

Edited by
János Fischer and
C. Robin Ganellin

**Analogue-based Drug
Discovery II**

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Analogue-based Drug Discovery II



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Abbreviations

ABC	ATP binding cassette
ABDD	analogue-based drug discovery
ABPM	ambulatory blood pressure monitoring
ACAT	acyl-CoA:cholesterol acyltransferase
ACE	angiotensin-converting enzyme
ACTH	adrenocorticotrophic hormone
ADMET	absorption, distribution, metabolism, excretion and toxicity
AFC	7-amino-4-trifluoromethylcoumarin
AIDS	acquired immunodeficiency syndrome
ALT	alanine aminotransferase
ALL	acute lymphoblastic leukemia
AMP	amprenavir
cAMP	cyclic 3',5'-adenosine monophosphate
ANDA	Abbreviated New Drug Application
α -APA	α -anilinophenylacetamide
APV	amprenavir
AR	androgen receptor
ATP	adenosine triphosphate
AUC	area under the curve
AZT	azidothymidine
BBB	blood-brain-barrier
Bcr-Abl	Breakpoint cluster region - Abelson
BG	blood glucose
b.i.d.	twice a day (from Latin <i>bis in die</i>)
BOC	t-butoxycarbonyl
CBF	cerebral blood flow
CC ₅₀	50% cytotoxic concentration
β -CCE	ethyl β -carboline-3-carboxylate
CGI	Clinical Global Impressions Scale
CHB	chronic hepatitis B
CK	creatine kinase
CL	clearance
CL _R	renal clearance

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XXII | Abbreviations

CL _T	total clearance
CLV	clevudine
CLV-TP	clevudine triphosphate
CML	chronic myelogenous leukemia
CMR _{glc}	cerebral metabolic rate of glucose
CNS	central nervous system
COBP	chronic obstructive broncho-pneumopathies
COPD	chronic obstructive pulmonary disease
COX-1	cyclooxygenase-1
COX-2	cyclooxygenase-2
CPI/r	comparator protease inhibitor boosted with ritonavir
CPT	camptothecin
CRC	colorectal cancer
CYP	cytochrome P450 isoenzyme
DA	dopamine
10-DAB	10-deacetyl-baccatin
DAPY	diarylpyrimidine
DATA	diaryltriazine
dCK	deoxycytidine kinase
DNA	desoxyribonucleic acid
cDNA	complementary deoxyribonucleic acid
cccDNA	covalently closed circular DNA
mtDNA	mitochondrial DNA
DOC	deoxycorticosterone
DOCA	deoxycorticosterone acetate
DPP-4	dipeptidyl peptidase 4
DSM-III	Diagnostic and Statistical Manual of Mental Disorders, third edition
EBV	Epstein-Barr virus
EC ₅₀	effective concentration 50
ED	erectile dysfunction
EFS	electric field stimulation
EGFR	epidermal growth factor receptor
EMA	European Medicines Agency
EPA	Environmental Protection Agency
EPO	European Patent Office
EPS	extrapyramidal side effect
Erk	extracellularly regulated kinase
ETC	emtricitabine
FAAH	fatty acid amide hydrolase
FBDD	fragment-based drug design
FDA	Food and Drug Administration
L-FAEU	1-(2'-deoxy-2'-fluoro-β-L-arabinofuranosyl)-5-ethyluridine
FEV	forced expiratory volume
L-FMAU	L-2'-fluoro-5-methyl-β-L-arabinofuranosyluracil

GABA _A	gamma-aminobutyric acid A
GAD	generalized anxiety disorder
GI	growth inhibition
GIP	glucose-dependent insulintropic polypeptide
GLP-1	glucagon-like peptide-1
cGMP	cyclic 3',5'-guanosine monophosphate
GP1Ib/IIIa	glycoprotein IIb/IIIa
HA	heavy atom
HAART	Highly Active Antiretroviral Therapy
HA/ACTH	histamine-induced adrenocorticotrophic hormone
HAM-A	Hamilton Anxiety Taring Scale
HAM-D	Hamilton Depression Rating Scale
HbA _{1c}	glycosylated haemoglobin
HBV	hepatitis B virus
HBcAg	hepatitis B core antigen
HBeAg	hepatitis B e antigen
HbsAg	hepatitis B surface antigen
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HDV	hepatitis delta virus
hERG	human ether-a-go-go-related gene
HFB	human foreskin fibroblast
HIAA	5-hydroxy-indole acetic acid
HIV	human immunodeficiency virus
HIV PR	HIV protease
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
5-HT	5-hydroxytryptamine (serotonin)
5-HTP	5-hydroxytryptophan
HTS	high-throughput screening
IBMX	isobutylmethylxanthine
IC ₅₀	inhibitory concentration 50
pIC ₅₀	-log IC ₅₀
ICS	inhaled corticosteroids
IDR	idarubicin
IDV	indinavir
i.m.	intramuscular
IND	Investigational New Drug
INN	International Nonproprietary Name
IOPY	iodophenoxypyridone
i.p.	intraperitoneal
i.v.	intravenous
K _i	inhibitory constant
LABA	long-acting β_2 -agonist
Lck	lymphocyte specific kinase
hLck	human Lck

XXIV | Abbreviations

mLck	murine Lck
LDL-C	low-density lipoprotein-cholesterol
LE	ligand efficiency
LPV	lopinavir
LVEF	left ventricular ejection fraction
MADRS	Montgomery-Asberg Depression Rating Scale
MAOI	monoamine oxidase inhibitor
M ₁	muscarinic receptor M ₁ subtype
MAP	mitogen-activated protein
rMD	restrained molecular dynamics
MDD	major depressive disorder
MDR	multidrug resistance
MED	minimal effective dose
MES	maximal electroshock seizure
MIC	minimal inhibitory concentration
MR	mineralocorticoid receptor
MRP	multidrug resistance-associated protein
MTD	maximum tolerated dose
NAPQI	N-acetyl-p-benzoquinone imine
NCE	New Chemical Entity
NCI	National Cancer Institute
NDA	New Drug Application
NE	norepinephrine
NMR	nuclear magnetic resonance
NNRTI	nonnucleoside reverse transcriptase inhibitor
NO	nitric oxide
NPs	natural products
NPC1L1	Niemann-Pick C1-Like-1
NRIs	norepinephrine reuptake inhibitors
NRTI	nucleoside reverse transcriptase inhibitor
NSAIDs	nonsteroidal anti-inflammatory drugs
NSCLC	non-small cell lung cancer
OADs	oral antidiabetic drugs
OC	ovarian cancer
OCD	obsessive-compulsive disorder
OGTT	oral glucose tolerance test
PCA	p-chloroamphetamine
PCF	plant cell fermentation
PCT	Patent Cooperation Treaty
PDEs	phosphodiesterases
PDGFR	platelet derived growth factor receptor
PEP	prolyl endopeptidase
PGE ₁	prostaglandin E ₁
PGE ₂	prostaglandin E ₂
P-gp	permeability glycoprotein

Ph (+)	Philadelphia chromosome positive
PK	pharmacokinetic
PKG	protein kinase G
POMS	profile of mood state
PPCE	postproline cleaving enzyme
PR	progesterone receptor
QSAR	quantitative structure-activity relationship
q.d. or QD	once a day (from Latin <i>quaque die</i>)
RBA	relative binding affinity
RGD	arginine-glycine-aspartic acid
RNA	ribonucleic acid
RNApol	RNA polymerase
mRNA	messenger RNA
RT	reverse transcriptase
RTV	ritonavir
SAR	structure-activity relationship
SBDD	structure-based drug design
s.c.	subcutaneous
SCID	severe combined immunodeficient
SCLC	small-cell lung cancer
SEDDS	self-emulsifying drug delivery system
SEF	sodium excreting factor
SI	selectivity index
SIV	simian immunodeficiency virus
SMC	smooth muscle cell
SNRI	serotonin/norepinephrine reuptake inhibitor
SQV	saquinavir
Src	sarcoma
SRI	serotonin reuptake inhibitor
SSRIs	selective serotonin reuptake inhibitors
TCR	T-cell antigen receptor
TDF	tenofovir disoproxil fumarate
TGF α	transforming growth factor- α
TI	tumor inhibition
TIBO	4,5,6,7-tetrahydro-5-methylimidazo[4,5,1-jk]benzodiazepin-2 (1 <i>H</i>)-one
t.i.d.	three times daily
TK	thymidine kinase
TMPK	thymidylate kinase
TPV	tipranavir
TPV/r	tipranavir/ritonavir combination
TPT	topotecan
TRIPs	Trade-related Aspects of Intellectual Property Rights
TTP	time to progression
UDP	uridine diphosphate

XXVI | *Abbreviations*

UGT	uridine diphosphate glucuronyl transferase
USAN	United States Adopted Names
VEGFR	vascular endothelial growth factor receptor
VMS	vasomotor symptoms
VSMC	vascular smooth muscle cell
V _{ss}	steady-state volume
WBC	white blood cell
WHcAg	woodchick hepatitis virus core antigen
WHsAg	woodchuck hepatitis virus surface antigen
WHV	woodchuck hepatitis virus
WTO	World Trade Organization

14

Liraglutide, a GLP-1 Analogue to Treat Diabetes

Lotte B. Knudsen

14.1

Introduction

The therapeutic potential of glucagon-like peptides (GLPs), particularly glucagon-like peptide-1 (GLP-1), is only now beginning to be realized at the start of the twenty-first century. It was, however, the discovery of secretin in 1902 by Bayliss and Starling [1] that initiated interest in the endocrine function of the gut and pancreas. These scientists speculated that signals arising from the gut could elicit an endocrine response affecting carbohydrate disposal. In 1929, Zunz and LaBarre described an intestinal extract that could produce hypoglycemia [2], and in a separate paper, LaBarre used the term “incretin” to describe activity in the gut that might stimulate pancreatic endocrine secretions [3]. Despite initial interest in “incretin,” research virtually stopped in this area due to the outbreak of World War II and the publication of several negative papers by Ivy and colleagues [4–6]. Twenty-five years later, McIntyre suggested that a humoral substance was released from the jejunum during glucose absorption, acting in concert with glucose to stimulate insulin release from pancreatic β -cells [7]. In 1969, Unger and Eisentraut referred to the gut–pancreas association as the enteroinsular axis [8], and this axis was subsequently described as involving nutrient, neural, and hormonal signals from the gut to the pancreatic islet cells. To be termed an “incretin,” any substance acting on this pathway must be secreted in response to nutrient stimuli and must stimulate glucose-dependent insulin secretion [9].

A second incretin hormone, following the discovery of glucose-dependent insulinotropic polypeptide (GIP), was postulated to exist as a consequence of the cloning of cDNAs encoding the preproglucagon gene in anglerfish pancreas [10–12]. Habener and colleagues conducted some of the very early work to characterize preproglucagon, but it was Bell who first identified GLP-1(1–37) [13]. In 1986/1987, it was discovered that the truncated forms, GLP-1(7–37) and (7–36)amide, were the active insulinotropic isoforms of GLP-1 [14, 15]. Lowering of blood glucose with GLP-1 was first shown in three studies by Nathan, Nauck, and Kreymann [16–18], with the

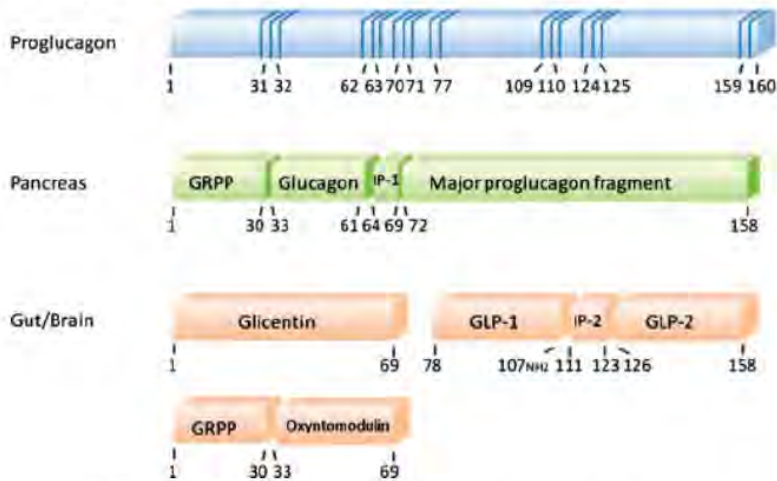


Figure 14.1 Differential post-translational processing of proglucagon in the pancreas and in the gut and brain. The numbers indicate amino acid positions in the 160-amino acid proglucagon sequence. The vertical lines

indicate positions of basic amino acid residues, typical cleavage sites. GRPP, glucin-related pancreatic polypeptide; IP-1, intervening peptide-1; IP-2, intervening peptide-2. Reproduced with permission from Holst et al. [19].

therapeutic application of GLP-1 realized in 1993 with the observation that GLP-1 could normalize blood glucose levels in patients suffering from type 2 diabetes [17].

The GLP-1(7-37) isoforms, together with the GLP-1(7-36)amide, are the insulinotropic peptides derived from the preproglucagon gene, products of the post-translational processing of proglucagon (Figure 14.1).

The amino acid sequence of GLP-1 is highly conserved across animal species [20]. This shows not only how important this hormone is but also how vital the particular amino acid sequence is (Figure 14.2).

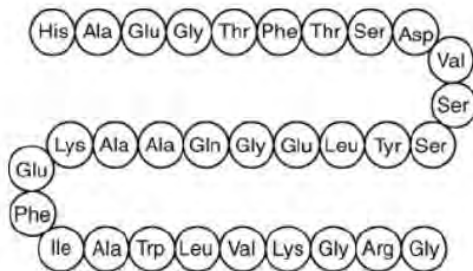


Figure 14.2 Amino acid sequence of native, truncated GLP-1(7-37).

The binding affinity and biological activity are particularly affected by His on position 7, Gly on position 10, Phe on position 12, Thr on position 13, Asp on position 15 (all directly involved in receptor interaction), Phe on position 28, and Ile on position 29 [21]. The conformation of GLP-1 includes an N-terminal random coil and two helical segments joined by a linker region; this closely resembles the structure of glucagon [22].

The preproglucagon gene is expressed in several cell types in the body. The pancreas contains α - and β -cells: α -cells process proglucagon and therefore secrete glucagon. Only small quantities of GLP-1 have been found secreted from pancreatic α -cells [23]. Proglucagon and its fragments are furthermore secreted in the small and large bowels. Intestinal L-cells that process proglucagon are the major source of GLP-1; these are mainly situated in the distal jejunum and ileum and also throughout the whole intestine. Proglucagon processing occurs in the central nervous system: GLP-1 is therefore an important neurotransmitter in the brain [24].

GLP-1 activity is mediated by the GLP-1 receptor, a class 2, G-protein-coupled receptor [25]. This receptor is found in many organs including the pancreas, stomach, intestines, and parts of the peripheral and central nervous systems, and these are the main therapeutic targets [26, 27]. GLP-1 receptors have been found in other tissues that may be relevant for its therapeutic effect, namely, the kidneys, endothelium, small blood vessels, and heart [28–31]. Due to the organ systems that GLP-1 acts on, it is an attractive therapeutic target for type 2 diabetes mellitus and obesity.

For example, in pancreatic β -cells, GLP-1 receptor activation increases adenylate cyclase activity that leads to glucose-dependent insulin secretion [14, 18]; in addition, GLP-1 exerts a glucose-dependent, suppressive effect on glucagon secretion [32]. Both effects act to lower blood glucose levels. Indeed, glucagon antagonism has long been suggested as a treatment for both type 1 and type 2 diabetes [33]. Together with glucose, GLP-1 acts on the β -cell to promote insulin gene transcription and therefore promotes insulin synthesis [34]; furthermore, GLP-1 can restore the β -cell's sensitivity to glucose, thereby improving β -cell function [35]. In addition, there are animal studies that support a "protective" β -cell effect of GLP-1, with apoptosis suppression, neogenesis and proliferation stimulation, and increases in β -cell mass [36, 37].

Furthermore, GLP-1 has advantageous gastrointestinal effects: gastric emptying is slowed following meal consumption [38] and it reduces gastric acid secretion [39], thus enabling food to be processed slowly. Studies have shown that GLP-1 infusion can reduce hunger sensations [40]. Consequently, a beneficial effect on body weight may be expected with any therapeutic application of GLP-1 [41].

An additional effect of GLP-1 that is of potential therapeutic interest is its actions on the cardiovascular system. Mostly based on animal data, it is thought that GLP-1 improves myocardial and endothelial function [42, 43] and GLP-1 may also directly protect the myocardium [29].

With these above effects, it is obvious to see why research in type 2 diabetes mellitus has focused on GLP-1 as an exciting therapeutic possibility (Figure 14.3).

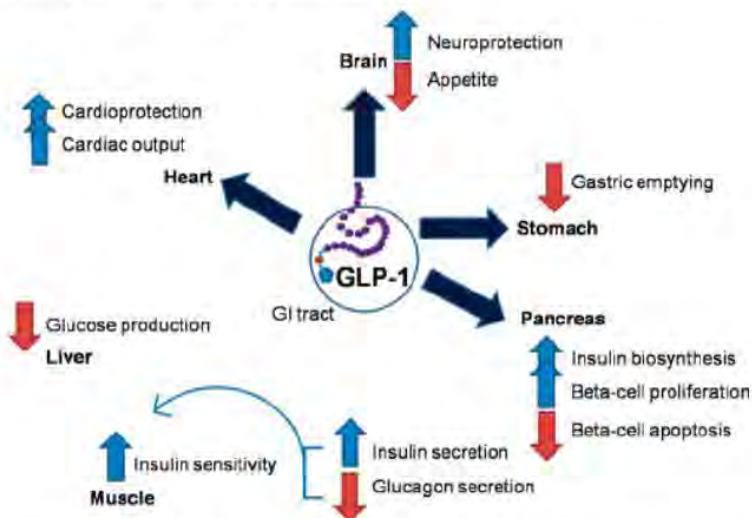


Figure 14.3 GLP-1 actions in peripheral tissues. GLP-1 acts directly on the endocrine pancreas, heart, stomach, and brain, whereas the actions on liver and muscle are indirect. Reproduced with permission from Drucker [44].

14.2

Discussion

14.2.1

Physiology of Native GLP-1

GLP-1 is secreted, primarily in response to food intake, throughout the distal small intestine and colon [45]. However, it is only oral glucose ingestion that stimulates the secretion of GLP-1 [46]; intravenous infusion of glucose in humans does not result in an increase in basal GLP-1 levels [46]. GLP-1 is secreted in a biphasic pattern: the first phase occurs within 15 min of eating, with peak GLP-1 levels seen around 90 min after eating in healthy individuals [47]. It is thought that the vagus nerve has a role to play in the first phase of GLP-1 secretion, as the majority of L-cells are located in the distal small intestine, secretion cannot be stimulated purely by the contact of food here [48]. The second, longer phase of GLP-1 secretion is most likely caused by food-stimulating intestinal L-cells [49].

GLP-1 is rapidly metabolized into GLP-1(9-37) and GLP-1(9-36)NH₂ by the enzyme dipeptidyl peptidase-4 (DPP-4) [50, 51] and has a half-life of approximately 2 min [50]. DPP-4 is an ubiquitous enzyme and is found circulating as well as in many organs including the kidneys, lungs, pancreas, and liver. Furthermore, it is expressed in endothelial cells that are located very close to intestinal L-cells, thereby inactivating almost half the GLP-1 that enters from the portal circulation before it enters the systemic circulation [52].

GLP-1 and its metabolites are mainly cleared via the kidney through glomerular filtration and renal catabolism [53]. This renal elimination does mean that in patients with renal failure or insufficiency, accumulation of GLP-1 levels can occur [54, 55].

Fasting plasma levels of GLP-1 appear to range between 5 and 10 pmol/l, and in healthy individuals increase to approximately 30 pmol/l after food intake [47]. The postprandial increase in GLP-1 levels depends on the size of the meal [56]. Some data suggest GLP-1 secretion in patients with type 2 diabetes is impaired compared to healthy individuals [47, 56]: healthy individuals have a significantly greater and more prolonged GLP-1 response, with levels peaking at around 90–120 min [47]. However, the effect is small and contradicted in other studies, so this probably does not have much significance. Much more important is that the other main incretin, GIP, only induces a very small release of insulin in patients with diabetes compared to healthy individuals in whom GLP-1 and GIP have more equal effects [57]. Thus, in patients there is a lack of insulin secretion from GIP that pharmacological levels of GLP-1 may restore.

14.2.2

Development of Liraglutide: A GLP-1 Analogue

Given the pharmacokinetic profile of endogenous GLP-1, it is clear that any GLP-1 therapy would need to approach the relevant drug discovery process in two ways: prolongation of action of pharmacologically administered GLP-1 or a GLP-1 analogue, either by administering a sustained release formulation or by making long-acting analogues. On top of the rapid degradation of GLP-1 by DPP-4 and swift renal clearance, several other hurdles had to be overcome in developing a viable GLP-1-based therapy. GLP-1 is a peptide and therefore cannot be administered orally; it has to be delivered by injection or through another nonoral route. GLP-1 also has a tendency to fibrillate, making it difficult to handle in solution. These problems have been documented [58].

Novo Nordisk began research for developing a GLP-1-based therapy in 1991. A study by Nauck and colleagues, published in 1993, demonstrated the viability of normalizing fasting plasma glucose concentrations with exogenous administration of GLP-1 [17] and thus underpinned the research efforts.

Novo Nordisk's first approach was to produce a natural GLP-1 drug either from GLP-1(7-36)amide or GLP-1(7-37). Research focused on a subcutaneous formulation, and as part of this effort a clinical study was conducted to compare GLP-1 infusion over 16 h with infusion over 24 h [59]. This study, which was not published until 2001, found that GLP-1 had to be continuously infused to obtain the most optimal glycemic control [59]; the authors concluded that, "...Because of the short plasma half-life of native GLP-1, long-acting derivatives should be developed to make GLP-1 treatment clinically relevant." Consequently, a sustained release formulation was entered into preclinical development in 1994. However, it was early in 1996 that this formulation was withdrawn due to the high incidence of injection site reactions (in pigs).

During this time, Novo Nordisk conducted structure–activity relationship studies on GLP-1, publishing a systematic survey of a series of GLP-1 analogues using an

alanine scan [21]. This study investigated the replacement of each amino acid of GLP-1 with L-alanine in order to identify the side chain functional groups involved in the interaction of the GLP-1 receptor. The analogues that were identified to have the lowest receptor binding affinity were studied further, with far-UV CD spectroscopy, to identify if conformational changes were induced at these positions. The study demonstrated that the amino acids at positions 7, 10, 12, 13, and 15 of the GLP-1 molecule were involved in receptor binding while those at positions 28 and 29 were involved in mediating the correct conformation for binding.

With the realization that GLP-1 was metabolically unstable, Novo Nordisk carried out work on DPP-4 stabilized simple amino acid analogues of GLP-1 throughout 1994/1995 [60]: four GLP-1 analogues with N-terminal substitutions of threonine (Thr), glycine (Gly), serine (Ser), or α -aminoisobutyric acid (Aib) were synthesized and tested for stability. All analogues bound to the cloned human pancreatic GLP-1 receptor but with differing affinities (as measured by IC_{50}): the Aib and Gly analogues bound with an affinity (0.45 ± 0.05 and 2.8 ± 0.42 nmol/l, respectively) in the same range as GLP-1(7-36)amide (0.78 ± 0.29 nmol/l), but the Thr and Ser analogues had lower binding affinities (49 ± 3.7 and 9.0 ± 1.9 , respectively, both $p < 0.001$ versus GLP-1(7-36)amide). Probably due to the presence of the polar hydroxyl group in the serine and threonine residues, binding was impaired.

Stability of these peptides was measured by the group of Jens Holst at the University of Copenhagen by incubating the analogues in porcine plasma at 37 °C. GLP-1(7-36)amide had a $t_{1/2}$ of 28.1 ± 1.2 min, but all analogues had a significantly prolonged $t_{1/2}$ in comparison: Gly, 159 ± 12 min; Ser, 174 ± 12 min; Thr, 197 ± 14 min, all $p < 0.0001$. Pharmacokinetic parameters of these peptides were tested *in vivo*: i.v. infusion of the four analogues in anesthetized pigs resulted in N-terminal plasma $t_{1/2}$ values of between 3.3 and 3.9 min and C-terminal plasma $t_{1/2}$ values of between 3.5 and 4.4 min (Table 14.1), values that were significantly greater than for GLP-1(7-36)amide [60].

The ability of these analogues to stimulate insulin secretion and/or inhibit glucagon secretion was tested in an isolated perfused pancreas. The Aib analogue was at least as potent as GLP-1(7-36)amide in stimulating insulin and inhibiting glucagon secretion, and was also the most potent ($p < 0.05$) of all the analogues in reducing glucagon output. The Gly analogue was not significantly different from GLP-1(7-36)amide in stimulating insulin or inhibiting glucagon secretion, but it was more potent ($p < 0.05$) than the Ser and Thr analogues in inhibiting glucagon release. This study demonstrated that small alterations in the N-terminus of GLP-1 conferred resistance to the action of DPP-4, but with retained biological activity and an improved metabolic stability [60]. However, the $t_{1/2}$ of these analogues in pigs was approximately 4 min, still making them unsuitable drug candidates. Consequently, further research into extending the duration of action of the analogues was required.

Alongside the successful development at Novo Nordisk of a prolonged acting insulin analogue, modified with the addition of a fatty acid side chain, research for a viable GLP-1 therapy turned toward acylated analogues. The attachment of a fatty acid side chain to this subcutaneously administered insulin analogue was shown to significantly protract the action of insulin, by facilitating serum albumin binding [61–63].

Table 14.1 *In vivo* plasma $t_{1/2}$ for GLP-1(7-36)amide and N-terminally modified analogues calculated using N- and C-terminally directed RIAs.

Group	Analogue $t_{1/2}$ (min)		GLP-1(7-36)amide $t_{1/2}$ (min)	
	N-terminal	C-terminal	N-terminal	C-terminal
Thr ⁸ -GLP-1(7-37)	3.9 ± 0.2 ^{a),b)}	4.2 ± 0.4 ^{c)}	0.7 ± 0.05 ^{d)}	4.0 ± 0.1
Gly ⁸ -GLP-1(7-37)	3.3 ± 0.4 ^{a),b)}	3.5 ± 0.7 ^{c)}	0.9 ± 0.03 ^{d)}	4.3 ± 0.5
Ser ⁸ -GLP-1(7-36)amide	3.7 ± 0.4 ^{a),b)}	4.2 ± 0.2 ^{c)}	0.9 ± 0.06 ^{d)}	4.1 ± 0.2
Aib ⁸ -GLP-1(7-36)amide	NDI	4.4 ± 0.2 ^{c)}	1.1 ± 0.07 ^{d)}	4.3 ± 0.3

Values are mean ± SEM; $n = 4$. NDI: not determined due to lack of cross-reactivity. Reproduced with permission from Deacon *et al.* [60].

a) NS, $p > 0.05$ versus C-terminal $t_{1/2}$ for analogue.

b) $p < 0.01$ versus N-terminal $t_{1/2}$ for GLP-1(7-36)amide.

c) NS, $p > 0.05$ versus C-terminal $t_{1/2}$ for GLP-1(7-36)amide.

d) $p < 0.01$ versus C-terminal $t_{1/2}$ for GLP-1(7-36)amide.

Consequently, a series of potent fatty acid derivatives of GLP-1 were investigated for their structure–activity relationships and pharmacokinetic properties [64].

Fatty acids or fatty diacids were optionally extended with a “spacer” between the ϵ -amino group of the lysine side chain and the carboxyl group of the fatty acid. Acylation with simple fatty acids increased the net negative charge of the resulting molecule by 1, whereas peptides acylated with a γ -glutamoyl spacer or with diacids provided a net increase of the negative charge by 2. This was expected to increase the albumin binding affinity and to provide a higher solubility of the compound at physiological pH. The compounds investigated in this study and their potency for the cloned human GLP-1 receptor expressed in BHK (baby hamster kidney) cells are shown in Table 14.2. All compounds acylated with a fatty acid equal to or longer than 12 carbon atoms had a considerably longer half-life than GLP-1 (after s.c. administration, $t_{1/2}$ was 1.2 h) [64]. The $t_{1/2}$ values of some of the most potent analogues are described in Table 14.3.

This study demonstrated that long fatty acids could be attached to a variety of positions along the analogue at the C-terminal without loss of potency. It was interesting to observe that attaching a fatty acid to position 8 (at the N-terminal) resulted in a loss of potency, supporting the earlier research that amino acids at positions 7, 10, 12, 13, and 15 of the GLP-1 molecule are involved in receptor binding, while those at positions 28 and 29 are involved in the conformational binding of the molecule [21]. Altogether, this study identified several potent compounds with plasma half-lives of over 10 h that made them suitable for once-daily administration. From these compounds, liraglutide was selected for clinical development.

Another study was undertaken to look at the structure–activity relationships in a series of compounds based around liraglutide. In all compounds, acylation on position 26 was explored, using the GLP-1 analogue R34-GLP-1(7-37) [65]. The amino acid sequences of these compounds were very similar to that of native GLP-1, with only one substitution of Lys³⁴ to Arg. This substitution was chosen with the

Table 14.2 Potencies of the compounds tested for the cloned human GLP-1 receptor expressed in BHK cells.

Parent peptide	Acyl site	Acyl substituent	Potency (EC ₅₀ , pM)
GLP-1(7-37)		None	55 ± 19
K ⁸ R ^{26,34} -GLP-1(7-37)	K ⁸	γ-Glu-C16	1260 ± 210
K ¹⁸ R ^{26,34} -GLP-1(7-37)	K ¹⁸	γ-Glu-C16	35.2 ± 6.2
K ²³ R ^{26,34} -GLP-1(7-37)	K ²³	γ-Glu-C16	30.1 ± 3.3
R ³⁴ -GLP-1(7-37) (liraglutide)	K ²⁶	γ-Glu-C16	61.0 ± 7.1
K ²⁷ R ^{26,34} -GLP-1(7-37)	K ²⁷	γ-Glu-C16	36.3 ± 0.3
R ²⁶ -GLP-1(7-37)	K ³⁴	γ-Glu-C16	121 ± 26
K ³⁶ R ^{26,34} -GLP-1(7-36)	K ³⁶	γ-Glu-C16	36.4 ± 2.1
R ^{26,34} -GLP-1(7-38)	K ³⁸	γ-Glu-C16	53.0 ± 2.8
GLP-1(7-37)	K ^{26,34}	bis-C16-diacid	7000 ± 7
GLP-1(7-37)	K ^{26,34}	bis-γ-Glu-C16	16 700 ± 3 700
GLP-1(7-37)	K ^{26,34}	bis-γ-Glu-C14	3050 ± 350
GLP-1(7-37)	K ^{26,34}	bis-C12-diacid	177 ± 52
R ³⁴ -GLP-1(7-37)	K ²⁶	C16-diacid	154 ± 66
R ³⁴ -GLP-1(7-37)	K ²⁶	C14-diacid	72 ± 0.7
R ³⁴ -GLP-1(7-37)	K ²⁶	γ-Glu-C18	194 ± 24
R ³⁴ -GLP-1(7-37)	K ²⁶	γ-Glu-C14	22.0 ± 7.1
R ³⁴ -GLP-1(7-37)	K ²⁶	γ-Glu-C12	27.3 ± 8.4
Desamino-H ⁷ R ³⁴ -GLP-1(7-37)	K ²⁶	γ-Glu-C16	687 ± 129
R ³⁴ -GLP-1(7-37)	K ²⁶	GABA-C16	84.4 ± 22.1
R ³⁴ -GLP-1(7-37)	K ²⁶	β-Ala-C16	113 ± 3
R ³⁴ -GLP-1(7-37)	K ²⁶	Iso-Nip-C16	410 ± 120
Desamino-H ⁷ R ²⁶ -GLP-1(7-37)	K ³⁴	γ-Glu-C16	2360 ± 370
Desamino-H ⁷ R ²⁶ -GLP-1(7-37)	K ³⁴	C8	236 ± 66
Desamino-H ⁷ R ²⁶ -GLP-1(7-37)	K ³⁴	γ-Glu-C8	169 ± 1
K ³⁶ R ^{26,34} -GLP-1(7-36)	K ³⁶	C20-diacid	210 ± 14
K ³⁶ R ^{26,34} -GLP-1(7-36)	K ³⁶	C16-diacid	7.89 ± 1.21
K ³⁶ R ^{26,34} -GLP-1(7-36)	K ³⁶	γ-Glu-C18	116 ± 3
R ^{26,34} -GLP-1(7-38)	K ³⁸	C16-diacid	5.6 ± 3.5
R ^{26,34} -GLP-1(7-38)	K ³⁸	C12-diacid	4.19 ± 0.98
R ^{26,34} -GLP-1(7-38)	K ³⁸	γ-Glu-C18	115 ± 21
R ^{26,34} -GLP-1(7-38)	K ³⁸	γ-Glu-C14	54 ± 1
G ⁸ R ^{26,34} -GLP-1(7-38)	K ³⁸	γ-Glu-C16	328 ± 14
E ³⁷ R ^{26,34} -GLP-1(7-38)	K ³⁸	γ-Glu-C16	27.2 ± 0.1
E ³⁷ G ⁸ R ^{26,34} -GLP-1(7-38)	K ³⁸	γ-Glu-C16	135 ± 7
E ³⁷ G ⁸ R ^{26,34} -GLP-1(7-38)	K ³⁸	γ-Glu-C18	213 ± 30

K: lysine; R: arginine; E: glutamic acid; G: glycine.

Abbreviations used for acyl groups in lysine N^ε-acylated peptides: γ-Glu-C8 = γ-L-glutamoyl(N^ε-octanoyl); γ-Glu-C14 = γ-L-glutamoyl(N^ε-tetradecanoyl); γ-Glu-C16 = γ-L-glutamoyl(N^ε-hexadecanoyl); γ-Glu-C18 = γ-L-glutamoyl(N^ε-octadecanoyl); C8 = octanoyl; C12-diacid = ω-carboxyundecanoyl; C16-diacid = ω-carboxypentadecanoyl; C20-diacid = ω-carboxytridecanoyl; GABA-C16 = γ-aminobutyryl(N^ε-hexadecanoyl); iso-Nip-C16 = 1-(hexadecanoyl)piperidyl-4-carboxy. Data are given as mean ± SD of two individual experiments with triplicate samples. Reproduced with permission from Knudsen *et al.* [64].

Table 14.3 Plasma half-lives in pigs of GLP-1 and selected potent acylated compounds.

Compound	Plasma $t_{1/2}$ (h) mean $\pm$ SD
GLP-1(7-37)	1.2
K ²³ R ^{26,34} -GLP-1(7-37) (acyl substituent: γ -Glu-C16)	20 $\pm$ 2
R ³⁴ -GLP-1(7-37) (acyl substituent: γ -Glu-C16) (liraglutide)	14 $\pm$ 2
R ²⁶ -GLP-1(7-37) (acyl substituent: γ -Glu-C16)	13
K ³⁶ R ^{26,34} -GLP-1(7-36) (acyl substituent: γ -Glu-C16)	12 $\pm$ 1
R ³⁴ -GLP-1(7-37) (acyl substituent: γ -Glu-C12)	15 $\pm$ 3
R ³⁴ -GLP-1(7-37) (acyl substituent: GABA-C16)	31 $\pm$ 4
R ³⁴ -GLP-1(7-37) (acyl substituent: β -Ala-C16)	8.8 $\pm$ 1
K ³⁶ R ^{26,34} -GLP-1(7-36) (acyl substituent: C16-diacid)	13 $\pm$ 4
E ³⁷ G ⁸ R ^{26,34} -GLP-1(7-38) (acyl substituent: γ -Glu-C16)	11 $\pm$ 1

Reproduced with permission from Knudsen *et al.* [64].

original selection of R34-GLP-1(7-37) (acyl substituent: γ -Glu-C16) to allow mono-acylation. The compounds, potencies, and $t_{1/2}$ values from this study are presented in Table 14.4.

It appears that the chain length of the fatty acid affects the potency for the cloned receptor only if the length is longer than 16 carbon atoms: compound 6 had a potency of 170 pM compared to that of 39, 66, 29, 21, and 61 pM, respectively, for compounds 1–5. However, the length of the fatty acid does appear to be closely related to protraction *in vivo*. Compound 1 with a C10 fatty acid has a half-life of 0.8 h, compound 2 with a C11 fatty acid has a half-life of 5.1 h, compound 5 with a C16 fatty acid has a half-life of 16 h, and compound 6 with a C18 fatty acid has a half-life of 21 h. Figure 14.4 shows the pharmacokinetic profiles of compounds 2, 3, 5, and 6 in pigs. The same relationship between fatty acid chain length and protraction *in vivo* was seen in compounds 7–10 (these are similar to compounds 1–6, but the spacer is different: γ -aminobutyric acid) and compounds 11–13 (β -alanine as the spacer). For the same length fatty acid, the γ -Glu spacer resulted in the longest half-life *in vivo* (compound 2 = 5.1 h, compared to compound 8 = 1.7 h, and compound 11 = 1.2 h).

This study demonstrated that there was a straightforward relationship between structure and potency, with only very long fatty acids decreasing potency slightly, whereas protraction correlated directly with the length of the fatty acid chain within the same series. The spacer component was important for potency but did not affect protraction of the pharmacokinetic profile in pigs. In contrast, introducing hydrophilicity at the omega terminal of the fatty acid moiety did not affect protraction but tended to decrease potency.

From these compounds, R34-GLP-1(7-37) (acyl substituent: γ -Glu-C16), or liraglutide, was first synthesized in February 1997 and entered preclinical development in August the same year.

Alongside the development of liraglutide, it should be mentioned that there are other incretin-based therapies either already available or close to market. Another candidate GLP-1 analogue has been developed separately liraglutide: exenatide. It is a synthetic version of a component of the saliva of the gila monster lizard

Table 14.4 Compounds, their potency on human cloned GLP-1 receptor, and *in vivo* protraction in pigs.

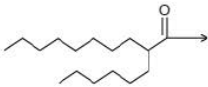
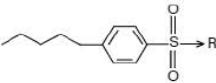
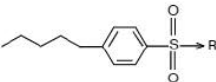
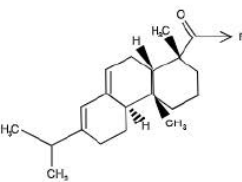
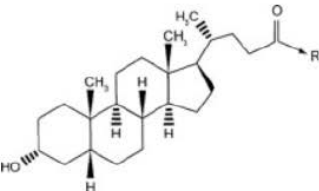
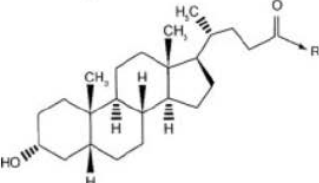
	Compound Acyl group	Spacer	Potency (pmol/l)	Half-life (h)
GLP-1	—	—	55 ± 19	1.2
1	-CO-(CH ₂) ₈ CH ₃	γ-Glu	39 ± 17	0.8
2	-CO-(CH ₂) ₉ CH ₃	γ-Glu	66 ± 23	5.1
3	-CO-(CH ₂) ₁₀ CH ₃	γ-Glu	29 ± 7	7.6
4	-CO-(CH ₂) ₁₂ CH ₃	γ-Glu	27 ± 2	9.0
5	-CO-(CH ₂) ₁₄ CH ₃ (liraglutide)	γ-Glu	61 ± 7	16
6	-CO-(CH ₂) ₁₆ CH ₃	γ-Glu	170 ± 40	21
7	-CO-(CH ₂) ₈ CH ₃	Gaba	28 ± 5	1.6
8	-CO-(CH ₂) ₉ CH ₃	Gaba	8.6 ± 3.6	1.7
9	-CO-(CH ₂) ₁₀ CH ₃	Gaba	19 ± 1	2.4
10	-CO-(CH ₂) ₁₂ CH ₃	Gaba	35 ± 11	4.6
11	-CO-(CH ₂) ₈ CH ₃	β-Ala	37 ± 6	1.2
12	-CO-(CH ₂) ₁₀ CH ₃	β-Ala	27 ± 3	2.8
13	-CO-(CH ₂) ₁₂ CH ₃	β-Ala	55 ± 15	6.5
14	-CO-(CH ₂) ₁₄ CH ₃	D-γ-Glu	74 ± 32	22
15	-CO-(CH ₂) ₁₄ CH ₃	α-Glu	76 ± 1	12
16	-CO-(CH ₂) ₁₄ CH ₃	—	4440 ± 440	16
17	-CO-(CH ₂) ₁₄ CH ₃	Triethylenglycol	1570 ± 60	13
18	-SO ₂ -(CH ₂) ₁₁ CH ₃	—	2110 ± 210	15
19	-SO ₂ -(CH ₂) ₁₁ CH ₃	β-Ala	350 ± 20	9.4
20	-CO-(CH ₂) ₇ CH=CH ₂	γ-Glu	36 ± 0.4	1.9
21	-CO-(CH ₂) ₁₀ NHCO-CH ₃	γ-Glu	160 ± 30	2.0
22	-CO-(CH ₂) ₁₀ NH ₂	γ-Glu	140 ± 30	1.1
23	-CO-(CH ₂) ₁₁ NH ₂	β-Ala	110 ± 20	2.0
24	-CO-(CH ₂) ₁₁ OH	γ-Glu	45 ± 8	2.6
25	-CO-(CH ₂) ₁₅ OH	β-Ala	65 ± 7	4.6
26	-CO-(CH ₂) ₁₀ SO ₃ H	β-Ala	110 ± 50	2.5
27	-CO-CH ₂ O-(CH ₂) ₈ CH ₃	γ-Glu	81 ± 19	1.7
28	-CO-(CH ₂) ₂ O-(CH ₂) ₉ CH ₃	γ-Glu	70 ± 20	3.1
29	-CO-CH ₂ O-(CH ₂) ₂ O-(CH ₂) ₂ O-(CH ₂) ₂ CH ₃	γ-Glu	320 ± 150	0.2
30	-CO-(CH ₂) ₈ (OH)CH ₂ (OH)(CH ₂) ₆ OH	γ-Glu	210 ± 20	2.3
31	-CO-(CH ₂) ₆ CH ₃	8-Aminooctanoyl	48 ± 4	2.5
32	-CO-(CH ₂) ₆ CH ₃	9-Aminononanoyl	64 ± 12	4.5
33	-CO-(CH ₂) ₆ CH ₃	10-Aminodecanoyl	39 ± 12	4.3
34	-CO-(CH ₂) ₄ NHCO-(CH ₂) ₄ CH ₃	5-Aminopentanoyl	74 ± 35	2.0
35	-CO-(CH ₂) ₁₀ NHCO-(CH ₂) ₂ CH ₃	β-Ala	48 ± 2	3.4
36		γ-Glu	400 ± 100	18
37	-CO-(CH ₂) ₉ phenyl	γ-Glu	31 ± 14	2.4
38	-CO-(CH ₂) ₄ cyclohexyl	γ-Glu	73 ± 1	2.0

Table 14.4 (Continued)

Compound	Acyl group	Spacer	Potency (pmol/l)	Half-life (h)
39		γ -Glu	26 ± 2	3.0
40		—	380	14
41		γ -Glu	260 ± 80	6.0
42		γ -Glu	18 ± 10	6.8
43		β -Ala	360 ± 150	8.8

The receptor potency data are given as mean $\pm$ SD of two or three individual experiments with triplicate samples. Protraction is expressed as the terminal half-life after subcutaneous administration. Reproduced with permission from Madsen *et al.* [65].

(*Heloderma suspectum*) and has been approved by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA). Exenatide is a 39-amino acid peptide amide. The amino acid sequence of exenatide is H-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH₂. It was originally identified as exendin-4 from gila monster venom, sharing 53% sequence homology with mammalian GLP-1; it acts as a high-potency agonist on the GLP-1 receptor [66].

In addition, there are DPP-4 inhibitors: sitagliptin and vildagliptin prevent the degradation of GLP-1, thereby increasing plasma levels of endogenous GLP-1 [67].

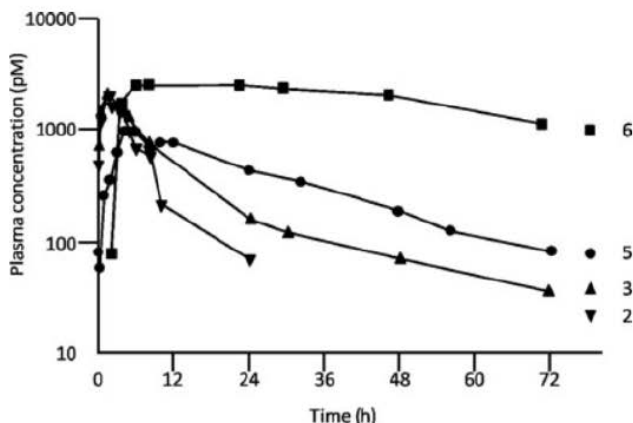


Figure 14.4 Pharmacokinetic profiles of compounds 2 ($-\text{CO}-(\text{CH}_2)_8\text{CH}_3$ [acyl spacer γ -Glu]), 3 ($-\text{CO}-(\text{CH}_2)_{10}\text{CH}_3$ [acyl spacer γ -Glu]), 5 ($-\text{CO}-(\text{CH}_2)_{14}\text{CH}_3$ (liraglutide) [acyl spacer γ -Glu]), and 6 ($-\text{CO}-(\text{CH}_2)_{16}\text{CH}_3$ [acyl spacer γ -Glu]) in pigs. Reproduced with permission from Madsen *et al.* [65].

Sitagliptin has been approved by FDA and EMEA, and vildagliptin has been approved by EMEA. The main difference between GLP-1 analogues and DPP-4 inhibitors is that the analogues have to be injected whereas the inhibitors are orally available. However, the GLP-1 analogues are also more efficacious, especially regarding body weight reduction.

14.2.3

The Pharmacology of Liraglutide

As outlined above, liraglutide has a molecular structure that is very similar to human GLP-1, with only two structural modifications: substitution of Lys³⁴ to Arg and the attachment of a 16C fatty acid side chain to Lys²⁶ via a glutamic acid linker (Figure 14.5). As a result, the amino acid sequence of liraglutide has 97% homology with native human GLP-1 [64].

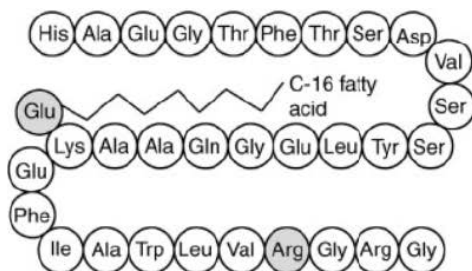


Figure 14.5 Amino acid structure of liraglutide.

Acylation of a molecule with a fatty acid has been shown to prolong the duration of action of a basal insulin analogue, insulin detemir, by causing reversible albumin binding, as well as by the molecule forming a hexamer [68]. The protraction mechanism for liraglutide is a result of albumin binding and self-association into heptamers. Liraglutide binds to albumin [69], resulting in a long plasma half-life, also following i.v. administration. The hydrophobic palmitate residues interact to form the core of the seven-molecule complex, resulting in slow dissociation and the inability to pass easily through capillary membranes following subcutaneous injection [70]. Furthermore, it is probable that some of the protraction in absorption of liraglutide is caused by reduced susceptibility to DPP-4 degradation.

The *in vitro* potency of liraglutide was confirmed in a study using plasma membranes from a cell line expressing the cloned human GLP-1 receptor: liraglutide was a full and potent agonist with an EC_{50} of 61 ± 7 pM [64]. Liraglutide is a selective agonist and has shown no cross-reactivity to 114 different receptors and ion channels, including the closely related glucagon receptor. In perfused mouse islets, liraglutide stimulated insulin secretion in a glucose-dependent and dose-dependent fashion with potency comparable to that of native GLP-1. Other *in vitro* testing of liraglutide has found that it not only prevents cytokine and free fatty acid-induced apoptosis in isolated rat pancreatic islets but also stimulates primary rat β -cell proliferation [37, 71].

The *in vivo* effects of liraglutide were explored in a series of studies investigating subchronic dosing regimens in different animal models of diabetes and obesity [72–76]. In diabetic ob/ob and db/db mice, liraglutide lowered blood glucose dose-dependently compared to a vehicle group [72]. In addition, an increase in pancreatic β -cell mass was seen [72]. Exendin-4 (exenatide) was also tested in this study: like liraglutide, exendin-4 decreased blood glucose levels compared to vehicle, although liraglutide displayed a longer duration of action. Furthermore, exendin-4 significantly increased the β -cell proliferation rate in the db/db mice; however, a statistically significant effect on β -cell mass was not seen in this study.

Increased β -cell mass and insulin release were seen following liraglutide administration in Zucker diabetic fatty rats [73]. Blood glucose levels were approximately 12 mM lower compared to vehicle animals ($p < 0.0002$) and plasma insulin levels were up to three times higher over 24 h ($p < 0.001$). This study showed that part of the blood glucose lowering effect was mediated by a reduction in food intake.

Liraglutide was studied extensively in pigs as a model in which pharmacokinetic parameters usually correlate well with humans. One month of treatment with once-daily liraglutide in streptozotocin-induced β -cell-reduced diabetic Goettingen minipigs led to a significant lowering of blood glucose. Hyperglycemic clamp studies showed increased glucose utilization and insulin release and decreased plasma glucagon levels following $2 \mu\text{g/kg}$ i.v. administration of liraglutide [74]. Both the insulin release and the glucagon-lowering effect were glucose-dependent.

Liraglutide lowered body weight in Sprague Dawley rats given access to candy and normal rat fodder [75]. Candy and fodder feeding increased body weight, fat mass, and feeding-associated energy expenditure. Twelve weeks of liraglutide treatment reduced body weight to the level of normal-weight control rats. The weight lost was fat mass. The DPP-4 inhibitor, vildagliptin, was used as an active comparator, but this

drug had no effect on body weight, food intake, or energy expenditure. Despite weight loss, liraglutide-treated rats did not experience a decrease in energy expenditure compared to candy-fed controls. Most interestingly, liraglutide led to an altered food preference. The animals treated with liraglutide selectively had a lower candy intake and a higher chow intake.

Obese pigs are a good model of human obesity as pigs have eating patterns like humans. They eat meals during daylight and sleep during darkness. Liraglutide markedly reduced food intake from 18.4 ± 0.6 to 7.3 ± 0.3 MJ/day in obese Goettingen minipigs [76]. Body weight (mean loss of -4.3 ± 1.2 kg, 4–5% after 7 weeks of treatment) was reduced following liraglutide treatment.

The newest studies support the role of liraglutide in cardiovascular protection. A study in mice showed liraglutide-treated animals had better survival rates after an induced myocardial infarct [77]. Another study using a human endothelial cell-line showed liraglutide reduced both cytokine and glucose-induced inflammatory responses [78].

Clinical pharmacology studies have supported and developed further the preclinical results. After subcutaneous injection, liraglutide is absorbed slowly into the human circulation: $T_{\max}$ occurs between 9 and 12 h following dosing and the mean elimination half-life is between 11 and 15 h [79]. There appears to be no single organ responsible for primary degradation or clearance of liraglutide. Liraglutide can be degraded *in vitro* by endogenous peptidases, including DPP-4 [80]. However, in the body this does not appear to be an important pathway for liraglutide clearance, in contrast to GLP-1 degradation. Consequently, liraglutide has no renal or hepatic metabolism or excretion. This does suggest that in patients with renal or hepatic impairment there will be a low risk of accumulation and/or drug interactions via that route with liraglutide [81, 82].

Of the other GLP-1 analogues, exenatide is dosed twice daily; it reaches peak plasma concentrations in approximately 2 h and has a terminal half-life of approximately 2.5 h in human subjects [83]. Nonclinical studies have shown that exenatide is predominantly eliminated by glomerular filtration, with subsequent proteolytic degradation [84–86].

The pharmacokinetic profile of liraglutide, measured in a randomized, double-blind study of 64 healthy men, demonstrated detection in plasma over 24 h, therefore suggesting suitability for once-daily dosing [79]. Steady state was reached after 3 days of once-daily dosing of liraglutide (1.25 – 12.5 $\mu\text{g/kg}$) in a study of 30 healthy volunteers [87]. A placebo-controlled, crossover study in 13 patients with type 2 diabetes confirmed the once-daily dosing of liraglutide with decreased plasma glucose concentrations over 24 h following subcutaneous administration [88].

A 14-week double-blind, placebo-controlled study in 165 patients with type 2 diabetes has confirmed the blood glucose-lowering efficacy of liraglutide (administered once daily at 0.65, 1.25, or 1.9 mg as monotherapy) [89]. Significant reductions in HbA_{1c} of -0.98 , -1.40 , and -1.45% from baseline were seen in the three dose groups, respectively, whereas HbA_{1c} increased by $+0.29\%$ with placebo ($p < 0.0001$ against all liraglutide treatment groups). Other measurements of glycemic control were significantly reduced with liraglutide treatment compared to placebo. In all

treatment groups, body weight decreased, with the greatest loss (–3 kg) seen in the 1.9 mg dose group (difference of –1.21 kg versus placebo, $p = 0.039$). Liraglutide was well tolerated, with no reported hypoglycemic episodes. Data on cardiovascular outcomes and β -cell function from the same study have also been reported [90, 91]. Liraglutide treatment improved certain biomarkers associated with increased cardiovascular risk [90]; there were also improvements in first- and second-phase insulin secretion, following liraglutide administration, together with improvements in arginine-stimulated insulin secretion during hyperglycemia [91].

14.2.4

Clinical Evidence with Liraglutide

Following the success of liraglutide in phase II clinical testing, a series of phase III placebo-controlled clinical trials (the LEAD, Liraglutide Effect and Action in Diabetes, program) were conducted to investigate the efficacy and safety of liraglutide administered as monotherapy or in combination with other oral antidiabetic drugs. Ranging in duration from 26 to 52 weeks, six trials examined liraglutide dosed at 1.2 or 1.8 mg daily to patients with type 2 diabetes as follows: LEAD 1, in combination with sulfonylurea versus thiazolidinedione plus sulfonylurea; LEAD 2, in combination with metformin versus sulfonylurea plus metformin; LEAD 3, monotherapy versus sulfonylurea; LEAD 4, in combination with metformin and thiazolidinedione versus metformin plus thiazolidinedione; LEAD 5, in combination with metformin and sulfonylurea versus insulin glargine plus metformin and sulfonylurea; and LEAD 6, in combination with metformin and/or sulfonylurea versus exenatide with metformin and/or sulfonylurea [92–97].

In this series of clinical trials, HbA_{1c} reductions of up to 1.6% were seen with liraglutide treatment, significantly greater than the reductions seen in the comparison groups. For example, HbA_{1c} was reduced by –1.6% from baseline when used as monotherapy in patients previously treated only with diet and exercise and no antidiabetic medication. HbA_{1c} in these patients was reduced to below 7.0% and sustained at this level for the 52-week duration of the study. This was a significantly greater reduction than achieved with sulfonylurea monotherapy (–0.88%, $p < 0.0001$) [94]. Similar improvements in other parameters of glycemic control such as fasting plasma glucose and postprandial plasma glucose were also seen across studies.

In these studies, major hypoglycemia was a rare occurrence with liraglutide treatment (6 from 2505 patients experienced a major episode, with only 1 patient requiring medical assistance) as a result of its glucose-dependent mechanism of action [92, 96]. The incidence of minor hypoglycemic events was also low (0.03–1.93 events per patient per year) [92, 94, 95, 97].

The beneficial effect on body weight seen in the animal studies has been realized in the clinical trials: as monotherapy, liraglutide treatment has resulted in a weight loss of around –2.5 kg. In the same study, patients treated with glimepiride gained +1.12 kg, leading to a difference in weight between liraglutide and glimepiride treatment of –3.6 kg ($p < 0.0001$) [94]. Greater body weight reductions of almost –3 kg have been seen when liraglutide was administered in combination with

metformin [93]. Nausea and other similar side effects have been reported with liraglutide, presumably related to its delay in gastric emptying: transient nausea has been experienced by 10.5–40% of patients [92–97]. Nausea, however, disappears over 4 weeks in most patients [94] and is considered mild with a Visual Analogue Score of 1.5 on a scale of 1 to 7 [98].

Advantageous effects on β -cell function and reductions in systolic blood pressure (of up to -6.7 mmHg) have been noted in patients treated with liraglutide in these trials [97, 99].

Liraglutide (1.8 mg/day dosed once daily) has been compared with exenatide (2×10 μ g dosed twice daily) in a clinical trial over 26 weeks in 464 patients with type 2 diabetes [97]. Both GLP-1 agonists were administered with metformin and/or sulfonylureas. HbA_{1c} reductions were significantly greater with liraglutide than with exenatide (-1.12% versus -0.79% , $p < 0.0001$), and both treatments reduced body weight in patients from baseline (-3.24 kg with liraglutide in comparison to -2.87 kg with exenatide). With liraglutide, the percentage of patients reporting nausea was 10% after 5 weeks, 4% after 10 weeks, and 2.5% after 26 weeks, whereas the frequency with exenatide was 18, 13, and 8.6%, respectively.

An additional clinical benefit of liraglutide over exenatide is related to their amino acid sequences: liraglutide has 97% homology with human GLP-1 whereas exenatide has only 53% homology. Rates of antibody formation against liraglutide of between 0 and 12.7% have been reported in the clinical trials described above [92, 93, 96], with no apparent effect on glycemic control. However, antibody formation against exenatide appears to be higher with approximately 50% of patients developing antibodies in clinical trials [100–102]. In a subset of patients on exenatide (3%), the antibodies demonstrated neutralizing effects and there was no blood glucose lowering [103]. For liraglutide, there has been no association between antibodies and clinical response.

14.3

Summary

It has taken over 100 years since the initial discovery of “secretin” for the therapeutic potential of GLP-1-based treatments to be realized. It was in the 1980s that research into the preproglucagon gene resulted in the discovery of GLP-1(7-37), the insulinotropic form of GLP-1. Studies looking into the effects of GLP-1 infusion in patients with diabetes demonstrated the therapeutic promise of such a related treatment, and research into developing GLP-1 analogues with suitable binding affinities and biological activities began. One of the major obstacles that had to be overcome was rapid degradation of native GLP-1 by DPP-4 enzymes in the circulation. Novo Nordisk undertook research into acylated GLP-1 analogues. From the resulting compounds that had increased self-association, albumin binding, and resistance to DPP-4 degradation, liraglutide, a once-daily human GLP-1 analogue, was developed. Preclinical pharmacology investigations demonstrated that liraglutide treatment has many of the beneficial effects of human GLP-1: improved glycemic control, reduced

body weight, and improved pancreatic β -cell function, the characteristics that made GLP-1 such an attractive target for the treatment of type 2 diabetes and obesity in the first place. Clinical trials on healthy volunteers and patients with diabetes have now confirmed these interesting results with liraglutide treatment. Furthermore, reduced systolic blood pressure has consistently been seen for liraglutide.

References

- 1 Bayliss, W.M. and Starling, E.H. (1902) Mechanism of pancreatic secretion. *J. Physiol.*, **28**, 235–334.
- 2 Zunz, E. and LaBarre, J. (1929) Contributions a l'etude des variations physiologiques de la secretion interne du pancreas: relations entre les secretions externe et interne du pancreas. *Arch. Int. Physiol. Biochim.*, **31**, 20–44.
- 3 LaBarre, J. and Still, E.U. (1930) Studies on the physiology of secretin. *Am. J. Physiol.*, **91**, 649–653.
- 4 Loew, E.R., Gray, J.S., and Ivy, A.C. (1939) The effect of duodenal instillation of hydrochloric acid upon the fasting blood sugar of dogs. *Am. J. Physiol.*, **126**, 270–276.
- 5 Loew, E.R., Gray, J.S., and Ivy, A.C. (1940) Is a duodenal hormone involved in carbohydrate metabolism? *Am. J. Physiol.*, **129**, 659–663.
- 6 Loew, E.R., Gray, J.S., and Ivy, A.C. (1940) The effect of acid stimulation of the duodenum upon experimental hyperglycemia and utilization of glucose. *Am. J. Physiol.*, **128**, 298–308.
- 7 McIntyre, N., Holdsworth, C.D., and Turner, D.A. (1964) New interpretation of oral glucose tolerance. *Lancet*, **2** (7349), 20–21.
- 8 Unger, R.H. and Eisentraut, A.M. (1969) Entero-insular axis. *Arch. Intern. Med.*, **123**, 261–266.
- 9 Creutzfeldt, W. (1979) The incretin concept today. *Diabetologia*, **16**, 75–85.
- 10 Lund, P.K., Goodman, R.H., and Habener, J.F. (1981) Intestinal glucagon mRNA identified by hybridization to a cloned islet cDNA encoding a precursor. *Biochem. Biophys. Res. Commun.*, **100**, 1659–1666.
- 11 Lund, P.K., Goodman, R.H., Dee, P.C., and Habener, J.F. (1982) Pancreatic preproglucagon cDNA contains two glucagon-related coding sequences arranged in tandem. *Proc. Natl. Acad. Sci. USA*, **79**, 345–349.
- 12 Lund, P.K., Goodman, R.H., Montminy, M.R., Dee, P.C., and Habener, J.F. (1983) Angler fish islet pre-proglucagon II. Nucleotide and corresponding amino acid sequence of the cDNA. *J. Biol. Chem.*, **258**, 3280–3284.
- 13 Bell, G.I., Santerre, R.F., and Mullenbach, G.T. (1983) Hamster proglucagon contains the sequence of glucagon and two related peptides. *Nature*, **302**, 716–718.
- 14 Mojsov, S., Weir, G.C., and Habener, J.F. (1987) Insulinotropin: glucagon-like peptide-1(7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. *J. Clin. Invest.*, **79**, 616–619.
- 15 Holst, J.J., Orskov, C., Van Nielsen, O., and Schwartz, T.W. (1987) Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. *FEBS Lett.*, **211**, 169–173.
- 16 Nathan, D.M., Schreiber, E., Fogel, H., Mojsov, S., and Habener, J.F. (1992) Insulinotropic action of glucagonlike peptide-1(7-37) in diabetic and nondiabetic subjects. *Diabetes Care*, **15** (2), 270–276.
- 17 Nauck, M.A., Kleine, N., Orskov, C., Holst, J.J., Willms, B., and Creutzfeldt, W. (1993) Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1(7-36 amide) in type 2 (non-insulin-dependent) diabetic patients. *Diabetologia*, **36** (8), 741–744.
- 18 Kreymann, B., Williams, G., Ghatel, M.A., and Bloom, S.R. (1987) Glucagon-like peptide-1 7-36: a physiological

- incretin in man. *Lancet*, 2 (8571), 1300–1304.
- 19 Holst, J.J. (2007) The physiology of glucagon-like peptide-1. *Physiol. Rev.*, 87, 1409–1439.
 - 20 Kieffer, T.J. and Habener, J.F. (1999) The glucagon-like peptides. *Endocr. Rev.*, 20 (6), 876–913.
 - 21 Adelhorst, K., Hedegaard, B.B., Knudsen, L.B., and Kirk, O. (1994) Structure–activity studies of glucagon-like peptide-1. *J. Biol. Chem.*, 269 (9), 6275–6278.
 - 22 Thornton, K. and Gorenstein, D.G. (1994) Structure of glucagon-like peptide (7–36) amide in a dodecylphosphocholine micelle as determined by 2D NMR. *Biochemistry*, 33 (12), 3532–3539.
 - 23 Mojsov, S., Kopiczynski, M.G., and Habener, J.F. (1990) Both amidated and nonamidated forms of glucagon-like peptide I are synthesized in the rat intestine and the pancreas. *J. Biol. Chem.*, 265 (14), 8001–8008.
 - 24 Shimizu, I., Hirota, M., Ohboshi, C., and Shima, K. (1987) Identification and localization of glucagon-like peptide-1 and its receptor in rat brain. *Endocrinology*, 121 (3), 1076–1082.
 - 25 Thorens, B. (1992) Expression cloning of the pancreatic beta cell receptor for the gluco-incretin hormone glucagon-like peptide 1. *Proc. Natl. Acad. Sci. USA*, 89 (18), 8641–8645.
 - 26 Wei, Y. and Mojsov, S. (1995) Tissue-specific expression of the human receptor for glucagon-like peptide-I: brain, heart and pancreatic forms have the same deduced amino acid sequences. *FEBS Lett.*, 358 (3), 219–224.
 - 27 Bullock, B.P., Heller, R.S., and Habener, J.F. (1996) Tissue distribution of messenger ribonucleic acid encoding the rat glucagon-like peptide-1 receptor. *Endocrinology*, 137 (7), 2968–2978.
 - 28 Körner, M., Stöckli, M., Waser, B., and Reubi, J.C. (2007) GLP-1 receptor expression in human tumors and human normal tissues: potential for *in vivo* targeting. *J. Nucl. Med.*, 48 (5), 736–743.
 - 29 Ban, K., Noyan-Ashraf, M.H., Hoefer, J., Bolz, S.S., Drucker, D.J., and Husain, M. (2008) Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. *Circulation*, 117 (18), 2340–2350.
 - 30 Liu, H., Hu, Y., Simpson, R.W., and Dear, A.E. (2008) Glucagon-like peptide-1 attenuates tumour necrosis factor- α -mediated induction of plasminogen activator inhibitor-1 expression. *J. Endocrinol.*, 196 (1), 57–65.
 - 31 Schlatter, P., Beglinger, C., Drewe, J., and Gutmann, H. (2007) Glucagon-like peptide 1 receptor expression in primary porcine proximal tubular cells. *Regul. Pept.*, 141 (1–3), 120–128.
 - 32 Orskov, C., Holst, J.J., and Nielsen, O.V. (1988) Effect of truncated glucagon-like peptide-1 [proglucagon-(78–107) amide] on endocrine secretion from pig pancreas, antrum, and nonantral stomach. *Endocrinology*, 123 (4), 2009–2013.
 - 33 Unger, R.H. and Orci, L. (1975) The essential role of glucagon in the pathogenesis of diabetes mellitus. *Lancet*, 1, 14–16.
 - 34 Drucker, D.J., Philippe, J., Mojsov, S., Chick, W.L., and Habener, J.F. (1987) Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. *Proc. Natl. Acad. Sci. USA*, 84 (10), 3434–3438.
 - 35 Holz, G.G., IV, Kühtreiber, W.M., and Habener, J.F. (1993) Pancreatic beta-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1(7–37). *Nature*, 361 (6410), 362–365.
 - 36 Xu, G., Stoffers, D.A., Habener, J.F., and Bonner-Weir, S. (1999) Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. *Diabetes*, 48 (12), 2270–2276.
 - 37 Bregenholt, S., Møldrup, A., Blume, N., Karlsen, A.E., Friedrichsen, B.N., Tornhave, D., Knudsen, L.B., and Petersen, J.S. (2005) The long-acting glucagon-like peptide-1 analogue, liraglutide, inhibits beta-cell apoptosis *in*

- vitro. Biochem. Biophys. Res. Commun.*, **330**, 577–584.
- 38 Wettergren, A., Schjoldager, B., Mortensen, P.E., Myhre, J., Christiansen, J., and Holst, J.J. (1993) Truncated GLP-1 (proglucagon 78-107-amide) inhibits gastric and pancreatic functions in man. *Dig. Dis. Sci.*, **38** (4), 665–673.
 - 39 Schjoldager, B.T., Mortensen, P.E., Christiansen, J., Orskov, C., and Holst, J.J. (1989) GLP-1 (glucagon-like peptide 1) and truncated GLP-1, fragments of human proglucagon, inhibit gastric acid secretion in humans. *Dig. Dis. Sci.*, **34** (5), 703–708.
 - 40 Flint, A., Raben, A., Astrup, A., and Holst, J.J. (1998) Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *J. Clin. Invest.*, **101** (3), 515–520.
 - 41 Zander, M., Madsbad, S., Madsen, J.L., and Holst, J.J. (2002) Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. *Lancet*, **359** (9309), 824–830.
 - 42 Nikolaidis, L.A., Mankad, S., Sokos, G.G., Miske, G., Shah, A., Elahi, D., and Shannon, R.P. (2004) Effects of glucagon-like peptide-1 in patients with acute myocardial infarction and left ventricular dysfunction after successful reperfusion. *Circulation*, **109**, 962–965.
 - 43 Nyström, T., Gutniak, M.K., Zhang, Q., Zhang, F., Holst, J.J., Åhrén, B., and Sjöholm, A. (2004) Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary artery disease. *Am. J. Physiol. Endocrinol. Metab.*, **287** (6), E1209–E1215.
 - 44 Drucker, D.J. (2006) The biology of incretin hormones. *Cell Metab.*, **3** (3), 153–165.
 - 45 Mortensen, K., Christensen, L.L., Holst, J.J., and Orskov, C. (2003) GLP-1 and GIP are colocalized in a subset of endocrine cells in the small intestine. *Regul. Pept.*, **114** (2–3), 189–196.
 - 46 Herrmann, C., Göke, R., Richter, G., Fehmann, H.C., Arnold, R., and Göke, B. (1995) Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. *Digestion*, **56** (2), 117–126.
 - 47 Vilsbøll, T., Krarup, T., Deacon, C.F., Madsbad, S., and Holst, J.J. (2001) Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes*, **50** (3), 609–613.
 - 48 Rocca, A.S. and Brubaker, P.L. (1999) Role of the vagus nerve in mediating proximal nutrient-induced glucagon-like peptide-1 secretion. *Endocrinology*, **140** (4), 1687–1694.
 - 49 Roberge, J.N. and Brubaker, P.L. (1991) Secretion of proglucagon-derived peptides in response to intestinal luminal nutrients. *Endocrinology*, **128** (6), 3169–3174.
 - 50 Deacon, C.F., Nauck, M.A., Toft-Nielsen, M., Pridal, L., Willms, B., and Holst, J.J. (1995) Both subcutaneously and intravenously administered glucagon-like peptide 1 are rapidly degraded from the NH₂-terminus in type II diabetic patients and in healthy subjects. *Diabetes*, **44** (9), 1126–1131.
 - 51 Kieffer, T.J., McIntosh, C.H., and Pederson, R.A. (1995) Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 *in vitro* and *in vivo* by dipeptidyl peptidase IV. *Endocrinology*, **136** (8), 3585–3596.
 - 52 Hansen, L., Deacon, C.F., Orskov, C., and Holst, J.J. (1999) 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*, **140** (11), 5356–5363.
 - 53 Ruiz-Grande, C., Pintado, J., Alarcón, C., Castilla, C., Valverde, I., and López-Novoa, J.M. (1990) Renal catabolism of human glucagon-like peptides 1 and 2. *Can. J. Physiol. Pharmacol.*, **68** (12), 1568–1573.
 - 54 Orskov, C., Andreasen, J., and Holst, J.J. (1992) All products of proglucagon are elevated in plasma from uremic patients. *J. Clin. Endocrinol. Metab.*, **74** (2), 379–384.

- 55 Meier, J.J., Nauck, M.A., Kranz, D., Holst, J.J., Deacon, C.F., Gaeckler, D., Schmidt, W.E., and Gallwitz, B. (2004) Secretion, degradation, and elimination of glucagon-like peptide 1 and gastric inhibitory polypeptide in patients with chronic renal insufficiency and healthy control subjects. *Diabetes*, **53** (3), 654–662.
- 56 Vilsbøll, T., Krarup, T., Sonne, J., Madsbad, S., Vølund, A., Juul, A.G., and Holst, J.J. (2003) Incretin secretion in relation to meal size and body weight in healthy subjects and people with type 1 and type 2 diabetes mellitus. *J. Clin. Endocrinol. Metab.*, **88** (6), 2706–2713.
- 57 Nauck, M.A., Heimesaat, M.M., Orskov, C., Holst, J.J., Ebert, R., and Creutzfeldt, W. (1993) 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. *J. Clin. Invest.*, **91** (1), 301–307.
- 58 Clodfelter, D.K., Pekar, A.H., Rebhun, D.M., Destrampe, K.A., Havel, H.A., Myers, S.R., and Brader, M.L. (1998) Effects of non-covalent self-association on the subcutaneous absorption of a therapeutic peptide. *Pharm. Res.*, **15** (2), 254–262.
- 59 Larsen, J., Hylleberg, B., Ng, K., and Damsbo, P. (2001) Glucagon-like peptide-1 infusion must be maintained for 24 h/day to obtain acceptable glycemia in type 2 diabetic patients who are poorly controlled on sulphonylurea treatment. *Diabetes Care*, **24** (8), 1416–1421.
- 60 Deacon, C.F., Knudsen, L.B., Madsen, K., Wiberg, F.C., Jacobsen, O., and Holst, J.J. (1998) Dipeptidyl peptidase IV resistant analogues of glucagon-like peptide-1 which have extended metabolic stability and improved biological activity. *Diabetologia*, **41** (3), 271–278.
- 61 Kurtzhals, P., Havelund, S., Jonassen, I., Kiehr, B., Larsen, U.D., Ribøl, U., and Markussen, J. (1995) Albumin-binding of insulins acylated with fatty-acids – characterization of the ligand protein-interaction and correlation between binding-affinity and timing of the insulin effect *in-vivo*. *Biochem. J.*, **312**, 725–731.
- 62 Markussen, J., Havelund, S., Kurtzhals, P., Andersen, A.S., Halstøm, J., Hasselager, E., Larsen, U.D., Ribøl, U., Schaffer, L., Vad, K., and Jonassen, I. (1996) Soluble, fatty-acid acylated insulins bind to albumin and show protracted action in pigs. *Diabetologia*, **39**, 281–288.
- 63 Kurtzhals, P., Havelund, S., Jonassen, I., Kiehr, B., Ribøl, U., and Markussen, J. (1996) Albumin-binding and time action of acylated insulins in various species. *J. Pharm. Sci.*, **85**, 304–308.
- 64 Knudsen, L.B., Nielsen, P.F., Huusfeldt, P.O., Johansen, N.L., Madsen, K., Pedersen, F.Z., Thøgersen, H., Wilken, M., and Agersø, H. (2000) Potent derivatives of glucagon-like peptide-1 with pharmacokinetic properties suitable for once daily administration. *J. Med. Chem.*, **43** (9), 1664–1669.
- 65 Madsen, K., Knudsen, L.B., Agersø, H., Nielsen, P.F., Thøgersen, H., Wilken, M., and Johansen, N.L. (2007) Structure–activity and protraction relationship of long-acting glucagon-like peptide-1 derivatives: importance of fatty acid length, polarity, and bulkiness. *J. Med. Chem.*, **50** (24), 6126–6132.
- 66 Goke, R., Fehmann, H.-C., Linn, T., Schmidt, H., Krause, M., Eng, J., and Goke, B. (1993) 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. *J. Biol. Chem.*, **268**, 19650–19655.
- 67 Drucker, D.J. and Nauck, M.A. (2006) The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet*, **368** (9548), 1696–1705.
- 68 Havelund, S., Plum, A., Ribøl, U., Jonassen, I., Vølund, A., Markussen, J., and Kurtzhals, P. (2004) The mechanism of protraction of insulin detemir, a long-acting, acylated analog of human insulin. *Pharm. Res.*, **21** (8), 1498–1504.
- 69 Knudsen, L.B., Knudsen, S.M., Colding-Jørgensen, M., Plum, A., Ribøl, U., Wilken, M., Agersø, H., and Hansen, K.T. (2003) Plasma protein binding of NN2211 (liraglutide), a long-acting

- derivative of glucagon-like peptide-1, is important for its efficacy. *Diabetologia*, 46 (Suppl. 2), A284.
- 70 Steensgaard, D.B., Thomsen, J.K., Olsen, H.B., and Knudsen, L.B. (2008) The molecular basis for the delayed absorption of the once-daily human GLP-1 analogue, Liraglutide. *Diabetes*, 57 (Suppl. 1), A164.
 - 71 Friedrichsen, B.N., Neubauer, N., Lee, Y.C.L., Gram, V.K., Blume, N., Petersen, J.S., Nielsen, J.H., and Møldrup, A. (2006) Stimulation of pancreatic beta-cell replication by incretins involves transcriptional induction of cyclin D1 via multiple signalling pathways. *J. Endocrinol.*, 188, 481–492.
 - 72 Rolin, B., Larsen, M.O., Gotfredsen, C.F., Deacon, C.F., Carr, R.D., Wilken, M., and Knudsen, L.B. (2002) The long-acting GLP-1 derivative NN2211 ameliorates glycemia and increases beta-cell mass in diabetic mice. *Am. J. Physiol. Endocrinol. Metab.*, 283, E745–E752.
 - 73 Sturis, J., Gotfredsen, C.F., Rømer, J., Rolin, B., Ribel, U., Brand, C.L., Wilken, M., Wassermann, K., Deacon, C.F., Carr, R.D., and Knudsen, L.B. (2003) GLP-1 derivative liraglutide in rats with b-cell deficiencies: influence of metabolic state on b-cell mass dynamics. *Br. J. Pharm.*, 140, 123–132.
 - 74 Ribel, U., Larsen, M.O., Rolin, B., Carr, R.D., Wilken, M., Sturis, J., Westergaard, L., Deacon, C.F., and Knudsen, L.B. (2002) NN2211: a long-acting glucagon-like peptide-1 derivative with antidiabetic effects in glucose-intolerant pigs. *Eur. J. Pharm.*, 451, 217–225.
 - 75 Raun, K., von Voss, P., Gotfredsen, C.F., Golozoubova, V., Rolin, B., and Knudsen, L.B. (2007) Liraglutide, a long-acting glucagon-like peptide-1 analog, reduces body weight and food intake in obese candy-fed rats, whereas a dipeptidyl peptidase-IV inhibitor, vildagliptin, does not. *Diabetes*, 56, 8–15.
 - 76 Raun, K., von Voss, P., and Bjerre Knudsen, L. (2007) The once-daily human GLP-1 analog liraglutide minimizes food intake in severely obese minipigs. *Obesity*, 15, 1710–1716.
 - 77 Noyan-Ashraf, M.H., Momen, M.A., Ban, K., Sadi, A.M., Zhou, Y.Q., Riaz, A.M., Baggio, L.L., Henkelman, R.M., Husain, M., and Drucker, D.J. (2009) GLP-1R agonist liraglutide activates cytoprotective pathways and improves outcomes after experimental myocardial infarction in mice. *Diabetes*, 58 (4), 975–983.
 - 78 Liu, H., Dear, A.E., Knudsen, L.B., and Simpson, R.W. (2009) A long-acting glucagon-like peptide-1 analogue attenuates induction of plasminogen activator inhibitor type-1 and vascular adhesion molecules. *J. Endocrinol.*, 201 (1), 59–66.
 - 79 Elbrønd, B., Jakobsen, G., Larsen, S., Agersø, H., Jensen, L.B., Rolan, P., Sturis, J., Hatorp, V., and Zdravkovic, M. (2002) 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*, 25 (8), 1398–1404.
 - 80 Bjørnsdottir, I. et al. (2008) Metabolism and excretion of the once-daily human GLP-1 analogue liraglutide in healthy subjects and its *in vitro* degradation by dipeptidyl peptidase IV and neutral endopeptidase. *Diabetologia*, 51 (Suppl. 1), S356 (Abstract 891).
 - 81 Flint, A., Nazzari, K., Jagielski, P., Segel, S., and Zdravkovic, M. (2007) Influence of hepatic impairment on pharmacokinetics of the long-acting human GLP-1 analogue liraglutide. *Diabetes*, 56 (Suppl. 1), A145:0545-P.
 - 82 Jacobsen, L.V., Hindsberger, C., Robson, R., and Zdravkovic, M. (2007) Pharmacokinetics of the long-acting human GLP-1 analogue liraglutide in subjects with renal impairment. *Diabetes*, 56 (Suppl. 1), A137:0513-P.
 - 83 Kolterman, O.G., Kim, D.D., Shen, L., Ruggles, J.A., Nielsen, L.L., Fineman, M.S., and Baron, A.D. (2005) Pharmacokinetics, pharmacodynamics, and safety of exenatide in patients with type 2 diabetes mellitus. *Am. J. Health Syst. Pharm.*, 62 (2), 173–181.
 - 84 Simonsen, L., Holst, J.J., and Deacon, C.F. (2006) Exendin-4, but not glucagon-

- like peptide-1, is cleared exclusively by glomerular filtration in anaesthetized pigs. *Diabetologia*, **49**, 706–712.
- 85 Parkes, D., Jodka, C., Smith, P., Nayak, S., Rinehart, L., Gingerich, R., Chen, K., and Young, A. (2001) Pharmacokinetic actions of exendin-4 in the rat: comparison with glucagon-like peptide-1. *Drug Dev. Res.*, **53**, 260–267.
 - 86 Copley, K., McCowen, K., Hiles, R., Nielsen, L.L., Young, A., and Parkes, D.G. (2006) Investigation of exenatide elimination and its *in vivo* and *in vitro* degradation. *Curr. Drug Metab.*, **7**, 367–374.
 - 87 Agerse, H., Jensen, L.B., Elbrønd, B., Rolan, P., and Zdravkovic, M. (2002) The pharmacokinetics, pharmacodynamics, safety and tolerability of NN2211, a new long-acting GLP-1 derivative, in healthy men. *Diabetologia*, **45** (2), 195–202.
 - 88 Degn, K.B., Juhl, C.B., Sturis, J., Jakobsen, G., Brock, B., Chandramouli, V., Rungby, J., Landau, B.R., and Schmitz, O. (2004) One week's treatment with the long-acting glucagon-like peptide 1 derivative liraglutide (NN2211) markedly improves 24-h glycemia and alpha- and beta-cell function and reduces endogenous glucose release in patients with type 2 diabetes. *Diabetes*, **53** (5), 1187–1194.
 - 89 Vilsbøll, T., Zdravkovic, M., Le-Thi, T., Krarup, T., Schmitz, O., Courrèges, J.P., Verhoeven, R., Bugánová, I., and Madsbad, S. (2007) 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*, **30** (6), 1608–1610.
 - 90 Courrèges, J.P., Vilsbøll, T., Zdravkovic, M., Le-Thi, T., Krarup, T., Schmitz, O., Verhoeven, R., Bugánová, I., and Madsbad, S. (2008) Beneficial effects of once-daily liraglutide, a human glucagon-like peptide-1 analogue, on cardiovascular risk biomarkers in patients with type 2 diabetes. *Diabet. Med.*, **25** (9), 1129–1131.
 - 91 Vilsbøll, T., Brock, B., Perrild, H., Levin, K., Lervang, H.H., Kølendorf, K., Krarup, T., Schmitz, O., Zdravkovic, M., Le-Thi, T., and Madsbad, S. (2008) 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. *Diabet. Med.*, **25** (2), 152–156.
 - 92 Marre, M., Shaw, J., Brandle, M., Bebakar, W.M., Kamaruddin, N.A., Strand, J., Zdravkovic, M., Le Thi, T.D., Colagiuri, S. *et al.* (2009) 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). *Diabet. Med.*, **26**, 268–278.
 - 93 Nauck, M.A., Frid, A., Hermansen, K., Shah, N.S., Tankova, T., Mitha, I.H. *et al.* (2009) Efficacy and safety comparison of liraglutide, glimepiride, and placebo, all in combination with metformin in type 2 diabetes mellitus (LEAD-2 Met). *Diabetes Care*, **32**, 84–90.
 - 94 Garber, A., Henry, R., Ratner, R., Garcia-Hernandez, P.A., Rodriguez-Pattzi, H., Olvera-Alvarez, I. *et al.* (2009) Liraglutide versus glimepiride monotherapy for type 2 diabetes (LEAD-3 Mono): randomised, 52-week, phase III, double-blind, parallel-treatment trial. *Lancet*, **373** (9662), 473–481.
 - 95 Zinman, B., Gerich, J., Buse, J., Lewin, A., Schwartz, S.L., Raskin, P. *et al.* (2009) Efficacy and safety of the human glucagon-like peptide-1 analog/liraglutide in combination with metformin and thiazolidine-dione in patients with type 2 diabetes mellitus (LEAD-4 Met + TZD). *Diabetes Care*, **32** (7) 1224–1230.
 - 96 Russell-Jones, D., Vaag, A., Schmitz, O., Sethi, B., Lalic, N.M., Antic, S. *et al.* Liraglutide Effect and Action in Diabetes 5 (LEAD-5) met+su study group. (2009) Liraglutide vs. insulin glargine and placebo in combination with metformin and sulphonylurea therapy in type 2 diabetes mellitus (LEAD-5 met+su): a

- randomised controlled trial. *Diabetologia*, **52** (10), 2046–2055.
- 97 Buse, J.B., Rosenstock, J., Sesti, G., Schmidt, W.E., Montanya, E., Brett, J.H., Zychra, M., and Blonde, L. (2009) 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). *Lancet*, **374** (9683), 39–47.
 - 98 Horowitz, M., Vilsbøll, T., Zdravkovic, M., Hammer, M., and Madsbad, S. (2008) Patient-reported rating of gastrointestinal adverse effects during treatment of type 2 diabetes with the once-daily human GLP-1 analogue, liraglutide. *Diabetes Obes. Metab.*, **10** (7), 593–596.
 - 99 Colagiuri, S., Frid, A., Zdravkovic, M., Duyen Le Thi, T., and Vaag, A. (2008) The once-daily human glucagon-like peptide-1 analog liraglutide reduces systolic blood pressure in patients with type 2 diabetes. *Diabetologia*, **51** (Suppl. 1), S360 (Abstract 899).
 - 100 Buse, J.B., Henry, R.R., Han, J., Kim, D.D., Fineman, M.S., Baron, A.D., and Exenatide-113 Clinical Study Group (2004) Effects of exenatide (exendin-4) on glycemic control over 30 weeks in sulfonylurea-treated patients with type 2 diabetes. *Diabetes Care*, **27** (11), 2628–2635.
 - 101 DeFronzo, R.A., Ratner, R.E., Han, J., Kim, D.D., Fineman, M.S., and Baron, A.D. (2005) Effects of exenatide (exendin-4) on glycemic control and weight over 30 weeks in metformin-treated patients with type 2 diabetes. *Diabetes Care*, **28** (5), 1092–1100.
 - 102 Kendall, D.M., Riddle, M.C., Rosenstock, J., Zhuang, D., Kim, D.D., Fineman, M.S., and Baron, A.D. (2005) 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*, **28** (5), 1083–1091.
 - 103 Byetta Package Insert. <http://pi.lilly.com/us/byetta-pi.pdf>. Accessed 09.04.09.

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