Incretin hormones and beta cell function in chronic pancreatitis

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1. Knop FK, Vilsbøll T, Larsen S, Højberg PV, Vølund A, Madsbad S, Holst JJ, Krarup T: Increased postprandial responses of GLP-1 and GIP in patients with chronic pancreatitis and steatorrhea following pancreatic enzyme substitution. Am J Physiol Endocrinol Metab 2007;292(1):E324-30.

2. Knop FK, Vilsbøll T, Højberg PV, Larsen S, Madsbad S, Vølund A, Holst JJ, Krarup T: Reduced incretin effect in type 2 diabetes - cause or consequence of the diabetic state? Diabetes 2007;56:1951-9.

3. Knop FK, Vilsbøll T, Højberg PV, Larsen S, Madsbad S, Holst JJ, Krarup T: The insulinotropic effect of GIP is impaired in patients with chronic pancreatitis and secondary diabetes mellitus as compared to normal glucose tolerant patients with chronic pancreatitis. Submitted for publication in Regulatory Peptides 2007;144:123-30.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is the result of genetic disposition combined with sedentary life-style and obesity[1]. It is now well established that T2DM is characterised not only by insulin resistance, but also by beta cell dysfunction[2;3]. Furthermore, the pathophysiology of T2DM has been shown to be characterised by a severely reduced incretin effect[4]. The incretin effect refers to the phenomenon of oral glucose eliciting a higher insulin response than intravenous (iv) glucose at identical plasma glucose profiles (isoglycaemia). The incretin effect is conveyed by the two incretin hormones: glucagon-like peptide-1 (GLP-1), and glucose-dependent insulinotropic polypeptide (GIP)[5]. Both hormones are secreted from small intestinal mucosal endocrine cells in response to ingestion of nutrients[5;6], and are highly insulinotropic in a strictly glucose-dependent fashion[5]. The reduced incretin effect in T2DM has been shown to be characterised by a reduced postprandial GLP-1 response[7;8] as well as a reduced insulinotropic potency of GLP-1[9]. In addition, and of crucial importance, the insulinotropic effect of GIP has been shown to be almost absent in patients with T2DM[10]. It is unknown whether the incretin deficiencies in T2DM are primary events leading to T2DM or consequences of the diabetic state.

To elucidate this, we have studied the incretin hormones and their impact on beta cell function in patients with chronic pancreatitis (CP). CP is a chronic progressive inflammatory condition that, over time, results in exocrine and endocrine pancreatic insufficiencies. Eventually, patients with CP develop diabetes mellitus (DM) secondary to their inflammatory condition[11-14]. This enables us to describe the different pathophysiological traits of DM with regard to cause or consequence of the diabetic state; if patients with CP and secondary DM exhibit the characteristic incretin deficiencies of T2DM and patients with CP and normal glucose tolerance are normal in that regard, it is more likely that these deficiencies are consequences of the diabetic state rather than primary events leading to T2DM. On the other hand, if the incretin physiology is preserved independently of the diabetic state of patients with CP, the deficiencies in T2DM could represent primary pathogenetic defects, as suggested in a study of first-degree relatives of patients with T2DM[15].

TYPE 2 DIABETES MELLITUS

T2DM comprises 90 % of people with DM around the world. The World Health Organization (WHO) estimates that more than 180 million people worldwide have DM, and as the western lifestyle is making its entry into the developing countries, this number is likely to more than double by 2030[16]. Over time, DM results in micro- and macrovascular complications damaging the heart, blood vessels, eyes, kidneys, and nerves. In 2000, approximately 2.9 million deaths worldwide were attributable to DM and its complications[17].

It is well established that beta cell dysfunction and insulin resistance are two central defects in the pathophysiology of T2DM[2;3]. Furthermore, it has been demonstrated that T2DM is a progressive disease, due to an almost linear decline in beta cell function over time[18]. Thus, it seems that T2DM evolves as the

beta cells lose the ability to respond adequately to the insulin need[19]. However, the primacy of beta cell dysfunction as compared to insulin resistance is controversial[20]. Both genetic and environmental factors, such as lack of physical exercise and hypercaloric nutrition, play major roles in the pathophysiological process, although the precise mechanisms for T2DM development remain largely unknown. Interestingly, *Sladek et al.* recently confirmed the known association between T2DM and the gene variant of the transcription factor *TCF7L2* and, on top of this, identified four loci containing variants that confer T2DM risk, including a polymorphism in the zinc transporter *SLC30A8,* which is expressed exclusively in pancreatic beta cells[21]. Thus, the genetic background of T2DM represents a complex scenario with interacting effects of multiple genetic variations. Despite the genetic complexity and its interaction with environmental factors, the weight of current evidence supports the view that insulin resistance and beta cell dysfunction play major roles in the pathogenesis of T2DM. Furthermore, evidence for inappropriate secretion of glucagon being a hallmark of T2DM is accumulating[22]; fasting and postprandial hyperglucagonaemia in T2DM have been shown to result in increased glucagon-induced hepatic glucose production, which again contributes to fasting hyperglycaemia and exaggerated postprandial glucose excursions[23-26]. Lastly, a severely reduced incretin effect has been demonstrated to characterise T2DM[4]. Our group has been investigating the incretin defect in T2DM extensively over the last 15 years. In the studies in this thesis, we expand our investigations to investigate patients with CP with and without secondary DM, hoping to provide clarification of the incretin defect and its role in the pathogenesis of T2DM.

CHRONIC PANCREATITIS

CP is a chronic inflammatory condition in the pancreas, which results in a progressive destruction of the pancreatic cells (leaving necrosis and fibrosis combined with pancreatic calcifications behind), and secondly, development of exocrine (malassimilation) and endocrine pancreatic insufficiency (secondary DM)[14]. Malassimilation results in steatorrhea and weight loss and is treated with pancreatic enzyme supplementation (PES). Secondary DM is treated with diet, sulphonylurea or insulin, depending on the degree of hyperglycaemia. In Europe, the onset of CP occurs in the 3rd or 4th decade, the major etiological factor being excessive alcohol consumption (~80%). In Denmark, the prevalence of CP has been found to be 0.028%, and the incidence rate between 8 and 9 per 100,000 persons per year[27;28].

In a large Danish cohort, one-third of the patients with CP had normal glucose tolerance, one-third had impaired glucose tolerance or DM not requiring insulin, and one-third had insulin-dependent DM[13], in accordance with other published findings[29-31]. No good estimates for the time from diagnosis of CP to the onset of secondary DM have been published (a cumulative rate of DM has been reported to be 83% 25 years after the clinical onset of CP[32]); but beta cell function and glucose tolerance decline along with increasing disease duration[11-13]. Whether genetic components contribute to secondary DM in patients with CP is unknown, but several of the known pathophysiological features of T2DM can be demonstrated in patients with DM secondary to CP[11-13]. The response of insulin to various secretogogues (such as oral and iv glucose, iv tolbutamide, iv glucagon, iv arginine and iv secretin) has consistently been demonstrated to be blunted in DM secondary to CP[33-36]. Studies investigating insulin sensitivity in patients with CP and secondary DM by means of the hyperinsulinaemic euglycaemic clamp technique have yielded contradicting results[37;38]. *Nosadini et al*. showed increased insulin sensitivity and clearance rate in comparison with type 1 diabetes mellitus[37], whereas *Yki-Järvinen et al*. demonstrated marked insulin resistance in patients with CP as compared to patients with type 1 diabetes mellitus and to healthy control subjects[38]. No obvious explanation for these divergent results exists, but considerable interindividual variation of factors affecting insulin sensitivity, as observed in normal man[39-41], may contribute, underlining the need for further investigations of insulin sensitivity in patients with CP.

Basal pancreatic glucagon concentrations have been found to be reduced[31], normal[42-44], or elevated[45] in patients with CP and secondary glucose intolerance. Likewise, results regarding glucagon responses to oral glucose, meal ingestion, iv arginine or iv alanine are conflicting[43;45-48]. The variable data on glucagon levels in CP probably reflect: 1) the mutual intra-islet relationship between insulin secretion (which varies a great deal in patients with CP) and glucagon secretion; and 2) varying specificity of previously employed glucagon assays, particularly with respect to glucagon-containing proglucagon products from the gut. Studies on glucagon responses following iv administration of glucose in patients with DM are scarce[49]; and no consistent data are available from patients with CP (with or without secondary DM).

Long-term complications of DM secondary to CP are very similar to those found in other forms of DM, including progressive capillary basement membrane thickening[50], retinopathy[51], nephropathy, and neuropathy[51;52] - all signs of diabetic microvascular disease.

Today, in the County of Copenhagen, Denmark, DM not requiring insulin secondary to CP is treated with diet alone or with diet in combination with oral sulphonylurea therapy. Sulphonylureas increase insulin secretion by closing the adenosine 5'-triphosphate (ATP) sensitive K^+ channels in the membrane of the pancreatic beta cell, causing membrane depolarization. The subsequent activation of calcium channels and increase in intracellular calcium levels lead to insulin exocytosis[53]. In CP patients with HbA_1c values above 6.5% on diet and maximal sulphonylurea dose, the treatment regimen is changed to insulin. Since most patients with CP are lean and potentially insulin sensitive, biguanides are generally not used in the County of Copenhagen. However, the uncertainty of preserved or reduced insulin sensitivity in patients with CP[37;38] in combination with recent observations indicating that lean (insulin sensitive) patients with T2DM may benefit from biguanides[54] make this practice open to debate.

The progressive nature of secondary DM in CP and the dissimilarity from T2DM with regard to its etiology, provide a model to distinguish between pathophysiological traits in T2DM likely to be affected by, or even resulting from, the diabetic state per se, and pathophysiological traits that appear solely in T2DM, which, therefore, must be considered to be independent of the diabetic (hyperglycaemic) state. Therefore, we decided to investigate the incretin effect and its underlying mechanisms in patients with CP and secondary DM and in patients with CP and normal glucose tolerance.

THE INCRETIN EFFECT

As mentioned, the incretin effect refers to the phenomenon of oral ingestion of glucose eliciting a significantly higher insulin response than isoglycaemic iv glucose. The scientific history of the incretin effect extends back more than 100 years, and the scientific interest surrounding it has only intensified over time. In 1906, extracts of mucosa from porcine small intestine were used by *Moore et al*. as a treatment for DM, hoping that "the pancreas secretion might be stimulated by the substance of the nature of a hormone yielded by the duodenal mucosa membrane"[55]. In 1928, *Zunz and LaBarre* described a hypoglycaemic effect following injection of "secretin" extracted from small intestinal mucosa and, using cross-circulation experiments, they were able to show that the effect was mediated through the pancreas[56]. Four years later, in 1932, *LaBarre* named the unidentified substance thought to exert this effect "incretin"[57]. In 1964, *McIntyre et al*. and *Elrick et al*. demonstrated that orally administered glucose evokes a greater insulin response than does intravenously administered glucose, and both groups hypothesized that gut-derived factors could have potentiating effects on insulin secretion after oral ingestion of glucose[58;59]. A few years later, in 1967, this finding was confirmed by *Perley and Kipnis*, who administered oral glucose; and, on a separate day, copied the oral glucose curve with an isoglycaemic iv glucose infusion in obese and normal weight patients with DM and in healthy control subjects[60]. They concluded that the insulin response to isoglycaemic iv glucose administration only amounted to 30-40% of that seen after oral glucose.

Today, the isoglycaemic method used by *Perley and Kipnis* is widely accepted as the method of choice to measure the incretin effect (Figure 1).

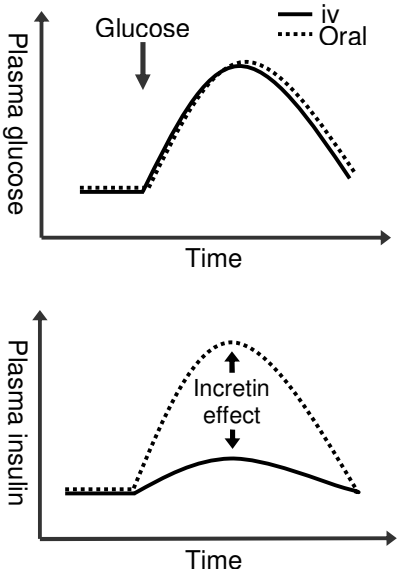

Figure 1. Upper panel: Glucose concentrations following administration of oral glucose and isoglycaemic intravenous (iv) glucose infusion, respectively. Lower panel: The corresponding insulin responses in a healthy subject.

The incretin effect is defined as the beta cell secretory response evoked by factors other than glucose itself, and is represented by the difference in integrated incremental responses (over basal) of plasma insulin or plasma C-peptide, between oral glucose ingestion and isoglycaemic iv glucose infusion. The difference in integrated beta cell secretory responses (∫SR) is usually expressed as the percentage of the response to oral glucose using the following formula[4;61]:

$$\text{Incretin effect (\%)} = 100\% \times \frac{\int SR_{oral} - \int SR_{iv}}{\int SR_{oral}}$$

The result of this formula answers the question: "What percentage of the insulin response following oral ingestion of glucose can be ascribed to the incretin effect?" When using ∫SR based on C-peptide concentrations, differences in hepatic insulin extraction during the two circumstances are eliminated[62] (unlike insulin, C-peptide is not extracted by the liver and has a constant peripheral clearance under various physiological circumstances) and - some would say - a more accurate estimate is obtained. In order to obtain an even more accurate estimate of the incretin effect, we have calculated the actual prehepatic insulin secretion rate (ISR) for each time point during the two administration forms[63]. ISR is estimated from plasma levels of C-peptide using a two-compartment mathematical model to account for C-peptide distribution and degradation[64] - mathematical deconvolution[65]. In this procedure, application of population-based parameters for C-peptide kinetics was used as described previously[66-68], thus circumventing the need to obtain a decay curve in each subject. This method of calculation takes into account glucose tolerance, body surface area, sex and age, and results in mean ISRs that differ by an average of 10-12% from those obtained with individual derived parameters[68]. ISR is expressed as pmol insulin secreted per minute per kilogram body weight, and the integrated ISR response represents the total amount of insulin secreted per kilogram body weight in an individual for a given time interval.

In 1976, *Jensen et al*. quantified the incretin effect in pigs before and after heterotopic pancreatico-duodenal allotransplantation and total pancreatectomy[69]. In these particular studies, the incretin effect was quantified by comparing insulin responses (incremental area under curve (AUC)) to oral (50 g) and isoglycaemic iv glucose administration. The following formula was used to calculate the incretin effect:

$$\text{Incretin effect} = \frac{\text{Incremental } AUC_{oral} - \text{Incremental } AUC_{iv}}{\text{Incremental } AUC_{iv}}$$

No difference before and after surgery was observed (and it was concluded that the incretin effect did not involve innervation of the pancreas - a finding that has been confirmed in the human species by *Nauck et al*.[70]), but the result of this method of calculation satisfactorily answered the question "how much is the glucose-induced insulin secretion amplified by the incretin effect?" This formula was later used in several studies investigating the incretin effect in humans[71-75].

THE INCRETIN HORMONES

In 1970, gastric inhibitory polypeptide, secreted from small intestinal endocrine K cells in response to ingestion of nutrients, was discovered[76] and eventually, the 42-amino acid polypeptide was shown to be insulinotropic at elevated glucose concentrations - a true incretin hormone - and renamed glucose-dependent insulinotropic polypeptide (GIP)[77-79]. Later, experimental and clinical studies suggested that the gut produces more than a single insulinotropic hormone[80;81]. In 1983, the gene encoding the human pancreatic hormone, glucagon, was cloned, and the structure of its precursor, proglucagon, was surprisingly shown to include the sequence of two glucagon-like peptides in addition to glucagon itself[82]. As expected, the gene was found to be expressed in both pancreatic alpha cells and mucosal endocrine L cells in the small intestine[83]. The primary transcripts and translation products of the gene in the two types of cells are identical[84]; but, as illustrated in Figure 2, the post-translational processing was shown to differ in the two tissues[83;85;86]: In the pancreas, proglucagon is cleaved by prohormone convertase 2 to glucagon, glicentin-related pancreatic peptide (GRPP) and a major proglucagon fragment[85-87].

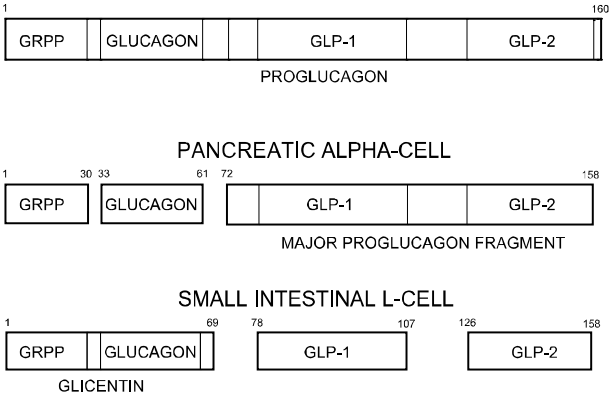

Figure 2. Proglucagon processing in human pancreatic alpha cells and in mucosal endocrine L cells in the small intestine.

Apart from glucagon, these fragments seem to be biologically inactive[88]. In contrast, in the intestinal L cells, proglucagon is processed by prohormone convertase 1 to GLP-1, glucagon-like peptide-2 (GLP-2)[89] and glicentin[90]. The 30-amino acid peptide, GLP-1, was found to be secreted in response to ingestion of nutrients and to be strongly insulinotropic[91;92] - a true incretin hormone - and GLP-2, also secreted in response to ingestion of nutrients, to be a key regulator of small intestinal growth[93]. The bioactive forms of GLP-1, amidated and glycine extended GLP-1, are designated GLP-1 7-36 amide and GLP-1 7-37.

Many hormones have been suspected to be responsible for the incretin effect[94]; but today there is ample evidence to suggest that the incretin effect mainly is conveyed by the two incretin hormones: GIP and GLP-1[5].

SECRETION AND DEGRADATION OF THE INCRETIN HORMONES

Both hormones are secreted almost immediately in response to ingestion of nutrients, with lipids and simple carbohydrates being potent stimulators of secretion[5;6]. Peak concentrations of GIP and GLP-1 are reached as soon as 15-30 and 30-45 minutes, respectively, after ingestion of e.g. glucose[95]. The rapid secretion following ingestion of nutrients - long before the substrates ingested are present in the small intestine - has led to the notion of vagus-mediated stimulation of secretion[96;97]. However, identification of glucokinase expression in the K cells[98] and glucose-stimulated GLP-1 secretion and firing of action potentials, via mechanisms involving closure of ATP sensitive K^+ channels, in GLUTag cells (an L cell model)[99] provide evidence for a direct relationship between absorption of nutrients and secretion of GIP and GLP-1. Furthermore, secretion of GLP-1 after uptake of the nonmetabolizable monosaccharide methyl-α-glucopyranoside through sodium-glucose co-transporters in GLUTag cells has been observed[100]. The direct relationship between absorption of nutrients and secretion of GLP-1 is further supported by the observation of intact GLP-1 responses following ileal instillation of carbohydrates and lipids[101]. In addition, a recent study performed on anaesthetized pigs showed no effect of electrical stimulation of the vagal trunks at the level of the diaphragm[102]. As mentioned, GLP-1 is secreted from the L cells, which are most numerous in the distal part of the small intestine, whereas GIP is released from the K cells, situated primarily in the duodenum and proximal jejunum[103;104]. Recent observations, however, indicate that GIP and GLP-1 are co-localized in a subset of endocrine cells throughout the gastrointestinal tract[105-107]. This finding may explain the fast secretory responses following ingestion of nutrients, but other mechanisms - for instance, paracrine interaction between the two incretin hormones as indicated by data in dogs[108], and intrinsic neuroendocrine mechanisms[6] - may be involved.

After the secretion of GIP and GLP-1, both hormones are degraded by the enzyme dipeptidyl peptidase 4 (DPP 4)[109-112]. This enzyme, in addition to its localization at sites such as the intestinal and renal brush border membranes, is also found on capillary surfaces and in a soluble form in plasma[113]. It cleaves off the two N-terminal amino acids of peptides with a penultimate proline or alanine residue, and for the incretin hormones, this abolishes their insulinotropic activity[109-112]. While GLP-1 is rapidly degraded in the circulation, resulting in a clearance which exceeds cardiac output and an apparent half-life of 1-1.5 minute[109;114], GIP is degraded more slowly, with a half-life for the intact hormone of 7 minutes[110;115]. The truncated metabolites are eliminated more slowly through the kidneys, with half-lives of 4-5 and 17 minutes, respectively[109;110;114;115].

INSULINOTROPIC EFFECT OF THE INCRETIN HORMONES

Specific receptors for GIP and GLP-1 are found in the pancreatic beta cell plasma membrane. Both receptors belong to the glucagon subfamily of G protein-coupled receptors. Following binding and subsequent activation of adenylate cyclase, intracellular accumulation of cyclic adenosine mono-phosphate, closure of ATP-sensitive K^+ channels and elevation of cytosolic calcium concentrations, mobilization and exocytosis of insulin-containing granules occur[116;117].

In studies in humans, where GIP and GLP-1 were infused together with iv glucose to concentrations approximately corresponding to

those observed during oral glucose tolerance tests, both hormones powerfully enhanced insulin secretion[118;119]. In other experiments involving clamping of blood glucose at fasting and postprandial levels and exact copying of meal-induced concentrations of GIP and GLP-1, the resulting insulin responses indicated that the hormones are active with respect to enhancing insulin secretion from the beginning of a meal, and that they contribute almost equally to postprandial insulin responses, but with the effect of GLP-1 predominating at higher glucose levels[120]. The effects of the two hormones with respect to insulin secretion have been shown to be additive in humans[121]. From studies in mice with targeted lesions of both GIP and GLP-1 receptors, it was concluded that the hormones are essential for a normal glucose tolerance and that the effect of deletion of one receptor was "additive" to the effect of deleting the other[122]. Thus, there is little doubt that the incretin effect plays an important role in postprandial insulin secretion and, therefore, glucose tolerance in humans and animals.

OTHER EFFECTS OF THE INCRETIN HORMONES

In addition to the glucose-dependent insulinotropic effect of the incretin hormones (the incretin effect), GLP-1 has been shown to enhance all steps of insulin biosynthesis as well as insulin gene transcription[123]. Activation of the transcription factor PDX-1, a key regulator of islet growth and insulin gene transcription may be involved[124]. In addition, GLP-1 up-regulates the genes for the cellular machinery involved in insulin secretion, such as the glucokinase and GLUT-2 genes[125]. Importantly, GLP-1 also has trophic effects on beta cells[126]. It stimulates beta cell proliferation[127;128]; and enhances the differentiation of new beta cells from progenitor cells in the pancreatic duct epithelium[129]. Most recently, GLP-1 has been shown to be capable of inhibiting apoptosis of beta cells, including human beta cells[130]. Furthermore, GLP-1 inhibits glucagon secretion and hepatic glucose production[131], and decreases gastrointestinal motility, thereby curtailing postprandial glucose excursions[132], and promotes satiety[133], probably via activation of GLP-1 receptors in the brain in combination with decreased gastrointestinal motility. GLP-1 receptors are also found in the heart; and, recently, a physiological role for these was shown in mice lacking the GLP-1 receptor[134]. These mice exhibit impaired left ventricular contractility and diastolic functions, as well as impaired responses to exogenous epinephrine. Recent studies indicate that GLP-1 protects the ischaemic and reperfused myocardium in rats[135], improves the ejection fraction in patients treated with angioplasty after acute myocardial infarction[136], and improves left ventricular function and systemic haemodynamics in dogs with induced dilated cardiomyopathy[137]. In addition, GLP-1 has been found to reduce the postprandial rise in triglycerides and lower the concentration of free fatty acids in healthy subjects[138], and improve endothelial dysfunction in patients with T2DM and coronary heart disease[139]. Finally, GLP-1 has been associated with improved learning in rats and has also displayed neuroprotective effects[140;141].

Regarding the other incretin hormone, GIP, a number of studies provide evidence for a role of the hormone in lipid metabolism: Lipids are strong stimulators of GIP secretion; 24-h GIP profiles parallel plasma concentrations of triglycerides[142]; and functional GIP receptors are found on adipocytes[143]. Furthermore, administration of GIP has been reported to increase chylomicron clearance in dogs[144], lower postprandial triglyceride levels in rats[145], increase glucose transport in rat adipocytes[146], increase fatty acid synthesis in adipocytes[147-149], and to increase lipoprotein lipase activity in rat adipose tissue explants[150]. Interestingly, mice with a deletion of the GIP receptor gene become slightly glucose intolerant[151]; and, unlike wild type controls, they do *not* become obese when given a high fat diet[152]. Like GLP-1, GIP has been shown to play a role in the maintenance of beta cell mass by stimulating cellular proliferation and decreasing apoptotic activity in beta cell lines[153]. Whereas GLP-1 inhibits glucagon secretion[131], GIP has been shown to stimulate pancreatic glucagon secretion[154;155]. Lastly, the discrepancy between plasma insulin and C-peptide responses to oral glucose, as compared to an iv glucose load[156;157], and the higher increment of insulin concentrations, as compared to the rise of C-peptide levels during infusion of GIP[158], have been attributed to an effect of GIP on insulin extraction. However, this notion is questionable, since a mere change in the ratio of insulin and C-peptide concentrations in the peripheral blood may be explained by their different plasma half-lives[159].

INCRETIN HORMONES AND TYPE 2 DIABETES MELLITUS

In 1986, *Nauck et al*. showed that the incretin effect was severely reduced in patients with T2DM[4]. Subsequent investigations have yielded a more detailed description of the reduced incretin effect in patients with T2DM[160]. *Vilsbøll et al*. and *Toft-Nielsen et al.* found that the postprandial (mixed meal) secretion of GLP-1 was significantly reduced in these patients[7;8], while the postprandial secretion of GIP was found to be intact[8]. With regard to the insulinotropic effects of the two hormones in patients with T2DM, *Krarup et al.* reported a negligible beta cell response to GIP[161], and *Vilsbøll et al*. showed that while GLP-1 may almost normalize glucose-induced insulin secretion (although its insulinotropic potency is reduced[9]), the insulinotropic effect of GIP has virtually disappeared[10].

In addition to the reduced insulinotropic effect of the incretin hormones, a recent study from our group suggests that an alpha cell defect may contribute to the reduced incretin effect in patients with T2DM[95]. In another study we showed that the loss of incretin effect also applies to obese subjects with T2DM (those studied by *Nauck et al*. were relatively lean); and we observed that the amount of iv glucose required to copy the oral glucose response was similar to the oral dose, another indication that in these patients, the route of administration did not result in different handling of the glucose (*Knop et al. unpublished*). We found that the impact of gastrointestinal (GI) factors on glucose disposal following oral ingestion of glucose as compared to isoglycaemic iv glucose infusion can be described conveniently using the following formula:

$$\text{GI-mediated glucose disposal (\%)} = 100\% \times \frac{glucose_{oral}\,(g) - glucose_{iv}\,(g)}{glucose_{oral}\,(g)}$$

This method of calculation answers the question "What percentage of glucose tolerance is caused by the oral route of glucose administration as compared to the iv route?" Thus, it describes the impact of the incretin effect on glucose disposal and includes not only insulinotropic substances released upon intestinal stimulation but takes into account all factors affecting glucose disposal during the two administration forms (e.g. differences in glucagon

secretion). This is of interest, since we[95] and others{Meier, 2007 906 /id}, as mentioned, recently reported a paradoxical difference in glucagon suppression during oral and isoglycaemic iv glucose infusion. In healthy control subjects, glucagon concentrations were equally suppressed during the two administration forms, whereas patients with T2DM exhibited a delayed (by 45 minutes) and reduced suppression during oral ingestion of glucose. Suppression during isoglycaemic iv glucose infusion (as compared to matched healthy control subjects), however, was completely normal. GI-mediated glucose disposal, calculated as indicated above, was almost completely lost in obese patients with T2DM (reduced from 50% in healthy subjects to 5% in patients with T2DM), whereas a comparison of insulin responses indicated that the incretin effect was reduced from approximately 70% in healthy subjects to approximately 35% in patients with T2DM. This difference might be explained by the abnormal glucagon suppression during oral ingestion of glucose[95]. Thus, the dampened glucagon suppression during oral glucose would be expected to result in inadequate suppression of hepatic glucose production, pulling the plasma glucose curve upwards. This, in turn, means that an increased amount of glucose is required to obtain isoglycaemic glucose excursions during the iv infusion, eventually approaching the amount of glucose given orally.

New data from our group suggest that in patients with T2DM undergoing super-regulation of their blood sugar using a rigorous insulin therapy regimen the insulinotropic potencies of GLP-1[162] and GIP are partly restored (*Højberg et al. unpublished - personal communication*). This suggests that the impaired insulinotropic effects of the hormones are consequences of a hyperglycaemic (diabetic) state. On the other hand, *Meier et al.* demonstrated a reduced insulinotropic effect of GIP in first-degree relatives of patients with T2DM, suggesting this deficiency to be a primary pathogenetic factor in the development of T2DM[15]. Despite this, the same group observed a preserved incretin effect in first-degree relatives of patients with T2DM[163] and, recently, *Meier at al.* reported a normal insulin secretory response to GIP in women with a history of gestational diabetes and therefore at high risk of developing T2DM[164]. *Muscelli et* al. recently showed that the incretin effect is affected in subjects with impaired glucose tolerance and, therefore, at high risk for developing T2DM[165]. This observation could imply a primary role for the reduced incretin effect in T2DM, but, on the other hand, the finding could also be interpreted as the incretin defect being an early consequence of a blunted glucose homeostasis. Thus, it remains to be established whether the severely reduced incretin effect in patients with T2DM is a consequence of the diabetic state or a primary event leading to the disease. Likewise, the lost insulinotropic effect of GIP remains to be fully characterised with regard to being cause or consequence of the diabetic state.

INCRETIN HORMONES AND CHRONIC PANCREATITIS

Few data on the secretion and effect of the incretin hormones in CP are available. In one study, the effects of GLP-1 administered subcutaneously in patients with CP and secondary DM not requiring exogenous insulin (serving as a model for DM with preserved insulin sensitivity according to the homeostatic model assessment (HOMA)) were assessed. These patients resisted hypoglycaemia after administration of 1.5 nmol GLP-1/kg body weight (maximally tolerated dose) given simultaneously with an iv glucose bolus[166]. In healthy subjects, this procedure causes overt reactive hypoglycaemia[167]. GLP-1 elicited insulin responses in patients with CP and secondary DM similar to those seen in lean patients with T2DM, and the glucagon-inhibiting effect of GLP-1 observed in patients with T2DM was reproduced in the patients with CP and DM. *Hiroyoshi et al*. studied the secretion of GLP-1 following oral glucose ingestion in patients with CP, and found that plasma GLP-1 responses were significantly elevated in patients with secondary DM as compared to patients with CP and normal or impaired glucose tolerance[168]. However, the assay employed by *Hiroyoshi et al*. is suboptimal due to the lack of specificity in regard to N- and C-terminally extended forms of GLP-1, and, as a result hereof, yields artificially high basal and stimulated concentrations. Postprandial secretion of GLP-1 has never been investigated in patients with CP. In GLP-1 infusion studies, in patients with CP and secondary DM, a glucose-lowering effect of GLP-1 was observed, which was accompanied by an increase in plasma C-peptide concentrations and a decrease in plasma glucagon concentrations[169].

There is more information regarding GIP in patients with CP, but the results are often conflicting. The secretion of GIP following ingestion of nutrients has been reported to be increased[170;171], normal[172;173] and decreased[174;175]. Furthermore, conclusions regarding the association between the secretion of GIP and the degree of glucose intolerance in these patients have been contradictory[171;173;174]. In the study performed by *Ebert et al*.[171], comparing three subgroups of patients with CP selected by the degree of pancreatic endocrine insufficiency, there was no difference in postprandial secretion of GIP between patients with near-normal and patients with reduced postprandial insulin responses. Significantly elevated GIP responses were, however, observed in the moderately impaired insulin response group as compared to the two other groups. This led to the suggestion that elevated secretion of GIP could be due to lack of negative feedback inhibition by insulin in the patients with moderately impaired insulin responses. The observation that patients with reduced insulin responses did not exhibit elevated GIP responses was explained by the fact that these patients also had the greatest degree of steatorrhea and, therefore, a reduced stimulus for GIP release. In a later study, *Ebert et al*. tested the hypothesis that GIP release is dependent upon the rate of assimilation of nutrients, and found that PES could increase the postprandial response of GIP in patients with CP and pancreatic exocrine insufficiency[176]. The increased postprandial GIP response following PES was accompanied by a significantly greater insulin response and a significant increase in postprandial glucose tolerance. The relationship between assimilation of nutrients and secretion of GLP-1 has never been investigated.

The insulinotropic effect of GIP has been studied in DM with different aetiologies including a small group of patients with CP and DM[177]. In that study, the GIP effect was poor in all groups; however, no group of glucose tolerant patients with CP was included for comparison.

HYPOTHESIS

In order to characterize the type 2 diabetic incretin deficiency with respect to it being a cause or a consequence of the diabetic state, we studied patients with CP. As aforementioned, patients with CP eventually develop DM secondary to the degenerative changes in the pancreas[11-14]. If patients with CP and secondary DM exhibit the same incretin-related pathophysiological charac-

teristics as patients with T2DM, and patients with CP and normal glucose tolerance are normal in that regard, it is likely that the incretin deficiencies are consequences of the diabetic state (the hyperglycaemic environment and the resulting glucotoxicity) and are, therefore, obvious therapeutic targets. On the other hand, if the incretin physiology is preserved independently of the diabetic state of patients with CP, the incretin deficiencies observed in T2DM could be primary defects and possibly genetically determined. Such defects would be obvious targets for intensified genetic investigations in the future.

Therefore, we decided to investigate the incretin effect and the insulinotropic actions of GLP-1 and GIP in CP patients with normal glucose tolerance and in CP patients with secondary DM not requiring exogenous insulin. However, in order to determine whether patients with CP and pancreatic exocrine insufficiency have a preserved postprandial response of GLP-1 (and GIP, as observed by *Ebert et al*.[176]), and to establish a possible relationship between assimilation of nutrients and secretion of GLP-1 (and GIP, as observed by *Ebert et al*.[176]), liquid meal tests were investigated initially.

SELECTION OF PATIENTS WITH CHRONIC PANCREATITIS

It should be noted that all patients with CP included in the following studies were without clinical or biochemical signs (amylase, C-reactive protein and leukocyte counts within normal limits) of acute inflammatory activity in the pancreas, did not drink alcohol on a daily basis (no clinical or biochemical signs (albumin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and coagulation factors II, V, VII and X within normal limits x 1.5) of affected liver function), and met the diagnostic criteria of CP according to *Layer et al*.[14]: After a thorough diagnostic evaluation to exclude other causes of chronic abdominal pain or steatorrhea, diagnosis of CP was established if a score of 4 or more was achieved using the following scoring system:

4	Pancreatic calcifications
4	Typical histological changes
3	Characteristic findings on endoscopic retrograde cholangiopancreatography (ERCP)
2	Pancreatic exocrine insufficiency (steatorrhea by abnormal qualitative or quantitative fecal fat excretion (>7 g/day) or abnormal cholecystokinin test result)
2	Attacks of pancreatitis and/or chronic upper abdominal pain
1	Secondary DM

It should be mentioned that nowadays in the County of Copenhagen, ERCP and the cholecystokinin test are very seldom used in patients suspected of CP. Therefore, the abovementioned diagnostic criteria were slightly modified to encompass definite morphological changes evident by ultrasonography, computed tomography scan or magnetic resonance cholangiopancreatography (3 points), and pancreatic exocrine insufficiency was defined as reduced meal-stimulated duodenal concentrations of lipase and amylase (Lundh's meal test) (2 points). Additionally, in all patients, unequivocal morphological changes of the pancreas were evident by ultrasonography or computed tomography scan, according to the Cambridge classification[178]. The Cambridge classification uses imaging tests to provide a grading of the severity of the disease, and is useful as a staging system once the diagnosis is made. The glucose tolerance of each subject was determined according to the criteria of WHO[1] which state that DM can be diagnosed in three ways and each must be confirmed, on a subsequent day, by any one of the following three methods: 1) symptoms of DM plus casual plasma glucose concentration ≥11.1 mM; 2) fasting plasma glucose concentration ≥ 7.0 mM; or 3) 2-h postload value ≥11.1 mM following 75-g oral glucose tolerance test. Impaired glucose tolerance is diagnosed if the 2-h postload value is ≥7.8 mM and ≤11.1 mM (assessed by two separate tests); and normal glucose tolerance is defined as postload values <7.8 mM. In all patients, glucose intolerance, if present, developed after the diagnosis of CP had been established; and none of the patients had first-degree relatives with DM. All CP patients were negative with regard to islet cell autoantibodies and glutamate decarboxylase-65-autoantibodies. Additionally, all patients were normotensive and none had impaired renal function (normal plasma creatinine levels and no albuminuria).

MEAL STUDIES IN PATIENTS WITH CHRONIC PANCREATITIS

As mentioned before, postprandial GLP-1 responses in patients with CP have never been investigated; and studies on the secretion of GIP following ingestion of nutrients in such patients have yielded contradicting results. We, therefore, examined patients with CP and matched healthy control subjects using liquid meal tests. Another objective was to determine a possible relationship between assimilation of nutrients and secretion of GLP-1 and GIP. Therefore, 8 patients with CP and steatorrhea due to exocrine pancreatic insufficiency (one of these exhibited normal glucose tolerance, three impaired glucose tolerance and the remaining patients exhibited secondary DM not requiring insulin) were examined twice, with and without PES, and 8 healthy control subjects were studied for comparison[179].

This study showed that patients with CP have preserved postprandial incretin responses as compared to matched healthy subjects. Furthermore, we were able to show that PES increases the postprandial responses of the two incretin hormones in patients with CP and exocrine pancreatic insufficiency. Increased level of glucagon and a trend towards increased serum triglycerides following the meal with PES, point towards an enhanced absorption of amino acids and fat, respectively, compared to the meal without PES. Thus, the increased incretin responses following PES suggest not only that the secretion of incretin hormones, including GLP-1, is regulated by the mere presence of nutrients in the small intestine, but also that the assimilation of such nutrients is involved as well. However, it should be noted that even though plasma levels of intact and total GIP, as well as total GLP-1, increased significantly after PES, there was only a trend towards increased levels of intact GLP-1 following PES. The significance of this is unclear, but GLP-1 is known to be subject to degradation by DPP 4 almost immediately upon its release[180], so that only 10-15 % of intact GLP-1 actually reaches the systemic circulation[181], thereby reducing the possibility of detecting the response in the peripheral circulation. Importantly, the total concentrations are indicators of the overall levels of secretion, whereas the intact levels (prior to DPP 4 degradation) are indicators of the exact concentrations of active hormones in the peripheral circulation and, therefore, the endocrine impact on the beta cells.

A secondary objective of the study was to determine whether PES-induced changes in incretin responses (particularly GLP-1) were reflected in the endocrine performance of the beta cells, as

assessed by beta cell responsiveness to glucose. Beta cell responsiveness to glucose is a well-defined estimate of the effect of glucose on insulin secretion, and is determined in a two-step approach. First, the ISR for each time point is estimated by deconvolution of plasma C-peptide concentrations[66-68], in order to quantify prehepatic insulin secretion. Thereafter, ISR is cross-correlated with the concomitant plasma glucose concentration. The slope of this usually linear relationship is used as an index of beta cell responsiveness to glucose, expressed as pmol insulin secreted per minute per kilogram body weight per 1 mM change in plasma glucose[66;67;182;183]. Beta cell responsiveness to glucose is a composite index; and its strength is that it provides an overall measure of postprandial beta cell responsiveness to glucose. Its weakness is that it does not allow the glucose effect per se and non-glucose potentiation of postprandial insulin secretion (i.e., by incretin hormones) to be individually evaluated when applied to meal tests. We hypothesized that an increased response of one or both of the two incretin hormones following PES would enhance beta cell responsiveness to glucose. Surprisingly, however, only a minor and non-significant improvement in beta cell responsiveness to glucose was observed. This is somewhat in contrast to the study by *Ebert et al*.[176] showing a robust and significant increase in the postprandial insulin:glucose-ratio in patients with CP and pancreatic exocrine insufficiency following acute PES treatment. This discrepancy may be attributed to the fact that none of the patients with CP in that study had "overt" diabetes and, therefore, harbored a large number of incretin-sensitive beta cells. Thus, the lack of effect of PES on our secondary endpoint (beta cell responsiveness to glucose) is best explained by a secondary impairment of GIP activity, and perhaps GLP-1 activity, due to the hyperglycaemic state of the majority of the patients. However, we found a significantly increased insulin secretion during the meal supplemented with pancreatic enzymes, as compared to the meal alone (relative increase: 20%), but without a concomitant improvement in glucose tolerance. Our current data cannot elucidate in detail the mechanisms underlying the greater insulin response following administration of PES; but it is likely explained by the increase in digestive capacity and the enhanced absorption of nutrients. The increased insulin secretion might also explain why postprandial glucose excursions were similar during the two conditions, even though assimilation was enhanced after PES. It is currently unclear whether increased administration of PES in patients with CP and pancreatic exocrine insufficiency has potential therapeutic effects on postprandial hyperglycaemia in these patients.

One may suggest that studying patients with CP and pancreatic exocrine insufficiency without secondary impairment of glucose tolerance could lead to more conclusive results in regard to beta cell responsiveness to glucose. This might be true, but since very few patients with CP and pancreatic exocrine insufficiency maintain a normal glucose tolerance[11-13], it is very difficult to study exocrine and endocrine insufficiencies independently of each other - as reflected by the fact that only one subject with CP and pancreatic exocrine insufficiency in this particular study had a normal glucose tolerance. To our knowledge, the heterogenic endocrine status of the group of patients with CP and pancreatic exocrine insufficiency in question, very much reflects the clinical reality[13].

The design of the study (two groups of subjects; one group (patients with CP) tested with two meal tests on separate days with and without PES, respectively, and one group (healthy control subjects) tested with only one meal test without PES) could have been analysed by means of a mixed analysis of variance (ANOVA) model. This would, however, have required additional assumptions about variance homogenity within and between subjects. With only two groups and treatment/no treatment in only one of them, such an ANOVA is equivalent to group comparisons (unpaired) and paired tests of the treated group. Therefore, comparisons were performed directly by standard statistical methods. This statistical approach was preferred because it required fewer apriori assumptions and led directly to the endpoints of the study.

In conclusion, patients with CP and exocrine pancreatic insufficiency have preserved postprandial incretin responses, as compared to healthy control subjects and exhibit increased postprandial incretin responses and increased insulin secretion following administration of PES. However, no significant effect on postprandial beta cell responsiveness to glucose was observed, suggesting that the increased assimilation of nutrients is responsible for the potentiated insulin response following PES.

THE INCRETIN EFFECT IN PATIENTS WITH CHRONIC PANCREATITIS

Until now, the incretin defect in patients with T2DM has been considered a possible candidate for a primary deficiency in T2DM[15]. However, in subsequent studies, *Nauck et al*. estimated the incretin effect in first-degree relatives of patients with T2DM and found it to be similar to that of matched healthy subjects, suggesting the deficiency to be a consequence of the diabetic state[163]. In support of this, the incretin effect has been found to be reduced in individuals with type 1 diabetes mellitus (positive islet cell autoantibodies) and the unique metabolic profile of normal fasting glucose levels but diabetic oral glucose tolerance[184].

In order to further characterize the reduced incretin effect observed in patients with T2DM in regard to it being a cause or consequence of the diabetic state, we applied the classical isoglycaemic technique to gauge the incretin effect in 8 patients with CP and normal glucose tolerance and in 8 patients with CP and secondary DM not requiring exogenous insulin. Eight healthy subjects and 8 patients with T2DM were studied for comparison. The incretin effect was shown to be preserved in patients with CP and normal glucose tolerance, whereas it was strongly reduced in patients with CP and secondary DM. This suggests that the reduced incretin effect is a consequence of the diabetic state and not a primary event leading to T2DM. Furthermore, we showed that the reduced incretin effect in patients with CP and secondary DM not requiring treatment with insulin, and in patients with T2DM, cannot be explained by reduced levels of circulating incretin hormones.

The two groups of patients with CP differed with respect to glucose tolerance, but were very similar in regard to other CP-related pathologies. All patients with CP and secondary DM had a relatively well regulated glucose homeostasis on diet and/or oral antidiabetic drugs (none of them were treated with insulin), suggesting preservation of a substantial number of functional beta cells for the incretin hormones to exert their actions upon. Therefore, a difference in incretin effect between the two groups of patients with CP is most likely to be attributed to their different glycaemic control. Nonetheless, one could argue that a reduced incretin effect reflects a reduction in beta cell mass. However,

because the incretin effect is calculated using a within-subject comparison, the abnormality cannot be explained simply by a decrease in beta cell mass and is likely due to a functional abnormality of the beta cell. No matter how the incretin effect was calculated, a lower incretin effect was seen in patients with CP and secondary DM (decreased to the level of patients with T2DM (35%) or even lower (30%)) as compared to patients with CP and normal glucose tolerance (68%), who exhibited a similar incretin effect to that of healthy subjects (60%). Likewise, the amount of glucose required to mimic the oral glucose curves during the iv glucose infusion (reflecting all of the mechanisms facilitating glucose disposal after oral as opposed to iv administration of glucose) was increased similarly in the two diabetic groups, reflecting the equally dramatic impairment of GI-mediated glucose handling in these groups. As mentioned, it is possible that inappropriate glucagon secretion during oral glucose, as opposed to a normal suppression of glucagon during iv glucose contributed to this impairment. Therefore, we investigated whether our recent finding that the regulation of alpha cell secretion in patients with T2DM is different during oral glucose and isoglycemic iv glucose infusion[95] could be reproduced in patients with CP and DM and, therefore, could be attributed to the diabetic state per se, or whether it might be a primary event leading to T2DM. Interestingly, we observed that glucagon secretion was differentially regulated during oral glucose and isoglycaemic iv glucose infusion, respectively, in patients with CP and secondary DM and, to a lesser extent, in normal glucose tolerant patients with CP. The mechanism of this pathophysiological phenomenon is currently unclear. However, since GIP has been shown to possess glucagonotropic properties[154;155], a GIP-mediated mechanism could be involved. In our study, the patients with CP and secondary DM exhibited significantly greater responses of total and intact GIP following oral glucose (compared to the other groups) and actually hypersecreted glucagon during the first hour following ingestion, supporting a GIP-mediated mechanism. Clearly, further studies are needed to establish the underlying mechanisms of the different glucagon responses during oral and isoglycaemic iv glucose infusion.

In order to evaluate mechanisms underlying the reduced incretin effect, we measured intact and total plasma concentrations of GIP and GLP-1 during both experimental days. In all groups, the responses (AUC) of the total forms (indicators of the overall levels of secretion) were significantly higher during oral glucose, as compared to the isoglycaemic iv glucose infusion, as were the responses of intact GIP. Responses of intact GLP-1 were significantly greater during oral glucose, as compared to isoglycaemic iv glucose, only among patients with CP and secondary DM, and among healthy control subjects. The corresponding differences in the remaining two groups (patients with CP and normal glucose tolerance and patients with T2DM) failed to reach statistical significance. The latter observation is probably due to the fact that GLP-1 is subject to degradation by DPP 4 almost immediately upon its release[180], as outlined earlier. Interestingly, no differences in incretin hormone responses between the four groups could explain the different magnitude of the incretin effect in glucose tolerant and glucose intolerant subjects. Therefore, we set out to determine whether a deterioration of the effects of the incretin hormones developing along with the deteriorating glucose homeostasis in CP patients could be responsible for the dramatic impairment in incretin effect among patients with CP and secondary DM.

THE INSULINOTROPIC EFFECT OF GIP AND GLP-1 IN PATIENTS WITH CHRONIC PANCREATITIS

The insulinotropic effect of the incretin hormones was determined in 8 patients with CP and normal glucose tolerance and in 8 patients with CP and secondary DM not requiring insulin by hyperglycaemic (15 mM) clamp experiments, with concomitant infusion of supraphysiological doses of GLP-1, GIP, or saline. In this study, we showed that patients with CP and secondary DM seem to have lost the insulinotropic effect of GIP (especially on the late-phase insulin response) as compared to normal glucose tolerant patients with CP. This finding supports the notion that the reduced incretin effect in DM mainly is caused by a reduced insulinotropic effect of GIP, and that this deficiency seems to be a consequence of the diabetic state, rather than a primary event in the pathogenesis of T2DM.

As was the case in the prior study, the two groups of patients with CP exhibited different glucose tolerances, but were very similar in regard to other CP-related pathologies. Likewise, in this study, the patients with CP and secondary DM had a relatively well-regulated glucose homeostasis without receiving exogenous insulin, suggesting preservation of enough functional beta cells for the incretin hormones to exert their actions upon. Thus, differences in the effects of the incretin hormones between the two groups of patients with CP are likely to be attributed to the difference in glucose homeostasis.

The amounts of glucose needed to maintain the hyperglycaemic clamps at 15 mM during the GLP-1 and GIP infusions differed dramatically between the two groups. A significant increase in the amount of glucose used during GIP, as compared to saline infusion, was observed among patients with CP and normal glucose tolerance, whereas no significant difference was observed between the corresponding amounts in patients with CP and secondary DM. This discrepancy is likely to be due to a difference in the insulinotropic effect of GIP. In accordance with this notion, patients with CP and normal glucose tolerance responded to GLP-1 and GIP with significantly increased insulin and C-peptide concentrations, as compared to saline, throughout the period of hyperglycaemia (15 mM). However, patients with CP and secondary DM were able to increase their insulin and C-peptide concentrations continuously during GLP-1 infusion, and not during infusion of GIP. The discrepancy between the late-phase insulin responses to GIP in the two groups of patients with CP (normal glucose tolerance vs. secondary DM) is similar to the GIP defect observed in patients with T2DM as compared to healthy subjects[10]. This suggests that the reduced insulinotropic effect of GIP develops alongside the deterioration of glucose tolerance, suggesting in turn that it is a consequence of the diabetic state and not a primary event leading to T2DM.

Regarding the insulinotropic effect of GLP-1, the insulin and C-peptide responses to the hyperglycaemic clamp with concomitant infusion of GLP-1 showed similar fractional increases, as compared to the clamp with infusion of saline, in both groups. However, the absolute responses of insulin and C-peptide during the GLP-1 infusion clamp were 30-50 times greater in patients with CP and normal glucose tolerance as compared to patients with CP and secondary DM. This enormous difference is probably due to a reduction in the number of functional beta cells (i.e. beta cells with fully preserved sensitivity to the potentiating effect of GLP-1 on glucose-stimulated insulin secretion) in patients with CP and DM. Interestingly, however, the insulin and C-peptide responses

evoked by GLP-1 in patients with CP and secondary DM were equal to, or higher than, those observed in patients with CP and normal glucose tolerance during the 15-mM hyperglycaemic clamp with infusion of saline alone. This indicates, in accordance with findings in patients with T2DM[9;10;162], that infusion of a low, but supraphysiological, dose of GLP-1 in patients with CP and secondary DM not requiring exogenous insulin is capable of normalizing beta cell responsiveness to iv glucose.

It should be mentioned that two patients with CP and normal glucose tolerance experienced major hypoglycaemic episodes following termination of the hyperglycaemic clamp with concomitant infusion of GLP-1; this occurred in spite of food and fruit juice ingestion immediately after the experiments. Due to these two episodes, and difficulties encountered in previous protocols[10] (the almost impossible task to clamp a healthy, yet overweight, subject at a plasma glucose level of 15 mM during infusion of GLP-1 at the rate of 1 pmol/kg body weight/min) combined with the even higher risk of causing hypoglycaemia in lean, insulin sensitive, healthy subjects, we found it unethical to recruit a healthy control group.

In conclusion, the results of this study suggest that the loss of insulinotropic effect of GIP can not be ascribed to a primary event leading to T2DM, but should rather be interpreted as a consequence of deteriorating glucose tolerance.

CONCLUSIONS

We have established that patients with CP, regardless of pancreatic exocrine functionality, have normal postprandial responses of GLP-1 and GIP. These responses seem to be dependent not only on the mere presence of nutrients in the small intestine, but also on the assimilation of such nutrients. The increased (PES-induced) assimilation of nutrients in the small intestine does not result in increased postprandial glucose excursions in patients with CP and pancreatic exocrine insufficiency. This somewhat surprising finding is most likely explained by an accompanying increase in postprandial insulin secretion that may originate from the PES-induced increase in postprandial incretin responses. As mentioned above, it is currently unclear whether increased administration of PES in patients with CP and pancreatic exocrine insufficiency has a potential therapeutic effect on postprandial hyperglycaemia in these patients; but a treatment that increases assimilation of nutrients without incurring decreased glucose tolerance must be considered worthwhile for patients with CP exhibiting pancreatic endocrine and exocrine insufficiencies.

The incretin effect, as assessed by the classical isoglycaemic oral and iv glucose experiments, was shown to be preserved in patients with CP and normal glucose tolerance, whereas it was strongly reduced in patients with CP and secondary DM not requiring insulin. This suggests that the reduced incretin effect is a consequence of the diabetic state and not a primary event leading to T2DM. Lastly, our results suggest that the impaired incretin effect in patients with DM cannot be explained by reduced basal or stimulated levels of circulating incretin hormones as compared to glucose tolerant subjects. This finding prompted us to proceed with experiments describing the effects of the incretin hormones in patients with CP; and in those studies we showed that patients with CP and secondary DM not requiring exogenous insulin seem to have lost the insulinotropic effect of GIP, as compared to normal glucose tolerant patients with CP, which underscores the GIP defect as a consequence of the diabetic state per se in patients with DM.

One might speculate as to whether it is possible to reestablish the incretin effect and correct the impaired insulinotropic effect of GIP in patients with DM by near-normalization of plasma glucose for a longer period. That this might be feasible is supported by our recent finding that, in patients with T2DM, four weeks of strict glycaemic control during insulin treatment improved beta cell responsiveness to GIP (*Højberg et al. unpublished - personal communication*) and, to some extent, to GLP-1[162]. Preliminary data, from a study of women with gestational diabetes mellitus, suggest, that the incretin effect is reduced during pregnancy in these women; and data from a follow-up study of this cohort performed two months following delivery (when normal glucose tolerance has reemerged) are being awaited with great interest (*Kosinski et al. unpublished - personal communication*). Furthermore, studies investigating the incretin effect and the impaired insulinotropic effect of GIP before and after weight loss-induced remission of T2DM (up to 64% of severely obese patients with T2DM treated with laparoscopic adjustable gastric banding exhibit complete remission of T2DM one year after surgery[185]) are ongoing and will certainly bring about new knowledge of the potential reversibility of the incretin defects, which characterize patients with DM.

PERSPECTIVES

Regarding the pathophysiology of the incretin defect, our studies in patients with CP and secondary DM not requiring insulin clearly show a parallel to T2DM; and, since the observed deficiencies seem to be directly related to deteriorating glucose homeostasis, the results automatically bring up the question: “Can we correct the incretin deficiencies by applying incretin-based therapies to patients with CP and secondary DM?” As has been mentioned, the antidiabetic treatment of choice for DM not requiring exogenous insulin secondary to CP in the County of Copenhagen, Denmark, is diet alone or diet in combination with oral sulphonylurea therapy (to our knowledge, international treatment recommendations are currently unavailable). One may speculate that the increased work load inflicted upon the beta cells by sulphonylureas leads to increased beta cell stress and, therefore, to the immune system being exposed to a higher level of beta cell antigens, which in turn leads to a progression of the inflammatory condition in pancreas[186]. This notion is supported by studies demonstrating increased beta cell apoptosis following exposure of beta cell lines, rodent islets[187] and isolated human islets to sulphonylurea[188]. It is, therefore, obvious that an approach capable of protecting beta cells and, thereby, possibly breaking the otherwise inevitable progression of DM secondary to CP is desirable. The present results indicate that patients with CP and secondary DM not requiring exogenous insulin might benefit from incretin-based antidiabetic therapy. However, GLP-1 analogues have consistently been shown to cause reduced appetite and food intake leading to weight-loss in patients with T2DM. Since patients with CP often work hard to keep *up* their body weight, this option of treatment should be considered inexpedient in these patients. However, the physiologically rapid degradation of GIP and GLP-1 by the enzyme DPP 4 offers the possibility of increasing the endogenous concentrations of both circulating hormones by inhibiting DPP 4 without causing weight loss[189].

Importantly, our studies provide evidence for preserved postprandial incretin hormone responses in patients with CP[179], suggesting that inhibition of DPP 4 will result in increased levels of intact incretin hormones in these patients. Results from previous studies in patients with T2DM demonstrate that DPP 4-inhibition effectively prevents deterioration of glycaemic control in patients inadequately treated with metformin[190]. Interestingly, the amelioration of glycaemic control caused by DPP 4-inhibition is attained without significant changes in 24-hour or postprandial insulin levels[191], indicating less beta cell stress as opposed to sulphonylurea treatment. Thus, the involvement of several targets in the treatment of DM secondary to CP could minimize beta cell stress and thereby hinder progression of the inflammatory condition. DPP 4 inhibition in streptozotocin(STZ)-induced diabetic rats (with pancreatic necrosis and fibrosis due to the beta cell specific toxin STZ) causes enhanced islet neogenesis, beta cell survival, and insulin biosynthesis[192]. Recently, *Mu et al*. showed that 2-3 months of DPP 4 inhibition in high-fat diet, STZ-induced diabetic mice increased the number of insulin-positive beta cells in islets (leading to normalization of beta cell mass and beta cell-to-alpha cell ratio), increased islet insulin content, and improved glucose-stimulated insulin secretion in isolated islets, whereas sulphonylurea treatment had no effect on these parameters[193]. Lastly, DPP 4 inhibition has recently been shown to improve glycaemic control by improved suppression of glucagon in patients with T2DM (*Azuma et al. unpublished - personal communication*). These findings suggest that DPP 4 inhibitors may offer long-lasting efficacy in the treatment of DM and possibly modify the course of the disease. Furthermore, a dose-dependent prevention of STZ-induced apoptotic cell-death in the human beta cell line INS-1, by both GLP-1 and GIP, supports the role for incretins as beta cell protecting agents[192]. This should also be evaluated as a possible strategy to prevent deterioration of the secondary DM in patients with CP.

ABBREVIATIONS

ANOVA	Analysis of variance
ATP	Adenosine 5'-triphosphate
AUC	Area under curve
CP	Chronic pancreatitis
DM	Diabetes mellitus
DPP 4	Dipeptidyl peptidase 4
ERCP	Endoscopic retrograde cholangiopancreatography
GI	Gastrointestinal
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide-1
GLP-2	Glucagon-like peptide-2
GRPP	Glicentin-related pancreatic peptide
HOMA	Homeostatic model assessment
ISR	Insulin secretion rate
iv	Intravenous
PES	Pancreatic enzyme supplementation
∫SR	Integrated beta cell secretory response
T2DM	Type 2 diabetes mellitus
WHO	The World Health Organization

SUMMARY

Type 2 diabetes mellitus (T2DM) has been shown to be characterised by an almost abolished incretin effect. The incretin effect refers to the phenomenon of oral glucose eliciting a higher insulin response than intravenous glucose at identical plasma glucose profiles. It is conveyed by the two insulinotropic incretin hormones: glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). GLP-1 and GIP are secreted from the small intestines in response to ingestion of nutrients. The incretin defect of T2DM has been characterised by a virtually lost insulinotropic effect of GIP. It is unknown whether the incretin defect is a primary event leading to T2DM or arises as a consequence of the diabetic state. To investigate this we studied patients with chronic pancreatitis (CP). Over time, CP leads to secondary diabetes mellitus (DM). If patients with CP and secondary DM exhibit the characteristic type 2 diabetic incretin deficiencies and patients with CP and normal glucose tolerance are normal in that regard, it is more likely that these deficiencies are consequences of the diabetic state rather than primary events leading to T2DM. On the other hand, if incretin physiology is preserved independently of the endocrine status of patients with CP, the incretin defect could represent a primary pathogenetic defect. Three protocols have been employed to investigate this. In a study investigating postprandial incretin responses in 8 patients with CP and exocrine pancreatic insufficiency, with and without pancreatic enzyme supplementation (PES), we observed preserved incretin responses as compared to matched healthy subjects; and, further, that PES increased postprandial incretin responses in these patients. This suggests not only that the secretion of incretin hormones is regulated by the mere presence of nutrients in the small intestine, but also that the assimilation of such nutrients is involved, as well. Furthermore, we gauged the incretin effect in 8 patients with CP and normal glucose tolerance and in 8 patients with CP and secondary DM. Eight healthy subjects and 8 patients with T2DM were studied for comparison. The incretin effect was shown to be preserved in normal glucose tolerant patients with CP, whereas it was strongly reduced in patients with CP and secondary DM, suggesting the incretin defect to be a consequence of the diabetic state. Lastly, we investigated the insulinotropic effect of the incretin hormones in 8 patients with CP and normal glucose tolerance and in 8 patients with secondary DM, and observed that patients with CP and secondary DM exhibit an impaired insulinotropic effect of GIP, and that this most likely occurs as a consequence of the diabetic state. In conclusion, we suggest that: 1) the postprandial secretion of incretin hormones is preserved among patients with CP; 2) assimilation of nutrients stimulates secretion of GIP and GLP-1; and 3) the characteristic incretin deficiencies of T2DM most likely are consequences of a deteriorating glucose homeostasis, rather than primary events leading to T2DM.

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