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Effects of GLP-1 receptor agonists on pituitary and adrenal hormones

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CONTENTS

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II Heinla K, Vasar E, Sedman T, Volke V. A GLP-1 Receptor Agonist Inhibits Aldosterone Release in Healthy Volunteers. Horm Metab Res. 2021; 53(6):402–407. doi: 10.1055/a-1498-7098.

III Heinla K, Vasar E, Reppo I, Sedman T, Volke V. GLP-1 Receptor Agonists Induce Growth Hormone Secretion in Healthy Volunteers. Diabetes Ther. 2023; 14(4): 777–786. doi:10.1007/s13300-023-01381-w.

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ABBREVIATIONS

AC	Adenylate cyclase
ACE	Angiotensin-converting enzyme
ACTH	Adrenocorticotropic hormone
ADH	Antidiuretic hormone
Akt	Protein kinase B
AMPK	Adenosine monophosphate-activated protein kinase
ANOVA	Analysis of variance
AP	Area postrema
ARB	Angiotensin II type-1 receptor blocker
ARC	Arcuate nucleus
AS160	Akt substrate of 160 kDa
AVP	Arginine vasopressin
BBB	Blood-brain-barrier
BID	Twice a day
BP	Blood pressure
cAMP	Cyclic adenosine monophosphate
CAR	Cortisol awakening response
CNS	Central nervous system
CRF	Corticotropin-releasing factor
CRH	Corticotropic releasing hormone
DPP-4	Dipeptidyl peptidase-4
eGFR	Estimated glomerular filtration rate
EMA	European Medicines Agency
ESRD	End-stage renal disease
GCG	Proglucagon
GGIT	Graded glucose infusion test
GH	Growth hormone
GHRH	Growth hormone-releasing hormone
GI	Gastrointestinal
GLP-1	Glucagon-like peptide 1
GLP-2	Glucagon-like peptide 2
GLP-1R	Glucagon-like peptide 1 receptor
GLP-1 RA	Glucagon-like peptide 1 receptor agonist
GPCR	G protein-coupled receptor
GRPP	Glicentin-related pancreatic polypeptide
Glut-4	Glucose transporter type 4
GST	Glucagon stimulation test
HbA1c	Haemoglobin A1c
HPA axis	Hypothalamic-pituitary-adrenal axis
HR	Heart rate
IGF-1	Insulin-like growth factor 1
IP-1	Intervening peptide 1

IP-2	Intervening peptide 2
ITT	Insulin tolerance test
LH	Luteinising hormone
MACE	Major adverse cardiovascular events
MRA	Mineralocorticoid receptor antagonist
mRNA	Messenger ribonucleic acid
NTS	Nucleus tractus solitarius
PI3K	Phosphoinositide 3-kinase
PKA	Protein kinase A
PPG	Preproglucagon
PVN	Paraventricular nucleus
p.o	Orally
QD	Once daily
QTc interval	QT corrected for heart rate interval
QW	Once weekly
RAAS	Renin-angiotensin-aldosterone system
SD	Standard deviation
s.c	Subcutaneously
SmPC	Summary of Product Characteristics
SST	Somatostatin
$t_{1/2}$	Half-life
T2DM	Type 2 diabetes mellitus
TBC1D	Tre-2/BUB2/cdc 1 domain family
t_{max}	Time to peak drug concentration
VAS	Visual analogue scale

1. INTRODUCTION

Glucagon-like peptide -1 (GLP-1) belongs to a class of incretin hormones. The other related hormone is glucagon-like peptide-2, which is also a proglucagon derived peptide playing a multifaceted role within the intestine (Baldassano et al., 2014).

GLP-1 is secreted in response to food intake from the L-cells located in the epithelium of the gastrointestinal tract (Eissele et al., 1992). It was first isolated in 1986 from porcine intestinal mucosa (Nauck et al., 1986). Enteroendocrine L-cells are found throughout the jejunum, ileum, and colon (Eissele et al., 1992). Preproglucagon is expressed in pancreatic α-cells and enteroendocrine L-cells, and its expression is controlled by cAMP-activation of protein kinase A (PKA) (Philippe et al., 1991, Mojsov et al., 1986, Nian et al., 1999, Drucker et al., 1989). GLP-1 is a posttranslational cleavage product of Gcg. Moreover, a small population of preproglucagon-positive neurons are located in the nucleus tractus solitarius (NTS) in the brainstem, being the primary source of endogenous GLP-1 in the brain (Holt et al., 2019).

GLP-1 secretion is strongly stimulated by ingestion of nutrients, especially carbohydrates (Layer et al., 1995). GLP-1 plasma concentrations, being in the low picomolar range in fasting state, start to rise a few minutes after nutrient intake, reach a peak after ~1 hour, and return to basal concentrations after several hours (Nauck et al., 2016). GLP-1 has a major role in postprandial insulin release, also known as the incretin effect. It is stimulating insulin release in a glucose-dependent manner, thereby reducing risk for hypoglycemia (Nauck et al., 1986).

It has been demonstrated that the incretin effect is significantly impaired in subjects with type 2 diabetes (T2DM) (Nauck, Stockmann et al., 1986). Incretin deficiency is therefore considered one of the important pathogenetic factors behind impaired glucose tolerance – a hallmark for T2DM. GLP-1 receptor agonists are a novel drug class for the treatment of T2DM, with many additional effects beyond glucose control.

The main aim of this work was to provide a deeper insight into pharmacodynamic effects of GLP-1 RAs. This thesis adds to the growing body of research indicating that GLP-1 RAs have a distinct effect on the renin-angiotensin-aldosterone system, as well as other major neuroendocrine systems, such as the hypothalamic-pituitary-adrenal and somatotropic axis.

2. REVIEW OF THE LITERATURE

2.1 GLP-1 as an incretin hormone

Gastric inhibitory polypeptide (GIP) was the first incretin hormone to be identified, when in 1971 it was isolated from porcine small intestine (Brown 1971). GLP-1 was not identified until the 1980s, although earlier evidence suggested that intestinal and pancreatic glucagon-like material had a stimulating effect on the release of insulin (Müller et al., 2019). GLP-1 is a 30- or 31-amino-acid-long peptide hormone derived from tissue-specific posttranslational processing of the proglucagon peptide. It is mainly produced and secreted by intestinal enteroendocrine L-cells in response to nutrient ingestion. In addition, it is also produced by specific neurons in the brainstem in the nucleus of the solitary tract (NTS) (Baggio et al., 2007, Campbell et al., 2013).

The proglucagon gene is located on the long arm of chromosome 2. The coding sequence for GLP-1 is contained within exon 4. Mammalian proglucagon gene transcription results in a single messenger RNA (mRNA) transcript that is structurally identical in different cell types (i.e., intestine, brain and pancreas) (Baggio et al., 2007). A schematic of the structure of proglucagon is shown in Figure 1.

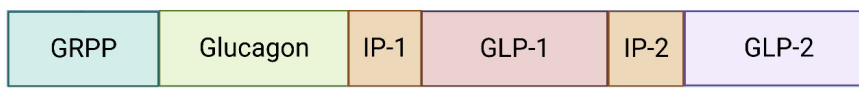

Figure 1. Proglucagon structure.
GRPP – Glicentin-related pancreatic polypeptide; IP-1 – Intervening peptide 1; GLP-1– Glucagon-like peptide 1; IP-2 – Intervening peptide 2; GLP-2 – Glucagon-like peptide 2

Nutrient ingestion is the primary regulator of intestinal proglucagon gene expression. The level of intracellular cAMP and activation of cAMP/PKA signalling are important determinants of both pancreatic and intestinal proglucagon gene expression (Baggio et al., 2007).

Proglucagon mRNA is translated into a 180-amino-acid-long precursor protein that is subjected to tissue-specific posttranslational processing, yielding specific peptide profiles in the pancreas, intestine, and brain. In the pancreas, the predominant posttranslational products are glicentin-related polypeptide, glucagon, intervening peptide-1, and the major proglucagon fragment. In the enteroendocrine L-cells and CNS, posttranslational processing of proglucagon results in glicentin, oxyntomodulin, GLP-1, intervening peptide-2, and GLP-2 (Baggio et al., 2007).

There are several GLP-1 forms: in humans, the majority of circulating GLP-1 is GLP-1(7-36)NH_2. GLP-1 undergoes rapid degradation by the ubiquitous

proteolytic enzyme dipeptidyl peptidase-4 (DPP-4), resulting in the half-life of bioactive form on GLP-1 being less than 2 minutes. GLP-1 and its metabolites are mainly eliminated by kidneys. Elimination rates are similar in healthy, obese and T2DM patients (Baggio et al., 2007).

Enteroendocrine L-cells are located mainly in the distal ileus and colon. The L-cells directly contact luminal nutrients via its apical surface, and neural and vascular tissue through its basolateral surface (Mortensen et al., 2003, Theodorakis et al., 2006). GLP-1 is secreted from the L-cells in response to a variety of nutrient, neural and endocrine factors. As mentioned before, meal ingestion, especially meals rich in carbohydrates and fats, is the primary physiological stimulus for GLP-1 secretion (Brubaker et al., 2006). GLP-1 secretion occurs in a biphasic pattern: an early phase at 10–15 minutes is followed by a second phase (30–60 min) that is longer (Herrmann et al., 1995). In addition to direct nutrient contact with the L-cell, the vagus nerve is an important mediator of nutrient-induced GLP-1 secretion (Rocca et al., 1999). It has also been shown mainly in animal models that leptin can stimulate GLP-1 secretion (Anini et al., 2003). On the other hand, it has been demonstrated *in vitro* and *in vivo* that insulin, somatostatin, and neuropeptide galanin might inhibit GLP-1 secretion from intestinal L-cells (Baggio et al., 2007).

2.2 GLP-1 receptors

The GLP-1 receptor belongs to class B G-protein coupled receptors (GPCR). This class includes receptors whose endogenous ligands are peptide hormones (Hoare et al., 2005, Wu et al., 2020). These include an N-terminal 120–160 residue extracellular domain and a C-terminal transmembrane domain, both crucial for peptide hormone binding and activation. The recognition of the peptide hormone triggers G-protein uncoupling and activation of the downstream signalling cascade (Wu et al., 2020). Activation of adenylate cyclase leads to an increase in intracellular cAMP and thus activation of protein kinase A (Müller et al., 2019, Mayo et al., 2003, Sonoda et al., 2008).

In addition to expression in the pancreatic islet cells, GLP-1 receptors are expressed in peripheral tissues, such as the kidneys, stomach, and heart, as well as in the central nervous system (CNS) (Wei et al., 1995, Mayo et al., 2003). It has been found that GLP-1R mRNA is expressed in the cerebral cortex, hippocampus, hypothalamus, thalamus, caudate putamen and globus pallidum (Alvarex et al., 2005). There is a high concentration of GLP-1 receptors in circumventricular organs and in nuclei involved in the regulation of energy balance and glucose metabolism (Knudsen et al., 2019, Secher et al., 2014).

2.3 GLP-1 receptors in pancreas, GI tract and CV system

GLP-1 potentiates glucose-induced insulin secretion from the pancreas, and also exerts a proliferative and anti-apoptotic effect on the β-cells (Drucker et al., 1987, Gromada et al., 2004, Farilla et al., 2002, Li et al., 2003, Kawamori et al., 2017, Jhala et al., 2003). GLP-1 also inhibits the release of glucagon from the pancreatic α-cells through induction of somatostatin release from pancreatic δ-cells or indirectly via its insulinotropic effect on the β-cells (de Heer et al., 2008, Orskov et al., 1988, Müller et al., 2019, Drucker 2018). In addition to endocrine effects, GLP-1 reduces postprandial glucose excursions by inhibiting gastric emptying, gastric acid secretion, and intestinal motility, thereby slowing down the rate at which glucose is absorbed into the circulatory system (Little et al., 2006, Gutniak et al., 1992, Thazhath et al., 2016).

In mice, GLP-1R mRNA is expressed in cardiomyocytes of the atrium, endocardium, and microvascular endothelium, and abundantly in smooth muscle cells of coronary vessels (Richards et al., 2014, Ban et al., 2008). GLP-1 has beneficial cardiovascular effects via both direct (such as inhibition of apoptosis in cardiomyocytes, improvement of endothelial function, reduction of blood pressure) and indirect (such as decrease in body weight and improved lipid metabolism) mechanisms (Noyan-Ashraf et al., 2009, Nystrom et al., 2004, Ceriello et al., 2011, Sun et al., 2015, Ussher et al., 2012, Marso et al., 2016). In addition, GLP-1 influences heart rate and BP, but these effects are species-related. In humans, GLP-1R agonists have been shown to cause a mild reduction in diastolic BP and an increase in heart rate (Sun et al., 2015, Katout et al., 2014).

2.4 GLP-1 receptors in kidneys

GLP-1 affects fluid balance and renal function by inhibiting water intake, but also by natriuretic and diuretic effects (Gutzwiller et al., 2006, Gutzwiller et al., 2004, Tonneijck et al., 2016). It has been hypothesized that the mechanisms behind these effects are inhibitions of sodium reabsorption in the proximal tubule and, quite possibly, changes in renal hemodynamics (Skov et al., 2013, Moreno et al., 2002). In addition, various studies have indicated that GLP-1R is expressed in renal vasculature (Pyke et al., 2014).

2.5 GLP-1 receptors in the central and peripheral nervous systems

GLP-1 is primarily released in the central nervous system by neurons in the nucleus solitary tract, and activates neurons in the hindbrain, the arcuate nucleus (ARC), the paraventricular nucleus (PVC) in the hypothalamus, and the central nucleus of the amygdala, thereby affecting feeding circuits in the central

nervous system (CNS) and reducing food intake (Turton et al., 1996, Shughrue et al., 1996, Rupprecht et al., 2013).

GLP-1-producing neurons located in the nucleus tractus solitarius (NTS) are the predominant source of endogenous GLP-1 in the brain. These neurons do not take part in primary food intake regulation, but are rather involved in a secondary satiation and satiety circuit, activated by psychogenic stress and large meals. It is important to note that ablation or inhibition of nucleus tractus solitarius GLP-1 neurons increases refeeding after a fast and inhibits stress-induced hypophagia (Holt et al., 2019).

Leptin and gastric distension additionally stimulate central GLP-1 release (GLP-1 neurons in the NTS). In contrast, peripherally released GLP-1 does not have such an effect (Vrang et al., 2003, Hisadome et al., 2010). In addition, 5-hydroxytryptamine (5-HT, serotonin) signalling influences NTS GLP-1 neuronal activity, and consequently metabolism (Holt et al., 2017).

It has been hypothesized that peripheral GLP-1 activates GLP-1 receptors located in the termini in the gut or the hepatic portal bed. These termini of vagal, afferent sensory nerve fibres, in turn, arise from the nodose ganglion (Nakagawa et al., 2004, Vahl et al., 2007, Holst 2013). These neurons stimulate neuronal activity in the NTS, activating the ARC and PVC in the hypothalamus.

It has been demonstrated via in situ hybridization histochemistry that GLP-1 receptors are expressed in various cerebral areas, such as the cerebral cortex, hypothalamus, thalamus, hippocampus, caudate putamen, and globus pallidum (Alvarez et al., 2005). Furthermore, it has been suggested that circulating GLP-1 and some of its agonists are able to reach the brain in areas where the blood-brain-barrier (BBB) is absent, such as the subfornical organ and area postrema (Orskov et al., 1996). The hypothalamus seems to be one of the primary targets of centrally administered GLP-1 RAs. In other words, the hypothalamus is one of the main parts of the brain that mediates the effects of GLP-1 and GLP-1 RAs on energy balance and glucose metabolism (Kabahizi et al., 2022). Since peripherally secreted endogenous GLP-1 undergoes rapid degradation by DPP-4, and therefore does not enter the brain in meaningful concentrations, the most probable source of endogenous GLP-1 that binds with GLP-1Rs expressed in the brain is primarily derived from hindbrain GLP-1-expressing neurons (Holst 2007). Similarly, hypothalamic GLP-1 receptors are likely primarily targeted by NTS GLP-1 neurons (Müller et al., 2019).

There is ample evidence to support the hypothesis that many of the GI-derived satiation signals primarily act through a paracrine vagal mechanism (Grill et al., 2012). Since endogenous GLP-1 has a very limited circulating half-life, endogenous GLP-1 is thought to primarily act in a paracrine fashion, stimulating GLP-1 receptors expressed on the dendritic terminals of celiac and gastric branches of the vagal afferents innervating the intestine. This vagal activation mediated by GLP-1 reduces food intake via vagal-to-NTS glutamatergic signalling, and via vago-vagal reflex-mediated insulin release (Hayes et al., 2010).

2.6 GLP-1 receptor agonists

Due to swift inactivation by the ubiquitous proteolytic enzyme dipeptidyl peptidase-4 (DPP-4) and renal elimination, the half-life of endogenous bioactive GLP-1 in the circulation is less than 2 minutes. GLP-1 analogues have been chemically modified to enhance its exposure and time of action. GLP-1 RAs are successfully used for the treatment of type 2 diabetes and obesity (Deacon et al., 1995, Meier et al., 2004).

GLP-1 RAs increase insulin secretion in a glucose-dependent manner, decrease inappropriate glucagon secretion, delay gastric emptying, and increase satiety. These properties lead to effective decrease in glycated hemoglobin (A1C) values and body weight, with minimal risk of hypoglycemia. Almost all GLP-1 RAs are administered via subcutaneous injection, except the oral formulation of semaglutide.

In terms of A1C lowering and effect on weight loss, s.c. semaglutide seems to be the most efficient, based on head-to-head studies between GLP-1 RAs (Truillo et al., 2021).

Of note, formulations that are based on the exendin-4 molecule (i.e. exenatide and lixisenatide) have only ~50% identity with native human GLP-1, whereas liraglutide, albiglutide, dulaglutide and semaglutide have ~90–97% identity (Madsbad et al., 2016, Sorli et al., 2017).

2.6.1 Pharmacokinetics (Sfairopoulos et al., 2018, Tirzepatide SmPC)

The clinical effects and pharmacokinetic/pharmacodynamic differences of GLP-1 RAs are presented in Table 1.

Table 1. Clinical effects and pharmacokinetic/pharmacodynamic differences of GLP-1 RAs.

GLP-1 RA	Dose	$t_{1/2}$	Features	Renal insufficiency
Exenatide (Byetta)	5–10 µg BID	2.4 h	First GLP-1 RA on the market	Not recommended with ESRD or severe renal impairment (eGFR < 30 mL/min/1.73 m^2).
Exenatide extended release (Bydureon)	2 mg QW	t_{max} 2.1–5.1 h Similar half-life to Byetta	High prevalence of antibody formation	Prolonged-release exenatide is not recommended for use in patients with end-stage renal disease or severe renal impairment (eGFR < 30 mL/min/1.73 m^2).
Liraglutide (Victoza)	1.2–1.8 mg QD	13 h	Superior in HbA1c lowering than short-acting exenatide and exenatide extended release.	No dose adjustment is required for patients with mild, moderate, or severe renal impairment. There is no therapeutic experience with patients with ESRD.
(Saxenda)	Up to 3 mg QD	13 h	More efficacy in weight loss. Approved as a therapy for obese or overweight patients with or without T2DM	No dose adjustment is required for patients with mild or moderate renal impairment (creatinine clearance ≥30 ml/min). Not recommended for use in patients with severe renal impairment (creatinine clearance <30 ml/min), including patients with ESRD.
Lixisenatide (Lyxumia)	20 µg QD	3 h	High level postprandial glucose control. High prevalence of antibody formation.	No dose adjustment is required for patients with mild or moderate renal impairment (creatinine clearance ≥30 ml/min). Not recommended for use in patients with severe renal impairment (creatinine clearance <30 ml/min), including patients with ESRD.
Albiglutide (Eperzan)	30–50 mg QW	5 d	Withdrawn from market.	
Dulaglutide (Trulicity)	0.75–1.5 mg QW	4.7 d	Non-inferior to liraglutide in HbA1c lowering.	No dose adjustment is required in patients with mild, moderate, or severe renal impairment. No experience in patients with ESRD.

GLP-1 RA	Dose	$t_{1/2}$	Features	Renal insufficiency
Semaglutide (Ozempic)	0.5–1.0 mg QW	1 wk	Superior to exenatide extended release QW in improving glycemic control and reducing body weight. CV benefits.	No dose adjustment is required in patients with mild, moderate, or severe renal impairment. No experience in patients with ESRD.
Semaglutide (Rybelsus) (Wegowy)	7–14 mg QD	1 wk	First oral GLP-1 treatment for T2DM.	
	Up to 2.4 mg QW	1 wk	Approved as a therapy for obese or overweight patients with or without T2DM.	
Tirzepatide (Mounjaro)	2.5–15 mg QW	Approx. 5 d	Long-acting dual GIP and GLP-1 receptor agonist.	No dose adjustment is required for patients with renal impairment including end stage renal disease (ESRD).

BID – Twice a day; d – Day(s); eGFR – Estimated glomerular filtration rate; ESRD – End-stage renal disease; GLP-1 RA – Glucagon-like peptide 1 receptor agonist; h – Hour(s); HbA1c – Haemoglobin A1c; QD – Once daily; QW – Once weekly; t1/2 – Half-life; tmax – Time to peak drug concentration; T2DM – Type 2 diabetes mellitus; wk – Week(s)

2.6.2 Pharmacodynamics

Pancreas and insulin release

Incretins play a crucial role in regulating postprandial insulin secretion in humans. The insulinotropic activity of GLP-1RAs is strictly glucose-dependent. GLP-1 receptors are located on the cell membrane of beta cells. The primary mediator of GLP-1-induced insulin secretion is cAMP, which in turn mediates its stimulatory effect through two distinct mechanisms: PKA-dependent phosphorylation of downstream targets, and PKA-independent activation of cAMP-binding proteins designated as cAMP-regulated guanine nucleotide exchange factors (cAMPGEFs, also known as Epac) (Holst 2007, Baggio et al., 2007).

Gastrointestinal tract

Endogenous GLP-1 secretion reduces gastric motility, leading to an increase in fasting and postprandial gastric volumes, and thereby a reduction in postprandial glycemia. This effect is also observed with the administration of GLP-1 RAs. It has been suggested that this regulation of gastroduodenal motility is not direct, but rather regulated by vagal inhibitory input through the release of nitric oxide and cholinergic pathways (Schirra et al., 2009). There is also evidence that GLP-1 might interact both with central (i.e brainstem neurons) and peripheral (i.e vagal nerve fibres) pathways (Göke et al., 1995, van Djik et al., 1996). However, it is important to note that GLP-1-induced deceleration of gastric emptying is subject to rapid tachyphylaxis at the level of vagal nervous activation, i.e the effect on gastric motility is significantly attenuated after chronic exposure to the drug (Nauck et al., 2011; Jelsing et al., 2012). In addition, GLP-1 attenuates small bowel motility, resulting in more time for enzymatic nutrient digestion and absorption (Drucker et al., 2023).

Liver

GLP-1 RAs have an indirect effect on gluconeogenesis via the stimulation of insulin release, but it has also been hypothesised that it may possess a direct hepatic effect, one of the possible pathways being Wnt signalling (Jin et al., 2016).

Nervous system

It has been demonstrated in preclinical studies that some of the peripherally administered GLP-1 analogues with smaller molecular size, such as exendin-4, liraglutide, and lixisenatide, have the ability to cross the BBB, and to exert physiological effects in the brain (Hunter et al., 2012). It has been hypothesized that long-acting GLP-1 RAs penetrate incomplete BBB in certain brain regions (Dong et al., 2022). For example, evidence supports that peripherally administered liraglutide accesses hypothalamus, and directly influences the orexigenic and anorexigenic pathways in the ARC (Secher et al., 2014).

Cardiovascular system

Although GLP-1R mRNA expression has been demonstrated in atria (mice, humans), ventricles (humans), and the sinoatrial node (monkeys), there remains much ambiguity on the exact localisation of GLP-1 receptors, and direct effects of GLP-1R stimulation (Baggio et al., 2017, Nauck et al., 2017).

In the animal models of cardiac injury, administration of GLP-1 leads to improvements in cardiac function, as evidenced by an increase in myocardial glucose uptake and improvement in left ventricular function. Although there are indications that GLP-1 may improve cardiac output in post-ischemic conditions, the opposite is true under non-pathological conditions.

In addition, GLP-1 receptor agonism inhibits cardiomyocyte survival via the inhibition of cell apoptosis.

GLP-1R agonism also affects heart rate (HR) and blood pressure (BP). A moderately stimulating effect on HR has been reported in the literature. Although some studies have concluded that GLP-1 RAs do not have any significant effect on BP, others have demonstrated that long-term administration of GLP-1 RAs elicit a modest decrease in BP in both healthy and diabetic subjects (Müller et al., 2019, Holman et al., 2017, Marso et al., 2016).

Kidneys

GLP-1 RAs can alter renal function via multiple mechanisms. It has been extensively demonstrated that GLP-1 and GLP-1 RAs inhibit water intake independently of food intake. Preclinical studies indicate that peripherally administered GLP-1 affects drinking behaviour through CNS-dependent mechanisms (Tang-Christensen et al., 1996, Gutzwiller et al., 2006, McKay et al., 2011). On the other hand, there is a possibility that GLP-1 RAs may affect kidney function by its effect on stimulation of natriuretic peptide (ANP) secretion (Müller et al., 2019). It has been demonstrated that liraglutide treatment induces natriuresis and diuresis (von Scholten et al., 2015, Lovshin et al., 2014). Moreover, treatment with GLP-1 RAs can modestly reduce albuminuria in patients with T2DM (von Scholten et al., 2015).

Although there is solid evidence that GLP-1 exhibits acute natriuretic effect in humans, a longer clinical trial has been suggested that this effect might be transient (Tonneijck et al., 2016).

Musculoskeletal system

In muscle cells, GLP-1 RAs induce glucose uptake by skeletal muscle cells via a molecular pathway that is different from the insulin stimulated PI3K/Akt/AS160 signalling cascade, and mediated by AMPK and TBC1D1 phosphorylation and subsequent Glut-4 translocation to the plasma membrane. Therefore, GLP-1 RAs have the capability to increase glucose uptake in muscle tissue in the absence of insulin (Andreozzi et al., 2016).

2.6.3 Clinical use

There are currently ten approved GLP-1-based therapies available in the European Union. Of note, albiglutide, a once-weekly GLP-1 RA, was discontinued in 2017 and withdrawn from use. GLP-1 RAs approved by the European Medicines Agency (EMA) for the treatment of T2DM with their respective authorisation years are presented in Figure 2. Classification of GLP-1 RAs according to their duration of action (short- versus long-acting) are shown in Table 2.

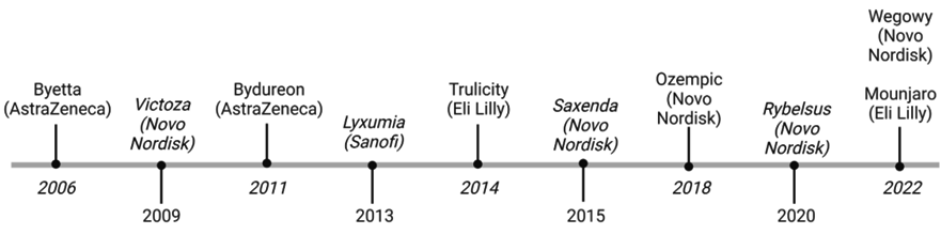

Figure 2. Timeline of GLP-1 RAs approved by the EMA for the treatment of type 2 diabetes.

Table 2. GLP-1 RAs classification according to duration of action.

Short-acting GLP-1 RAs	Exenatide Lixisenatide
Long-acting GLP-1 RAs	Dulaglutide Liraglutide Modified-release exenatide Semaglutide Tirzepatide

Glucose lowering effects

It is known that long-acting GLP-1 RAs are more effective in reducing HbA1c and fasting plasma glucose than short-acting GLP-1 analogues (Huthmacher et al., 2020) In addition, a recent meta-analysis by Yeh et al. (2023) investigated the effect on FDA-approved long-acting GLP-1 RAs (including liraglutide, once-weekly exenatide, dulaglutide, albiglutide, and semaglutide) on glycemic control in adults. The analysis confirmed that long-acting GLP-1 RAs significantly reduced HbA1c (Yeh et al., 2023) In contrast, short-acting GLP-1 RAs may have a distinct place in clinical practice: they produce greater reductions in postprandial glucose levels (Guo 2016, Drucker et al., 2023).

2.6.4 Effects beyond glucose lowering

2.6.4.1 Body weight and adipose tissue

One of the most notable and beneficial extrapancreatic effects of GLP-1R agonists is their ability to reduce body weight via inhibition of food intake. High-molecular-weight GLP-1 RAs, such as albiglutide and dulaglutide, are clearly inferior in terms of weight loss. It is currently not clear whether it is due to different uptake into the CNS, differences in transport or activity, or other factors (Baggio et al., 2014).

Larger meta-analyses have demonstrated that long-acting GLP-1 RAs, either used alone or in combination with insulin, have better efficacy in weight reduction than short-acting GLP-1 RAs. With higher doses of semaglutide, the weight loss observed is up to 13 kilograms compared to placebo (Wilding et al., 2021).

Adipose tissue

Adipose tissue macrophages produce inflammatory cytokines, playing a significant role in chronic inflammatory responses seen in obesity and T2DM. It has been hypothesised that the imbalance in the M1/M2 macrophages (i.e macrophages regulating inflammatory/anti-inflammatory responses) leads to chronic inflammation in adipose tissue, the accumulation of M1 macrophages being associated with insulin resistance. Studies in rodents and cell cultures indicate that GLP-1 might suppress macrophage-mediated adipose tissue inflammation, and therefore alleviate insulin resistance (Guo et al., 2016).

2.6.4.2 Neuroprotection

It has been demonstrated that central GLP-1R signalling pathways yield neuroprotective effects, e.g protection against apoptosis (Perry et al., 2002). Treatment with GLP-1 RAs has been shown to have beneficial effects in various neurological conditions, such as Huntington's disease, Alzheimer's disease and Parkinson's disease (Chang et al., 2018, Qi et al., 2016, McClean et al., 2011, Li et al., 2009, Athauda et al., 2017, Foltynie et al., 2014). In addition, there are reports from non-clinical studies that indicate the beneficial effect of GLP-1 RAs in alcohol use disorder (Marty et al., 2020, Egecioglu et al., 2013).

2.6.4.3 Bone metabolism

In mice, it has been shown that GLP-1 RAs have the potential to increase bone formation under conditions where bone loss is anticipated (e.g during weight loss or after an ovariectomy) (Iepsen et al., 2015, Lu et al., 2015, Pereira et al., 2015). So far, clinical trials have not succeeded in unequivocally confirming these results.

2.6.4.4 Cardiovascular and renal protection

Several major randomized placebo-controlled trials have investigated the safety of GLP-1 RAs in T2DM patients. It has been demonstrated that GLP-1 RAs are beneficial in preventing major cardiovascular events (MACE) including stroke and cardiovascular mortality, as well as all-cause mortality in this patient population (Malhorta et al., 2020, Monami et al., 2017). In addition, GLP-1 RAs are associated with reductions in adverse renal outcomes, which are mainly explained by the antiproteinuric effects (Kristensen et al., 2019, Gorriz et al., 2020, Marso et al., 2016).

2.6.5 Safety profile

The potential adverse effects of GLP-1 RAs are generally mild and transient. The most common adverse effects are nausea and diarrhea, followed by vomiting, constipation, abdominal pain, and dyspepsia. These adverse effects are usually more pronounced at the beginning of the treatment, gradually decreasing as therapy continues (Filippatos et al., 2014).

There have been concerns regarding a potential association between treatment with GLP-1 RAs and pancreatitis based on evidence from animal studies (Lee et al., 2011, Ayoub et al., 2010, Nakata et al., 2012, Yu et al., 2012, Rouse et al., 2014). In humans, although there is some evidence that treatment with GLP-1 RAs increases amylase and/or lipase levels, as well as the risk of developing acute pancreatitis compared to non-users, no direct cause-and-effect relationship has been established between GLP-1 RAs and pancreatitis (Steinberg et al., 2014, Singh et al., 2013, Giorda et al., 2014). In addition, T2DM and hypertriglyceridemia are both independent risk factors for pancreatitis (Scheen 2013, Filippatos, Elisaf et al., 2014). However, when considering treatment with GLP-1 RAs, consideration should be given to patients with pre-existing risk factors for pancreatitis.

It has been established that albiglutide, liraglutide, and exenatide do not cause any clinically relevant increase in the QTc interval. This is true for exenatide even at supratherapeutic concentrations (Darpo et al., 2014, Chatterjee et al., 2009, Darpo et al., 2013).

Since GLP-1 RAs are synthetic peptides usually administered as a subcutaneous injection, this may lead to antibody formation. The immunogenic potential of different GLP-1 RAs is variable, with exenatide and lixisenatide being the most immunogenic (Buse et al., 2011, Exenatide SmPC, Lixisenatide SmPC). Generally, the efficacy of the treatment is quite similar between antibody-positive and antibody-negative patients, but it has been demonstrated in a small subset of patients on exenatide that high titers of treatment-emergent antibodies lead to diminished efficacy (Fineman et al., 2012).

Injection site reactions, such as rash, itching at the injection site, and erythema, are a common side effect of GLP-1 RAs. Long-acting GLP-1 RAs give more injection site reactions than short-acting analogues. These reactions

are usually transient and do not cause discontinuation of the treatment (Madsbad et al., 2011).

GLP-1 RAs slow down the motility of the gastrointestinal (GI) tract, including that of gallbladder emptying. A recent meta-analysis demonstrated that the use of GLP-1 RAs is associated with increased risk of developing gallbladder or biliary diseases, the risk being higher with higher doses, longer treatment duration, and when being used for weight loss (Smits et al., 2016, He et al., 2022).

2.6.6 Non-canonical effects of GLP-1 RAs on other hormonal systems

Hypothalamic-pituitary-adrenal (HPA) axis

One of the multiple effects of GLP-1 receptor stimulation is a change in the HPA axis. Several studies have shown that acute administration of GLP-1 agonists stimulates the HPA axis. Central and peripheral administration of GLP-1 RAs significantly increases circulating glucocorticoid levels in both rodents and humans (Gil-Lozano et al., 2010, Malendowicz et al., 2003). In addition, tolerance does not develop towards corticosterone release after chronic treatment with the GLP-1 RAs exenatide or liraglutide (Krass et al., 2015).

Renin-angiotensin-aldosterone system (RAAS)

Several studies have dissected the effects of GLP-1 and GLP-1RAs on distinct levels of RAAS. It has been demonstrated that peripheral administration of GLP-1 agonists significantly increases aldosterone levels in rodents (Gil-Lozano et al., 2010). It has been shown that GLP-1 infusion and single dose of GLP-1 RA liraglutide decrease plasma angiotensin II concentration in both healthy subjects and patients with T2DM (Skov et al., 2013, Skov et al., 2016). In addition, although there is evidence that GLP-1 inhibits renin secretion (Asmar et al., 2015), other studies show no such effect (Skov et al., 2013). Similar controversy exists regarding the effect of GLP-1 RAs on aldosterone concentration: some groups have found that GLP-1 infusion decreases aldosterone levels in healthy subjects (Baretic et al., 2018), whereas other studies point out that GLP-1 RAs do not alter aldosterone levels (Skov et al., 2013, Asmar et al., 2021).

Growth hormone (GH)

GLP-1 RAs possess several extrapancreatic effects, including a potential effect on GH secretion. It has been speculated that somatotropic axis might play a role in mediating beneficial metabolic effects of GLP-1 RAs (Cignarelli et al., 2022).

Reproductive system

Due to its wide range of actions and based on results from preclinical studies, it has been speculated that GLP-1 RAs might modulate the reproductive system. It has been demonstrated in rodents that intracerebroventricular (icv) injection of

GLP-1 acts on the gonadal axis, and influence reproductive efficiency (Outeirino-Iglesias et al., 2015). However, evidence from clinical trials is conflicting. Some reports indicate that GLP-1 administration leads to a reduction in testosterone secretion in healthy men, whereas other studies have demonstrated no such effect (Jeibmann et al., 2005, Izzi-Engbeava et al., 2020). Considering the potent effects of GLP-1 RAs on glucose metabolism and body weight, and the fact that altered incretin secretion has been associated with polycystic ovary syndrome (PCOS), use of GLP-1 RAs might expand the treatment options available to patients with PCOS. However, it is not entirely clear whether such beneficial effects can be attributed solely to the weight loss effects of GLP-1 RAs, or if there are additional effects on the reproductive system (Cena et al., 2020).

Other hormones

The effect of GLP-1 RAs on other hormones has not been investigated thoroughly. There are some studies reporting effects on other pituitary hormones. For example, according to Tee et al. (2023), use of exenatide reduces serum thyrotropin (TSH) levels and improves central sensitivity to thyroid hormones. However, this effect is mediated via weight loss (Tee et al., 2023).

Copeptin is considered a surrogate marker for arginine vasopressin, and has been associated with cardiovascular risk in patients with PCOS (Karbek et al., 2014). However, Frøssing et al. (2014) demonstrated in a placebo-controlled randomized controlled trial investigating the effect of liraglutide on the cardiovascular biomarkers (including copeptin) in women with PCOS that there was no change in copeptin levels compared to placebo (Frøssing et al., 2014). On the other hand, nausea and vomiting are common side effects of GLP-1RAs, and a recent study showed that nausea and vomiting cause an increase in copeptin levels (Brooks et al., 2021).

2.7 Hypothalamic-pituitary-adrenal axis

2.7.1 Anatomy and physiology

Hypothalamic-pituitary-adrenal (HPA) axis is a major neuroendocrine system, controlling glucocorticoid hormone production and secretion (Joels et al., 2009). Three distinct temporal patterns can be distinguished in the activity of the HPA axis: basal ultradian pulses, basal circadian fluctuation, and stimulus-induced activity (Dickmeis et al., 2013, Spiga et al., 2014). Humans have pronounced daily rise in cortisol peaking around dawn, and an additional sharp surge in cortisol secretion immediately upon awakening (the cortisol awakening response, CAR) that usually peaks 30 minutes after awakening (Stalder et al., 2015). Since glucocorticoid hormones regulate all mammalian physiological systems in a dynamic and complex way, numerous pathological conditions are connected with dysregulation of HPA axis, such as Cushing's syndrome and

adrenal insufficiency, but also depression, hypertension, schizophrenia, fibromyalgia, etc. (Walker et al., 2013, Wingenfeld et al., 2008, Hammen 2005, Raff et al., 2015, Martin-Grace et al., 2020). These pathological conditions may manifest as changes in basal hormone secretion patterns, as well as alterations in the response to acute stressor challenge.

HPA axis and positive/negative feedback loops are presented in Figure 3.

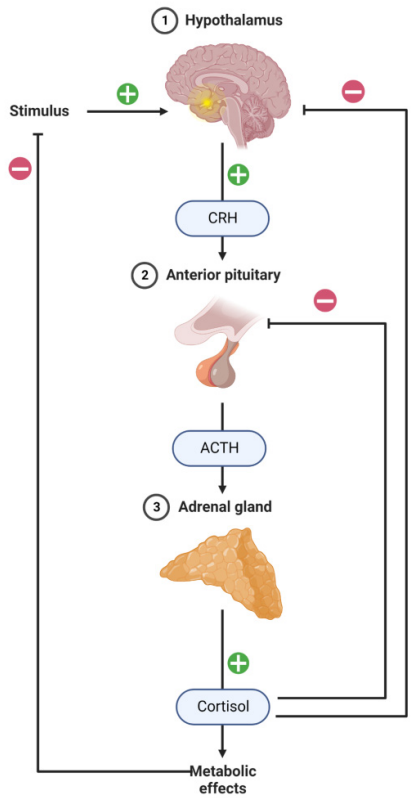

Figure 3. HPA axis and positive/negative feedback loops.
CRH – Corticotropic releasing hormone; ACTH – Adrenocorticotropic hormone

The HPA axis consists of the following key elements:

1. The paraventricular nucleus (PVN) of the hypothalamus. The PVN contains neuroendocrine neurons that synthesize and secrete arginine vasopressin (AVP) and corticotropin-releasing factor (CRF; also known as corticotropic releasing hormone or CRH). Hypothalamic CRF neurons receive direct and indirect neural input from other brain regions, and are directly regulated by glucocorticoids – thus being the primary site of action for glucocorticoid negative feedback (Herman et al., 2003, Watts et al., 2005).
2. The anterior lobe of the pituitary gland. AVP and CRH released from the PVN stimulate the secretion of adrenocorticotropic hormone (ACTH) from

anterior pituitary endocrine cells, known as corticotrophs. Under normal physiological conditions, corticotrophs have minimal intrinsic activity, and the exocytosis of ACTH is primarily controlled by CRF. Therefore, ACTH secretion depends on upstream CRF neuron activity (Dallman et al., 1987).

3. The adrenal cortex, which is responsible for production of the glucocorticoid hormones (cortisol). Cortisol synthesis is triggered by ACTH stimulation, and takes place in cells located primarily in the zona fasciculata layer of the adrenal cortex. Since cortisol-producing cells have very low intrinsic activity in the absence of stimulation by ACTH, and due to cortisol being lipid soluble and therefore being passively diffused out of cells after its formation, cortisol secretion also is reliant on upstream CRF neuron activity (Spiga et al., 2015, Dallman et al., 1987).

2.7.2 Stimulation tests (Karaca et al., 2021)

In primary adrenal insufficiency, pathology affects the adrenal gland itself. Secondary adrenal insufficiency occurs when there is a decreased level of adrenocorticotrophin hormone (ACTH) released from the pituitary gland. Primary adrenal insufficiency is usually a relatively easy diagnosis to make. In contrast, the diagnosis of secondary adrenal insufficiency may be challenging. Since the clinical presentation in adrenal insufficiency can be quite variable, HPA axis should be investigated in the presence of pituitary or hypothalamic pathology.

Following dynamic tests are being used in the diagnosis of pituitary disorders: the insulin tolerance test (ITT), ACTH stimulation test, glucagon stimulation test (GST), and metyrapone stimulation test.

In the insulin tolerance test, patients are administered insulin until hypoglycemia is reached (below 2.2 mmol/l). In healthy patients, this should lead to counteractive release of GH, ACTH and, in turn, cortisol. ITT is considered the gold standard for assessing the integrity of the HPA axis. On the other hand, this test has several contraindications (e.g ischemic heart disease), as well as considerable side effects (including risk of seizures and myocardial infarction). Moreover, it is time-consuming and needs to be performed in a specialized medical centre.

The ACTH stimulation test, also known as the short Synacthen test, Cosyntropin stimulation test, or rapid ACTH stimulation test, is an alternative to the ITT. Administration of synthetic ACTH directly stimulates the adrenal gland, leading to the release of glucocorticoids and sex steroids. However, although the test is very sensitive to primary adrenal insufficiency, it is considerably less sensitive in detecting secondary adrenal insufficiency, and additional testing is sometimes required.

In the glucagon stimulation test, glucagon is administered to assess the cortisol response, since it is able to stimulate both GH and HPA axes. It is therefore used as an alternative test to ITT for patients with contraindications, and who require assessment of both HPA and GH axes.

The metyrapone stimulation test is characterized by a negative feedback stimulus instead of direct stimulation, as with the ITT and GST. Metyrapone induces a reduction in the circulating cortisol levels, which leads to stimulation of the HPA axis, but at the same time does not lead to effective suppression of ACTH secretion from the pituitary. However, this test has not demonstrated clear superiority over other tests, and its lack of universal availability sets limits in its use in routine clinical practice.

The corticotropin-releasing hormone test involves intravenous administration of human CRH and measurements of cortisol and ACTH levels. However, it is expensive and not readily available.

In conclusion, there is no diagnostic test for secondary adrenal insufficiency available today which is simultaneously safe, cheap, sensitive, specific, and reproducible.

2.8 Renin-angiotensin-aldosterone system

2.8.1 Anatomy and physiology

The renin-angiotensin-aldosterone system (RAAS) is a hormonal system playing a crucial role in the regulation of BP, fluid and electrolyte balance and systemic vascular resistance.

Angiotensinogen is the precursor to all angiotensins. Renin, the enzyme catalyzing the first and rate-limiting step in the RAAS cascade, converts angiotensinogen into angiotensin I. Next, angiotensin-converting enzyme (ACE) converts angiotensin I into angiotensin II, the main effector peptide of RAAS. Effects mediated by the RAAS include, for example, vasoconstriction, sodium and water retention, aldosterone synthesis, and pro-inflammatory effects (Mirabito et al., 2019). A schematic overview of RAAS is presented in Figure 4.

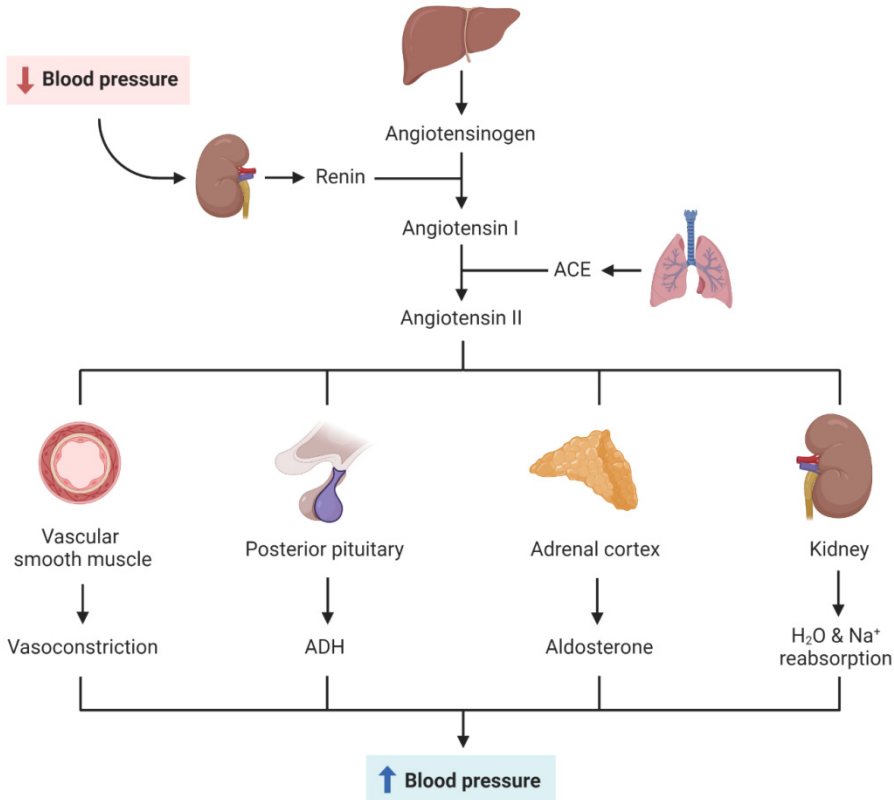

Figure 4. The Renin-Angiotensin-Aldosterone System.
ACE – Angiotensin converting enzyme; ADH – Antidiuretic hormone

2.8.2 Renal and cardiovascular effects

In the early stages of cardiovascular and renal diseases, RAAS activation can be compensatory, but its long-term activation is maladaptive. Chronic activation of RAAS promotes and sustains syndromes, such as congestive heart failure, systemic hypertension, and chronic kidney disease. Therefore, RAAS suppression is decisive in the treatment of chronic cardiovascular and renal diseases. It is achieved through the administration of angiotensin converting enzyme inhibitors (ACEIs), Angiotensin II type-1 receptor blockers (ARBs) and mineralocorticoid receptor antagonists (MRAs), which have been a backbone for the treatment of such diseases for the last three decades (Mirabito et al., 2019).

Primary aldosteronism (PA) is characterized by the autonomous secretion of aldosterone. The plasma aldosterone–renin ratio has been widely used in clinical practice as a screening test. As a rule, patients who test positive are recommended to undergo at least one confirmatory test. Although the current evidence does not identify a gold standard confirmatory test for PA, four testing procedures (oral sodium loading, the saline infusion test, fludrocortisone suppression test, and captopril challenge test) are in common use. Of those listed, the

fludrocortisone suppression test is considered the most sensitive for PA confirmation (Funder et al., 2016).

2.9 Hypothalamic-pituitary-somatotropic axis

2.9.1 Anatomy and physiology

The hypothalamic-pituitary-somatotropic (HPS) axis is an endocrine system which includes secretion of growth hormone (GH) from the somatotropic cells of the pituitary into systemic circulation. Subsequently, insulin-like growth factor 1 (IGF-1) is produced and released from the liver. Other hormones of the HPS axis include growth hormone-releasing hormone (GHRH) and somatostatin (SST), all of which play a role in controlling GH secretion from the pituitary gland. Physiological secretion of GH, which accounts for ~85% of total daily GH secretion, from the anterior pituitary is episodic and pulsatile. Serum GH levels are highly variable, being extremely low between pulses. GH levels are affected by various factors, such as gender, nutrition, sleep, physical activity, and other metabolic and hormonal signals from the endocrine system. GH secretion is regulated by a feedback mechanism involving IGF-1, insulin, glucose and free fatty acids. Ghrelin is a strong stimulator of GH release. IGF-1 is the primary mediator of peripheral GH effects (Gunawardane et al., 2000, Muller et al., 1999). The hypothalamic-pituitary-somatotropic axis and its feedback mechanisms are schematically presented in Figure 5.

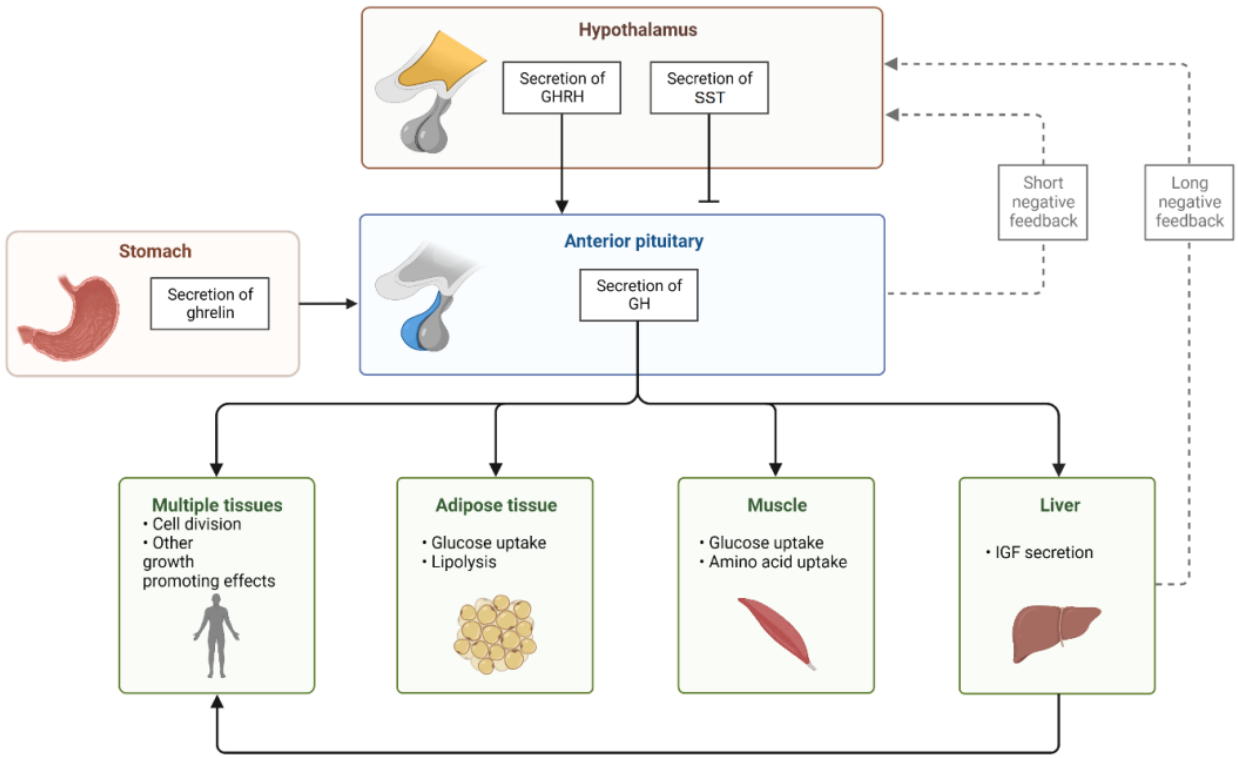

Figure 5. Hypothalamic–pituitary–somatotropic axis.
GHRH – Growth hormone-releasing hormone; GH – Growth hormone; IGF – Insulin-like growth factor; SST – Somatostatin

2.9.2 Stimulation tests

As IGF-1 secretion is tightly connected to GH, and IGF-1 has longer circulatory half-life, measurement of IGF-1 levels provides an integrated measure of GH secretion. When a patient needs additional testing, the insulin tolerance test is considered the gold standard test for diagnosing adult growth hormone deficiency (Yuen et al., 2019). The pros and cons of this diagnostic test were previously discussed in section 2.7.2.

The glucagon stimulation test (GST) is considered a potential alternative to the ITT. The use of GST for the assessment of GH was first described over 50 years ago by Mitchell et al. (Mitchell et al., 1969). Although the exact mechanism by which glucagon induces GH release is not entirely understood, it is hypothesized that glycemic fluctuations during the test, generation of a peptidyl fragment associated with the GH- and ACTH-releasing activity, and induction of noradrenaline secretion may be the contributing factors (Yuen, 2011).

2.10 Summary of the literature review

GLP-1 is an incretin hormone secreted in response to food intake from the L-cells located in epithelium of the gastrointestinal tract (Eissele et al., 1992). GLP-1 is expressed in the pancreas, enteroendocrine L-cells, and the nucleus tractus solitarius in the brainstem (Eissele et al., 1992, Philippe et al., 1991, Mojsov et al., 1986, Nian et al., 1999, Drucker et al., 1989, Holt et al., 2019). GLP-1 has a major role in postprandial insulin release, stimulating insulin release in a glucose-dependent manner, thereby avoiding the risk of hypoglycemia (Nauck et al., 1986). Since the incretin effect is significantly impaired in subjects with type 2 diabetes, GLP-1 receptor agonists are a novel drug class for the treatment of T2DM with plethora of effects beyond glucose control (Nauck, Stockmann et al., 1986).

The GLP-1 receptor belongs to class B G-protein coupled receptors. In addition to expression in the pancreatic islet cells, GLP-1 receptors are expressed in peripheral tissues, such as the kidneys, stomach, and heart, as well as in the central nervous system (Wei et al., 1995, Mayo et al., 2003, Alvarex et al., 2005).

GLP-1 potentiates glucose-induced insulin secretion from the pancreas, and also exerts proliferative and anti-apoptotic effect on the β-cells (Drucker et al., 1987, Gromada et al., 2004, Ferilla et al., 2022, Li et al., 2003, Kawamori et al., 2017, Jhala et al., 2003). In addition to endocrine effects, GLP-1 reduces postprandial glucose excursions by inhibiting gastric emptying and intestinal motility (Little et al., 2006, Gutniak et al., 1992, Thazhath et al., 2016). GLP-1 has beneficial cardiovascular effects via both direct and indirect mechanisms (Noyan-Ashraf et al., 2009, Nystrom et al., 2004, Ceriello et al., 2011, Sun et al., 2015, Ussher et al., 2012, Marso et al., 2016). In humans, GLP-1R agonists have been shown to cause a mild reduction in diastolic BP, but an increase in

heart rate (Sun et al., 2015, Katout et al., 2014). GLP-1 affects fluid balance and renal function by inhibiting water intake, as well as via natriuretic and diuretic effects (Gutzwiller et al., 2006, Gutzwiller et al., 2004, Tonneijck et al., 2016). GLP-1 is released in the central nervous system from neurons in the nucleus solitary tract, affecting feeding circuits in the central nervous system and reducing food intake (Turton et al., 1996, Shughrue et al., 1996, Rupprecht et al., 2013).

Due to fast inactivation by DPP-4 and renal elimination, the half-life of endogenous bioactive GLP-1 in the circulation is less than 2 minutes. GLP-1 analogues have been chemically modified to enhance its exposure and time of action. GLP-1 RAs are successfully used for the treatment of type 2 diabetes and obesity (Deacon et al., 1995, Meier et al., 2004). At the moment, there are ten approved GLP-1-based therapies available in the European Union.

One of the most notable and beneficial extrapancreatic effects of GLP-1R agonists is their ability to reduce body weight via inhibition of food intake. It has been demonstrated that long-acting GLP-1 RAs, either used alone or in combination with insulin, have better efficacy in weight reduction than short-acting GLP-1 RAs (Wilding et al., 2021). In addition, GLP-1 RAs potentially possess neuro-, cardio- and renoprotective properties (Perry et al., 2002, Chang et al., 2018, Qi et al., 2016, McClean et al., 2011, Li et al., 2009, Athauda et al., 2017, Foltynie et al., 2014, Malhorta et al., 2020, Monami et al., 2017, Kristensen et al., 2019, Gorriz et al., 2020, Marso et al., 2016). The safety profile of GLP-1 RAs is favourable, the most common adverse effects being nausea and diarrhea, which are transient in nature (Filippatos et al., 2014).

One of the multiple effects of GLP-1 receptor stimulation are the change to the HPA axis and RAAS (Gil-Lozano et al., 2010, Malendowitz et al., 2003, Krass et al., 2015). In addition, it has been suggested that GLP-1 RAs might influence GH secretion and the gonadal axis (Wilson et al., 2018).

3. AIMS OF THE STUDY

This study's aims were the following:

1) To find out if acute or chronic administration of GLP-1 RA influences renin and aldosterone levels in humans.
2) To examine the effects of GLP-1 RAs on the HPA axis in humans, to elucidate whether acute administration of GLP-1 RA may be used as a novel test for central adrenal failure.
3) To determine whether GLP-1 RAs affect the secretion of GH and other hormones.

4. MATERIALS AND METHODS

4.1 Study approvals

The clinical trials were conducted in accordance with the Declaration of Helsinki.

Each volunteer read and signed a written informed consent form before they were included in the study. In the liraglutide trial, participants also received thorough training about handling and using the liraglutide injection device.

The protocols were approved by the Research Ethics Committee of the University of Tartu (236/T-10 and 270/T-13) and the Estonian Agency of Medicines (RKU-4/18 and RKU-4/28). The study protocols were also registered at clinical.trials.gov (NCT02089256 and NCT03160261) and EU Clinical Trial Register (2014-000238-43 and 2017-001437-12).

4.2 Materials and laboratory analyses

A commercially available liraglutide solution (Victoza, NovoNordisk, Bagsværd, Denmark) was used. Liraglutide was administered using a prefilled injection pen and Novofine needles (NovoNordisk, Bagsværd, Denmark). During the graded glucose infusion test, 20% glucose solution (Braun, Meldbungen, Germany) was used.

A commercially available exenatide solution (0.25mg/ml; 10 µg in 40 µl) in prefilled injection pens (Byetta, AstraZeneca Macclesfield, Cheshire, UK) was used. Each dose contained 10 micrograms of exenatide in 40 microlitre doses.

All laboratory analyses were obtained from the University of Tartu Laboratory using standard techniques. Glucose was measured using the enzymatic reference method, with hexokinase (Cobas Glucose HK test). Potassium and sodium were measured using an indirect method with ion-selective electrode (Cobas ISE indirect Na-K-Cl for Gen. 2). Electrochemiluminescence immunoassay was used for measuring cortisol (Cobas Cortisol II immunoassay), C-peptide (Cobas C-peptide immunoassay) and adrenocorticotropic hormone (Cobas ACTH immunoassay, all from Roche Diagnostics GmbH, Mannheim, Germany). Chemiluminescence technology was used for measuring aldosterone (IDS-iSYS Aldosterone assay) and renin (IDS-iSYS Direct Renin assay; Immunodiagnostic Systems Ltd, Boldon, England). Chemiluminescence technology was used to measure GH levels (IDSiSYS system, Human Growth Hormone [hGH] assay; Immunodiagnostic Systems, East Bolden, UK) and IGF-1 (IDS-iSYS, Insulin like Growth Factor-I Assay; Immunodiagnostic Systems), LH (Elecsys LH assay; Roche Diagnostics, Indianapolis, IN, USA), prolactin (Elecsys Prolactin II assay; Roche Diagnostics) and testosterone (Elecsys Testosterone II assay; Roche Diagnostics). TRACE technology (Time-Resolved Amplified Cryptate Emission) was used for measuring copeptin (B.R.A.H.M.STM Copeptin proAVP; Thermo Fisher Scientific, Waltham, MA, USA).

4.3 Statistical analysis

All data were analyzed using Statistica version 7 (StatSoft, Inc, USA) and GraphPad Prism 5.0 (GraphPad Software, Inc, USA). Data are presented as mean ± SEM, mean ± SD or as median values with range. A P value of <0.05 was considered statistically significant.

The D'Agostino-Pearson normality test was used to verify normal distribution. Data were statistically examined using one-way analysis of variance (ANOVA) with time as repeated measure, or using a T-test for dependent samples when appropriate. The Newman Keuls post hoc analysis was used after statistically significant ANOVA. The Friedman test and Dunn's post hoc analysis was used when appropriate. The Mann-Whitney U-test was used to compare ACTH and cortisol levels before and during GGIT at baseline, and after acute and chronic administration of liraglutide. Data that did not have a normal distribution were analysed using the Wilcoxon signed-rank and Kruskal-Wallis tests. The time series data was analysed using Friedman Repeated Analysis of Variance or ANOVA repeated measures test.

4.4 Clinical trial with liraglutide (I)

4.4.1 Study design and procedures

The study design is shown in Figure 6. This study was a single-group, open-label clinical trial. The primary purpose of the study was to evaluate liraglutide pharmacodynamics in acute versus chronic settings in healthy subjects. The primary outcome was to investigate the development of potential tolerance towards the glucose-lowering effect of liraglutide after chronic administration. The secondary outcome was the potential effect of chronic administration of liraglutide on the regulation of adrenal hormones, i.e. aldosterone and cortisol.

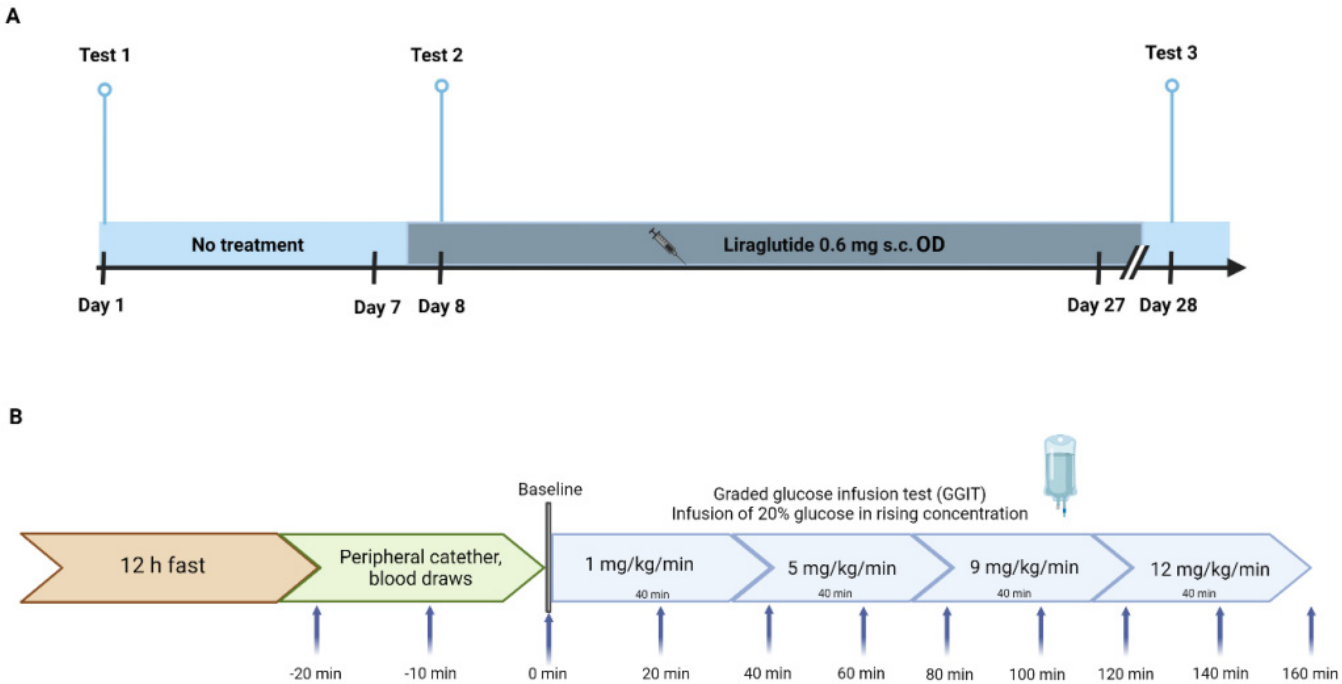

Figure 6. (A) General study design in clinical trial with liraglutide. (B) Procedure flow during each test (i.e., test 1, 2 and 3).

Each participant was tested three times: without treatment; 12 hours after the first dose of liraglutide (0.6 mg); and after three weeks on liraglutide (0.6 mg/day). The drug was administered 12 hours before testing, as the maximum plasma concentration after liraglutide administration is reached after approximately 10–14 hours.

During the study, volunteers self-administered liraglutide subcutaneously from self-injection pens for 21 consecutive days. The injection was administered between 9:00–11:00 PM. A graded glucose infusion test (GGIT) was performed three times on each participant. Every participant served as his/her control. The first GGIT was performed seven days before the first liraglutide injection was administered; the second GGIT was performed 12 hours after the first liraglutide injection; and the third GGIT was performed 12 hours after the last, i.e. 21st, liraglutide injection.

Graded glucose infusion tests were carried out in the morning between 8:00–10:00 AM. Subjects were asked to fast 12 hours before the beginning of the test and to avoid extreme physical activity at the previous day. A peripheral venous catheter was placed on both arms. One catheter was used to obtain blood for analysis and the other was used to administer the glucose infusion. Two blood samples were taken (10 minutes apart) for baseline levels of glucose. One of them (taken 20 minutes before the start of the GGIT) was also analyzed for hormone markers. 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 minutes. Blood samples for glucose and C-peptide were drawn every 20 minutes, and samples for measuring hormones were taken before the initiation of the infusion and at the 120-minute time point. During the first 120 minutes, 600 mg/kg of glucose and 3 ml/kg of glucose solution was administered. During the test study subjects were in a semi-supine position, avoided physical activity, and did not eat or drink.

4.4.2 Study participants

Ten healthy volunteers, both male (n=7) and female (n=3), were recruited. The inclusion criteria were the following: 1) age 18–50 years; 2) body weight 50–100 kilograms.

The exclusion criteria were the following: 1) underlying chronic illnesses; 2) use of daily medications; 3) pregnancy or lactation; 4) fasting glucose > 6 mmol/l.

4.5 Clinical trial with exenatide (II)

4.5.1 Study design and procedures

The study design is shown in Figure 7. The study was a single-group, open-label clinical trial. The aim of the study was to test the hypothesis of whether a single dose of exenatide (10 µg subcutaneously (s.c)) could be used as a stimulation test to interrogate the function of the pituitary-adrenal axis. The primary outcome was the maximal level of cortisol after the administration of exenatide.

Tests were carried out in the morning between 08:00–11:00. Subjects were asked to fast 12 hours before the beginning of the test and to avoid extreme physical activity on the previous day. A peripheral venous catheter was placed in one arm of the volunteer between 8:00–8.10. The first blood sample was collected 20 minutes later and exenatide solution (10 µg in 40 microliters) was administered subcutaneously. The volunteers remained comfortably sitting during the study. The blood samples were taken at the following time points: baseline (i.e. 0 minutes), and 30, 60, 90, 120 and 150 minutes after administration of the drug. After the collection of the first blood sample, every volunteer received 10 µg of exenatide solution, administered subcutaneously by the study nurse. In addition, blood pressure, heart rate and nausea were measured at the above-mentioned time points. Every subject used a visual analogue scale (VAS) to assess the presence and intensity of nausea (i.e. value 0 indicating no nausea, with 10 corresponding to the worst nausea imaginable).

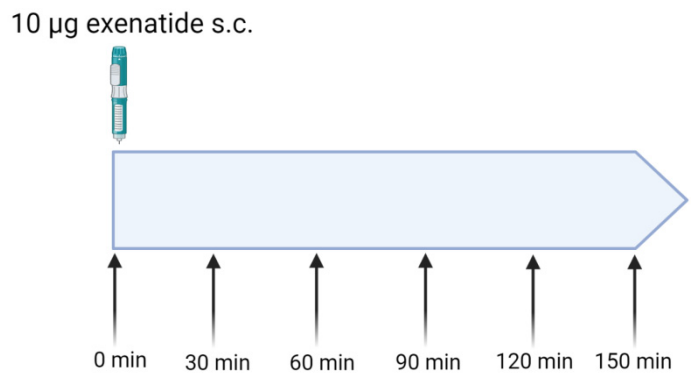

Figure 7. Study design in clinical trial with exenatide.

4.5.2 Study participants

Ten healthy volunteers (male=9, female=1) were recruited. The inclusion criteria were the following: 1. age 18–50 years; 2. body weight >65 kg.

The exclusion criteria were the following: 1. presence of chronic illness; 2. use of daily medicines; 3. pregnancy, lactation; 4. use of oral contraceptives during the previous 2 months.

5. RESULTS

5.1 Liraglutide Treatment May Affect Renin and Aldosterone Release (clinical trial I)

Effect of liraglutide on renin and aldosterone secretion

Ten healthy volunteers were enrolled in the clinical trial to examine the effects of acute and chronic treatment with a long-acting GLP-1 RA liraglutide on the HPA axis in humans. The baseline characteristics of study participants are given in Table 3. The design of the study is shown in Figure 6. GGIT was conducted three times on every participant to evaluate the effect of acute and chronic liraglutide treatment compared to baseline (no treatment) conditions. Otherwise, all tests were carried out under the same conditions.

Table 3. Baseline characteristics of study participants.

Sex (males/females)	**3/7**
Age (mean ± SD)	28.2 ± 1.9 years
Weight (mean ± SD)	77.0 ± 2.6 kg
Fasting glucose (mean ± SD)	4.8 ± 0.2 mmol/l

There was no significant effect of liraglutide on renin concentration after acute administration, although there was a trend to decrease, followed by increased levels with chronic dosing ($p<0.05$; Newman-Keuls test, acute vs. chronic liraglutide, Figure 8A). The effect on aldosterone levels closely followed the renin change ($p<0.05$; Dunn's test, acute vs. chronic liraglutide, Figure 8B).

Effect of liraglutide on Na and K

Treatment with liraglutide had no effect on Na and K levels (Figure 8C).

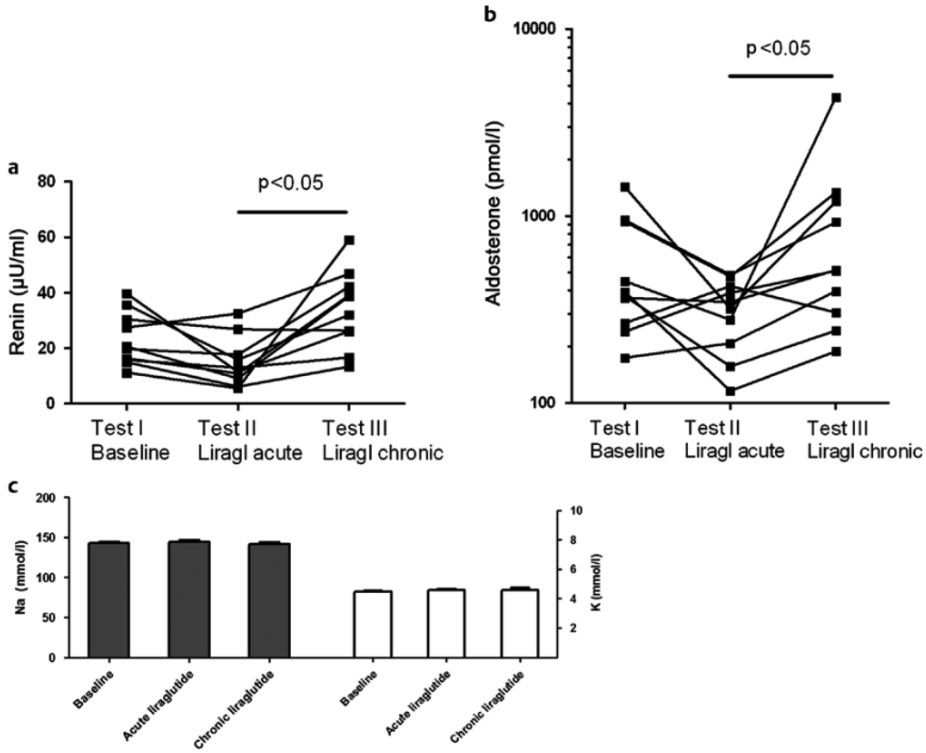

Figure 8. Effects of acute and chronic treatment with liraglutide 0.6 mg/day s.c. on renin (a), aldosterone (b), and Na and K levels (c). Data represent pre-GGIT values. Reference values: renin 5.3–99.1 µU/ml; aldosterone 111–860 pmol/l; K 3.4–4.8 mmol/l; Na 136–145 mmol/l.

The hormones were also measured at 120 minutes of GGIT and followed the same pattern (Figure 9A and 9B). Chronic treatment with liraglutide caused a statistically significant increase in renin levels compared to acute treatment ($p<0.05$; Newman-Keuls test). Changes in aldosterone levels did not reach statistical significance.

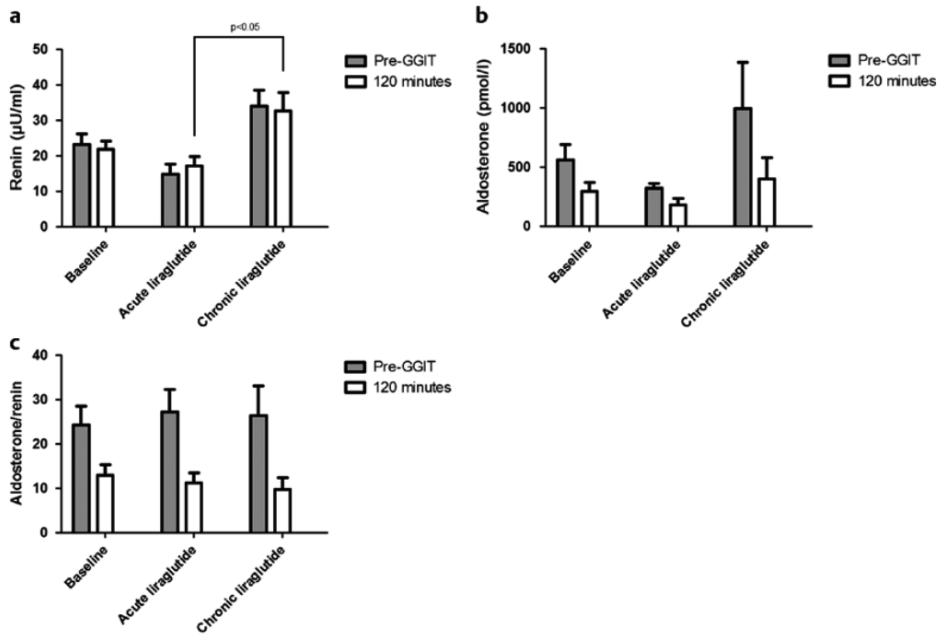

Figure 9. Effects of acute and chronic treatment with liraglutide 0.6 mg/day s.c on renin (A) and aldosterone levels (B) and aldosterone-to-renin ratio (C). Reference values: renin 5.3–99.1 µU/ml; aldosterone 111–860 pmol/l. Grey bars represent pre-GGIT values (A and B same data as Figure 8); white bars represent values measured during GGIT.

The aldosterone/renin ratio remained unaltered during the experiment (Figure 9C).

Effect of liraglutide on ACTH and cortisol secretion

Treatment with liraglutide did not change the levels of ACTH and cortisol measured before GGIT (Figure 10).

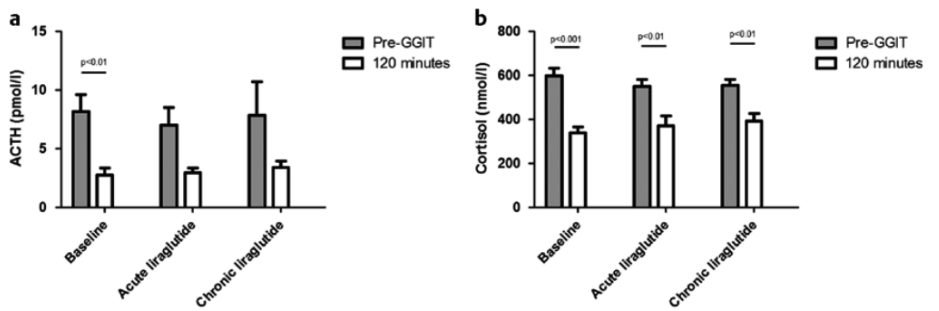

Figure 10. Effects of acute and chronic treatment with liraglutide 0.6 mg/day s.c on ACTH (A) and cortisol levels (B). Reference values: ACTH 1.6–13.9 pmol/l; cortisol 172–497 nmol/l. Grey bars represent pre-GGIT values (A and B same data as Figure 9); white bars represent values measured during GGIT.

During the GGIT, cortisol levels were suppressed in all three tests ($p<0.001$, $p<0.01$, and $p<0.01$, respectively; Mann-Whitney U-test). ACTH was significantly suppressed in test 1 under basal conditions ($p<0.01$; Mann-Whitney U-test); the change was close to statistical significance after acute liraglutide administration ($p=0.059$).

This response to glucose infusion represents a normal physiological response.

5.2 A GLP-1 RA Exenatide affects the Release of Adrenal Hormones in Healthy Volunteers (clinical trial II)

Ten healthy volunteers were recruited to examine if acute administration of a short-acting GLP-1 RA exenatide may be used as a novel test for central adrenal failure. The anthropometric characteristics of study participants are given in Table 4. After collecting baseline blood samples, 10 µg of exenatide was administered to the subject. Blood samples were collected in regular intervals until 150 minutes after administration of the study drug. In addition, BP, heart rate and nausea were measured.

Table 4. Anthropometric characteristics of study participants.

Age in years (mean (SD))	33.0 (7.6)
Sex – male (N/Total)	9/10
Sex – female (N/Total)	1/10
Weight in kg (mean (SD))	84.7 (8.9)
Height in cm (mean (SD))	177.7 (8.8)
Body mass index in kg/m2 (mean (SD))	26.9 (3.1)

Cortisol, ACTH, and glucose

A single dose of exenatide (10 µg s.c.) resulted in an increase of peak cortisol levels compared to untreated values (pre-treatment 375±74 nmol/l; treated 424±83 nmol/l; $p=0.03$; Figure 11B). However, mean cortisol levels at different time points did not show a statistical difference (Figure 11D). ACTH peak levels were also increased, and the change reached statistical significance (untreated median value 5 pmol/l, with range 15.6 pmol/l; treated median value 6.9 pmol/l with range 15.4 pmol/l; $p=0.01$; Figure 11A). Here, on the other hand, median ACTH levels at different time points attained statistical difference, in particular baseline (0 min) versus 150 minutes post-baseline ($p=0.03$; Figure 11C).

The administration of exenatide led to a significant decrease in blood glucose levels ($p<0.01$; Figure 11E and 11F).

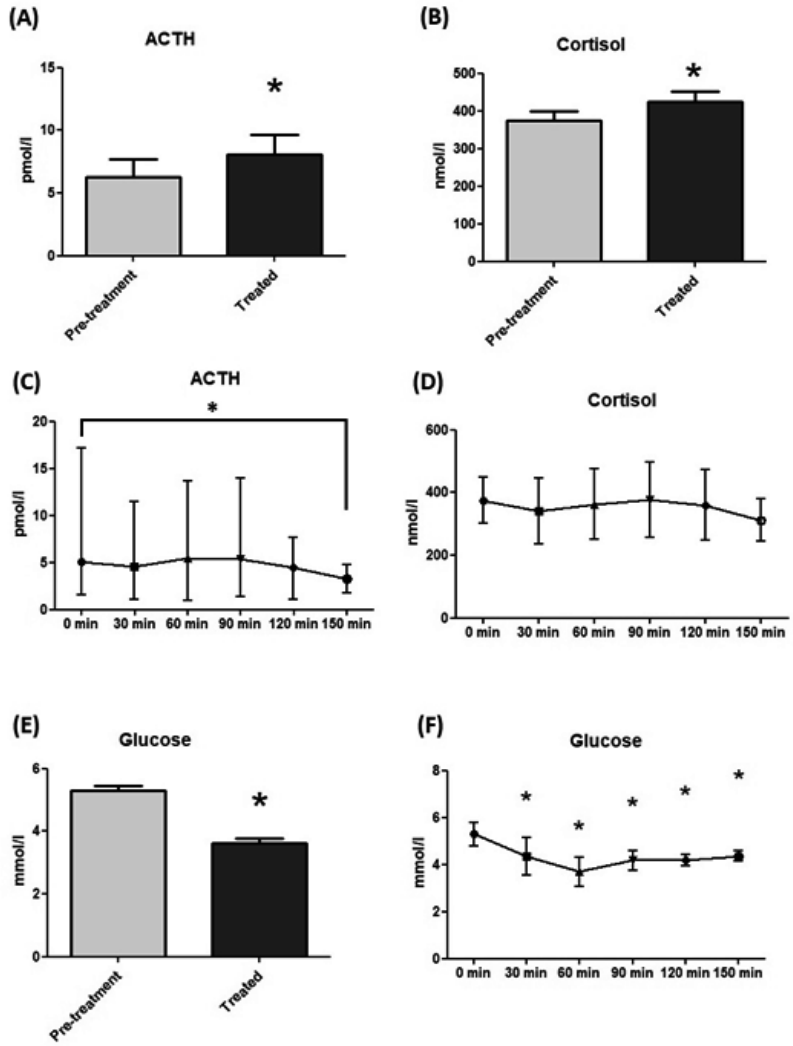

Figure 11. Blood levels of ACTH (A – peak value, C – values at pre-specified sampling points depicted as timeline; Reference values: 1.6–13.9 pmol/l), cortisol (B – peak value, D – values at pre-specified sampling points depicted as timeline; Reference values: 172–497 nmol/l) and glucose (E – minimum value, F – values at pre-specified sampling points depicted as timeline; Reference values: 4.5–6 mmol/l) before and after administration of exenatide 10 µg s.c. * $p<0.05$. Cortisol and glucose data are depicted as mean/SD; ACTH data as median/range.

Renin, aldosterone, electrolytes

Surprisingly, levels of both renin and aldosterone decreased abruptly (untreated 24.0±14.7 mIU/l; treated minimum level 11.5±5.5 mIU/l; p=0.003, and untreated 12.4±7.4 ng/dl; treated minimum level 5.4±3.0 ng/dl; p=0.002, respec-

tively; Figures 12A-B). In addition, mean aldosterone levels at baseline and 120 minutes also differed significantly, as did mean renin levels at 120 minutes vs baseline and vs 30 minutes (p=0.003 and p=0.004, respectively; Figures 12C-D). Sodium and potassium levels remained stable during the experiment (Figures 12E and 12F).

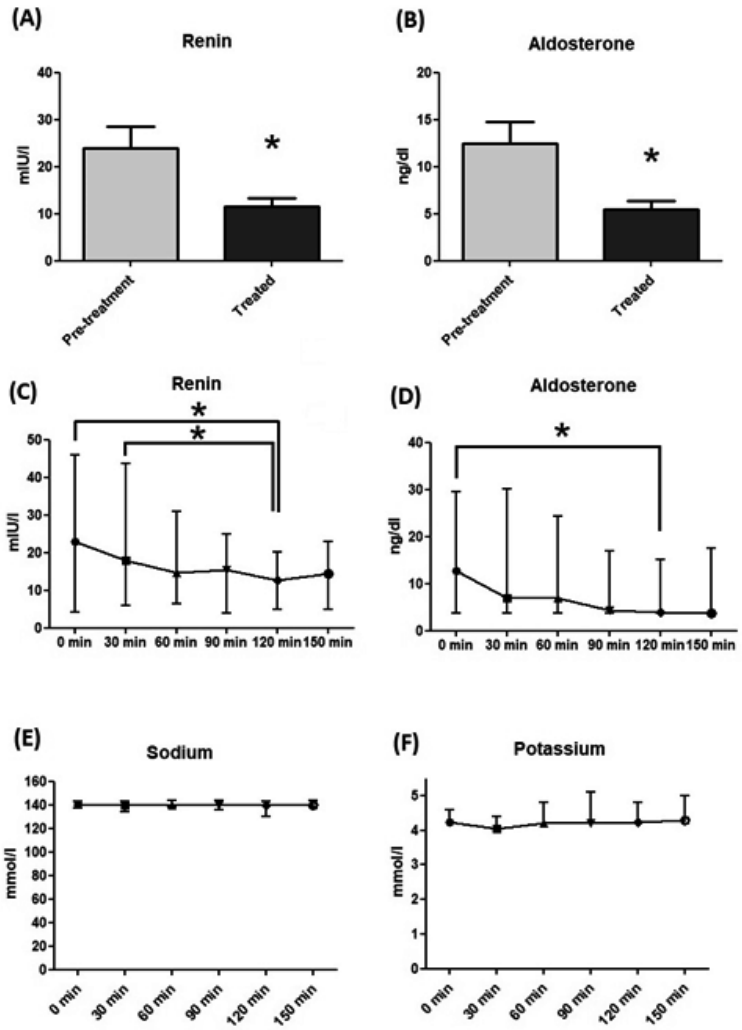

Figure 12. Blood levels of renin (A – minimum value, C – values at pre-specified sampling points depicted as timeline; Reference values: 5.3–99.1 mIU/l), aldosterone (B – minimum value, D – values at pre-specified sampling points depicted as timeline; Reference values: 3.7–43.2 ng/dl), sodium (E – values at pre-specified sampling points depicted as timeline; Reference values: 136–145 mmol/l) and potassium (F – values at pre-specified sampling points depicted as timeline; Reference values: 3.4–4.8 mmol/l) before and after administration of exenatide 10 µg s.c. * p<0.01. Aldosterone and renin data are depicted as mean/SD; sodium and potassium data as median/range.

Safety
We did not observe any significant change in systolic or diastolic BP or heart rate (Table 5, $p>0.05$). 7 of 10 subjects reported nausea with median maximal intensity of 3.5 in 10 points on the VAS. The maximal intensity of nausea was 5 points, occurring in one subject at the 60-minute time point.

Table 5. Blood pressure, heart rate and intensity of nausea.

	0 min	30 min	60 min	90 min	120 min	150 min
Systolic BP (mmHg) mean (SD)	127 (10)	131 (14)	136 (10)	135 (13)	132 (14)	131 (11)
Diastolic BP (mmHg) mean (SD)	80 (11)	80 (8)	83 (11)	83 (10)	80 (10)	82 (12)
Heart rate min^{-1} mean (SD)	63 (14)	63 (7)	65 (5)	65 (6)	64 (7)	65 (7)

5.3 GLP-1 RA Exenatide Induces GH Secretion in Healthy Volunteers (clinical trials I and II)

We conducted additional analyses of the results of the clinical trials described above. Both studies included adult female and male volunteers with no chronic illnesses or use of daily medicines.

Effects on GH and IGF-1
After a single dose of 10 μg exenatide, we observed a statistically significant peak in GH levels compared to pre-treatment values ($p < 0.05$; Figure 13A). The GH peak occurred most frequently between 60 and 90 minutes (Figure 13B).

GH levels were significantly higher after chronic administration of 0.6 mg liraglutide compared to baseline values (Figure 13C). After administration of 0.6 mg liraglutide, IGF-1 levels did not change compared to the baseline, but were slightly lower after chronic treatment compared to acute administration of the drug ($p < 0.05$; Figure 13D).

In addition, there was no correlation between change in glucose levels and GH peak values after administration of exenatide (Pearson correlation coefficient 0.437; 95% CI -0.266 to 0.835; $p = 0.207$; Figure 14).

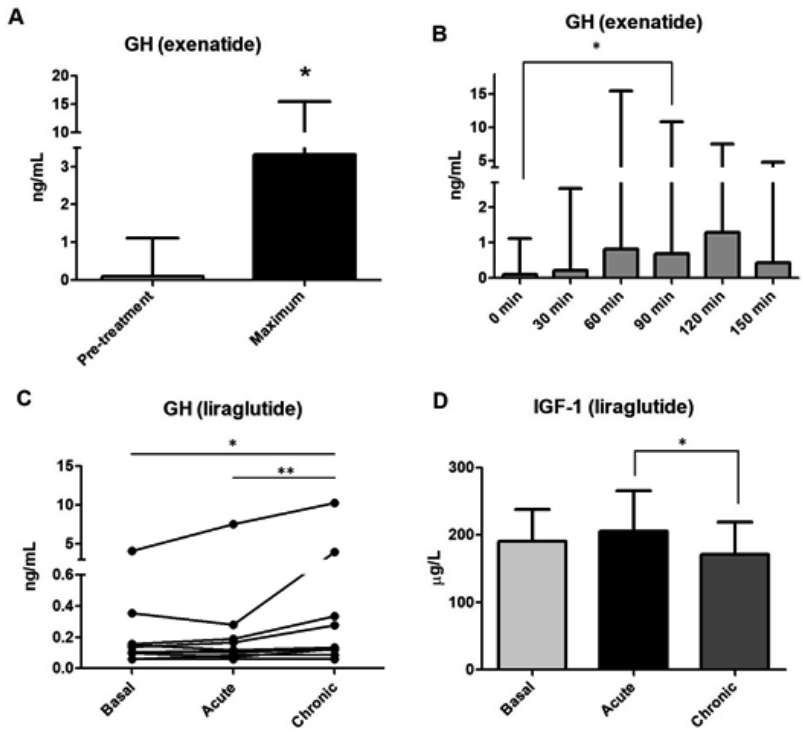

Figure 13. A, GH pre-treatment and peak value after administration of 10 µg exenatide s.c. * $p < 0.05$ (Wilcoxon signed-rank test). B, GH values at pre-specified sampling points depicted as timeline. * $p < 0.05$ (Dunn's test). C**,** GH levels at baseline, and after acute and chronic treatment with 0.6 mg liraglutide s.c. * $p < 0.05$, ** $p < 0.01$ (Dunn's test). D, IGF-1 levels at baseline, and after acute and chronic treatment with 0.6 mg liraglutide s.c. * $p < 0.05$ (Tukey test). GH data are depicted as median with range; IGF-1 data as mean with SD. Reference values: GH < 5 ng/mL; IGF-1 116–353 µg/L.

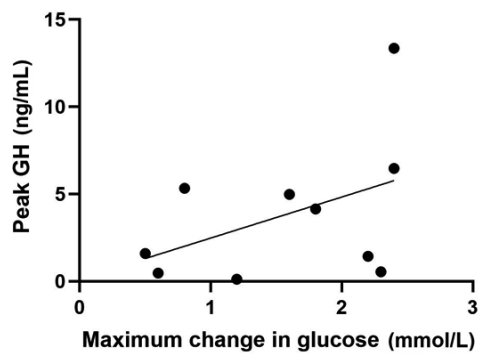

Figure 14. Correlation between GH peak values and change in glucose levels in the exenatide experiment. Pearson correlation $p = 0.207$.

LH and Testosterone

Analysis included only male subjects (n = 8). Testosterone was significantly suppressed 120 minutes after administration of 10 µg exenatide ($p < 0.05$). LH levels followed a similar trend: LH levels were significantly lower at 120 minutes compared to baseline ($p = 0.02$; Figure 15).

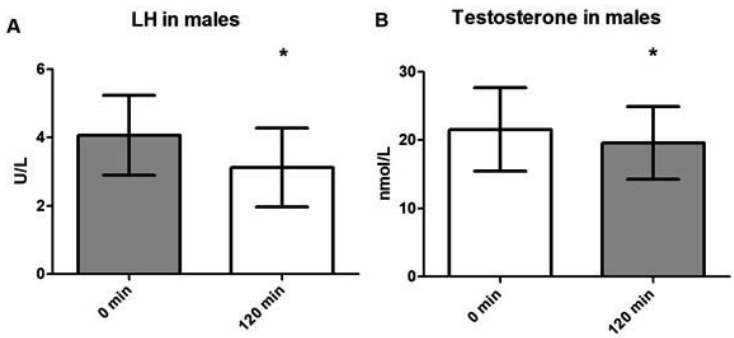

Figure 15. A: LH levels at baseline and 120 minutes after administration of 10 µg exenatide. B: Testosterone levels at baseline and 120 minutes after administration of 10 µg exenatide. * $p < 0.05$ (Tukey test). Data are depicted as mean with SD. Reference values: LH 1.7–8.6 U/L; testosterone 6.68–29.0 nmol/L.

Prolactin

Administration of a single dose of 10 µg exenatide resulted in a statistically significant fall at both 60 and 120 minutes compared to baseline ($p < 0.05$; Figure 16A).

Copeptin

Blood samples were available for six subjects. The change in the concentration of copeptin after administration of 10 µg exenatide was not significant ($p = 0.22$) (Figure 16B).

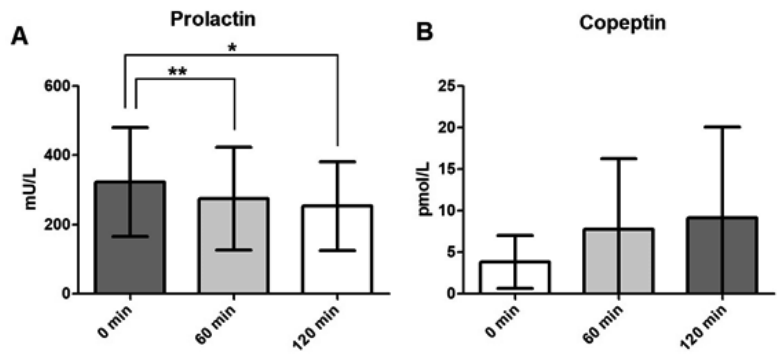

Figure 16. Change in Prolactin (A) and Copetin (B) levels. * $p < 0.05$ and ** $p < 0.01$ (Tukey test). Data are depicted as mean with SD. Reference values: Prolactin (males) 86 – 324 mU/L; (females) 102–496 mU/L; copeptin 1.0–28.2 pmol/L.

6. DISCUSSION

As discussed in the literature review, GLP-1 RAs possess an outstanding number of effects in different organ systems. The current dissertation adds and widens the knowledge about the pleiotropic effects of GLP-1 RAs, mostly focusing on their actions on other hormonal systems. The two key questions about the effects described in the study are whether they contribute to the still largely unexplained cardioprotective and renoprotective properties of these drugs, and whether they may pose safety problems in long-term use.

Effect of GLP-1 RA-s on Renin-Angiotensin-Aldosterone System (RAAS)

RAAS is one of the cardinal mechanisms regulating blood volume, electrolyte levels, and blood pressure. Suppression of RAAS is one of the cornerstones of the treatment of chronic cardiovascular and renal disease (Mirabito et al., 2019). As GLP-1 and GLP-1 RA-s have been shown to increase ACTH levels, and aldosterone is also partly controlled by ACTH stimulation, one would expect that GLP-1 RAs will increase aldosterone levels (Kinzig et al., 2003, Gil-Lozano et al., 2010).

However, in our study with the chronic dosing of long-acting GLP-1 RA, using liraglutide, the acute effect seemed to be a fall in both renin and aldosterone concentrations. We used a low, subclinical dose of liraglutide (0.6 mg s.c.) and 12 hours after administration, there was an acute suppression of renin and aldosterone levels, although the change was not statistically significant. On the other hand, when liraglutide was administered during a longer period of time (i.e. 21 consecutive days), we observed a rebound in renin levels, followed by an increase in aldosterone levels. These changes in aldosterone levels were clearly driven by renin, as renin levels followed the same pattern, and the aldosterone-to-renin ratio remained stable. Thus, the study indicated that GLP-1 RAs may affect the regulation of renin and aldosterone after acute and chronic dosing. To confirm the observation, we studied the effects of a short-acting GLP-1 RA, exenatide, in healthy volunteers. In the clinical trial examining the acute effect of full therapeutic dose of exenatide (10 μg s.c.), we observed a significant decrease in aldosterone levels in the blood, which was accompanied by a drop in renin levels.

These findings may have important clinical implications. Firstly, if (some) GLP-1 RAs change aldosterone levels, this must be taken into account when testing hypertensive patients for primary hyperaldosteronism. Secondly, as aldosterone antagonists are effective drugs for several cardiovascular disorders, a change in aldosterone levels after chronic treatment may potentially affect this important outcome.

So far, there are several studies demonstrating effect of GLP-1 or GLP-1 RAs on distinct steps of the RAAS system with considerable variability.

Aldosterone was suppressed after GLP-1 infusion in a placebo-controlled trial in healthy volunteers, but that was not statistically significant compared to

the placebo group. Meanwhile, renin levels remained unaffected during the study (Baretić et al., 2018).

There have been several other human studies reporting no effect by GLP-1 receptor agonists on plasma renin and aldosterone levels (von Scholten et al., 2015, Skov et al., 2013, von Scholten et al., 2017, Ferdinand et al., 2014). Von Scholten et al. (2017) observed no statistically significant changes in renin, angiotensin II, and aldosterone levels after 12 weeks of treatment with liraglutide (final dose 1.8 mg/day) compared to placebo. However, it is important to point out that the latter trial was conducted in patients with type 2 diabetes and 100 % of participants were taking RAAS-inhibiting drugs (von Scholten et al., 2017). However, the effect of liraglutide on renal physiology and blood pressure is rather complex, as shown by a study by von Scholten et al. (von Scholten et al., 2015). A study in Japanese patients demonstrated that 12 weeks of treatment with liraglutide (0.9 mg) did not change levels of renin and aldosterone (Kamei et al., 2017).

In another study, infusion of GLP-1 resulted in increased BP and decreased renin concentration (Karaza et al., 2011). Unfortunately, aldosterone levels were not reported in that study. A recent small trial supports the existence of a GLP-1-mediated gut-renal axis in the regulation of renal sodium excretion (Asmar et al., 2019). GLP-1 infusion did not change renin or aldosterone levels compared to placebo in the background of intravenous infusion of 0.9 % NaCl, but decreased Angiotensin II levels (Asmar et al., 2019). The exact mechanism as to how GLP-1 RAs affect renin/aldosterone levels is currently unclear.

It has been proposed that as GLP-1 decreases sodium reabsorption, renin secretion might be inhibited by the resulting activation of the tubuloglomerular feedback mechanism. Moreover, GLP-1 RAs increase natriuresis in the proximal tubule apical membrane, thus regulating sodium and water balance. The effect of liraglutide on renal physiology and BP depends on treatment duration, as shown by a study by von Scholten et al. (von Scholten et al., 2015). Animal and human studies have shown that GLP-1 receptor agonists induce diuresis and natriuresis (Gutzwiller et al., 2004, Gil-Lozano et al., 2013).

In both trials reported here, plasma concentrations of potassium and sodium remained unaltered, largely excluding secondary changes due to changed electrolyte levels. The change in BP is another possible mechanism mediating the effect of GLP-1 RAs. A low dose of liraglutide significantly increased BP 3 days after starting the treatment, followed by a decrease after prolonged treatment (von Scholten et al., 2015). Accordingly, one possibility is that the renin/aldosterone changes observed in our studies are merely secondary to the changes in blood pressure. We did observe a small increment of systolic BP in our trials, but the change did not reach statistical significance.

The exact localization of GLP-1 receptors in the kidney remains poorly characterized (Skov 2014), thus a direct effect of GLP-1 RAs on juxtaglomerular cells cannot be ruled out. In both of our studies, the activity of renin changed in parallel with aldosterone, indicating that GLP-1 RAs mediated their effect via a change in kidney endocrine function.

It has been hypothesized that the disbalance in RAAS activity is connected to cardiovascular complications of T2DM, as well as to diabetic nephropathy (Patel et al., 2012, Ruggenenti et al., 2010). Therefore, it would be important to further dissect the chronic effects of distinct GLP-1 RAs to understand whether some of the beneficial effects of GLP-1 RAs observed in clinical trials and clinical practice relate to the modification of RAAS system.

The renoprotective effect of GLP-1 RA may also be the result of down-regulation of angiotensin II production at the tissue level (Puglisi et al., 2021).

It has been demonstrated that GLP-1 RAs exert cardiovascular benefits and reduce incidence of MACE in T2DM patients. Although underlying mechanisms are not entirely clear, it has been proposed that GLP-1 RAs might have a direct effect on the juxtaglomerular apparatus, which ultimately leads to reductions in renin and aldosterone levels, as observed in our experiments. As far as we are aware, our study with exenatide was the first to demonstrate that a clinically used GLP-1 receptor agonist suppresses aldosterone levels. On the other hand, GLP-1 RAs are known to reduce body weight and blood pressure in the long term, both effects being renoprotective. All in all, the effects observed in these two clinical trials with exenatide and liraglutide stress the positive effect of GLP-1 RAs on the RAAS, confirming the overall beneficial effects observed in larger trials examining CV benefits. A schematic overview of the effects and potential clinical implications of GLP-1 RA-s on RAAS is presented in Figure 17.

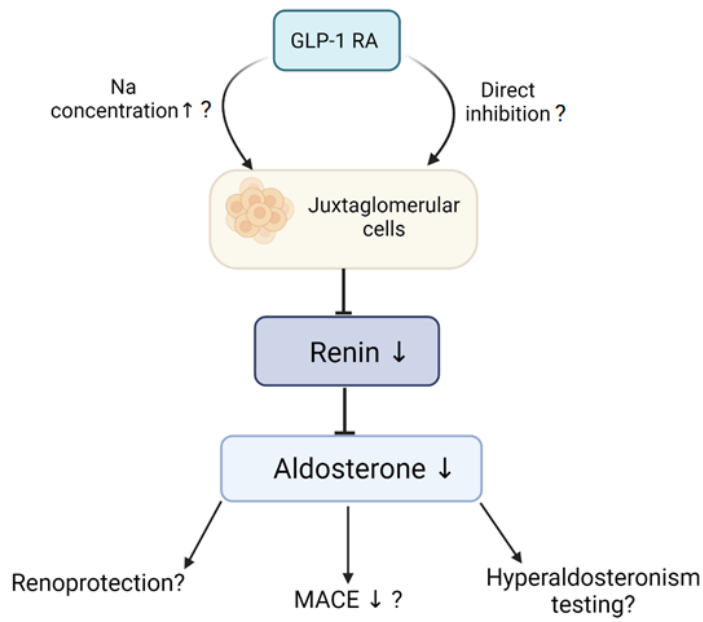

Figure 17. Effects and potential clinical implications of GLP-1 RA-s on RAAS.

Effect of GLP-1 RAs on Hypothalamic-pituitary-adrenal axis

The best-described action of GLP-1 RAs on other hormonal systems is related to the increase of glucocorticoid levels after treatment. The acute stimulating effect of GLP-1 RAs has been shown several times in animal and human studies (Gil-Lozano et al., 2010, Malendowitz et al., 2003, Krass et al., 2015).

Surprisingly, we did not see any change in ACTH/cortisol levels in our clinical trial after acute or chronic treatment with liraglutide. The lack of effect in our liraglutide study may be related to the low dose of liraglutide used or to the fact that the effect on cortisol does not last 12 h after administration of the drug. The maximum plasma concentration after liraglutide administration was reached at approximately 10–14 h (Agersø et al., 2002), which does not necessarily correlate with the maximum effect on hormone levels. We also measured hormones in one time point during glucose infusion. Administration of liraglutide did not change the regulation of cortisol and aldosterone release during glucose and volume loading.

In a recent placebo-controlled clinical trial with dulaglutide, the authors also failed to demonstrate any effect on cortisol release after a thorough assessment using unstimulated and stimulated release of cortisol (Winzeler et al., 2019). One must take into account that dulaglutide is a large molecule and unlikely to reach the potential targets in CNS. A study on patients with T2DM who had newly been started on liraglutide examined the effect of long-term exposure to GLP-1 RA. It was concluded that 12 weeks of treatment with liraglutide resulted in significant increase in cortisol/ACTH ratio, and at the same time, a decrease in both ACTH and cortisol levels (Kamei et al., 2017). Thus, one may conclude that the potential interference of GLP-1 RAs with cortisol release after chronic dosing is either absent or leads to suppression of cortisol levels. The summary of results with different GLP-RAs is given in Table 6. Still, there is anecdotal evidence that GLP-1 RA treatment may interfere with the dexamethasone suppression test by giving false-positive findings (Nagai et al., 2019).

However, our clinical trial with the full therapeutic dose of exenatide revealed the expected pattern on the ACTH/cortisol axis. The study confirmed that acute administration of exenatide has a modest stimulating effect on the hypothalamic-pituitary-adrenal axis. Exenatide did increase the release of ACTH and cortisol, but the effect was far less than expected. While the first time-point of the cortisol measurement seemed to be contaminated by a stress response related to trial participation, the maximum level of cortisol obtained during the test was relatively low, ranging from 313–564 nmol/L. Moreover, exenatide did not stimulate cortisol release in 4 out of 10 subjects (Publication II). Overall, our data are broadly similar to those obtained with the glucagon stimulation test. Thus, in 55 healthy volunteers, the lowest peak cortisol level obtained was 251 nmol/L (Karaca et al., 2011). Quite different lower cut-off points have been proposed for the glucagon stimulation test. For example, Hamrahian et al. (2016) proposed 243 and 309 nmol/L as lower and upper cutoffs for the 1 mg glucagon test (Hamrahian et al., 2016). In our study, 9/10 and 7/10 healthy subjects exceeded these values. Whether subcutaneous admi-

nistration of exenatide or another GLP-1 RA holds promise as a stimulation test for adrenal function needs further evaluation in a larger sample. While the effect of the GLP-1 agonist on cortisol release is clearly central, i.e., mediated by an increase in ACTH levels, the mechanism beyond this effect remains elusive. Administration of exenatide decreased glucose levels and, therefore, the drop in glucose concentration, may well underlie the counter-response of ACTH and cortisol. Further studies are needed to evaluate whether the GLP-1 RA can increase ACTH/cortisol release under isoglycaemic conditions. The modest effect of the clinically used dose of exenatide is in line with our clinical trial of a low dose of liraglutide on cortisol levels.

The cortisol-stimulating effect of peripherally administered GLP-1 RA has been previously demonstrated in both rodents and humans (Gil-Lozano et al., 2010, Malendowicz, Neri et al., 2003, Malendowicz, Nussdorfer et al., 2003, Krass et al., 2015, Khoo et al., 2010. Lerche et al., 2009). However, the mechanism behind this effect is not entirely clear. It is thought that the effect of GLP-1 on the HPA axis is independent of its insulinotropic effect and is most probably central (Gil-Lozano et al., 2013, Gil-Lozano et al., 2014). Although the exact mechanism of how GLP-1 RAs influence ACTH and cortisol levels in blood remains to be elucidated, GLP-1 signalling pathways have been identified in the CNS. It has been demonstrated that GLP-1 neurons in the NTS project directly to the PVN. Moreover, there is evidence that there might be synaptic connections between the GLP-1-containing terminals and corticotropin-releasing factor (CRF)-containing neurons in the PVN (Katsurada et al., 2014, Alhadeff et al., 2012).

On the other hand, other studies suggest that GLP-1 RAs do not affect ACTH levels, but indicate that they might be involved in chronic stress-induced facilitation of corticosterone responses to a novel stressor (Tauchi et al., 2008).

Ultimately, although we observed an effect on the HPA axis, its clinical implications are probably minor, as the levels of both ACTH and cortisol remained only moderately affected.

Table 6. Effects of different GLP-1 RAs on HPA axis.

GLP-1 RA	Duration of action	Acute administration	Chronic administration
Liraglutide	Long-acting	Cortisol ∅, ACTH ∅ (Publication I)	Cortisol ∅, ACTH ∅ (Publication I) Cortisol ↓, ACTH ↓, Cortisol/ACTH ↑↑ (Kamei et al., 2017)
Exenatide	Short-acting	Cortisol ↑, ACTH ↑ (Publication II)	Not tested
Dulaglutide	Long-acting		Cortisol ∅ (Winzeler et al., 2019)

∅ - no effect

Effect of GLP-1 RA-s on growth hormone

One of our most striking findings was that acute administration of 10 μg exenatide led to a peak in GH levels in most subjects. To the best of our knowledge, this effect by GLP-1 RAs on GH secretion has not been described previously. Therefore, not much is currently known about the possible mechanism of action of GLP-1 agonists on the somatotropic axis. However, a direct effect by GLP-1 RA on the hypothalamic-GH axis seems to be the most probable mechanism underlying the stimulation of GH release.

A subtherapeutic dose of liraglutide did not alter GH levels after acute administration. On the other hand, GH levels did increase after chronic treatment with liraglutide, confirming the effect we observed in the exenatide trial.

Currently, there is only limited indirect evidence on the possible interaction of incretin hormones and GH secretion. The dipeptidyl peptidase-4 inhibitor sitagliptin, which increases endogenous levels of both GLP-1 and gastric inhibitory polypeptide (GIP), has been shown to potentiate stimulated GH secretion in women (Wilson et al., 2018). On the other hand, Teti et al. (Teti et al., 2020) reported that dipeptidyl-peptidase 4 (DPP-4) inhibition did not alter the GH/IGF-1 axis in adults with type 2 diabetes. In addition, GLP-RAs do not interfere with the secretion of hypoglycaemia-induced counter-regulatory hormones, including GH. Intravenous exenatide infusion did not alter the secretion of GH during hyperinsulinaemic hypoglycaemic clamp in healthy male volunteers (Degn et al., 2004).

Similarly, Almby et al. (Almby et al., 2019) investigated the effects of GLP-1 receptor activation on counter-regulatory hormones (i.e., glucagon, catecholamines, cortisol, and GH) during hyperinsulinaemic hypoglycaemic clamp in patients who had undergone gastric bypass surgery. These authors concluded that neither basal levels nor the rise in GH during hypoglycaemia differed between groups receiving placebo or infusion with GLP-1RA (Almby et al., 2019).

It is currently unclear whether GLP-1RAs act directly on the release of GH, GH-releasing hormone (GHRH), or somatostatin, or whether they have an indirect effect mediated by some of the known regulators of the GH axis. There is electrophysiological evidence that GLP-1 can act on ghrelin-sensitive neurons in the nucleus arcuatus (ARC) (Riediger et al., 2010); specifically, ghrelin-excited ARC neurons were concordantly stimulated by GLP-1 and, to a lesser degree, inhibitory effects were also observed in this population of cells (Riediger et al., 2010). Accordingly, GLP-1 Ras may at least partially share the pathway with ghrelin in stimulating GHRH/GH release. Regarding possible indirect mechanisms of action, a change in metabolites (amino acids, free fatty acids) seems an unlikely mediator, as our experiment was conducted under fasting conditions. Ghrelin is known to be a potent stimulant of GH secretion. However, distinct GLP-1RAs have been reported to exert a neutral or inhibitory effect on the release of ghrelin after acute or chronic administration (Hagemann et al., 2007, Farr et al., 2016, Beti et al., 2019, Garg et al., 2017).

Another potential mechanism worth discussing is decrease of glucose level as a stimulus to GH release. Insulin induced hypoglycemia is a classical test to

confirm GH reserve. However, it is important to note that achievement of adequate hypoglycemia, with serum blood glucose < 2.2 mmol/L, is necessary to evoke a GH response (Cheer et al., 2014).

In our experiments, glucose levels decreased but no hypoglycaemic values were detected, and the change in glucose levels did not correlate with the observed GH release. Liraglutide clearly decreased the glucose values during the graded glucose infusion test and robustly enhanced insulin secretion (Sedman, Vasar et al., 2017). However, all subjects had glucose levels well above the hypoglycemic threshold. In the exenatide experiment, glucose levels decreased, but no hypoglycaemic values were detected, and the change in glucose levels did not correlate with the observed GH release. It has been established that GLP-1RAs induce insulin secretion (Sedman, Vasar et al., 2017), but insulin itself seems to have a neutral or inhibitory effect in healthy subjects (Sharp et al., 1984, Lanzi et al., 1997).

Effect of GLP-1 RAs on other hormones

We also tested whether exenatide may change the levels of other pituitary hormones. An overview of the results is presented in Table 7.

Interestingly, LH and testosterone levels decreased slightly following the administration of a single dose of exenatide. Although these changes were statistically significant, they were small and their functional significance remains to be determined. Prolactin levels fell during the study as well, but this decrease reflects the circadian rhythm and stress response during the initiation of blood sampling.

We also observed an increase in copeptin levels, which did not reach statistical significance. However, data were only available for six subjects, and thus the possible effect must be scrutinised in further studies. A recent study showed that nausea and vomiting, which are common side effects of GLP-1RAs, cause an increase in copeptin levels. Consequently, these side effects should be taken into account in further studies (Brooks et al., 2021).

Unfortunately, other hormones were not measured during our liraglutide trial.

Table 7. Effect of GLP-1 RA on other hormones.

GLP-1 RA	Effect on other hormones			
Exenatide	LH ↓	Testosterone ↓	Prolactin§	Copeptin*

§ Normal physiological response; * Statistically insignificant result

Potential clinical implications

RAAS

The renin-angiotensin-aldosterone system is a crucial regulator of blood volume, electrolyte balance, and systemic vascular resistance, and responsible for acute and chronic alterations of arterial BP. It plays an important role in maintaining normal cardiovascular functions and contributes to a variety of cardio-

vascular diseases. According to the classical understanding of RAAS, it comprises three significant compounds: renin, angiotensin II, and aldosterone (Wu et al., 2018). Individuals with type 2 diabetes are at high risk for developing cardiovascular diseases, including myocardial infarction, stroke, heart failure, and cardiovascular death. According to current guideline recommendations and recent cost-effectiveness analyses, GLP-1 RAs with proven cardiovascular benefit are recommended in patients with T2D and atherosclerotic cardiovascular disease or those at high risk of cardiovascular events (Marx et al., 2022). Therefore, the results from our studies might have important clinical implications in this context. Firstly, increased aldosterone biosynthesis (as defined by an elevated aldosterone to renin ratio) or primary aldosteronism is a key phenotype that is present in up to 15% of individuals with hypertension (Lim et al., 1999). Therefore, if both acute and chronic administration of GLP-1 RAs influence aldosterone levels, this must be considered when testing patients with hypertension for primary hyperaldosteronism. In addition, if chronic treatment with GLP-1 RAs changes aldosterone and renin levels, it might modify the cardiovascular outcomes of patients. On the other hand, the studies examining the effect of GLP-1 RAs on the RAAS system are of variable design, and present conflicting results. Unfortunately, we do not have data on possible chronic effect of exenatide on aldosterone levels. In addition, using healthy volunteers with no evidence of RAAS dysfunction versus diabetic patients with or without hypertension may also be a contributing factor. All in all, the beneficial effects of GLP-1 RAs on cardiovascular system and kidneys are outweighing the potential changes in aldosterone and renin levels. However, the underlying pathophysiological mechanisms are well worth further studying.

HPA

As several lines of evidence pointed to the fact that GLP-1 RAs may stimulate cortisol release centrally, i.e., via CRF and/or ACTH release, we hypothesized that acute administration of a full dose of short-acting GLP-1 RA exenatide may be used as a novel test for central adrenal failure. Although we did observe a modest effect on the HPA axis, it is probably of minor clinical relevance after chronic administration. This is consistent with the results with our liraglutide trial. Furthermore, the lack of effect on cortisol levels after chronic treatment is positive considering the deleterious effect of hypercortisolemia on many organ systems.

GH

So far, only a few studies have examined interactions between GLP-1 RAs and GH levels, and the results are somewhat conflicting. In our experiment with liraglutide, acute administration of a subtherapeutic dose of a long-acting GLP-1 RA did not influence GH levels. On the other hand, we did observe a peak in GH levels after acute administration of a short-acting GLP-1 RA exenatide, and the GH level was also higher after chronic treatment with liraglutide.

Firstly, our data support the potential of GLP-1 RAs to develop a diagnostic test to interrogate pituitary function. Secondly, the chronic effect of these drugs on GH may have multifaceted health implications in patients with overweight and/or diabetes with potential harmful effects also involved.

The potential clinical use in diagnostic testing of hypopituitarism is presented in Figure 18.

Although the differences in GH levels at baseline and post-administration were clinically marginal, these findings warrant further investigation in larger sample size and various patient populations.

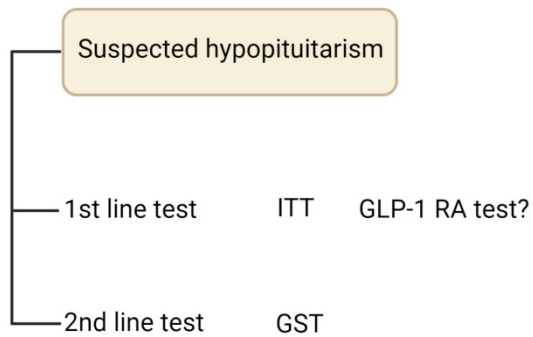

Figure 18. Potential clinical use of GLP-1 RA test in diagnosing hypopituitarism. GST – Glucagon stimulation test; ITT – Insulin Tolerance Test

Other hormones

Interestingly, there was a slight decrease in testosterone levels after the administration of a single dose of exenatide. Although this change was statistically significant, they were small, and thus its functional significance remains to be determined. In addition, the levels of testosterone follow a circadian rhythm, and therefore these changes might have been a reflection of fall in testosterone levels after peaking in morning hours.

The change in prolactin levels reflected the circadian rhythm and stress response during the initiation of blood sampling, and therefore the possible change cannot be interpreted without further studies.

In addition, there was a small increase in copeptin levels. Taken together with other available evidence from the literature, this effect can reflect nausea – a common side effect of GLP-1 RAs.

Study strengths and limitations

The common limitation with both liraglutide and exenatide studies was their small sample size. Accordingly, some effects may have remained undetected due to the lack of statistical power. In addition, neither of the studies had a placebo arm, and most subjects were male. In the liraglutide trial, due to the

primary aim of the clinical study, the dose of liraglutide was fixed and not escalated to the usual clinical dose of 1.2 mg/day. As the renin-aldosterone system is sensitive to body position, we cannot exclude that the drop in the activity was related to positioning the subjects from upright to sitting in the beginning of the exenatide experiment. As to the GH results, the studies reported represented a post hoc analysis of original studies.

On the other hand, the strength of our studies was that healthy volunteers were used with no interference from any disease or concomitantly used drug.

Concluding remarks

GLP-1 agonists have gained tremendous popularity in recent years due to their beneficial side effect of weight loss. Considering the array of possible additional indications which have reached late stages of clinical trials, such diabetic kidney disease, peripheral artery disease, neurodegenerative disorders, or even non-alcoholic steatohepatitis (NASH), clinical trials investigating mechanistic pathways are important in understanding the complexity of GLP-1 actions.

It is possible that the effects we observed in these two pilot clinical trials do not have robust clinical significance, since the safety information gathered from a large sample of patients and provided in the SmPCs does not reflect these changes. However, such academic clinical trials examining possible pathways are helpful in providing in-depth knowledge about drug pharmacodynamics. Additionally, most of the data gathered from large-scale clinical trials and real-world evidence is from patients with T2DM, and much less data is available in patients with obesity, let alone healthy subjects.

Much is still unknown about the exact mechanisms of action of the GLP-1 RAs, and our studies helped to shed some light on them. In the long run, these findings could help identify subgroups of patients who are better candidates for GLP-1-based therapies.

7. CONCLUSIONS

The conclusions of this study were the following:

1. Administration of a GLP-1 RA led to decreases in both renin and aldosterone levels, i.e. down-regulation of RAAS. These findings could provide explanation for the reno- and cardioprotective effects of GLP-1 RAs.
2. Administration of a GLP-1 RA exhibited a modest stimulating effect on the hypothalamic-pituitary-adrenal axis, the effect being central.
3. Administration of a GLP-1 RA elicited a peak in growth hormone levels. This effect represents most likely a direct stimulation of hypothalamic/GH axis. GLP-1 RAs may have a potential to be used as a pituitary stimulation test to detect ACTH and/ or GH deficiency.

SUMMARY IN ESTONIAN

GLP-1 retseptori agonistide toimed hüpofüüsi ja neerupealiste hormoonidele

Sissejuhatus:
Glükagooni-sarnane peptiid 1 (GLP-1) on inkretiinhormoon, millel on avastatud ootamatult palju toimeid. GLP-1 sekreteeritakse seedetraktis L-rakkude poolt ning selle vabanemist stimuleerib söömine. Enteroendokriinseid L-rakke leidub peen- ja jämesooles. Pankrease alfa-rakud ja enteroendokriinsed L-rakud ekspresseerivad preproglükagooni; GLP-1 tekib preproglükagoonist translatsioonijärgse spetsiifilise lõikamise tagajärjel. Lisaks leidub ajutüves *nucleus tractus solitarius*es (NTS) väike hulk preproglükagoon-positiivseid neuroneid, mis on ajus peamine endogeense GLP-1 allikaks.

GLP-1 sekretsiooni kõige olulisemaks stimuleerijaks on toitainete, eriti süsivesikute, seedeprotsess. GLP-1 plasmasisaldus on paastuseisundis väga väike. GLP-1 sisaldus plasmas suureneb kiiresti paari minuti jooksul pärast söömist, jõudes maksimaalse kontsentratsioonini ligikaudu ühe tunni jooksul. GLP-1 stimuleerib insuliini vabanemist glükoosist sõltuval viisil, mistõttu on sellel oluline roll söögijärgse insuliini vabanemise puhul.

On näidatud, et 2. tüüpi diabeeti põdevatel isikutel on nn inkretiini efekt märkimisväärselt vähenenud. Seetõttu peetakse inkretiinide puudulikkust üheks oluliseks glükoositaluvuse vähenemisega seotud patogeneetiliseks teguriks. GLP-1 retseptori agonistid on võrdlemisi uus 2. tüüpi diabeedi ravimite rühm, millel on lisaks vere glükoosisisalduse kontrollimisele märkimisväärne hulk teisi organsüsteeme mõjutavaid toimeid.

Käesoleva uurimistöö eesmärgid olid:
1) määrata GLP-1 RA akuutse ja/või kroonilise manustamise mõju inimese reniini ja aldosterooni sisaldusele veres;
2) kvantifitseerida GLP-1 RA mõju inimese HPA telje osadele hindamaks, kas GLP-1 RA akuutne manustamine omab potentsiaali tsentraalse neerupealiste puudulikkuse diagnostilise testina;
3) iseloomustada GLP-1 RA manustamise mõju teistele vähemuuritud hormoonsüsteemidele.

Uurimistöö meetodid:
Doktoritöö raames viisime läbi kaks kliinilist uuringut tervetel vabatahtlikel:
1) Kliiniline uuring liraglutiidiga:
Tegemist oli ühe rühmaga avatud uuringuga, milles hinnati liraglutiidi farmakodünaamikat tervetel vabatahtlikel akuutse ja kroonilise manustamise puhul. Peamiseks eesmärgiks oli hinnata potentsiaalse tolerantsuse teket liraglutiidi vere glükoosisisaldust vähendava toime suhtes kroonilise manustamise puhul. Lisaks soovisime hinnata liraglutiidi kroonilise manustamise võimalikku mõju neerupealiste hormoonide (st aldosterooni ja kortisooli) regulatsioonile.

2) Kliiniline uuring eksenatiidiga:
Tegemist oli ühe rühmaga avatud uuringuga, milles hinnati, kas eksenatiidi ühekordne annus on rakendatav hüpofüüsi-neeupealiste telje funktsionaalsuse diagnostilise testina. Esmaseks tulemusnäitajaks oli kortisooli maksimaalne sisaldus veres pärast eksenatiidi manustamist.

Tulemused ja järeldused:

1) GLP-1 retseptori agonisti manustamine vähendas reniini ja aldosterooni sisaldust veres tervetel täiskasvanutel, st toimus reniin-angiotensiin-aldosterooni süsteemi supressioon. Antud leiud võivad aidata selgitada GLP-1 retseptori agonistide reno- ja kardioprotektiivseid toimeid.
2) GLP-1 retseptori agonisti ühekordne annus tõi kaasa hüpotalamuse-hüpofüüsi-neerupealise telje mõõduka tsentraalse stimulatsiooni.
3) GLP-1 retseptori agonisti ühekordne annus kutsus esile kasvuhormooni sisalduse järsu tõusu. Tõenäoliselt on see toime tsentraalne, st eksenatiid mõjutas otseselt hüpotaalamuse-kasvuhormooni telge.

GLP-1 retseptori manustamine võib olla kliiniliselt kasutatav hüpofüüsi puudulikkuse testimiseks.

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