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Metabolic responses and benefits of GLP-1 receptor ligands

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Abstract

Glucagon-like peptide-1 (GLP-1) is an incretin hormone that has undergone a revolutionary turnaround from discovery to clinically approved therapeutic. Rapid progress in drug design and formulation has led from initial development of short- and long-acting drugs suitable for daily or weekly parenteral administration, respectively, through to the most recent approval of an orally active GLP-1 agent. The current review outlines the biological action profile of GLP-1 including the various beneficial metabolic responses in pancreatic and extrapancreatic tissues, including the gastrointestinal tract, liver, bone and kidney as well as the reproductive cardiovascular and central nervous systems. We then briefly consider clinically approved GLP-1 receptor ligands and recent advances in this field. Given sustained evolution in the area of GLP-1 drug development and excellent safety profile, as well as the plethora of metabolic benefits, clinical approval for use in diseases beyond diabetes and obesity is very much conceivable.

Abbreviations

ApoE, apolipoprotein E

ANP, atrial natriuretic hormone

CAMKII, Ca^{2+}/calmodulin-dependent kinase II,

CaSR, calcium-sensing receptor (CaSR)

CCK, cholecystokinin

FFA, free fatty acid

GIP, gastric inhibitory polypeptide

GIPR, gastric inhibitory polypeptide receptor

GIT, gastrointestinal tract

GLP-1, glucagon-like peptide-1

GLP-1R, glucagon-like peptide-1receptor

GPBAR1, bile acid receptor 1

HR, heart rate

PEPT1, peptide transporter 1

Pdx1, pancreatic and duodenal homeobox 1

SNAC, sodium N-(8-[2-hydroybenzoyl] amino) caprylate (SNAC)

SSTR, somatostatin receptor

T2DM, type 2 diabetes mellitus

1 Introduction

The physiological role of the gastrointestinal tract (GIT) was traditionally thought to involve nutrient digestion and absorption, but it is now known to be the source of a plethora of peptide hormones involved in the regulation of metabolism and other body functions [Baggio and Drucker, 2007]. Seminal work in the late 1960s led to the identification of peptide hormones with glucagon-like immunoreactivity following GIT stimulation by glucose [Samols & Marks 1967]. Since then, two major GIT-derived hormones involved in regulation of postprandial glucose have been identified, namely glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), secreted from L-cells and K-cells of the GIT, respectively. Collectively, these two hormones account for 50-70% of insulin secretion in response to a meal [Baggio and Drucker, 2007], with this action termed "the incretin effect". Given the glucose-dependent nature of GLP-1 induced insulin secretion and retention of bioactivity in type 2 diabetes [Nauck et al, 1993a], drugs based on the biological action of this hormone were rapidly translated to benefits in humans [Baggio and Drucker, 2007]. Thus, the amino acid peptide sequence of GLP-1 was first discovered by Habener and colleagues in the early 1980s through decoding of recombinant cDNA clones in anglerfish [Lund et al, 1982], and subsequently found to enhance insulin secretion in the perfused rat pancreas [Mojsov et al, 1987], with clinical approval of GLP-1 mimetic for the treatment of type 2 diabetes mellitus (T2DM) following in 2005 [Kolterman et al, 2005]. Although this original approval was largely based on the potent glucose-dependent insulinotropic properties of GLP-1 receptor (GLP-1R) activation on pancreatic beta-cells, it is now clear that the GLP-1R is expressed on various other metabolically active tissues eliciting a range of biological effects across diverse organ systems [Figure 1].

2 GLP-1 secretion

In terms of endogenous secretion, GLP-1 producing L-cells are predominantly located along the ileum and colon of the GIT [Eissele et al, 1992]. With the apical surface of the L-cell in contact with the gut lumen, GLP-1 secretion is stimulated by the presence of intestinal nutrients [Eissele et al, 1992], albeit via distinct mechanisms. Thus, glucose absorption within the L-cell leads to ATP production, subsequent closure of K_{ATP} channels and opening of voltage-gated Ca^{2+} channels, a process known to be linked to sodium-coupled glucose transporters (SGLT1) that sense ingested glucose [Parker et al, 2012]. The resulting Ca^{2+} influx triggers exocytosis of GLP-1 containing vesicles into the circulation. Alternatively, free fatty acids bind to and activate their respective free fatty acid L-cell receptors, for example FFA1, FFA4, GPR120 and GPR40, to increase intracellular Ca^{2+} via Gq signalling pathways that stimulate protein kinase C (PKC) signalling leading to GLP-1 secretion [Tolhurst et al, 2011]. In particular, activation of the G-protein-coupled receptor (GPCR), bile acid receptor 1 (GPBAR1), increases L-cell differentiation and elicits substantial GLP-1 secretion [Lund et al, 2020].

In addition to this, proteins and amino acids are consistently shown to elicit L-cell GLP-1 secretion *in vitro* [Tolhurst et al, 2011], in rodents [Clemmensen et al, 2013] and in humans [Lejeune et al, 2006]. This action is mediated by activation of Ca^{2+}/calmodulin-dependent kinase II (CAMKII), calcium-sensing receptor (CaSR) and peptide transporter 1 (PEPT1) leading to a rise in intracellular Ca^{2+} and subsequent GLP-1 secretion [Diakogiannaki et al, 2013]. Furthermore, intestinal L-cells are situated in close proximity to enteric neurons and microvasculature, suggesting GLP-1 secretion is also influenced by neuronal and endocrine factors [Anini et al, 2002]. As such, GIP and cholecystokinin (CCK) are the two gut-derived hormones implicated in GLP-1 secretion. In rodents, intravenous treatment with GIP is associated with an increase in glucagon-like immunoreactivity via a GIP-GLP-1 vagal axis [Rocca et al, 1999]. Whether such a GIP-GLP-1 axis operates in humans is questionable, given that pharmacological doses of GIP fail to elicit GLP-1 secretion in man [Mentis et al, 2011].

In addition to these pharmacological stimuli, murine L-cells display a circadian pattern of GLP-1 secretion that peaks prior to the onset of feeding periods [Biancolin et al, 2020].

Contrary to extensive and growing knowledge around the stimulation of GLP-1 secretion from enteroendocrine L-cells, much less is known about potential mediators that provide feedback inhibition to GLP-1 secretion. In this regard, the neuropeptide galanin has been shown to inhibit GLP-1 secretion via action of the G_i-linked GAL_1 receptor expressed on L-cells [Psichas et al, 2015]. Likewise, somatostatin has been shown to inhibit GLP-1 secretion, likely via modulation of the somatostatin receptor 5 (SSTR5) [Chisholm and Greenberg, 2002]. Exploiting this knowledge, selective SSTR5 antagonists have been shown to augment circulating GLP-1 levels in mice [Farb et al, 2017]. Indeed, more recent evidence reveals that selective stimulation of colonic L-cells leads to significant improvements in metabolic control, with obvious possible therapeutic implications [Lewis et al, 2020].

Once secreted, GLP-1 has a short duration of biological action due enzymatic degradation by the ubiquitous enzyme dipeptidyl peptidase-4 (DPP-4) and efficient renal clearance, resulting in an *in vivo* $t_{½}$ of around 5-10 minutes [Deacon et al, 1996]. It is suggested that up to 75% of secreted GLP-1 is degraded within the gut, with an additional 50% then degraded in the liver, before even entering the general circulation [Deacon et al, 1996]. Within the circulation, GLP-1 binds and activates the GLP-1R expressed on various sites throughout the body. The GLP-1R is a membrane bound GPCR, coupled to Gαs that activates adenylyl cyclase (AC) to increase cyclic adenine monophosphate (cAMP) and triggers intracellular cascades leading to various responses within each cell type [Mayo et al, 2003]. However, there has been some controversy around the specificity of commercially available antibodies directed against the GLP-1R [Pyke and Knudsen, 2013], generating debate on the exact location of GLP-1R expression in the body [Pyke and Knudsen, 2013]. Fortunately, use of molecular biology techniques, alongside recent advances in monoclonal antibody development, has allowed for

clearer identification of the GLP-1R, distinct from that of GLP-2R, GIPR and glucagon receptors [Biggs et al, 2018; Pyke et al, 2014;]. Further to this, development of fluorescent probes, such as LUXendin645, allow for super-resolution microscopic detection of the GLP-1R both *in vitro* and *in vivo* [Ast et al, 2020]. As such, monoclonal antibodies with improved selectivity for GLP-1R have now been developed confirming true GLP-1R expression in the pancreas, brain, kidney, lung, heart and stomach [Pyke et al. 2014]. Furthermore, transgenic mice expressing fluorescent markers in tissues that express the GLP-1R largely confirm these findings [Richards et al. 2014]. In addition, mRNA expression of the GLP-1R has been observed in osteoblastic cell lines [Pacheco-Pantoja et al, 2011], but there is limited evidence for presence of GLP-1R on human bone. Centrally, the GLP-1R is expressed within the following brain regions; cerebral cortex, hypothalamus, hippocampus, thalamus, caudate-putamen and globus pallidum [Alvarez et al, 2005]. Finally, GLP-1R mimetic therapy has consistently shown to improve liver disease, possibly indirectly via anti-inflammatory and weight-reducing actions.

3 GLP-1 and the endocrine pancreas

Glucose-stimulated insulin release from pancreatic beta-cells is a tightly regulated process, that involves many complementary pathways. In the case of GLP-1, activation of the GLP-1R on beta-cells triggers an intracellular signalling cascade that potentiates glucose-stimulated insulin secretion, whilst also exerting more longer-term benefits on beta-cell growth and survival, ultimately leading to improvements in overall beta-cell sensitivity and insulin production [Figure 2; Campbell and Drucker, 2013]. Advances in islet cell lineage tracing technologies have also highlighted the importance of GLP-1R in maintaining beta-cell identity and preventing beta-cell de-differentiation under situations of pancreatic islet stress [Tanday et al, 2020]. In terms of the pancreatic alpha-cell, GLP-1 consistently suppresses glucagon secretion

[Hare et al, 2009]. Indeed, this glucagonostatic action is suggested to account for 50% of the blood glucose lowering ability of GLP-1 [Hare, 2010]. The exact mechanisms underlying this action are uncertain, however there are two major theories [Figure 2]. The "direct" theory, relies on alpha-cells expressing the GLP-1R with GLP-1 exerting a direct inhibitory action on alpha-cells [De Marinis et al, 2010]. However, even with the use of more specific antibodies and probes to accurately detect the GLP-1R, there are still conflicting reports on whether alpha-cells express GLP-1R. Studies have demonstrated that only a small proportion, at most approximately 10-12%, of mouse alpha-cells express the GLP-1R [Ast et al, 2020], whilst others have failed to detect the GLP-1R on human alpha-cells [Waser et al, 2015]. Whether GLP-1R present on alpha-cells make any meaningful contribution to the glucagonostatic effects of GLP-1R mimetics is questionable. Due to the lack of clear evidence regarding alpha-cell GLP-1R expression, a second "indirect" theory has emerged [Figure 2]. This indirect theory ascribes to the idea that GLP-1 mediates its glucagonostatic effect indirectly through stimulation of somatostatin secretion from pancreatic delta-cells, that express functional GLP-1R [Ørgaard and Holst, 2017]. In this regard, somatostatin consistently inhibits glucagon, insulin and GLP-1 across all species. Given that GLP-1 is known to stimulate delta-cell secretions [Ørgaard and Holst, 2017], it is feasible that somatostatin exerts a paracrine inhibitory effect on neighbouring alpha-cells. Indeed, in a perfused mouse pancreas model, administration of GLP-1 suppressed glucagon secretion, with this inhibitory effect annulled in the presence of a specific somatostatin receptor 2 (SSTR2) antagonist [Ørgaard and Holst, 2017]. In similar fashion, the ability of liraglutide to reduce dapagliflozin-induced hyperglucagonemia is abolished in somatostatin receptor knockout mice [Saponaro et al, 2019]. Collectively, these findings present strong evidence for the indirect theory of GLP-1 mediated glucagon inhibition [Figure 2]. In reality, GLP-1 induced inhibition of glucagon secretion is a complicated process that requires further investigation, especially since GLP-1 is known to

stimulate release of amylin [Gedulin et al, 1997], GABA [Wendt et al, 2004] and zinc [Zhou et al, 2007], that can all independently modulate glucagon secretion. Nonetheless, promotion of glucose-dependent insulin secretion, coupled with reduced glucagon release, represents an ideal paradigm for diabetes therapy.

In contrast to GLP-1 actions on alpha-cells, the molecular actions underpinning potentiation of beta-cell insulin secretion have been explored in depth [Figure 2]. Upon GLP-1R binding and activation, the enzyme AC increases cAMP levels which in turn stimulates protein kinase A (PKA) and Epac activity [Holz, 2004]. PKA closes the beta-cell ATP-sensitive K^+ (K_{ATP}) channel to depolarise the cell membrane [Light et al, 2002]. This depolarisation opens voltage-dependant Ca^{2+} channels leading to increased Ca^{2+} influx, essential for exocytosis of insulin granules [MacDonald et al, 2003]. In harmony with this, Epac proteins sensitise the K^+ channel, lowering its ATP threshold for activation and further act on the endoplasmic reticulum to release Ca^{2+} cellular stores [Doyle and Egan, 2007]. These pathways are critical for GLP-1 mediated insulin secretory activity, as blocking cAMP accumulation [Härndahl et al, 2002] or PKA activity [Wang et al, 2001], eliminates GLP-1 induced insulin secretion.

As well as stimulating glucose dependent insulin secretion, GLP-1 exerts additional effects of pancreatic beta-cells with obvious therapeutic benefits in diabetes. As such, GLP-1 is able to slow the loss of beta-cell mass in diabetes through its ability to increase proliferation [Arakawa et al, 2009] and protect against apoptosis [Li et al, 2003]. Specifically, activation of PKA by GLP-1 leads to an increase in Pdx1, a transcription factor critical for maintenance of beta-cell function and protein kinase B (Akt) induced beta-cell proliferation [Wang et al, 2001]. However, it should be noted that adult human beta-cells appear to have somewhat limited proliferative capacity, when compared to juvenile mouse or human cells [Dai et al, 2017], with important therapeutic implications. Interestingly, upregulation of beta-cell Pdx1 expression is

also attributed to GLP-1 mediated benefits on the maintenance of beta-cell identity and prevention of beta-cell de-differentiation in situations of islet stress [Tanday et al, 2020]. In addition to this, GLP-1R-mediated intracellular beta-cell signalling also leads to upregulation of anti-apoptotic proteins, such as B-cell lymphoma 2 (Bcl2) and B-cell lymphoma-extra large (Bcl-XL), as well as inhibition of caspase activation and nuclear factor-κB (NF-κB) to ultimately encourage beta-cell survival and resistance to endoplasmic reticulum stress [Tsunekawa et al, 2007]. Taken together, the compilation of these GLP-1 induced benefits on pancreatic islet cells highlight the clinical benefits of GLP-1 mimetics in diabetes.

3.1 GLP-1 and the GIT

Activation of the GLP-1R within the CNS reduces gut contractility, slowing gastric motility and emptying [Goyal et al, 2019]. By reducing gastric motility, nutrients are absorbed into the circulation at a slower rate, decreasing the postprandial spike in blood glucose [Smits et al, 2016], with obvious benefit in diabetes. The mechanism behind this action is multifaceted and involves vagal innervation, adrenergic innervation and nitric oxide (NO) signalling [Tolessa et al, 1998]. This GIT effect is consistent across species, is observed in healthy and diabetic [Meier et al, 2003] humans, and amplifies the antidiabetic actions of GLP-1.

3.2 GLP-1 and the cardiovascular system

Type 2 diabetes is strongly associated with increased cardiovascular disease (CVD) risk [Marso et al, 2016a; Marso et al, 2016b], and there has been recent strong emphasis on the ability current and future antidiabetic drugs to reduce CVD mortality in diabetes [Marso et al, 2016a; Marso et al, 2016b]. Notably, expression of GLP-1R has been detected within all four chambers of the heart, sinoatrial node and arteriole smooth muscle cells [Baggio et al, 2018; Pyke et al, 2014]. In this regard, GLP-1 infusion has been shown to improve endothelial function

[Nystrom et al, 2004], with GLP-1 mimetic therapy known to reduce blood pressure [Sun et al, 2015] and offer overall cardiomyocyte protection [Wang et al, 2013; Sjoberg et al, 2014; Asmar et al, 2017]. Collectively these actions benefit cardiovascular health, as has been demonstrated in recent cardiovascular outcome trials using various GLP-1 mimetics [Marso et al, 2016a; Marso et al, 2016b]. Specifically, in the SUSTAIN-6 trial, patients with type 2 diabetes and CVD risk had a reduced rate of cardiovascular death, non-fatal myocardial infarction and non-fatal stroke when treated with the recently approved GLP-1 mimetic semaglutide [Marso et al, 2016a]. Likewise, the LEADER trial concluded that a related GLP-1 mimetic, namely liraglutide, was beneficial in reducing the rate of non-fatal myocardial infarction, stroke and first occurrence of death from cardiovascular causes [Marso et al, 2016b]. However, this CVD benefit may be GLP-1 mimetic specific, given that other trials using GLP-1 mimetics with shorter half-lives and lower homology to native GLP-1, such as lixisenatide (ELIXSA) or exenatide (EXSCEL), failed to show a significant CVD benefit [Holman et al 2017]. Despite this variation, a more recent meta-analysis of all these trials confirmed the beneficial actions of GLP-1 mimetics through a 13% relative risk reduction in CVD mortality, 12% risk reduction in all-cause mortality and 10% relative risk reduction in cardiovascular death, non-fatal myocardial infarction and non-fatal stroke [Bethel et al, 2018]. Ultimately, clinical trials such as these have confirmed that, similar to sodium glucose co-transporters 2 (SGLT2) inhibitors, GLP-1 mimetics have established CVD benefits in diabetes.

The mechanisms underpinning the cardiovascular benefit of GLP-1 mimetics are multifaceted. GLP-1 mimetics can reduce traditional CVD risk factors such as obesity, whilst also exerting anti-inflammatory and anti-atherosclerotic effects as well as having positive direct modulatory effects on endothelial, cardiac and renal function [Garg et al, 2019]. In T2DM, GLP-1R activation has been shown to reduce blood pressure, due to a direct vasodilatory action combined with indirect actions on lowering body weight and inducing kidney natriuresis

[Asmar et al, 2019]. In all four major cardiovascular outcome trials, namely SUSTAIN-6, LEADER, ELIXA, EXCSEL, systolic blood pressure was significantly reduced with GLP-1 mimetic therapy, with the greatest mean reduction (5.4 mmHg) associated with 1 mg once-weekly semaglutide treatment [Marso et al, 2016a]. Similarly, all four trials highlighted GLP-1 mimetic induced weight loss, again with the greatest effect (4.3 kg weight loss) observed in the semaglutide treated group [Marso et al, 2016a]. GLP-1 mimetics, such as liraglutide and exenatide, have also been shown to reduce low-density lipoprotein (LDL) cholesterol, total cholesterol and triglyceride levels [Sun et al, 2015]. Collectively, these actions account for at least some of the CVD benefits observed with GLP-1 mimetic therapy.

Given that the GLP-1R is expressed on all four chambers of the heart as well as the sinoatrial node, it is likely that GLP-1 and its mimetics exert a direct action on cardiac cells [Baggio et al, 2018]. In agreement with this, GLP-1 therapy has a protective effect on cardiomyocytes during myocardial infarction in mice [Nikolaidis et al, 2004]. Interestingly, this beneficial action is still present in cardiac-specific GLP-1 receptor knockout mice, implying possible important indirect effects [Ussher et al, 2014]. GLP-1 and associated mimetics have also been shown to increase heart rate (HR) [Robinson et al, 2013] via direct receptor mediated actions [Baggio et al., 2017], but this effect was variable between mimetics. As such, in a head-to-head study with lixisenatide and liraglutide, the shorter-acting agent lixisenatide produced a modest, transient 1-3 beat per minute increase in HR, whilst the longer-acting GLP-1 mimetic liraglutide was associated a more distinct and sustained 6-10 beat per minute beat per minute elevation [Meier et al, 2015]. The potential impact of elevated HR in patients with heart failure does need to be carefully considered [Marso et al, 2016a]. Furthermore, heart rate is well known to increase postprandially, and GLP-1 mimetic mediated elevations of heart rate could also be a compensatory consequence of GLP-1-induced

vasodilation in specific tissues [Asmar et al. 2017], but such a GLP-1 mimetic effect still needs to be confirmed.

3.3 GLP-1 and inflammation

Further to this, GLP-1 therapy has also been shown to augment anti-inflammatory and anti-atherosclerotic processes, demonstrated by their ability to reduce occurrence of myocardial infarctions and strokes [Tanaka et al, 2018]. In this regard, GLP-1 mimetics have been shown to impede inflammatory responses and reduce atherosclerosis development [Figure 3A]. Specifically, in animal models of atherosclerosis, namely $ApoE^{-/-}$ and LDL $receptor^{-/-}$ knockout mice, GLP-1 treatment reduced plaque size [Bjørnholm et al, 2020]. The GLP-1 mimetics exendin-4 and semaglutide have also been shown to reduce cerebrovascular infarct size in rodent models of cerebral ischaemia [Basalay et al, 2019]. In patients with acute myocardial infarction, administration of GLP-1 or its mimetics improved ventricular function and reduced reperfusion injury [Nikolaidis et al, 2004]. Improvement of endothelial dysfunction is another mechanism through which GLP-1 mimetics exert their CVD benefit [Nystrom et al, 2004]. As such, liraglutide is known to ameliorate vascular endothelial dysfunction via suppression of oxidative stress and direct promotion of endothelial-derived nitic oxide synthase (eNOS) mediated NO production and vasodilation [Figure 3A; Li et al, 2020]. However, it should be noted that others have failed to observe clear beneficial effects of GLP-1 mimetics on endothelial function [Faber et al, 2015].

GLP-1 and associated mimetics also have established anti-inflammatory benefits beyond cardiovascular disease [Lee and Jun, 2016]. As such, intraepithelial lymphocytes (IELs) of the GIT express GLP-1R, with activation resulting in reduced cytokine production to positively control innate immunity and gut barrier function [Yusta et al, 2015]. In this regard, liraglutide improves aspects of inflammatory skin diseases, such as psoriasis, through

beneficial anti-inflammatory actions on immune cells [Hogan et al, 2011]. Likewise, liraglutide has been shown to reduce lung fibrosis in a bleomycin-induced rodent model of lung disease, by directly decreasing the expression of pro-fibrotic cytokines markers [Fandiño et al, 2020]. Collectively these studies highlight the potential of GLP-1 mimetics for the treatment of diseases and disorders driven by chronic inflammation.

3.4 GLP-1 and the kidney

GLP-1 and associated mimetics elicit actions on the renal system, but effects may be dependent on species studied, renal health status and concentration of GLP-1 mimetic employed [Hviid and Sorensen, 2020]. In rodents, GLP-1 acts on kidney GLP-1Rs located on renal vascular smooth muscle [Pyke et al, 2014], to increase renal plasma flow (RPF) and glomerular filtration rate (GFR) as well as diuresis and natriuresis whilst reducing renal inflammation, fibrosis and oxidative stress, via cAMP and PKA signalling pathways [Lee and Jun, 2016]. The presence of SGLT1 on L-cells of the GIT [Parker et al, 2012] may also represent sodium sensing capability within the gut and suggest important gut-kidney crosstalk in relation to regulation of ingested sodium [Asmar et al. 2020]. Tensive status also seems to be an important factor in determining the renal actions of GLP-1. As such, when compared to normotensive rats, hypertensive rats exhibit reduced renal GLP-1R expression and related effects [Ronn et al, 2017]. In addition, renal output is closely associated with cardiac function, with GLP-1R activation at both sites determining overall responses. In humans, GLP-1 infusion has recently been shown to exert a natriuretic effect as well as suppress angiotensin II release independent of RPF and GFR [Asmar et al, 2021], but the specific site of GLP-1R expression required for these effects still to be determined. Alternatively, in rodents a GLP-1/atrial natriuretic peptide (ANP) axis exists, whereby activation of the GLP-1R in cardiac tissue stimulates ANP secretion to evoke renal natriuresis and reduced blood pressure [Kim et al, 2013]. GLP-1 has also been shown to exert positive actions on the renal system in the treatment of

both diabetic and non-diabetic kidney disease [Roscioni et al, 2014]. In addition to cytoprotective and anti-inflammatory actions, GLP-1 also acts to increase RPF, GFR, renal interstitial fluids and urinary flow rate, whilst reducing tubular necrosis [Skov et al, 2013]. The importance of GLP-1R action within the kidney is exemplified in GLP-1 receptor knockout mice that display increased renal oxidative stress [Fujita et al, 2014]. Together, these actions highlight the potential of GLP-1R mimetics for treating kidney disease and improving kidney function in diabetes.

3.5 GLP-1 and bone

T2DM is associated with increased bone fracture risk, but the mechanisms behind this effect are still to be fully elucidated [Mabilleau et al, 2018a]. Diabetic animal models present with a loss of bone mineral density which can be restored through administration of GLP-1R mimetics [Mansur et al, 2015; Mansur et al 2019a]. In addition, exenatide has been shown to stimulate osteoblast activation and restore bone formation in an ovariectomy-induced model of bone loss [Mabilleau et al, 2013]. Whether these beneficial effects are linked to direct activation of GLP-1R on bone remains to be determined, given conflicting reports GLP-1R expression on bone [Jeon et al, 2014]. One theory suggests that GLP-1 acts on bone marrow stromal cells, with transcription of genes to promote osteoblast differentiation and inhibit adipocyte differentiation to ultimately favour bone formation, although this concept and presence of GLP-1R on marrow stromal cells still requires further clarification [Figure 3B]. GLP-1R mediated improvements in bone strength and quality have also been demonstrated in various distinct forms of diabetes including insulin-deficient type 1 diabetic mice [Mansur et al, 2015], insulin-resistant high fat fed diabetic mice [Mansur et al, 2019a] as well as genetically-induced type 2 diabetic animal models [Sun et al, 2016a]. The observed positive actions of sitagliptin on bone are likely due to the elevations of both GLP-1 and GIP levels [Mansur et al, 2019b], given that GIP has well documented benefits on bone in animals and humans [Gobron et al, 2020; Mabilleau et al,

2016; 2018b; Stensen et al, 2020; Vyavahare et al, 2020]. In the clinic, recent reports show exenatide to have no impact on bone fractures, whilst lixisenatide and liraglutide reduce fracture occurrence [Cheng et al, 2019].

3.6 GLP-1 and liver

Despite conflicting reports on whether hepatocytes express the GLP-1R [Gupta et al, 2010; Pyke et al, 2014], GLP-1 has been shown to positively impact hepatic gluconeogenesis, glycogen synthesis and glycolysis [Gupta et al, 2010]. In this regard, the impact of GLP-1 mimetics on liver function is likely to be linked to activation of GLP-1R on immune macrophages to attenuate T-cell mediated inflammation [Nagashima et al, 2011]. In disease states, GLP-1R mimetics reduce hyperlipidaemia, liver fibrosis and inflammation in non-alcoholic fatty liver disease (NAFLD) [Armstrong et al, 2016; Newsome et al, 2020] as well as liver fat content in type 2 diabetes [Petit et al, 2017]. Similarly, in animal models GLP-1 also imparts beneficial effects on the liver, with exendin-4 reducing oxidative stress and improving hepatic steatosis and inflammation in diabetic and atherosclerotic animal models, respectively [Sharma et al, 2011]. In animal models of both acute and chronic liver injury, liraglutide protected against hepatotoxicity, associated with a reduction in oxidative stress, improved liver mitochondrial function and insulin resistance [Guo et al, 2018; Wang et al, 2017]. Further research is required to demonstrate whether these hepatic benefits are mediated through direct GLP-1R action on hepatocytes or indirectly though GLP-1R induced weight loss, reduction in HbA1c, and augmented lipid metabolism and insulin sensitivity.

3.8 GLP-1 and fertility

Gut hormones, including GLP-1, have been shown to impact the reproductive system and effect fertility [Moffett and Naughton, 2020]. Thus, GLP-1R signalling increases menstrual

frequency and chance of pregnancy in women with polycystic ovary syndrome (PCOS) [Liu et al, 2017]. Additional actions have been identified in animal studies showing that GLP-1 mimetics can restore ovarian morphology [Sun et al, 2016b] and improve development of ovarian follicles [Yang and Wang 2016]. Moreover, GLP-1 mimetics can reduce testicular inflammation, leading to improved sperm motility and activity in diet-induced obese mice [Zhang et al, 2015]. Further to this, and although not directly related to GLP-1R mediated effects on fertility, expansion in beta-cell mass that occurs during pregnancy is linked to pancreatic alpha-cell production of GLP-1, which exerts a positive paracrine effect on neighbouring beta-cells to encourage growth and proliferation [Moffett et al, 2014]. In harmony with these findings, GLP-1R knockout mice exhibit delayed puberty, irregular oestrus cycles, impaired fertility and reduced litter sizes [MacLusky et al, 2000]. The actions of GLP-1 on fertility have yet to be fully exploited in the clinic, and further research is required to develop a suitable treatment options in respect to PCOS and infertility.

3.9 GLP-1 and the brain

The GLP-1R is expressed throughout many regions of the brain including the brainstem, cerebellum, cerebral cortex, hippocampus, hypothalamus, substantia nigra and thalamus [Cork et al, 2015]. As a result, GLP-1 receptors have important, and potential pharmacologically exploitable, effects within the central nervous system (CNS). Discussion of GLP-1 receptor mediated CNS actions, including aspects of neuroprotection, hypothalamic regulation of food intake, stress response as well as locally produced GLP-1 is largely outside the scope of our current review and is covered in detail within other reviews in this themed issue.

4 Clinically approved GLP-1R ligands

The extensive biological action profile of GLP-1 detailed above, with notable benefits in various disease states, promotes the wide therapeutic use of enzymatically stable, longer-acting GLP-1 analogues. However, to date use of GLP-1R based therapies has only been approved in the treatment of obesity and diabetes. In this regard, GLP-1R drugs can be subdivided pharmacologically by their duration of action into short-acting and long-acting classes [Aroda, 2018]. Short-acting GLP-1R ligands, namely exenatide and lixisenatide, provide shorter elevations in circulating GLP-1 levels (2-3 hours) that act quickly to delay gastric emptying and reduce postprandial blood glucose levels [Nauck et al, 2011]. Whereas, long-acting GLP-1R ligands, namely liraglutide, albiglutide, dulaglutide and exenatide-LAR, lead to more prolonged periods of GLP-1R activation (>24 hours) to reduce fasting blood glucose [Buse et al, 2009]. This more consistent elevation in plasma GLP-1 levels, and therefore potential for an uninterrupted receptor activation profile, appears to result in greater improvements in HbA1c levels when compared to short-acting GLP-1 compounds [Buse et al, 2009]. However, longer-acting GLP-1R ligands have been shown to induce some tachyphylaxis, and as such have a more limited impact on gastric motility and are therefore unable to reduce postprandial hyperglycaemia as effectively as their short-acting counterparts [Nauck et al, 2011]. Both short and long-acting GLP-1R ligands induce weight loss, confirming that this action is not secondary to delaying gastric motility, but rather due to direct actions within the CNS and hypothalamus. In addition, although highly likely, it is still unknown whether a more consistent GLP-1R activation profile is observed with longer-acting GLP-1R mimetics.

4.1 Early progress with clinically approved GLP-1R mimetics

Progress with developing new and enhanced clinically approved GLP-1R mimetics has been frequent over the years [Figure 4]. As such, exenatide was the first GLP-1R ligand drug approved for type 2 diabetes in 2005 as a twice-daily preparation [Nielsen et al, 2004], and was

swiftly followed by approval of once-daily liraglutide [Drucker et al, 2010]. Exenatide extended-release (exenatide-LAR) was the first approved once-weekly GLP-1R mimetic in 2012, followed by albiglutide, dulaglutide and semaglutide [Dhillon, 2018], with lixisenatide also gaining approval in 2016 as another once-daily administered drug option [Heimburger et al, 2019]. It should however be noted that albiglutide was globally withdrawn from the market in July 2018 for economic reasons. Nonetheless, all these GLP-1 have proven clinical effectiveness, but each requires parenteral administration due to peptidic nature of GLP-1, with obvious patient compliance issues. To date, liraglutide is the only GLP-1 mimetic approved for the treatment of obesity, albeit at a slightly increased dose than that used for diabetes therapy [Figure 4].

4.2 Recent advance with GLP-1R mimetics

Semaglutide represents the most recently approved once-weekly formulation, first gaining clinical approval in 2017. Semaglutide is comprised of human GLP-1 molecule with C-18 acylation at Lys^{26} and, amino acid substitution of Ala^{2} with Abu^{2} to impart full DPP-4 resistance and an additional Lys^{34} for Arg^{34} amino acid replacement [Buckley et al, 2018]. Collectively these alterations result in a biological half-life of approximately 7 days allowing for once-weekly administration [Dhillon, 2018]. More strikingly, recent advances in peptide formulation and delivery led to the generation of a semaglutide drug that that can be delivered orally. As such, oral semaglutide (Rybelsus) was FDA approved in 2019 (with EMA approval in early 2020) as the first non-injectable GLP-1 mimetic suitable for once-daily oral administration in humans [Hedrington, 2019]. Orally active semaglutide is formulated with an absorption enhancer, namely sodium *N*-(8-[2-hydroybenzoyl] amino) caprylate (SNAC), to encourage transcellular absorption of intact semaglutide through the gastric membrane by causing causes a localised increase in pH [Buckley et al, 2018]. This represents a major

milestone for GLP-1 therapeutics and will likely herald further unprecedented progress in this field. As a monotherapy, oral semaglutide promotes dose-dependent reductions in HbA1c and weight loss [Aroda et al, 2019]. Compared to injectable liraglutide, oral semaglutide was equally effective at reducing HbA1c during a 26 week treatment regimen, and actually exhibited superior efficacy over liraglutide following 52 weeks treatment [Pratley et al, 2019]. Moreover, in a similar head-to-head comparison (SUSTAIN-10), oral semaglutide was superior to liraglutide in reducing weight and HbA1c, but was associated with gastrointestinal adverse events [Capehorn et al, 2020]. In that regard, GIT related side effects associated with oral semaglutide resulted in 6-7% of patients discontinuing during these trials [Aroda et al, 2019; Pratley et al, 2019], but this is similar to other previously approved GLP-1 mimetics. Further to its clinical use in diabetes, once-weekly injection of 2.4 mg semaglutide has shown significant promise as an anti-obesity agent by reducing body weight by up to 15% in overweight adults [Wilding et al, 2021]. Additional clinical trials, namely PIONEER 11 and PIONEER 12, are currently ongoing to assess the safety and efficacy of oral semaglutide monotherapy or when combined with sitagliptin respectively, with data expected in 2021.

Other recent notable advances in the area of GLP-1 therapy relate to simultaneously supplementing GLP-1R signalling with activation of receptors for related hormones, that exhibit complementary mechanisms of action [Irwin and Flatt, 2009a]. The most obvious companion for GLP-1 in this regard is its sister incretin hormone GIP [Stumvoll and Tschöp, 2018]. Thus, like GLP-1, GIP exhibits prominent glucose-dependent insulinotropic actions in addition to numerous other beneficial extrapancreatic glucose-lowering actions [Irwin and Flatt, 2015]. Initially the hypoglycaemic effectiveness of GIP was believed to be severely impaired in patients with T2DM [Nauck et al, 1993b], with preclinical studies in animal models of diabetes revealing limited additive positive effects of combination therapy using long-acting, enzymatically stable GIP and GLP-1 compounds [Irwin et al, 2007a; 2007b]. However, clinical

studies clearly demonstrated that GIP insensitivity in T2DM is surmountable [Højberg et al. 2009], suggesting potential for additive antidiabetic benefits of GIP alongside GLP-1 in humans. This area of research was ultimately brought to the fore by the generation of single peptide molecules capable of co-activating GIP and GLP-1 receptors, dubbed the dual-acting 'twincretin' unimolecular drugs [Finan et al. 2013; Gault et al, 2013]. One such dual-acting drug developed by Lilly, namely tirzepatide, with bias towards the GIP receptor (GIPR) over GLP-1R [Coskun et al. 2018], is currently in Phase 3 clinical trials. In this regard, tirzepatide appears to exert remarkable positive effects on glycaemic control and body weight loss in T2DM, with benefits well beyond that observed in patients treated with GLP-1R mimetic therapy alone [Frías, 2020].

Interestingly, there is also a suggestion that inhibition of GIPR signalling can induce benefits in obesity and related diabetes [Irwin and Flatt, 2009b]. As such, activation of GIPR leads to accumulation of lipids in peripheral tissues [Irwin et al, 2020]. It follows that blockade of GIPR action could counter insulin resistance and improve metabolic status through prevention of fat deposition. Indeed, a recent observation reveals that sustained GIPR agonism actually leads to desensitisation of the GIPR to impart metabolic benefits [Killion et al, 2020]. The therapeutic benefits of combined GLP-1R agonism and GIPR antagonism have also been investigated, with largely positive outcomes observed in preclinical studies [Irwin et al, 2009; Killion et al, 2018]. Encouragingly, several other dual-, or even triple-acting, compounds with a GLP-1 backbone have been produced, and many of these reveal clear metabolic benefits over GLP-1R agonism alone [Bhat et al, 2013; Hasib et al, 2018; Irwin et al, 2015; Jall et al, 2017; Khajavi et al, 2018; Pathak et al, 2018]. In brief, it appears that combinatorial unimolecular therapies, that incorporate GLP-1R benefits together with the metabolic advantages of other related GIT-derived hormones, have unmistakeable therapeutic potential for obesity, diabetes and beyond.

5 Closing remarks

It is somewhat hard to fathom that a single gut-derived hormone like GLP-1 can exert such significant beneficial actions across multiple organ systems, with clear therapeutic potential. Correctly utilising this hormone to take full advantage of all such biological actions has the potential to treat multiple pathologies and provide benefit to many patients. To date, approval for use of GLP-1 mimetics has only be gained in diabetes and obesity. However, additional positive effects of GLP-1R activation in the GIT, liver, bone and kidney as well as the reproductive cardiovascular and central nervous systems, whether direct or indirect, suggests further readily exploitable clinical potential. Finally, significant advancements in the pharmaceutical development of GLP-1 based drugs, leading from initial generation of injectable short- and long-acting mimetics to now orally active GLP-1 receptor ligands, opens up the therapeutic benefits of this class of drugs to a much wider cohort of patients. It is clear that GLP-1R mimetics have had a dramatic and positive impact on diabetes and obesity treatment regimens within a relatively short time period, and we await further progress on the therapeutic utility of GLP-1 based drugs with real optimism. This may ultimately involve exploitation with other metabolically active gut hormones in the form of unimolecular dual or triple acting receptor agonists.

Conflict of Interest

NI and PRF hold patents for exploitation of gut peptide based therapeutics. PRF also serves on scientific advisory boards and has received speaker's honoraria and research support from several companies with interests in glucose-lowering drugs and incretin-based therapies. NT declares no conflict of interest.

Author Contributions

All authors contributed equally to conception and design, analysis and interpretation of data. NT drafted the manuscript, with PRF and NI revising it critically for important intellectual content. All authors approved the final version of the manuscript.

References

Alvarez, E., Martínez M.D., Roncero, I., Chowen, J.A., García-Cuartero, B., Gispert, J.D., Sanz, C., … Desco, M. (2005). The expression of GLP-1 receptor mRNA and protein allows the effect of GLP-1 on glucose metabolism in the human hypothalamus and brainstem. *Journal of Neurochemistry*, **92**, 798-806. https://doi.org/10.1111/j.1471-4159.2004.02914.x

Anini, Y., Hansotia, T., & Brubaker, P.L. (2002). Muscarinic receptors control postprandial release of glucagon-like peptide-1: in vivo and in vitro studies in rats. *Endocrinology*, **143**, 2420-2426. https://doi.org/10.1210/endo.143.6.8840

Arakawa, M., Ebato, C., Mita, T., Hirose, T., Kawamori, R., Fujitani, Y., & Watada, H. (2009). Effects of exendin-4 on glucose tolerance, insulin secretion, and beta-cell proliferation depend on treatment dose, treatment duration and meal contents. *Biochemical and Biophysical Research Communications*, **390**, 809-814. https://doi.org/10.1016/j.bbrc.2009.10.054

Armstrong, M.J., Gaunt, P., Aithal, G.P., Barton, D., Hull, D., Parker, R., … Stocken, D. (2016). Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *The Lancet*, **387**, 679-690. https://doi.org/10.1016/S0140-6736(15)00803-X

Aroda, V.R. (2018). A review of GLP-1 receptor agonists: evolution and advancement, through the lens of randomised controlled trials. *Diabetes, Obesity and Metabolism*, **20**, 22-33.

Aroda, V.R., Rosenstock, J., Terauchi, Y., Altuntas, Y., Lalic, N.M., Villegas, E.C.M., … Haluzík, M. (2019). PIONEER 1: randomized clinical trial of the efficacy and safety of oral semaglutide monotherapy in comparison with placebo in patients with type 2 diabetes. *Diabetes Care*, **42**, 1724-1732. https://doi.org/10.2337/dc19-0749

Asmar, A., Asmar, M., Simonsen, L., Madsbad, S., Holst, J.J., Hartmann, B., … Bülow, J. (2017). Glucagon-like peptide-1 elicits vasodilation in adipose tissue and skeletal muscle in healthy men. *Physiological Reports*, **5**, e13073. https://doi.org/10.14814/phy2.13073

Asmar, A., Cramon, P.K., Asmar, M., Simonsen, L., Sorensen, C.M., Madsbad, S., … Bülow, J. (2021). The renal extraction and the natriuretic action of GLP-1 in humans depend on interaction with the GLP-1 receptor. *The Journal of Clinical Endocrinology & Metabolism*, **106**, e11-e19. https://doi.org/10.1210/clinem/dgaa643

Asmar, A., Cramon, P.K., Asmar, M., Simonsen, L., Sorensen, C.M., Madsbad, S., & Bülow, J. (2020). Increased oral sodium chloride intake in humans amplifies selectively

postprandial GLP-1 but not GIP, CCK, and gastrin in plasma. *Physiological Reports*, **8**, e14519. https://doi.org/10.14814/phy2.14519.

Asmar, A., Cramon, P.K., Simonsen, L., Asmar, M., Sorensen, C.M., Madsbad, S., … Bülow, J. (2019). Extracellular Fluid Volume Expansion Uncovers a Natriuretic Action of GLP-1: A Functional GLP-1–Renal Axis in Man. *The Journal of Clinical Endocrinology & Metabolism*, **104**, 2509-2519. https://doi.org/10.1210/jc.2019-00004

Ast, J., Arvaniti, A., Fine, N.H., Nasteska, D., Ashford, F.B., Stamataki, Z., … Sasaki, S. (2020). Super-resolution microscopy compatible fluorescent probes reveal endogenous glucagon-like peptide-1 receptor distribution and dynamics. *Nature Communications*, **11**, 1-18. https://doi.org/10.1038/s41467-020-14309-w

Baggio, L.L., & Drucker, D.J. (2007). Biology of incretins: GLP-1 and GIP. *Gastroenterology*, **132**, 2131-2157. https://doi.org/10.1053/j.gastro.2007.03.054

Baggio, L.L., Ussher, J.R., McLean, B.A., Cao, X., Kabir, M.G., Mulvihill, E.E., & Drucker, D.J. (2017). The autonomic nervous system and cardiac GLP-1 receptors control heart rate in mice. *Molecular Metabolism*, **6**, 1339-1349. https://doi.org/10.1016/j.molmet.2017.08.010.

Baggio, L.L., Yusta, B., Mulvihill, E.E., Cao, X.., Streutker, CJ., Butany, J., … Drucker, D.J. (2018). GLP-1 receptor expression within the human heart. *Endocrinology*, **159**, 1570-1584. https://doi.org/10.1210/en.2018-00004

Basalay, M.V., Davidson, S.M., & Yellon, D.M. (2019). Neuroprotection in Rats Following Ischaemia-Reperfusion Injury by GLP-1 Analogues—Liraglutide and Semaglutide. *Cardiovascular Drugs and Therapy*, **33**, 661-667. https://doi.org/10.1007/s10557-019-06915-8

Bethel, M.A., Patel, R.A., Merrill, P., Lokhnygina, Y., Buse, J.B., Mentz, R.J., … Maggioni, A.P. (2018). Cardiovascular outcomes with glucagon-like peptide-1 receptor agonists in patients with type 2 diabetes: a meta-analysis. *The Lancet Diabetes & Endocrinology*, **6**, 105-113. https://doi.org/10.1016/s2213-8587(17)30412-6

Bhat, V.K., Kerr, B.D., Vasu, S., Flatt, P.R., and Gault, V.A. (2013). A DPP-IV-resistant triple-acting agonist of GIP, GLP-1 and glucagon receptors with potent glucose-lowering and insulinotropic actions in high-fat-fed mice. *Diabetologia*, **56**: 1417-1424. https://doi.org/10.1007/s00125-013-2892-2

Biancolin, A.D., Martchenko, A., Mitova, E., Gurges, P., Michalchyshyn, E., Chalmers, J.A., … Gribble, F.M. (2020). The core clock gene, Bmal1, and its downstream target, the

SNARE regulatory protein secretagogin, are necessary for circadian secretion of glucagon-like peptide-1. *Molecular Metabolism*, **31**, 124-137. https://doi.org/10.1016/j.molmet.2019.11.004

Biggs, E.K., Liang, L., Naylor, J., Madalli, S., Collier, R., Coghlan, M.P., … Gribble, F.M. (2018). Development and characterisation of a novel glucagon like peptide-1 receptor antibody. *Diabetologia*, **61**, 711-721. https://doi.org/10.1007/s00125-017-4491-0

Bjørnholm, K.D., Skovsted, G.F., Mitgaard-Thomsen, A., Rakipovski, G., Tveden-Nyborg, P., Lykkesfeldt, J., & Povlsen G.K. (2020). Liraglutide treatment improves endothelial function in the Ldlr−/− mouse model of atherosclerosis and affects genes involved in vascular remodelling and inflammation. *Basic & Clinical Pharmacology & Toxicology*, 2020. https://doi.org/10.1111/bcpt.13486

Buckley, S.T., Bækdal, T.A., Vegge, A., Maarbjerg, S.J., Pyke, C., Ahnfelt-Rønne, J., … Pedersen, B.L. (2018). Transcellular stomach absorption of a derivatized glucagon-like peptide-1 receptor agonist. *Science Translational Medicine*, **10**, 467. https://doi.org/10.1126/scitranslmed.aar7047

Buse, J.B., Rosenstock, J., Sesti, G., Schmidt, W.E., Montanya, E., Brett, J.H., … LEAD-6 Study Group. (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). *The Lancet*, **374**, 39-47. https://doi.org/10.1016/s0140-6736(09)60659-0

Campbell, J.E., & Drucker, D.J. (2013). Pharmacology, physiology, and mechanisms of incretin hormone action. *Cell Metabolism*, **17**, 819-837. https://doi.org/10.1016/j.cmet.2013.04.008

Capehorn, M.S., Catarig, A.M., Furberg, J.K., Janez, A., Price, H.C., Tadayon, S., … Marre, M. (2020). Efficacy and safety of once-weekly semaglutide 1.0 mg vs once-daily liraglutide 1.2 mg as add-on to 1–3 oral antidiabetic drugs in subjects with type 2 diabetes (SUSTAIN 10). *Diabetes & Metabolism*, **46**, 100-109. https://doi.org/10.1016/j.diabet.2019.101117

Cheng, L., Hu, Y., Li, Y.Y., Cao, X., Bai, N., Lu, T.T., … Mao, X.M. (2019). Glucagon-like peptide-1 receptor agonists and risk of bone fracture in patients with type 2 diabetes: A meta-analysis of randomized controlled trials. *Diabetes/Metabolism Research and Reviews*, **35**, e3168. https://doi.org/10.1002/dmrr.3168

Chisholm, C., & Greenberg, G.R. (2002). Somatostatin-28 regulates GLP-1 secretion via somatostatin receptor subtype 5 in rat intestinal cultures. *American Journal of Physiology-Endocrinology and Metabolism*, **282**, E311-E317. https://doi.org/10.1152/ajpendo.00434.2001

Clemmensen, C., Smajilovic, S., Smith, E.P., Woods, S.C., Bräuner-Osborne, H., Seeley, R.J., … Ryan, K.K. (2013). Oral L-arginine stimulates GLP-1 secretion to improve glucose tolerance in male mice. *Endocrinology*, **154**, 3978-3983. https://doi.org/10.1210/en.2013-1529

Cork, S.C., Richards, J.E., Holt, M.K., Gribble, F.M., Reimann, F., & Trapp, S. (2015). Distribution and characterisation of Glucagon-like peptide-1 receptor expressing cells in the mouse brain. *Molecular Metabolism*, **4**, 718-731. https://doi.org/10.1016/j.molmet.2015.07.008

Coskun, T., Sloop, K.W., Loghin, C., Alsina-Fernandez, J., Urva, S., Bokvist, K.B., … Kuchibhotla, U. (2018). LY3298176, a novel dual GIP and GLP-1 receptor agonist for the treatment of type 2 diabetes mellitus: from discovery to clinical proof of concept. *Molecular Metabolism*, **18**, 3-14. https://doi.org/10.1016/j.molmet.2018.09.009

Dai, C., Hang, Y., Shostak, A., Poffenberger, G., Hart, N., Prasad, N., … Bottino, R. (2017). Age-dependent human β cell proliferation induced by glucagon-like peptide 1 and calcineurin signalling. *The Journal of Clinical Investigation*, **127**, 3835-3844. https://doi.org/10.1172/jci91761

Deacon, C.F., Pridal, L., Klarskov, L., Olesen, M., & Holst, J.J. (1996). Glucagon-like peptide 1 undergoes differential tissue-specific metabolism in the anesthetized pig. *American Journal of Physiology-Endocrinology and Metabolism*, **271**, E458-E464. https://doi.org/10.1152/ajpendo.1996.271.3.e458

De Marinis, Y.Z., Salehi, A., Ward, C.E., Zhang, Q., Abdulkader, F., Bengtsson, M., … Habib, A.M. (2010). GLP-1 inhibits and adrenaline stimulates glucagon release by differential modulation of N-and L-type Ca2+ channel-dependent exocytosis. *Cell Metabolism*, **11**, 543-553. https://doi.org/10.1016/j.cmet.2010.04.007

Dhillon, S. (2018). Semaglutide: first global approval. *Drugs*, **78**, 275-284. https://doi.org/10.1007/s40265-018-0871-0

Diakogiannaki, E., Pais, R., Tolhurst, G., Parker, H.E., Horscroft, J., Rauscher, B., … Reimann, F. (2013). Oligopeptides stimulate glucagon-like peptide-1 secretion in mice through proton-coupled uptake and the calcium-sensing receptor. *Diabetologia*, **56**, 2688-2696. https://doi.org/10.1007/s00125-013-3037-3

Doyle, M.E. & Egan, J.M. (2007). Mechanisms of action of glucagon-like peptide 1 in the pancreas. *Pharmacology & Therapeutics*, **113**, 546-593. https://doi.org/10.1016/j.pharmthera.2006.11.007

Drucker, D.J., Dritselis, A., & Kirkpatrick, P. (2010). Liraglutide. *Nature Reviews Drug Discovery*, **9**, 267-268. https://doi.org/10.1038/nrd3148

Eissele, R., Göke, R., Willemer, S., Harthus, H.P., Vermeer, H., Arnold, R.E., & Göke, B. (1992). Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. *European Journal of Clinical Investigation*, **22**, 283-291. https://doi.org/10.1111/j.1365-2362.1992.tb01464.x

Faber, R., Zander, M., Pena, A., Michelsen, M.M., Mygind, N.D., & Prescott, E. (2015). Effect of the glucagon-like peptide-1 analogue liraglutide on coronary microvascular function in patients with type 2 diabetes–a randomized, single-blinded, cross-over pilot study. *Cardiovascular Diabetology*, **14**, 41. https://doi.org/10.1186/s12933-015-0206-3

Fandiño, J., Toba, L., González-Matías, L.C., Diz-Chaves, Y. & Mallo, F. (2020). GLP-1 receptor agonist ameliorates experimental lung fibrosis. *Scientific Reports*, **10**, 1-15. https://doi.org/10.1038/s41598-020-74912-1

Farb, T.B., Adeva, M., Beauchamp, T.J., Cabrera, O., Coates, D.A., Meredith, T.D… Martinez-Grau, M.A. (2017). Regulation of endogenous (male) rodent GLP-1 secretion and human islet insulin secretion by antagonism of somatostatin receptor 5. *Endocrinology*, **158**, 3859-3873. https://doi.org/10.1210/en.2017-00639

Finan, B, Ma, T., Ottaway, N., Müller, T.D., Habegger, K.M., Heppner, K.M., … Lockie, S.H. (2013). Unimolecular dual incretins maximize metabolic benefits in rodents, monkeys, and humans. *Science Translational Medicine*, **5**, 209ra151. https://doi.org/10.1126/scitranslmed.3007218

Frías, J.P. (2020). Tirzepatide: a glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) dual agonist in development for the treatment of type 2 diabetes. *Expert Review of Endocrinology & Metabolism*, **15**, 379-394. https://doi.org/10.1080/17446651.2020.1830759

Fujita, H., Morii, T., Fujishima, H., Sato, T., Shimizu, T., Hosoba, M., … Seino, Y. (2014). The protective roles of GLP-1R signaling in diabetic nephropathy: possible mechanism and therapeutic potential. *Kidney International*, **85**, 579-589. https://doi.org/10.1038/ki.2013.427

Garg, V., Verma, S., & Connelly, K. (2019). Mechanistic insights regarding the role of SGLT2 inhibitors and GLP1 agonist drugs on cardiovascular disease in diabetes. *Progress in Cardiovascular Diseases*, **62**, 349-357. https://doi.org/10.1016/j.pcad.2019.07.005

Gault, V.A., Bhat, V.K., Irwin, N., & Flatt, P.R. (2013). A novel glucagon-like peptide-1 (GLP-1)/glucagon hybrid peptide with triple-acting agonist activity at glucose-dependent

insulinotropic polypeptide, GLP-1, and glucagon receptors and therapeutic potential in high fat-fed mice. *Journal of Biological Chemistry*, **288**, 35581-35591. https://doi.org/10.1074/jbc.m113.512046

Gedulin, B.R., Rink, T.J., & Young, A.A. (1997). Dose-response for glucagonostatic effect of amylin in rats. *Metabolism*, **46**, 67-70. https://doi.org/10.1016/s0026-0495(97)90170-0

Gobron, B., Bouvard, B., Vyavahare, S., Blom, L.V., Pedersen, K.K., Windeløv, J.A., … Wice, B. (2020). Enteroendocrine K Cells Exert Complementary Effects to Control Bone Quality and Mass in Mice. *Journal of Bone and Mineral Research*, **35**, 1363-1374. https://doi.org/10.1002/jbmr.4004

Goyal, R.K., Guo, Y. & Mashimo, H. (2019). Advances in the physiology of gastric emptying. *Neurogastroenterology & Motility*, **31**, e13546. https://doi.org/10.1111/nmo.13546

Guo, J., Li, C., Yang, C., Li, B., Wei, J., Lin, Y., … Li, J. (2018). Liraglutide reduces hepatic glucolipotoxicity-induced liver cell apoptosis through NRF2 signaling in Zucker diabetic fatty rats. *Molecular Medicine Reports*, **17**, 8316-8324. https://doi.org/10.3892/mmr.2018.8919

Gupta, N.A., Mells, J., Dunham, R.M., Grakoui, A., Handy, J., Saxena, N.K., & Anania, F.A. (2010). Glucagon-like peptide-1 receptor is present on human hepatocytes and has a direct role in decreasing hepatic steatosis in vitro by modulating elements of the insulin signaling pathway. *Hepatology*, **51**, 1584-1592. https://doi.org/10.1002/hep.23569

Hare, K.J. (2010). Role of GLP-1 induced glucagon suppression in type 2 diabetes mellitus. *Danish Medical Bulletin*, **57**, B4181.

Hare, K.J., Knop, F.K., Asmar, M., Madsbad, S., Deacon, C.F., Holst, J.J., & Vilsbøll, T. (2009). Preserved inhibitory potency of GLP-1 on glucagon secretion in type 2 diabetes mellitus. *The Journal of Clinical Endocrinology & Metabolism*, **94**, 4679-4687. https://doi.org/10.1210/jc.2009-0921

Härndahl, L., Jing, X.J., Ivarsson, R., Degerman, E., Ahrén B., Manganiello, V.C., R… Holst, L.S. (2002). Important role of phosphodiesterase 3B for the stimulatory action of cAMP on pancreatic β-cell exocytosis and release of insulin. *Journal of Biological Chemistry*, **277**, 37446-37455. https://doi.org/10.1074/jbc.m205401200

Hasib, A., Ng, M.T., Khan, D., Gault, V.A., Flatt, P.R., & Irwin, N. (2018). A novel GLP-1/xenin hybrid peptide improves glucose homeostasis, circulating lipids and restores GIP sensitivity in high fat fed mice. *Peptides* **100**, 202-211. https://doi.org/10.1016/j.peptides.2017.10.015

Hedrington, M.S., & Davis, S.N. (2019). Oral semaglutide for the treatment of type 2 diabetes. *Expert Opinion on Pharmacotherapy*, **20**, 133-141. https://doi.org/10.1080/14656566.2018.1552258

Heimbürger, S.M, Brønden, A., Johansen, N.J., Dejgaard, T.F., Vilsbøll, T., & Knop, F.K. (2019). The efficacy and safety of exenatide once weekly in patients with type 2 diabetes. *Expert Opinion on Pharmacotherapy*, **20**, 501-510. https://doi.org/10.1080/14656566.2019.1571040

Hogan, A.E., Tobin, A.M., Ahern, T., Corrigan, M.A., Gaoatswe, G., Jackson, R., … Kirby, B, (2011). Glucagon-like peptide-1 (GLP-1) and the regulation of human invariant natural killer T cells: lessons from obesity, diabetes and psoriasis. *Diabetologia*, **54**, 2745-2754. https://doi.org/10.1007/s00125-011-2232-3

Højberg, P.V., Vilsbøll, T., Rabøl, R., Knop, F.K., Bache, M., Krarup, T., … Madsbad, S. (2009). Four weeks of near-normalisation of blood glucose improves the insulin response to glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide in patients with type 2 diabetes. *Diabetologia*, **52**,199-207. https://doi.org/10.1007/s00125-008-1195-5

Holman, R.R., Bethel, M.A., Mentz, R.J., Thompson, V.P., Lokhnygina, Y., Buse, J.B., … Maggioni, A.P. (2017) Effects of once-weekly exenatide on cardiovascular outcomes in type 2 diabetes. *New England Journal of Medicine*, **377**, 1228-1239. https://doi.org/10.1056/nejmoa1612917

Holz, G.G. (2004). Epac: a new cAMP-binding protein in support of glucagon-like peptide-1 receptor-mediated signal transduction in the pancreatic β-cell. *Diabetes*, **53**, 5-13. https://doi.org/10.2337/diabetes.53.1.5 https://dx.doi.org/10.2147%2FCOPD.S175145

Hviid, A.V.R., & Sørensen, C.M. (2020). Glucagon-like peptide-1 receptors in the kidney: impact on renal autoregulation. *American Journal of Physiology-Renal Physiology*, **318**, F443-F454. https://doi.org/10.1152/ajprenal.00280.2019

Irwin, N., & Flatt, P.R. (2009a). Therapeutic potential for GIP receptor agonists and antagonists. *Best Practice & Research Clinical Endocrinology & Metabolism*, **23**, 499-512. https://doi.org/10.1016/j.beem.2009.03.001

Irwin, N. & Flatt, P.R. (2009b). Evidence for beneficial effects of compromised gastric inhibitory polypeptide action in obesity-related diabetes and possible therapeutic implications. *Diabetologia*, **52**, 1724-1731. https://doi.org/10.1007/s00125-009-1422-8

Irwin, N., & Flatt, P.R. (2015). New perspectives on exploitation of incretin peptides for the treatment of diabetes and related disorders. *World Journal of Diabetes*, **6**, 1285. https://doi.org/10.4239/wjd.v6.i15.1285

Irwin, N., Gault, V.A., O'Harte, F.P. & Flatt, P.R. (2020). Blockade of gastric inhibitory polypeptide (GIP) action as a novel means of countering insulin resistance in the treatment of obesity-diabetes. *Peptides*, **125**, 170203. https://doi.org/10.1016/j.peptides.2019.170203

Irwin, N, McClean, P.L., Cassidy, R.S., O'Harte, F.P., Green, B.D., Gault, V.A., … Flatt, P.R. (2007a). Comparison of the anti-diabetic effects of GIP-and GLP-1-receptor activation in obese diabetic (ob/ob) mice: studies with DPP IV resistant N-AcGIP and exendin (1–39) amide. *Diabetes/Metabolism Research and Reviews*, **23**, 572-579. https://doi.org/10.1002/dmrr.729

Irwin, N., McClean, P.L., Hunter, K. & Flatt, P.R. (2009). Metabolic effects of sustained activation of the GLP-1 receptor alone and in combination with background GIP receptor antagonism in high fat–fed mice. *Diabetes, Obesity and Metabolism*, **11**, 603-610. https://doi.org/10.1111/j.1463-1326.2009.01036.x

Irwin, N., McClean, P.L., and Flatt, P.R. (2007b). Comparison of the subchronic antidiabetic effects of DPP IV–resistant GIP and GLP-1 analogues in obese diabetic (ob/ob) mice. *Journal of Peptide Science: An Official Publication of the European Peptide Society*, **13**, 400-405. https://doi.org/10.1002/psc.861

Irwin, N., Pathak, V., & Flatt, P.R. (2015). A novel CCK-8/GLP-1 hybrid peptide exhibiting prominent insulinotropic, glucose-lowering, and satiety actions with significant therapeutic potential in high-fat–fed mice. *Diabetes*, **64**, 2996-3009. https://doi.org/10.2337/db15-0220

Jall, S., Sachs, S., Clemmensen, C., Finan, B., Neff, F., DiMarchi, R.D., … Hofmann, S.M. (2017). Monomeric GLP-1/GIP/glucagon triagonism corrects obesity, hepatosteatosis, and dyslipidemia in female mice. *Molecular Metabolism*, **6**, 440-446. https://doi.org/10.1016/j.molmet.2017.02.002

Jeon, Y.K., Bae, M.J., Kim, J.I., Kim, J.H., Choi, S.J., Kwon, S.K., … Kim, I.J. (2014). Expression of glucagon-like peptide 1 receptor during osteogenic differentiation of adipose-derived stem cells. *Endocrinology and Metabolism*, **29**, 567-573. https://doi.org/10.3803/enm.2014.29.4.567

Khajavi, N., Finan, B., Kluth, O., Müller, T.D., Mergler, S., Schulz, A., … Tschöp, M.H. (2018). An incretin-based tri-agonist promotes superior insulin secretion from murine

pancreatic islets via PLC activation. *Cellular Signalling*, **51**, 13-22. https://doi.org/10.1016/j.cellsig.2018.07.006

Killion, E.A., Chen, M., Falsey, J.R., Sivits, G., Hager, T., Atangan, L., … Cheng, Y. (2020). Chronic glucose-dependent insulinotropic polypeptide receptor (GIPR) agonism desensitizes adipocyte GIPR activity mimicking functional GIPR antagonism. *Nature Communications*, **11**, 1-17. https://doi.org/10.1038/s41467-020-18751-8

Killion, E.A., Wang, J., Yie, J., Shi, S.D.H., Bates, D., Min, X., … Lu, S.C. (2018). Anti-obesity effects of GIPR antagonists alone and in combination with GLP-1R agonists in preclinical models. *Science Translational Medicine*, **10**, eaat3392. https://doi.org/10.1126/scitranslmed.aat3392

Kim, M., Platt, M.J., Shibasaki, T., Quaggin, S.E., Backx, P.H., Seino, S., … Drucker, D.J. (2013). GLP-1 receptor activation and Epac2 link atrial natriuretic peptide secretion to control of blood pressure. *Nature Medicine*, **19**, 567-575. https://doi.org/10.1038/nm.3128

Kolterman, O.G., Kim, D.D., Shen, L., Ruggles, J.A., Nielsen, L.L., Fineman, M.S., & Baron, A.D. (2005). Pharmacokinetics, pharmacodynamics, and safety of exenatide in patients with type 2 diabetes mellitus. *American Journal of Health-System Pharmacy*, **62**, 173-181. https://doi.org/10.1093/ajhp/62.2.173

Lee, Y.S., & Jun, H.S. (2016). Anti-inflammatory effects of GLP-1-based therapies beyond glucose control. *Mediators of Inflammation*, **2016**, 3094642. https://doi.org/10.1155/2016/3094642

Lejeune, M.P, Westerterp, K.R., Adam, T.C., Luscombe-Marsh, N.D., & Westerterp-Plantenga, M.S. (2006). Ghrelin and glucagon-like peptide 1 concentrations, 24-h satiety, and energy and substrate metabolism during a high-protein diet and measured in a respiration chamber. *The American Journal of Clinical Nutrition*, **83**, 89-94. https://doi.org/10.1093/ajcn/83.1.89

Lewis, J.E., Miedzybrodzka, E.L., Foreman, R.E., Woodward, O.R., Kay, R.G., Goldspink, D.A., ... Reimann, F. (2020). Selective stimulation of colonic L cells improves metabolic outcomes in mice. *Diabetologia*, **63**, 1396-1407. https://doi.org/10.1007/s00125-020-05149-w

Li, X., Wu, W., Wang, Y., Zhang, X., Feng, X., & Liu, R. (2020). GLP-1 Agonists Liraglutide Improved Vascular Endothelial Function in Type 2 Diabetes Rats. *Diabetes Research: Open Access*, **2**, 46. https://doi.org/10.36502/2020/droa.6168

Li, Y., Hansotia, T., Yusta, B., Ris, F., Halban, P.A., & Drucker, D.J. (2003). Glucagon-like peptide-1 receptor signaling modulates β cell apoptosis. *Journal of Biological Chemistry*, **278**, 471-478. https://doi.org/10.1074/jbc.m209423200

Light, P.E., Manning Fox, J.E., Riedel, M.J., & Wheeler, M.B. (2002). Glucagon-like peptide-1 inhibits pancreatic ATP-sensitive potassium channels via a protein kinase A-and ADP-dependent mechanism. *Molecular Endocrinology*, **16**, 2135-2144. https://doi.org/10.1210/me.2002-00844

Liu, X., Zhang, Y., Zheng, S.Y., Lin, R., Xie, Y.J., Chen, H., … Xu, W. (2017). Efficacy of exenatide on weight loss, metabolic parameters and pregnancy in overweight/obese polycystic ovary syndrome. *Clinical Endocrinology*, **87**, 767-774. https://doi.org/10.1111/cen.13454

Lund, M.L., Sorrentino, G., Egerod, K.L., Kroone, C., Mortensen, B., Knop, F.K., … Schoonjans, K. (2020). L-Cell Differentiation Is Induced by Bile Acids Through GPBAR1 and Paracrine GLP-1 and Serotonin Signaling. *Diabetes*, **69**, 614-623. https://doi.org/10.2337/db19-0764

Lund, P.K., Goodman, R.H., Dee, P.C., Habener, J.F. (1982). Pancreatic preproglucagon cDNA contains two glucagon-related coding sequences arranged in tandem. *Proceedings of the National Academy of Sciences of the United States of America*, **79**, 345-359. https://doi.org/10.1073/pnas.79.2.345.

Mabilleau, G., Gobron, B., Mieczkowska, A., Perrot, R., & Chappard, D. (2018b). Efficacy of targeting bone-specific GIP receptor in ovariectomy-induced bone loss. *Journal of Endocrinology*, **239**, 215-227. https://doi.org/10.1530/joe-18-0214

Mabilleau, G., Mieczkowska, A., Irwin, N., Flatt, P.R., & Chappard, D. (2013). Optimal bone mechanical and material properties require a functional glucagon-like peptide-1 receptor. *Journal of Endocrinology*, **219**, 59-68. https://doi.org/10.1530/joe-13-0146

Mabilleau, G, Pereira, M., & Chenu, C. (2018a). Novel skeletal effects of glucagon-like peptide-1 (GLP-1) receptor agonists. *Journal of Endocrinology*, **236**, R29-R42. https://doi.org/10.1530/JOE-17-0278

Mabilleau, G., Perrot, R., Mieczkowska, A., Boni, S., Flatt, P.R., Irwin, N., & Chappard, D. (2016). Glucose-dependent insulinotropic polypeptide (GIP) dose-dependently reduces osteoclast differentiation and resorption. *Bone*, **91**, 102-112. https://doi.org/10.1016/j.bone.2016.07.014

MacDonald, P.E., & Wheeler, M.B. (2003). Voltage-dependent K+ channels in pancreatic beta cells: role, regulation and potential as therapeutic targets. *Diabetologia*, **46**, 1046-1062. https://doi.org/10.1007/s00125-003-1159-8

MacLusky, N.J., Cook, S., Scrocchi, L., Shin, J., Kim, J., Vaccarino, F., … Drucker, D.J. (2000). Neuroendocrine function and response to stress in mice with complete disruption of glucagon-like peptide-1 receptor signaling. *Endocrinology*, **141**, 752-762. https://doi.org/10.1210/endo.141.2.7326

Mansur, S.A., Mieczkowska, A., Bouvard, B., Flatt, P.R., Chappard, D., Irwin, N., & Mabilleau, G. (2015). Stable incretin mimetics counter rapid deterioration of bone quality in type 1 diabetes mellitus. *Journal of Cellular Physiology*, **230**, 3009-3018. https://doi.org/10.1002/jcp.25033

Mansur, S.A., Mieczkowska, A., Flatt, P.R., Chappard, D., Irwin, N., & Mabilleau, G. (2019a). The GLP-1 receptor agonist exenatide ameliorates bone composition and tissue material properties in high fat fed diabetic mice. *Frontiers in Endocrinology*, **10**, 51. https://doi.org/10.3389/fendo.2019.00051

Mansur, S.A., Mieczkowska, A., Flatt, P.R., Chappard, D., Irwin, N., & Mabilleau, G. (2019b). Sitagliptin Alters Bone Composition in High-Fat-Fed Mice. *Calcified Tissue International*, **104**, 437-448. https://doi.org/10.1007/s00223-018-0507-0

Marso, S.P., Bain, S.C., Consoli, A., Eliaschewitz, F.G., Jódar, E., Leiter, L.A., … Woo, V. (2016a) Semaglutide and cardiovascular outcomes in patients with type 2 diabetes. *New England Journal of Medicine*, **375**, 1834-1844. https://doi.org/10.1056/nejmoa1607141

Marso, S.P., Daniels, G.H., Brown-Frandsen, K., Kristensen, P., Mann, J.F., Nauck, M.A., … Steinberg, W.M. (2016b). Liraglutide and cardiovascular outcomes in type 2 diabetes. *New England Journal of Medicine*, **375**, 311-322. https://doi.org/10.1056/nejmoa1603827

Mayo, K.E., Miller, L.J., Bataille, D., Dalle, S., Göke, B., Thorens, B., & Drucker, D.J. (2003). International Union of Pharmacology. XXXV. The glucagon receptor family. *Pharmacological Reviews*, **55**, 167-194. https://doi.org/10.1124/pr.55.1.6

Meier, J.J., Gallwitz, B., Salmen, S., Goetze, O., Holst, J.J., Schmidt, W.E., & Nauck, M.A. (2003). Normalization of glucose concentrations and deceleration of gastric emptying after solid meals during intravenous glucagon-like peptide 1 in patients with type 2 diabetes. *The Journal of Clinical Endocrinology & Metabolism*, **88**, 2719-2725. https://doi.org/10.1210/jc.2003-030049

Meier, J.J., Rosenstock, J., Hincelin-Méry, A., Roy-Duval, C., Delfolie, A., Coester, H.V., … Kapitza, C. (2015). Contrasting effects of lixisenatide and liraglutide on postprandial

glycemic control, gastric emptying, and safety parameters in patients with type 2 diabetes on optimized insulin glargine with or without metformin: a randomized, open-label trial. *Diabetes Care*, **38**, 1263-1273. https://doi.org/10.2337/dc14-1984

Mentis, N., Vardarli, I., Köthe, L.D., Holst, J.J., Deacon, C.F., Theodorakis, M., … Nauck, M.A. (2011). GIP does not potentiate the antidiabetic effects of GLP-1 in hyperglycemic patients with type 2 diabetes. *Diabetes*, **60**, 1270-1276. https://doi.org/10.2337/db10-1332

Moffett, R.C, & Naughton, V. (2020). Emerging role of GIP and related gut hormones in fertility and PCOS. *Peptides*, **125**, 170233. https://doi.org/10.1016/j.peptides.2019.170233

Moffett, R.C., Vasu, S., Thorens, B., Drucker, D.J., & Flatt, P.R. (2014). Incretin receptor null mice reveal key role of GLP-1 but not GIP in pancreatic beta cell adaptation to pregnancy. *PLoS One*, **9**, e96863. https://doi.org/10.1371/journal.pone.0096863

Mojsov, S., Weir, G.C., & Habener, J.F. (1987). Insulinotropin: glucagon-like peptide I (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. *The Journal of Clinical Investigation*, **79**, 616-619. https://doi.org/10.1172/JCI112855

Nagashima, M., Watanabe, T., Terasaki, M., Tomoyasu, M., Nohtomi, K., Kim-Kaneyama, J., Miyazaki, A., Hirano, T. (2011) Native incretins prevent the development of atherosclerotic lesions in apolipoprotein E knockout mice. *Diabetologia*, **54**, 2649-2659. https://doi.org/10.1007/s00125-011-2241-2.

Nauck, M.A., Heimesaat, M.M., Orskov, C., Holst, J.J., Ebert, R., & Creutzfeldt, W. (1993b). 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*, **91**, 301-307. https://doi.org/10.1172/jci116186

Nauck, M.A., Kleine, N., Ørskov, C., Holst, J.J., Willms, B., & Creutzfeldt, W. (1993a). Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7-36 amide) in type 2 (non-insulin-dependent) diabetic patients. *Diabetologia*, **36**, 741-744. https://doi.org/10.1007/bf00401145

Nauck, M.A., Kemmeries, G., Holst, J.J., & Meier, J.J. (2011). Rapid tachyphylaxis of the glucagon-like peptide 1–induced deceleration of gastric emptying in humans. *Diabetes*, **60**, 1561-1565. https://doi.org/10.2337/db10-0474

Newsome, P.N., Buchholtz, K., Cusi, K., Linder, M., Okanoue, T., Ratziu, V., … Harrison, S.A. (2020). A placebo-controlled trial of subcutaneous semaglutide in nonalcoholic steatohepatitis. *New England Journal of Medicine*, Epub ahead of print. https://doi.org/10.1056/nejmoa2028395

Nielsen, L.L., Young, A.A., & Parkes, D.G. (2004). Pharmacology of exenatide (synthetic exendin-4): a potential therapeutic for improved glycemic control of type 2 diabetes. *Regulatory Peptides*, **117**, 77-88. https://doi.org/10.1016/j.regpep.2003.10.028

Nikolaidis, L.A., Mankad, S., Sokos, G.G., Miske, G., Shah, A., Elahi, D., & 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. https://doi.org/10.1161/01.cir.0000120505.91348.58

Nystrom, T., Gutniak, M.K., Zhang, Q., Zhang, F., Holst, J.J., Ahrén, B., & Sjoholm, A. (2004). Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary artery disease. *American Journal of Physiology-Endocrinology and Metabolism*, **287**, E1209-E1215. https://doi.org/10.1152/ajpendo.00237.2004

Ørgaard, A., & Holst, J.J. (2017). The role of somatostatin in GLP-1-induced inhibition of glucagon secretion in mice. *Diabetologia*, **60**, 1731-1739. https://doi.org/10.1007/s00125-017-4315-2

Pacheco-Pantoja, E.L., Ranganathm L.R., Gallagherm J.A., Wilsonm P.J., & Fraserm W.D. (2011). Receptors and effects of gut hormones in three osteoblastic cell lines. *BMC Physiology*, **11**, 1-14. https://doi.org/10.1186/1472-6793-11-12

Parker, H.E., Adriaenssens, A., Rogers, G., Richards, P., Koepsell, H., Reimann, F., & Gribble, F.M. (2012). Predominant role of active versus facilitative glucose transport for glucagon-like peptide-1 secretion. *Diabetologia*, **55**, 2445-2455. https://doi.org/10.1007/s00125-012-2585-2

Pathak, N.M., Pathak, V., Gault, V.A., McClean, S., Irwin, N., & Flatt, P.R. (2018). Novel dual incretin agonist peptide with antidiabetic and neuroprotective potential. *Biochemical Pharmacology*, **155**, 264-274. https://doi.org/10.1016/j.bcp.2018.07.021

Petit, J.M., Cercueil, J.P., Loffroy, R., Denimal, D., Bouillet, B., Fourmont, C., … Verges, B. (2017). Effect of liraglutide therapy on liver fat content in patients with inadequately controlled type 2 diabetes: the Lira-NAFLD study. *The Journal of Clinical Endocrinology & Metabolism*, **102**, 407-415. https://doi.org/10.1210/jc.2016-2775

Pratley, R., Amod, A., Hoff, S.T., Kadowaki, T., Lingvay, I., Nauck, M., … Meier, J.J. (2019). Oral semaglutide versus subcutaneous liraglutide and placebo in type 2 diabetes (PIONEER 4): a randomised, double-blind, phase 3a trial. *The Lancet*, **394**, 39-50. https://doi.org/10.2337/dc19-0749

Psichas, A., Sleeth, M.L., Murphy, K.G., Brooks, L., Bewick, G.A., Hanyaloglu, A.C., … Frost, G. (2015). The short chain fatty acid propionate stimulates GLP-1 and PYY secretion

via free fatty acid receptor 2 in rodents. *International Journal of Obesity*, **39**, 424-429. https://doi.org/10.1038/ijo.2014.153

Pyke, C., Heller, R.S., Kirk, R.K., Ørskov, C., Reedtz-Runge, S., Kaastrup, P., … Knudsen, L.B. (2014). GLP-1 receptor localization in monkey and human tissue: novel distribution revealed with extensively validated monoclonal antibody. *Endocrinology*, **155**, 1280-1290. https://doi.org/10.1210/en.2013-1934

Pyke, C., & Knudsen, L.B. (2013). The glucagon-like peptide-1 receptor—or not? *Endocrinology*, **154**, 4-8. https://doi.org/10.1210/en.2012-2124

Richards, P., Parker, H.E., Adriaenssens, A.E., Hodgson, J.M., Cork, S.C., Trapp, S., Gribble, F.M., Reimann, F (2014). Identification and characterization of GLP-1 receptor-expressing cells using a new transgenic mouse model. *Diabetes*, **63**, 1224-33. https://doi.org/10..2337/db13-1440.

Robinson, L.E., Holt, T.A., Rees, K., Randeva, H.S., & O'Hare, J.P. (2013). Effects of exenatide and liraglutide on heart rate, blood pressure and body weight: systematic review and meta-analysis. *BMJ Open*, **3**, e001986. https://doi.org/10.1136/bmjopen-2012-001986

Rocca, A.S., & Brubaker, P.L. (1999). Role of the vagus nerve in mediating proximal nutrient-induced glucagon-like peptide-1 secretion. *Endocrinology*, **140**, 1687-1694. https://doi.org/10.1210/endo.140.4.6643

Ronn, J., Jensen, E.P., Wewer Albrechtsen, N.J., Holst, J.J. & Sorensen, C.M. (2017). Glucagon-like peptide-1 acutely affects renal blood flow and urinary flow rate in spontaneously hypertensive rats despite significantly reduced renal expression of GLP-1 receptors. *Physiological Reports*, **5**, e13503. https://doi.org/10.14814/phy2.13503

Roscioni, S.S., Heerspink, H.J.L., & De Zeeuw, D. (2014). The effect of RAAS blockade on the progression of diabetic nephropathy. *Nature Reviews Nephrology*, **10**, 77. https://doi.org/10.1038/nrneph.2013.251

Samols, E., & Marks, V. (1967). New conceptions on the functional significance of glucagon (pancreatic and extra-pancreatic). Journees Annuelles de Diabetologie de l'Hotel-Dieu, **7**, 43–66.

Saponaro, C., Gmyr, V., Thévenet, J., Moerman, E., Delalleau, N., Pasquetti, G., … Vantyghem, M.C. (2019). The GLP1R agonist liraglutide reduces hyperglucagonemia induced by the SGLT2 inhibitor dapagliflozin via somatostatin release. *Cell Reports*, **28**, 1447-1454.
https://doi.org/10.1016/j.celrep.2019.07.009

Sharma, S., Mells, J.E., Fu, P.P., Saxena, N.K., & Anania, F.A. (2011). GLP-1 analogs reduce hepatocyte steatosis and improve survival by enhancing the unfolded protein response and promoting macroautophagy. *PloS One*, **6**, e25269. https://doi.org/10.1371/journal.pone.0025269

Sjøberg, K.A., Holst, J.J., Rattigan, S., Richter, E.A. & Kiens, B., 2014. GLP-1 increases microvascular recruitment but not glucose uptake in human and rat skeletal muscle. *American Journal of Physiology-Endocrinology and Metabolism*, **306**, E355-E362. https://doi.org/10.1152/ajpendo.00283.2013

Skov, J., Dejgaard, A., Frøkiær, J., Holst, J.J., Jonassen, T., Rittig S., & Christiansen, J.S. (2013). Glucagon-like peptide-1 (GLP-1): effect on kidney hemodynamics and renin-angiotensin-aldosterone system in healthy men. *The Journal of Clinical Endocrinology & Metabolism*, **98**, E664-E671. https://doi.org/10.1210/jc.2012-3855

Smits, M.M., Tonneijck, L., Muskiet, M.H.A., Kramer, M.H.H., Cahen, D.L., & van Raalte, D.H. (2016). Gastrointestinal actions of glucagon-like peptide-1-based therapies: glycaemic control beyond the pancreas. *Diabetes, Obesity and Metabolism*, **18**, 224-235. https://doi.org/10.1111/dom.12593

Stensen, S., Gasbjerg, L.S., Helsted, M.M., Hartmann, B., Christensen, M.B., & Knop, F.K. (2020). GIP and the gut-bone axis–Physiological, pathophysiological and potential therapeutic implications. *Peptides*, **125**, 170197. https://doi.org/10.1016/j.peptides.2019.170197

Stumvoll, M. & Tschöp, M. (2018). Twice the benefits with twincretins?. *The Lancet*, **392**, 2142-2144. https://doi.org/10.1016/S0140-6736(18)32466-8

Sun, F., Wu, S., Guo, S., Yu, K., Yang, Z., Li, L., … Zhan, S. (2015). Impact of GLP-1 receptor agonists on blood pressure, heart rate and hypertension among patients with type 2 diabetes: a systematic review and network meta-analysis. *Diabetes Research and Clinical Practice*, **110**, 26-37. https://doi.org/10.1016/j.diabres.2015.07.015

Sun, H.X., Lu, N., Liu, D.M., Zhao, L., Sun, L.H., Zhao, H.Y., … Tao, B. (2016a). The bone-preserving effects of exendin-4 in ovariectomized rats. *Endocrine*, **51**, 323-332. https://doi.org/10.1007/s12020-015-0667-x

Sun, L., Ji, C., Jin, L., Bi, Y., Feng, W., Li, P., … Zhu, D. (2016b). Effects of Exenatide on metabolic changes, sexual hormones, inflammatory cytokines, adipokines, and weight change in a DHEA-treated rat model. *Reproductive Sciences*, **23**, 1242-1249. https://doi.org/10.1177/1933719116635278

Tanaka, A., & Node, K. (2018). Clinical application of glucagon-like peptide-1 receptor agonists in cardiovascular disease: lessons from recent clinical cardiovascular outcomes trials. *Cardiovascular Diabetology*, **17**, 1-6. https://doi.org/10.1186/s12933-018-0731-y

Tanday, N., Flatt, P.R., Irwin, N., & Moffett, R.C. (2020). Liraglutide and sitagliptin counter beta-to alpha-cell transdifferentiation in diabetes. *Journal of* Endocrinology, **245**, 53-64. https://doi.org/10.1530/joe-19-0451

Tolessa, T., Gutniak, M., Holst, J.J., Efendic, S., & Hellström, P.M. (1998). Inhibitory effect of glucagon-like peptide-1 on small bowel motility. Fasting but not fed motility inhibited via nitric oxide independently of insulin and somatostatin. *The Journal of Clinical Investigation*, **102**, 764-774. https://doi.org/10.1172/jci942

Tolhurst, G., Zheng, Y., Parker, H.E., Habib, A.M., Reimann, F., & Gribble, F.M. (2011). Glutamine triggers and potentiates glucagon-like peptide-1 secretion by raising cytosolic Ca2+ and cAMP. *Endocrinology*, **152**, 405-413. https://doi.org/10.1210/en.2010-0956

Tsunekawa, S., Yamamoto, N., Tsukamoto, K., Itoh, Y., Kaneko, Y., Kimura, T., … Niki, I. (2007). Protection of pancreatic β-cells by exendin-4 may involve the reduction of endoplasmic reticulum stress; in vivo and in vitro studies. *Journal of Endocrinology*, **193**, 65-74. https://doi.org/10.1677/joe-06-0148

Ussher, J.R., Baggio, L.L., Campbell, J..E., Mulvihill, EE., Kim, M., Kabir, M.G., … Drucker, D.J. (2014). Inactivation of the cardiomyocyte glucagon-like peptide-1 receptor (GLP-1R) unmasks cardiomyocyte-independent GLP-1R-mediated cardioprotection. *Molecular Metabolism*, **3**, 507-517. https://doi.org/10.1016/j.molmet.2014.04.009

Vyavahare, S.S., Mieczkowska, A., Flatt, P.R., Chappard, D., Irwin, N., & Mabilleau, G. (2020). GIP analogues augment bone strength by modulating bone composition in diet-induced obesity in mice. *Peptides*, **125**, 170207. https://doi.org/10.1016/j.peptides.2019.170207

Wang, D., Luo, P., Wang, Y., Li, W., Wang, C., Sun, D., … Wang, H. (2013). Glucagon-like peptide-1 protects against cardiac microvascular injury in diabetes via a cAMP/PKA/Rho-dependent mechanism. *Diabetes*, **62**, 1697-1708. https://doi.org/10.2337/db12-1025

Wang, X., Zhou, J., Doyle, M.E., & Egan, J.M. (2001). Glucagon-like peptide-1 causes pancreatic duodenal homeobox-1 protein translocation from the cytoplasm to the nucleus of pancreatic β-cells by a cyclic adenosine monophosphate/protein kinase A-dependent mechanism. *Endocrinology*, **142**, 1820-1827. https://doi.org/10.1210/endo.142.5.8128

Wang, Z., Hou, L., Huang, L., Guo, J., & Zhou, X. (2017). Exenatide improves liver mitochondrial dysfunction and insulin resistance by reducing oxidative stress in high fat

diet-induced obese mice. *Biochemical and Biophysical Research Communications*, **486**, 116-123. https://doi.org/10.1016/j.bbrc.2017.03.010

Waser, B., Blank, A., Karamitopoulou, E., Perren, A., & Reubi, JC. (2015). Glucagon-like-peptide-1 receptor expression in normal and diseased human thyroid and pancreas. *Modern Pathology*, **28**, 391-402. https://doi.org/10.1038/modpathol.2014.113

Wendt, A., Birnir, B., Buschard, K., Gromada, J., Salehi, A., Sewing, S., … Braun, M. (2004). Glucose inhibition of glucagon secretion from rat α-cells is mediated by GABA released from neighboring β-cells. *Diabetes*, **53**, 1038-1045. https://doi.org/10.2337/diabetes.53.4.1038

Wilding, J.P., Batterham, R.L., Calanna, S., Davies, M., Van Gaal, L.F., Lingvay, I., McGowan, B.M., Rosenstock, J., Tran, M.T., Wadden, T.A. and Wharton, S. (2021) Once-Weekly Semaglutide in Adults with Overweight or Obesity. *New England Journal of Medicine*. Epub ahead of print. https://doi.org/10.1056/nejmoa2032183

Yang, Q., & Wang, F. (2016). Successful pregnancy after improving insulin resistance with the glucagon-like peptide-1 analogue in a woman with polycystic ovary syndrome: a case report and review of the literature. *Gynecologic and Obstetric Investigation*, **81**, 477-480. https://doi.org/10.1159/000446951

Yusta, B., Baggio, L.L., Koehler, J., Holland, D., Cao, X., Pinnell, L.J., … Sherman, P.M. (2015). GLP-1R agonists modulate enteric immune responses through the intestinal intraepithelial lymphocyte GLP-1R. *Diabetes*, **64**, 2537-2549. https://doi.org/10.2337/db14-1577

Zhang, E., Xu, F., Liang, H., Yan, J., Xu, H., Li, Z., … Weng, J. (2015). GLP-1 receptor agonist exenatide attenuates the detrimental effects of obesity on inflammatory profile in testis and sperm quality in mice. *American Journal of Reproductive Immunology*, **74**, 457-466. https://doi.org/10.1111/aji.12420

Zhou, H., Zhang, T., Harmon, J.S., Bryan, J., & Robertson, R.P. (2007). Zinc, not insulin, regulates the rat α-cell response to hypoglycemia in vivo. *Diabetes*, **56**, 1107-1112. https://doi.org/10.2337/db06-1454

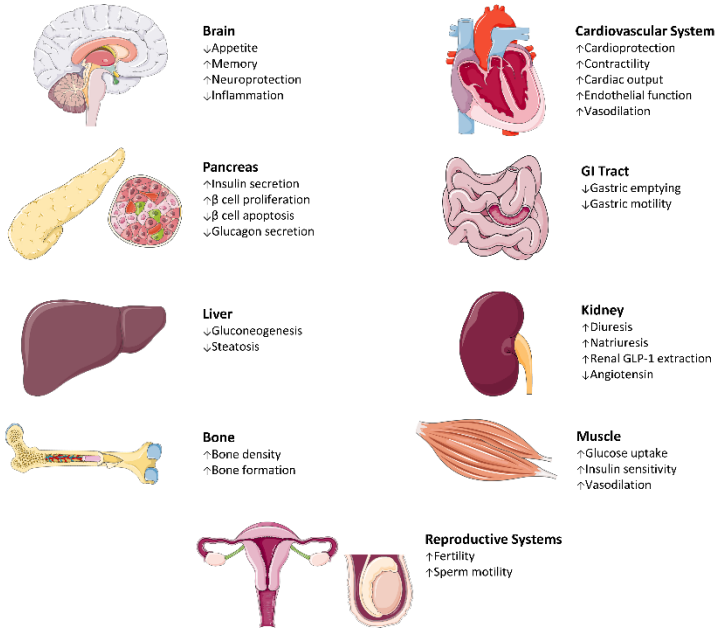

Figure 1. The metabolic actions of GLP-1 across diverse organs including the pancreas, brain, gastrointestinal (GI) tract, liver, muscle, bone, kidney as well as the cardiovascular and reproductive systems.

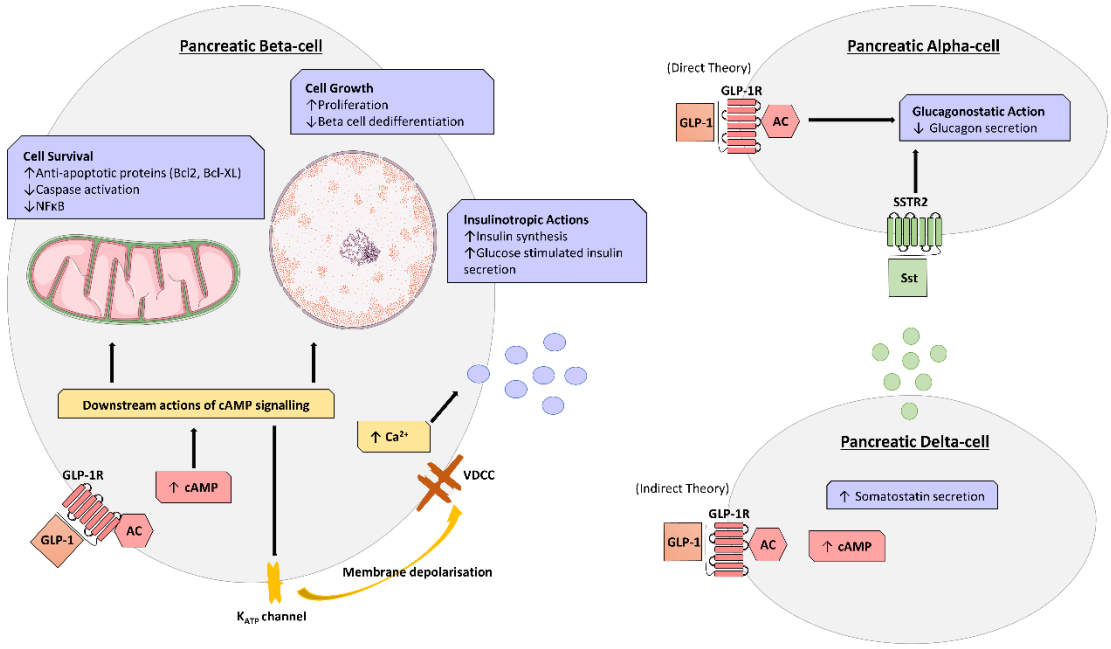

Figure 2. Actions of GLP-1 within pancreatic alpha-, beta- and delta-cells. In beta-cells, GLP-1 binding to its receptor triggers intracellular signalling cascades that positively influence insulin synthesis and secretion as well as beta-cell proliferation and survival. The direct and indirect effects, mediated through the delta-cell, of GLP-1R activation on inhibition of alpha-cell derived glucagon is also considered.

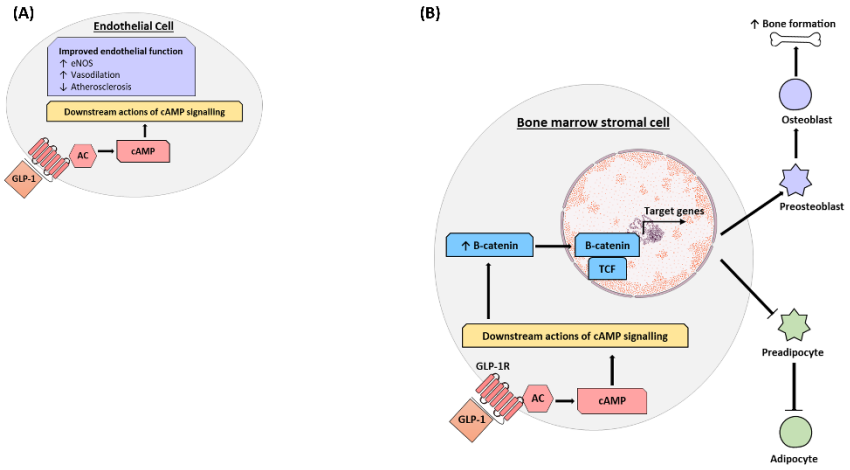

Figure 3. Actions of GLP-1 on (A) endothelial cells and (B) bone. (A) GLP-1 triggers a signalling cascade inside endothelial cells to mediate vasodilation and reduce atherosclerosis, to collectively improve cardiovascular health. (B) GLP-1 binds to its receptor on bone marrow stromal cells to activate intracellular signalling cascades that prevent the breakdown of B-catenin. This augments gene expression to favour differentiation of the bone marrow stromal cell into pre-osteoblasts, rather than pre-adipocytes, and increase bone formation.

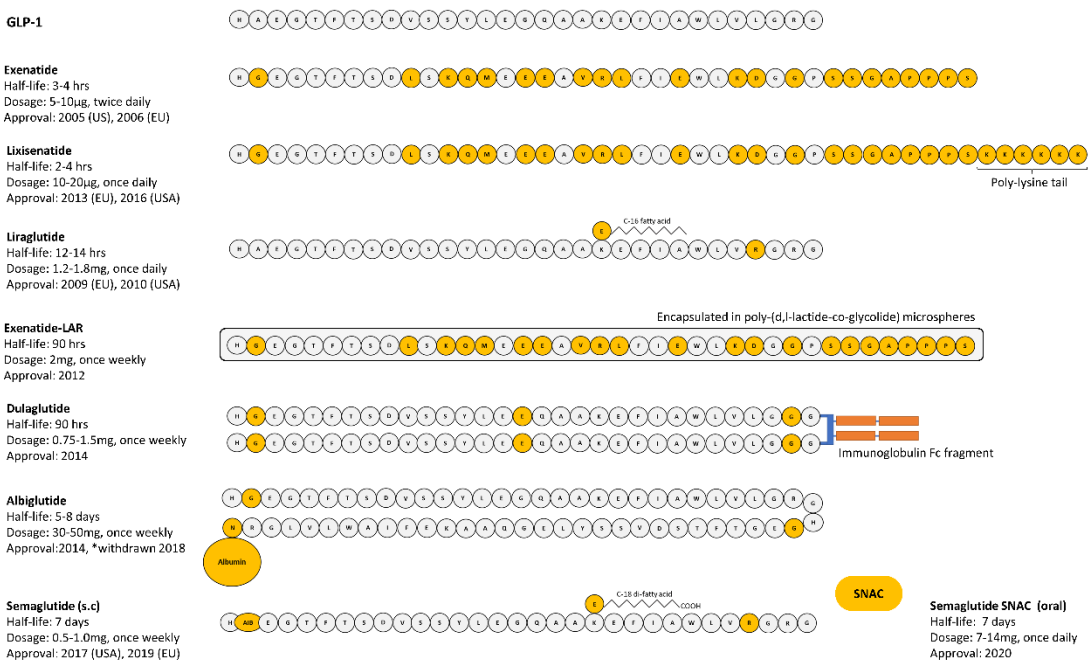

Figure 4. Clinically approved GLP-1 mimetics prescribed for the treatment of diabetes. Structural modifications of GLP-1 mimetics, compared to native GLP-1, are highlighted in gold. Information on drug half-lives, dosage and date of initial approval is also included. *withdrawn from the market in 2018.