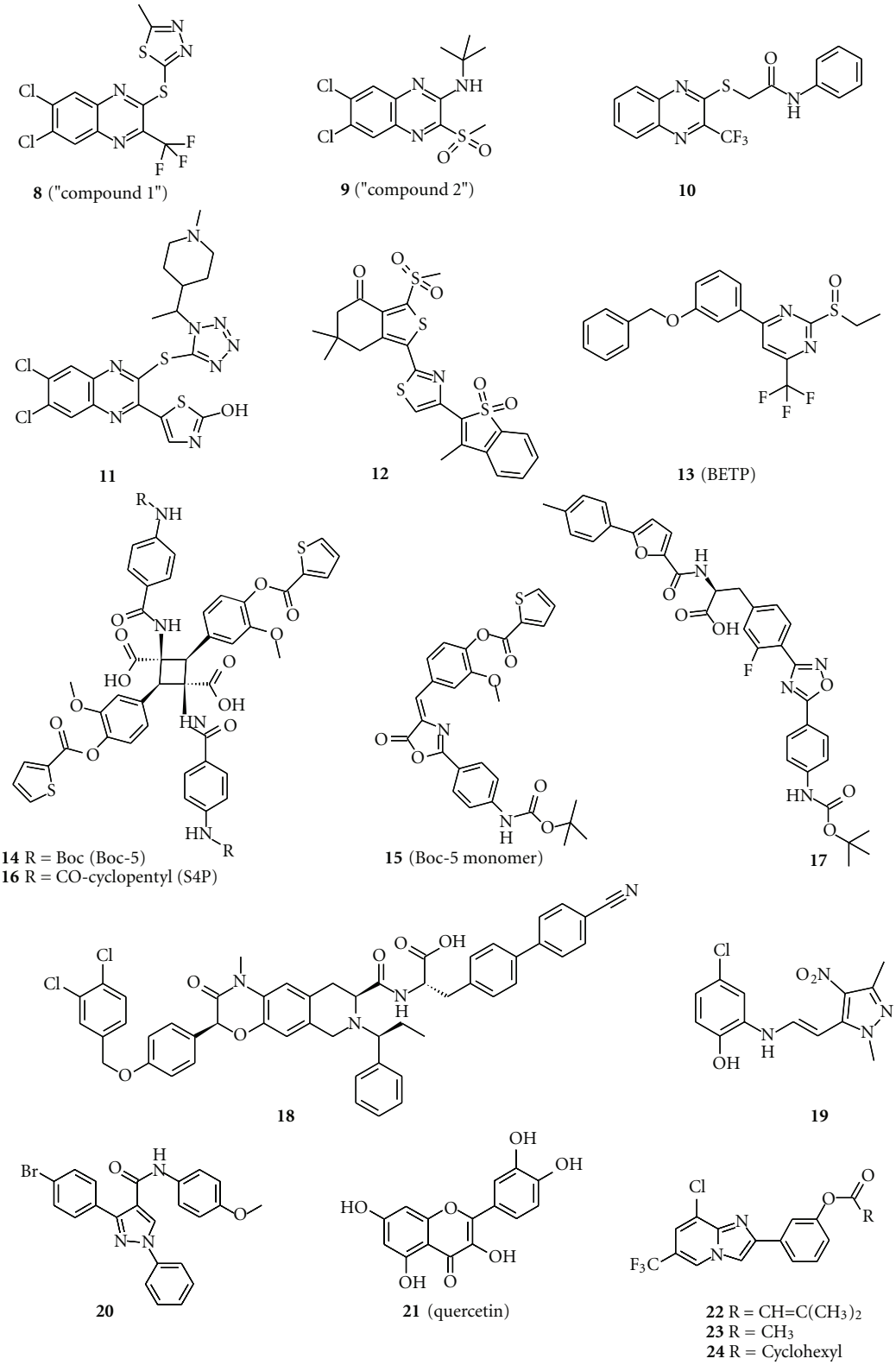


Figure 2: Chemical structures of GLP-1 receptor agonists. Representative depictions of (**8–11**) quinoxalines, (**12**) thiophene, (**13**) pyrimidine, (**14–16**) Boc-5 and derivatives, (**17**) phenylalanine, (**18**) azoanthracene, (**19**) pyrazole, (**20**) pyrazole-carboxamide, (**21**) flavonoid, and (**22–24**) imidazopyridines.

and enhances glucose disposal in a glucose tolerance test [30].

Following the disclosure of compounds **8** and **9**, further SAR around the quinoxalines was conducted [31]. A sulfone, sulfoxide, or thioether in position 2 of the bicycle is essential for activity, and quinoxalines are superior to other heterocycles as GLP-1 receptor agonists. The investigation also shows the need for electron-withdrawing groups on the quinoxaline with 6,7-dichloroquinoxaline being the best core. It is noted in the SAR describing the optimization of compound **9** that the quinoxaline analogs are chemically unstable in the presence of nucleophiles and have high microsomal metabolism. It can be speculated that the labile sulfur-containing side chain is responsible for the instability of these compounds to nucleophiles. The poor chemical stability precludes longer-term *in vivo* studies with this compound.

There have been other efforts aimed at exploring this scaffold. Scientists from the New England Medical Center claimed a series of 2-thiosubstituted quinoxalines, represent-ed by compound **10** (Figure 2 (**10**) 2-(3-methylquinoxalin-2-yl)sulfanyl-*N*-phenyl-acetamide), as weak GLP-1 receptor agonists [21]. Zydus also studied analogs of compound **8** but did not show improved activity over this compound in glucose dependent insulin secretion assays using RINmF5 insulinoma cells [32]. Recent reports from the Dong-A Pharmaceutical Company [33, 34] describe identification of a new series of 2-thioquinoxaline analogs of **8**. Compound **11** (Figure 2 (**11**) (5-[6,7-dichloro-3-[1-[1-(1-methyl-4-piperidyl)ethyl]tetrazol-5-yl]sulfanyl-quinoxalin-2-yl]thiazol-2-ol), disclosed as a racemic mixture, has 100 nM potency in a cAMP response element (CRE)-luciferase reporter assay. This molecule stimulates insulin secretion in INS-1E insulinoma cells and is selective against other class B1 GPCRs. Importantly, oral dosing of this compound enhances insulin secretion in a mouse intravenous glucose tolerance test model [33, 34]. It appears the Dong-A compound may overcome some of the instability issues observed with the initial quinoxalines to achieve effective oral exposure.

3.2. Thiophenes. Besides the quinoxaline compounds, Novo Nordisk also disclosed a second family of GLP-1 receptor agonists, a series of sulfonyl-thiophenes represented by compound **12** (Figure 2) [35]. No biological data are provided, and these compounds share a common feature with the quinoxalines: a sulfonyl group attached to an aromatic ring. While in this case, the thiophene is an electron-rich ring and thus less prone to nucleophilic attack, one or two strong electron-withdrawing carbonyl groups occur in all of the examples, leading to speculation that the sulfonyl group in these systems is also labile.

3.3. Pyrimidines. Our group identified a series of pyrimidine based ago-allosteric modulators of the GLP-1 receptor exemplified by racemic compound **13** (Figure 2, (**13**) 4-(3-benzyloxyphenyl)-2-ethylsulfinyl-6-(trifluoromethyl) pyrimidine; BETP) [13]. The parent molecule of this series was found by screening a small library, generated from three-dimensional pharmacophore models, using HEK293 cells stably cotransfected with the human GLP-1 receptor and a CRE-luciferase reporter. Compound **13** shows GLP-1 receptor dependent activity in both CRE-luciferase and cAMP accumulation assays in HEK293 cells, and the molecule stimulates glucose dependent insulin secretion in *ex vivo* assays of both rodent and human islet preparations. In combination experiments with GLP-1, compound **13** is not competitive with ^{125}I-GLP-1 but can act in an additive manner to enhance GLP-1 induced cAMP signaling and insulin secretion. Consistent with these findings, and similar to the quinoxalines, compound **13** action is not blocked by exendin-$4_{(9-39)}$. Importantly, our studies show compound **13** induces insulin secretion *in vivo*. The molecule is active in animals undergoing either the intravenous glucose tolerance test or the hyperglycemic clamp assay. While these *in vivo* results are encouraging, significant improvement in various metabolic liabilities of this compound are necessary before longer term studies can be explored. Although compound **13** is stable upon incubation in plasma, it shows chemical instability in the presence of nucleophiles.

3.4. Boc-5. A significant advance in the development of small molecule GLP-1 receptor agonists is the discovery of substituted cyclobutanes, exemplified by compound **14** (known as Boc-5) (Figure 2 (**14**) 1,3-bis [[4-(*tert*-butoxycarbonylamino)benzoyl]amino]-2,4-bis [3-methoxy-4-(thiophene-2-carbonyloxy)phenyl]cyclobutane-1,3-dicarboxylic acid). Boc-5 was discovered using a high throughput CRE-luciferase screen for activators of the rat GLP-1 receptor [36]. Serendipity played a role in this discovery as the original compound selected for testing was an olefin that is half of the size of Boc-5. It was soon realized that the olefins (depicted as compound **15**, monomer of Boc-5) dimerize in the DMSO solution to become cyclobutanes, represented by Boc-5 and S4P (compound **16**), that are the real actives. While lacking structural characteristics of drug-like molecules (Boc-5 violates all of the Lipinsky and Veber rules), Boc-5 provides a useful proof of concept molecule for nonpeptide GLP-1 receptor agonists. Pharmacologically, Boc-5 is a full agonist in the CRE-luciferase assay, while the closely related molecule S4P is a partial agonist. In a cAMP accumulation assay, however, both Boc-5 and S4P are partial agonists. Importantly, both S4P and Boc-5 are functionally antagonized by exendin-$4_{(9-39)}$ and displace ^{125}I-GLP-1 in receptor binding assays. Inhibition of ^{125}I-GLP-1 binding does not appear saturable [36], possibly suggesting an allosteric binding mechanism; however, this has yet to be elucidated.

Boc-5 dose dependently stimulates glucose dependent insulin secretion in isolated rat islets [36]. Paradoxically, 10 μM of Boc-5 is more efficacious than a saturating concentration of GLP-1 in inducing insulin secretion. It is worth noting that these studies were performed at an abnormally high glucose concentration of 25 mM. Boc-5 is reported to have a plasma half life of approximately 8 hours following intraperitoneal dosing of the compound. Acute administration of Boc-5 shows an anorectic effect in C57/B6 mice that lasts over 12 hours in the intraperitoneally

dosed group and 90 minutes in orally administered animals [36]. Although no exposure is reported for the oral study, the large difference in food intake observed in oral versus intraperitoneal administration probably indicates poor oral bioavailability of the compound. Chronic intraperitoneal administration to *db*/*db* mice lowers HbA1c, reduces food intake, lowers body weight, and enhances insulin secretion and glucose excursion (using an intraperitoneal glucose tolerance test) [36]. Importantly, acute effects of Boc-5 are entirely abrogated by coadministration of exendin-$4_{(9-39)}$, suggesting a GLP-1 receptor dependent action [36]. Follow-up studies confirmed many of these findings using a diet-induced obesity mouse model [37]. A limited SAR around Boc-5 has been reported despite synthetic challenges of these molecules [38]. While some improvement in potency is shown, the new molecules share the poor physicochemical properties with S4P and Boc-5, which presumably preclude these compounds from being oral drugs.

3.5. Phenylalanines. Argusina, Inc. disclosed phenylalanine derivatives as GLP-1 receptor modulators, represented by compound **17**, (Figure 2 (**17**) 3-[4-[5-[4-(tertbutoxycarbonylamıno)phenyl]-1,2,4-oxadiazol-3-yl]-3-fluoro-phenyl]-2-[[5-(*p*-tolyl)furan-2-carbonyl]amino]propanoic acid) [39]. The molecules are disclosed as racemic mixtures. One can envision the Argusina compounds as an optimization of the monomer of Boc-5. Thorough pharmacological evaluation of these compounds has not been disclosed, although the initial report suggests that compounds can modulate cAMP mobilization and insulin secretion in cell culture systems [39]. From a pharmacokinetic perspective, these molecules violate the Lipinski and Veber rules (large molecular weight and high lipophilicity; high PSA), so an improvement of the physicochemical properties of the scaffold would likely be required to achieve an orally active agent.

3.6. Azoanthracenes. Several patent disclosures from Transtech Pharmaceuticals (TTP) report identification of a number of azoanthracene and oxadiazoanthracene derivatives as GLP-1 receptor agonists [40–43], exemplified by compound **18**, (Figure 2 (**18**) (*S*)-3-(4′-cyano-biphenyl-4-yl)-2-{[(3*S*, 7*S*)-3-[4-(3,4-dichloro-benzyloxy)-phenyl]-1-methyl-2-oxo-6-((*S*)-1-phenyl-propyl)-2,3,5,6,7,8-hexahydro-1*H*-4-oxa-1,6-diaza-anthracene-7-carbonyl]-amino}propionic acid) [43]. Molecules described in these disclosures have nanomolar potencies for the GLP-1 receptor in recombinant cell assays of cAMP, and there is some indication from the data that the compounds may be partial agonists [42]. A recent publication disclosed that TTP molecules are effective antidiabetic agents in preclinical rodent models of T2DM, stimulating glucose dependent insulin secretion in rodent islets and improving glucose excursion in an oral glucose tolerance test [44]. The leading molecule in this class, TTP054 (structure not disclosed) is reported to currently be under evaluation in Phase II clinical trials as an oral drug [44]. All of the molecules disclosed by TTP in these patents are of large molecular weight and high lipophilicity (**18**: MW ~ 880 Da, calculated log P = 10, 13 rotatable bonds) compared to typical orally administered medications, suggesting high doses of the compound would likely be required to achieve efficacious exposure.

3.7. Pyrazoles. A series of pyrazoles represented by compound **19** (Figure 2 (**19**) 4-chloro-2-[[(*E*)-2-(2,5-dimethyl-4-nitro-pyrazol-3-yl)vinyl]amino]phenol) were identified by Kopin and Beinborn as weak agonists of the GLP-1 receptor [21]. The compound has micromolar activity for the GLP-1 receptor in recombinant cell assays of cAMP [21]. No other studies have been disclosed with this kind of molecule.

3.8. Pyrazole-Carboxamides. A recent publication reported discovery of small molecule GLP-1 receptor potentiators as an ancillary outcome of efforts to identify glucagon receptor antagonists [45]. The authors used virtual screening of a library of commercially available drug-like compounds to search for compounds with physicochemical similarities to known glucagon receptor antagonists. This was followed by a homology model based docking approach. Compound **20**, (Figure 2 (**20**) 3-(4-bromophenyl)-*N*-(4-methoxyphenyl)-1-phenyl-pyrazole-4-carboxamide), identified as a potential candidate for glucagon receptor antagonism, does not show functional activity at this receptor, but it is observed to potentiate an EC_{20} concentration of GLP-1 induced cAMP production in TC6 cells [45]. The authors claim to have discovered a novel small molecule chemotype that potentiates the GLP-1 receptor. If these data are confirmed in recombinant cell systems where GLP-1 receptor dependence can be more definitively ascribed, this would be a breakthrough discovery with respect to GLP-1 receptor allosteric modulators. The ability to significantly potentiate either the affinity or efficacy (the data in this report do not distinguish between these possibilities) of GLP-1 would be highly desirable characteristics of a GLP-1 receptor targeted small molecule. Moreover, this would represent an important computational and operational approach to discovering class B1 GPCR positive allosteric modulators.

3.9. Flavonoids. Sexton et al. characterized a series of quercetin-like flavonoids, represented by quercetin (Figure 2 (**21**) 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-chromen-4-one), with positive allosteric modulator activity on the GLP-1 receptor [28, 46]. These compounds were originally identified by Domain Therapeutics as GLP-1 receptor modulators [46]. A thorough analysis of this chemical scaffold demonstrates this class of molecules positively modulates the affinity and efficacy of GLP-1 receptor peptide ligands. The quercetin series appears to show ligand bias in that it is specific for GLP-1 receptor mediated Ca^{2+} mobilization but not cAMP accumulation. Typical of polyphenolic compounds, a lack of robust functional effects, polypharmacology, and flat SAR limits the usefulness of this chemotype to *in vitro* studies and precludes optimization for pharmacological purposes. However, the discovery and analysis of this series of molecules represent a further proof of concept for allosteric modulators of the GLP-1 receptor.

3.10. Imidazopyridines. There is one published report describing a series of imidazopyridine based molecules. The

initial hit, compound **22** (Figure 2 (**22**) [3-(8-chloro-6-methyl-imidazo [1,2-a]pyridin-2-yl)phenyl] 3-methylbut-2-enoate), was identified from a library of 10,000 compounds using a GLP-1 receptor functional assay. The strategy used for optimization was to design a pharmacophore model from three known agonists (compounds **9** and **12** and the monomer of S4P, a close analog of monomer **15**), although there is no evidence of structural similarity between these three series. Nevertheless, analogs of compound **22** that fit additional features of the pharmacophore were designed.

Both compounds **23** and **24**, (Figure 2 (**23**) 3-[8-chloro-6-(trifluoromethyl)imidazo [1,2-a]pyridin-2-yl]phenyl] acetate and (**24**) [3-[8-chloro-6-(trifluoromethyl)imidazo [1,2-a]pyridin-2-yl]phenyl] cyclohexanecarboxylate), show induc-tion of GLP-1 receptor signaling in GLP-1 receptor expressing CHO or HEK293 cells [47]. These molecules are shown to have some agonist activity in heterologous cell lines expressing the GLP-1 receptor, but further investigation in other systems is not reported. While these are very small molecules, and pass the Lipinski and Veber rules, further optimization of the activity of the compounds against the GLP-1 receptor, and SAR work to move away from the labile ester of the phenol, is likely necessary for these compounds to become useful for oral studies.

4. Ligand Biased Signaling

Studies to more fully characterize several of the small molecule ligands of the GLP-1 receptor are needed to advance the field. An emerging concept in GPCR pharmacology is that of functional selectivity (also known as ligand bias or stimulus bias) [48, 49]. It is now appreciated that GPCR ligands can stabilize distinct receptor conformations, which in some instances, lead to differential modulation of signal transduction pathways [48]. To date, there is a single study reporting that the weak GLP-1 receptor peptide agonists, oxyntomodulin and glucagon, are biased toward $G\alpha_s$ over β-arrestin for coupling to the GLP-1 receptor [50]. These peptides show low potency and partial agonism (>50% of GLP-1 efficacy) for β-arrestin recruitment at the GLP-1 receptor yet are full agonists for cAMP accumulation. In support of this, another report describes oxyntomodulin as having stimulus bias for the ERK1/2 activation pathway relative to cAMP or Ca^{2+} mobilization [11]. Further work is necessary to understand whether the observed *in vitro* functional selectivity of the various GLP-1 receptor peptide ligands is physiologically relevant *in vivo*. Such studies may be complicated by the polypharmacology of oxyntomodulin as it is a dual agonist of both glucagon and GLP-1 receptors and by receptor reserve phenomena that often lead to the classification of partial agonists as biased ligands [51].

Sexton et al. demonstrated two GLP-1 receptor ligands are functionally selective. Quercetin (**21**) and a subset of related naturally occurring flavonoids exhibit functional selectivity as these molecules potentiate GLP-1 peptide signaling in Ca^{2+} mobilization but not cAMP production [11, 28]. Similarly, the flavonoids also display probe dependence in that the molecules modulate both GLP-1 and exendin-4 action but not oxyntomodulin signal transduction. While a variety of flavonoid cores were tested in these studies, only the 3-hydroxyflavone core displayed functional activity. Unfortunately, flavonoids exert multiple pharmacologic effects at the concentrations required for the activation of the GLP-1 receptor. Thus, these are not good starting points for the identification of more potent allosteric ligands.

An SAR analysis of related compounds identified the polyphenolic natural product, catechin (**7**), as a probe dependent, functionally selective, and negative allosteric modulator of the GLP-1 receptor [28]. Both *trans* enantiomers of this compound are known as catechin, but it is not clear from this publication if the racemic compound or one *trans* enantiomer was used in this study. Catechin decreases the efficacy of GLP-1 signaling via cAMP but does not modulate non-cAMP signaling by the GLP-1 receptor peptide agonists nor does it significantly alter the pharmacology of exendin-4 or oxyntomodulin signaling. Analogously, the small molecule quinoxaline **8** [12] displays a complex profile of activity consistent with functional selectivity and probe dependence. Compound **8** shows affinity driven positive allosteric modulator activity for oxyntomodulin, and to a lesser extent, GLP-1 but not toward exendin-4. This activity is only observed for the cAMP pathway and not for the Ca^{2+} or ERK pathways, demonstrating probe dependence whereby allosteric modulation of the GLP-1 receptor is dependent on the species of the bound orthosteric agonist.

These studies are seminal as they provide proof of concept that allosteric modulation of the GLP-1 receptor can engender pathway specific modulation of signal transduction outcomes. It would be informative to identify small molecule ligands of the GLP-1 receptor with biased signaling to delineate the *in vivo* consequences of selective modulation of specific molecular signal transduction mechanisms. Importantly, this would provide a better understanding of the molecular mechanisms of the antidiabetic effects of GLP-1 receptor agonism. At this time, these *in vitro* studies demonstrate that probe dependence of allosteric modulators can occur at the GLP-1 receptor and identify critical aspects to be considered when optimizing small molecule GLP-1 receptor agonists or modulators. Future efforts also should be directed at mapping the structural determinants of allosteric ligand binding and at evaluating interaction between allosteric and orthosteric sites.

5. Conclusions

Despite 20 years of research following the molecular identification and cloning of the GLP-1 receptor [52, 53], no orally available small molecule GLP-1 receptor activator has been developed for therapeutic use. Encouragingly, however, the pace of identifying small molecule GLP-1 receptor ligands is increasing. In addition to the molecules discussed herein, the field anxiously awaits disclosures from both Addex Pharmaceuticals S.A. and Vivia Biotech S.L. that have recently presented data from their small molecule GLP-1 receptor modulator programs (see Cambridge Healthtech Institute, Discovery On Target 2011, Cambridge, MA, USA, Nov 2–4, 2011). Although the mechanisms of peptide binding and receptor activation of the GLP-1 receptor are

complex and likely difficult to mimic with low molecular weight compounds, examples of small molecules working via an allosteric mode provide compelling evidence that medicinal chemistry strategies for this target should be considered. Further, the application of advanced structural biology methodologies and more sophisticated assay systems and testing schemes, including work to understand biased signaling for GLP-1 receptor ligands, will likely be needed to advance drug-like molecules.

Disclosure

While this paper was under editorial review, a patent application from Receptos Inc. (WO2011/156655 A2) was published disclosing novel compounds with allosteric modulator activity at the GLP-1 receptor.

Author's Contribution

F. S. Willard, A. B. Bueno, and K. W. Sloop contributed equally to this paper.

Conflict of Interests

All authors are employees of Eli Lilly and Company and may own company stock or possess stock options.

References

[1] J. J. Holst, "Glucagon-like peptide-1: from extract to agent. The claude bernard lecture, 2005," *Diabetologia*, vol. 49, no. 2, pp. 253–260, 2006.

[2] J. A. Lovshin and D. J. Drucker, "Incretin-based therapies for type 2 diabetes mellitus," *Nature Reviews Endocrinology*, vol. 5, no. 5, pp. 262–269, 2009.

[3] D. E. Grigoriadis, S. R. J. Hoare, S. M. Lechner, D. H. Slee, and J. A. Williams, "Drugability of extracellular targets: discovery of small molecule drugs targeting allosteric, functional, and subunit-selective sites on GPCRs and ion channels," *Neuropsychopharmacology*, vol. 34, no. 1, pp. 106–125, 2009.

[4] S. R. Hoare, "Mechanisms of peptide and nonpeptide ligand binding to Class B G-protein-coupled receptors," *Drug Discovery Today*, vol. 10, no. 6, pp. 417–427, 2005.

[5] V. P. Jaakola, M. T. Griffith, M. A. Hanson et al., "The 2.6 angstrom crystal structure of a human A2A adenosine receptor bound to an antagonist," *Science*, vol. 322, no. 5905, pp. 1211–1217, 2008.

[6] T. Warne, M. J. Serrano-Vega, J. G. Baker et al., "Structure of a β1-adrenergic G-protein-coupled receptor," *Nature*, vol. 454, no. 7203, pp. 486–491, 2008.

[7] T. Warne, R. Moukhametzianov, J. G. Baker et al., "The structural basis for agonist and partial agonist action on a beta(1)-adrenergic receptor," *Nature*, vol. 469, no. 7329, pp. 241–244, 2011.

[8] V. Cherezov, D. M. Rosenbaum, M. A. Hanson et al., "High-resolution crystal structure of an engineered human β2-adrenergic G protein-coupled receptor," *Science*, vol. 318, no. 5854, pp. 1258–1265, 2007.

[9] E. Y. Chien, W. Liu, Q. Zhao et al., "Structure of the human dopamine D3 receptor in complex with a D2/D3 selective antagonist," *Science*, vol. 330, no. 6007, pp. 1091–1095, 2010.

[10] T. Shimamura, M. Shiroishi, S. Weyand et al., "Structure of the human histamine H 1 receptor complex with doxepin," *Nature*, vol. 475, no. 7354, pp. 65–72, 2011.

[11] C. Koole, D. Wootten, J. Simms et al., "Allosteric ligands of the glucagon-like peptide 1 receptor (GLP-1R) differentially modulate endogenous and exogenous peptide responses in a pathway-selective manner: implications for drug screening," *Molecular Pharmacology*, vol. 78, no. 3, pp. 456–465, 2010.

[12] L. B. Knudsen, D. Kiel, M. Teng et al., "Small-molecule agonists for the glucagon-like peptide 1 receptor," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 3, pp. 937–942, 2007.

[13] K. W. Sloop, F. S. Willard, M. B. Brenner et al., "Novel small molecule glucagon-like peptide-1 receptor agonist stimulates insulin secretion in rodents and from human islets," *Diabetes*, vol. 59, no. 12, pp. 3099–3107, 2010.

[14] W. P. Walters and M. A. Murcko, "Prediction of "drug-likeness," *Advanced Drug Delivery Reviews*, vol. 54, no. 3, pp. 255–271, 2002.

[15] C. A. Lipinski, "Drug-like properties and the causes of poor solubility and poor permeability," *Journal of Pharmacological and Toxicological Methods*, vol. 44, no. 1, pp. 235–249, 2000.

[16] D. F. Veber, S. R. Johnson, H. Y. Cheng, B. R. Smith, K. W. Ward, and K. D. Kopple, "Molecular properties that influence the oral bioavailability of drug candidates," *Journal of Medicinal Chemistry*, vol. 45, no. 12, pp. 2615–2623, 2002.

[17] A. Gronberg, E.-R. Sol, A. Danielsson et al., "A low molecular weight non-peptide antagonist of glucagon-like peptide-1," *Diabetes*, vol. 49, supplement 1, p. A251, 2000.

[18] E. C. Tibaduiza, C. Chen, and M. Beinborn, "A small molecule ligand of the glucagon-like peptide 1 receptor targets its amino-terminal hormone binding domain," *Journal of Biological Chemistry*, vol. 276, no. 41, pp. 37787–37793, 2001.

[19] S. Runge, H. Thøgersen, K. Madsen, J. Lau, and R. Rudolph, "Crystal structure of the ligand-bound glucagon-like peptide-1 receptor extracellular domain," *Journal of Biological Chemistry*, vol. 283, no. 17, pp. 11340–11347, 2008.

[20] C. R. Underwood, P. Garibay, L. B. Knudsen et al., "Crystal structure of glucagon-like peptide-1 in complex with the extracellular domain of the glucagon-like peptide-1 receptor," *Journal of Biological Chemistry*, vol. 285, no. 1, pp. 723–730, 2010.

[21] A. S. Kopin and M. Beingorn, *WO2004/203310 Methods and Compositions for the Treatment of Metabolic Disorders*, 2004.

[22] L. K. Truesdale et al., "US 6,469,021 B1 Non-Peptide Antagonists Of GLP-1 Receptor And Methods Of Use," U.S. Patent, Editor, Agouron Pharmaceuticals, San Diego, Calif, USA, 2002.

[23] D. R. Gehlert, A. Cippitelli, A. Thorsell et al., "3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-8-(1-ethylpropyl)-2, 6-dimethyl-imidazo[1,2-b]pyridazine: a novel brain-penetrant, orally available corticotropin-releasing factor receptor 1 antagonist with efficacy in animal models of alcoholism," *Journal of Neuroscience*, vol. 27, no. 10, pp. 2718–2726, 2007.

[24] J. Lau, G. Behrens, U. G. Sidelmann et al., "New β-alanine derivatives are orally available glucagon receptor antagonists," *Journal of Medicinal Chemistry*, vol. 50, no. 1, pp. 113–128, 2007.

[25] J. L. Duffy, B. A. Kirk, Z. Konteatis et al., "Discovery and investigation of a novel class of thiophene-derived antagonists of the human glucagon receptor," *Bioorganic and Medicinal Chemistry Letters*, vol. 15, no. 5, pp. 1401–1405, 2005.

[26] D. M. Shen, F. Zhang, E. J. Brady et al., "Discovery of novel, potent, and orally active spiro-urea human glucagon receptor

antagonists," *Bioorganic and Medicinal Chemistry Letters*, vol. 15, no. 20, pp. 4564–4569, 2005.

[27] C. Sinz, J. Chang, A. R. Lins et al., "Discovery of cyclic guanidines as potent, orally active, human glucagon receptor antagonists," *Bioorganic and Medicinal Chemistry Letters*, vol. 21, no. 23, pp. 7131–7136, 2011.

[28] D. Wootten, J. Simms, C. Koole et al., "Modulation of the glucagon-like peptide-1 receptor signaling by naturally occurring and synthetic flavonoids," *Journal of Pharmacology and Experimental Therapeutics*, vol. 336, no. 2, pp. 540–550, 2011.

[29] M. Teng, L. K. Truesdale, and D. Bhumralkar, *WO2000/42026 Non-Peptide GLP-1 Agonists*, 2000.

[30] N. Irwin, P. R. Flatt, S. Patterson, and B. D. Green, "Insulin-releasing and metabolic effects of small molecule GLP-1 receptor agonist 6,7-dichloro-2-methylsulfonyl-3-N-tertbutyl-aminoquinoxaline," *European Journal of Pharmacology*, vol. 628, no. 1–3, pp. 268–273, 2010.

[31] M. Teng, M. D. Johnson, C. Thomas et al., "Small molecule ago-allosteric modulators of the human glucagon-like peptide-1 (hGLP-1) receptor," *Bioorganic and Medicinal Chemistry Letters*, vol. 17, no. 19, pp. 5472–5478, 2007.

[32] R. H. Bahekar, M. R. Jain, A. A. Gupta et al., "Synthesis and antidiabetic activity of 3,6,7-trisubstituted-2-(1H-imidazol-2-ylsulfanyl)quinoxalines and quinoxalin-2-yl isothioureas," *Archiv der Pharmazie*, vol. 340, no. 7, pp. 359–366, 2007.

[33] H. S. Moon et al., *WO2011/122815 A2 Novel Quinoxilane Derivatives*, 2011, Edited by WIPO.

[34] M. K. Kim et al., "590-P Orally active small molecule GLP-1R agonist, DA-15864 increases insulin secretion in mice," in *Proceedings of the 70th Scientific Sessions of Annual Meeting of American Diabetes Association*, Orlando, Fla, USA, 2010.

[35] S. M. Knudsen et al., "*WO2006/003096 Condensed Thiophene Dernatives And Their Use As Cyclic GLP-1 Agonists*," 2006.

[36] D. Chen, J. Liao, N. Li et al., "A nonpeptidic agonist of glucagon-like peptide 1 receptors with efficacy in diabetic db/db mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 3, pp. 943–948, 2007.

[37] M. He, H. Su, W. Gao et al., "Reversal of obesity and insulin resistance by a non- peptidic glucagon-like peptide-1 receptor agonist in diet-induced obese mice," *Plos ONE*, vol. 5, no. 12, Article ID e14205, 2010.

[38] Q. Liu, N. Li, Y. Yuan et al., "Cyclobutane derivatives as novel non-peptidic small molecule agonists of glucagon-like peptide-1 receptor," *Journal of Medicinal Chemistry*, vol. 55, no. 1, pp. 250–267, 2012.

[39] J. Liao et al., "US2011/023482 Phenylalanine Derivatives And Their Use As Non-Peptide GLP-1 Receptor Modulators," U.S.P. Application, Editor, Argusina Inc, San Diego, Claif, USA, 2011.

[40] M. Rao, PCT/US2009/039905 Ligands For The Glp-1 Receptor And Methods For Discovery Thereof, I.P. Application, Editor, TransTech Pharma, 2009.

[41] D. R. Polisetti et al., "PCT/US2010/047661 Solid Compositions Comprising An Oxadiazoanthracene Compound And Methods Of Making And Using The Same, I.P. Application, Editor, TransTech Pharma, 2009".

[42] A. M. M. Mjallli et al., PCT/US2009/036333 Oxadiazoanthracene Compounds For The Treatment Of Diabetes, I.P. Application, Editor, TransTech Pharm, 2009.

[43] D. R. Polisett, E. Benjamin, J. C. Quada et al., WO2011/031620 Solid Composition Comprising an Oxadiazoanthracene Compound and Methods of Making and Using the Same, 2011.

[44] C. Valcarce et al., "Oral glucagon-like peptide-1 (GLP-1) receptor agonists for the treatment of type 2 diabetes," in *Proceedings of the 4th International Congress on Prediabetes and the Metabolic Syndrome*, Madrid, Spain, April 2011.

[45] C. de Graaf, C. Rein, D. Piwnica et al., "Structure-based discovery of allosteric modulators of two related class B G-protein-coupled receptors," *ChemMedChem*, vol. 6, no. 12, pp. 2159–2169, 2011.

[46] S. Schann, S. Mayer, M. Frauli et al., "Sounds of silence: innovative approach for identification of novel GPCR-modulator chemical entities," in *Proceedings of the 238th American Chemical Society National Meeting*, Washington, DC, USA, 2009.

[47] Y. Gong, H. G. Cheon, T. Lee, and N. S. Kang, "A Novel 3-(8-Chloro-6-(trifluoromethyl)imidazo[1,2-a]pyridine-2-yl) phenyl acetate skeleton and pharmacophore model as glucagon-like peptide 1 receptor agonists," *Bulletin of the Korean Chemical Society*, vol. 31, no. 12, pp. 3760–3764, 2010.

[48] T. Kenakin, "Functional selectivity and biased receptor signaling," *Journal of Pharmacology and Experimental Therapeutics*, vol. 336, no. 2, pp. 296–302, 2011.

[49] P. Keov, P. M. Sexton, and A. Christopoulos, "Allosteric modulation of G protein-coupled receptors: a pharmacological perspective," *Neuropharmacology*, vol. 60, no. 1, pp. 24–35, 2011.

[50] R. Jorgensen, V. Kubale, M. Vrecl, T. W. Schwartz, and C. E. Elling, "Oxyntomodulin differentially affects glucagon-like peptide-1 receptor β-arrestin recruitment and signaling through Gαs," *Journal of Pharmacology and Experimental Therapeutics*, vol. 322, no. 1, pp. 148–154, 2007.

[51] S. Rajagopal, S. Ahn, D. H. Rominger et al., "Quantifying ligand bias at seven-transmembrane receptors," *Molecular Pharmacology*, vol. 80, no. 3, pp. 367–377, 2011.

[52] B. Thorens, "Expression cloning of the pancreatic β cell receptor for the gluco- incretin hormone glucagon-like peptide 1," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 89, no. 18, pp. 8641–8645, 1992.

[53] M. P. Graziano, P. J. Hey, D. Borkowski, G. G. Chicchi, and C. D. Strader, "Cloning and functional expression of a human glucagon-like peptide-1 receptor," *Biochemical and Biophysical Research Communications*, vol. 196, no. 1, pp. 141–146, 1993.

Hindawi Publishing Corporation
Experimental Diabetes Research
Volume 2012, Article ID 635472, 9 pages
doi:10.1155/2012/635472

Review Article

Cardiovascular Benefits of GLP-1-Based Therapies in Patients with Diabetes Mellitus Type 2: Effects on Endothelial and Vascular Dysfunction beyond Glycemic Control

Thomas Forst,[1,2] Matthias M. Weber,[2] and Andreas Pfützner[1]

[1] *Institute for Clinical Research and Development, 55116 Mainz, Germany*
[2] *First Medical Department, Johannes Gutenberg University, Mainz, Germany*

Correspondence should be addressed to Thomas Forst, thomasf@ikfe.de

Received 4 January 2012; Accepted 6 February 2012

Academic Editor: Matteo Monami

Type 2 diabetes mellitus (T2DM) is a progressive multisystemic disease accompanied by vascular dysfunction and a tremendous increase in cardiovascular mortality. Numerous adipose-tissue-derived factors and beta cell dysfunction contribute to the increased cardiovascular risk in patients with T2DM. Nowadays, numerous pharmacological interventions are available to lower blood glucose levels in patients with type 2 diabetes. Beside more or less comparable glucose lowering efficacy, some of them have shown limited or probably even unfavorable effects on the cardiovascular system and overall mortality. Recently, incretin-based therapies (GLP-1 receptor agonists and DPP-IV inhibitors) have been introduced in the treatment of T2DM. Beside the effects of GLP-1 on insulin secretion, glucagon secretion, and gastrointestinal motility, recent studies suggested a couple of direct cardiovascular effects of GLP-1-based therapies. The goal of this paper is to provide an overview about the current knowledge of direct GLP-1 effects on endothelial and vascular function and potential consequences on the cardiovascular outcome in patients with T2DM treated with GLP-1 receptor agonists or DPP-IV inhibitors.

1. Introduction

The global prevalence of T2DM has been estimated at 171 million people and is projected to more than double by 2030 [1]. The epidemiological establishment of T2DM as a coronary artery disease equivalent has been confirmed in several investigations [2–4]. Type 2 diabetes mellitus (T2DM) is a progressive multisystemic disease accompanied by endothelial dysfunction [5–7] and an increased cardiovascular mortality [3, 8]. Also several mechanistic pathways linking glucose metabolism with endothelial dysfunction are postulated; recent studies aimed to investigate the beneficial role of strict glycemic control using conventional treatment algorhythms failed to provide beneficial effects on cardiovascular mortality [9–11]. The accumulation and interaction of several metabolic and cardiovascular risk factors merge in the pathophysiology of diabetic vascular disease and the development of micro- and macrovascular complications. Diabetic vascular disease has been associated with an upregulation of reactive oxygen species and chronic inflammatory and hypercoagulable states, and as such with the pathogenesis of atherosclerosis and cardiovascular disease.

2. Adipose Tissue, Insulin Resistance, and Beta Cell Function in Vascular Pathology

Adipose tissue is assumed to play an integral role in the pathogenesis of vascular dysfunction and the development of T2DM [12]. As maturing pre-adipocytes differentiate to become adult adipocytes, they acquire the ability to synthesize hundreds of proteins. Adipose tissue releases a large number of cytokines and bioactive mediators with endocrine, autocrine, juxtacrine, and paracrine activity. These proinflammatory adipocytokines include TNF-α, IL-6, leptin, plasminogen activator inhibitor 1 (PAI-1), angiotensinogen, resistin, and c-reactive protein, all of them well known to interfere with insulin sensitivity, blood

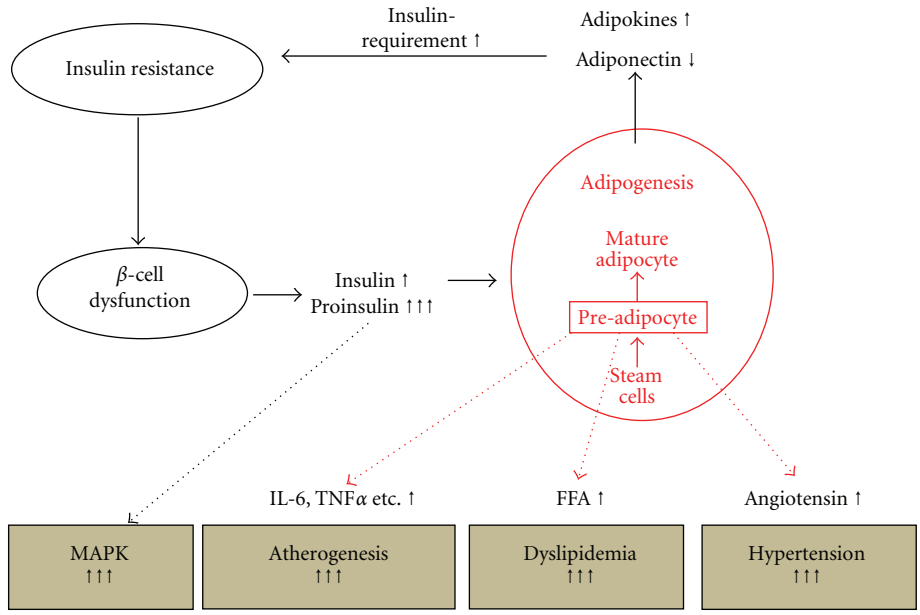


Figure 1: Schematic illustration of the central role of visceral adipose tissue in the generation of the atherogenic environment in obese patients. Visceral adipose tissue induces insulin resistance, thereby increasing insulin and proinsulin release from the beta cell. Unphysiological levels of insulin and proinsulin promote atherogenesis through the activation of endothelial MAPK. Preadipocytes secrete numerous adipocytokines involved in the pathogenesis of hypertension, dyslipidemia, and inflammation. (MAPK: mitogen-activated protein kinase, IL-6: interleukin-6, TNFα: tumor necrosis factor α, FFA: free fatty acids).

pressure regulation, lipid metabolism, and inflammation (Figure 1). On the other hand, the release of adiponectin, an anti-inflammatory and vasoprotective adipokine, is reduced in insulin-resistant obese patients and in patients with T2DM [13]. In addition, it is likely that many more undiscovered fat cell-derived mediators are causally involved in cardiovascular health, insulin resistance, and the development of T2DM.

Epidemiological evidence suggests that cardiovascular risk begins to develop many years before the diagnosis of T2DM [14]. Increasing insulin resistance, associated with increasing visceral body fat, is associated with an increased risk for cardiovascular disease and is commonly comorbid with hypertension, dylipidemia, obesity, and a prothrombotic state [15–17]. As long as the pancreatic beta cells compensate for increasing insulin resistance with an augmented release of insulin, blood glucose will stay controlled, and the patient will not present, as per definition, with frank diabetes mellitus. There are strong prospective data that, even before deranging glucose levels, obesity and insulin resistance are associated with atherosclerosis and coronary heart disease [18, 19]. However, it is the progressively deteriorating function of the beta cell, which ultimately leads to hyperglycemia and is critical for the manifestation of T2DM. There is increasing evidence that islet cell polymorphisms my account for the individual risk for beta cell breakdown and the manifestation of T2DM as defined by an increase in fasting and/or postprandial blood glucose levels [20, 21]. The insulin/proinsulin ratio is used as a marker for the capability of the β cell to convert intact proinsulin into insulin and C-peptide [21]. Preclinical and clinical studies of type 2 diabetes have identified proinsulin both as an indicator of decreasing beta cell function and a predictor of increased beta cell loss due to apoptosis and/or diminished neogenesis [21, 22]. Furthermore, a direct role of this prohormone in the development of cardiovascular disease has been suggested by numerous experimental and epidemiological studies. Increased intact proinsulin levels were found to be closely associated with the development of coronary heart disease involving subjects with and without diabetes [23–27]. In fact, the atherogenic potential of proinsulin was highlighted some years ago in a clinical trial, investigating the therapeutic potential of human proinsulin given as subcutaneous injections. In that study, an eightfold increase in cardiovascular events was observed during treatment with human proinsulin versus human regular insulin [28]. More recently, an association between increased plasma concentrations of proinsulin and the severity of angiographically characterized coronary heart disease has been reported [29]. Even the exact mechanism how proinsulin is involved in the pathogenesis of atherosclerosis is not completely recognized, it was already shown that PAI-1 activity increases after proinsulin administration in vitro [30]. Increased expression of PAI-1 and vascular adhesion molecules have been associated with hyperglycemia-related endothelial cell dysfunction and a predisposition to accelerated atherogenesis [31]. There is increasing evidence that the atherogenic effects of proinsulin might, at least in part, be mediated by increasing PAI-1 levels with subsequent inhibition of fibrinolysis and an augmented thrombogenic potency [32–34]. In accordance with this finding, the reduction of intact proinsulin levels during treatment with a PPARγ agonist in T2DM was shown

to be associated with a decrease in intima media thickness of the arteria carotis communis [35].

3. Endothelial Dysfunction in Diabetes Mellitus

Vascular wall dysfunction is a critical mediator of atherogenesis in patients with T2DM. The response to injury hypotheses of atherosclerosis supposed that the initial damage affects the arterial endothelium leading to endothelial dysfunction [36]. Endothelial dysfunction in patients with obesity and T2DM is characterized by an imbalance between endothelium-dependent vasodilatation and vasoconstriction as well as antithrombotic and prothrombotic factors. Nitric oxide (NO) maintains the vasodilatation and vasoprotective property of the endothelium and opposes the effects of vasoconstrictors such as endothelin 1 or angiotensin II [6, 37]. It inhibits leucocyte and platelet activation and helps to maintain the endothelium as smooth nonthrombotic barrier. Thus endothelial dysfunction is a prominent feature at various stages of atherogenesis, vessel occlusion, and tissue infarction [12]. In a study by Goldfine et al., endothelial function and NO bioavailability was evaluated in 38 individuals without a history of T2DM [38]. In this study, 19 patients were offspring of type 2 diabetes parents, while the other 19 had no first degree relatives with either T2DM or cardiovascular disease. Patients with a family history of diabetes were found to have significantly reduced endothelial function and nitric oxide (NO) bioavailability.

Beside the well-known metabolic effects of insulin, the hormone exerts important vascular effects through the activation of opposing intracellular signaling pathways. Under normal conditions, insulin provides vasodilatation and vasoprotective effects through the activation of the Phosphoinositol-3-Kinase pathway (PI-3 K) and promoting the release of NO. In contrast, in case of insulin resistance and an unphysiological release of insulin and proinsulin from the beta cells, the signal shifts from the vasoprotective PI-3 K pathway to the mitogen activated proteinkinase (MAPK) pathway (Figure 2). In this case, the vasodilating and beneficial effects of insulin turn over into the vasoconstrictive and mitogenic properties of insulin [6, 39].

4. Effects of GLP-1 on Body Weight and Beta Cell Function

Nowadays, numerous pharmacological interventions are available to lower blood glucose levels in patients with type 2 diabetes. Beside more or less comparable glucose lowering efficacy, some of them have shown limited or probably even unfavorable effects on the cardiovascular system and overall mortality [40–44]. Therefore, treatments in T2DM should be reevaluated not solely by judging their glucose lowering potency, but, moreover, on their overall effects on the cardiovascular risk profile and overall mortality in patients with T2DM.

Recently, incretin-based therapies have been introduced in the treatment of T2DM. Incretins, gut-derived hormones, predominantly glucagon peptide 1 (GLP-1), and gastric inhibitory polypeptide (GIP), are released in response to an ingested meal. They reduce blood glucose concentrations by enhancing the insulin release from pancreatic beta cells [45] and inhibiting postprandial glucagon secretion [46, 47] and gastric emptying after a meal [48]. However, only GLP-1 seemed to be suitable for the treatment of type 2 diabetic patients since the action of GIP was found to be grossly impaired in patients with T2DM [49]. Following its release from the L-cells into the blood stream, GLP-1 [7–36] is quickly cleaved by the enzyme dipeptidyl-peptidase-IV (DPP-IV) into the split product GLP-1 [9–39], which results in a half-life time of GLP-1 [7–39] of only 2 minutes. Therefore, strategies have been developed to increase the therapeutical window of GLP-1-based treatments by inhibiting the degrading enzyme DPP-IV or by providing exogenous agonists of GLP-1, which are resistant to the degradation by DPP-IV. At present, four DPP-IV inhibitors (Sitagliptin, Vildagliptin, Saxagliptin, and Linagliptin) and two GLP-1 receptor agonists (Exenatide and Liraglutide) are approved for the treatment of T2DM. In several studies, treatment with DPP-IV inhibitors and GLP-1 receptor agonists was shown to improve blood glucose control in type 2 diabetic patients without increasing the risk of hypoglycemia. While treatment with DPP-IV inhibitors was shown to be more or less weight neutral, treatment with the GLP-1 receptor agonists reduced body weight in the majority of patients with T2DM [50]. Beside these overall weight reducing effects of GLP-1 receptor agonists a pronounced reduction in visceral body fat was shown during treatment with Liraglutide [51]. In another study, treatment with the GLP-1 receptor agonist Exenatide resulted in a reduction in body weight, waist circumference, total body, and truncal fat mass by 6%, 5%, 11%, and 13%, respectively [52]. In addition, exenatide increased total adiponectin by 12% and reduced high-sensitive CRP by 61%. Treatment with GLP-1 receptor agonists has been consistently demonstrated to reduce blood pressure, as observed in clinical trials with exenatide or liraglutide [53–57]. Of interest is, that the reduction of blood pressure in most studies occurred even before weight reduction could be achieved, indicating direct effects of GLP-1 on blood pressure. The mechanisms beyond the blood pressure lowering effects of GLP-1 are still unclear. Beside changes in the release of adipokines from the adipose tissue, direct vasodilatory effects [58] and renal natriuretic effects [59] of GLP-1 might cause the beneficial effects of GLP-1 on blood pressure.

Recently, it has been reported that the vulnerability of the beta cell to release the prohormone intact proinsulin, instead of insulin and C-peptide, is linked to beta cell polymorphisms observed in three different risk alleles [22]. The mechanisms by which these risk alleles (HHEX, CDKN2A/B, and IGF2BP2) influence proinsulin processing are still a matter of debate. Loos et al. demonstrated that the genes of pro-protein convertases 1 and 2, which are key proteins in the conversion from proinsulin to insulin, exhibit binding sites for the *T*-cell transcription factor [60]. Therefore, an interaction with these pro-protein convertases may be a mechanism leading to increased proinsulin levels in carriers of the risk alleles [22]. As GLP-1 infusion

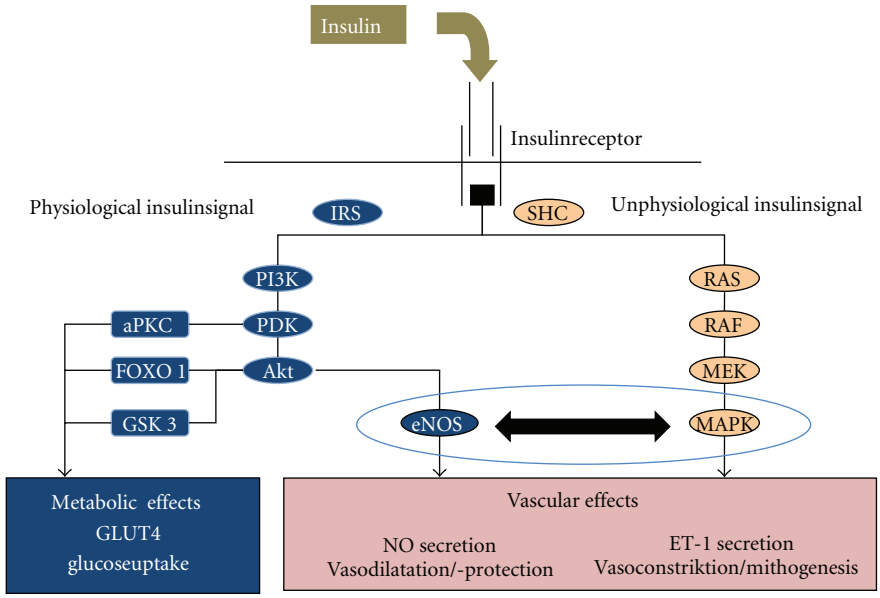


Figure 2: Schematic illustration of intracellular insulin signalling (aPKC: atypical Protein-Kinase C, eNOS: endothelial Nitric Oxide Synthase, ET1: Endothelin 1, FOX01: Forkhead Box01, IRS: Insulin Receptor Substrate, MAPK: Mitogen Activated Proteinkinase, MEK: Mitogen Activated Proteinkinase Kinase, NO: Nitric Oxide, PDK: Phosphoinositide-Dependent Kinase, PI3K: Phosphatidylinostol 3 Kinase, RAS, RAF: Proto-Oncogenes, SHC: Adapter Protein; adapted to [6]).

is able to normalize reduced proinsulin conversion, and GLP-1 signaling is impaired in carriers of the risk alleles in TCF7L2, it is conceivable that an attenuated GLP-1 signaling might lead to an impaired proinsulin processing [61]. Several clinical studies have shown an improvement in the Proinsulin/Insulin Ratio during treatment with GLP-1 receptor agonists [62, 63] and DPP-IV inhibitors [64–66]. In a cross-sectional study, postprandial intact proinsulin levels were significantly higher in sulfonylurea-treated patients compared to insulin and DPP-IV-inhibitor-treated patients and a non-diabetic control group [67]. As shown in Figure 3, the proinsulin/insulin ratio was comparable in between the DPP-IV-inhibitor-treated group and the nondiabetic control group, while it was evaluated in the sulfonylurea- and the insulin-treated group. In a recent study, the introduction of liraglutide treatment caused a pronounced decrease in intact proinsulin levels, which was found in parallel with a reduction in E-Selectin, asymmetric-dimethyl-arginine (ADMA), and PAI-1 levels, and an improvement in endothelial function [68]. Therefore, it seems conceivable that GLP-1-based treatments do not only improve beta cell function by an induction of the proinsulin convertases but also reduce vascular risk by the reduction in circulating absolute proinsulin levels.

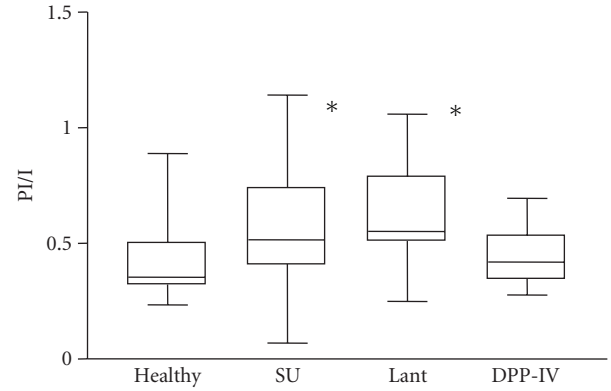


Figure 3: Postprandial proinsulin/insulin ratio in patients treated with sulfonylurea, insulin Glargine, or DPP-IV inhibitors compared to nondiabetic healthy controls (*: $P < 0.05$ versus nondiabetic controls adapted to [67]).

5. Effects of GLP-1 on Endothelial and Cardiovascular Function

GLP-1 acts through a distinct heptahelical G-protein-coupled receptor, which has been located not only in beta cells and the gastrointestinal tract, but also in the nervous system, heart, vascular smooth muscle cells, endothelial cells, and macrophages [45, 58, 70, 71]. GLP-1 appears to modulate a wide range of physiological effects, not only regulating blood glucose and metabolic control, but also directly affecting several cardiovascular pathways involved in atherogenesis. Binding of GLP-1 to the GLP-1 receptor in the myocardium leads to an increase in the production of cyclic adenosine monophosphate (cAMP) and an activation of protein kinase A (PKA), which results in an increase in glucose uptake and the inotropic effects in myocardial tissue. GLP-1 knockout mice exhibit reduced resting heart rate, elevated left ventricular and diastolic pressure, and increased left ventricular thickness compared with normal controls

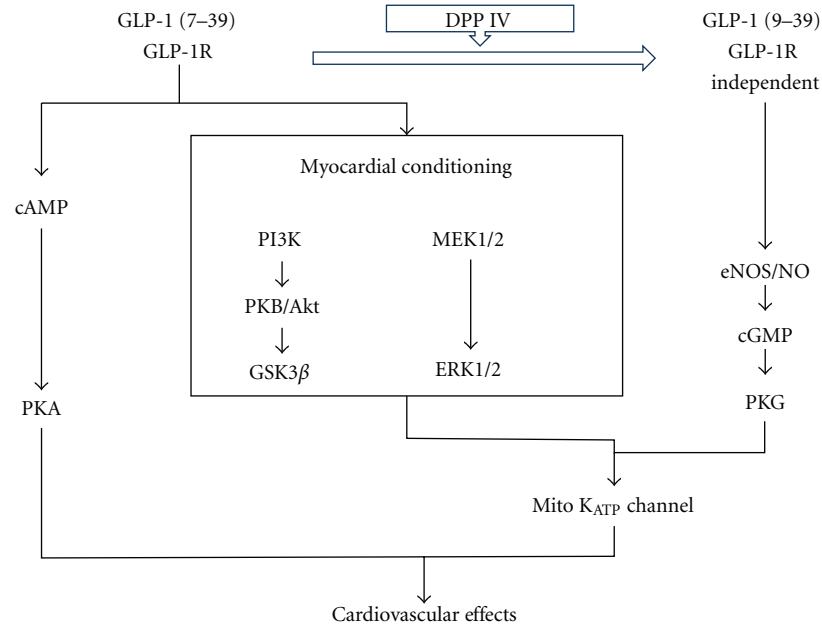


Figure 4: Schematic illustration of GLP-1-receptor-dependent and GLP-1-receptor-independent pathways in the signalling of GLP-1 associated cardiovascular effects (PI3K: phosphatidylinositol 3 kinase; cAMP: cyclic adenosine monophosphate, PKA: protein kinase A, PKB: protein kinase B, GSK3ß: glycogen synthase kinase 3 beta, MEK1/2: components of the MAP kinase cascade, ERK: extracellular-signal-regulated kinase, NO: nitric oxide, eNOS: endothelial nitric oxide synthase, cGMP: cyclic guanosine monophosphate, PKG: protein kinase G; adapted to [69]).

[58]. In agreement with this findings, treatment with GLP-1 or the GLP-1 receptor agonists was found to improve left ventricular function [72, 73] and to reduce circulating levels of brain natriuretic peptide (BNP) [74, 75]. GLP-1 increased functional cardiomyocyte viability in an isolated mouse heart reperfusion model but, unexpectedly, many of these actions were preserved in the GLP-1 knockout mouse model, suggesting cardiovascular effects that are independent of the GLP-1-receptor-mediated activation of cAMP/PKA [50]. Ban et al. studied the myocardial effects of GLP-1 (7-39) and GLP-1(9-39) under postischemic conditions in both wild-type and GLP1r (−/−) (GLP-1 receptor knockout mice) [58]. In wild-type mice, some of the effects of GLP-1 (7-39) could be reproduced by the truncated peptide GLP-1 (9-39). Those effects could also be induced by GLP1 (7-39) and GLP-1 (9-39) in the GLP1r (−/−) knockout mouse model. In addition, the beneficial effects of GLP-1 in the knockout mouse model could be abolished by the addition of the DPP-IV inhibitor Sitagliptin and by the administration of the NO-synthase blocker L-NNA (N^G-nitro-arginine). This leads to the hypothesis of a two pathway mechanism for the protective cardiovascular action of GLP-1. One that depends on the GLP-1 receptor mediated action with inotropic, glucose uptake stimulating, ischemic preconditioning effects, and one, receptor independent vasodilatatory pathway, which is mediated by NO (Figure 4).

Growing lines of evidence demonstrate beneficial effects of GLP-1 on endothelium and vascular smooth muscle cells [58, 76–78]. In human vascular endothelial cells, liraglutide caused eNOS phosphorylation and potentiated eNOS activity with an increased nitric oxide production [79]. In endothelial cells, isolated from human coronary arteries, GLP-1 rapidly activates endothelial nitric oxide synthase (eNOS), promotes cell proliferation, and inhibits glucolipoapoptosis [80]. In healthy, nondiabetic, subjects, GLP-1 infusion enhanced the acetylcholine-mediated increase in forearm blood flow, while no such effect could be observed on endothelial-independent vasodilatation after sodium nitroprusside [78]. Interestingly, in that study, the beneficial effects of GLP-1 on endothelial function were damped by the addition of glyburide, but not by glimepiride. In type 2 diabetic patient with coronary artery disease, infusion of GLP-1 increased flow-mediated vasodilatation in the brachial artery, affirming the NO-dependent mechanism of GLP-1 in the vascular system [81]. In this study, no such effect could be observed in nondiabetic healthy controls.

6. Other Potential Cardiovascular Benefits

Treatment with the GLP-1 receptor agonists, liraglutide and exenatide, was shown to reduce PAI-1 levels in patients with T2DM [68, 74, 75, 82]. In cultured human vascular endothelial cells, Liraglutide inhibited the expression of tumor necrosis factor-α (TNF α) and the hyperglycemia-mediated induction of VCAM-1 and PAI-1 [74, 80]. This may be of relevance as elevated PAI-1 levels have been implicated in endothelial cell dysfunction [83]. In a previous study, 14 weeks of treatment with liraglutide significantly decreased PAI-1 levels by 25% [74]. Complementing the

beneficial effect on PAI-1 levels, liraglutide attenuates the induction of PAI-1 and vascular adhesion molecules in vitro [31].

A recent metaanalysis of the 6 trials from the LEAD program revealed that treatment with the GLP-1 receptor agonist liraglutide decreased hsCRP levels by 23% from baseline till 6 months [84]. In a study comparing exenatide and glargine treatment, treatment with exenatide over a period of 12 months reduces hsCRP levels by 61% [52].

In addition to the effects on myocardium and the endothelial cells, GLP-1 may also have effects on atherogenesis through direct interactions with monocytes or macrophages. Treatment with Exenatide significantly inhibited monocyte adhesion in the aorta of C57BL/6 mice [85]. In apoE(−/−) mice, the same treatment reduced monocyte adhesion to the endothelium and suppressed atherogenesis.

As pointed out by Zilversmit many years ago, atherosclerosis could be considered to be a prandial phenomenon [86]. Therefore, GLP-1 might serve as a metabolic and vasoprotective factor evolving its main effects after the ingestion of a meal. In a study by Koska et al. postprandial endothelial function was investigated in IGT subjects and in patients with recently diagnosed T2DM by reactive hyperemia peripheral arterial tonometry [87]. In that study, a single dose of Exenatide resulted in a significant improvement in postprandial endothelial function compared with placebo administration. In another study, intravenous GLP (7-39) infusion resulted in a significant improvement of postprandial FMD during an OGT and during a hyperglycemic clamp procedure [88]. The improvement in FMD was paralleled by a reduced postprandial increase in nitrotyrosine and 8-iso-PGF2a levels. These results suggest that GLP-1 has the potential to reduce glucose load, oxidative stress and to improve endothelial function especially in the postprandial state.

How far all the previously observed pleiotropic effects of GLP-1 will translate in a reduction of micro- and/or macrovascular complications in patients with T2DM is still not established. In a retrospective analysis of the LifeLink database, patients treated with the GLP-1 receptor agonist Exenatide were less likely to have a CVD event (HR 0.81, $P = 0.01$) and lower rates of CVD-related hospitalization (HR 0.88, $P = 0.02$) [89]. A recent meta-analysis assessing the cardiovascular outcome of GLP-1-receptor agonists in clinical trials up to November 2010 revealed a significant lower rate of major cardiovascular events in GLP-1-receptor-agonist-treated patients compared to placebo [90]. Ongoing randomized prospective clinical studies will provide more evidence about potential clinical long-term effects of GLP-receptor agonist or DPP-IV inhibitor treatment in patients with T2DM at cardiovascular risk.

Funding

T. Forst, M. M. Weber, and A. Pfützner received research funds, speaker and advisory fees from Novo Nordisk, E. Lilly, Novartis, and Boehringer Ingelheim.

References

[1] S. Wild, G. Roglic, A. Green, R. Sicree, and H. King, "Global prevalence of diabetes: estimates for the year 2000 and projections for 2030," *Diabetes Care*, vol. 27, no. 5, pp. 1047–1053, 2004.

[2] A. Juutilainen, S. Lehto, T. Rönnemaa, K. Pyörälä, and M. Laakso, "Type 2 diabetes as a "coronary heart disease equivalent": an 18-year prospective population-based study in Finnish subjects," *Diabetes Care*, vol. 28, no. 12, pp. 2901–2907, 2005.

[3] S. M. Haffner, S. Lehto, T. Rönnemaa, K. Pyörälä, and M. Laakso, "Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction," *The New England Journal of Medicine*, vol. 339, no. 4, pp. 229–234, 1998.

[4] L. Whiteley, S. Padmanabhan, D. Hole, and C. Isles, "Should diabetes be considered a coronary heart disease risk equivalent? Results from 25 years of follow-up in the Renfrew and Paisley survey," *Diabetes Care*, vol. 28, no. 7, pp. 1588–1593, 2005.

[5] J. Calles-Escandon and M. Cipolla, "Diabetes and endothelial dysfunction: a clinical perspective," *Endocrine Reviews*, vol. 22, no. 1, pp. 36–52, 2001.

[6] T. Forst, C. Hohberg, and A. Pfützner, "Cardiovascular effects of disturbed insulin activity in metabolic syndrome and in type 2 diabetic patients," *Hormone and Metabolic Research*, vol. 41, no. 2, pp. 123–131, 2009.

[7] E. Cosson, I. Pham, P. Valensi, J. Pariès, J. R. Attali, and A. Nitenberg, "Impaired coronary endothelium-dependent vasodilation is associated with microalbuminuria in patients with type 2 diabetes and angiographically normal coronary arteries," *Diabetes Care*, vol. 29, no. 1, pp. 107–112, 2006.

[8] T. K. Schramm, G. H. Gislason, L. Køber et al., "Diabetes patients requiring glucose-lowering therapy and nondiabetics with a prior myocardial infarction carry the same cardiovascular risk: a population study of 3.3 million people," *Circulation*, vol. 117, no. 15, pp. 1945–1954, 2008.

[9] W. T. Friedewald, J. B. Buse, J. T. Bigger et al., "Effects of intensive glucose lowering in type 2 diabetes," *The New England Journal of Medicine*, vol. 358, no. 24, pp. 2545–2559, 2008.

[10] A. Patel, S. MacMahon, J. Chalmers et al., "Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes," *The New England Journal of Medicine*, vol. 358, no. 24, pp. 2560–2572, 2008.

[11] W. Duckworth, C. Abraira, T. Moritz et al., "Glucose control and vascular complications in veterans with type 2 diabetes," *The New England Journal of Medicine*, vol. 360, no. 2, pp. 129–139, 2009.

[12] D. C. W. Lau, B. Dhillon, H. Yan, P. E. Szmitko, and S. Verma, "Adipokines: molecular links between obesity and atheroslcerosis," *American Journal of Physiology*, vol. 288, no. 5, pp. H2031–H2041, 2005.

[13] N. Klöting, M. Fasshauer, A. Dietrich et al., "Insulin-sensitive obesity," *American Journal of Physiology*, vol. 299, no. 3, pp. E506–E515, 2010.

[14] F. B. Hu, M. J. Stampfer, S. M. Haffner, C. G. Solomon, W. C. Willett, and J. E. Manson, "Elevated risk of cardiovascular disease prior to clinical diagnosis of type 2 diabetes," *Diabetes Care*, vol. 25, no. 7, pp. 1129–1134, 2002.

[15] N. Ishizaka, Y. Ishizaka, E. Takahashi et al., "Association between insulin resistance and carotid arteriosclerosis in subjects with normal fasting glucose and normal glucose

tolerance," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 23, no. 2, pp. 295–301, 2003.

[16] G. M. Reaven, "Insulin resistance, the insulin resistance syndrome, and cardiovascular disease," *Panminerva Medica*, vol. 47, no. 4, pp. 201–210, 2005.

[17] K. J. Ausk, E. J. Boyko, and G. N. Ioannou, "Insulin resistance predicts mortality in nondiabetic individuals in the U.S," *Diabetes Care*, vol. 33, no. 6, pp. 1179–1185, 2010.

[18] H. C. McGill Jr., C. A. McMahan, E. E. Herderick et al., "Obesity accelerates the progression of coronary atherosclerosis in young men," *Circulation*, vol. 105, no. 23, pp. 2712–2718, 2002.

[19] E. Cho, J. E. Manson, M. J. Stampfer et al., "A prospective study of obesity and risk of coronary heart disease among diabetic women," *Diabetes Care*, vol. 25, no. 7, pp. 1142–1148, 2002.

[20] S. Cauchi, S. D. Guerra, H. Choquet et al., "Meta-analysis and functional effects of the SLC30A8 rs13266634 polymorphism on isolated human pancreatic islets," *Molecular Genetics and Metabolism*, vol. 100, no. 1, pp. 77–82, 2010.

[21] A. Fritsche, A. Madaus, N. Stefan et al., "Relationships among age, proinsulin conversion, and β-cell function in nondiabetic humans," *Diabetes*, vol. 51, supplement 1, pp. S234–S239, 2002.

[22] K. Kirchhoff, F. Machicao, A. Haupt et al., "Polymorphisms in the TCF7L2, CDKAL1 and SLC30A8 genes are associated with impaired proinsulin conversion," *Diabetologia*, vol. 51, no. 4, pp. 597–601, 2008.

[23] M. Alssema, J. M. Dekker, G. Nijpels, C. D. A. Stehouwer, L. M. Bouter, and R. J. Heine, "Proinsulin concentration is an independent predictor of all-cause and cardiovascular mortality: an 11-year follow-up of the Hoorn study," *Diabetes Care*, vol. 28, no. 4, pp. 860–865, 2005.

[24] J. Y. Oh, E. Barrett-Connor, and N. M. Wedick, "Sex differences in the association between proinsulin and intact insulin with coronary heart disease in nondiabetic older adults: the Rancho Bernardo study," *Circulation*, vol. 105, no. 11, pp. 1311–1316, 2002.

[25] M. Wohlin, J. Sundström, J. Ärnlöv, B. Andrén, B. Zethelius, and L. Lind, "Impaired insulin sensitivity is an independent predictor of common carotid intima-media thickness in a population sample of elderly men," *Atherosclerosis*, vol. 170, no. 1, pp. 181–185, 2003.

[26] B. Zethelius, H. Lithell, C. N. Hales, and C. Berne, "Insulin sensitivity, proinsulin and insulin as predictors of coronary heart disease. A population-based 10-year, follow-up study in 70-year old men using the euglycaemic insulin clamp," *Diabetologia*, vol. 48, no. 5, pp. 862–867, 2005.

[27] B. Zethelius, L. Byberg, C. N. Hales, H. Lithell, and C. Berne, "Proinsulin is an independent predictor of coronary heart disease: report from a 27-year follow-up study," *Circulation*, vol. 105, no. 18, pp. 2153–2158, 2002.

[28] J. A. Galloway, S. A. Hooper, C. T. Spradlin et al., "Biosynthetic human proinsulin: review of chemistry, in vitro and in vivo receptor binding, animal and human pharmacology studies, and clinical trial experience," *Diabetes Care*, vol. 15, no. 5, pp. 666–692, 1992.

[29] E. Z. Jia, Z. J. Yang, T. B. Zhu et al., "Proinsulin is an independent predictor of the angiographical characteristics of coronary atherosclerosis," *Cardiology*, vol. 110, no. 2, pp. 106–111, 2008.

[30] T. K. Nordt, D. J. Schneider, and B. E. Sobel, "Augmentation of the synthesis of plasminogen activator inhibitor type-1 by precursors of insulin: a potential risk factor for vascular disease," *Circulation*, vol. 89, no. 1, pp. 321–330, 1994.

[31] H. Liu, A. E. Dear, L. B. Knudsen, and R. W. Simpson, "A long-acting glucagon-like peptide-1 analogue attenuates induction of plasminogen activator inhibitor type-1 and vascular adhesion molecules," *Journal of Endocrinology*, vol. 201, no. 1, pp. 59–66, 2009.

[32] T. K. Nordt, C. Bode, and B. E. Sobel, "Stimulation in vivo of expression of intra-abdominal adipose tissue plasminogen activator inhibitor type I by proinsulin," *Diabetologia*, vol. 44, no. 9, pp. 1121–1124, 2001.

[33] A. Festa, R. D'Agostino Jr., L. Mykkänen et al., "Relative contribution of insulin and its precursors to fibrinogen and PAI-1 in a large population with different states of glucose tolerance: the Insulin Resistance Atherosclerosis Study (IRAS)," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 19, no. 3, pp. 562–568, 1999.

[34] C. J. Lyon and W. A. Hsueh, "Effect of plasminogen activator inhibitor-1 in diabetes mellitus and cardiovascular disease," *American Journal of Medicine*, vol. 115, no. 8, supplement 1, pp. 62S–68S, 2003.

[35] T. Forst, C. Hohberg, S. D. Fuellert et al., "Pharmacological PPARγ stimulation in contrast to beta cell stimulation results in an improvement in adiponectin and proinsulin intact levels and reduces intima media thickness in patients with type 2 diabetes," *Hormone and Metabolic Research*, vol. 37, no. 8, pp. 521–527, 2005.

[36] R. Ross, "The pathogenesis of atherosclerosis: a perspective for the 1990s," *Nature*, vol. 362, no. 6423, pp. 801–809, 1993.

[37] S. Verma and T. J. Anderson, "Fundamentals of endothelial function for the clinical cardiologist," *Circulation*, vol. 105, no. 5, pp. 546–549, 2002.

[38] A. B. Goldfine, J. A. Beckman, R. A. Betensky et al., "Family history of diabetes is a major determinant of endothelial function," *Journal of the American College of Cardiology*, vol. 47, no. 12, pp. 2456–2461, 2006.

[39] K. Mather, T. J. Anderson, and S. Verma, "Insulin action in the vasculature: physiology and pathophysiology," *Journal of Vascular Research*, vol. 38, no. 5, pp. 415–422, 2001.

[40] D. J. Margolis, O. Hoffstad, and B. L. Strom, "Association between serious ischemic cardiac outcomes and medications used to treat diabetes," *Pharmacoepidemiology and Drug Safety*, vol. 17, no. 8, pp. 753–759, 2008.

[41] D. T. Eurich, F. A. McAlister, D. F. Blackburn et al., "Benefits and harms of antidiabetic agents in patients with diabetes and heart failure: systematic review," *British Medical Journal*, vol. 335, no. 7618, pp. 497–501, 2007.

[42] A. D. Rao, N. Kuhadiya, K. Reynolds, and V. A. Fonseca, "Is the combination of sulfonylureas and metformin associated with an increased risk of cardiovascular disease or all- cause mortality?" *Diabetes Care*, vol. 31, no. 8, pp. 1672–1678, 2008.

[43] S. Bolen, L. Feldman, J. Vassy et al., "Systematic review: comparative effectiveness and safety of oral medications for type 2 diabetes mellitus," *Annals of Internal Medicine*, vol. 147, no. 6, pp. 386–399, 2007.

[44] M. J. Davies, "Insulin secretagogues," *Current Medical Research and Opinion*, vol. 18, supplement 1, pp. s22–s30, 2002.

[45] T. J. Kieffer and J. F. Habener, "The glucagon-like peptides," *Endocrine Reviews*, vol. 20, no. 6, pp. 876–913, 1999.

[46] T. Matsuyama, R. Komatsu, M. Namba, N. Watanabe, H. Itoh, and S. Tarui, "Glucagon-like peptide-1 (7-36 amide): a potent glucagonostatic and insulinotropic hormone," *Diabetes Research and Clinical Practice*, vol. 5, no. 4, pp. 281–284, 1988.

[47] G. C. Weir, S. Mojsov, G. K. Hendrick, and J. F. Habener, "Glucagonlike peptide I (7-37) actions on endocrine pancreas," *Diabetes*, vol. 38, no. 3, pp. 338–342, 1989.

[48] A. Wettergren, B. Schjoldager, P. E. Mortensen, J. Myhre, J. Christiansen, and J. J. Holst, "Truncated GLP-1 (proglucagon 78-107-amide) inhibits gastric and pancreatic functions in man," *Digestive Diseases and Sciences*, vol. 38, no. 4, pp. 665–673, 1993.

[49] M. A. Nauck, M. M. Heimesaat, C. Orskov, J. J. Holst, R. Ebert, and W. Creutzfeldt, "Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type- 2 diabetes mellitus," *The Journal of Clinical Investigation*, vol. 91, no. 1, pp. 301–307, 1993.

[50] M. H. Davidson, "Cardiovascular effects of glucagonlike peptide-1 agonists," *American Journal of Cardiology*, vol. 108, no. 3, supplement, pp. 33B–41B, 2011.

[51] J. Jendle, M. A. Nauck, D. R. Matthews et al., "Weight loss with liraglutide, a once-daily human glucagon-like peptide-1 analogue for type 2 diabetes treatment as monotherapy or added to metformin, is primarily as a result of a reduction in fat tissue," *Diabetes, Obesity and Metabolism*, vol. 11, no. 12, pp. 1163–1172, 2009.

[52] M. C. Bunck, M. Diamant, B. Eliasson et al., "Exenatide affects circulating cardiovascular risk biomarkers independently of changes in body composition," *Diabetes Care*, vol. 33, no. 8, pp. 1734–1737, 2010.

[53] D. D. Mafong and R. R. Henry, "The role of incretins in cardiovascular control," *Current Hypertension Reports*, vol. 11, no. 1, pp. 18–22, 2009.

[54] D. C. Klonoff, J. B. Buse, L. L. Nielsen et al., "Exenatide effects on diabetes, obesity, cardiovascular risk factors and hepatic biomarkers in patients with type 2 diabetes treated for at least 3 years," *Current Medical Research and Opinion*, vol. 24, no. 1, pp. 275–286, 2008.

[55] M. Marre, J. Shaw, M. Brändle et al., "Liraglutide, a once-daily human GLP-1 analogue, added to a sulphonylurea over 26 weeks produces greater improvements in glycaemic and weight control compared with adding rosiglitazone or placebo in subjects with Type 2 diabetes (LEAD-1 SU)," *Diabetic Medicine*, vol. 26, no. 3, pp. 268–278, 2009.

[56] J. B. Buse, J. Rosenstock, G. Sesti et al., "Liraglutide once a day versus exenatide twice a day for type 2 diabetes: a 26-week randomised, parallel-group, multinational, open-label trial (LEAD-6)," *The Lancet*, vol. 374, no. 9683, pp. 39–47, 2009.

[57] T. Okerson, P. Yan, A. Stonehouse, and R. Brodows, "Effects of exenatide on systolic blood pressure in subjects with type 2 diabetes," *American Journal of Hypertension*, vol. 23, no. 3, pp. 334–339, 2010.

[58] K. Ban, M. H. Noyan-Ashraf, J. Hoefer, S. S. Bolz, D. J. Drucker, and M. Husain, "Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways," *Circulation*, vol. 117, no. 18, pp. 2340–2350, 2008.

[59] J. P. Gutzwiller, S. Tschopp, A. Bock et al., "Glucagon-like peptide 1 induces natriuresis in healthy subjects and in insulin-resistant obese men," *The The Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 6, pp. 3055–3061, 2004.

[60] R. J. F. Loos, P. W. Franks, R. W. Francis et al., "TCF7L2 polymorphisms modulate proinsulin levels and β-cell function in a British europid population," *Diabetes*, vol. 56, no. 7, pp. 1943–1947, 2007.

[61] M. Stumvoll, A. Fritsche, N. Stefan, E. Hardt, and H. Häring, "Evidence against a rate-limiting role of proinsulin processing for maximal insulin secretion in subjects with impaired glucose tolerance and β-cell dysfunction," *The Journal of Clinical Endocrinology and Metabolism*, vol. 86, no. 3, pp. 1235–1239, 2001.

[62] T. Vilsbøll, B. Brock, H. Perrild et al., "Liraglutide, a once-daily human GLP-1 analogue, improves pancreatic B-cell function and arginine-stimulated insulin secretion during hyperglycaemia in patients with Type 2 diabetes mellitus," *Diabetic Medicine*, vol. 25, no. 2, pp. 152–156, 2008.

[63] R. A. DeFronzo, R. E. Ratner, J. Han, D. D. Kim, M. S. Fineman, and A. D. Baron, "Effects of exenatide (exendin-4) on glycemic control and weight over 30 weeks in metformin-treated patients with type 2," *Diabetes Care*, vol. 28, no. 5, pp. 1092–1100, 2005.

[64] S. Del Prato, A. H. Barnett, H. Huisman, D. Neubacher, H.-J. Woerle, and K. A. Dugi, "Effect of linagliptin monotherapy on glycaemic control and markers of β-cell function in patients with inadequately controlled type 2 diabetes: a randomized controlled trial," *Diabetes, Obesity and Metabolism*, vol. 13, no. 3, pp. 258–267, 2011.

[65] R. E. Pratley, A. Schweizer, J. Rosenstock et al., "Robust improvements in fasting and prandial measures of β-cell function with vildagliptin in drug-naïve patients: analysis of pooled vildagliptin monotherapy database," *Diabetes, Obesity and Metabolism*, vol. 10, no. 10, pp. 931–938, 2008.

[66] I. Raz, M. Hanefeld, L. Xu, C. Caria, D. Williams-Herman, and H. Khatami, "Efficacy and safety of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy in patients with type 2 diabetes mellitus," *Diabetologia*, vol. 49, no. 11, pp. 2564–2571, 2006.

[67] S. Pscherer, M. Larbig, B. von Stritsky et al., "In type 2 diabetic patients, insulin glargine is associated with lower postprandial release of intact compared with sulfonyurea treatment," *Journal of Diabetes Science and Technology*. In press.

[68] T. Forst, G. Michelson, F. Ratter et al., "Addition of liraglutide in patients with Type 2 diabetes well controlled on metformin monotherapy improves several markers of vascular function," *Diabetic Medicine*. In press.

[69] K. Ban, S. Hui, D. J. Drucker, and M. Husain, "Cardiovascular consequences of drugs used for the treatment of diabetes: potential promise of incretin-based therapies," *Journal of the American Society of Hypertension*, vol. 3, no. 4, pp. 245–259, 2009.

[70] S. M. Gardiner, J. E. March, P. A. Kemp, T. Bennett, and D. J. Baker, "Possible involvement of GLP-1(9-36) in the regional haemodynamic effects of GLP-1(7-36) in conscious rats," *British Journal of Pharmacology*, vol. 161, no. 1, pp. 92–102, 2010.

[71] E. Tomas and J. F. Habener, "Insulin-like actions of glucagon-like peptide-1: a dual receptor hypothesis," *Trends in Endocrinology and Metabolism*, vol. 21, no. 2, pp. 59–67, 2010.

[72] L. A. Nikolaidis, S. Mankad, G. G. Sokos et al., "Effects of glucagon-like peptide-1 in patients with acute myocardial infarction and left ventricular dysfunction after successful reperfusion," *Circulation*, vol. 109, no. 8, pp. 962–965, 2004.

[73] G. G. Sokos, L. A. Nikolaidis, S. Mankad, D. Elahi, and R. P. Shannon, "Glucagon-like peptide-1 infusion improves left ventricular ejection fraction and functional status in patients with chronic heart failure," *Journal of Cardiac Failure*, vol. 12, no. 9, pp. 694–699, 2006.

[74] J. P. Courrèges, T. Vilsbøll, M. Zdravkovic et al., "Beneficial effects of once-daily liraglutide, a human glucagon-like peptide-1 analogue, on cardiovascular risk biomarkers in

patients with type 2 diabetes," *Diabetic Medicine*, vol. 25, no. 9, pp. 1129–1131, 2008.

[75] R. M. Bergenstal, C. Wysham, L. MacConell et al., "Efficacy and safety of exenatide once weekly versus sitagliptin or pioglitazone as an adjunct to metformin for treatment of type 2 diabetes (DURATION-2): a randomised trial," *The Lancet*, vol. 376, no. 9739, pp. 431–439, 2010.

[76] H. A. Golpon, A. Puechner, T. Welte, P. V. Wichert, and C. O. Feddersen, "Vasorelaxant effect of glucagon-like peptide-(7-36)amide and amylin on the pulmonary circulation of the rat," *Regulatory Peptides*, vol. 102, no. 2-3, pp. 81–86, 2001.

[77] G. Richter, O. Feddersen, U. Wagner, P. Barth, R. Goke, and B. Goke, "GLP-1 stimulates secretion of macromolecules from airways and relaxes pulmonary artery," *American Journal of Physiology*, vol. 265, no. 4, pp. L374–L381, 1993.

[78] A. Basu, N. Charkoudian, W. Schrage, R. A. Rizza, R. Basu, and M. J. Joyner, "Beneficial effects of GLP-1 on endothelial function in humans: dampening by glyburide but not by glimepiride," *American Journal of Physiology*, vol. 293, no. 5, pp. E1289–E1295, 2007.

[79] Y. Hattori, T. Jojima, A. Tomizawa et al., "A glucagon-like peptide-1 (GLP-1) analogue, liraglutide, upregulates nitric oxide production and exerts anti-inflammatory action in endothelial cells," *Diabetologia*, vol. 53, no. 10, pp. 2256–2263, 2010.

[80] H. Liu, Y. Hu, R. W. Simpson, and A. E. Dear, "Glucagon-like peptide-1 attenuates tumour necrosis factor-α-mediated induction of plasmogen activator inhibitor-1 expression," *Journal of Endocrinology*, vol. 196, no. 1, pp. 57–65, 2008.

[81] T. Nyström, M. K. Gutniak, Q. Zhang et al., "Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary artery disease," *American Journal of Physiology*, vol. 287, no. 6, pp. E1209–E1215, 2004.

[82] T. Vilsbøll, M. Zdravkovic, T. Le-Thi et al., "Liraglutide, a long-acting human glucagon-like peptide-1 analog, given as monotherapy significantly improves glycemic control and lowers body weight without risk of hypoglycemia in patients with type 2 diabetes," *Diabetes Care*, vol. 30, no. 6, pp. 1608–1610, 2007.

[83] S. M. Nicholl, E. Roztocil, and M. G. Davies, "Plasminogen activator system and vascular disease," *Current Vascular Pharmacology*, vol. 4, no. 2, pp. 101–116, 2006.

[84] J. Plutzky, A. J. Garber, A. Falahati et al., "The once daily human GLP-1-analogue, liraglutide significantly reduces markers of cardiovascular risk in type 2 diabetes: a metaanalysis of six clinical trials," *European Heart Journal*, vol. 30, pp. 917–919, 2009.

[85] M. Arakawa, T. Mita, K. Azuma et al., "Inhibition of monocyte adhesion to endothelial cells and attenuation of atherosclerotic lesion by a glucagon-like peptide-1 receptor agonist, exendin-4," *Diabetes*, vol. 59, no. 4, pp. 1030–1037, 2010.

[86] D. B. Zilversmit, "Atherogenesis: a postprandial phenomenon," *Circulation*, vol. 60, no. 3, pp. 473–485, 1979.

[87] J. Koska, E. A. Schwartz, M. P. Mullin, D. C. Schwenke, and P. D. Reaven, "Improvement of postprandial endothelial function after a single dose of exenatide in individuals with impaired glucose tolerance and recent-onset type 2 diabetes," *Diabetes Care*, vol. 33, no. 5, pp. 1028–1030, 2010.

[88] A. Ceriello, K. Esposito, R. Testa, A. R. Bonfigli, M. Marra, and D. Giugliano, "The possible protective role of glucagon-like peptide1 on endothelium during the meal and evidence for an "endothelial resistance" to glucagon-like peptide 1 in diabetes," *Diabetes Care*, vol. 34, no. 3, pp. 697–702, 2011.

[89] J. H. Best, B. J. Hoogwerf, W. H. Herman et al., "Risk of cardiovascular disease events in patients with type 2 diabetes prescribed the Glucagon-Like Peptide 1 (GLP-1) receptor agonist exenatide twice daily or other glucose-lowering therapies: a retrospective analysis of the lifelink database," *Diabetes Care*, vol. 34, no. 1, pp. 90–95, 2011.

[90] M. Monami, F. Cremasco, C. Lamanna et al., "Glucagon-like peptide-1 receptor agonists and cardiovascular events: a meta-analysis of randomized clinical trials," *Experimental Diabetes Research*, vol. 2011, Article ID 215764, 10 pages, 2011.