Incretin hormones and maturity onset diabetes of the young – pathophysiological implications and anti-diabetic treatment potential

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The following 3 papers form the basis of the thesis:

I.
Incretin Effect and Glucagon Responses to Oral and Intravenous Glucose in Patients with Maturity Onset Diabetes of the Young – Type 2 and Type 3. Signe H. Østoft, Jonatan I. Bagger, Torben Hansen, Oluf Pedersen, Jens J. Holst, Filip K. Knop and Tina Vilsbøll. Diabetes. March 2014. PMID:24677712.

II.
Postprandial incretin and islet hormone responses and dipeptidyl-peptidase 4 enzymatic activity in patients with maturity onset diabetes of the young. Signe H. Østoft, Jonatan I. Bagger, Torben Hansen, Bolette Hartmann, Oluf Pedersen, Jens J. Holst, Filip K. Knop and Tina Vilsbøll. European Journal of Endocrinology. August 2015. PMID:25953829.

III.
Glucose Lowering Effects and Low Risk of Hypoglycemia in Patients With Maturity Onset Diabetes of the Young When Treated With a Glucagon-like Peptide-1 Receptor Agonist – A Double-blind, Randomized, Cross-over Trial. Signe H. Østoft, Jonatan I. Bagger, Torben Hansen, Oluf Pedersen, Jens Faber, Jens J. Holst, Filip K. Knop and Tina Vilsbøll. Diabetes Care. July 2014. PMID:24929431

Introduction

Diabetes is a common disease with more than 300,000 patients in Denmark. Maturity onset diabetes of the young (MODY) designates monogenic forms of diabetes causing approximately 1-2% of all cases of diabetes[1]. This means that there are 3,000-6,000 patients with MODY in Denmark and probably as many who do not know they have diabetes.
MODY is characterised by the following clinical features: 1) early onset of diabetes, often before 25 years of age, 2) family history of diabetes, 3) no history of ketoacidosis, 4) normal insulin sensitivity, 5) measurable C-peptide levels in plasma and 6) absence of islet cell and glutamic acid decarboxylase autoantibodies at the time of diagnosis[2,3]. Mutations in more than 8 different genes explain the genetically and clinically heterogeneous forms of MODY. The most common forms are MODY3 and MODY2 with mutations in the transcription factor hepatocyte nuclear factor 1α (HNF1A) (HNF1A-diabetes) and the glucokinase (GCK) (GCK-diabetes) genes, and responsible for up to 60% and 20% of all patients with MODY, respectively[4,5]. Strict glycaemic control is crucial in HNF1A-diabetes, as patients have the same risk for diabetic micro- and macrovascular complications as patients with type 2 diabetes. Patients with HNF1A-diabetes are very sensitive to treatment with sulphonylureas (SU)[6]. This, in combination with a normal insulin sensitivity, leads treatment with even low doses of SU often to be associated with a high risk of hypoglycaemia[6,7]. In contrast, patients with GCK-diabetes have only mildly elevated plasma glucose (PG) caused by altered glucokinase activity resulting in a glucose sensing defect, but preserved ability to secrete insulin at a higher threshold of glucose[8]. These patients seem to be without risk of diabetic complications, why treatment is unnecessary.

The incretin hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), are intestinal hormones essential for glucose metabolism. The incretins exert insulinotropic effects on the beta cell and GLP-1 glucagonostatic effect on the alpha cell acting via specific receptors in the cell membrane. The insulinotropic effect is strictly glucose dependent, hence the effect ceases when plasma glucose decreases[9–11]. The circulating incretins are rapidly degraded by the enzyme dipeptidyl-peptidase 4 (DPP-4)[12,13]. Patients with type 2 diabetes are known to have a defective incretin hormone system with a

reduced incretin effect[14–17]. Whether this reduced incretin effect is the cause or a consequence of the diabetic state has been disputed. Nevertheless, patients with type 2 diabetes benefit from incretin-based treatments, including 1) GLP-1 receptor agonists (GLP-1RAs) and 2) DPP-4 inhibitors.

Until now, the incretin system in MODY patients has only been examined sparsely[18–20], and the incretin effect has never been studied in these patients and the glucagon secretion has also only been investigated in one study[7]. Furthermore, no randomised prospective studies have evaluated the treatment potential of incretin-based therapy in patients with HNF1A-diabtes[21–23].

The objectives of this PhD thesis were to examine the incretin effect and glucagon response (Study I) and the postprandial incretin response (Study II) in patients with GCK-diabetes and HNF1A-diabetes, and to investigate the efficacy and associated side effects of GLP-1RA treatment in patients with HNF1A-diabetes (Study III).

Maturity onset diabetes of the young – MODY

MODY is a clinically and genetically heterogeneous group of diabetes types, caused by mutations in single well-described genes. Today known mutations in more than 8 different genes are associated with specific types of MODY. These mutations mainly affect beta cell function. In spite of genetic heterogenicity, patients with MODY are often characterised by the following clinical features[2,24,25]:

- Family history of diabetes
- Young age at onset (typically before the age of 40)
- Functional beta cells at the time of diagnosis
- Absence of beta cell autoimmunity
- Normal or increased insulin sensitivity

Diagnosing MODY is often quite a challenge for clinicians. Distinguishing individuals with MODY from those with type 1 diabetes or type 2 diabetes can be difficult due to several similarities regarding phenotypes. Therefore, patients with MODY are often misclassified as having type 1 diabetes or type 2 diabetes[6,26]. Furthermore, gene sequencing is currently rather expensive and therefore not used for diagnostic purposes on a regular basis.

The clinical phenotype is closely dependent on the specific defect in the genes. Most cases of MODY (Table 1) are caused by mutations in transcription factor genes, of which HNF1A (MODY3) and hepatocyte nuclear factor 4α (HNF4A) (MODY1) are the most common, constituting approximately 60% and 10%, respectively[4,5,27]. Another common form of MODY is caused by mutations in the GCK gene (MODY2), causing approximately 20% of all cases of MODY. The rest of the known types of MODY are rare, and more mutations will be identified as more patients are subjected to medical gene sequencing in the future. This thesis focuses on the two most common types of MODY: GCK-diabetes and HNF1A-diabetes.

Gene defect	Percentage of all MODY	Debut	Fasting plasma glucose	Risk of diabetic complications	Treatment
HNF4A (MODY1)	4	Puberty/ young adult	High (variable)	Yes	Sulphonylurea (insulin)
GCK (MODY2)	22	At birth	Slightly elevated	No	No
HNF1A (MODY3)	60	Puberty/ young adult	High (variable)	Yes	Sulphonylurea (insulin)

Table 1. Characteristics of the three most common types of MODY

HNF1A-diabetes

HNF1A-diabetes (MODY3) is the most common form of MODY[4,5,27]. It is estimated that approximately 2,000 patients in Denmark have this type of diabetes although many patients may not have been correctly diagnosed or are not even diagnosed with diabetes. The HNF1A defect is causing an altered glucose metabolism in the beta cell involving a reduced pyruvate kinase activity and a defective mitochondrial oxidation of metabolic substrates resulting in a reduced generation of adenosine triphosphate (ATP). The interaction of ATP with ATP-sensitive potassium (K+) channels (KATP-channel) is hereby reduced, leading to impaired glucose-induced depolarisation of the cell membrane, with subsequent impaired influx of calcium (Ca2+) and finally a reduced insulin secretion resulting in glucose intolerance[28,29]. Patients with HNF1A-diabetes are often diagnosed during adolescence. More than 50% of mutation carriers will develop diabetes before 25 years of age, and the lifetime risk of developing diabetes is higher than 95%[24]. The typical course of disease is characterised by a rapid progression from impaired glucose tolerance to diabetes. After the diagnosis of diabetes, glucose tolerance is further impaired due to a continuous decline of beta cell function and beta cell mass[6]. HNF1A-diabetes often develops suddenly with classic hyperglycaemic symptoms such as polyuria and polydipsia, which is why this form of diabetes is often misclassified as type 1 diabetes[6,26]. Furthermore, mutation carriers are known to have a low renal threshold for glucose due to a reduced expression of the sodium-glucose transporter-2 (SGLT2) in the renal proximal tubule[30]. Therefore, glycosuria is present even before glucose intolerance. In addition, HNF1A expression has been demonstrated in mouse alpha cells[31], but the role of a mutated HNF1A in the alpha cell remains unknown.

In spite of the majority of patients being of normal weight, patients with HNF1A-diabetes have a similar risk of developing micro- and macrovascular complications to diabetes as patients with type 2 diabetes[32,33] and the frequencies of neuropathy and hypertension seem to be similar to type 1 diabetes, which is why strict glycaemic control and proper screening for complications is crucial for an improved prognosis. Interestingly, it seems like patients with HNF1A-diabetes have elevated levels of HDL cholesterol compared to patients with type 2 diabetes, which may be useful when distinguishing between HNF1A-diabetes, type 2 diabetes and other types of MODY[34,35].

Patients with HNF1A-diabetes are very sensitive to SU, whereas metformin has very limited effect[6,36,37]. Patients who are

misclassified as type 1 diabetes can – if some beta cell function is maintained – often stop their insulin treatment without the risk of ketoacidosis and treatment can be replaced with SU when MODY is diagnosed correctly (by gene sequencing)[38]. A disadvantage of the marked sensitivity to SU is a rather high risk of hypoglycaemia in SU-treated patients with HNF1A-diabetes. The risk of hypoglycaemia during acute treatment with the SU glibenclamide and nateglinide in patients with HNF1A-diabetes has previously been investigated[7]. Forty per cent of the patients experienced hypoglycaemia after glibenclamide administration, while no events of hypoglycaemia occurred after nateglinide administration. Further, SU therapy may lead to accelerated loss of beta cell function and/or beta cell mass which may cause treatment failure[39,40]. Despite this SU therapy is often preferred over insulin therapy. In most patients a continuous loss of beta cells and thereby beta cell function eventually result in treatment failure with SU, why treatment with insulin is often required some years after diagnosis. Incretin based treatment has been reported with positive effects on a casuistic level, with DPP-4 inhibitors[21,22] and GLP-1RA[23].

GCK-diabetes

It is estimated that GCK-diabetes affects approximately 1,000 patients in Denmark. The mutations compromise the activity of GCK, which under normal circumstances catalyses rate-limiting glucose phosphorylation, enabling the beta cells (and hepatocytes) to respond appropriately to hyperglycaemia[41]. Thus, patients with GCK-diabetes have a glucose-sensing defect resulting in a higher glucose threshold for secreting insulin (plus 1-3 mmol/l) and therefore a higher fasting plasma glucose (FPG) level. An adequate insulin secretion occurs after oral glucose intake which will lower plasma glucose to the same extent as in normal glucose tolerant individuals[8]. The mild hyperglycaemia is present from birth and does not worsen with time. The mildly elevated glucose levels only increases glycated haemoglobin A1c (HbA1c) up to 8% (64 mmol/mol)[42], and patients have a very low risk of complications with this type of diabetes[43]. Because patients with GCK-diabetes do not have symptoms of hyperglycaemia and because their overall risk of typical complications is low, the majority of patients do not require glucose lowering treatment[2]. One exception is during pregnancy where insulin treatment may be required to prevent excess foetal growth[44].

Incretin hormones and glucagon

Physiology of the incretin hormones

The incretin hormones, GIP and GLP-1 are secreted into circulation in response to presence of nutrients in the gut [10,11], especially in response to meals rich in fat and carbohydrate (Figure 1). GIP is a 42 amino acid polypeptide (GIP1-42) encoded by the preproGIP gene expressed in the endocrine K cells primarily located in the mucosa of duodenum and upper jejunum[45]. GLP-1 is a 30 amino acid polypeptide (GLP-17-37) produced by posttranslational processing of proglucagon, which is synthesized in the endocrine L cells of the mucosa primarily in the distal part of the small intestine and colon[46,47]. GCK is present in both K and L cells, but does not seem to be the main glucose sensor in these cells[19]. This has been investigated in patients with GCK-diabetes, where normal secretion of GIP and GLP-1 was demonstrated.

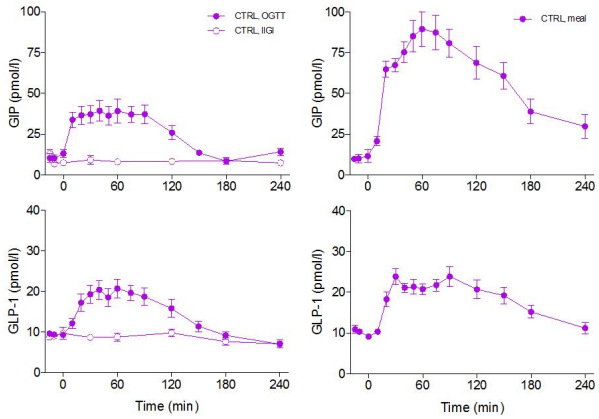

Figure 1. Responses of total GIP and GLP-1 in healthy individuals (CTRL) to oral glucose tolerance test (OGTT) and isoglycaemic iv glucose infusion (IIGI) (Study 1, Appendix I) and test meal (Study 2, Appendix II).

Biologically active GIP and GLP-1 (i.e. the intact forms of the hormones) are rapidly and extensively metabolised by the enzyme DPP-4 found in plasma and many organs[13,48,49]. DPP-4 cleaves off the N-terminal dipeptide and thereby inactivates the hormones resulting in half-lives of 7 minutes (intact GIP) and 1-2 minutes (intact GLP-1)[9,13,48,50]. The elimination of the degraded, inactive forms of the hormones (GIP3-42, GLP-19-36amidie and GLP-19-37 takes place by renal extraction and degradation[48,50,51]. Because of the DPP-4 mediated degradation only a minor part of the circulating hormones represent biologically active forms[11]. Interestingly, HNF1A is necessary for promoter activation of the DPP-4 gene in enterocyte (caco-2 cells) and hepatocyte (HepG2 cells) cell lines[52]. However, the potential impact of defects in HNF1A on human incretin physiology has not been investigated.

GIP and GLP-1 interact with specific receptors in the cell membrane of the pancreatic beta cell[53,54]. When binding to their respective receptor both GIP and GLP-1 evoke activation of adenylat cyclase subsequently increasing intracellular cyclic adenosine monophosphate (cAMP)[55,56] and activation of protein kinase A. Concurrent release of Ca2+ from intracellular stores and increasing Ca2+ influx through voltage-gated Ca2+ channels cause exocytosis of insulin-containing granules[53,54] with release of insulin to the blood. Both cAMP and activated protein kinase A may influence secretory events distal to generation of ATP by glucose metabolism[54,57,58]. The insulinotropic effect of both hormones may be observed already from the beginning of a meal [11,59], and they appear to contribute equally to the postprandial glucose-induced insulin secretion, although GLP-1 is more effective at higher glucose levels[50,59]. The insulinotropic effect is strictly glucose-dependent and ceases at PG concentrations below 4 mmol/l[60]. The mechanisms behind this glucose-dependency of the insulinotropic effect are not clear but are thought to be linked to the glucose-dependent generation of ATP[57,61]. Furthermore, the incretin hormones have been shown to improve insulin gene expression and insulin biosynthesis[62], and both hormones show beta cell trophic and beta cell protective actions[63,64]. Both incretin hormones also have strictly glucose-dependent effects on the alpha cell. Especially GIP acts glucose-dependently, since the peptide has a stimulating

effect on glucagon secretion at low glucose concentrations with this effect ceasing at high concentrations[65,66]. GLP-1 strongly and dose-dependently inhibits glucagon secretion in a glucose-dependent fashion[67–69] further contributing to the blood glucose lowering effect of GLP-1[70], whereas GIP may actually stimulate secretion even at elevated glucose levels[71]. The incretin hormones have been reported to have several other functions including promotion of fat deposition in fat tissue (GIP), inhibition of hepatic glucose production (probably indirect), increasing glucose up-take in muscle (controversial), increasing fatty acid synthesis and inhibiting hepatic insulin extraction[56,72,73] (also controversial). GLP-1 seems to promote a feeling of satiety through activation of GLP-1 receptors in the brain and also inhibits gastrointestinal motility via inhibition of the vagal efferent activity[74–76].

The incretin effect

The incretin effect describes the amplification of insulin response (Figure 2) after an oral glucose load compared to glucose administered intravenously to obtain equal levels of glycaemia. It is mediated mainly by GIP and GLP-1[10,15]. The incretin effect accounts for up to 70% of insulin secretion in response to oral glucose intake in healthy individuals[68], and varies with the size of the oral glucose load[68,77]. Thus, healthy individuals are able to increase the incretin effect with increasing loads of oral glucose to maintain plasma glucose within the normal range. The incretin effect can be estimated by relating the difference in integrated beta cell secretory responses (area under the curve (AUC) for insulin, C-peptide or insulin secretion rate (ISR)) between stimulation with OGTT and isoglycaemic intravenous glucose infusion (IIGI) to the response after OGTT[14]:

$$\text{Incretin effect (\%)} = 100\% \times (AUC_{OGTT} - AUC_{IIGI}) / AUC_{OGTT}$$

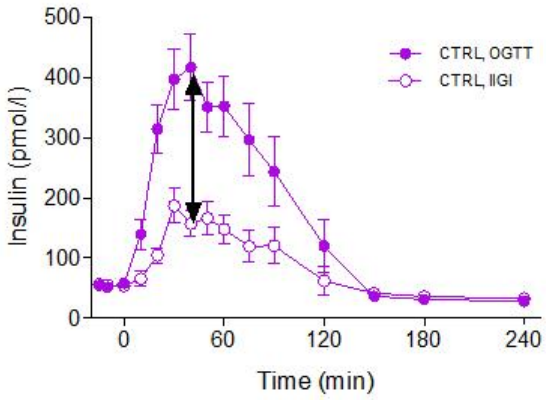

Figure 2. The incretin effect (indicated by the black arrow); based on plasma insulin in healthy individuals (CTRL) (Study 1, appendix I). The illustrated incretin effect is 48±5% (mean±SEM). OGTT: oral glucose tolerance test; IIGI: isoglycaemic iv infusion.

Incretin-related pathophysiology

It has been known for decades that patients with type 2 diabetes have an impaired incretin effect – reduced from 70% in healthy individuals to 30% in type 2 diabetes[14], which has been shown in later studies as well[16,17]. The incretin effect seems to decrease with higher body mass index (BMI) and with the severity of glucose intolerance, with each parameter independently affecting the incretin effect on beta cell function[17,71]. This means that a reduced incretin effect might play a primary role in type 2 diabetes, or it could be due to early consequences of an altered glucose homeostasis[78,79]. However, genetic predisposition does not seem to influence the incretin effect, since a normal incretin effect has been found in normal glucose tolerant (NGT) first degree relatives of patients with type 2 diabetes[80]. Studies showing reversibility of the incretin effect in individuals with gestational diabetes mellitus (GDM) after normalisation of glucose homeostasis[81] and demonstrating attenuation of incretin effect in patients with diabetes secondary to chronic pancreatitis[16] support the hypothesis that the impaired incretin effect is a consequence of the diabetes state. Since the incretin hormones GIP and GLP-1 are considered the primary contributors to the incretin effect, these hormones have been investigated regarding potential deficiencies with respect to secretion, metabolism and/or effect.

Studies of GIP secretion in patients with type 2 diabetes have shown both increased[82] and decreased secretion[83], hence no clear association exists. A recent meta-analysis found preserved GIP secretion in patients with type 2 diabetes, although a reduced secretion may be associated with increasing age and HbA1c, while high BMI seems to be associated with increased responses[84]. Decreased levels of postprandial GLP-1 secretion has been found in some studies of patients with type 2 diabetes[17,83,85], whereas others have found preserved secretion[86–88]. A meta-analysis evaluating the GLP-1 secretion in patients with type 2 diabetes found an overall preserved secretion following OGTT and meals, but with an association between decreased secretion levels and deteriorating glycaemic control[89]. Furthermore, a reduced GLP-1 secretion has been found in obese, NGT individuals[90–92], which could be related to or aggravated by the insulin resistance characterising obese individuals[17,93], which is further supported by the normalisation of GLP-1 secretion following weight loss[94], hence increasing insulin sensitivity.

Secretion of the incretin hormones has only been studied sparsely in MODY patients. A single study found normal secretion of both GIP and GLP-1 in patients with GCK-diabetes[19], and another study demonstrated normal secretion of both incretin hormones in patients with HNF1A-diabetes[20].

The effect of the incretin hormones in patients with type 2 diabetes has been investigated in several studies during both fasting and postprandial conditions. An almost absolute loss of late-phase insulin secretion during GIP infusions in different doses indicate a marked reduced insulinotropic effect of GIP regardless of dose[71,95,96]. A reduced insulinotropic effect of GIP has also been demonstrated in HNF1A-diabetes as well as in patients with other types of diabetes (latent autoimmune diabetes of adults (LADA) and newly diagnosed patients with type 1 diabetes), whereas GLP-1 enhanced both early and late phase insulin secretion[18]. Similar findings were made in patients with diabetes secondary to chronic pancreatitis[16,97]. In contrast, in women with prior GDM who are known to have increased risk of developing type 2 diabetes[98] and in first degree NGT relatives of patients with type 2 diabetes a preserved insulinotropic effect of GIP was found[80,99], suggesting that this pathophysiological trait is a consequence of the diabetic state rather than a cause leading to diabetes. In contrast to GIP, an insulinotropic effect of GLP-1 is preserved in patients with type 2 diabetes[96], although the potency is decreased[100]. Studies have demonstrated that it

is possible to restore the beta cell responsiveness to intravenous (iv) glucose during iv infusion of GLP-1 in pharmacological doses[100,101]. Furthermore, improvement of beta cell responsiveness to both GIP and GLP-1 after near-normalisation of glucose homeostasis by intense insulin treatment in patients with type 2 diabetes has been demonstrated[102]. These findings suggest that the impaired insulinotropic effects of both hormones are a consequence of the hyperglycaemic/dysmetabolic state in patients with type 2 diabetes and furthermore, that the insulinotropic effect of the incretin hormones can be regained.

Physiology of glucagon

Glucose homeostasis is closely regulated by glucagon, which is a peptide hormone secreted from alpha cells in the pancreas. Glucagon serves to maintain plasma glucose concentration within the normal range and counteracts the glucose lowering effect of insulin, GLP-1 and GIP. In alpha cells prohormone convertase 2 processes proglucagon[103] into glucagon, glicentin-related pancreatic polypeptide and the major proglucagon fragment – the latter two are probably both biologically inactive[104]. Hypoglycaemia is probably the most potent stimulator of glucagon secretion. Other known stimulators are amino acids, fatty acids, the autonomic nervous system, glucagon-like peptide 2 (GLP-2) and GIP (during fasting and hypoglycaemia[65]), while inhibitors of glucagon secretion include somatostatin and GLP-1. The beta cell products insulin, amylin and zinc may also inhibit secretion although this is controversial.

The specific mechanism of the inhibiting effect of low glucose on glucagon secretion is still to be discovered, but one hypothesis describes ATP-sensitive K+-channels in the alpha cell membrane, which like in the beta cell is closed by glucose-induced ATP leading to a depolarisation of the membrane. In contrast to the beta cell this depolarisation inactivates Ca2+-channels and consequently inhibits glucagon secretion[105] explaining the inhibitory effect of glucose. Another theory, known as the 'intra-islet'-hypothesis, describes a direct inhibition of glucagon secretion by insulin. When plasma glucose declines, insulin concentrations decline hereby lifting the inhibition of glucagon secretion, and vice versa when plasma glucose rises[106]. Other hypotheses include involvement of the incretin hormones and GLP-2 in regulation of glucagon secretion, disputing the 'intra-islet'-hypothesis[107–109]. The secreted glucagon acts via binding to specific G protein-coupled receptors in different tissues - most importantly in the liver. Here glucagon activates adenylat cyclase resulting in increasing cAMP levels[110], stimulating hepatic glucose production via gluconeogenesis and glycogenolysis resulting in plasma glucose elevation. Glucagon receptors are also present in the heart, pancreas, brain, kidney and smooth muscle, where a physiological role of glucagon is yet to be discovered[109].

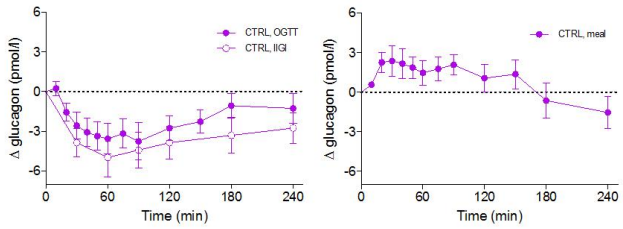

Figure 3. Glucagon responses in healthy individuals (CTRL) to oral glucose tolerance test (OGTT) and isoglycaemic iv glucose infusion (IIGI) (Study 1, Appendix I), and test meal (Study 2, Appendix II). Data are baseline subtracted.

Pathophysiology of glucagon

Patients with type 2 diabetes are characterised by fasting and postprandial hyperglucagonaemia clearly contributing to type 2 diabetic hyperglycaemia[111,112]. Surprisingly, patients with type 2 diabetes are able to suppress glucagon normally during IIGI, indicating that the exaggerated secretion of glucagon after oral glucose is gastrointestinally mediated[111]. Similar findings were made in patients with type 1 diabetes with no residual insulin secretion, indicating that a defect 'intra-islet' interaction is not the cause of the hyperglucagonaemia[107]. In addition, fasting hyperglucagonaemia has been found in individuals with IGT and impaired fasting glucose (IFG)[113], and a positive correlation between insulin resistance and hyperglucagonaemia has been demonstrated in individuals with NGT[114], in individuals with IGT and in patients with type 2 diabetes[115].
A single study has examined postprandial glucagon levels in patients with HNF1A-diabetes treated with a single dose of nateglinide, glibenclamide and placebo, respectively. Impaired postprandial suppression of glucagon was observed with both active treatments and placebo[7].

Blood glucose lowering treatment in relation to this thesis

Sulphonylurea (SU)

Drugs from the class of SU increase insulin secretion by binding to a membrane protein which is a subunit of the KATP-channel in the beta cell, hereby closing the channel[116]. Closure of the KATP-channel causes membrane depolarisation leading to opening of voltage-gated Ca2+-channels increasing intracellular Ca2+-concentration leading to exocytosis of insulin-containing granules. In addition, SUs increase peripheral insulin sensitivity and increase the glucose transporter (GLUT4) in muscle and fat tissue, resulting in increased glucose uptake[117]. They are used for treatment of type 2 diabetes and monogenic forms of diabetes. Glimepiride is the most commonly prescribed SU in Denmark. It is administered orally once–daily and has a half-life of 5-8 hours and peak plasma concentration is reached after 2.5 hours.

Incretin-based treatment

Two types of incretin based therapy for treatment of obese patients with type 2 diabetes have been on the market since 2006 in Europe: 1) Incretin mimetics (stable GLP-1RA) and 2) incretin enhancers (DPP-4-inhibitors). The incretin mimetics have more potent glucose lowering effects[118] than the incretin enhancers, but are administered as subcutaneous injections in contrast to the oral administration of the incretin enhancers. In addition, GLP-1RAs are known – in preclinical settings – to reduce beta cell apoptosis[64]. Liraglutide is a GLP-1 analogue with 97% sequence homology to native GLP-1. Liraglutide has a pharmacokinetic and pharmacodynamic profile suitable for once-daily administration due to a protracted action profile (plasma half-life of 12-13 hours) based on three mechanisms: self-association resulting in slow absorption, binding to albumin and higher enzymatic stability towards DPP-4. Liraglutide acts via binding to the GLP-1 receptor, thus stimulating insulin secretion (glucose-dependently), inhibit-

ing glucagon secretion and gastric emptying, as well as having positive effects on satiety causing weight loss[118–120].

Objectives and hypotheses

The objectives of this thesis were to elucidate the pathophysiology of GCK-diabetes and HNF1A-diabetes by investigating the incretin effect, the incretin hormone responses to food ingestion and to evaluate the potential of GLP-1RA treatment in patients with HNF1A-diabetes. The hypotheses were that since patients with HNF1A-diabetes clinically resemble patients with type 2 diabetes in many ways they would also be characterised by impaired incretin effect and possibly altered postprandial hormone responses. Therefore, they may as patients with type 2 diabetes benefit from incretin-based treatment – especially the low risk of hypoglycaemia. The patients with GCK-diabetes resemble healthy individuals more than other types of diabetes, with their mild glucose intolerance and no need for treatment. Still, due to the mild diabetic state in GCK-diabetes, an impaired incretin effect and altered hormone responses could be expected.

The experimental work includes three studies. In Study I (Appendix I) we examined the incretin effect and the responses of incretin hormones and pancreatic islet hormones to OGTT and IIGI in patients with GCK-diabetes and HNF1A-diabetes, compared to healthy individuals (CTRLs). In Study II (Appendix II) we examined glucose excursions and secretion of insulin, glucagon and incretin hormones – including both total and intact hormone levels and DPP-4 enzymatic activity following a standardised test meal in patients with GCK-diabetes and HNF1A-diabetes, compared to healthy CTRLs. Finally, in Study III (Appendix III) we examined the effect of liraglutide (GLP-1RA) compared to glimepiride (SU) on fasting plasma glucose (FPG) in patients with HNF1A-diabetes. The study was a randomised, double-blind crossover trial evaluating the effect of 6 weeks' treatment with each drug, including evaluation of the frequency of hypoglycaemia, islet hormone responses and counter-regulatory hormone responses following a test meal.

Thus, ***Study I*** and ***Study II*** elucidate the pathophysiology in the two most frequent types of MODY patients providing a foundation for evaluating the treatment potential of GLP-1RA in HNF1A-diabetes in ***Study III***.

***Study I*: Incretin effect and glucagon responses to oral and intravenous glucose in patients with maturity onset diabetes of the young – type 2 and type 3**

We evaluated the incretin effect and the glucagon and incretin hormone responses during OGTT and IIGI in patients with HNF1A-diabetes and GCK-diabetes compared to a group of BMI and age matched CTRLs. Nine patients with GCK-diabetes, 10 patients with HNF1A-diabetes and 9 CTRLs were enrolled in the study. Patients were recruited via Steno Diabetes Center, where they had been diagnosed by genetic sequencing. To estimate the incretin effect (see above) the study included 2 experimental days preceded by 1 week's wash-out of blood glucose lowering drugs and an overnight fast:

1. A 4 hour 50 g-oral glucose tolerance test (OGTT)
2. A 4 hour isoglycaemic intravenous glucose infusion (IIGI)

Paracetamol was added to the OGTT to evaluate gastric emptying rates from the paracetamol plasma concentration curve[121,122].

FPG and PG responses were significantly higher in patients with GCK-diabetes and HNF1A-diabetes compared to CTRLs and mostly so in the patients with HNF1A-diabetes. The patients with HNF1A-diabetes exhibited significantly lower insulin and C-peptide responses and peak values during both OGTT and IIGI compared to CTRLs and patients with GCK-diabetes. No significant differences were demonstrated between patients with GCK-diabetes and CTRLs. The incretin effect was similar in CTRLs and patients with GCK-diabetes, whereas patients with HNF1A-diabetes exhibited a significantly reduced incretin effect. Normal insulin sensitivity according to homeostatic model assessment (HOMA-IR) and Matsuda index was found in all groups. Similar fasting values of glucagon were observed in all groups. CTRLs and patients with GCK-diabetes suppressed glucagon immediately following both stimuli. In contrast, in patients with HNF1A-diabetes, glucagon secretion initially increased during OGTT and later decreased to values that were lower than those observed during IIGI, which then resulted in immediate suppression as observed in the other groups. The levels of the incretin hormones GIP and GLP-1 increased during OGTT, but remained at fasting levels during IIGI, with no differences between the groups on either experimental day. Gastric emptying was similar in all groups.

The investigated groups of MODY patients represented different specific mutations within GCK and HNF1A genes and both exhibited glucose intolerance. However, this was only associated with a markedly reduced incretin effect among the patients with HNF1A-diabetes but not among the patients with GCK-diabetes. The causality of the impaired incretin effect in patients with diabetes is still debated.

We demonstrated an intact secretory insulin capacity in patients with GCK-diabetes, supporting the interpretation that GCK-diabetes patients solely have a glucose-sensing defect and apart from that a normal beta cell function. In spite of impaired glucose tolerance in these patients we demonstrated a normal incretin effect, which is consistent with an overall normal functioning beta cell not requiring blood glucose lowering treatment[123]. In contrast, patients with HNF1A-diabetes showed severe beta cell dysfunction with reduced insulin secretion as shown previously[123,124]. Noting the findings of normal insulin sensitivity in patients with HNF1A-diabetes which has been shown previously[125,126], it seems that insulin resistance is not involved in the reduced incretin effect in this specific group of patients. This is supported by the findings of a slightly impaired insulin sensitivity in patients with GCK-diabetes as also demonstrated previously[42,126] in combination with normal incretin effect. In contrast, our findings indicate that the reduced incretin effect is a result of the beta cell defect involving glucose metabolism and insulin secretion in combination with a reduced sensitivity of the beta cell to the incretin hormones as indicated previously[18]. It is still an open question whether the reduced sensitivity in type 2 diabetes is specific for this disease or a consequence of the general beta cell defect.

Our findings of normal incretin hormone secretion support findings in previous studies in patients with GCK-diabetes (OGTT)[19] and in patients with HNF1A-diabetes (mixed meal)[127]. Thus, the

findings of a reduced incretin effect in combination with normal responses of incretin hormones indicate impaired sensitivity to the incretin hormones in patients with HNF1A-diabetes, which could be caused by the impaired metabolism and ATP formation in the beta cell, hence impairing the effect of the incretin hormones.

Glucagon suppression in response to meal or oral glucose intake is reduced in patients with type 2 diabetes[111,128–130]. The mechanisms behind this dysfunction is debated[131,132]. The theory of an intra-islet interaction of insulin and glucagon regulating PG has been proposed to explain this. In patients with diabetes insulin secretion ceases causing lack of inhibition of glucagon, consequently leading to hyperglycaemia. However, this is not compatible with the preserved suppression during IIGI where some insulin is still secreted, and the lack of suppression during OGTT where much more is secreted, even in diabetes patients. Instead, gastrointestinal factors may be responsible[108]. In fact, previous studies from our group in patients with type 2 diabetes have shown that infusions of gut hormones on top of an IIGI, reaching similar concentrations as during OGTT, produces a similar abnormal glucagon response[108]. Normal glucagon suppression during a hyperglycaemic clamp has been shown in patients with HNF1A-diabetes[18] in agreement with our findings of normal suppression following IIGI, and the delayed suppression following OGTT is similar to what has been found in patients with type 2 diabetes. The beta cell defect and the resulting reduced insulin secretion to appropriate stimuli in patients with HNF1A-diabetes may explain this delayed glucagon suppression, but a reduced sensitivity of the beta cell to the incretin hormones could be involved as well. In contrast, patients with GCK-diabetes exhibited completely normal suppression of glucagon in response to both iv and oral glucose, supporting the interpretation that patients with GCK-diabetes resemble healthy individuals – apart from having a higher glucose threshold.

In conclusion, patients with GCK-diabetes exhibit preserved incretin physiology while patients with HNF1A-diabetes are characterised by reduced incretin effect, beta cell dysfunction and inappropriate glucagon response to OGTT and as such resemble patients with type 2 diabetes, but in contrast to these have normal insulin sensitivity. It remains possible, however, that the HNF1A mutations may also affect alpha cell function.

***Study II*: Postprandial incretin and islet hormone responses in patients with maturity onset diabetes of the young – type 2 and type 3**

We examined fasting and postprandial circulating levels of incretin hormones, glucagon, and insulin and DPP-4 enzymatic activity in patients with GCK-diabetes, in patients with HNF1A-diabetes and in matched healthy individuals. Ten patients with HNF1A-diabetes, 10 patients with GCK-diabetes and 10 CTRLs were included in the study. Patients were recruited as described above (Study I). Each participant was examined with a standardised test meal after a 10 hour overnight fast. For evaluation of gastric emptying, paracetamol was added. Blood glucose lowering drugs were paused for 1 week prior to the experimental day. Glucose intolerance (according to AUC) was demonstrated in both types of MODY although most evident in patients with HNF1A-diabetes, who also exhibited lower postprandial responses of insulin and C-peptide compared to patients with GCK-diabetes and CTRLs. Insulin resistance was similar in all groups according to HOMA-IR. No differences in fasting glucagon values were found between the groups, but pronounced postprandial hyperglucagonaemia was demonstrated in patients with HNF1A-diabetes when compared to patients with GCK-diabetes and CTRLs. Similar baseline and peak values of the incretin hormones were found in all groups. Patients with GCK-diabetes exhibited higher responses of both total and intact GIP when compared to patients with HNF1A-diabetes and CTRLs. No differences between the groups were found for total and intact GLP-1 (baseline, peak and postprandial responses). The plasma DPP-4-activity, however, was higher in patients with HNF1A-diabetes compared to CTRLs. Gastric emptying was similar in all groups.

One study examined patients with HNF1A using a test meal and a single dose of nateglinide, glibenclamide and placebo, respectively, and found impaired postprandial suppression of glucagon with both active treatments and placebo[7]. The postprandial hyperglucagonaemia found in our study correlates well with these previous findings. The HNF1A defect may as discussed above be indirectly responsible for the hyperglucagonaemia because of the defective beta cell glucose metabolism resulting in reduced insulin secretion[124]. The role of HNF1A in the glucose metabolism in the alpha cell is uncertain. Given that high glucose levels lead to inhibited glucagon secretion by stimulated ATP production, then inhibited metabolism with reduced ATP production would expectedly cause both fasting and postprandial hyperglucagonaemia. Thus, the combination of reduced release of insulin and perhaps a decreased effect of GIP and GLP-1 on both alpha and beta cells may contribute to the inappropriate glucagon response. In line with their normal intracellular metabolism, patients with GCK-diabetes showed normal suppression of glucagon and responded to the meals with increased insulin secretion.

A single study has investigated postprandial responses of the incretin hormones in patients with HNF1A-diabetes exhibiting normal postprandial GIP and GLP-1 responses[127], which is in line with our findings and with some findings in patients with type 2 diabetes[84,88,133]. Incretin hormone responses to mixed meal-stimulation have not previously been studied in patients with GCK-diabetes. However, such patients had normal responses of both GLP-1 and GIP following a 2h 75g-OGTT[19], which is in agreement with our findings of normal postprandial incretin hormone responses.

Due to the involvement of HNF1A in the expression of the DPP-4 gene[52] potentially influencing the activity of DPP-4, increased levels of intact GLP-1 and GIP might be expected in patients with HNF1A-diabetes. However, we could not demonstrate elevated ratios of intact-to-total incretin hormone levels. On the contrary, an exaggerated DPP-4-activity was found in plasma of patients with HNF1A-diabetes. The circulating intact incretin hormones do not seem to be affected by the gene defect, although further studies are required to elucidate the regulation of DPP-4-activity in HNF1A-diabetes.

In conclusion, patients with GCK-diabetes and HNF1A-diabetes resemble healthy individuals regarding postprandial secretion of GIP and GLP-1. In contrast, patients with HNF1A-diabetes are characterised by increased DPP-4-activity and marked postprandial hyperglucagonaemia.

***Study III*: Glucose lowering effects and low risk of hypoglycaemia in patients with maturity onset diabetes of the young when treated with a glucagon-like peptide-1 receptor agonist – a double-blind, randomized, crossover trial**

We investigated the effect on FPG and the risk of hypoglycaemia of 6 weeks' treatment with the GLP-1RA, liraglutide compared with the SU, glimepiride in a randomised, double-blind, crossover trial in patients with HNF1A-diabetes.

Sixteen patients were included in the trial, but one patient withdrew 4 days after randomisation during initiation of liraglutide (first treatment period) due to intolerable diarrhoea and vomiting. Patients were recruited as described above (Study I). The primary endpoint of the trial was FPG after 6 weeks of treatment. Secondary endpoints were the following: Number and severity of episodes with hypoglycaemia; Serum fructosamine; Responses of pancreatic hormones (insulin, C-peptide and glucagon) and counter-regulatory hormones (growth hormone, cortisol, epinephrine and norepinephrine) following a test meal and during a 30 minutes bicycling test.

After 1 week's wash-out of blood glucose lowering drugs, patients were randomised to receive A) once-daily injections of liraglutide plus placebo tablets, or B) once-daily injections of placebo plus glimepiride tablets for 6 weeks. After the end of the first treatment period and followed by 1 week's washout the patients received the other treatment. Patients were initiated on a 0.5 mg lower dose of their regular daily dose of glimepiride (or analogue dose of other SU), and up-titrated with 0.5 mg glimepiride/placebo daily every week in a treat-to-target fashion (mean FPG in the range of 5.0-5.9 mmol/l). Liraglutide/placebo was initiated at 0.6 mg once-daily and up-titrated with 0.6 mg daily every week to the target dose of 1.8 mg once-daily. During the trial the patients self-monitored blood glucose (SMBG) and recorded episodes of hypoglycaemia and trial medication dosages in a diary. Clinical visits took place on 3 occasions; at baseline (after 1 week's wash-out of blood glucose lowering drugs) and at the end of each treatment period. After an overnight fast the patients ingested a standardised test meal (paracetamol added to evaluate gastric emptying). Trial medication was administered 30 minutes before ingestion of the test meal (no medication at the baseline visit). After 150 minutes a light bicycling test was performed. Symptoms of hypoglycaemia were monitored, PG concentrations were measured at pre-specified time points and in case of symptoms of hypoglycaemia, and blood samples were drawn at regular intervals for 4 hours.

Both treatments resulted in significantly lower FPG compared to baseline, but FPG tended to be lower during glimepiride than during treatment with liraglutide. Glucose responses (AUC) were different from baseline, but no difference between the treatments was found. For fructosamine, HbA1c and HOMA-IR no differences were found from baseline or between the treatments. In total 19 events of hypoglycaemia were reported: 1 event during treatment with liraglutide and 18 events during treatment with glimepiride. All episodes of hypoglycaemia were classified as mild and distributed with 17 events in the blood glucose range of 3.1-3.9 mmol/land 2 events in the range of 2.0-3.0 mmol/L (both during glimepiride treatment). Ten patients (67%) experienced hypoglycaemia during glimepiride treatment and in contrast only one patient (7%) experienced 1 event of hypoglycaemia during liraglutide. Six episodes occurred during the cycling test, including the single event during liraglutide treatment. Peak values and responses of insulin and C-peptide were higher with both treatments compared to baseline with no differences between treatments. Glimepiride treatment elicited a higher ISR response than liraglutide treatment and glimepiride generally exhibited greater insulin secretory response than liraglutide, although the differences were not statistically significant. No differences between baseline and treatments or among treatments were found according to fasting- and peak glucagon values and glucagon responses including during the bicycle test. Gastric emptying was similar at baseline and during both treatments, with no differences between treatments. Preserved counter-regulatory responses were found with both treatments. Apart from the 19 events of hypoglycaemia 6 adverse events were reported: reduced appetite (2 reports), nausea (1 report), heartburn (1 report), tiredness (1 report), and vomiting and diarrhoea (1 report; withdrawal of patient). All events were considered related to trial medication. All non-hypoglycaemic events occurred during liraglutide treatment, except for the report on tiredness and one report on reduced appetite.

Because of the low prevalence of HNF1A-diabetes a crossover design was used, and the rather short treatment duration (6 weeks) was chosen to minimize inadequate compliance and patient withdrawal due to lack of glucose lowering effect or to potential side effects. The treatment periods were preceded by a 1-week washout period to reduce a potential carry-over effect.

We demonstrated reduction in FPG and postprandial glucose excursions with treatment with both SU and GLP-1RA, although to the greatest extent during glimepiride treatment. These results support the theory that glimepiride bypasses the glucose dependency of insulin secretion, whereas the effect of GLP-1 is glucose dependent. GLP-1 may still amplify the weaker signals generated in HNF1A-diabetes[61], since the peptide may enhance the sensitivity of the KATP-channels to ATP. However, GLP-1 may also have a direct effect on the KATP-channel[134,135]. Furthermore, GLP-1 may, as mentioned, have effects on beta cell secretion downstream of the KATP-channels. The postprandial ISR response was more pronounced during glimepiride treatment, which also reflects the differential effects of glimepiride and liraglutide.

We chose to use the long-acting SU, glimepiride in our trial, because it is the most commonly prescribed SU to patients with HNF1A-diabetes. We found a considerably higher risk of hypoglycaemia during glimepiride treatment compared to liraglutide treatment. However, the use of short-acting oral anti-diabetic drugs like nateglinide or repaglinide may reduce this risk.

We expected reduced glucagon responses during treatment with liraglutide due to the inhibitory effects of GLP-1 on glucagon secretion. In Study I we showed that patients with HNF1A-diabetes suppress glucagon normally following intravenous glucose, but have an inappropriate hyperglucagonaemic response to oral glucose[136] similar to patients with type 2 diabetes[111,112]. None of the treatments had any significant reduction in glucagon responses, however. This finding could indicate that the HNF1A-defect has a specific role in the alpha cell, whereas the mechanisms remain unknown.

The use of GLP-1RAs in patients with HNF1A-diabetes has only been reported in a few case stories. Beneficial effects of DPP-4

inhibitors in combination with other oral glucose lowering drugs[21,22], or liraglutide as adjunct therapy to SU and basal insulin[23] were described. Our findings are in agreement with these reports. However, it is not clear from our trial whether monotherapy with GLP-1RA is effective enough to maintain an acceptable glycaemic regulation in patients with HNF1A-diabetes. Treatment with GLP-1RAs could be considered in patients who are particularly prone to hypoglycaemia or are gaining weight. In addition, GLP-1RAs might slow down the rate of beta cell loss in HNF1A-diabetes, since inhibitory effects of GLP-1RAs on beta cell apoptosis have been demonstrated in preclinical settings[64].

In conclusion, GLP-1RAs may have a place in treatment of patients with HNF1A-diabetes, especially when hypoglycaemia is a problem.

Conclusions

In this thesis we have elucidated aspects of the pathophysiology of GCK-diabetes and HNF1A-diabetes with regards to the incretin effect, the physiological response to food ingestion. Further, the potential of GLP-1RA as a glucose lowering therapy in patients with HNF1A-diabetes was elucidated. Patients with GCK-diabetes have preserved incretin physiology regarding the incretin effect and the postprandial secretion of GIP and GLP-1 hereby resembling healthy individuals. In addition, glucagon secretion was normal in response to both oral and iv glucose stimuli as well as postprandially. In contrast, patients with HNF1A-diabetes were characterised by a reduced incretin effect, marked beta cell dysfunction and an inappropriate glucagon response to oral glucose and to meal ingestion, and as such resemble patients with type 2 diabetes. Patients with HNF1A-diabetes showed normal insulin sensitivity and exhibited normal secretion of GIP and GLP-1 following oral glucose stimulus and postprandially in spite of an increased plasma DPP-4-activity.

Investigating the treatment potential of GLP-1RAs as glucose-lowering agents in patients with HNF1A-diabetes demonstrated that 6 weeks' treatment with glimepiride or liraglutide lowered FPG and postprandial glucose excursions in these patients. The glucose lowering effect was greater with glimepiride compared to liraglutide, although insignificant, but at the expense of a considerably higher risk of hypoglycaemia (predominantly mild). Thus, GLP-1RAs may have a place in the treatment of patients with HNF1A-diabetes, especially when hypoglycaemia is a problem.

Perspectives and future research

Several important questions concerning MODY, glucagon and incretin hormones await clarification in the future:

- We find it rather surprising that patients with GCK-diabetes exhibit normal incretin effect in spite of apparent glucose intolerance, while the suggested resemblance of HNF1A-diabetes with type 2 diabetes was confirmed. In Study I the experiments were performed at different FPG levels in the three studied groups. It would be very interesting to study the incretin effect and hormone responses with a 75g OGTT and following IIGI preceded by equalizing FPG in all groups after overnight normalisation of FPG with infusion of exogenous insulin. In such a setting, the effect of elevated FPG on the incretin effect and the responses of incretin hormones and glucagon will be diminished, and the ability to improve the incretin effect can be evaluated. Besides investigating patients with GCK-diabetes and HNF1A-diabetes, patients with HNF4A-diabetes could be included as well.

- The effects of the incretin hormones in patients with MODY are still not clear. Gutniak et al[135] previously demonstrated a reinforcement of insulin secretion by the combination of glibenclamide (SU) and GLP-1 in patients with type 2 diabetes with secondary failure to SU. The combination of GLP-1 and SU may have a synergistic effect on insulin secretion. De Heer et al[61] demonstrated an uncoupling of the glucose-dependency of GLP-1 by tolbutamide (SU) showing insulinotropic effect of GLP-1 at PG of 3.0 mmol/l. In addition, clinical trials in patients with type 2 diabetes have shown increased risk of hypoglycaemia when combining GLP-1RA and SU[137,138]. A mechanistic trial investigating the insulinotropic and glucagonostatic responses of oral SU in combination with infusions of GLP-1, GIP and saline could be performed during a hyperglycaemic clamp with continuous glucose infusion. Evaluation of the effects of the incretin hormones in combination with SU would also be interesting during euglycaemic and hypoglycaemic clamps hereby estimating the glucose dependency.

- To estimate the potential risk of hypoglycaemia during combination therapy, a trial where administration of acute dosage of: repaglinide, repaglinide + GLP-1RA and repaglinide + DPP-4 inhibitor, respectively, followed by a meal test and bicycle test – similar to our experimental day in Study III and the design demonstrated by Tuomi et al[7] could be performed. This trial might be carried out before initiating any of the prospective trials mentioned below.

- A randomised, double-blind, crossover trial with a longer duration (at least 16 weeks) would be more clinical relevant than our 6-week trial. Treatment with SU was superior to GLP-1RA, but at the expense of a higher risk of hypoglycaemia (Study III). We chose the most frequently prescribed SU glimepiride for treatment of HNF1A-diabetes, but the shorter acting oral anti-diabetic drugs such as repaglinide have a lower risk of hypoglycaemia[7]. Future trials where repaglinide monotherapy (+ placebo injection) is compared to the combination of repaglinide and low dose GLP-1RA (e.g. liraglutide), and to the combination of repaglinide and medium dose GLP-1RA, investigating the risk of hypoglycaemia are warranted in patients with HNF1A-diabetes. The trials should include long washout periods (3 weeks) to minimize possible carry-over effects. After 3 weeks of dose escalation of GLP-1RA, repaglinide is administered in a treat-to-target manner with the aim of reaching a mean FPG of 5 mmol/l and no episodes of hypoglycaemia. The initial dose of repaglinide should be low, e.g., 0.5 mg with the greatest meal of the day, followed by slow up-titration. It may be a challenge to do a proper blinding in this setting, however. Our hypothesis is that a low dose of SU in combination with a low dose of GLP-1RA will allow the beta cell to exploit the smaller amounts of ATP better and thereby reinforce the insulin secretion while still retaining some glucose dependency. In this way, patients who are gaining weight or have secondary fail-

ure to SU might stay longer without insulin therapy. Patients especially prone to hypoglycaemia might benefit from GLP-1RA monotherapy. Since the majority of patients with HNF1A-diabetes are characterised by normal weight, these patients do not require the weight reducing effects of GLP1-RAs, why a low dose may be sufficient.

- A similar trial could be performed with a DPP-4 inhibitor instead of a GLP-1RA. Taking into consideration the increased DPP-4-activity found in HNF1A-diabetes in Study II, DPP-4 inhibitors may be another safe and efficacious alternative both as mono- and combination therapy, and should be preferred to expensive injection therapy if equally effective.

Summary

Maturity onset diabetes of the young (MODY) designates monogenic forms of non-autoimmune diabetes characterised by autosomal dominant inheritance, non-insulin dependent diabetes at onset and diagnosis often before 25 years of age. MODY constitutes genetically and clinically heterogeneous forms of diabetes. More than 8 different genes are known to cause MODY, among which hepatocyte nuclear factor 1α (HNF1A) (MODY3) and glucokinase (GCK) (MODY2) mutations are the most common. Both forms of MODY are characterised by specific beta cell dysfunction, with patients with HNF1A-diabetes having a reduced insulin secretory capacity, while patients with GCK-diabetes have a glucose-sensing defect, but preserved insulin secretory capacity. Patients with MODY are effectively treated with sulphonylurea (SU) due to very high sensitivity to these drugs, but they are also prone to develop hypoglycaemia.

The objectives of this thesis were to study the pathophysiology of GCK-diabetes and HNF1A-diabetes by investigating the incretin effect, the physiological response to food ingestion and to estimate the treatment potential of a glucagon-like peptide-1 receptor agonist (GLP-1RA) in patients with HNF1A-diabetes. In Study 1 we investigated the incretin effect and the responses of islet hormones and incretin hormones to oral glucose tolerance test (OGTT) and isoglycaemic iv glucose infusion (IIGI) in patients with GCK-diabetes, in patients with HNF1A-diabetes, and in BMI and age matched healthy individuals (CTRLs). In Study II we investigated responses of islet hormones and incretin hormones to a more physiological stimulus consisting of a standardised meal test in patients with GCK-diabetes, in patients with HNF1A-diabetes, and in BMI and age matched CTRLs. In Study III we conducted a randomised, double-blind, crossover trial investigating the glucose lowering effect and risk of hypoglycaemia during 6 weeks of treatment with the GLP-1RA, liraglutide compared to the SU, glimepiride in 16 patients with HNF1A-diabetes. At baseline and at the end of each treatment period a standardised meal test followed by a light bicycling test was performed.

The results of the studies showed that patients with HNF1A-diabetes were less glucose tolerant than patients with GCK-diabetes, but both groups were more glucose intolerant than CTRLs. In spite of glucose intolerance patients with GCK-diabetes showed normal incretin effect, whereas patients with HNF1A-diabetes showed an impaired incretin effect. Patients with HNF1A-diabetes were also characterised by an inappropriate glucagon response to OGTT and test meal compared to patients with GCK-diabetes and CTRLs. Both groups of diabetes patients showed normal suppression of glucagon in response to intravenous glucose infusion, and exhibited normal responses of incretin hormones to OGTT, IIGI and test meal. Furthermore patients with HNF1A-diabetes showed an increased dipeptidyl peptidase-4 (DPP-4) activity, and a severe beta cell dysfunction with reduced insulin responses. Normal insulin sensitivity was found in both groups of diabetes patients. In the prospective intervention trial a glucose lowering effect on fasting plasma glucose (FPG) was demonstrated with both treatments without significant difference between the treatments. The postprandial plasma glucose responses were also lower with both glimepiride and liraglutide compared to baseline without significant difference between treatments. In spite of these findings glimepiride seems to have superior glucose lowering effects according to both FPG and postprandial glucose responses. Hypoglycaemic events (plasma glucose ≤ 3.9 mM) occurred 18 times during glimepiride treatment and once during liraglutide treatment. No differences between treatments were demonstrated according to insulin and glucagon responses and gastric emptying, and counter-regulatory responses were preserved during both treatments. No effect of either treatment was seen on fructosamine or HbA1c.

In conclusion, patients with GCK-diabetes show normal incretin and glucagon physiology, thus resembling healthy individuals, in spite of fasting hyperglycaemia and subtle glucose intolerance. In contrast, patients with HNF1A-diabetes exhibited noticeable glucose intolerance, beta cell dysfunction, impaired incretin effect, and inappropriate glucagon response to oral stimuli, hence resembling patients with type 2 diabetes. However, normal responses of incretin hormones and normal insulin sensitivity were found in patients with HNF1A-diabetes. Six weeks of treatment with glimepiride or liraglutide demonstrated glucose lowering effects. This effect was greater with glimepiride, although insignificant, but at the expense of a higher risk of hypoglycaemia (predominantly mild). GLP-1RAs may have a place in treatment of patients with HNF1A-diabetes, especially when hypoglycaemia is a problem. Future studies are required to clarify this.

List of abbreviations

ATP	Adenosine triphosphate
AUC	Area under the curve
BMI	Body mass index
cAMP	Cyclic adenosine monophosphate
CTRL	Healthy control subject/healthy individual
FPG	Fasting plasma glucose
GCK	Glucokinase
DPP-4	Dipeptidyl-peptidase 4
GDM	Gestational diabetes mellitus
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide-1
GLP-2	Glucagon-like peptide-2
GLP-1RA	GLP-1 receptor agonists
GLUT4	The glucose transporter in muscle and fat tissue
HbA_{1c}	Glycated haemoglobin A1c
HNF1A	Hepatocyte nuclear factor 1α
HOMA-IR	Insulin resistance by homeostatic model assessment
IGT	Impaired glucose tolerance

IFG	Impaired fasting glucose
IIGI	Isoglycemic intravenous glucose infusion
ISR	Insulin secretion rate
iv	Intravenous
K_{ATP}-channel	ATP-sensitive potassium (K^+) channel
LADA	Latent autoimmune diabetes of adults
MODY	Maturity onset diabetes of the young
MODY1	Hepatocyte nuclear factor 4α (HNF4A)-diabetes
MODY2	Glucokinase-diabetes
MODY3	Hepatocyte nuclear factor 1α (HNF1A)-diabetes
NGT	Normal glucose tolerance
OGTT	Oral glucose tolerance test
PG	Plasma glucose
SGLT2	The sodium-glucose transporter-2
SMBG	Self-monitored blood glucose
SU	Sulphonylurea

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