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Department of Physiology, Institute of Biomedicine and Translational Medicine, Faculty of Medicine, University of Tartu

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III Sedman, T., Rünkorg, K., Krass, M., Luuk, H., Plaas, M., Vasar, E., & Volke, V. (2016). Exenatide is an effective antihyperglycaemic agent in a mouse model of Wolfram Syndrome 1. *Journal of Diabetes Research*, *2016*. https://doi.org/10.1155/2016/9239530

The author participated in designing the studies, performing the animal experiments (I, III), conducting the clinical trial (II), analysing the results, and writing the manuscripts.

ABBREVIATIONS

AC	Adenylate cyclase
ACTH	Adrenocorticotropic hormone
ANOVA	Analysis of variance
ATF6	Transcription factor 6
ATP	Adenosine triphosphate
ARRIVE	Animal Research: Reporting of In Vivo Experiments
AUC	Area under the curve
cAMP	Cyclic adenosine monophosphate
CNS	Central nervous system
CVOT	Cardiovascular outcome trials
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPP4	Dipeptidyl-peptidase 4
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
ER	Endoplasmic reticulum
ERK	Extracellular signal-related kinase
FDA	Food and Drug Administration
GABA	Gamma-aminobutyric acid
GCG	Proglucagon
GCG	Proglucagon gene
GEF	Guanine nucleotide exchange factor
GGIT	Graded glucose infusion test
GIP	Glucose-dependent insulinotropic polypeptide
GI	Gastrointestinal
GLP1	Glucagon-like peptide 1
GLP2	Glucagon-like peptide 2
GLP1R	Glucagon-like peptide 1 receptor
GLP1RA	Glucagon-like peptide 1 receptor agonist
GLUT2	Glucose transporter 2
GRPP	Glicentin-related pancreatic polypeptide
GSIS	Glucose-stimulated insulin secretion
HbA1c	Haemoglobin A1c
HNF1	Hepatocyte nuclear factor 1
HPA	Hypothalamic-pituitary-adrenal
i.c.v	Intracerebroventricular
i.p	Intraperitoneal
IPGTT	Intraperitoneal glucose tolerance test
IP1	Intervening peptide 1
IR	Immediate-release
IRE1	Inositol-requiring protein 1
ISR	Insulin secretion rate

LAR	Long-acting release
MACE	Major adverse cardiovascular events
MAPK	Mitogen-activated protein kinase
MODY	Maturity-onset diabetes of the young
MPGF	Major proglucagon fragment
NASH	Non-alcoholic fatty liver disease
NFAT	Nuclear factor of activated T cells
NIH	National Institutes of Health
NTS	Nucleus tractus solitarius
PERK	Protein-kinase RNA-like ER kinase
PC	Prohormone convertase
Pdx1	Pancreas duodenum homeobox 1
PI3K	Phosphatidylinositol 3 kinase
PKA	Protein kinase A
p.o	Orally
RNA	Ribonucleic acid
s.c	Subcutaneously
SEM	Standard error of the mean
SGLT2	Sodium-glucose cotransporter 2
T2DM	Type 2 diabetes mellitus
T1DM	Type 1 diabetes mellitus
UPR	Unfolded protein response
VDCC	Voltage-dependent Ca^{2+} channels
WFS1	Wolfram syndrome 1 gene in humans
Wfs1	Wolfram syndrome 1 gene in mice
WFS1	Wolfram syndrome 1 protein (wolframin)
WS	Wolfram syndrome
WSD	Wolfram syndrome-related diabetes

1. INTRODUCTION

Glucagon-like peptide 1 (GLP1) is an important regulator of homeostatic processes in the alimentary and central nervous systems. GLP1 receptor agonists (GLP1RA) have become a popular tool for treating type 2 diabetes mellitus (T2DM), displaying benefits beyond their anti-hyperglycaemic action. The key benefits of GLP1RAs include a low risk of hypoglycaemia and modest weight loss, with some of them having been proven to be protective agents against cardiovascular events. Moreover, unconventional therapeutic effects have been proposed for the drug class, e.g., neuroprotective effect in Parkinson's (Athauda et al., 2017) and Alzheimer's disease (Perry & Greig, 2005), alcohol dependence (Thomsen et al., 2017), chemotherapy-induced vascular toxicity (Altieri et al., 2017), and septic complications (Steven et al., 2017). GLP1RAs seem to affect both peripheral and central targets. The appetite-suppressing and weight-reducing effects of GLP1 analogues are a combination of GLP1 receptor effects on the vagus nerve and in the area postrema, as well as in the underlying nucleus of the solitary tract (Secher et al., 2014), and the administration of GLP1RAs has been demonstrated to reduce food-induced brain responses (van Bloemendaal et al., 2014).

There are several GLP1RAs approved for the treatment of T2DM. Unlike many other anti-diabetic drug classes, distinct GLP1RAs have different therapeutic profiles, primarily due to their different pharmacokinetic properties. For example, one of the effects of GLP1RA administration is decreased gastric motility. It has been demonstrated that tolerance to that effect develops with long-acting GLP1RAs but not with the short-acting molecule (Jelsing et al., 2012). Furthermore, it is well known that gastrointestinal adverse effects, such as nausea and vomiting, subside with prolonged treatment (Gamble et al., 2015). As there is clear evidence that some effects of GLP1RAs are subject to tachyphylaxis/tolerance development, it is logical to ask whether the core effect of these drugs on glucose regulation may also be affected. We have studied whether tolerance develops toward the glucose-lowering effect of GLP1RAs.

GLP1RAs have been successfully used to treat certain forms of monogenic diabetes, which are traditionally managed by sulfonylureas. We have tested whether a rare type of diabetes associated with Wolfram syndrome may respond to GLP1 receptor agonists.

2. LITERATURE REVIEW

2.1. Glucagon-like peptide 1

2.1.1. Incretins and "incretin effect"

The term "incretin" was coined in 1932 by the Belgian physiologist Jean La Barre after discovering that an isolated fraction of the gut extract decreased the blood glucose level in experimental animals (La Barre, 1932). Since then, many decades have passed in the search for those glucose-lowering gut substances. In the early 1960s, thanks to the development of radioimmunoassays, several nearly simultaneous reports demonstrated that insulin secretion is much greater in response to oral glucose administration than intravenous glucose administration (Elrick et al., 1964; Mcintyre et al., 1964). The phenomenon became known as "the incretin effect" (Rehfeld, 2018). Gastric inhibitory peptide (GIP) was discovered in 1970 and named based on its ability to inhibit gastric acid secretion (Brown et al., 1970). However, later studies demonstrated that GIP could stimulate insulin secretion under physiological conditions (Dupre et al., 1973); the hormone was renamed glucose-dependent insulinotropic polypeptide (GIP) to retain the acronym. In 1983 the human glucagon gene was cloned and sequenced, which led to the discovery that the prohormone proglucagon contained, in addition to the pancreatic glucagon, two novel peptides. The peptides were named glucagon-like peptide 1 and glucagon-like peptide 2 (GLP2) because of the structural similarities with glucagon (Bell et al., 1983). Although the original definition suggests that incretin is any gut hormone that stimulates the secretion of hormones from the pancreatic endocrine cells, the narrower definition is used nowadays. Incretins are the gut hormones that, in a glucose-dependent manner, stimulate insulin secretion. To date, only GIP and GLP1 fulfil the definition; they additively stimulate insulin secretion and contribute equally to the incretin effect (Baggio & Drucker, 2007).

2.1.2. GLP1 synthesis, secretion, and clearance

Synthesis: GLP1 is produced by the proteolytic cleavage of its precursor proglucagon (Fig. 1). Proglucagon, precursor molecule for various hormones, is encoded by the proglucagon gene (*GCG*), which is expressed in multiple cells, prevailingly in pancreas alpha-cells, intestine enteroendocrine L-cells, and in brainstem neurons (Drucker & Asa, 1988; Mojsov et al., 1986; Nian et al., 1999). Proglucagon proteolytic cleavage is a tissue-specific process because of selective expression of the prohormone convertase (PC) and results in different products in different locations. In neurons and enteroendocrine L-cells, mostly prohormone convertase 1 (PC1) is expressed; therefore, the cleavage products are GLP1, GLP2, oxyntomodulin, glicentin, and intervening peptide 1 (IP1). In pancreatic alpha cells, PC2 is expressed, and proglucagon is cleaved into

glucagon, IP1, major proglucagon fragment (MPGF), and glicentin-related pancreatic polypeptide (GRPP) (Holst et al., 1994; Rouille et al., 1995).

Several proglucagon-derived peptides have important functions in regulating metabolism, whereas others seem inactive (Holst et al., 1994). GLP1 is produced as a larger inactive molecule $GLP1_{1-37}$, which is amidated or cleaved to the bioactive "truncated" isoform-amidated GLP1 ($GLP1_{7-36}$) or glycine-extended GLP1 ($GLP1_{7-37}$) soon after synthesis. Both "truncated" isoforms are equally potent in stimulating insulin secretion (Holst et al., 1987). The ratio of GLP1 isoforms differs significantly between species. In humans, $GLP1_{7-36}$ contributes about 80% of synthesised GLP1, and $GLP1_{7-37}$ contributes about 20% (Ørskov et al., 1989). In rats, the ratio is 50/50, whereas, in mice, the GLP1 is exclusively amidated (Kuhre et al., 2014).

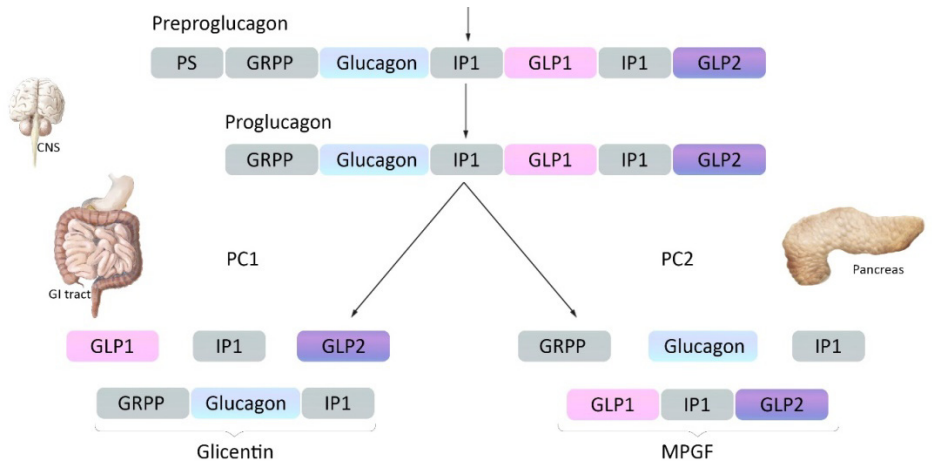

Figure 1. Tissue-specific proglucagon processing. GLP1 is encoded by the proglucagon gene (*GCG*) and cleaved by its precursor's preproglucagon and glucagon (Baggio & Drucker, 2007; Müller et al., 2019). Proglucagon post-translational processing is a tissue-specific process mediated by PC1 (in the brain and intestine) or PC2 (in pancreatic alpha-cells). IP1 – intervening peptide 1, GRPP – glicentin-related pancreatic polypeptide; PC – prohormone convertase, MPGF – major proglucagon fragment.

Secretion: GLP1 is produced in the gastrointestinal tract, nervous system, and pancreas. Most GLP1 is made in the intestine by enteroendocrine L-cells as a response to food ingestion. L-cells are located in the intestinal epithelium, with higher density in the ileum and colon (Bryant et al., 1983). GLP1 secretion occurs as a response to food intake in a biphasic pattern starting with an early phase (within 10–15 minutes) and followed by the prolonged second phase (30-60 minutes). Food consumption is the most potent stimulus for GLP1 secretion. Early rapid secretion is indirectly mediated by combining endocrine and neural

signals and the prolonged phase directly by contact between nutrients and L-cells (Eissele et al., 1992; Herrmann et al., 1995).

GLP1 is also produced in the pancreas, mainly in alpha-cells. In physiological conditions, pancreatic alpha-cells mainly produce glucagon, whereas, during metabolic stress, more GLP1 is produced (Nie et al., 2000). The mechanism seems essential to compensate for increased beta-cell functional demand under insulin resistance or cellular stress (Thyssen et al., 2006).

In the central nervous system (CNS) GLP1 is produced by the neurons' specific population. Those neurons are mainly located in the nucleus tractus solitarius (NTS) and have projections to numerous regions of the brain (Drucker & Asa, 1988; Larsen et al., 1997). GLP1-producing neurons store GLP1 in the axon terminal as a neurotransmitter (Zheng et al., 2015).

Clearance: Native GLP1 half-life is short, approximately 1-2 minutes (Hui et al., 2002). It is hydrolysed by the enzyme dipeptidyl-peptidase 4 (DPP4) or eliminated by the kidneys. DPP4 cleaves $GLP1_{7-36}$ and $GLP1_{7-37}$ at the N-terminal dipeptide. During the hydrolysis, two metabolites are generated, $GLP1_{9-36}$ or $GLP1_{9-37}$, both low-affinity ligands to the GLP1 receptor. Inactive metabolites are cleared from the circulation via renal elimination (Kieffer et al., 1995; Mentlein et al., 1993). The clearance of GLP1 varies in different species. Compared to humans, in mice, the half-life of GLP1 is even lower (Windeløv et al., 2017).

2.1.3. GLP1 receptor

GLP1 receptor (GLP1R) belongs to the secretin/glucagon receptors' superfamily and is encoded by the *GLP1R* gene. It is a heterotrimeric G-protein coupled receptor located in the cell membrane (Mayo, Miller, Bataille, 2003). A single structurally identical GLP1R has been identified. GLP1R has several endogenous ligands, such as GLP1 and glucagon. In contrast, GLP1 exclusively binds to GLP1R (Thorens, 1992).

The GLP1Rs have been discovered in various tissues, including the pancreas, nervous system, heart, kidney, lung, adipose tissue, and smooth muscle (Andersen et al., 2018). In the pancreatic islets, *GLP1R* is robustly expressed in beta-cells, moderately in delta-cells, and absent or expressed only in the small proportion in alpha-cells (Adriaenssens et al., 2016; Gray et al., 2020). Also, *GLP1R* is expressed in the pancreatic acinar cells (Hou et al., 2016).

GLP1 is produced only in a small population of neurons, whereas GLP1 receptors are widely distributed throughout the central nervous system. The exceptionally high expression of GLP1 receptors has been shown in the ventromedial hypothalamus, nucleus paraventricularis, nucleus arcuatus, hippocampus, thalamus, amygdala, putamen caudatum, and globus pallidum (Muscogiuri et al., 2017).

2.1.4. Physiological effects of GLP1

Endocrine pancreas: GLP1 produces several biological actions in the different organ systems (Fig. 2). The best-known action is stimulation of insulin secretion in pancreatic beta-cell and suppression of glucagon release in alpha-cell. The action is glucose-dependent and leads to decreased blood glucose levels (Scrocchi et al., 1996).

GLP1 binding to its receptor on pancreatic beta-cells leads to activation of adenylate cyclase (AC) and production of cyclic adenosine monophosphate (cAMP). Subsequently, cAMP mediates its effects by (1) increasing intracellular Ca^{2+}, adenylate cyclase, and phospholipase C and (2) activation of protein kinase A (PKA), protein kinase C, phosphatidylinositol 3 kinase (PI3K), Epac2, and MAPK signal transduction pathways (Nauck et al., 1993).

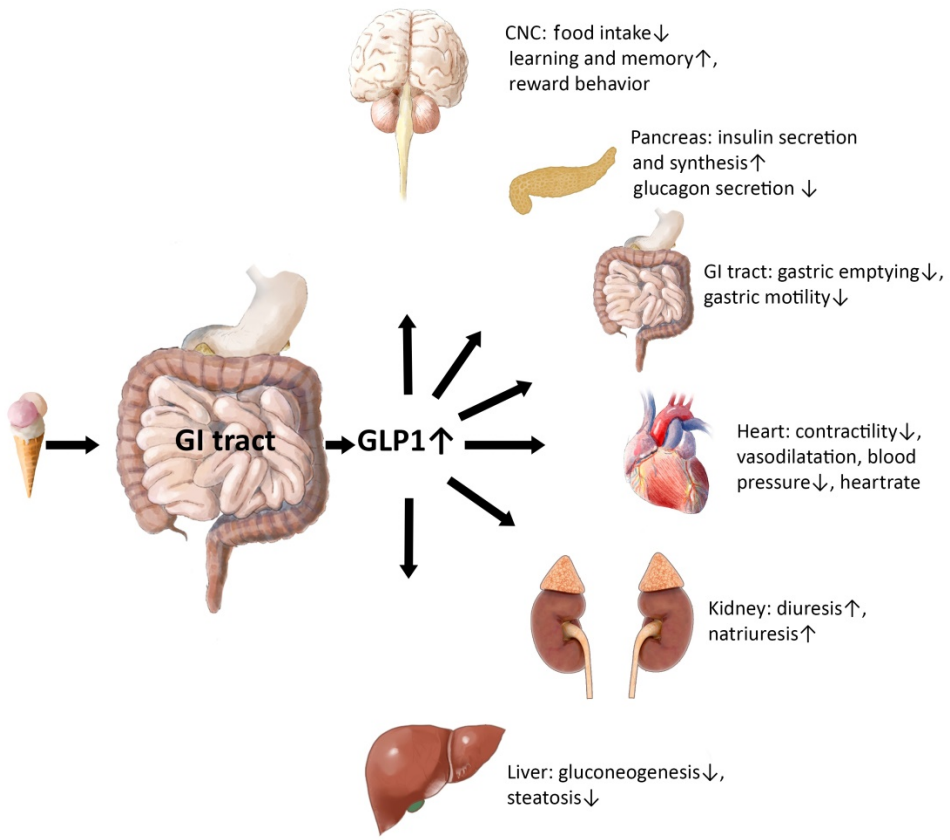

Figure 2. Physiological effects of GLP1. GLP1 is secreted from the GI tract as a response to food consumption. GLP1 has several metabolic effects. GLP1 mediates its effects by an endocrine (or paracrine) regulator or in the CNC as a neuromediator.

GLP1 also acts synergistically with glucose to promote insulin gene transcription and biosynthesis. Those mechanisms contribute to the refilling of beta-cell insulin stores. Different mechanisms have been identified, such as activating nuclear factor of activated T cells (NFAT) and pancreas duodenum homeobox 1 (Pdx1), transcription factors which are essential for pancreatic development and functioning. Moreover, the receptor-ligand binding activates early gene expression mechanisms, which lead to cell proliferation and survival (Mayo, Miller, Bataille, 2003).

GLP1 also inhibits glucagon and stimulates somatostatin secretion. The stimulatory effect of GLP1 on somatostatin secretion is caused by direct interaction with somatostatin-secreting pancreatic delta-cell (Fehmann & Habener, 1991). On the other hand, the inhibitory effect on glucagon secretion seems to be indirect since GLPR gene expression is absent in pancreatic alpha-cells (Adriaenssens et al., 2016; Gray et al., 2020). Moreover, multiple products secreted from beta-cells (insulin, Zn, GABA) inhibit glucagon secretion and might contribute to the GLP1R-dependant inhibition of alpha-cell secretory activity (Drucker, 2018).

Gastrointestinal tract: GLP1 decelerates gastric motility and emptying, which contributes to postprandial glucose control. The mechanisms underlying those actions involve central and peripheral nervous system communication, especially the activation of ascending vagal afferents (Imeryüz et al., 1997). Moreover, GLP1 regulates acid secretion in the stomach and secretion of digestive enzymes in the exocrine pancreas. GLP1 receptors are expressed in the stomach on gastric parietal cells and directly regulate gastric acid secretion (Schmidtler et al., 1994). In the pancreatic acinar cells, where GLP1 receptors are also expressed, GLP1 regulates digestive enzyme secretion by increasing the enzyme concentration in the pancreatic secrete (Hou et al., 2016).

Nervous system: GLP1 receptors and GLP1-containing neurons are present in CNS regions regulating various homeostatic functions such as feeding behaviour, gastric motility, glucose regulation, and cardiovascular function. Moreover, GLP1 signalling is associated with regulating multiple neurological and cognitive functions. Although the GLP1 molecule can diffuse passively throw the blood-brain barrier, its' rapid degradation makes direct action of peripherally secreted GLP1 in the CNS unlikely. There are three different ways in which GLP1 might affect the nervous system: (1) indirectly, by peripherally-secreted GLP1 activating the vagus nerve (Rüttimann et al., 2009); (2) by accessing the brain via the circumventricular organs, such as the area postrema, subfornical organ, median eminence and choroid plexus (Muscogiuri et al., 2017; Ørskov et al., 1996); and (3) as a neuromediator when centrally produced GLP1 binds to the GLP1 receptors in different parts of the nervous system.

GLP1 mediates inhibition of food intake, which may lead to weight loss. GLP1 anorexic effects are most likely mediated by a combination of direct and indirect actions. Peripherally secreted GLP1 binds to the receptors in enteric and

vagal sensory neurons that transmit signals to the brain stem and hypothalamus appetite-regulating centres (Hayes, Kanoski, et al., 2011; Rüttimann et al., 2009). Also, centrally produced GLP1 mediates its direct actions as a neuromediator by activating neurons in brain areas expressing GLP1R. For example, activation of GLP1 receptors in the hindbrain and cortical food reward centres in the mesolimbic reward system leads to decreased food intake and weight loss (Alhadeff et al., 2012; Hayes, Leichner, et al., 2011). Moreover, GLP1 is produced in the oral taste cells, and GLP1 receptors are expressed on taste nerve fibres, which leads to the assumption that GLP1 is associated with mediating taste sensation, mechanism, which may also contribute to the food consumption regulation (Muscogiuri et al., 2017; van Bloemendaal et al., 2014). Studies demonstrate that GLP1-producing neurons are also activated in stress situations and, therefore, may play a part in a risk assessment system (McLean et al., 2020).

GLP1R signalling has been associated with neuroprotective processes, such as anti-apoptotic actions and enhanced neuronal differentiation and proliferation (Perry, Haughey, et al., 2002; Perry, Lahiri, et al., 2002). Moreover, GLP1R-dependent pathways may be involved in memory and learning processes as these are expressed widely in the corresponding brain areas (During et al., 2003).

Other organs: In addition to regulating glucose metabolism, gastric emptying, and food intake, emerging evidence shows GLP1 involvement in various other physiological effects. In the kidney GLP1 produces natriuretic and diuretic responses which are associated with increase in glomerular filtration rate and inhibition of sodium reabsorption in the proximal tubule.(Gutzwiller et al., 2004; Kodera et al., 2011). GLP1 has been related to the activity of the hypothalamic-pituitary-adrenal axis and is known to increase corticosterone, aldosterone, and adrenocorticotropin levels (Gil-Lozano et al., 2010; Herman, 2018). In addition, GLP1 signalling has been implicated in cardiac development and functioning (Gros et al., 2003), apoptosis, inflammation, and adipogenesis, to name a few (Bose et al., 2005; Buteau et al., 2004; Challa et al., 2012; Li et al., 2003).

2.2. GLP1 receptor agonists

2.2.1. Drug development

In 1932 when La Barre coined the term “incretin”, he also suggested that this concept of gut hormones lowering the blood glucose levels could be used to treat diabetes mellitus (Barre, 1936). Thus, the idea of incretin-based therapy is nearly a century old. GLP1 signalling system has been a fruitful drug target for the treatment of T2DM. Given in physiological and supraphysiological concentrations, GLP1 stimulates insulin secretion, inhibits glucagon release, delays gastric emptying, and reduces food intake. Due to its short half-life, GLP1, as

the natural peptide, is not a beneficial therapeutic agent (Ritzel et al., 1995). Two main classes of drugs have been developed based on the incretin system: GLP1 receptor agonists (GLP1RA) and DPP4 inhibitors. Clinically used drugs are modified GLP1 analogues with improved pharmacokinetic properties (Deacon et al., 1998) and DDP4 inhibitors as "incretin enhancers", which inhibit the enzyme DPP4 resulting in the extended circulation of endogenous GLP1 and GIP (Holst & Deacon, 1998). The first GLP1RA, exenatide, was introduced to the clinical practice in 2005; the first DPP4 inhibitor, sitagliptin, was a year later (Drucker & Nauck, 2006). Since then, multiple GLP1RAs have been approved by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA), and several molecules are in development. One avenue of further drug development is investigating molecules that address not only GLP1 receptors but a second or even third receptor, such as glucagon, GIP, or peptide YY receptor (Baggio & Drucker, 2020; Frias et al., 2018).

2.2.2. Pharmacokinetics

Compared to native GLP1, all GLP1RAs are resistant to DPP4 enzymatic degradation. Also, they have structural differences that lead to slower elimination and a longer half-life. Depending on the drug's pharmacokinetics, GLP1RAs are classified as short-acting and long-acting GLP1RAs (Drucker & Nauck, 2006). Short-acting GLP1RAs, exenatide, and lixisenatide have a half-life of about 60–90 minutes. They are administered twice daily (exenatide) or once daily (lixisenatide) and are effective in far less than 24 hours. Long-acting GLP1RAs have a significantly longer half-life and are administered once-daily (liraglutide, semaglutide p.o form) or once-weekly (dulaglutide, exenatide LAR, albiglutide, semaglutide s.c form) (Tran et al., 2017). Different mechanisms are used to achieve a prolonged half-life: embedding drug into biodegradable microspheres (exenatide LAR), increasing affinity for albumin binding by adding a fatty acid chain (liraglutide, semaglutide s.c, albiglutide) or an immunoglobulin fragment (dulaglutide, efpeglenatide) to the drug molecule (Ryan et al., 2013; Tran et al., 2017). Moreover, implantable subdermal osmotic minipump ITCA 650 has been developed to provide continuous exenatide release for up to 12 months (Henry et al., 2014).

GLP1RAs are generally not suitable for oral administration as peptide molecules and are administered as subcutaneous injections. Semaglutide, the newest GLP1 in the market, has an advanced oral administration formula, but the bioavailability is still very low (0,4–1%) (Brayden et al., 2020; Hall et al., 2018). Subcutaneous bioavailability of GLP1RAs is relatively high, reaching from 62% for exenatide to 94% for semaglutide. Clearance of the GLP1RAs occurs primarily by glomerular filtration with tubular proteolysis (exenatide, lixisenatide) or through the metabolic pathway of large plasma proteins (long-acting GLP1RAs) (Hall et al., 2018; Handelsman et al., 2018). The key characteristics of GLP1RAs are provided in Table 1.

Table 1. Characteristics of glucagon-like peptide 1 receptor agonists (Hall et al., 2018; Henry et al., 2014; Nauck et al., 2020; Prato et al., 2018). IR – immediate-release, LAR – long-acting release, FDA – Food and Drug Administration, EMA – European Medicines Agency, p.o – orally, s.c – subcutaneously.

GLP1	Approved	Half-life	Structure	Administration
Exenatide IR	2005 (FDA)/ 2006 (EMA)	2.4 h	Exendin 4 analogue	Twice daily, s.c
Liraglutide	2009 (EMA)/ 2010 (FDA)	13 h	GLP1 analogue with free fatty acid	Once daily, s.c
Exenatide LAR	2011 (EMA)/ 2012 (FDA)	2 weeks	Exendin 4 analogue encapsulated in microspheres	Once weekly, s.c
Albiglutide	2014	5 days	GLP1 analogue with albumin	Once weekly, s.c
Dulaglutide	2014	5 days	GLP1 analogue with immunoglobulin Fc fragment	Once weekly, s.c
Lixisenatide	2013 (EMA)/ 2016 (FDA)	3 h	Exendin 4 analogue with poly-lysine tail	Once daily, s.c
Semaglutide	2017 (FDA)/ 2019 (EMA)	1 week	GLP1 analogue with free fatty acid	Once weekly, s.c
Semaglutide oral	2019	1 week	GLP1 analogue with free fatty acid	Once daily, p.o
Efpeglenatide	In development	1 week	GLP1 analogue with immunoglobulin Fc fragment	Once weekly, s.c

2.2.3. Pharmacodynamics

2.2.3.1. Mechanism of glucose-dependent stimulation of insulin secretion

All GLP1 receptor agonists have a common mechanism of action – binding and activating the GLP1 receptor. The GLP1 receptor is widely expressed in the pancreas, especially in beta-cells. The activation of the pancreatic beta-cell GLP1 receptor leads to increased insulin secretion and exocytosis in a glucose-dependent manner. Moreover, chronic activation of the GLP1 receptor leads to stimulation of insulin gene transcription, islet cell growth, and neogenesis (Müller et al., 2019).

Mechanisms of insulin release: GLP1 exerts its insulinotropic effects by stimulating the formation of cAMP, its primary effector, and activating down-stream pathways coupled to protein kinase A (PKA) and PKA-independent activation of guanine nucleotide exchange factor (GEF). Many different mecha-nisms have been described how GLP1 synergises with glucose and stimulates insulin secretion, whereas the exact functioning of the system and pathways

involved are yet to be discovered. Glucose-stimulated insulin secretion (GSIS) is regulated by several ionic (K_{ATP}-dependent) and non-ionic (K_{ATP}-independent) pathways:

K_{ATP}-dependent pathways: GLP1 stimulates insulin secretion by regulating the activity of several ion channels in the beta-cell membrane involved in K_{ATP}-dependent pathways. The mechanisms are: (1) inhibiting K_{ATP} channels leading to beta-cell membrane depolarisation; (2) increasing intracellular Ca^{2+} levels by increasing ion influx, and (3) mobilising intracellular Ca^{2+} storage; (4) closing K^+ channels preventing membrane repolarisation (MacDonald et al., 2002; Meloni et al., 2013).

K_{ATP}-independent pathways: Glucose can exert a stimulatory effect on insulin exocytosis, independent of its actions initiated by K_{ATP} channels' inhibition. Those mechanisms involve several signals that act on non-ionic targets, particularly the distal steps of insulin exocytosis. For this stimulatory effect, glucose metabolism is required and is mediated by ATP, cAMP, PKA, glutamate, and malonyl-CoA (MacDonald et al., 2002; Portha et al., 2011; Rowlands et al., 2018).

Effects on other islet cells: Also, GLP1 inhibits glucagon and stimulates somatostatin secretion. The stimulatory effect on somatostatin is likely related to direct interaction with GLP1R on somatostatin-secreting pancreatic delta-cells (Fehmann & Habener, 1991). In contrast, mechanisms for inhibiting glucagon secretion are less clear.

2.2.3.2. Tolerance toward GLP1RAs effects

Whether and how tolerance toward GLP1RAs develops is not fully understood. *In vitro* experiments have shown that the GLP1 receptor has the capacity for rapid desensitisation (Fehmann et al., 1991; Gromada et al., 1996; Widmann et al., 1996). In theory, GLP1RAs with dramatically prolonged exposure to GLP1 receptor, compared to native GLP1, may have an enhanced capacity to desensitise the receptors. Likewise, the phenomenon of tolerance has been demonstrated in the case of gastric effects – sustained GLP1R agonism leads to rapid receptor desensitisation (tachyphylaxis) and diminished inhibition of gastric motility (Jelsing et al., 2012; Nauck et al., 2011). Evidence for GLP1RAs tolerance toward glucose-lowering effect is vague. In our previous studies in mice, it seemed that some degree of tolerance might develop toward the glucose-lowering effect, but not toward the corticosterone stimulating effect of exenatide and liraglutide (Krass et al., 2015). Nauck et al. demonstrated that the effect of GLP1 infusion on postprandial glucose was already diminished after 6 hours, but the change was probably mediated by gastric emptying (Nauck et al., 2011). Another study reported a trend of weakening of anti-hyperglycaemic effects of GLP1RAs after chronic dosing, but authors attributed it to the change in food intake (Baggio et al., 2004). Tudurí et al. demonstrated that, in mice,

acute activation of brain GLP1 receptors enhanced glucose-stimulated insulin secretion, whereas chronic activation did not affect insulin secretion or glucose tolerance (Tudurí et al., 2015). In addition, Rolin et al. demonstrated in diabetic mice models GLP1RAs diminished glucose-lowering effect after prolonged dosing (Rolin et al., 2002).

2.2.3.3. Clinical effects of GLP1RAs

Glucose control in T2DM: Anti-hyperglycaemic effect is achieved by multiple mechanisms, including glucose-dependent stimulation of insulin release, inhibition of glucagon release, and decreased gastric emptying. The treatment of T2DM with GLP1RAs results in improved glycaemic control, as indicated by reduced haemoglobin A1c (HbA1C) and fasting glucose levels (Zander et al., 2002). Treatment with GLP1RAs achieves the lowering of HbA1c by 0.5–1.5% (Gamble et al., 2015). Short-acting GLP1RAs are inferior compared to long-acting GLP1RAs in reducing HbA1c and fasting plasma glucose levels, whereas they have a comparable effect on postprandial glucose levels (Madsbad, 2016).
GLP1 inhibits beta-cell death, induces beta-cell proliferation, and promotes beta-cell mass expansion in experimental models of diabetes; it is speculated that sustained treatment with GLP1RAs might preserve and enhance beta-cell function, thereby resulting in a disease-modifying activity. Although in clinical trials modest improvements in beta-cell function have been occasionally reported, these have not been robust, and thus, current data do not prove the hypothesis (Drucker, 2018).

Effect on food consumption and body weight: Treatment with GLP1RA is associated with anorexigenic effects, food consumption reduction, and weight loss in diabetic and non-diabetic overweight individuals (Drucker & Nauck, 2006). In long-term clinical trials, the average weight loss using GLP1RAs is approximately 2–3 kg (Vilsbøll et al., 2012). The weight loss is dose-dependent and progressive, independent of nausea and other gastrointestinal adverse effects (Amori et al., 2007). Interestingly, the weight loss can be further augmented by increasing the dose of GLP1RAs above the doses used in diabetes treatment (Nauck et al., 2016; Pi-Sunyer et al., 2015). Although GLP1RAs activate brown fat tissue in preclinical experiments and increase energy expenditure in rodents (Osaka et al., 2005), clinical trials with human subjects do not show a significant increase in fat oxidation or energy expenditure using GLP1RAs (Harder et al., 2004; Horowitz et al., 2012). The available evidence suggests that weight loss in humans is mainly connected to reducing food consumption (Faerch et al., 2015).

Cardiovascular benefits: Treatment with GLP1RAs is associated with improvements in several cardiovascular risk factors, such as blood pressure and serum lipid concentration (Bethel et al., 2018; Marso et al., 2016), and in large-

scale cardiovascular outcome trials (CVOT), some of the long-acting GLP1RAs (liraglutide, dulaglutide, semaglutide) have shown a positive effect in reducing major adverse cardiovascular events (MACE) (Goldman, 2020; Marso et al., 2016). Interestingly, the effects do not expand to all GLP1RAs as lixisenatide had no effect on MACE, and trials with exenatide long-acting release (LAR) did not reach clear statistical significance in principal outcome (Holman et al., 2017; Pfeffer et al., 2015). Whether the cardiovascular effects are mediated directly by the activation of GLP1R in the heart, found in the left ventricle, the sinoatrial node, and endothelium, or indirectly by modifying cardiovascular risk factors (weight, blood pressure, lipid-profile) or anti-inflammatory pathways have not been fully identified (Cornell, 2020). However, GLP1RAs increase the heart rate of non-diabetic humans, and no apparent adverse outcomes have been detected in individuals with T2DM (Cornell, 2020; Smits et al., 2017).

Actions in the liver: Although GLP1 receptors are not expressed in hepatocytes and expression in other non-hepatocyte liver cells is uncertain, GLP1RAs have many effects on the liver (Pyke et al., 2014). Preclinical and clinical data have demonstrated GLP1RAs positive impact in non-alcoholic steatohepatitis as reducing hepatic steatosis and decreasing its progression (Panjwani et al., 2013; Petit et al., 2016). As hepatocytes do not express GLP1R, the actions are probably indirect and mediated by neural circuits, GLP1R-dependant reduction in postprandial lipidaemia, glycaemia, and inflammation or, given the importance of weight loss in the treatment of non-alcoholic fatty liver disease (NASH), is mediated by the weight loss (Drucker, 2018).

Actions in the kidney: Collective evidence suggests that GLP1RAs have direct actions in the kidney. GLP1RAs induce diuresis and natriuresis and are associated with renoprotective effects beyond modulating metabolic risk factors for kidney disease (Crajoinas et al., 2011; Gutzwiller et al., 2004; Kodera et al., 2011; Steven P. Marso et al., 2016; Tuttle et al., 2018). However, the possible renoprotective mechanisms are not entirely understood. It is hypothesized that GLP1 receptor activation can protect the vascular endothelium reducing oxidative stress and local inflammation responses, ameliorating albuminuria and glomerular sclerosis (Cornell, 2020; Kodera et al., 2011; Sarafidis et al., 2019).

Actions in the nervous system: GLP1RAs are associated with anorexigenic effect, reward processing system, memory and learning, and range of neuroprotective abilities. Moreover, peripherally administered GLP1RAs stimulate the hypothalamic-pituitary-adrenal (HPA) axis and increase circulating corticosterone, aldosterone, and adrenocorticotropic hormone (ACTH) levels (Gil-Lozano et al., 2010). GLP1R signalling is also associated with memory and learning. Central signalling of GLP1R has been demonstrated to have neuroprotective properties (Perry, Haughey, et al., 2002; Perry, Lahiri, et al., 2002). So far, neuroprotective effects have been demonstrated only in *in vivo* experiments and animal studies. If those findings would be translatable to human subjects, they

could be potentially helpful in neurodegenerative diseases, such as Alzheimer's disease.

2.2.3.4. Adverse effects, safety, and tolerability

The most common adverse effects using GLP1RAs are gastrointestinal disturbances, namely, mild to moderate nausea, diarrhoea, and vomiting. Up to 50% of individuals experience gastrointestinal disturbances during the treatment with GLP1RAs (Drucker & Nauck, 2006). Gastrointestinal adverse effects are thought to be due to the inhibition of gastric motility and emptying. Both short- and long-acting GLP1s are associated with actions in the gastrointestinal tract; however, long-acting GLP1RAs effects seem to diminish over time due to the rapid development of tachyphylaxis and GLP1 receptor desensitisation (Nauck et al., 2011). Those adverse effects are dose-dependent, peaking during the initial weeks and decline after that (Gamble et al., 2015). Using titration regimes with slower escalation to effective doses decreases the rates of nausea and vomiting. As GLP1RAs may delay gallbladder emptying, they are associated with a higher risk of gallbladder diseases, such as cholecystitis (Pizzimenti et al., 2016). Hypoglycaemia, a common and dangerous adverse effect in many anti-diabetic drugs, is uncommon in GLP1RAs since the drug class's insulin-releasing effect is glucose-dependent.

Since the introduction of GLP1RAs in clinical practice, concerns about the drug class safety have been raised. GLP1RAs have been associated with pancreatitis and increased malignancy risk (pancreatic cancer, medullar thyroid cancer, colorectal neoplasms). Although anecdotal evidence from small clinical studies and preclinical experiments raised some concerns, no large-scale safety studies have confirmed the causal link between GLP1RA use and pancreatic diseases or malignancy (Egan et al., 2014).

2.2.4. Clinical use

Over the past 15 years, the popularity of GLP1RAs has tremendously increased as medications for T2DM and obesity. GLP1RAs are not yet a first-line treatment for T2DM in patients who are inadequately controlled with diet and exercise. Usually, GLP1RAs are the preferred drugs after metformin in patients with established cardiovascular disease or high cardiovascular risk factors (Buse et al., 2020). In some guidelines, GLP1RAs are the first-line treatment for patients with T2DM and comorbid obesity (Cosentino et al., 2020). According to international recommendations, 30–60% of patients with T2DM would qualify for treatment with GLP1RA. However, in reality, only 1–10% of patients receive the treatment. Limitations for the broader use of GLP1RAs are probably the relatively high price and administration route (Nauck et al., 2020). In addition to treatment for adult patients with T2DM, liraglutide is approved for treating obesity in adults and adolescents since 2014 and the treatment of

T2DM in children since 2019 (Nuffer & Trujillo, 2015; Tamborlane et al., 2019).

Interest in using GLP1RAs goes beyond T2DM and obesity because of their favourable safety profile and wide range of beneficial effects. GLP1RAs appear promising for treating non-alcoholic steatohepatitis (Panjwani et al., 2013; Petit et al., 2016). GLP1RAs, associated with neuroprotection, are investigated to treat neurodegenerative diseases, such as Alzheimer's and Parkinson's disease (Athauda et al., 2017; Perry & Greig, 2005). In psychiatry, the positive effects of GLP1RAs have been shown in animal models of psychosis and alcohol dependence (Dixit et al., 2013; Marty et al., 2020) and in pilot clinical trials for eating disorders (Da Porto et al., 2020). In treatment optimisation, a new approach is combining GLP1RAs with another glucose-lowering agent for improved cardiovascular outcomes. Such combinations have been studied with SGLT2 inhibitors (Frías et al., 2016).

2.3. Wolfram syndrome

2.3.1. The clinical manifestations of Wolfram syndrome

Wolfram syndrome (WS) is a rare genetic disorder affecting multiple organ systems. The syndrome was described and named by Dr. Wolfram in 1938 when he presented a study of diabetes mellitus and optic nerve atrophy in four siblings (Wolfram, 1938). Wolfram syndrome is also known by the acronym DIDMOAD, indicating its main clinical manifestations – developing diabetes insipidus, diabetes mellitus, optic nerve atrophy, and deafness (Barrett & Bundey, 1997). The prevalence of Wolfram syndrome is estimated to be between 1/700,000 in the UK and Japan 1/1,000,0000 in North America and Italy (Barrett et al., 1995; Lombardo et al., 2014; Matsunaga et al., 2014; Rigoli et al., 2020).

WS is a progressive neurodegenerative and endocrine condition. The main clinical manifestations are described in Table 2. The childhood manifestations are usually diabetes mellitus and optic nerve atrophy. During the second decade of life, diabetes insipidus occurs; third-decade renal tract abnormalities; and fourth-decade neurological complications such as cerebellar ataxia and myoclonus. Comorbidity with psychiatric diseases is high, whereas depression and affective disorders are the most common. The prognosis is poor; life expectancy is about 30–40 years. Death occurs by respiratory failure due to brain stem atrophy and neurodegeneration (Minton et al., 2003). Suicide is more common in patients with WS than in the general population (Sequeira et al., 2003).

Table 2. The clinical manifestations of Wolfram syndrome (Barrett et al., 1995; Minton et al., 2003; Pallotta et al., 2019). T1DM – type 1 diabetes mellitus, HbA1c – haemoglobin A1c.

Major clinical sign	Clinical sign description	The average age of diagnosis	Frequency
Diabetes mellitus	Compared to T1DM slower progression, longer duration of remission, lower insulin requirements, and HbA1c level, ketoacidosis is rare; episodes of severe hypoglycaemia are frequent	6 years	100%
Optic nerve atrophy	Progressive decrease of visual acuity, colour vision defect	10–11 years	100%
Diabetes insipidus	Cranial form	14–15 years	40-75%
Hearing loss	Sensorineural, progressive, affecting high frequencies	16 years	50-60%
Neurological manifestations	Cerebellar ataxia, dysarthria, dysphagia, areflexia, epilepsy	15 years	60%
Urinary tract problems	Incontinence, urinary infections, renal dysfunction	20 years	60%
Psychiatric symptoms	depression, psychosis, anxiety	20–30 years	60%
Autosomal dysfunction	central apnoea, dysphagia, areflexia, gastroparesis	20 years	60%

WS is classified as an endoplasmic reticulum (ER) disease. The disease transmission occurs in an autosomal recessive manner, but autosomal dominant mutations responsible for WS-related disorders have also been described (Pallotta et al., 2019). Two causative genes (*WFS1* and *WFS2*) have been identified. The *WFS1* gene is associated with Wolfram syndrome (sometimes referred to as Wolfram syndrome 1) and *WFS2* with Wolfram syndrome 2. Wolfram syndrome 2 is similar to childhood-onset diabetes mellitus and optic atrophy, with distinctive gastrointestinal ulcers and bleeding and absence of diabetes insipidus (Al-Sheyyab et al., 2001). Even though dominant *WFS1* mutations are rare, genome-wide association studies have shown that carriers of heterozygous *WFS1* mutations may have an increased risk for the development of type 2 diabetes and other Wolfram syndrome-associated disorders (psychiatric disease, hearing loss) (Chaussenot et al., 2011; Sandhu et al., 2007).

2.3.2. *WFS1* gene and WFS1 protein

In 1998, two separate groups identified the Wolfram Syndrome gene. Inoue group from Japan called the gene *WFS1,* and Strom's group from Germany named it wolframin (Inoue et al., 1998; Strom et al., 1998). *WFS1* gene comprises eight

exons, spanning 33.4kb of genomic DNA, and is located in the short arm of the 4^{th} chromosome in humans and the long arm of the 5^{th} chromosome in mice. The gene is widely expressed in various tissues, such as the pancreas, heart, brain, lung, liver, kidney, skeletal muscle, and placenta. The human *WFS1* gene has 87% homology with the mouse *Wfs1* gene (Inoue et al., 1998; Strom et al., 1998).

The *WFS1* gene encodes the protein WFS1 (wolframin). WFS1 is a membrane glycoprotein that is primarily localised in the ER membrane. It is an 890-amino acid protein and has nine transmembrane segments across the ER membrane (Takeda et al., 2001).

In Wolfram syndrome, a wide range of mutations in the *WFS1* gene have been described to produce dysfunctional protein. Over 200 mutations have been reported. Identified mutations are distributed along the whole coding sequence, with no apparent hotspots or clusters identified (Piccinno et al., 2014).

WFS1 functions: The protein WFS1, located in the ER membrane, has an important role in ER functioning, whereas Wolfram syndrome is associated with ER dysfunction. WFS1 seems to have a significant role within the ER in transporting proteins, lipids, and ions and, in addition, regulating insulin gene expression and cell apoptotic mechanisms (Ariyasu et al., 2017).

ER is the cellular organelle with an essential role in cell survival. It is essential to store Ca^{2+} and is responsible for folding and posttranslational modification of secretory proteins, cell surface receptors, and integral membrane proteins. Different physiological and pathological conditions may perturb the folding environment of ER, leading to exceeded folding capacity and accumulation of unfolded/misfolded proteins, a process defined as “ER stress”. To retrieve homeostasis, a pathway called “unfolded protein response” (UPR) is activated. In high or continuous ER stress conditions, UPR cannot retrieve homeostasis, and ER stress leads to cell apoptosis. UPR reduces ER stress by activating three different signalling proteins: inositol-requiring protein 1 (IRE1), protein-kinase RNA-like ER kinase (PERK), and transcription factor 6 (ATF6). Activating those proteins can culminate in both survival-adaptive and death responses (Pallotta et al., 2019).

WFS1 seems to be a negative regulator of the UPR pathway by preventing ATF6 activity; therefore, it has anti-apoptotic properties. WFS1 regulates Ca^{2+} signal transduction process and influences the storage of Ca^{2+} in the ER, consequently, cell apoptosis. Moreover, WFS1 is a crucial component of proinsulin folding and processing in the beta-cell ER (Fonseca et al., 2005; Pallotta et al., 2019).

In patients with Wolfram syndrome, WFS1 functioning is inefficient, leading to dysfunctional activation of the genes that regulate insulin gene expression and promote apoptosis of beta-cells and neurons. Depending on the cell, those dysfunctional mechanisms lead to the development of Wolfram syndrome clinical manifestations. Considering the WS symptoms, the most detrimental effects of dysfunctional WFS1 seem to be in the pancreatic beta-cells and neurons.

2.3.3. Wolframin deficient mouse model

Monogenic diabetes is a heterogeneous group of disorders representing attractive models to study glucose metabolism and diabetes mechanisms. Moreover, for rare diseases like WS, animal models are essential for evaluating the potential treatment effects because of the small patient population. Several groups have developed and characterised the mouse model of Wolfram syndrome (Ishihara et al., 2004; Noormets et al., 2011; Riggs et al., 2005). *Wfs1*-deficient mice generated in the University of Tartu have the 8th exon at the C-terminal end replaced by NLSLacZ-Neo expression cassette. In contrast, the N-terminal domain of the wolframin remains functional (Luuk et al., 2009).

Wolframin-deficient mice develop diabetes; however, the phenotype has been slightly different between various knockout lines. *Wfs1*-deficient mice developed in the University of Tartu have lower body weight, lower insulin secretion, and severe glucose intolerance since two months of age. In contrast, an increase in fasting blood glucose levels does not develop until the age of 24 months (Noormets et al., 2011).

2.3.4. Treatment of Wolfram syndrome

Curative therapy is not available for the treatment of Wolfram syndrome. The main treatment goal is to treat the symptoms and delay the disease's progression (Urano, 2016). Diabetes is usually managed by insulin treatment (Reschke et al., 2021).

2.4. Summary of the literature review

Diabetes is one of the most prevalent chronic diseases globally, affecting more than 8,5% of the adult population. As most of the complications of diabetes are avoided or delayed with appropriate treatment, new, safe, and effective antidiabetic medications are needed (Sarwar et al., 2010). One of the newest antidiabetic drug classes is GLP1RAs. They are popular tools in the treatment of diabetes, displaying cardiovascular benefits beyond antihyperglycemic action, low risk of hypoglycemia, and modest weight loss. The adverse effects, most frequently nausea, are generally mild and subside with time. GLP1RAs affect multiple organ systems and may hold promise as a new treatment modality for other chronic disorders besides diabetes.

Even though GLP1RAs have been widely studied, many important knowledge gaps remain to be filled.

One of the effects of GLP1RA administration is decelerated gastric motility. Interestingly, it is well known that this effect subsides with prolonged treatment. As there is clear evidence that some effects of GLP1RAs are subject to tachyphylaxis/tolerance development, it is logical to ask whether the core effect of these drugs on glucose regulation may also be affected. We hypothesised that tolerance may develop toward GLP1RAs' glucose-lowering effect. To test it,

we designed a comprehensive study including animal experiments as well as a pilot clinical trial in healthy volunteers.

Wolfram syndrome is a rare progressive neurodegenerative and endocrine condition. Wolfram syndrome's main clinical manifestations are diabetes insipidus, diabetes mellitus, optic nerve atrophy, and deafness, and it is classified as an endoplasmic reticulum disease. Curative treatment is not available for the syndrome. Usually, the treatment is aimed to alleviate and treat the symptoms, whereas WS diabetes is traditionally managed by insulin treatment. We have asked whether this rare type of diabetes associated with Wolfram syndrome may respond to GLP1RAs.

3. AIMS OF THE STUDY

The aims of this study were the following:

1) To elucidate whether tolerance towards GLP1RAs glucose-lowering effect develops in mice and humans.
2) To investigate GLP1RAs glucose-lowering effect in the animal model of Wolfram syndrome.

4. METHODS

4.1. Animal experiments (I, III)

4.1.1. Drugs and chemicals

In all experiments, commercially available liraglutide (Victoza, Novo Nordisk, Bagsværd, Denmark) and exenatide solution (Byetta, AstraZeneca AB, Cambridge, London or Eli Lilly, Houten, Netherlands) were used. In animal experiments, the drug solution was diluted in saline (154 mmol/l NaCl). Glipizide (Sigma-Aldrich, Missouri, USA) was dissolved in a few DMSO drops and then diluted to the final concentration using saline. In the intraperitoneal glucose tolerance test (IPGTT), a 20% glucose solution was used (Braun, Melsbungen, Germany). All injections were carried out in a volume of 10 ml/kg.

4.1.2. Animals

All animal procedures were conducted according to standards set forth by the NIH guidelines on the care and use of animals and had the permission of the Estonian National Committee for Ethics in Animal Experimentation (No. 13, June 2009; No 80, June 2011). Animal studies are reported in compliance with the ARRIVE guidelines (Kilkenny et al., 2011; McGrath & Lilley, 2015). Male C57Bl/6J mice (Harlan, Horst, The Netherlands) aged 6–8 months and weighing 28–38g were used (I). Wfs1-deficient mice (*Wfs1*$^{-/-}$), their wild type (*Wfs1*$^{+/+}$), and heterozygous (*Wfs1*$^{+/-}$) littermates were used (III). Wfs1-deficient mice were F2 hybrids ([129S6/SvEvTac × C57BL/6] × [129S6/SvEvTac x C57BL/6]) (Kõks et al., 2009), and breeding and genotyping were performed in the Department of Physiology, Institute of Biomedicine and Translational Medicine, University of Tartu. Male mice aged 6–7 months weighing 17–26 g were used.

All mice were kept eight per cage in an animal house under standard conditions (temperature 20–22˚C, 12 h/12 h light-dark cycle) and fed standard chow and water ad libitum (unless otherwise stated).

4.1.3. Animal procedures

In paper I, animal experiments were conducted to test the glucose-lowering effect of GLP1RA in acute, sub-chronic, and chronic settings. The general design of the study is shown in Fig. 3. The effects of treatment with liraglutide (600 μg/kg once a day s.c.) or exenatide (10 μg/kg twice a day s.c.) were tested in separate experiments. According to our previous experiments, the doses of drugs were selected to possess an equipotent effect on glucose level (Krass et al., 2012). Drugs (or saline) were given for 18 days (chronic group) or 11 days (sub-chronic group). All injections were performed at the same time every day.

The treatment effect on the non-fasting glucose level was measured seven days (subchronic group) or 14 days (chronic group) after the initiation of treatment (Test I). The control group received saline once or twice daily. Liraglutide (600 µg/kg s.c.) or exenatide (10 µg/kg s.c.) was injected 60 min before the glucose measurement, during that time animals were in their home cages without access to food.

An intraperitoneal glucose tolerance test (IPGTT) (Test II) was performed on the 11th or 18th day (subchronic and chronic group, respectively). Mice were fasted overnight for 12 h. A glucose solution (2 g/kg) was administered by intraperitoneal injection (at time point 0 min). Liraglutide (600 µg/kg s.c), exenatide (10 µg/kg s.c), or saline was injected 30 min before the glucose administration. Blood glucose was monitored by tail bleed before the study and at time points 0, 30, 60, and 120 min. Blood for insulin measurements was collected at the 30 min time point during the study with liraglutide. All groups consisted of 10 (liraglutide study) or 12 animals (exenatide study).

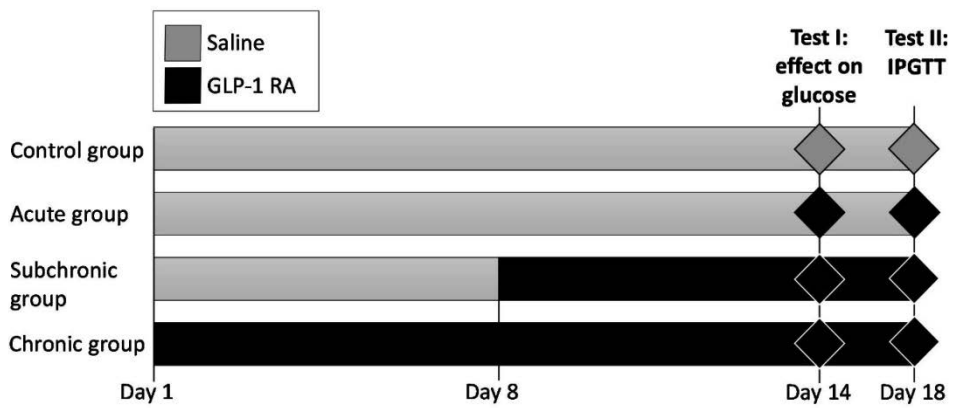

Figure 3. Design of animal experiment. During the study, four experimental groups were used for both exenatide and liraglutide: (1) control group; (2) acute treatment group; (3) subchronic treatment group; (4) chronic treatment group. Liraglutide 600 µg/kg once a day s.c and exenatide 10 µg/kg twice a day s.c. were used. Experiment with liraglutide, n=10; exenatide, n=12. The effect of drugs was tested on the non-fasting glucose level (Test I), and an intraperitoneal glucose tolerance test was conducted (Test II). Mice were injected with saline or liraglutide/exenatide before the testing.

In paper III, animal experiments were conducted to test the glucose-lowering effect of GLP1RAs in Wolframin knockout mice.

Experiment I – acute effects of exenatide and glipizide on the non-fasting glucose level. Exenatide was injected s.c. 90 minutes before the glucose

measurement, and glipizide was injected, i.p. 60 minutes before the measurement. All groups consisted of 8 animals.

Experiment II – acute effect of exenatide on glucagon secretion. Glucose was measured, and blood samples for insulin were taken before and 60 minutes after exenatide injection; after that, animals were killed, and the blood samples for glucagon were collected. All groups consisted of 8 animals.

Intraperitoneal glucose tolerance test (IPGTT). The mice were fasted overnight for 12 h. A glucose solution (2 g/kg) was administered by intraperitoneal injection (at time point 0 min). Exenatide (10 μg/kg, s.c.), glipizide (0.6 mg/kg or 2 mg/kg, i.p.) or saline was injected 30 minutes before the glucose administration (time point -30). Blood glucose was monitored at the following time points: -30, 0, 15, 30, 60, and 120 minutes. Blood for insulin measurements was collected at time points -30 and 60 minutes by the tail bleed. All groups consisted of 8 animals.

4.1.4. Biochemistry

Plasma insulin levels were determined using a mouse insulin ELISA kit (Crystal Chem, Illinois, US). Plasma glucagon levels were determined using a mouse glucagon ELISA kit (Kamiya Biomedical Company, Washington, USA). The samples were assayed in duplicate, and the guidelines of the manufacturers were followed. Blood glucose concentration was determined using an Abbott Optium Xceed glucometer (Abbott Diabetes Care, Alameda, CA, USA).

4.1.5. Statistical methods

Data are presented as mean ± SEM. A P value <0.05 was considered statistically significant. Data were statistically examined using one-way or repeated measures ANOVA (I) or two-way ANOVA (III) to test for the genotype and treatment effects. Duncan's multiple range post hoc test was used when applicable after statistically significant ANOVA. For the statistical analysis, STATISTICA 7 (StatSoft, Bedford, UK) was used. The area under the curve was calculated using the trapezoidal method using GraphPad Prism 8 (GraphPad Software Inc., San Diego, CA, USA).

4.2. Clinical trial (II)

4.2.1. Study design

The study design is shown in Figure 4. To study GLP1RAs effect in acute and chronic settings a single group, open-label clinical trial. The study's primary purpose was to investigate the development of tolerance to the glucose-lowering effect of liraglutide after chronic administration in healthy subjects. The

primary endpoint was a difference in the dose-response relationship between calculated insulin secretion rate (ISR) and blood glucose level, expressed as the slope of these curves.

4.2.2. Study approval

The clinical trial was approved by the Research Ethics Committee of the University of Tartu (236/T-10) and the Estonian Agency of Medicines (RKU-4/18). All clinical investigations were conducted according to the Declaration of Helsinki principles, and all study subjects provided written informed consent before their inclusion in the study.

4.2.3. Study participants

Ten healthy volunteers were recruited. The inclusion criteria were: 1) age 18–50 years; and 2) bodyweight 50–100 kg. The exclusion criteria were: 1) underlying chronic illnesses; 2) use of daily medications; 3) pregnancy or lactation; or 4) fasting glucose >6 mmol/l. Each volunteer read and signed a written informed consent form and received thorough training about handling and using the liraglutide injection device.

4.2.4. Study procedures

The study design is shown in Figure 4. Liraglutide pharmacodynamic effects were evaluated during a graded glucose infusion test (GGIT). Each subject underwent the test three times: the first was to assess the subject's normal state; the second to measure acute effects of the drug; the third to test for chronic effects. The study design made it possible to use every participant as his/her control. Subjects received treatment with liraglutide at a dose of 0.6 mg for 21 consecutive days. Liraglutide was self-administered subcutaneously in the stomach area using self-injection pens (Victoza pen) once daily between 9 PM and 11 PM. Seven days before the initiation of treatment, the first GGIT was performed. The second GGIT was performed 12 h after the first liraglutide injection, and the third GGIT was performed 12 h after the last liraglutide injection.

GGITs were carried out in the morning between 8 AM and 10 AM. Subjects were asked to fast for 12 h before the beginning of the test and avoid extreme physical activity during the previous day. A peripheral venous catheter was placed in both arms; one was used to obtain blood for analysis, and the other was used to administer the glucose infusion. Two blood samples were taken (10 min apart) for baseline levels of glucose and hormones. An intravenous infusion of 20% glucose was then started at the rate of 1 mg/kg/min, followed by 5, 9, and 12 mg/kg/min. Each rate was sustained for 40 min, and the total duration of the infusion was 160 min. Blood samples for glucose and C-peptide measure-

ment were drawn every 20 min. Study subjects were in a semi-supine position during the study, avoided physical activity, and did not eat or drink.

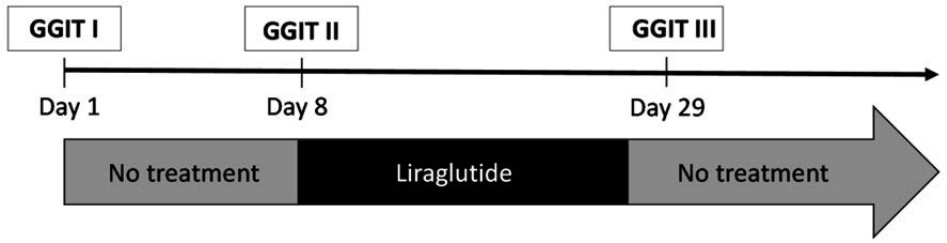

Figure 4. Clinical study design. Each participant underwent the GGIT three times: control study with no treatment, acute study after liraglutide (0.6mg s.c) single injection, chronic study after treatment with liraglutide for 21 consecutive days.

4.2.5. Materials and analysis

Materials Commercially available liraglutide solution (Victoza, Novo Nordisk, Bagsværd, Denmark) was administered using prefilled injection pens and Novofine needles (Novo Nordisk, Bagsværd, Denmark). During GGIT, 20% glucose solution (Braun, Meldbungen, Germany) was used.

Laboratory analysis: In the clinical trial, all the laboratory analyses were carried out by the accredited laboratory at Tartu University Hospital. Glucose was measured using the enzymatic reference method with hexokinase (Cobas Glucose HK test; Roche Diagnostics GmbH, Mannheim, Germany). C-peptide was measured using an electrochemiluminescence immunoassay (Cobas C-peptide immunoassay, Roche Diagnostics GmbH).

4.2.6. Calculations and statistics

The insulin secretion rate was calculated for each time interval during the GGIT, using the computer program ISEC version 3.4, obtained from the author (Hovorka et al., 1996). This program calculates pre-hepatic insulin secretion from plasma C-peptide measurements using a regression model to derive C-peptide kinetics parameters from a subject's gender, type (normal, obese, non-insulin-dependent diabetes mellitus), age, weight, and height (Hovorka & Jones, 1994). The relationship between glucose and insulin secretion rate (ISR) was used to describe the beta-cell responsiveness to glucose. Mean ISR was plotted against the corresponding glucose level, thereby establishing a dose-response relationship between the variables. The responsiveness to glucose was expressed as the slope of the linear regression line relating insulin secretion rate and plasma glucose concentration.

Data are presented as mean ± SEM. A *P* value of <0.05 was considered statistically significant. Data were statistically examined using one-way ANOVA. Duncan's multiple range post hoc test was used when applicable after statistically significant ANOVA. For the statistical analysis, STATISTICA 7 (StatSoft, Bedford, UK) was used. The regression analysis was performed, and the slope between the ISR and glucose relationship was calculated in R version 3.2.5 using the ggplot2 package (R Foundation for Statistical Computing, Vienna, Austria). The area under the curve (AUC) was calculated using the trapezoidal method using GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA, USA).

5. RESULTS

5.1. Tolerance toward GLP1RAs

5.1.1. Tolerance develops toward GLP1RAs glucose-lowering effect in mice

The glucose-lowering effect of liraglutide in mice weakened with prolonged dosing: The design of animal experiments is shown in Fig. 3. In the first part of the experiment (Test I), the glucose-lowering effect of the drugs was assessed after acute, sub-chronic (7 days of saline followed by seven days of GLP1 receptor agonist), or chronic (14 days of GLP1 receptor agonist) treatment with exenatide (10 µg/kg twice daily) or liraglutide (600 µg/kg once daily). Glucose was measured at baseline and 60 min after administration of liraglutide or exenatide in non-fasted mice. Both subchronic (11 days) and chronic (18 days) administration with liraglutide and exenatide significantly decreased the body weight compared to the control group (Fig. 5A and B). During the experiment, both liraglutide and exenatide significantly lowered blood glucose levels (Fig. 6, A and B). The absolute effect of liraglutide was weaker after subchronic and chronic administration compared to acute administration (Fig. 6C), indicating tachyphylaxis/tolerance development. However, the baseline values of glucose were numerically lower, although statistically not significant, in the chronic and subchronic groups than the acute group. Unlike liraglutide, exenatide was equally effective after acute, subchronic, and chronic administration (Fig. 6D).

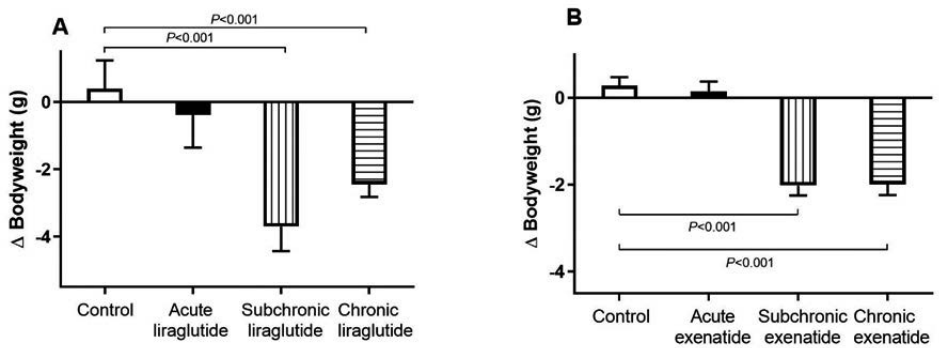

Figure 5. Effect of treatment with liraglutide (600 µg/kg s.c once daily: A) and exenatide (10 µg/kg s.c twice daily: B) on body weight after subchronic (11 days) and chronic (18 days) administration. One-way ANOVA with Duncan's post hoc test was used. Experiment with liraglutide, n=10; exenatide, n=12.

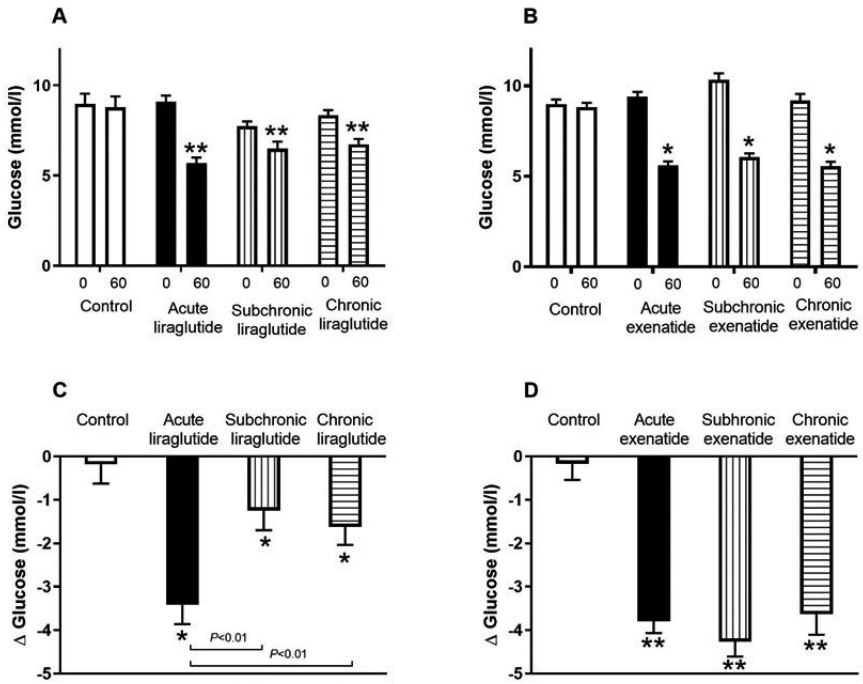

Figure 6. Glucose levels before and after administration (60 min) of liraglutide (600 μg/kg; A) and exenatide (10 μg/kg; B) (Test I). Decrease in glucose level from the baseline after administration of liraglutide (C) and exenatide (D). One-way ANOVA with Duncan's post hoc test was used. *$P < 0.05$, **$P < 0.01$ vs. baseline (A, B) or vs. control (C, D). Experiment with liraglutide, n=10; exenatide, n=12.

Both liraglutide and exenatide were less potent in intraperitoneal glucose tolerance tests after prolonged dosing: Four days after the first part of the experiment, an IPGTT was conducted (treatment duration 11 and 18 days, respectively, for subchronic and chronic groups). Mice were fasted for 12 h before the test. Blood glucose levels during IPGTT are shown in Fig. 7A and 7B. Predictably, both liraglutide and exenatide decreased area under the curve (AUC) for glucose compared to the control group (Fig. 7, C and D). Interestingly, with both liraglutide and exenatide, the AUC for glucose was noticeably more extensive in the chronic groups than in the acute groups. In the experiment with liraglutide, the smallest AUC value was in the acute treatment group; the subchronic group's difference was statistically significant and close to significant with the chronic group (Fig. 7C). Similarly, exenatide lowered the AUC of glucose during the IPGTT the most in acute settings than subchronic and chronic treatment (Fig. 7D).

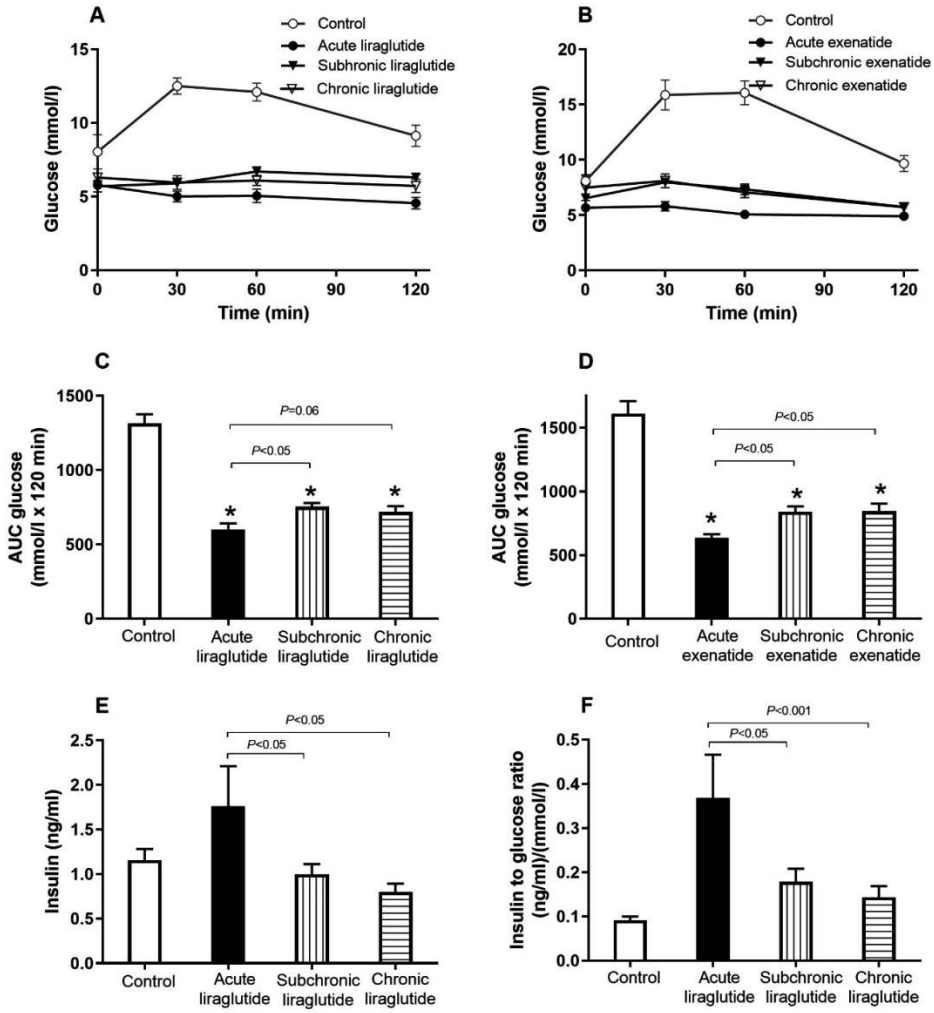

Figure 7. Intraperitoneal glucose tolerance test (Test II). Glucose levels during IPGTT and AUC for glucose with liraglutide (A, C) and exenatide (B, D). Liraglutide 600 μg/kg s.c or exenatide 10 μg/kg s.c was administered 30 min before glucose administration (2 g/kg i.p). The Control group received a saline injection. During the experiment with liraglutide, insulin level was measured 30 min after glucose administration (E), and the insulin-to-glucose ratio was calculated (F). Liraglutide or exenatide was injected before the experiment for 11 days (subchronic group) or 18 days (chronic group). One-way ANOVA with Duncan's post hoc test was used. *$P < 0.05$ vs. control. Experiment with liraglutide, n=10; exenatide, n=12.

Stimulation of insulin release declined after chronic treatment with liraglutide: During the IPGTT with liraglutide, the level of insulin was measured 30 minutes after the glucose administration. Insulin response to the glucose administration was augmented only in the acute treatment group, whereas the response in chronic groups was comparable to the control group insulin level (Fig. 7E). Since the glucose levels at the 30 min time point were markedly different between the groups, the insulin-to-glucose ratio was calculated. Again, the insulin-to-glucose ratio increase was significantly blunted after subchronic and chronic treatment (Fig. 7F).

5.1.2. Tolerance does not develop toward GLP1RAs glucose-lowering effect in humans

Effect of liraglutide remained unaltered during the 3-week treatment in healthy volunteers: We conducted a small clinical study to test whether tolerance develops in humans. Ten healthy volunteers were recruited. The baseline characteristics of study participants are given in Table 3. The general design of the study is shown in Figure 4. Three different GGITs were conducted on every participant to evaluate the effect of acute and chronic liraglutide treatment compared to no treatment conditions. All tests were carried out under the same conditions. As expected, treatment with liraglutide significantly lowered glucose levels and raised C-peptide levels and ISR during the GGIT (Fig. 8A–C). For all parameters, chronic liraglutide treatment was as effective as acute treatment (Fig. 8D–G). Slope values of calculated ISR vs. blood glucose level were: control group 1.6 ± 0.7, acute group 8.4 ± 2.7 ($P > 0.05$ vs. control), and chronic group 6.8 ± 2.2 ($P > 0.05$ vs. control). Hence, the study's primary endpoint (ISR/glucose slope) did not change after the drug's chronic administration.

Table 3. Baseline characteristics of study participants.

Characteristic	Value
Age (mean ± SD)	28.2 ± 1.9 years
Sex (males/females)	7/3
Weight (mean ± SD)	77.0 ± 2.6 kg
Body mass index (mean ± SD)	25.3 ± 0.8 kg/m^2
Fasting glucose (mean ± SD)	4.8 ± 0.2 mmol/l

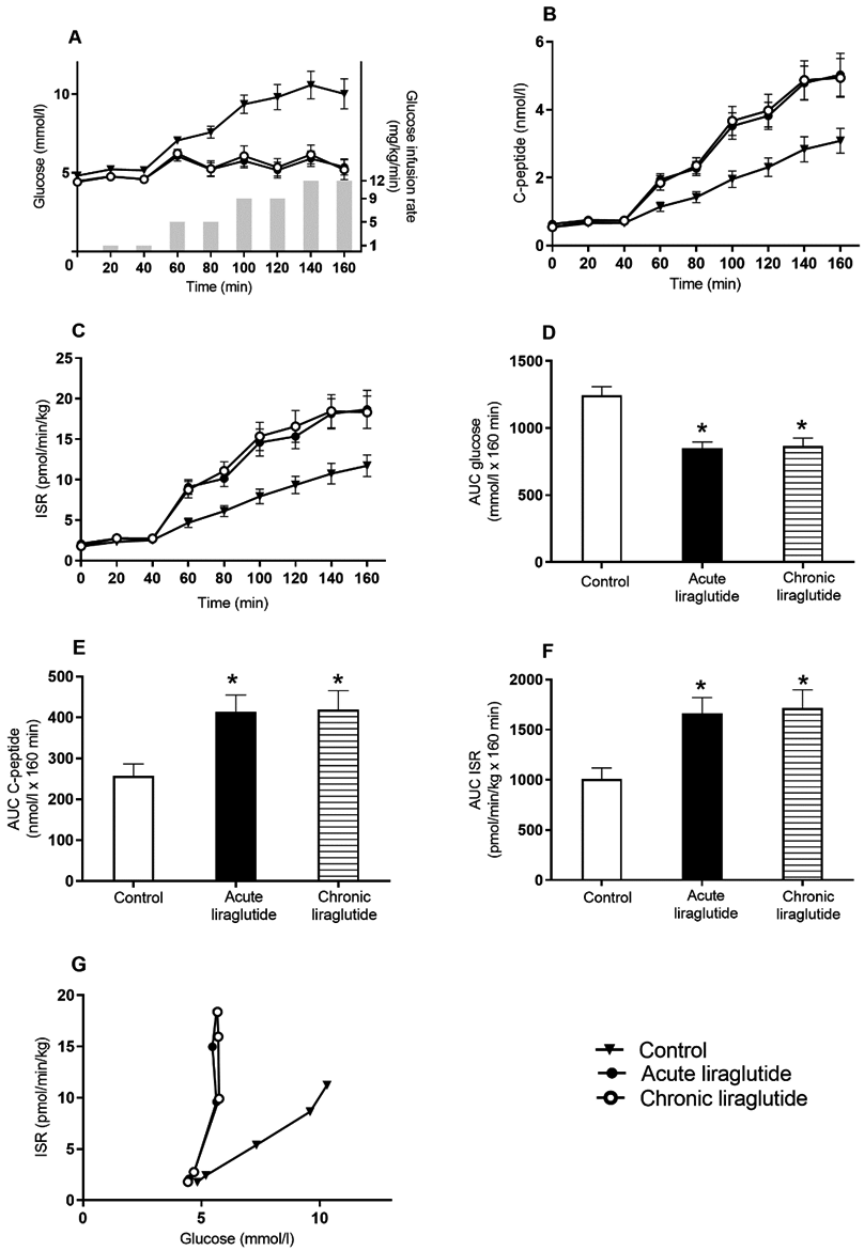

Figure 8. The effect of acute and chronic liraglutide treatment on the level of glucose (A), C-peptide (B), and calculated ISR (C) during GGIT in healthy participants; AUC for glucose (D), C-peptide (E), ISR (F) and ISR vs. glucose (G). The general design of the study is shown in figure 4. During GGIT, subjects received intravenous glucose infusion at a progressively increasing rate (1, 5, 9, 12 mg/kg/min), and each rate was administered for 40 min (A). One-way ANOVA with Duncan's post hoc test was used. *$P < 0.05$ vs. control study. n=10 in every group.

5.2. GLP1RAs are effective in the Wolfram syndrome model

Acute stress induces hyperglycaemia in *Wfs1*-deficient mice: During the intraperitoneal glucose tolerance test, all genotypes, irrespective of treatment group, experienced certain hyperglycaemia during the first 30 minutes as a response to acute stress (handling, blood sample collection, injection). ANOVA with time as a repeated measure indicated a significant effect of time ($F=48$; $p<0.001$) and close to a significant interaction of genotype x time ($F=2.9$; $p=0.068$). The post hoc Duncan test revealed that glucose levels were significantly higher at the time point of 30 min in all groups, and *Wfs1*-deficient mice had augmented hyperglycaemia compared to heterozygotes ($p<0.05$) or their wild-type littermates ($p<0.05$) (Fig. 9).

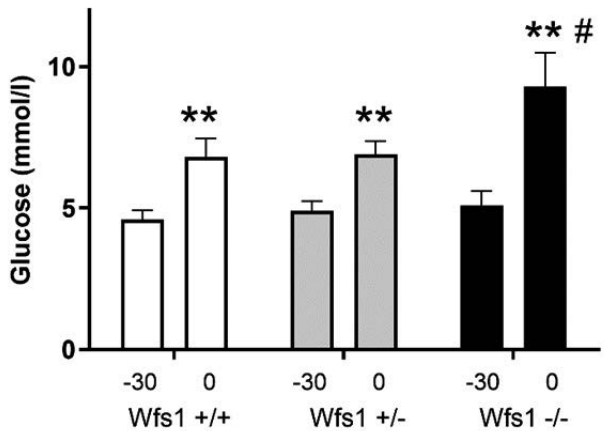

Figure 9. Stress-induced hyperglycaemia before IPGTT. $Wfs1^{+/+}$, white bars; $Wfs1^{+/-}$, gray bars; $Wfs1^{-/-}$, black bars. Repeated measures ANOVA, followed by the Duncan post hoc test, where ** $p<0.01$ vs. time point -30; # $p<0.05$ vs. other genotypes at time point 0. Data of saline-treated animals were pooled from two independent experiments. n=16 in all groups.

Exenatide lowers the blood glucose level in *Wfs1*-deficient mice; glipizide has no significant effect: Two-way ANOVA revealed a significant effect of treatment with exenatide on the blood level of glucose ($F=33.6$, $p<0.001$). An experiment with glipizide showed significant effects of genotype ($F=3.5$, $p<0.05$) and treatment ($F=29.1$, $p<0.001$). The post hoc Duncan test revealed that exenatide lowered glucose levels compared to saline in every genotype ($p<0.01$ in wild type and heterozygotes, $p<0.001$ in *Wfs1*-deficient mice) (Fig. 10A). Glipizide lowered the glucose level in wild-type mice ($p<0.001$) and heterozygotes ($p<0.001$) but had no significant effect in *Wfs1*-deficient mice ($p=0.174$) (Fig. 10B).

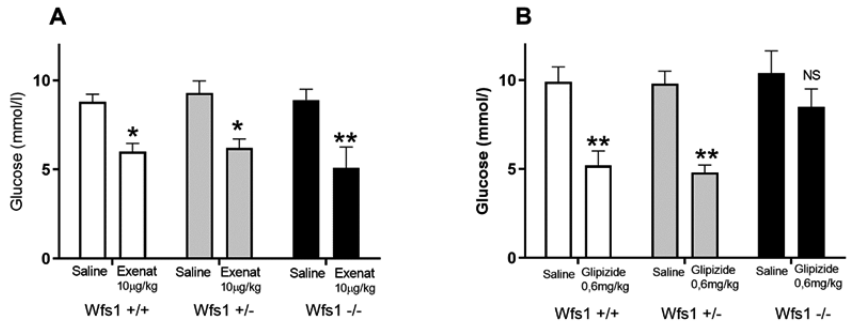

Figure 10. Effect of treatment with exenatide (A) and glipizide (B) on the blood glucose level. *Wfs1*$^{+/+}$, white bars; *Wfs1*$^{+/-}$, gray bars; *Wfs1*$^{-/-}$, black bars. Two-way ANOVA was used, followed by Duncan post hoc test, where * p<0.01 vs. saline; ** p<0.001 vs. saline. n=8 in all groups.

Exenatide has a potent glucose-lowering effect during IPGTT in *Wfs1*-deficient mice; glipizide has no significant effect: The administration of glucose (2 g/kg i.p.) induced a rise in blood glucose level in all genotypes despite the treatment. The maximum blood level peak was traceable 30 minutes after glucose administration (Fig. 11). Two-way ANOVA revealed that both exenatide and glipizide had a significant genotype effect on glucose AUC during IPGTT (exenatide F=19.3, p<0.001; glipizide F=18.9, p<0.001). Treatment had a significant effect in the exenatide experiment (F=14.9, p<0.001) and close to a significant effect in the glipizide group (F=3.7, p=0.063). Treatment with exenatide lowered AUC significantly in wild-type mice (p<0.05) and *Wfs1*-deficient animals (p<0.01), but the decrease was not statistically significant in heterozygotes (p=0.233); the biggest decline was in the *Wfs1*-deficient group (Fig. 12A). Treatment with glipizide decreased AUC significantly in wild-type mice (p<0.05), but the effect was not significant in heterozygotes and the *Wfs1*-deficient group (heterozygotes p=0.268 *Wfs1*-deficient group glipizide 0.6 mg/kg p=0.855 and glipizide 2 mg/kg p=0.09) (Fig. 12B). The higher dose of glipizide was only used in *Wfs1*-deficient animals.

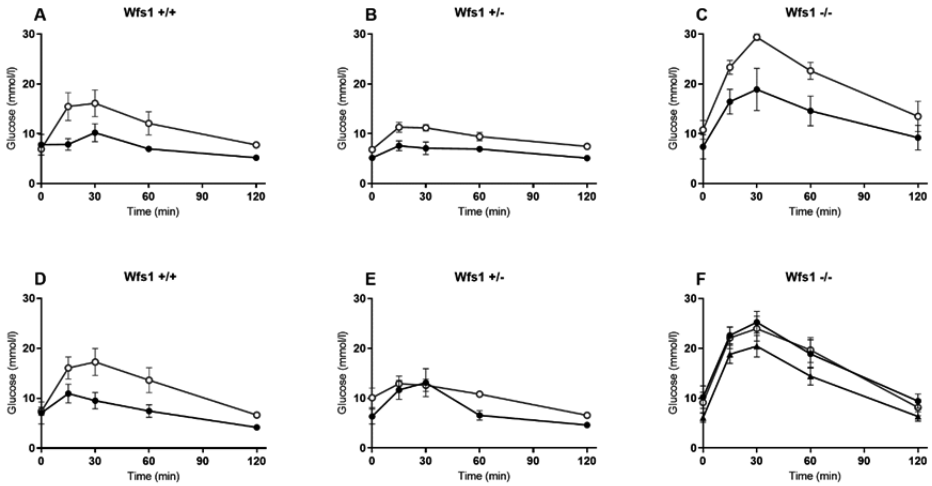

Figure 11. Glucose levels during IPGTT with exenatide in Wfs1$^{+/+}$ (A), Wfs1$^{+/-}$ (B), Wfs1$^{-/-}$ mice (C); saline (white dots), exenatide 10 μg/kg (black dots) and with glipizide in Wfs1$^{+/+}$ (D), Wfs1$^{+/-}$ (E), Wfs1$^{-/-}$ mice (F); saline (white dots), glipizide 0.6 mg/kg (black dots), glipizide 2 mg/kg (black triangles). n=8 in all groups.

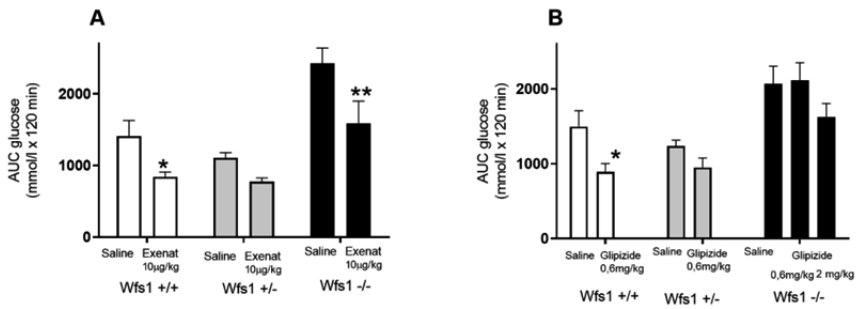

Figure 12. Effect of treatment with exenatide (A) and glipizide (B) on glucose AUC during IPGTT. Wfs1$^{+/+}$, white bars; Wfs1$^{+/-}$, gray bars; Wfs1$^{-/-}$, black bars. Two-way ANOVA was used, followed by Duncan post hoc test, where * p<0.05 vs. saline; ** p<0.01 vs. saline. n=8 in all groups.

Exenatide increases insulin-to-glucose ratio irrespective of genotype: During the IPGTT with exenatide treatment, there were no differences in basal levels of insulin. Exenatide tended to enhance the increase in insulin levels, but this change was not statistically significant (Fig. 13A). Since the glucose levels at the 60 min time point were markedly different between the groups, we calculated the insulin-to-glucose ratio. ANOVA with time as repeated measure indicated a significant effect of time x genotype x treatment with exenatide on the insulin-to-glucose ratio ($F=14.6$, $p<0.001$). In contrast to other groups, wolframin-deficient mice had a lower insulin-to-glucose ratio during the

IPGTT, indicating impaired insulin secretion. Exenatide increased the insulin-to-glucose ratio in all genotypes ($p<0.05$ in every group; Fig. 13B).

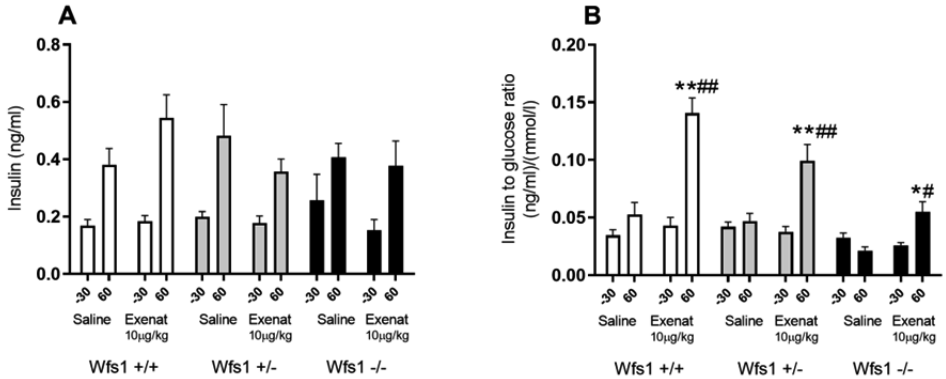

Figure 13. Effect of treatment with exenatide during IPGGT on insulin levels (A) and insulin-to-glucose ratio (B). $Wfs1^{+/+}$, white bars; $Wfs1^{+/-}$, gray bars; $Wfs1^{-/-}$ black bars. Repeated measures ANOVA was used to compare the insulin levels between baseline (-30 min) and the 60 minute time point, followed by the Duncan post hoc test, where * $p<0.05$ vs. baseline; ** $p<0.001$ vs. baseline. To compare the treatment effect at the 60-minute time point, two-way ANOVA was used, followed by the Duncan post hoc test, where # $p<0.05$ vs. saline at the same time point; ## $p<0.001$ vs. saline at the same time point. n=8 in all groups.

Exenatide had no significant effect on glucagon level: ANOVA indicated no effect of treatment or genotype on glucagon level. Sixty minutes after the treatment, the glucagon level was comparable in all groups irrespective of treatment or genotype (Table 4).

Table 4. Effect of treatment with exenatide on glucagon level. Glucagon was measured sixty minutes after the treatment. Two-way ANOVA was used to test for statistical differences. Data are mean±SEM. n=8 in all groups.

Genotype	Treatment	Glucagon (ng/ml)
Wild type	Saline	121.2 (±36.1)
	Exenatide (10 µg/kg)	111.3 (±14.7)
Wfs1-deficient	Saline	110.4 (±12.7)
	Exenatide (10 µg/kg)	103.9 (±17.7)

6. DISCUSSION

6.1. Tolerance toward GLP1RAs effects

Several studies have demonstrated tachyphylaxis/tolerance for gastric emptying with prolonged use of GLP1 (Näslund et al., 2004; Nauck et al., 2011; Umapathysivam et al., 2014) and long-acting GLP1 receptor agonists (Abdulreda et al., 2016; Jelsing et al., 2012). The attenuated gastric emptying delay has been shown after 8- and 24-hours of continuous GLP1 i.v infusion (Nauck et al., 2011; Umapathysivam et al., 2014) and five days of s.c infusion (Näslund et al., 2004). Similar effects have been demonstrated with liraglutide after 14 days of dosing (Jelsing et al., 2012). Furthermore, it is well known that most important gastrointestinal side effects of GLP1RAs, such as nausea and vomiting, subside with prolonged treatment (Bettge et al., 2017). For example, in case of liraglutide, nausea is initially reported in 25–30% of subjects, declining to 2–5% after the first 8–12 weeks (Garber et al., 2011).

As there is clear evidence that some effects of GLP1RAs are subject to tachyphylaxis/tolerance, it is unclear whether tachyphylaxis/tolerance may modulate GLP1RAs insular effects and diminish their glucose-lowering effect in prolonged use. Our previous studies in mice have indicated that GLP1RAs lower the blood glucose level more robustly after acute administration than after chronic dosing (Krass et al., 2012, 2015). Whether tolerance develops toward the glucose-lowering effect of GLP1RAs has never been formally studied. However, several studies have reported indirect evidence about GLP1RAs chronic effects on glucose control. Preclinical studies demonstrated that liraglutide lost efficacy after prolonged treatment in a humanised mouse model generated by intraocular islet transplantation (Abdulreda et al., 2016) and in diabetic mice models (Rolin et al., 2002). Nauck et al. demonstrated in a clinical trial that GLP1 induced rapid tachyphylaxis in gastric emptying, whereas postprandial glucose control was also diminished after GLP1 continuous administration (Nauck et al., 2011). The authors attributed the change in postprandial glucose control to the change in gastric emptying. Interestingly, Tuduri et al. showed a diminished effect of intracerebroventricular GLP1 infusion on glucose-stimulated insulin secretion during prolonged dosing in mice (Tudurí et al., 2015). The evidence about tolerance development using GLP1 and GLP1RAs is summarised in Table 5.

We tested the hypothesis of whether tolerance develops toward GLP1RAs' glucose-lowering effect in chronic use in mice and humans.

Table 5. Comparison of animal and human studies of the development of tolerance toward different pharmacological effects of GLP1RAs.

		Rodents	**Humans**
Gastric emptying	Short-acting GLP1RA	No tolerance (Jelsing et al., 2012)	No tolerance (Lorenz et al., 2013; Näslund et al., 2004)
	Long-acting GLP1RA	Tolerance (Jelsing et al., 2012)	Tolerance (GLP1 infusion) (Näslund et al., 2004; Nauck et al., 2011; Umapathysivam et al., 2014)
Glucose lowering	Short-acting GLP1RA	No tolerance (Baggio et al., 2004); Tolerance (Abdulreda et al., 2016; Rolin et al., 2002; Sedman et al., 2020)	Not tested
	Long-acting GLP1RA	Tolerance (Rolin et al., 2002; Sedman et al., 2020); Tolerance (i.c.v GLP1 infusion) (Tudurí et al., 2015)	No tolerance (Sedman et al., 2017)
Heart acceleration	Short-acting GLP1RA	Not tested	No tolerance (Nakatani et al., 2016)
	Long-acting GLP1RA	No tolerance (Simonds et al., 2019)	No tolerance (Jendle et al., 2018; Smits et al., 2017)

6.1.1. Tolerance in mice

In the current study, exenatide and liraglutide were used to represent short-acting and long-acting GLP1 receptor agonists, respectively. In the first part of the experiment, the drugs' glucose-lowering effect was assessed after different treatment periods (acute administration and treatment for 7 and 14 days) with exenatide (10 μg/kg twice daily) or liraglutide (600 μg/kg once daily). Exenatide and liraglutide similarly lowered blood glucose levels demonstrating that the doses were correct and comparable during the experiment. The absolute effect of liraglutide was weaker after chronic and sub-chronic administration compared to acute administration, indicating tachyphylaxis/tolerance development. However, the baseline values of glucose were numerically lower in the chronic and sub-chronic groups compared to the acute group, probably due to a carryover effect of the previous dose of liraglutide on glucose level or food consumption. This difference was not statistically significant, yet may offer an alternative explanation to tolerance development. Unlike liraglutide, exenatide was equally effective after acute, sub-chronic, and chronic administration. The

different propensity of short- and long-acting GLP1 receptor agonists to induce tolerance has also been demonstrated in the case of gastric emptying (Jelsing et al., 2012).

Four days after the experiment's first part, an IPGTT was conducted (treatment duration 11 and 18 days, respectively, for sub-chronic and chronic groups). Acute administration of liraglutide and exenatide were equally effective in decreasing the AUC for glucose compared to the control group, and both drugs lost efficacy with prolonged treatment. Thus, during the 11- or 18-day treatment with a GLP1 receptor agonist, tolerance to the glucose-lowering effect developed. Overall, our results from animal experiments support and broaden the previous reports of loss of efficacy of GLP1 receptor agonists after chronic treatment (Abdulreda et al., 2016; Jelsing et al., 2012; Tudurí et al., 2015). Importantly, tolerance developed toward the glucose-lowering effect of exenatide, demonstrating that short-acting molecules are not an exception. During the IPGTT with liraglutide, insulin response to the glucose administration was significantly blunted after sub-chronic and chronic treatment. Thus, the impaired stimulation of insulin release seems to be the leading mechanism of tolerance development. However, as we have not tested shorter exposures to GLP1 receptor agonists, we are currently unable to say how quickly the loss of efficacy appears.

Interestingly, the possible loss of the anti-hyperglycaemic effect after prolonged dosing was noted in an early preclinical study with liraglutide (Rolin et al., 2002). The authors explained the finding as loss of food intake lowering effect of both liraglutide and exendin 4 after four treatment days. We conducted the IPGTT after 12 hours fast. Accordingly, it seems clear that a change in food intake cannot explain the diminished effect of liraglutide and exenatide. Another remarkable aspect of the study mentioned above was that liraglutide and exenatide had a similar anti-hyperglycaemic effect pattern but divergent effects on beta-cell mass (Rolin et al., 2002).

In line with our results, Tudurí and colleagues demonstrated similar findings after acute or prolonged intracerebral dosing of GLP1 (Tudurí et al., 2015). They showed in mice that acute intracerebroventricular infusion of GLP1 resulted in significantly improved glucose tolerance and higher plasma insulin levels compared to saline in response to a glucose load, suggesting that central GLP1 regulates pancreatic beta-cells and potentiates insulin secretion. In contrast, after chronic i.c.v infusion of GLP1, the glucose levels did not differ from these of saline-treated animals. Interestingly, no differences in the expression of GLP1 receptor levels were observed in either the brainstem or the hypothalamus when comparing GLP1-treated mice with the saline group in both acute and chronic studies, suggesting that chronic intracerebral GLP1 infusion does not down-regulate the expression of GLP1 receptors. The authors speculated that increased DPP4 activity after chronic dosing might explain these findings. As we used GLP1RAs which are resistant to DPP4 and not native GLP1, this hypothesis does not explain our findings. However, the study by

Tudurí et al. raises the possibility that, at least in animals, central effects of GLP1RAs on glucose regulation may be subject to tolerance.

We have to admit that our study has several limitations regarding the exact mechanistic background of tolerance development. Insulin levels were measured only at one time-point with liraglutide, and we did not collect pancreatic samples. Thus, a more focused study is necessary to prove whether insulin release, change in pancreatic mass, insulin sensitivity, or CNS tolerance development is the leading mechanism.

6.1.2. GLP1RAs tolerance in humans

We conducted a small clinical trial to demonstrate whether the animal findings can be replicated in humans. Ten healthy volunteers were treated with a low dose of once-daily liraglutide (0.6 mg s.c) for three weeks, and insulin secretion parameters were measured by conducting GGIT. The trial's primary endpoint was a difference in the dose-response relationship between calculated insulin secretion rate (ISR) and blood glucose level, expressed as the slope of these curves.

The drug was administered 12 hours before testing as the maximum plasma concentration after liraglutide administration is reached after approximately 10-14 hours (Agersø et al., 2002).

As expected, treatment with liraglutide lowered glucose levels and raised C-peptide levels and ISR during the GGIT. For all parameters, chronic liraglutide treatment was as effective as acute treatment. Hence, the study's primary endpoint (ISR/glucose slope) did not change after chronic administration. Thus, we have demonstrated that tolerance does not develop in healthy volunteers towards the glucose-lowering effect of liraglutide after three weeks of treatment. Our finding does not support the previous results from the mouse studies (Abdulreda et al., 2016; Rolin et al., 2002; Sedman et al., 2020). Studies looking at gastric motility effects of GLP1 agonists have revealed consistent results in animal models and humans (Jelsing et al., 2012; Lorenz et al., 2013; Näslund et al., 2004; Nauck et al., 2011; Umapathysivam et al., 2014). This may not hold for the glucose-lowering effect of the drugs. The different results in mouse and human studies might be explained by the essential differences between human and mouse islet structure, function, and/or beta-cell regulation (Hart & Powers, 2019). Even animal studies have generated conflicting results about the chronic effect of GLP1 agonists on beta-cells. Thus, in diabetes models, treatment with various molecules has increased beta-cell mass and function (Jelsing et al., 2012). On the contrary, the treatment of normoglycaemic animals led to decreased beta-cell mass (Ellenbroek et al., 2013; Mondragon et al., 2014). The latter study even found reduced beta-cell mass after chronic treatment with liraglutide, but not with exenatide (Mondragon et al., 2014). Alternatively, it is possible that tolerance development in mice is through effects on the central nervous system. Tuduri et al. demonstrated a

significant difference in acute and chronic glucose-lowering effects of GLP1 when administered i.c.v (Tudurí et al., 2015). One may speculate that in human beings either the brain penetrance of GLP1RAs or the role of CNS system in glucose regulation is different from rodents explaining the lack of tolerance.

However, it is important to address some limitations. Firstly, the liraglutide dose was lower than the usual clinical dose of 1.2 mg per day. Stepwise introduction of the drug is used in clinical practice to ease gastrointestinal side effects. Such an approach would obscure the development of tolerance; hence, flat dosing was used in our study. As the drug's effect on glucose parameters and insulin release was robust in our experiment, the dose used does not seem to be a significant concern.

Secondly, the study used healthy volunteers instead of T2DM patients. It would be challenging to test this hypothesis in diabetes patients because the liraglutide-induced improvement in glucose levels may enhance beta-cell function via reduced glucotoxicity and mask the possible development of tolerance. While there have been speculations regarding whether GLP1RAs may directly improve beta-cell function, the clinical trial data in early T2DM patients did not support this hypothesis (Gudipaty et al., 2014).

Thirdly, in our clinical trial, the treatment time was three weeks. One may speculate that a more extended period of treatment is needed to induce tolerance. In previous clinical trials where the diminished effect on gastric motility was demonstrated, various treatment durations and drug administration routes were used. Thus, continuous intravenous GLP1 infusions have been used lasting 8- and 24-hours (Nauck et al., 2011; Umapathysivam et al., 2014). In contrast, similar results were obtained after five days of subcutaneous infusion of GLP1 administration (Näslund et al., 2004).

Despite these limitations, we find that our results largely refute the hypothesis of tolerance development toward glucose-lowering effect with prolonged use of liraglutide in humans.

6.2. Wolfram syndrome and potential of GLP1RAs as a treatment modality

We have further described the diabetic phenotype of mice lacking a functional wolframin gene. The perturbation of glucose metabolism was only apparent in homozygous mice. Mice with one copy of the functional wolframin gene had similar characteristics to control mice in all experiments. As in a previous report, wolframin-deficient mice had nearly normal fasting glucose levels but developed hyperglycaemia after the glucose challenge (Ishihara et al., 2004). Interestingly, fasted wolframin-deficient mice displayed an augmented hyperglycaemic response 30 minutes after relatively mild stress – blood sampling by tail bleed and the subcutaneous injection of saline. This kind of stress-induced hyperglycaemia has been previously demonstrated in another diabetes model – ob/ob mice (Surwit et al., 1984). However, in the previous report, a much more

stressful protocol (30 min of immobilisation followed by 5 min of shaking) was used. It has been previously shown that wolframin-deficient mice display an exaggerated corticosterone response (Luuk et al., 2009). Thus, we speculate that stress-induced hyperglycaemia results from a higher corticosterone response to stress combined with limited insulin availability.

Specific or disease-modifying therapy is not available in the treatment of WS, and therefore, insulin has been used as a mainstay of treatment of WSD. As WS is considered a prototype of endoplasmic reticulum disease, one possibility for the identification of novel therapies is directed to maintaining ER homeostasis and, thus, the regulation of calcium levels and protein folding. GLP1RAs, as ER stabilisers, may hold promise in patients with WS. In WS animal models, GLP1R signalling has been shown to alleviate the cellular stress and beta-cell function improvements (Kondo et al., 2018; Yusta et al., 2006). Moreover, GLP1RAs have shown an effect on beta-cell functioning in the mouse models of WS2 (Danielpur et al., 2016). Although preclinical data are promising, clinical trials studying the effect of GLP1RAs in patients with WS are so far limited to case studies and phase 1 clinical trials (Danielpur et al., 2016; Toppings et al., 2018).

Repurposing of existing drugs is an attractive line of developing new treatments for WS. Different treatment approaches and candidate drugs in development are shown in figure 14. The muscle relaxant dantrolene, T2DM medication pioglitazone, and immunosuppressants rapamycin, and macrolide may benefit WS as ER calcium level regulators. Molecular chaperones, a class of molecules assisting protein folding, may be beneficial by stabilising protein conformation in the ER (Abreu & Urano, 2019). The anticonvulsant and mood stabiliser valproic acid has shown neuroprotective properties in preclinical studies and promising results in WS models, most probably by modulating the ER stress response (Kakiuchi et al., 2009; Pallotta et al., 2019). Some of these drugs have severe adverse effects that limit their clinical use (Kim et al., 2011; Pallotta et al., 2019). One promising strategy to achieve cure in WS treatment is regenerative and gene therapy, which may replace pathogenic *WFS1* variants or damaged cells and tissues (Abreu & Urano, 2019).

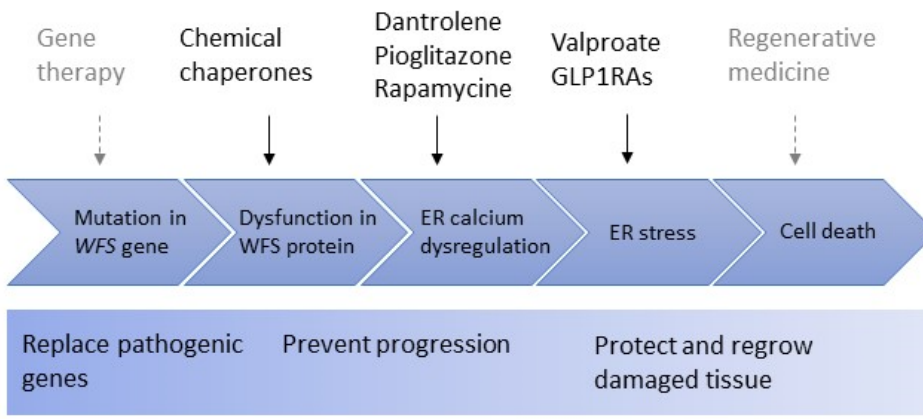

Figure 14. Pathophysiological steps in the development of WS and various therapeutic strategies and candidate drugs targeting them (Akiyama et al., 2009; Hara et al., 2014; Lu et al., 2014).

Our study characterised the effects of GLP1 receptor agonist and sulphonylurea on glucose regulation in wolframin knockout mice. Sulphonylureas, insulin secretagogues, have been effective in the treatment of some monogenic forms of diabetes, whereas the efficacy in WS has been unknown (Zhang et al., 2021). As expected, both exenatide (10 µg/kg s.c.) and glipizide (0.6 mg/kg i.p.) significantly decreased glucose levels in control mice and heterozygotes injected in a non-fasted state. Interestingly, in wolframin-deficient mice, sulphonylurea did not change glucose levels after acute administration. In contrast, exenatide at a dose of 10 µg/kg decreased glucose levels as effectively as in control mice. As the first experiment was performed with food available *ad libitum*, one can argue that the drug's anorexigenic effect may partly explain the GLP1 receptor agonist effect. Nevertheless, in the next set of experiments, the effects of drugs were studied in the intraperitoneal glucose tolerance test with fasting animals and no food availability. The results in the IPGTT were similar to the previous experiment: exenatide was effective in wolframin-deficient mice while the glipizide effect was diminished.

Thus, one can conclude that wolframin-deficient mice display contrasting sensitivity towards different insulin secretagogues, and GLP1 agonists may have potential in treating Wolfram syndrome diabetes. Previously, pioglitazone treatment has been shown to protect wolframin-deficient mice against diabetes (Akiyama et al., 2009). However, these data are not directly comparable to ours, as the model combined wolframin deletion with the introduction of an agouti lethal yellow mutation to promote insulin resistance.

We also studied the possible mechanisms beyond the effect of the GLP1RAs. There was no difference in glucagon levels after exenatide administration in any group of mice. The insulin levels tended to be higher in the glucose tolerance test after exenatide administration, but the difference was not statistically

significant. However, one must consider that glucose levels were much higher in wolframin-deficient animals after the glucose challenge. Thus, insulin-to-glucose ratios were also calculated. In contrast to other groups, wolframin-deficient mice had a lower insulin-to-glucose ratio during the IPGTT, indicating impaired insulin secretion. Exenatide increased the insulin-to-glucose ratio during the glucose tolerance test irrespective of genotype, demonstrating the ability to correct the impaired insulin secretion caused by wolframin deficiency.

Recent evidence from other groups supports the potential effect of GLP1RAs in the case of Wolfram syndrome-related diabetes. One of the final effectors in the pathway after GLP1 receptor activation is synaptotagmin 7, a molecule involved in the calcium-sensitive transmitter (incl. insulin) release (Wu et al., 2015). In line with that, a recent report using Wfs1-deficient beta cells as a model linked altered calcium homeostasis with cell death. Several molecules were able to protect beta cells in the model mentioned above, and GLP1 was one of them (Lu et al., 2014). Thus, we speculate that there is a defect in the calcium-dependent release of insulin in the case of WS, which may explain why GLP1RAs, but not sulfonylurea, were effective in the model. Furthermore, Seppa et al. demonstrated liraglutide's neuroprotective effect in the WS rat model – 6-month treatment with liraglutide reduced neuro-inflammation in the inferior olive and protected retinal ganglion cells from cell death and degeneration (Seppa et al., 2019).

Thus, we propose that GLP1RAs could be tried to treat (early) WSD patients. GLP1RAs have a solid background in terms of efficacy and safety in clinical studies. Exenatide has been successfully tried in a MODY3 (related to the defect in HNF-1alpha gene) patient (Ahluwalia et al., 2009). In a case study of autosomal dominant WFS1-related disorder, GLP1RA has shown efficiency in glycemic control (Scully & Wolfsdorf, 2021). GLP1 receptor agonist therapy's key benefits would be the lower risk of hypoglycemia and the lower number of injections needed with long-acting molecules.

As a general limitation, one must realise that islet structure, function, and beta-cell regulation are different between rodents and humans. For example, the chronic treatment of mice with liraglutide led to decreased beta-cell mass (Mondragon et al., 2014). Thus, our encouraging findings in the WS mouse model do not automatically guarantee clinical success in patients.

7. CONCLUSIONS

The conclusions of this study were the following:

(I) Tolerance toward GLP1 receptor agonists glucose-lowering effect develops in mice using both short- and long-acting molecules.

(II) Unlike in mice, GLP1 receptor agonists do not induce tolerance toward their effect on glucose regulation in humans.

(III) Different results in developing tolerance in humans and mice indicate the core differences in glucose regulation in different species and, therefore, indicate the difficulties in translating results from animal studies to clinical practice.

(IV) The glucose regulation pathway is selectively impaired in Wolfram syndrome, and the GLP1 receptor may have the potential to improve glucose regulation.

SUMMARY IN ESTONIAN

Uued suunad GLP1 retseptori agonistide kasutamises diabeedi ravis

Glükagoonilaadne peptiid 1 (GLP1) on peptiidhormoon, mis vabaneb soolestikust vastusena söömisele. GLP1-l on mitmeid toimeid erinevates elundsüsteemides. Inkretiinhormoonina osaleb see glükoosi ainevahetuses – stimuleerides insuliini ja inhibeerides glükagooni vabanemist vere glükoositase langeb. GLP1 toimel aeglustub mao motoorika ja tühjenemine. Kesknärvisüsteemis reguleerib GLP1 söögiisu ning seeläbi ka kehakaalu, osaleb mälu- ja õppimisprotsessis. Lisaks toimib GLP1 südame-veresoonkonnale, neerudele ja maksale.

GLP1 füsioloogilistel toimetel põhineb ravimklassi GLP1 retseptori agonistide kasutamine. GLP1 retseptori agonistid on diabeediravimid, mis lisaks veresuhkru taseme langetamisele vähendavad söögiisu ja alandavad kehakaalu. Ravimklass on võrreldes mitmete teiste diabeediravimitega ohutum, kuna hüpoglükeemia risk on väga väike. Nende ravimite kõrvaltoimed on pigem kerged, sagedasemateks iiveldus ja harvem oksendamine. Tänu efektiivsele põhitoimele, heale talutavusele ja soodsatele lisatoimetele on GLP1 retseptori agonistid muutunud populaarseks nii diabeedi ravis kui ka muudel näidustustel.

GLP1 retseptori agonistide mõnede toimete suhtes kujuneb välja tolerantsus – näiteks mao motoorika aeglustumine, mis ravi kestel väheneb. Samuti vähenevad ravimi korduval kasutamisel kõrvaltoimed, möödudes tavapäraselt esimeste ravinädalatega. Seda, kas GLP1 retseptori agonistide veresuhkru taset langetava toime suhtes tekib tolerantsus, ei ole seni teada.

GLP1 retseptori agoniste on kasutatud monogeensete diabeedivormide puhul. Wolframi sündroomi, geneetiline haigus, mille käigus areneb insuliinravi vajav diabeet, magediabeet ja silmanärvi kahjustus, spetsiifilist ravi pole seni leitud. Seda, kas GLP1 retseptori agonistidel on toime Wolframi sündroomi puhul, ei ole seni teada.

Käesoleva uurimistöö eesmärgid:

1) Võrrelda GLP1 retseptori agonistide toimet lühiajalisel- ja pikaajalisel kasutamisel nii hiirtel kui ka inimestel selgitamaks välja tolerantsuse teke;
2) Uurida GLP1 retseptori agonistide toimet Wolframi sündroomi loommudelil.

Uurimistöö meetodid ja tulemused:

Tolerantsuse uurimiseks hiirtel teostati erinevaid loomkatseid. Uuritavate ravimite (eksenatiid ja liraglutiid) toimet veresuhkru tasemele hinnati peale ühekordset manustamist ja peale 2-nädalast ravikuuri. Toime hindamiseks kasutati erinevaid eksperimente – paastuveresuhkru mõõtmine, glükoosi taluvuse proov. Mõlemad GLP1 retseptori agonistid langetasid veresuhkru taset ühekordse manustamise järgselt oluliselt rohkem kui pikemaajalise manustamise korral viidates tolerantsuse tekkele.

Tolerantsuse uurimiseks inimestel viidi läbi kliiniline ravimuuring. Uuringusse kaasati kümme tervet vabatahtlikku, kellel teostati kolmel korral astmelise glükoosi manustamise test. Esimene test teostati ilma ravimita, teine peale ühekordset liraglutiidi manustamist ja kolmas peale 21-päevast ravikuuri. Iga testi käigus manustati uuritavatele kahe tunni jooksul intravenoosselt glükoosilahust ning määrati veresuhkru ja C-peptiidi tase. Ravimi efekti hindamiseks arvutati veresuhkru ja C-peptiidi tasemete alusel insuliini sekretsiooni kiirus. Liraglutiid langetas efektiivselt veresuhkru taset nii ühekordse kui ka pikemaajalise manustamise järgselt, mis tähendas, et liraglutiidi põhitoime suhtes ei tekkinud tolerantsust.

GLP1 retseptori agonistide efektiivsuse uurimiseks Wolframi sündroomi ravis kasutati Wolframi sündroomi loommudelit – *Wfs1* geeni puudulikkusega hiir. GLP1 retseptori agonist eksenatiid langetas geenipuudulikkusega hiirtel veresuhkru taset, samas kui sulfonüüluurea preparaadil glipisiidil toime puudus. GLP1 retspetori agonistide antidiabeetiline toime Wolframi sündroomi mudelis viitab GLP1 retseptori osalusele Wolframi sündroomi patogeneesis.

Järeldused:

1) Hiirtel tekib glükoosi langetava toime suhtes tolerantsus nii pika- kui ka lühitoimeliste GLP1 retseptori agonistide puhul.
2) Erinevalt hiirtest ei teki inimestel GLP1 retseptori agonistide põhitoime suhtes tolerantsust.
3) Wolframi sündroomi puhul on glükoosi metabolism selektiivselt häiritud ning GLP1 retseptori mõjutamine võib omada selle haiguse puhul positiivset toimet.

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REFERENCES

Abdulreda, M. H., Rodriguez-Diaz, R., Caicedo, A., & Berggren, P. O. (2016). Liraglutide Compromises Pancreatic β Cell Function in a Humanized Mouse Model. *Cell Metab*, *23*(3), 541–546. https://doi.org/10.1016/j.cmet.2016.01.009

Abreu, D., & Urano, F. (2019). Current Landscape of Treatments for Wolfram Syndrome. In *Trends in Pharmacological Sciences* (Vol. 40, Issue 10, pp. 711–714). Elsevier Ltd. https://doi.org/10.1016/j.tips.2019.07.011

Adriaenssens, A. E., Svendsen, B., Lam, B. Y. H., Yeo, G. S. H., Holst, J. J., Reimann, F., & Gribble, F. M. (2016). Transcriptomic profiling of pancreatic alpha, beta and delta cell populations identifies delta cells as a principal target for ghrelin in mouse islets. *Diabetologia*. https://doi.org/10.1007/s00125-016-4033-1

Agersø, H., Jensen, L. B., Elbrønd, B., Rolan, P., & Zdravkovic, M. (2002). The pharmacokinetics, pharmacodynamics, safety and tolerability of NN2211, a new long-acting GLP-1 derivative, in healthy men. *Diabetologia*. https://doi.org/10.1007/s00125-001-0719-z

Ahluwalia, R., Perkins, K., Ewins, D., & Goenka, N. (2009). Exenatide-a potential role in treatment of HNF1-alpha MODY in obese patients? *Diabetic Medicine : A Journal of the British Diabetic Association*, *26*(8), 834–835. https://doi.org/10.1111/j.1464-5491.2009.02753.x

Akiyama, M., Hatanaka, M., Ohta, Y., & Ueda, K. (2009). Increased insulin demand promotes while pioglitazone prevents pancreatic beta cell apoptosis in Wfs1 knockout mice. *Diabetologia*, 653–663. https://doi.org/10.1007/s00125-009-1270-6

Al-Sheyyab, M., Jarrah, N., Younis, E., Shennak, M. M., Hadidi, A., Awidi, A., El-Shanti, H., & Ajlouni, K. (2001). Bleeding tendency in Wolfram syndrome: A newly identified feature with phenotype genotype correlation. *European Journal of Pediatrics*. https://doi.org/10.1007/s004310000704

Alhadeff, A. L., Rupprecht, L. E., & Hayes, M. R. (2012). GLP-1 neurons in the nucleus of the solitary tract project directly to the ventral tegmental area and nucleus accumbens to control for food intake. *Endocrinology*. https://doi.org/10.1210/en.2011-1443

Altieri, P., Murialdo, R., Barisione, C., Lazzarini, E., Garibaldi, S., Fabbi, P., Ruggeri, C., Borile, S., Carbone, F., Armirotti, A., Canepa, M., Ballestrero, A., Brunelli, C., Montecucco, F., Ameri, P., & Spallarossa, P. (2017). 5-fluorouracil causes endothelial cell senescence: potential protective role of glucagon-like peptide 1. *Br J Pharmacol*, *174*(21), 3713–3726. https://doi.org/10.1111/bph.13725

Amori, R. E., Lau, J., & Pittas, A. G. (2007). Efficacy and safety of incretin therapy in type 2 diabetes: Systematic review and meta-analysis. In *Journal of the American Medical Association*. https://doi.org/10.1001/jama.298.2.194

Andersen, A., Lund, A., Knop, F. K., & Vilsbøll, T. (2018). Glucagon-like peptide 1 in health and disease. In *Nature Reviews Endocrinology* (Vol. 14, Issue 7, pp. 390–403). Nature Publishing Group. https://doi.org/10.1038/s41574-018-0016-2

Ariyasu, D., Yoshida, H., & Hasegawa, Y. (2017). Endoplasmic reticulum (Er) stress and endocrine disorders. In *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms18020382

Athauda, D., Maclagan, K., Skene, S. S., Bajwa-Joseph, M., Letchford, D., Chowdhury, K., Hibbert, S., Budnik, N., Zampedri, L., Dickson, J., Li, Y., Aviles-Olmos, I., Warner, T. T., Limousin, P., Lees, A. J., Greig, N. H., Tebbs, S., & Foltynie, T. (2017). Exenatide once weekly versus placebo in Parkinson's disease: a randomised,

double-blind, placebo-controlled trial. *Lancet*, *390*(10103), 1664–1675. https://doi.org/10.1016/S0140-6736(17)31585-4

Baggio, L. L., & Drucker, D. J. (2007). Biology of Incretins: GLP-1 and GIP. *Gastroenterology*, *132*(6), 2131–2157. https://doi.org/10.1053/j.gastro.2007.03.054

Baggio, L. L., & Drucker, D. J. (2020). Glucagon-like peptide-1 receptor co-agonists for treating metabolic disease. In *Molecular Metabolism*. https://doi.org/10.1016/j.molmet.2020.101090

Baggio, L. L., Kim, J. G., & Drucker, D. J. (2004). Chronic exposure to GLP-1R agonists promotes homologous GLP-1 receptor desensitization in vitro but does not attenuate GLP-1R-dependent glucose homeostasis in vivo. *Diabetes*. https://doi.org/10.2337/diabetes.53.suppl_3.S205

Barre, J. (1936). La sécretine: Son rôle physiologique, ses propriétés thérapeutiques. *Masson et Cie Éditeurs*.

Barrett, T. G., & Bundey, S. E. (1997). Wolfram (DIDMOAD) syndrome. *Journal of Medical Genetics*, *34*(10), 838–841. https://doi.org/10.1136/jmg.34.10.838

Barrett, T. G., Bundey, S. E., & Macleod, A. F. (1995). Neurodegeneration and diabetes: UK nationwide study of Wolfram (DIDMOAD) syndrome. *The Lancet*, *346*(8988), 1458–1463. https://doi.org/10.1016/S0140-6736(95)92473-6

Bell, G. I., Santerre, R. F., & Mullenbach, G. T. (1983). Hamster preproglucagon contains the sequence of glucagon and two related peptides [20]. In *Nature*. https://doi.org/10.1038/302716a0

Bethel, M. A., Patel, R. A., Merrill, P., Lokhnygina, Y., Buse, J. B., Mentz, R. J., Pagidipati, N. J., Chan, J. C., Gustavson, S. M., Iqbal, N., Maggioni, A. P., Öhman, P., Poulter, N. R., Ramachandran, A., Zinman, B., Hernandez, A. F., & Holman, R. R. (2018). Cardiovascular outcomes with glucagon-like peptide-1 receptor agonists in patients with type 2 diabetes: a meta-analysis. *The Lancet Diabetes and Endocrinology*. https://doi.org/10.1016/S2213-8587(17)30412-6

Bettge, K., Kahle, M., Abd El Aziz, M. S., Meier, J. J., & Nauck, M. A. (2017). Occurrence of nausea, vomiting and diarrhoea reported as adverse events in clinical trials studying glucagon-like peptide-1 receptor agonists: A systematic analysis of published clinical trials. *Diabetes, Obesity and Metabolism*. https://doi.org/10.1111/dom.12824

Bose, A. K., Mocanu, M. M., Carr, R. D., Brand, C. L., & Yellon, D. M. (2005). Glucagon-like peptide 1 can directly protect the heart against ischemia/reperfusion injury. *Diabetes*. https://doi.org/10.2337/diabetes.54.1.146

Brayden, D. J., Hill, T. A., Fairlie, D. P., Maher, S., & Mrsny, R. J. (2020). Systemic delivery of peptides by the oral route: Formulation and medicinal chemistry approaches. In *Advanced Drug Delivery Reviews* (Vol. 157, pp. 2–36). Elsevier B.V. https://doi.org/10.1016/j.addr.2020.05.007

Brown, J. C., Mutt, V., & Pederson, R. A. (1970). Further purification of a polypeptide demonstrating enterogastrone activity. *The Journal of Physiology*. https://doi.org/10.1113/jphysiol.1970.sp009155

Bryant, M. G., Bloom, S. R., Polak, J. M., Hobbs, S., Domschke, W., Domschke, S., Mitznegg, P., Ruppin, H., & Demling, L. (1983). Measurement of gut hormonal peptides in biopsies from human stomach and proximal small intestine. *Gut*, *24*(2), 114–119. https://doi.org/10.1136/gut.24.2.114

Buse, J. B., Wexler, D. J., Tsapas, A., Rossing, P., Mingrone, G., Mathieu, C., D'Alessio, D. A., & Davies, M. J. (2020). 2019 update to: Management of hyperglycemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association

(ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care*. https://doi.org/10.2337/dci19-0066

Buteau, J., El-Assaad, W., Rhodes, C. J., Mosenberg, L., Joly, E., & Prentki, M. (2004). Glucagon-like peptide-1 prevents beta cell glucolipotoxicity. *Diabetologia*. https://doi.org/10.1007/s00125-004-1379-6

Challa, T. D., Beaton, N., Arnold, M., Rudofsky, G., Langhans, W., & Wolfrum, C. (2012). Regulation of Adipocyte Formation by GLP-1/GLP-1R Signaling. *The Journal of Biological Chemistry*, *287*(9), 6421. https://doi.org/10.1074/JBC.M111.310342

Chaussenot, A., Bannwarth, S., Rouzier, C., Vialettes, B., Mkadem, S. A. El, Chabrol, B., Cano, A., Labauge, P., & Paquis-Flucklinger, V. (2011). Neurologic features and genotype-phenotype correlation in Wolfram syndrome. *Annals of Neurology*. https://doi.org/10.1002/ana.22160

Cornell, S. (2020). A review of GLP-1 receptor agonists in type 2 diabetes: A focus on the mechanism of action of once-weekly agents. In *Journal of Clinical Pharmacy and Therapeutics* (Vol. 45, Issue S1, pp. 17–27). Blackwell Publishing Ltd. https://doi.org/10.1111/jcpt.13230

Cosentino, F., Grant, P. J., Aboyans, V., Bailey, C. J., Ceriello, A., Delgado, V., Federici, M., Filippatos, G., Grobbee, D. E., Hansen, T. B., Huikuri, H. V., Johansson, I., Juni, P., Lettino, M., Marx, N., Mellbin, L. G., Ostgren, C. J., Rocca, B., Roffi, M., … Chowdhury, T. A. (2020). 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. *European Heart Journal*. https://doi.org/10.1093/eurheartj/ehz486

Crajoinas, R. O., Oricchio, F. T., Pessoa, T. D., Pacheco, B. P. M., Lessa, L. M. A., Malnic, G., & Girardi, A. C. C. (2011). Mechanisms mediating the diuretic and natriuretic actions of the incretin hormone glucagon-like peptide-1. *American Journal of Physiology –Renal Physiology*, *301*(2), 355–363. https://doi.org/10.1152/ajprenal.00729.2010

Da Porto, A., Casarsa, V., Colussi, G., Catena, C., Cavarape, A., & Sechi, L. (2020). Dulaglutide reduces binge episodes in type 2 diabetic patients with binge eating disorder: A pilot study. *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*, *14*(4), 289–292. https://doi.org/10.1016/j.dsx.2020.03.009

Danielpur, L., Sohn, Y. S., Karmi, O., Fogel, C., Zinger, A., Abu-Libdeh, A., Israeli, T., Riahi, Y., Pappo, O., Birk, R., Zangen, D. H., Mittler, R., Cabantchik, Z. I., Cerasi, E., Nechushtai, R., & Leibowitz, G. (2016). GLP-1-RA corrects mitochondrial labile iron accumulation and improves β-cell function in type 2 wolfram syndrome. *Journal of Clinical Endocrinology and Metabolism*, *101*(10), 3592–3599. https://doi.org/10.1210/jc.2016-2240

Deacon, C. F., Knudsen, L. B., Madsen, K., Wiberg, F. C., Jacobsen, O., & 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. https://doi.org/10.1007/s001250050903

Dixit, T. S., Sharma, A. N., Lucot, J. B., & Elased, K. M. (2013). Antipsychotic-like effect of GLP-1 agonist liraglutide but not DPP-IV inhibitor sitagliptin in mouse model for psychosis. *Physiology and Behavior*, *114–115*, 38–41. https://doi.org/10.1016/j.physbeh.2013.03.008

DJ Wolfram, H. W. (1938). Diabetes mellitus and simple optic atrophy among siblings: Report of four cases. *Mayo Clin Proc*, *13*, 715–718.

Drucker, D. J., & Asa, S. (1988). Glucagon gene expression in vertebrate brain. *Journal of Biological Chemistry*. https://doi.org/10.1016/S0021-9258(18)68261-4

Drucker, Daniel J. (2018). Mechanisms of Action and Therapeutic Application of Glucagon-like Peptide-1. In *Cell Metabolism* (Vol. 27, Issue 4, pp. 740–756). Cell Press. https://doi.org/10.1016/j.cmet.2018.03.001

Drucker, Daniel J., & Nauck, M. A. (2006). The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. In *Lancet* (Vol. 368, Issue 9548, pp. 1696–1705). Lancet. https://doi.org/10.1016/S0140-6736(06)69705-5

Dupre, J., Ross, S. A., Watson, D., & Brown, J. C. (1973). Stimulation of insulin secretion by gastric inhibitory polypeptide in man. *Journal of Clinical Endocrinology and Metabolism*. https://doi.org/10.1210/jcem-37-5-826

During, M. J., Cao, L., Zuzga, D. S., Francis, J. S., Fitzsimons, H. L., Jiao, X., Bland, R. J., Klugmann, M., Banks, W. A., Drucker, D. J., & Haile, C. N. (2003). Glucagon-like peptide-1 receptor is involved in learning and neuroprotection. *Nature Medicine*. https://doi.org/10.1038/nm919

Egan, A. G., Blind, E., Dunder, K., de Graeff, P. A., Hummer, B. T., Bourcier, T., & Rosebraugh, C. (2014). Pancreatic Safety of Incretin-Based Drugs — FDA and EMA Assessment. *New England Journal of Medicine*, *370*(9), 794–797. https://doi.org/10.1056/nejmp1314078

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

Ellenbroek, J. H., Töns, H. A., van Meeteren, M. J., de Graaf, N., Hanegraaf, M. A., Rabelink, T. J., Carlotti, F., & de Koning, E. J. (2013). Glucagon-like peptide-1 receptor agonist treatment reduces beta cell mass in normoglycaemic mice. *Diabetologia*, *56*(9), 1980–1986. https://doi.org/10.1007/s00125-013-2957-2

Elrick, H., Stimmler, L., Hlad, C. J., & Arai, Y. (1964). Plasma insulin response to oral and intravenous glucose administration. *The Journal of Clinical Endocrinology and Metabolism*. https://doi.org/10.1210/jcem-24-10-1076

Faerch, K., Torekov, S. S., Vistisen, D., Johansen, N. B., Witte, D. R., Jonsson, A., Pedersen, O., Hansen, T., Lauritzen, T., Sandbaek, A., Holst, J. J., & Jørgensen, M. E. (2015). GLP-1 response to oral glucose is reduced in prediabetes, screen-detected type 2 diabetes, and obesity and influenced by sex: The ADDITION-PRO Study. *Diabetes*, *64*(7), 2513–2525. https://doi.org/10.2337/db14-1751

Fehmann, H. C., & Habener, J. F. (1991). Functional receptors for the insulinotropic hormone glucagon-like peptide-I(7-37) on a somatostatin secreting cell line. *FEBS Letters*. https://doi.org/10.1016/0014-5793(91)80182-3

Fehmann, H. C., Habener, J. F., & Fehmann, H. C. (1991). Homologous desensitization of the insulinotropic glucagon-like peptide-i(7-37) receptor on insulinoma (hit-t15) cells. *Endocrinology*. https://doi.org/10.1210/endo-128-6-2880

Fonseca, S. G., Fukuma, M., Lipson, K. L., Nguyen, L. X., Allen, J. R., Oka, Y., & Urano, F. (2005). WFS1 is a novel component of the unfolded protein response and maintains homeostasis of the endoplasmic reticulum in pacreatic β-cells. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M507426200

Frías, J. P., Guja, C., Hardy, E., Ahmed, A., Dong, F., Öhman, P., & Jabbour, S. A. (2016). Exenatide once weekly plus dapagliflozin once daily versus exenatide or dapagliflozin alone in patients with type 2 diabetes inadequately controlled with

metformin monotherapy (DURATION-8): a 28 week, multicentre, double-blind, phase 3, randomised control. *The Lancet Diabetes and Endocrinology*. https://doi.org/10.1016/S2213-8587(16)30267-4

Frias, J. P., Nauck, M. A., Van, J., Kutner, M. E., Cui, X., Benson, C., Urva, S., Gimeno, R. E., Milicevic, Z., Robins, D., & Haupt, A. (2018). Efficacy and safety of LY3298176, a novel dual GIP and GLP-1 receptor agonist, in patients with type 2 diabetes: a randomised, placebo-controlled and active comparator-controlled phase 2 trial. *The Lancet*. https://doi.org/10.1016/S0140-6736(18)32260-8

Gamble, J. M., Clarke, A., Myers, K. J., Agnew, M. D., Hatch, K., Snow, M. M., & Davis, E. M. (2015). Incretin-based medications for type 2 diabetes: an overview of reviews. *Diabetes Obes Metab*, *17*(7), 649–658. https://doi.org/10.1111/dom.12465

Garber, A., Henry, R. R., Ratner, R., Hale, P., Chang, C. T., & Bode, B. (2011). Liraglutide, a once-daily human glucagon-like peptide 1 analogue, provides sustained improvements in glycaemic control and weight for 2 years as monotherapy compared with glimepiride in patients with type 2 diabetes. *Diabetes, Obesity and Metabolism*, *13*(4), 348–356. https://doi.org/10.1111/j.1463-1326.2010.01356.x

Gil-Lozano, M., Pérez-Tilve, D., Alvarez-Crespo, M., Martís, A., Fernandez, A. M., Catalina, P. A. F., Gonzalez-Matias, L. C., & Mallo, F. (2010). GLP-1(7-36)-amide and exendin-4 stimulate the HPA axis in rodents and humans. *Endocrinology*. https://doi.org/10.1210/en.2009-0915

Goldman, J. D. (2020). Cardiovascular safety outcomes of once-weekly GLP-1 receptor agonists in people with type 2 diabetes. In *Journal of Clinical Pharmacy and Therapeutics* (Vol. 45, Issue S1, pp. 61–72). Blackwell Publishing Ltd. https://doi.org/10.1111/jcpt.13226

Gray, S. M., Xin, Y., Ross, E. C., Chazotte, B. M., Capozzi, M. E., El, K., Svendsen, B., Ravn, P., Sloop, K. W., Tong, J., Gromada, J., Campbell, J. E., & D'Alessio, D. A. (2020). Discordance between GLP-1R gene and protein expression in mouse pancreatic islet cells. *The Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.RA120.014368

Gromada, J., Dissing, S., & Rorsman, P. (1996). Desensitization of glucagon-like peptide 1 receptors in insulininsulinsecreting βTC3 cells: role of PKA-independent mechanisms. *British Journal of Pharmacology*, *118*(3), 769–775. https://doi.org/10.1111/j.1476-5381.1996.tb15466.x

Gros, R., You, X., Baggio, L. L., Golam Kabir, M., Muktafi Sadi, A., Mungrue, I. N., Parker, T. G., Huang, Q., Drucker, D. J., & Husain, M. (2003). Cardiac function in mice lacking the glucagon-like peptide-1 receptor. *Endocrinology*. https://doi.org/10.1210/en.2003-0007

Gudipaty, L., Rosenfeld, N. K., Fuller, C. S., Gallop, R., Schutta, M. H., & Rickels, M. R. (2014). Effect of exenatide, sitagliptin, or glimepiride on β-cell secretory capacity in early type 2 diabetes. *Diabetes Care*, *37*(9), 2451–2458. https://doi.org/10.2337/dc14-0398

Gutzwiller, J.-P., Tschopp, S., Bock, A., Zehnder, C. E., Huber, A. R., Kreyenbuehl, M., Gutmann, H., Drewe, J., Henzen, C., Goeke, B., & Beglinger, C. (2004). Glucagon-Like Peptide 1 Induces Natriuresis in Healthy Subjects and in Insulin-Resistant Obese Men. *The Journal of Clinical Endocrinology & Metabolism*, *89*(6), 3055–3061. https://doi.org/10.1210/jc.2003-031403

Hall, S., Isaacs, D., & Clements, J. N. (2018). Pharmacokinetics and Clinical Implications of Semaglutide: A New Glucagon-Like Peptide (GLP)-1 Receptor Agonist.

In *Clinical Pharmacokinetics* (Vol. 57, Issue 12, pp. 1529–1538). Springer International Publishing. https://doi.org/10.1007/s40262-018-0668-z

Handelsman, Y., Wyne, K., Cannon, A., Shannon, M., & Schneider, D. (2018). Glycemic efficacy, weight effects, and safety of once-weekly glucagon-like peptide-1 receptor agonists. *Journal of Managed Care and Specialty Pharmacy*, *24*(9), S14–S29. https://doi.org/10.18553/jmcp.2018.24.9-a.s14

Hara, T., Mahadevan, J., Kanekura, K., Hara, M., Lu, S., & Urano, F. (2014). Calcium efflux from the endoplasmic reticulum leads to β-cell death. *Endocrinology*, *155*(3), 758–768. https://doi.org/10.1210/en.2013-1519

Harder, H., Nielsen, L., Thi, T. D. T., & Astrup, A. (2004). The effect of liraglutide, a long-acting glucagon-like peptide 1 derivative, on glycemic control, body composition, and 24-h energy expenditure in patients with type 2 diabetes. *Diabetes Care*, *27*(8), 1915–1921. https://doi.org/10.2337/diacare.27.8.1915

Hart, N. J., & Powers, A. C. (2019). Use of human islets to understand islet biology and diabetes: progress, challenges and suggestions. *Diabetologia*, *62*(2), 212–222. https://doi.org/10.1007/s00125-018-4772-2

Hayes, M. R., Kanoski, S. E., de Jonghe, B. C., Leichner, T. M., Alhadeff, A. L., Fortin, S. M., Arnold, M., Langhans, W., & Grill, H. J. (2011). The common hepatic branch of the vagus is not required to mediate the glycemic and food intake suppressive effects of glucagon-like-peptide-1. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*. https://doi.org/10.1152/ajpregu.00356.2011

Hayes, M. R., Leichner, T. M., Zhao, S., Lee, G. S., Chowansky, A., Zimmer, D., De Jonghe, B. C., Kanoski, S. E., Grill, H. J., & Bence, K. K. (2011). Intracellular signals mediating the food intake-suppressive effects of hindbrain glucagon-like peptide-1 receptor activation. *Cell Metabolism*. https://doi.org/10.1016/j.cmet.2011.02.001

Henry, R. R., Rosenstock, J., Logan, D., Alessi, T., Luskey, K., & Baron, M. A. (2014). Continuous subcutaneous delivery of exenatide via ITCA 650 leads to sustained glycemic control and weight loss for 48 weeks in metformin-treated subjects with type 2 diabetes. *Journal of Diabetes and Its Complications*. https://doi.org/10.1016/j.jdiacomp.2013.12.009

Herman, J. P. (2018). Regulation of Hypothalamo-Pituitary-Adrenocortical Responses to Stressors by the Nucleus of the Solitary Tract/Dorsal Vagal Complex. In *Cellular and Molecular Neurobiology* (Vol. 38, Issue 1, pp. 25–35). Springer New York LLC. https://doi.org/10.1007/s10571-017-0543-8

Herrmann, C., Göke, R., Richter, G., Fehmann, H. C., Arnold, R., & Göke, B. (1995). Glucagon-like peptide-1 and glucose-dependent insulin- releasing polypeptide plasma levels in response to nutrients. *Digestion*. https://doi.org/10.1159/000201231

Holman, R. R., Bethel, M. A., Mentz, R. J., Thompson, V. P., Lokhnygina, Y., Buse, J. B., Chan, J. C., Choi, J., Gustavson, S. M., Iqbal, N., Maggioni, A. P., Marso, S. P., Öhman, P., Pagidipati, N. J., Poulter, N., Ramachandran, A., Zinman, B., & Hernandez, A. F. (2017). Effects of Once-Weekly Exenatide on Cardiovascular Outcomes in Type 2 Diabetes. *New England Journal of Medicine*. https://doi.org/10.1056/nejmoa1612917

Holst, J. J., Ørskov, C., Vagn Nielsen, O., & Schwartz, T. W. (1987). Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. *FEBS Letters*. https://doi.org/10.1016/0014-5793(87)81430-8

Holst, Jens J., Bersani, M., Johnsen, A. H., Kofod, H., Hartmann, B., & Ørskov, C. (1994). Proglucagon processing in porcine and human pancreas. *Journal of Biological Chemistry*. https://doi.org/10.1016/S0021-9258(17)32241-X

Holst, Jens J., & Deacon, C. F. (1998). Inhibition of the activity of dipeptidyl-peptidase IV as a treatment for type 2 diabetes. In *Diabetes* (Vol. 47, Issue 11, pp. 1663–1670). Diabetes. https://doi.org/10.2337/diabetes.47.11.1663

Horowitz, M., Flint, A., Jones, K. L., Hindsberger, C., Rasmussen, M. F., Kapitza, C., Doran, S., Jax, T., Zdravkovic, M., & Chapman, I. M. (2012). Effect of the once-daily human GLP-1 analogue liraglutide on appetite, energy intake, energy expenditure and gastric emptying in type 2 diabetes. *Diabetes Research and Clinical Practice*, *97*(2), 258–266. https://doi.org/10.1016/j.diabres.2012.02.016

Hou, Y., Ernst, S. A., Heidenreich, K., & Williams, J. A. (2016). Glucagon-like peptide-1 receptor is present in pancreatic acinar cells and regulates amylase secretion through cAMP. *American Journal of Physiology - Gastrointestinal and Liver Physiology*. https://doi.org/10.1152/ajpgi.00293.2015

Hovorka, R., & Jones, R. H. (1994). How to measure insulin secretion. *Diabetes Metab Rev*, *10*(2), 91–117. http://www.ncbi.nlm.nih.gov/pubmed/7956679

Hovorka, R., Soons, P. A., & Young, M. A. (1996). ISEC: a program to calculate insulin secretion. *Comput Methods Programs Biomed*, *50*(3), 253–264. http://www.ncbi.nlm.nih.gov/pubmed/8894385

Hui, H., Farilla, L., Merkel, P., & Perfetti, R. (2002). The short half-life of glucagon-like peptide-1 in plasma does not reflect its long-lasting beneficial effects. *European Journal of Endocrinology*, *146*(6), 863–869. https://doi.org/10.1530/eje.0.1460863

Imeryüz, N., Yeğen, B. Ç., Bozkurt, A., Coşkun, T., Villanueva-Penacarrillo, M. L., & Ulusoy, N. B. (1997). Glucagon-like peptide-1 inhibits gastric emptying via vagal afferent- mediated central mechanisms. *American Journal of Physiology – Gastrointestinal and Liver Physiology*, *273*(4 36-4). https://doi.org/10.1152/ajpgi.1997.273.4.g920

Inoue, H., Tanizawa, Y., Wasson, J., Behn, P., Kalidas, K., Bernal-Mizrachi, E., Mueckler, M., Marshall, H., Donis-Keller, H., Crock, P., Rogers, D., Mikuni, M., Kumashiro, H., Higashi, K., Sobue, G., Oka, Y., & Alan Permutt, M. (1998). A gene encoding a transmembrane protein is mutated in patients with diabetes mellitus and optic atrophy (Wolfram syndrome). *Nature Genetics*. https://doi.org/10.1038/2441

Ishihara, H., Takeda, S., Tamura, A., Takahashi, R., Yamaguchi, S., Takei, D., Yamada, T., Inoue, H., Soga, H., Katagiri, H., Tanizawa, Y., & Oka, Y. (2004). Disruption of the WFS1 gene in mice causes progressive β-cell loss and impaired stimulus - Secretion coupling in insulin secretion. *Human Molecular Genetics*. https://doi.org/10.1093/hmg/ddh125

Jelsing, J., Vrang, N., Hansen, G., Raun, K., Tang-Christensen, M., & Knudsen, L. B. (2012). Liraglutide: short-lived effect on gastric emptying – long lasting effects on body weight. *Diabetes Obes Metab*, *14*(6), 531–538. https://doi.org/10.1111/j.1463-1326.2012.01557.x

Jendle, J., Fang, X., Cao, Y., Bojö, L., Nilsson, B. K., Hedberg, F., Santos-Pardo, I., & Nyström, T. (2018). Effects on repetitive 24-hour ambulatory blood pressure in subjects with type II diabetes randomized to liraglutide or glimepiride treatment both in combination with metformin: a randomized open parallel-group study. *Journal of the American Society of Hypertension*, *12*(5), 346–355. https://doi.org/10.1016/j.jash.2018.02.003

Kakiuchi, C., Ishigaki, S., Oslowski, C. M., Fonseca, S. G., Kato, T., & Urano, F. (2009). Valproate, a mood stabilizer, induces WFS1 expression and modulates its interaction with ER stress protein GRP94. *PLoS ONE*, *4*(1). https://doi.org/10.1371/journal.pone.0004134

Kieffer, T. J., Mc Intosh, C. H. S., & 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. https://doi.org/10.1210/endo.136.8.7628397

Kilkenny, C., Browne, W., Cuthill, I. C., Emerson, M., Altman, D. G., for the Replacement, R. finemen., & of Amimals in Research, R. (2011). Animal research: reporting in vivo experiments – the ARRIVE guidelines. *J Cereb Blood Flow Metab*, *31*(4), 991–993. https://doi.org/10.1038/jcbfm.2010.220

Kim, J. Y., Chun, S., Bang, M. S., Shin, H. I., & Lee, S. U. (2011). Safety of low-dose oral dantrolene sodium on hepatic function. *Archives of Physical Medicine and Rehabilitation*, *92*(9), 1359–1363. https://doi.org/10.1016/j.apmr.2011.04.012

Kodera, R., Shikata, K., Kataoka, H. U., Takatsuka, T., Miyamoto, S., Sasaki, M., Kajitani, N., Nishishita, S., Sarai, K., Hirota, D., Sato, C., Ogawa, D., & Makino, H. (2011). Glucagon-like peptide-1 receptor agonist ameliorates renal injury through its anti-inflammatory action without lowering blood glucose level in a rat model of type 1 diabetes. *Diabetologia*. https://doi.org/10.1007/s00125-010-2028-x

Kõks, S., Soomets, U., Paya-Cano, J. L., Fernandes, C., Luuk, H., Plaas, M., Terasmaa, a, Tillmann, V., Noormets, K., Vasar, E., & Schalkwyk, L. C. (2009). Wfs1 gene deletion causes growth retardation in mice and interferes with the growth hormone pathway. *Physiological Genomics*, *37*(3), 249–259. https://doi.org/10.1152/physiolgenomics.90407.2008

Kondo, M., Tanabe, K., Amo-Shiinoki, K., Hatanaka, M., Morii, T., Takahashi, H., Seino, S., Yamada, Y., & Tanizawa, Y. (2018). Activation of GLP-1 receptor signalling alleviates cellular stresses and improves beta cell function in a mouse model of Wolfram syndrome. *Diabetologia*, *61*(10), 2189–2201. https://doi.org/10.1007/s00125-018-4679-y

Krass, M., Rünkorg, K., Vasar, E., & Volke, V. (2012). Acute administration of GLP-1 receptor agonists induces hypolocomotion but not anxiety in mice. *Acta Neuropsychiatr*, *24*(5), 296–300. https://doi.org/10.1111/j.1601-5215.2012.00648.x

Krass, M., Volke, A., Rünkorg, K., Wegener, G., Lund, S., Abildgaard, A., Vasar, E., & Volke, V. (2015). GLP-1 receptor agonists have a sustained stimulatory effect on corticosterone release after chronic treatment. *Acta Neuropsychiatr*, *27*(1), 25–32. https://doi.org/10.1017/neu.2014.36

Kuhre, R. E., Albrechtsen, N. W., Windeløv, J. A., Svendsen, B., Hartmann, B., & Holst, J. J. (2014). GLP-1 amidation efficiency along the length of the intestine in mice, rats and pigs and in GLP-1 secreting cell lines. *Peptides*, *55*, 52–57. https://doi.org/10.1016/j.peptides.2014.01.020

La Barre, J. (1932). Sur les possibilites d'un traitement du diabete par I'incretine. *Bull Acad Royal Med Belg*, *12:620-34*.

Larsen, P. J., Tang-Christensen, M., Holst, J. J., & Ørskov, C. (1997). Distribution of glucagon-like peptide-1 and other preproglucagon-derived peptides in the rat hypothalamus and brainstem. *Neuroscience*. https://doi.org/10.1016/S0306-4522(96)00434-4

Li, Y., Hansotia, T., Yusta, B., Ris, F., Halban, P. A., & Drueker, D. J. (2003). Glucagon-like peptide-1 receptor signaling modulates β cell apoptosis. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M209423200

Lombardo, F., Salzano, G., Di Bella, C., Aversa, T., Pugliatti, F., Cara, S., Valenzise, M., De Luca, F., & Rigoli, L. (2014). Phenotypical and genotypical expression of Wolfram syndrome in 12 patients from a Sicilian district where this syndrome might not be so infrequent as generally expected. *Journal of Endocrinological Investigation*, *37*(2), 195–202. https://doi.org/10.1007/s40618-013-0039-4

Lorenz, M., Pfeiffer, C., Steinsträßer, A., Becker, R. H. A., Rütten, H., Ruus, P., & Horowitz, M. (2013). Effects of lixisenatide once daily on gastric emptying in type 2 diabetes - Relationship to postprandial glycemia. *Regulatory Peptides*, *185*, 1–8. https://doi.org/10.1016/j.regpep.2013.04.001

Lu, S., Kanekura, K., Hara, T., Mahadevan, J., Spears, L. D., Oslowski, C. M., Martinez, R., Yamazaki-Inoue, M., Toyoda, M., Neilson, A., Blanner, P., Brown, C. M., Semenkovich, C. F., Marshall, B. a., Hershey, T., Umezawa, A., Greer, P. a., & Urano, F. (2014). A calcium-dependent protease as a potential therapeutic target for Wolfram syndrome. *Proceedings of the National Academy of Sciences*, *111*(49), E5292–E5301. https://doi.org/10.1073/pnas.1421055111

Luuk, H., Plaas, M., Raud, S., Innos, J., Sütt, S., Lasner, H., Abramov, U., Kurrikoff, K., Kõks, S., & Vasar, E. (2009). Wfs1-deficient mice display impaired behavioural adaptation in stressful environment. *Behavioural Brain Research*, *198*, 334–345. https://doi.org/10.1016/j.bbr.2008.11.007

MacDonald, P. E., El-kholy, W., Riedel, M. J., Salapatek, A. M. F., Light, P. E., & Wheeler, M. B. (2002). The multiple actions of GLP-1 on the process of glucose-stimulated insulin secretion. *Diabetes*, *51*(SUPPL. 3). https://doi.org/10.2337/diabetes.51.2007.s434

Madsbad, S. (2016). Review of head-to-head comparisons of glucagon-like peptide-1 receptor agonists. In *Diabetes, Obesity and Metabolism*. https://doi.org/10.1111/dom.12596

Marso, S P, Daniels, G. H., Brown-Frandsen, K., Kristensen, P., Mann, J. F., Nauck, M. A., Nissen, S. E., Pocock, S., Poulter, N. R., Ravn, L. S., Steinberg, W. M., Stockner, M., Zinman, B., Bergenstal, R. M., Buse, J. B., Committee, L. S., & Investigators, L. T. (2016). Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med*, *375*(4), 311–322. https://doi.org/10.1056/NEJMoa1603827

Marso, Steven P., Bain, S. C., Consoli, A., Eliaschewitz, F. G., Jódar, E., Leiter, L. A., Lingvay, I., Rosenstock, J., Seufert, J., Warren, M. L., Woo, V., Hansen, O., Holst, A. G., Pettersson, J., & Vilsbøll, T. (2016). Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes. *New England Journal of Medicine*. https://doi.org/10.1056/nejmoa1607141

Marty, V. N., Farokhnia, M., Munier, J. J., Mulpuri, Y., Leggio, L., & Spigelman, I. (2020). Long-Acting Glucagon-Like Peptide-1 Receptor Agonists Suppress Voluntary Alcohol Intake in Male Wistar Rats. *Frontiers in Neuroscience*, *14*. https://doi.org/10.3389/fnins.2020.599646

Matsunaga, K., Tanabe, K., Inoue, H., Okuya, S., Ohta, Y., Akiyama, M., Taguchi, A., Kora, Y., Okayama, N., Yamada, Y., Wada, Y., Amemiya, S., Sugihara, S., Nakao, Y., Oka, Y., & Tanizawa, Y. (2014). Wolfram syndrome in the japanese population; molecular analysis of wfs1 gene and characterization of clinical features. *PLoS ONE*, *9*(9). https://doi.org/10.1371/journal.pone.0106906

Mayo KE, Miller LJ, Bataille D, et al. I. (2003). The glucagon receptor family. Pharmacol Rev. *International Union of Pharmacology.*

McGrath, J. C., & Lilley, E. (2015). Implementing guidelines on reporting research using animals (ARRIVE etc.): new requirements for publication in BJP. *Br J Pharmacol*, *172*(13), 3189–3193. https://doi.org/10.1111/bph.12955

Mcintyre, N., Holdsworth, C. D., & Turner, D. S. (1964). New interpretation of oral glucose tolerance. *The Lancet*. https://doi.org/10.1016/S0140-6736(64)90011-X

McLean, B. A., Wong, C. K., Campbell, J. E., Hodson, D. J., Trapp, S., & Drucker, D. J. (2020). Revisiting the complexity of GLP-1 action-from sites of synthesis to receptor activation. *Endocrine Reviews*. https://doi.org/10.1210/endrev/bnaa032

Meloni, A. R., Deyoung, M. B., Lowe, C., & Parkes, D. G. (2013). GLP-1 receptor activated insulin secretion from pancreatic β-cells: Mechanism and glucose dependence. In *Diabetes, Obesity and Metabolism* (Vol. 15, Issue 1, pp. 15–27). Blackwell Publishing Ltd. https://doi.org/10.1111/j.1463-1326.2012.01663.x

Mentlein, R., Gallwitz, B., & Schmidt, W. E. (1993). Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7–36)amide, peptide histidine methionine and is responsible for their degradation in human serum. *European Journal of Biochemistry*, *214*(3), 829–835. https://doi.org/10.1111/j.1432-1033.1993.tb17986.x

Minton, J. A. L., Rainbow, L. A., Ricketts, C., & Barrett, T. G. (2003). Wolfram syndrome. In *Reviews in Endocrine and Metabolic Disorders* (Vol. 4, Issue 1, pp. 53–59). Kluwer Academic Publishers. https://doi.org/10.1023/A:1021875403463

Mojsov, S., Heinrich, G., Wilson, I. B., Ravazzola, M., Orci, L., & Habener, J. F. (1986). Preproglucagon gene expression in pancreas and intestine diversifies at the level of post-translational processing. *Journal of Biological Chemistry*. https://doi.org/10.1016/S0021-9258(18)67324-7

Mondragon, A., Davidsson, D., Kyriakoudi, S., Bertling, A., Gomes-Faria, R., Cohen, P., Rothery, S., Chabosseau, P., Rutter, G. a, & da Silva Xavier, G. (2014). Divergent effects of liraglutide, exendin-4, and sitagliptin on beta-cell mass and indicators of pancreatitis in a mouse model of hyperglycaemia. *PloS One*, *9*(8), e104873. https://doi.org/10.1371/journal.pone.0104873

Müller, T. D., Finan, B., Bloom, S. R., D'Alessio, D., Drucker, D. J., Flatt, P. R., Fritsche, A., Gribble, F., Grill, H. J., Habener, J. F., Holst, J. J., Langhans, W., Meier, J. J., Nauck, M. A., Perez-Tilve, D., Pocai, A., Reimann, F., Sandoval, D. A., Schwartz, T. W., … Tschöp, M. H. (2019). Glucagon-like peptide 1 (GLP-1). In *Molecular Metabolism* (Vol. 30, pp. 72–130). Elsevier GmbH. https://doi.org/10.1016/j.molmet.2019.09.010

Muscogiuri, G., DeFronzo, R. A., Gastaldelli, A., & Holst, J. J. (2017). Glucagon-like Peptide-1 and the Central/Peripheral Nervous System: Crosstalk in Diabetes. In *Trends in Endocrinology and Metabolism* (Vol. 28, Issue 2, pp. 88–103). Elsevier Inc. https://doi.org/10.1016/j.tem.2016.10.001

Nakatani, Y., Kawabe, A., Matsumura, M., Aso, Y., Yasu, T., Banba, N., & Nakamoto, T. (2016). Effects of GLP-1 receptor agonists on heart rate and the autonomic nervous system using holter electrocardiography and power spectrum analysis of heart rate variability. *Diabetes Care*, *39*(2), e22–e23. https://doi.org/10.2337/dc15-1437

Näslund, E., King, N., Mansten, S., Adner, N., Holst, J. J., Gutniak, M., & Hellström, P. M. (2004). Prandial subcutaneous injections of glucagon-like peptide-1 cause weight

loss in obese human subjects. *British Journal of Nutrition*, *91*(3), 439–446. https://doi.org/10.1079/bjn20031064

Nauck, M. A., Heimesaat, M. M., Orskov, C., Holst, J. J., Ebert, R., & 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. *Journal of Clinical Investigation*, *91*(1), 301–307. https://doi.org/10.1172/JCI116186

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*(5), 1561–1565. https://doi.org/10.2337/db10-0474

Nauck, Michael A., Petrie, J. R., Sesti, G., Mannucci, E., Courrèges, J. P., Lindegaard, M. L., Jensen, C. B., & Atkin, S. L. (2016). A phase 2, randomized, dose-finding study of the novel once-weekly human GLP-1 analog, semaglutide, compared with placebo and open-label liraglutide in patients with type 2 diabetes. *Diabetes Care*, *39*(2), 231–241. https://doi.org/10.2337/dc15-0165

Nauck, Michael A., Quast, D. R., Wefers, J., & Meier, J. J. (2020). GLP-1 receptor agonists in the treatment of type 2 diabetes – state-of-the-art. *Molecular Metabolism*. https://doi.org/10.1016/j.molmet.2020.101102

Nian, M., Drucker, D. J., & Irwin, D. (1999). Divergent regulation of human and rat proglucagon gene promoters in vivo. *American Journal of Physiology - Gastrointestinal and Liver Physiology*. https://doi.org/10.1152/ajpgi.1999.277.4.g829

Nie, Y., Nakashima, M., Brubaker, P. L., Li, Q. L., Perfetti, R., Jansen, E., Zambre, Y., Pipeleers, D., & Friedman, T. C. (2000). Regulation of pancreatic PC1 and PC2 associated with increased glucagon- like peptide 1 in diabetic rats. *Journal of Clinical Investigation*. https://doi.org/10.1172/JCI7456

Noormets, K., Kõks, S., Muldmaa, M., Mauring, L., Vasar, E., & Tillmann, V. (2011). Sex differences in the development of diabetes in mice with deleted wolframin (Wfs1) gene. *Experimental and Clinical Endocrinology and Diabetes*, *119*(5), 271–275. https://doi.org/10.1055/s-0030-1265163

Nuffer, W. A., & Trujillo, J. M. (2015). Liraglutide: A New Option for the Treatment of Obesity. *Pharmacotherapy*. https://doi.org/10.1002/phar.1639

Orskov, C., Bersani, M., Johnsen, A. H., Hojrup, P., & Holst, J. J. (1989). Complete sequences of glucagon-like peptide-1 from human and pig small intestine. *Journal of Biological Chemistry*, *264*(22), 12826–12829. https://doi.org/10.1016/S0021-9258(18)51561-1

Ørskov, C., Poulsen, S. S., Møller, M., & Holst, J. J. (1996). Glucagon-like peptide I receptors in the subfornical organ and the area postrema are accessible to circulating glucagon-like peptide I. *Diabetes*. https://doi.org/10.2337/diab.45.6.832

Osaka, T., Endo, M., Yamakawa, M., & Inoue, S. (2005). Energy expenditure by intravenous administration of glucagon-like peptide-1 mediated by the lower brainstem and sympathoadrenal system. *Peptides*, *26*(9), 1623–1631. https://doi.org/10.1016/j.peptides.2005.02.016

Pallotta, M. T., Tascini, G., Crispoldi, R., Orabona, C., Mondanelli, G., Grohmann, U., & Esposito, S. (2019). Wolfram syndrome, a rare neurodegenerative disease: From pathogenesis to future treatment perspectives. In *Journal of Translational Medicine*. https://doi.org/10.1186/s12967-019-1993-1

Panjwani, N., Mulvihill, E. E., Longuet, C., Yusta, B., Campbell, J. E., Brown, T. J., Streutker, C., Holland, D., Cao, X., Baggio, L. L., & Drucker, D. J. (2013). GLP-1 receptor activation indirectly reduces hepatic lipid accumulation but does not

attenuate development of atherosclerosis in diabetic male ApoE -/- mice. *Endocrinology*. https://doi.org/10.1210/en.2012-1937

Perry, TracyAnn, & Greig, N. (2005). Enhancing Central Nervous System Endogenous GLP-1 Receptor Pathways for Intervention in Alzheimers Disease. *Current Alzheimer Research*, *2*(3), 377–385. https://doi.org/10.2174/1567205054367892

Perry, Tracyann, Haughey, N. J., Mattson, M. P., Egan, J. M., & Greig, N. H. (2002). Protection and reversal of excitotoxic neuronal damage by glucagon-like peptide-1 and exendin-4. *Journal of Pharmacology and Experimental Therapeutics*, *302*(3), 881–888. https://doi.org/10.1124/jpet.102.037481

Perry, Tracyann, Lahiri, D. K., Chen, D., Zhou, J. I. E., Shaw, K. T. Y., Egan, J. M., & Greig, N. H. (2002). A novel neurotrophic property of glucagon-like peptide 1: A promoter of nerve growth factor-mediated differentiation in PC12 cells. *Journal of Pharmacology and Experimental Therapeutics*. https://doi.org/10.1124/jpet.300.3.958

Petit, J. M., Cercueil, J. P., Loffroy, R., Denimal, D., Bouillet, B., Fourmont, C., Chevallier, O., Duvillard, L., & Vergès, B. (2016). Effect of liraglutide therapy on liver fat content in patients with inadequately controlled type 2 diabetes. The Lira-NAFLD study. *J Clin Endocrinol Metab*, jc20162775. https://doi.org/10.1210/jc.2016-2775

Pfeffer, M. A., Claggett, B., Diaz, R., Dickstein, K., Gerstein, H. C., Køber, L. V., Lawson, F. C., Ping, L., Wei, X., Lewis, E. F., Maggioni, A. P., McMurray, J. J. V., Probstfield, J. L., Riddle, M. C., Solomon, S. D., & Tardif, J.-C. (2015). Lixisenatide in Patients with Type 2 Diabetes and Acute Coronary Syndrome. *New England Journal of Medicine*. https://doi.org/10.1056/nejmoa1509225

Pi-Sunyer, X., Astrup, A., Fujioka, K., Greenway, F., Halpern, A., Krempf, M., Lau, D. C. W., le Roux, C. W., Violante Ortiz, R., Jensen, C. B., & Wilding, J. P. H. (2015). A Randomized, Controlled Trial of 3.0 mg of Liraglutide in Weight Management. *New England Journal of Medicine*, *373*(1), 11–22. https://doi.org/10.1056/nejmoa1411892

Piccinno, E., Ortolani, F., Vendemiale, M., Tummolo, A., Masciopinto, M., Natale, M. P., De Luca, A., Agolini, E., Aloi, C., Salina, A., D'Annunzio, G., Fischetto, R., & Papadia, F. (2014). Novel homozygous mutation in exon 5 of WFS1 gene in an Apulian family with mild phenotypic expression of Wolfram syndrome. In *Clinical Genetics* (Vol. 86, Issue 2, pp. 197–198). Blackwell Publishing Ltd. https://doi.org/10.1111/cge.12260

Pizzimenti, V., Giandalia, A., Cucinotta, D., Russo, G. T., Smits, M., Cutroneo, P. M., & Trifirò, G. (2016). Incretin-based therapy and acute cholecystitis: A review of case reports and EudraVigilance spontaneous adverse drug reaction reporting database. In *Journal of Clinical Pharmacy and Therapeutics* (Vol. 41, Issue 2, pp. 116–118). Blackwell Publishing Ltd. https://doi.org/10.1111/jcpt.12373

Portha, B., Tourrel-Cuzin, C., & Movassat, J. (2011). Activation of the glp-1 receptor signalling pathway: A relevant strategy to repair a deficient beta-cell mass. In *Experimental Diabetes Research* (Vol. 2011). https://doi.org/10.1155/2011/376509

Prato, S. D. C., Kang, I. Y., JAHOON, TRAUTMANN, M. E., YOON, K.-H., & SORLI, C. H. (2018). Efpeglenatide, a Long-Acting Glucagon-Like Peptide-1 Receptor Agonist – Immunogenicity Profile Based on Preclinical and Clinical Studies. *Diabetes*, *67*(Supplement 1), 1097-P. https://doi.org/10.2337/db18-1097-p

Pyke, C., Heller, R. S., Kirk, R. K., Ørskov, C., Reedtz-Runge, S., Kaastrup, P., Hvelplund, A., Bardram, L., Calatayud, D., & Knudsen, L. B. (2014). GLP-1

receptor localization in monkey and human tissue: Novel distribution revealed with extensively validated monoclonal antibody. *Endocrinology*. https://doi.org/10.1210/en.2013-1934

Rehfeld, J. F. (2018). The origin and understanding of the incretin concept. In *Frontiers in Endocrinology* (Vol. 9, Issue JUL). Frontiers Media S.A. https://doi.org/10.3389/fendo.2018.00387

Reschke, F., Rohayem, J., Maffei, P., Dassie, F., Schwandt, A., de Beaufort, C., Toni, S., Szypowska, A., Cardona-Hernandez, R., Datz, N., Klee, K., & Danne, T. (2021). Collaboration for rare diabetes: understanding new treatment options for Wolfram syndrome. In *Endocrine* (Vol. 71, Issue 3, pp. 626–633). Springer. https://doi.org/10.1007/s12020-021-02622-3

Riggs, A. C., Bernal-Mizrachi, E., Ohsugi, M., Wasson, J., Fatrai, S., Welling, C., Murray, J., Schmidt, R. E., Herrera, P. L., & Permutt, M. A. (2005). Mice conditionally lacking the Wolfram gene in pancreatic islet beta cells exhibit diabetes as a result of enhanced endoplasmic reticulum stress and apoptosis. *Diabetologia*. https://doi.org/10.1007/s00125-005-1947-4

Rigoli, L., Aloi, C., Salina, A., Di Bella, C., Salzano, G., Caruso, R., Mazzon, E., Maghnie, M., Patti, G., D'Annunzio, G., & Lombardo, F. (2020). Wolfram syndrome 1 in the Italian population: genotype–phenotype correlations. *Pediatric Research*, *87*(3), 456–462. https://doi.org/10.1038/s41390-019-0487-4

Ritzel, R., Ørskov, C., Holst, J. J., & Nauck, M. A. (1995). Pharmacokinetic, insulinotropic, and glucagonostatic properties of GLP-1 [7-36 amide] after subcutaneous injection in healthy volunteers. Dose-response-relationships. *Diabetologia*, *38*(6), 720–725. https://doi.org/10.1007/BF00401846

Rolin, B., Larsen, M. O., Gotfredsen, C. F., Deacon, C. F., Carr, R. D., Wilken, M., & 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*(4), E745-52. https://doi.org/10.1152/ajpendo.00030.2002

Rouille, Y., Martin, S., & Steiner, D. F. (1995). Differential processing of proglucagon by the subtilisin-like prohormone convertases PC2 and PC3 to generate either glucagon or glucagon-like peptide. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.270.44.26488

Rowlands, J., Heng, J., Newsholme, P., & Carlessi, R. (2018). Pleiotropic Effects of GLP-1 and Analogs on Cell Signaling, Metabolism, and Function. *Frontiers in Endocrinology*, *9*, 672. https://doi.org/10.3389/fendo.2018.00672

Rüttimann, E. B., Arnold, M., Hillebrand, J. J., Geary, N., & Langhans, W. (2009). Intrameal hepatic portal and intraperitoneal infusions of glucagon-like peptide-1 reduce spontaneous meal size in the rat via different mechanisms. *Endocrinology*. https://doi.org/10.1210/en.2008-1221

Ryan, G. J., Moniri, N. H., & Smiley, D. D. (2013). Clinical effects of once-weekly exenatide for the treatment of type 2 diabetes mellitus. *American Journal of Health-System Pharmacy*. https://doi.org/10.2146/ajhp120168

Sandhu, M. S., Weedon, M. N., Fawcett, K. A., Wasson, J., Debenham, S. L., Daly, A., Lango, H., Frayling, T. M., Neumann, R. J., Sherva, R., Blech, I., Pharoah, P. D., Palmer, C. N. A., Kimber, C., Tavendale, R., Morris, A. D., McCarthy, M. I., Walker, M., Hitman, G., … Barroso, I. (2007). Common variants in WFS1 confer risk of type 2 diabetes. *Nature Genetics*. https://doi.org/10.1038/ng2067

Sarafidis, P., Ferro, C. J., Morales, E., Ortiz, A., Malyszko, J., Hojs, R., Khazim, K., Ekart, R., Valdivielso, J., Fouque, D., London, G. M., Massy, Z., Ruggenenti, P.,

Porrini, E., Wiecek, A., Zoccali, C., Mallamaci, F., & Hornum, M. (2019). SGLT-2 inhibitors and GLP-1 receptor agonists for nephroprotection and cardioprotection in patients with diabetes mellitus and chronic kidney disease. A consensus statement by the EURECA-m and the DIABESITY working groups of the ERA-EDTA. *Nephrology Dialysis Transplantation*, *34*(2), 208–230. https://doi.org/10.1093/ndt/gfy407

Sarwar, N., Gao, P., Kondapally Seshasai, S. R., Gobin, R., Kaptoge, S., Di Angelantonio, E., Ingelsson, E., Lawlor, D. A., Selvin, E., Stampfer, M., Stehouwer, C. D. A., Lewington, S., Pennells, L., Thompson, A., Sattar, N., White, I. R., Ray, K. K., Danesh, J., Tipping, R. W., … Wormser, D. (2010). Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: A collaborative meta-analysis of 102 prospective studies. *The Lancet*, *375*(9733), 2215–2222. https://doi.org/10.1016/S0140-6736(10)60484-9

Schmidtler, J., Dehne, K., Allescher, H. D., Schusdziarra, V., Classen, M., Holst, J. J., Polack, A., & Schepp, W. (1994). Rat parietal cell receptors for GLP-1-(7-36) amide: Northern blot, cross- linking, and radioligand binding. *American Journal of Physiology – Gastrointestinal and Liver Physiology*. https://doi.org/10.1152/ajpgi.1994.267.3.g423

Scrocchi, L. A., Brown, T. J., MacLusky, N., Brubaker, P. L., Auerbach, A. B., Joyner, A. L., & Drucker, D. J. (1996). Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. *Nature Medicine*. https://doi.org/10.1038/nm1196-1254

Scully, K. J., & Wolfsdorf, J. I. (2021). Efficacy of GLP-1 Agonist Therapy in Autosomal Dominant WFS1-Related Disorder: A Case Report. *Hormone Research in Paediatrics*, *93*(6), 409–414. https://doi.org/10.1159/000510852

Secher, A., Jelsing, J., Baquero, A. F., Hecksher-Sørensen, J., Cowley, M. A., Dalbøge, L. S., Hansen, G., Grove, K. L., Pyke, C., Raun, K., Schäffer, L., Tang-Christensen, M., Verma, S., Witgen, B. M., Vrang, N., & Bjerre Knudsen, L. (2014). The arcuate nucleus mediates GLP-1 receptor agonist liraglutide-dependent weight loss. *J Clin Invest*, *124*(10), 4473–4488. https://doi.org/10.1172/JCI75276

Sedman, T., Krass, M., Rünkorg, K., Vasar, E., & Volke, V. (2020). Tolerance develops toward GLP-1 receptor agonists' glucose-lowering effect in mice. *European Journal of Pharmacology*. https://doi.org/10.1016/j.ejphar.2020.173443

Sedman, T., Vasar, E., & Volke, V. (2017). Tolerance does not develop toward liraglutide's glucose-lowering effect. *Journal of Clinical Endocrinology and Metabolism*, *102*(7). https://doi.org/10.1210/jc.2017-00199

Seppa, K., Toots, M., Reimets, R., Jagomäe, T., Koppel, T., Pallase, M., Hasselholt, S., Krogsbæk Mikkelsen, M., Randel Nyengaard, J., Vasar, E., Terasmaa, A., & Plaas, M. (2019). GLP-1 receptor agonist liraglutide has a neuroprotective effect on an aged rat model of Wolfram syndrome. *Scientific Reports*, *9*(1), 1–13. https://doi.org/10.1038/s41598-019-52295-2

Sequeira, A., Kim, C., Seguin, M., Lesage, A., Chawky, N., Desautels, A., Tousignant, M., Vanier, C., Lipp, O., Benkelfat, C., Rouleau, G., & Turecki, G. (2003). Wolfram syndrome and suicide: Evidence for a role of WFS1 in suicidal and impulsive behavior. *American Journal of Medical Genetics - Neuropsychiatric Genetics*, *119 B*(1), 108–113. https://doi.org/10.1002/ajmg.b.20011

Simonds, S. E., Pryor, J. T., Koegler, F. H., Buch-Rasmussen, A. S., Kelly, L. E., Grove, K. L., & Cowley, M. A. (2019). Determining the effects of combined liraglutide and phentermine on metabolic parameters, blood pressure, and heart rate

in lean and obese Male mice. *Diabetes*, *68*(4), 683–695. https://doi.org/10.2337/db18-1149

Smits, M. M., Tonneijck, L., Muskiet, M. H. A., Hoekstra, T., Kramer, M. H. H., Diamant, M., & Van Raalte, D. H. (2017). Heart rate acceleration with GLP-1 receptor agonists in type 2 diabetes patients: An acute and 12-week randomised, double-blind, placebo-controlled trial. *European Journal of Endocrinology*. https://doi.org/10.1530/EJE-16-0507

Steven, S., Jurk, K., Kopp, M., Kröller-Schön, S., Mikhed, Y., Schwierczek, K., Roohani, S., Kashani, F., Oelze, M., Klein, T., Tokalov, S., Danckwardt, S., Strand, S., Wenzel, P., Münzel, T., & Daiber, A. (2017). Glucagon-like peptide-1 receptor signalling reduces microvascular thrombosis, nitro-oxidative stress and platelet activation in endotoxaemic mice. *Br J Pharmacol*, *174*(12), 1620–1632. https://doi.org/10.1111/bph.13549

Strom, T. M., Hörtnagel, K., Hofmann, S., Gekeler, F., Scharfe, C., Rabl, W., Gerbitz, K. D., & Meitinger, T. (1998). Diabetes insipidus, diabetes mellitus, optic atrophy and deafness (DIDMOAD) caused by mutations in a novel gene (wolframin) coding for a predicted transmembrane protein. *Human Molecular Genetics*. https://doi.org/10.1093/hmg/7.13.2021

Surwit, R. S., Feinglos, M. N., Livingston, E. G., Kuhn, C. M., & McCubbin, J. A. (1984). Behavioral manipulation of the diabetic phenotype in ob/ob mice. *Diabetes*, *33*, 616–618. https://doi.org/10.2337/diabetes.33.7.616

Takeda, K., Inoue, H., Tanizawa, Y., Matsuzaki, Y., Oba, J., Watanabe, Y., Shinoda, K., & Oka, Y. (2001). WFS1 (Wolfram syndrome 1) gene product: Predominant subcellular localization to endoplasmic reticulum in cultured cells and neuronal expression in rat brain. *Human Molecular Genetics*. https://doi.org/10.1093/hmg/10.5.477

Tamborlane, W. V., Barrientos-Pérez, M., Fainberg, U., Frimer-Larsen, H., Hafez, M., Hale, P. M., Jalaludin, M. Y., Kovarenko, M., Libman, I., Lynch, J. L., Rao, P., Shehadeh, N., Turan, S., Weghuber, D., & Barrett, T. (2019). Liraglutide in Children and Adolescents with Type 2 Diabetes. *New England Journal of Medicine*. https://doi.org/10.1056/nejmoa1903822

Thomsen, M., Dencker, D., Wörtwein, G., Weikop, P., Egecioglu, E., Jerlhag, E., Fink-Jensen, A., & Molander, A. (2017). The glucagon-like peptide 1 receptor agonist Exendin-4 decreases relapse-like drinking in socially housed mice. *Pharmacology Biochemistry and Behavior*, *160*, 14–20. https://doi.org/10.1016/j.pbb.2017.07.014

Thorens, B. (1992). Expression cloning of the pancreatic β cell receptor for the gluco-incretin hormone glucagon-like peptide 1. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.89.18.8641

Thyssen, S., Arany, E., & Hill, D. J. (2006). Ontogeny of regeneration of β-cells in the neonatal rat after treatment with streptozotocin. *Endocrinology*. https://doi.org/10.1210/en.2005-0396

Toppings, N. B., McMillan, J. M., Au, P. Y. B., Suchowersky, O., & Donovan, L. E. (2018). Wolfram Syndrome: A Case Report and Review of Clinical Manifestations, Genetics Pathophysiology, and Potential Therapies. *Case Reports in Endocrinology*, *2018*, 1–8. https://doi.org/10.1155/2018/9412676

Tran, K. L., Park, Y. I., Pandya, S., Muliyil, N. J., Jensen, B. D., Huynh, K., & Nguyen, Q. T. (2017). Overview of glucagon-like peptide-1 receptor agonists for the treatment of patients with type 2 diabetes. *American Health and Drug Benefits*, *10*(4), 178–187. https://pubmed.ncbi.nlm.nih.gov/28794822/

Tudurí, E., Beiroa, D., Porteiro, B., López, M., Diéguez, C., & Nogueiras, R. (2015). Acute but not chronic activation of brain glucagon-like peptide-1 receptors enhances glucose-stimulated insulin secretion in mice. *Diabetes, Obesity and Metabolism*, *17*(8), 789–799. https://doi.org/10.1111/dom.12488

Tuttle, K. R., Lakshmanan, M. C., Rayner, B., Busch, R. S., Zimmermann, A. G., Woodward, D. B., & Botros, F. T. (2018). Dulaglutide versus insulin glargine in patients with type 2 diabetes and moderate-to-severe chronic kidney disease (AWARD-7): a multicentre, open-label, randomised trial. *The Lancet Diabetes and Endocrinology*. https://doi.org/10.1016/S2213-8587(18)30104-9

Umapathysivam, M. M., Lee, M. Y., Jones, K. L., Annink, C. E., Cousins, C. E., Trahair, L. G., Rayner, C. K., Chapman, M. J., Nauck, M. A., Horowitz, M., & Deane, A. M. (2014). Comparative effects of prolonged and intermittent stimulation of the glucagon-like peptide 1 receptor on gastric emptying and glycemia. *Diabetes*, *63*(2), 785–790. https://doi.org/10.2337/db13-0893

Urano, F. (2016). Wolfram Syndrome: Diagnosis, Management, and Treatment. In *Current Diabetes Reports* (Vol. 16, Issue 1, pp. 1–8). Current Medicine Group LLC 1. https://doi.org/10.1007/s11892-015-0702-6

van Bloemendaal, L., IJzerman, R. G., Ten Kulve, J. S., Barkhof, F., Konrad, R. J., Drent, M. L., Veltman, D. J., & Diamant, M. (2014). GLP-1 receptor activation modulates appetite- and reward-related brain areas in humans. *Diabetes*, *63*(12), 4186–4196. https://doi.org/10.2337/db14-0849

Vilsbøll, T., Christensen, M., Junker, A. E., Knop, F. K., & Gluud, L. L. (2012). Effects of glucagon-like peptide-1 receptor agonists on weight loss: Systematic review and meta-analyses of randomised controlled trials. *BMJ (Online)*, *344*(7841). https://doi.org/10.1136/bmj.d7771

Widmann, C., Dolci, W., & Thorens, B. (1996). Heterologous desensitization of the glucagon-like peptide-1 receptor by phorbol esters requires phosphorylation of the cytoplasmic tail at four different sites. *Journal of Biological Chemistry*, *271*(33), 19957–19963. https://doi.org/10.1074/jbc.271.33.19957

Windeløv, J. A., Wewer Albrechtsen, N. J., Kuhre, R. E., Jepsen, S. L., Hornburg, D., Pedersen, J., Jensen, E. P., Galsgaard, K. D., Winther-Sørensen, M., Ørgaard, A., Deacon, C. F., Mann, M., Kissow, H., Hartmann, B., & Holst, J. J. (2017). Why is it so difficult to measure glucagon-like peptide-1 in a mouse? *Diabetologia*. https://doi.org/10.1007/s00125-017-4347-7

Wu, B., Wei, S., Petersen, N., Ali, Y., Wang, X., Bacaj, T., Rorsman, P., Hong, W., Südhof, T. C., & Han, W. (2015). Synaptotagmin-7 phosphorylation mediates GLP-1-dependent potentiation of insulin secretion from β-cells. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(32), 9996–10001. https://doi.org/10.1073/pnas.1513004112

Yusta, B., Baggio, L. L., Estall, J. L., Koehler, J. A., Holland, D. P., Li, H., Pipeleers, D., Ling, Z., & Drucker, D. J. (2006). GLP-1 receptor activation improves β cell function and survival following induction of endoplasmic reticulum stress. *Cell Metabolism*, *4*(5), 391–406. https://doi.org/10.1016/j.cmet.2006.10.001

Zander, M., Madsbad, S., Madsen, J. L., & Holst, J. J. (2002). Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and β-cell function in type 2 diabetes: A parallel-group study. *Lancet*, *359*(9309), 824–830. https://doi.org/10.1016/S0140-6736(02)07952-7

Zhang, H., Colclough, K., Gloyn, A. L., & Pollin, T. I. (2021). Monogenic diabetes: A gateway to precision medicine in diabetes. In *Journal of Clinical Investigation* (Vol.

131, Issue 3). American Society for Clinical Investigation. https://doi.org/10.1172/JCI142244

Zheng, H., Stornetta, R. L., Agassandian, K., & Rinaman, L. (2015). Glutamatergic phenotype of glucagon-like peptide 1 neurons in the caudal nucleus of the solitary tract in rats. *Brain Structure and Function*. https://doi.org/10.1007/s00429-014-0841-6

PUBLICATIONS

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Sedman, Tuuli; Krass, Maarja; Rünkorg, Kertu; Vasar, Eero; Volke, Vallo (2020). Tolerance develops toward GLP-1 receptor agonists' glucose-lowering effect in mice. European Journal of Pharmacology, 173443−173443. DOI: 10.1016/j.ejphar.2020.173443.

Sedman, Tuuli; Vasar, Eero; Volke, Vallo (2017). Tolerance Does Not Develop Toward Liraglutide's Glucose-Lowering Effect. The Journal of Clinical Endocrinology & Metabolism, 102 (7), 2335−2339. DOI: 10.1210/jc.2017-00199.

Sedman T, Heinla K, Vasar E, Volke V (2017). Liraglutide Treatment May Affect Renin and Aldosterone Release. Hormone and Metabolic Research, 49 (1), 5−9. DOI: 10.1055/s-0042-109065.

Sedman, Tuuli; Rünkorg, Kerttu; Krass, Maarja; Luuk, Hendrik; Plaas, Mario; Vasar Eero; Volke, Vallo (2016). Exenatide Is an Effective Antihyperglycaemic Agent in a Mouse Model of Wolfram Syndrome 1. Journal of Diabetes Research, 9239530, 1−7. DOI: 10.1155/2016/9239530.

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Sedman, Tuuli; Vasar, Eero; Volke, Vallo (2017). Tolerance Does Not Develop Toward Liraglutide's Glucose-Lowering Effect. The Journal of Clinical Endocrinology & Metabolism, 102 (7), 2335−2339. DOI: 10.1210/jc.2017-00199.

Sedman T, Heinla K, Vasar E, Volke V (2017). Liraglutide Treatment May Affect Renin and Aldosterone Release. Hormone and Metabolic Research, 49 (1), 5−9. DOI: 10.1055/s-0042-109065.

Sedman, Tuuli; Rünkorg, Kerttu; Krass, Maarja; Luuk, Hendrik; Plaas, Mario; Vasar Eero; Volke, Vallo (2016). Exenatide Is an Effective Antihyperglycaemic Agent in a Mouse Model of Wolfram Syndrome 1. Journal of Diabetes Research, 9239530, 1−7. DOI: 10.1155/2016/9239530.

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