

# On the role of the incretin hormones GIP and GLP-1 in the pathogenesis of Type 2 diabetes mellitus

Tina Vilsbøll

This review has been accepted as a thesis with twelve previously published papers, by the University of Copenhagen, April 30, 2004, and defended on September 17, 2004.

Department of Internal Medicine F and Clinical Pharmacology, Gentofte Hospital in corporation with the Department of Medical Physiology, The Panum Institute and the Department of Endocrinology, Hvidovre Hospital.

Correspondence: Correspondence: Tina Vilsbøll, Kratmosevej 11, 2950 Vedbæk, Denmark.

Official opponents: Michael Nauck, Germany, and Jørgen Vinten.

Dan Med Bull 2004;51:364-70.

## BACKGROUND

In 1906, extracts of mucosa from the porcine upper small intestine were used by Moore et al. as a treatment for diabetes, hoping that "the pancreas secretion might be stimulated by the substance of the nature of a hormone yielded by the duodenal mucosa membrane" (1). In 1932, La Barre named the unidentified substance thought to exert this effect "incretin" (2). Thirty years later, McIntyre et al. demonstrated that gut derived factors have a potentiating effect on insulin secretion after ingestion of glucose (3). Some years later, a polypeptide was discovered and named gastric inhibitory polypeptide (GIP) because of its inhibitory effect on gastric acid secretion in dogs (4). Eventually, it was shown to be insulinotropic at elevated glucose concentrations (5-7). Its gastric inhibitory effects were weak (8, 9) and it was, therefore, suggested that GIP should be renamed "glucose-dependent insulinotropic polypeptide" (5-7). Today, both names are still in use. Later, experimental and clinical studies suggested that the gut produces more than a single insulinotropic hormone (10, 11). In 1983, the gene encoding the human pancreatic

hormone, glucagon, was cloned, and the structure of its precursor, proglucagon, was deduced and shown to include the sequence of two glucagon-like peptides, in addition to glucagon itself (12). The gene was found to be expressed in both the pancreatic  $\alpha$ -cells and the intestinal L-cells. The primary transcripts and translation products of the gene in the two types of cells are identical (13), but the post-translational processing differs markedly in these two tissues (14-16). In the pancreas proglucagon is cleaved to glucagon, glicentin-related pancreatic peptide (GRPP) and a major proglucagon fragment (14). Apart from glucagon, all of these fragments seem to be biologically inactive (17). In contrast, in the intestinal L-cells, the molecule is processed to GLP-1 (glucagon-like peptide-1), GLP-2 (glucagon-like peptide-2) (18) and glicentin (19) (Figure 1). GLP-1 was found to be strongly insulinotropic (20, 21) and GLP-2 to be a key regulator of small bowel growth (22).

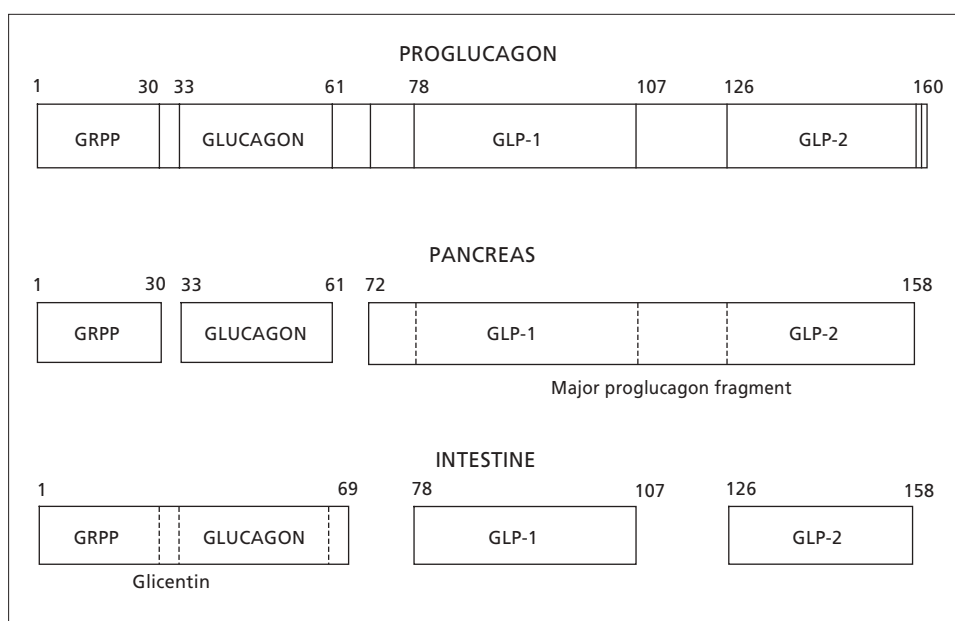
Today, therefore, we have two major incretin hormones: glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), which together are thought to be responsible for the incretin effect. The incretin effect is quantitated by comparison of the insulin response after oral and intravenous glucose, administered in such a way that identical blood glucose concentrations are obtained (23-26).

## GLUCOSE-DEPENDENT INSULINOTROPIC POLYPEPTIDE (GIP) AND GLUCAGON-LIKE PEPTIDE-1 (GLP-1)

GIP is a 42-amino acid polypeptide secreted from the endocrine K-cells of the duodenum and proximal jejunum after ingestion of carbohydrates, fat and amino acids, with fat being the most potent stimulator of GIP secretion (27, 28). GIP acts through a specific GIP-receptor in the  $\beta$ -cell plasma membrane (see below). The binding of GIP at the receptor on pancreatic  $\beta$ -cell enhances exocytosis of insulin containing granules (29). In addition to the insulinotropic effect on the  $\beta$ -cell, GIP also influences lipid metabolism (27, 30, 31).

In mammals, GLP-1 is a 30 amino acid peptide with approximately 50% homology to glucagon. The GLP-1 nomenclature has been confusing because different synonyms have been used for the same molecular forms. The bioactive forms, amidated and glycine extended GLP-1, are often designated GLP-1 7-36 amide and GLP-1 7-37, respectively, and it is now agreed that GLP-1 refers to these biologically active forms. GLP-1 is released in response to meal ingestion (32, 33), with lipids and carbohydrates being most potent in stimulating secretion (34). As for GIP, the mechanism of the insulinotropic action of GLP-1 involves interaction with a specific recep-

**Figure 1.** Post-translational processing of proglucagon in the pancreas and in the intestine.



tor belonging to the glucagon subfamily of G-protein-coupled receptors, located on the pancreatic  $\beta$ -cells, with subsequent activation of adenylate cyclase. The actions on the  $\beta$ -cell seem to be related to accumulation of cAMP within the cell and include closure of  $K^+$  channels, elevation of cytosolic  $Ca^{++}$  concentrations and mobilization of a pool of insulin containing granules (29, 35). Besides stimulating glucose induced insulin secretion, GLP-1 also increases insulin biosynthesis (36) and stimulates  $\beta$ -cell growth and proliferation (37). It also inhibits glucagon secretion (38), hepatic glucose production (39, 40), and gastrointestinal motility (41, 42), and promotes satiety and fullness, thereby reducing food intake (43, 44).

GLP-1 is strongly insulinotropic in humans, and administration of an antagonist to the GLP-1 receptor, exendin 9-39, in healthy subjects suggests that GLP-1 is essential for normal glucose tolerance in humans (45). Similarly, mice with a targeted deletion of the GLP-1 receptor become glucose intolerant and develop fasting hyperglycaemia (46). Impaired glucose tolerance is also seen in mice with a targeted deletion of the GIP receptor gene (47). Postprandial concentrations of GIP are much higher than postprandial GLP-1 concentrations. Some investigators have found GIP and GLP-1 to be equally potent with respect to insulin secretion (48). While, others found GLP-1 to be 3-5 times more potent than GIP (49-51). GIP has been reported not to stimulate insulin secretion significantly at fasting plasma glucose levels (49, 50, 52), on the other hand, as suggested by others, the GLP-1 concentration in the fasting state and during an oral glucose tolerance test may be too low to stimulate insulin secretion (50). Thus, the contributions of GIP and GLP-1 to the incretin effect under normal physiological conditions with small plasma glucose excursions in humans were unclear, and required further investigation.

The finding of an impaired or absent incretin effect in type 2 diabetic patients (49, 53) focused attention on the possible importance of GIP and GLP-1 in diabetes mellitus. An impaired incretin effect may contribute to the pathogenesis of type 2 diabetes mellitus. In healthy subjects, approximately 50-70% of the insulin response to oral glucose is due to the incretin hormones (54). Loss of their effect might contribute to the impaired postprandial insulin response in type 2 diabetic patients. However, GLP-1 was shown to retain insulinotropic action in diabetic patients (55, 56) without risk of hypoglycaemia when given subcutaneously to obese type 2 diabetic patients (57) and insulin sensitive normal weight type 2 diabetic patients (58). Thus, administration of an intravenous infusion of GLP-1 normalised blood glucose concentrations in type 2 diabetic patients – even in patients with long-standing disease and secondary failure of oral antidiabetic drugs (55).

An impaired incretin effect in type 2 diabetic patients could theoretically be caused by either a decreased secretion, an increased elimination or an impaired effect of the incretin hormones. Therefore, it is the aim of this review to evaluate the relative contribution of the incretin hormones and discuss their relation and their possible importance for the pathogenesis of diabetes mellitus, by analysis of their secretion and effects in such patients.

## SECRETION AND METABOLISM OF INCRETIN HORMONES IN DIABETES MELLITUS

After the secretion of GIP and GLP-1, from the K- and L-cells, respectively, in the intestine, both hormones are inactivated in the circulation by the enzyme dipeptidyl peptidase-IV (DPP-IV) (59-62). This enzyme, in addition to its localization at sites such as the intestinal and renal brush border membranes, is also found on the capillary surfaces and in a soluble form in plasma (63). It cleaves off the two N-terminal amino acids of peptides with a penultimate proline or alanine, and for both incretin hormones, this abolishes their biological activity (59-62, 64, 65). While GLP-1 is rapidly degraded in the circulation, resulting in a clearance which exceeds cardiac output and results in an apparent half-life of 1-1.5 min (60, 66). GIP is degraded more slowly, with a half-life for the intact hormone of 7

min (60). The truncated metabolites are eliminated more slowly, with half-lives of 4-5 and 17 min respectively (60, 66). In most studies, plasma GIP and GLP-1 concentrations have been measured with assays that do not distinguish between the intact hormones and their metabolites. Although these non-discriminating assays are useful for the estimation of the rate of secretion of the hormones (which is reflected in the sum of the concentrations of the intact hormones and their primary metabolites) they do *not* provide information about the level of the intact biologically active hormones. This raises the possibility of over- or underestimating their effect on pancreatic islet secretion, because this effect will only be related to the plasma concentrations of the intact hormones. Total GLP-1 concentrations have been reported to be increased (67) or reduced (68, 69) in obese subjects, to be higher in women than in men (70), and to be either increased (71, 72), decreased (70) or unchanged (73, 74) in subjects with impaired or diabetic glucose tolerance. The discrepancies between these studies may reflect the differences with respect to assay specificity. The early assays reacting with all GLP-1 containing moieties, including pancreatic forms, reflecting the well known hypersecretion of pancreatic proglucagon products in type 2 diabetes (72), and later assays measuring not only intact GLP-1 but also the inactive metabolites (GLP-1 (9-36)amide). Since the concentrations of the intact, biologically active hormones are unknown in type 2 diabetic patients, we measured plasma concentrations, using newly developed assays for intact GIP and intact GLP-1, after a mixed breakfast meal (566 kcal) in 12 middle aged, obese type 2 diabetic patients and 12 matched healthy subjects (75). Fasting levels and meal responses were similar between patients and healthy subjects for total GIP (intact+metabolite) as well as intact GIP, except for a significantly lower plasma concentration in the patients at 120 min. In contrast, the response of both the total and intact GLP-1 was characterized by an early rise (30-45 min) and a significantly reduced late phase (75-150 min) in the type 2 diabetic patients compared to the healthy subjects, supporting the hypothesis that a decreased plasma concentration of GLP-1 may contribute to the inappropriate insulin secretion in type 2 diabetes (75). Lugiari et al., in almost simultaneously published reports, described an absent GLP-1 response to a small meal (230 kcal) in both type 1 and type 2 diabetic patients (76). Possible explanations for the decreased GLP-1 secretion could include altered gastric emptying rate, resulting in increased absorption in the proximal intestine, and less food reaching the distal intestine where the GLP-1 producing L cells are more numerous. In agreement with this notion, increased exposure of the distal intestinal mucosa to carbohydrates, elicited by administration of  $\alpha$ -glucosidase inhibitors or accelerated gastric emptying, increase GLP-1 secretion (77, 78). However, the gastric emptying rate is not consistently changed in type 2 diabetes mellitus and obesity, although it has been reported as delayed (79, 80). The obese subjects may have increased proximal absorption rates (81) which could provide an explanation for the decreased GLP-1 secretion in obesity (because of the preferentially distal localisation of the L-cells) (69, 71, 82). It was therefore possible that ingestion of a small meal with a relatively higher proximal absorption than after a large meal would result in a relatively lower secretion of the distal incretin hormone, GLP-1.

We, therefore, evaluated postprandial concentrations of intact and total GIP and GLP-1 in type 1 and type 2 diabetic patients and in two groups of matched healthy subjects (lean and obese) in response to ingestion of a small and a large breakfast meal of 260 and 520 kcal, respectively (83). Significantly enhanced incretin responses in response to the large meal occurred in type 1 and 2 diabetic patients and in lean and obese healthy subjects compared to the small meal. The increased incretin response to the larger meal is probably best explained by the increased exposure of the incretin hormone-producing endocrine K and L-cells of the intestinal mucosa to nutrients, since there were no differences in gastric emptying rates during the large versus the small meal between the four groups or between

the two different meal tests. Our previous finding of a decreased GLP-1 response in type 2 diabetic patients compared to healthy controls was confirmed (75) suggesting that type 2 diabetic patients generally have an attenuated GLP-1 secretion, which may contribute to the impaired insulin response. In a recent paper, Lugari et al. found that even after ingestion of 700 kcal, a GLP-1 response could not be detected in type 2 diabetic patients (84). Their results are in contrast to the present study and many other studies, and the difference can only be explained by differences in methodology or by unexpected differences with respect to the type 2 diabetic patients. Our type 1 diabetic patients had a normal incremental total and intact GLP-1 response compared to lean healthy subjects. In agreement with our previous studies, the GIP responses were similar in diabetic patients and healthy subjects. Therefore, the conclusion in respect to secretion of incretin hormones in patients with diabetes mellitus is, that a decreased GLP-1 secretion, reflected in lower concentrations of both the intact and degraded hormone, in our study measured with two different assays, may contribute to the impaired insulin secretion in type 2 diabetes mellitus, whereas GIP and GLP-1 secretion is probably normal in type 1 diabetic patients.

The lower GLP-1 concentrations could be caused by either a decreased secretion of GLP-1 or an increased elimination of GLP-1 in diabetic patients compared to healthy subjects. The possibility of differences in metabolism between diabetic patients and matched healthy subjects was investigated in a separate study, involving intravenous bolus injections of increasing doses of GLP-1 (2.5 to 25 nmol GLP-1) (85). We found similar pharmacokinetic profiles for intact GLP-1 as well as the primary metabolite, after the four different doses of GLP-1, in type 2 diabetic patients and matched healthy subjects. The decreased plasma concentrations of GLP-1 seen after ingestion of a standard breakfast meal in type 2 diabetic patients is therefore, most likely, caused by a decreased secretion of GLP-1 in the patients (85). In identical twins discordant for diabetes, the total GLP-1 secretion profiles were lower in the diabetic twin (70). In first degree relatives of diabetic patients, the 24-h GLP-1 secretion was normal (86). A decreased secretion of GLP-1 therefore, seems to be secondary to the diabetic condition.

#### **THE EFFECT OF THE INCRETIN HORMONES IN PATIENTS WITH DIABETES MELLITUS**

It has been controversial which of the incretin hormones is the most important during normal physiological conditions in healthy subjects. We evaluated the effects of physiological concentrations of GLP-1 and GIP on insulin secretion at plasma glucose concentrations clamped stepwise at physiological levels to clarify their relative roles in potentiating glucose induced insulin secretion in healthy subjects. An underlying assumption of the study was that the glucose concentrations had to be clamped at the desired level (both during fasting and postprandial levels), because even the slightest stimulation of insulin secretion would be expected to lower plasma glucose concentrations and, thereby, remove the glucose stimulated insulin secretion that the incretin hormones are thought to potentiate (36, 87). The study was, therefore, designed as a stepwise glucose clamp, with the first step of the clamp at the individual fasting glucose level, which was increased to 6 and subsequently 7 mmol/l, with concomitant infusions of either saline or GIP or GLP-1, in amounts calculated to result in physiological postprandial elevation of plasma concentrations. The results were compared to the insulin and incretin hormone responses during a meal test (comprising 566 kcal) (88). The peptides appeared to have similar insulinotropic effects at fasting glucose concentrations and at 6 mmol/l. At 7 mmol/l, the GLP-1 infusion resulted in significantly higher insulin secretion. This could indicate that at higher glucose levels, the effect of GLP-1 may be more potentiated than the effect of GIP, but further studies are needed to substantiate this. The concentrations of total GIP and GLP-1 obtained during the meal amounted to approximately 100-110 and 20-25 pmol/l, respectively. Peak concentrations of the incre-

tin hormones observed during the infusions were 300-360 and 50-60 pmol/l, respectively, with similar levels being obtained at all three glucose levels. The levels obtained can be considered high in the physiological range, but not irrelevant, however, since larger meals consisting of 1.000-1.100 kcal have been shown to elicit higher GIP and GLP-1 concentrations, corresponding to those obtained in this study, when measured with the same assay which was employed in our investigation (33). The present meal contained only 566 kcal, and the plasma concentrations obtained reflect to the amount of kcal ingested. It is, therefore, reasonable to assume that the range of concentrations of the incretin hormones obtained covers the entire physiological spectrum. A similar relationship between meal induced concentrations and those resulting from infusion were observed with respect to the concentration of the intact hormones. It was concluded from the study that during normal physiological plasma glucose levels of up to 6 mmol/l, GIP and GLP-1 contribute nearly equally to the incretin effect in healthy subjects, whereas at higher glucose levels, GLP-1 may be more potent than GIP in stimulating insulin secretion (88). Notably, both hormones were insulinotropic in physiological concentrations at fasting glucose levels, indicating that both incretin hormones act to amplify insulin secretion almost from the beginning of a meal, which typically results in a rise in the concentrations of the incretin hormones after 10-15 minutes (75).

Although, the incretin hormones, therefore, may contribute equally to the incretin effect in healthy subjects, it remained to be established whether the impaired incretin effect found in patients with type 2 diabetes mellitus (49, 74) might result from an impaired insulinotropic effect of either GIP or GLP-1. The  $\beta$ -cell secretory capacity depends on 1) the total  $\beta$ -cell mass 2) the secretory capacity of the individual cells, and 3) the sensitivity of the individual cells to the applied stimulus. In diabetic patients, all of the three may be impaired. In type 2 diabetes particularly, insulin secretion to glucose stimulation is impaired or absent (89). In evaluating  $\beta$ -cell secretory capacity, it is, therefore, important to choose a stimulus to which  $\beta$ -cell sensitivity is preserved. To evaluate whether GLP-1 could be such a stimulus,  $\beta$ -cell secretory responses to GLP-1 in various doses and modes of administration, was compared with the responses to a meal, to glucagon and to arginine injected during a hyperglycaemic clamp (30 mmol/l) in obese type 2 diabetic patients and in matched healthy subjects (90). In the dose-response part of the study, we found that similar peak insulin and C-peptide concentrations could be obtained with a standard meal, 2.5 nmol of GLP-1 and 1 mg (287 nmol) of glucagon in the type 2 diabetic patients, indicating that GLP-1 is as efficient as glucagon as a stimulus for  $\beta$ -cell secretion. GLP-1 however, in this 100 fold lower dose, had markedly fewer side effects than glucagon. The results obtained in an extended group of patients (n=12) and healthy subjects were similar to those obtained in the initial dose-response study (90). However, significantly greater responses could be obtained with the higher doses of GLP-1, and a maximal response to a single injection of GLP-1, therefore, requires doses higher than 2.5 nmol (90). On the other hand, an increasing number of patients reported side effects with the higher doses. In the normal subjects, similar responses were obtained with all doses. The finding that a dose-response relationship existed for the patients, but not for the healthy subjects suggests that the sensitivity of the  $\beta$ -cell to GLP-1 may be reduced in the patients, a conclusion that was also reached by Kjems et al. (91) using an entirely different experimental approach.

In the same study (90), we compared the responses of a combined glucose (15 mmol/l) + GLP-1 stimulation (2.5 nmol as a bolus injection) to those obtained in response to a 30 mmol/l hyperglycaemic clamp plus arginine (by some authors called "the golden standard" when evaluating  $\beta$ -cell secretory capacity) as described by Ward et al. (92). The incremental insulin and C-peptide responses to GLP-1 and arginine + 30 mmol/l glucose were similar for diabetic patients, but both the plasma insulin and C-peptide concentrations increased

during the glucose clamp so that the absolute  $\beta$ -cell response was greater with arginine than with GLP-1. The effect of glucose on the  $\beta$ -cell during the 45 min hyperglycaemic clamp may explain the higher absolute insulin and C-peptide responses during the arginine injection. Thus, even in patients with type 2 diabetes, the maximal secretory capacity of the  $\beta$ -cell can only be elicited with a combination of very high glucose concentrations (i.e. much higher than the patients' daily glucose levels) and an additional, potent secretagogue which could be either GLP-1 or arginine. However, the amount of insulin secreted during normal daily life as e.g. after a mixed meal, may be gauged rapidly and conveniently and with little discomfort for the patients with as little as 2.5 nmol of GLP-1 i.v. This could be useful e. g. in situations when instigation of insulin therapy is considered in patients with type 2 diabetes. The test may also be useful in estimating residual  $\beta$ -cell capacity in patients with type 1 diabetes.

Previous studies have indicated that whereas GLP-1 is strongly insulinotropic in patients with type 2 diabetes mellitus, the effect of GIP is much weaker or absent (49, 74, 93). The effect of GIP in type 2 diabetic patients is an important issue, because a defect in GIP action could contribute to the impaired incretin function. We, therefore, investigated more closely the potentiating insulinotropic effects of GIP, using the preserved  $\beta$ -cell response to GLP-1 as a measure of the insulin secretory capacity (90). The study protocol included bolus injections of GIP and GLP-1 concomitant with elevation of plasma glucose to 15 mmol/l in obese type 2 diabetic patients and matched healthy subjects (94). The purpose was to develop a test which could be used in evaluating insulin responses after GIP and GLP-1 administration in different subgroups of diabetic patients, allowing characterization of their  $\beta$ -cell response to the two incretin hormones, and in turn, analysis of the epidemiology of impaired GIP responsiveness. Surprisingly, during this acute stimulation, we found a relative insulin response to GIP in obese type 2 diabetic patients which was exactly the same as in the healthy subjects (94). The conclusions of these "bolus-studies" were, therefore, that diabetic patients express a functional GIP-receptor and that the "early plasma insulin response" is the same in diabetic patients and matched healthy subjects during both GIP and GLP-1 stimulation. Therefore, these initial studies could not confirm the impaired insulinotropic effect of GIP in type 2 diabetes mellitus. We then changed the experimental design to include a more prolonged stimulation of the  $\beta$ -cell, in order to estimate both "early" and "late phase" insulin and C-peptide responses (94). Hyperglycaemic clamps (15 mmol/l) with infusions (per kg body weight/min) of either 1 pmol GLP-1 (7-36)amide, 4 pmol GIP, 16 pmol GIP or no incretin hormone, were performed in obese type 2 diabetic patients and matched healthy subjects. "Early phase" (0-20 minutes) insulin responses to glucose were delayed and almost absent in the patients, but were enhanced similarly by GIP and GLP-1, although not to normal levels. During the "late phase" (20-120 minutes) GLP-1 augmented insulin secretion to levels similar to those observed in healthy subjects during glucose alone. In other words, GLP-1 was capable of restoring completely the  $\beta$ -cell response to glucose. A similar result was obtained by Kjems et al. using an entirely different methodology (91). In contrast, there was no amplification of the "late phase" insulin response, compared to glucose alone, during infusion of GIP regardless of the dose. Accordingly, whereas a doubling of the glucose infusion rate was required during the GLP-1 stimulation in the "late phase" period (20-120 minutes), the glucose infusion rates required to maintain the hyperglycaemic clamp were similar between glucose alone and glucose plus GIP. Lack of GIP amplification of the late phase insulin response to glucose, which contrasts markedly the normalising effect of GLP-1, may therefore, be a key defect in insulin secretion in type 2 diabetic patients (94).

To investigate whether this is a primary defect or secondary to the diabetic state, we studied the GIP responsiveness of 5 groups of diabetic patients, with completely different aetiology: 1) patients with

diabetes mellitus secondary to chronic pancreatitis (CP) 2) lean type 2 diabetic patients (BMI <25 kg/m<sup>2</sup>), 3) patients with latent autoimmune diabetes in adults (LADA), 4) diabetic patients with mutations in the HNF-1 $\alpha$  gene (maturity-onset diabetes of the young (MODY3)) and 5) newly diagnosed type 1 diabetic patients. All participants underwent three hyperglycaemic clamps (2 hours, 15 mmol/l) with or without continuous infusion of incretin hormones (saline or 1 pmol GLP-1 (7-36)amide or 4 pmol GIP pmol/kg body weight/min) to provide a prolonged stimulation of the  $\beta$ -cell (95). In this study, we showed that the early phase (0-20 min) plasma insulin response tended to be enhanced by both GIP and GLP-1 compared to glucose alone in all 5 groups of diabetic patients. In contrast the "late-phase" (20-120 min) insulin response to GIP was attenuated compared to the insulin response to GLP-1 in all 5 groups. Accordingly, glucose infusion rates required to maintain the hyperglycaemic clamp were not significantly different between GIP and GLP-1 clamps in the "early-phase", whereas significantly higher infusion rates were required during the "late phase" of the GLP-1 stimulation compared to the GIP stimulation (95). We, therefore, concluded that the lack of GIP amplification of the late phase insulin response to glucose seems to appear as a consequence of the diabetic state (95).

The differential responsiveness of the  $\beta$ -cell to GIP and GLP-1 is surprising, because their intracellular signal transduction mechanisms involve many common steps, except for transmission via closely related, but highly specific membrane receptors (35, 96). Thus, when activated, these receptors generate identical changes of membrane potential, intracellular calcium responses, membrane currents, and cAMP responses (97, 98). Furthermore, the insulinotropic effects of both peptides are similarly potentiated by sulphonylurea treatment and similarly augmented in the genetically obese (*fa/fa*) Zucker rat (99). It has, therefore, been concluded that the two receptors are likely to activate the same intracellular machinery. The hypothesised lack of expression of a functional receptor as the cause of the impaired insulin response to GIP in type 2 diabetic patients (100, 101) can be rejected because of the similar early phase responses to GIP. Molecular genetic studies have revealed sequence variations in the human GIP receptor (102), but no devastating, clinically significant mutations. The present investigations (94, 95) show that GIP receptors are expressed on the pancreatic  $\beta$ -cell of the patients, since the early, relative insulin response to GIP compared to GLP-1 was the same as in healthy subjects. Then, what is the mechanism of the almost abolished  $\beta$ -cell "late-phase"-response to GIP in diabetic patients? The possibility of a decreased sensitivity of the GIP receptor during the "late phase" insulin response was examined using a very high dose of GIP (16 pmol/kg body weight/min) infused intravenously (94). However, in spite of pharmacological plasma concentrations of GIP (markedly exceeding those observed after meal ingestion), in an attempt to compensate not only for the apparent difference in potency of the two hormones, but also for a decreased affinity of GIP for its receptor in type 2 diabetes mellitus, it was still impossible to generate a significant "late-phase" response in the type 2 diabetic patients. Furthermore, the results obtained during a 4 hour clamp in the first study (94) were qualitatively similar to those obtained with the 2 hour clamp, indicating that a delayed response to GIP in the patients could not explain the difference in the 2 hour clamp experiments. For GLP-1, insulin secretion was continuously increasing during the 2 hour clamp experiments, whereas in the 4 hour clamp, a plateau was eventually reached after 150 minutes (94).

In a recent study, a reduced insulinotropic effectiveness of GIP in 50% of glucose tolerant, first-degree relatives of type 2 diabetic patients in comparison to healthy subjects was found (103). Thus, it was hypothesised that the phenotypic abnormality in such subjects might be genetically determined (103). Such a genetically determined defective response to GIP may contribute to the pathogenesis of diabetes mellitus, but since a similar defect is seen in 6 different

groups of diabetic patients with completely different aetiology, one of them even being secondary diabetes (chronic pancreatitis), the impaired response in the diabetic patient is probably not a primary defect causing diabetes, but rather a defect that is secondary to the metabolic disturbances of diabetes. Further studies are needed to clarify this, e.g. studies involving strict metabolic control of diabetic patients, which may be able to improve or normalise the  $\beta$ -cell responsiveness to GIP.

## CONCLUSION

The available evidence suggests that the incretin effect plays a major role in the regulation of glucose metabolism and that a deficient incretin function is an important factor in the pathogenesis of diabetes mellitus. Decreased plasma concentrations of GLP-1 are seen in type 2 diabetic patients, probably due to an impaired secretion of this incretin hormone. However, a severe impairment of the insulinotropic effects of the other incretin hormone, GIP, probably further contributes to the decreased incretin effect in the patients, and, therefore, both incretin hormones may be involved in the pathogenesis of type 2 diabetes, one because of an inadequate secretion, and the other because of a decreased effect.

## SUMMARY

Glucose dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are the two major incretin hormones, which together are responsible for the so-called incretin effect. Type 2 diabetic patients have an impaired incretin effect, which could theoretically be due to impaired secretion, increased elimination or an impaired effect of the incretin hormones. Decreased concentrations of both total and intact GLP-1 are seen in type 2 diabetic patients. This is probably due to decreased secretion, since elimination of GLP-1 was shown to be similar in type 2 diabetic patients and healthy subjects. The decreased secretion is probably due to diabetes per se, and not a primary cause of diabetes, since normal GLP-1 secretion is seen in first degree relatives of diabetic patients, and since only the diabetic twin of identical twins discordant for diabetes had impaired GLP-1 secretion. The two incretin hormones were shown to contribute nearly equally to the incretin effect in healthy subjects, but in diabetic patients, the effect of GIP is severely impaired. In various subgroups of diabetic patients, including lean and obese type 2 diabetic patients, patients with diabetes mellitus secondary to chronic pancreatitis, LADA-patients, MODY3 patients, and newly diagnosed type 1 diabetic patients, GIP was incapable of generating a significant "late phase" insulin response. This defective response to GIP seems to be a post-receptor defect of the intracellular machinery that seems to be secondary to diabetes per se, perhaps overlying a genetic defect as indicated by the finding of a decreased insulinotropic effect of GIP in first degree relatives of type 2 diabetes mellitus.

Therefore, in conclusion, both GLP-1 and GIP may be involved in the pathogenesis of type 2 diabetes, GLP-1 because of an inadequate secretion and GIP because of a decreased effect.

## REFERENCES

1. Moore B, Edie ES, Abram JH: On the treatment of diabetes mellitus by acid extracts of duodenal mucosa membrane. *Biochem.J.* 28-38, 1906.
2. La Barre J: Sur les possibilites d'un traitement du diabetes par l'incrétin. *Bull Acad R Med Belg* 12:620-634, 1932.
3. McIntyre N, Turner DS, Holdsworth CD: New Interpretation of Oral Glucose Tolerance. *Lancet* 2:20-21, 1964.
4. Brown JC, Mutt V, Pederson RA: Further Purification of A Polypeptide Demonstrating Enterogastrone Activity. *Journal of Physiology-London* 209:57-64, 1970.
5. Dupre J, Ross SA, Watson D, Brown JC: Stimulation of Insulin-Secretion by Gastric Inhibitory Polypeptide in Man. *Journal of Clinical Endocrinology and Metabolism* 37:826-828, 1973.
6. Andersen DK, Elahi D, Brown JC, Tobin JD, Andres R: Oral Glucose Augmentation of Insulin-Secretion - Interactions of Gastric Inhibitory Polypeptide with Ambient Glucose and Insulin Levels. *Journal of Clinical Investigation* 62:152-161, 1978.

7. Elahi D, Andersen DK, Brown JC, Debas HT, Hershcopf RJ, Raizes GS, Tobin JD, Andres R: Pancreatic Alpha-Cell and Beta-Cell Responses to GIP Infusion in Normal Man. *American Journal of Physiology* 237:E185-E191, 1979.
8. Brown JC: Gastric Inhibitory Polypeptide. New York, Springer-Verlag, 1982.
9. Maxwell V, Shulkes A, Brown JC, Solomon TE, Walsh JH, Grossman MI: Effect of Gastric-Inhibitory Polypeptide on Pentagastrin-Stimulated Acid-Secretion in Man. *Digestive Diseases and Sciences* 25:113-116, 1980.
10. Creutzfeldt W: Incretin Concept Today. *Diabetologia* 16:75-85, 1979.
11. Lauritsen KB, Moody AJ, Christensen KC, Jensen SL: Gastric-Inhibitory Polypeptide (GIP) and Insulin Release After Small Bowel Resection in Man. *Scandinavian Journal of Gastroenterology* 15:833-840, 1980.
12. Bell GI, Sanchez PR, Laybourn PJ, Najarian RC: Exon duplication and divergence in the human proglucagon gene. *Nature* 304:368-371, 1983.
13. Novak U, Wilks A, Buell G, McEwen S: Identical Messenger-Rna for Pre-proglucagon in Pancreas and Gut. *European Journal of Biochemistry* 164:553-558, 1987.
14. Mojsov S, Heinrich G, Wilson IB, Ravazzola M, Orzi L, Habener JF: Pre-proglucagon Gene-Expression in Pancreas and Intestine Diversifies at the Level of Posttranslational Processing. *Journal of Biological Chemistry* 261:11880-11889, 1986.
15. Ørskov C, Holst JJ, Knuhtsen S, Baldissera FG, Poulsen SS, Nielsen OV: Glucagon-like peptides GLP-1 and GLP-2, predicted products of the glucagon gene, are secreted separately from pig small intestine but not pancreas. *Endocrinology* 119:1467-1475, 1986.
16. Ørskov C, Holst JJ, Poulsen SS, Kirkegaard P: Pancreatic and intestinal processing of proglucagon in man. *Diabetologia* 30:874-881, 1987.
17. Ørskov C, Andreasen J, Holst JJ: All products of proglucagon are elevated in plasma from uremic patients. *J.Clin.Endocrinol.Metab.* 74:379-384, 1992.
18. Ørskov C, Bersani M, Johnsen AH, Hojrup P, Holst JJ: Complete sequences of glucagon-like peptide-1 from human and pig small intestine. *J.Biol.Chem.* 264:12826-12829, 1989
19. Thim L, Moody AJ: The Primary Structure of Porcine Glicentin (Proglucagon). *Regulatory Peptides* 2:139-150, 1981
20. Holst JJ, Ørskov C, Nielsen OV, Schwartz TW: Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. *FEBS Lett.* 211:169-174, 1987
21. Mojsov S, Weir GC, Habener JF: Insulinotropic - Glucagonlike Peptide-I (7-37) Co-Encoded in the Glucagon Gene Is A Potent Stimulator of Insulin Release in the Perfused Rat Pancreas. *Journal of Clinical Investigation* 79:616-619, 1987.
22. Drucker DJ: Glucagon-like peptide 2. *Journal of Clinical Endocrinology and Metabolism* 86:1759-1764, 2001.
23. Creutzfeldt W, Ebert R: New developments in the incretin concept. *Diabetologia* 28:565-573, 1985.
24. Creutzfeldt W, Nauck M: Gut hormones and diabetes mellitus. *Diabetes Metab.Rev.* 8:149-177, 1992.
25. Kreyman B, Williams G, Ghatei MA, Bloom SR: Glucagon-like peptide-1 7-36: a physiological incretin in man. *Lancet* 2:1300-1304, 1987.
26. Perley MJ, Kipnis DM: Plasma Insulin Responses to Oral and Intravenous Glucose - Studies in Normal and Diabetic Subjects. *Journal of Clinical Investigation* 46:1954-1962, 1967.
27. Morgan LM: The role of gastrointestinal hormones in carbohydrate and lipid metabolism and homeostasis: effects of gastric inhibitory polypeptide and glucagon-like peptide-1. *Biochem.Soc.Trans.* 26:216-222, 1998.
28. O'Harte FP, Abdel-Wahab YH, Conlon JM, Flatt PR: Amino terminal glycation of gastric inhibitory polypeptide enhances its insulinotropic action on clonal pancreatic B-cells. *Biochim.Biophys.Acta* 1425:319-327, 1998.
29. Ding WG, Renstrom E, Rorsman P, Buschard K, Gromada J: Glucagon-like peptide I and glucose-dependent insulinotropic polypeptide stimulate  $Ca^{2+}$ -induced secretion in rat alpha-cells by a protein kinase A-mediated mechanism. *Diabetes* 46:792-800, 1997.
30. Miyawaki K, Yamada Y, Ban N, Ihara Y, Tsukiyama K, Zhou HY, Fujimoto S, Oku A, Tsuda K, Toyokuni S, Hiai H, Mizunoya W, Fushiki T, Holst JJ, Makino M, Tashita A, Kobara Y, Tsubamoto Y, Jinnouchi T, Jomori T, Seino Y: Inhibition of gastric inhibitory polypeptide signaling prevents obesity. *Nature Medicine* 8:738-742, 2002.
31. Yip RG, Boylan MO, Kieffer TJ, Wolfe MM: Functional GIP receptors are present on adipocytes. *Endocrinology* 139:4004-4007, 1998.
32. Elliott RM, Morgan LM, Tredger JA, Deacon S, Wright J, Marks V: Glucagon-like peptide-1 (7-36)amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns. *J.Endocrinol.* 138:159-166, 1993.
33. Ørskov C, Wettergren A, Holst JJ: Secretion of the incretin hormones glucagon-like peptide-1 and gastric inhibitory polypeptide correlates with insulin secretion in normal man throughout the day. *Scand.J.Gastroenterol.* 31:665-670, 1996.

34. Layer P, Holst JJ, Grandt D, Goebell H: Ileal release of glucagon-like peptide-1 (GLP-1). Association with inhibition of gastric acid secretion in humans. *Dig.Dis.Sci.* 40:1074-1082, 1995.
35. Gromada J, Holst JJ, Rorsman P: Cellular regulation of islet hormone secretion by the incretin hormone glucagon-like peptide 1. *Pflugers Arch.* 435:583-594, 1998.
36. Fehmann HC, Goke R, Goke B: Cell and molecular biology of the incretin hormones glucagon-like peptide-I and glucose-dependent insulin releasing polypeptide. *Endocr.Rev.* 16:390-410, 1995.
37. Stoffers DA, Kieffer TJ, Hussain MA, Drucker DJ, Bonner-Weir S, Habener JF, Egan JM: Insulinotropic glucagon-like peptide 1 agonists stimulate expression of homeodomain protein IDX-1 and increase islet size in mouse pancreas. *Diabetes* 49:741-748, 2000.
38. Nauck MA, Heimesaat MM, Behle K, Holst JJ, Nauck MS, Ritzel R, Hufner M, Schmiegel WH: Effects of glucagon-like peptide 1 on counter-regulatory hormone responses, cognitive functions, and insulin secretion during hyperinsulinemic, stepped hypoglycemic clamp experiments in healthy volunteers. *Journal of Clinical Endocrinology and Metabolism* 87:1239-1246, 2002.
39. Hvidberg A, Nielsen MT, Hilsted J, Øskov C, Holst JJ: Effect of glucagon-like peptide-1 (proglucagon 78-107amide) on hepatic glucose production in healthy man. *Metabolism* 43:104-108, 1994.
40. Larsson H, Holst JJ, Ahren B: Glucagon-like peptide-1 reduces hepatic glucose production indirectly through insulin and glucagon in humans. *Acta Physiol.Scand.* 160:413-422, 1997.
41. Wettergren A, Schjoldager B, Mortensen PE, Myhre J, Christiansen J, Holst JJ: Truncated GLP-1 (proglucagon 78-107-amide) inhibits gastric and pancreatic functions in man. *Dig.Dis.Sci.* 38:665-673, 1993.
42. Willms B, Werner J, Holst JJ, Øskov C, Creutzfeldt W, Nauck MA: Gastric emptying, glucose responses, and insulin secretion after a liquid test meal: effects of exogenous glucagon-like peptide-1 (GLP-1)-(7-36) amide in type 2 (noninsulin-dependent) diabetic patients. *J.Clin.Endocrinol.Metab.* 81:327-332, 1996.
43. Flint A, Raben A, Astrup A, Holst JJ: Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *J.Clin.Invest.* 101:515-520, 1998.
44. Gutzwiller JP, Drewe J, Goke B, Schmidt H, Rohrer B, Lareida J, Beglinger C: Glucagon-like peptide-1 promotes satiety and reduces food intake in patients with diabetes mellitus type 2. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* 276:R1541-R1544, 1999.
45. Edwards CMB, Todd JF, Mahmoudi M, Wang ZL, Wang RM, Ghatei MA, Bloom SR: Glucagon-like peptide 1 has a physiological role in the control of postprandial glucose in humans - Studies with the antagonist exendin 9-39. *Diabetes* 48:86-93, 1999.
46. Scrocchi LA, Brown TJ, McClusky N, Brubaker PL, Auerbach AB, Joyner AL, Drucker DJ: Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. *Nat.Med.* 2:1254-1258, 1996.
47. Miyawaki K, Yamada Y, Yano H, Niwa H, Ban N, Ihara Y, Kubota A, Fujimoto S, Kajikawa M, Kuroe A, Tsuda K, Hashimoto H, Yamashita T, Jomori T, Tashiro F, Miyazaki J, Seino Y: Glucose intolerance caused by a defect in the entero-insular axis: a study in gastric inhibitory polypeptide receptor knockout mice. *Proc.Natl.Acad.Sci.U.S.A* 96:14843-14847, 1999.
48. Jia X, Brown JC, Ma P, Pederson RA, McIntosh CHS: Effects of glucose-dependent insulinotropic polypeptide and glucagon-like peptide-I-(7-36) on insulin-secretion. *American Journal of Physiology-Endocrinology and Metabolism* 31:E645-E651, 1995.
49. Elahi D, McAloon-Dyke M, Fukagawa NK, Meneilly GS, Sclater AL, Minaker KL, Habener JF, Andersen DK: The insulinotropic actions of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (7-37) in normal and diabetic subjects. *Regul.Pept.* 51:63-74, 1994.
50. Nauck MA, Bartels E, Øskov C, Ebert R, Creutzfeldt W: Additive insulinotropic effects of exogenous synthetic human gastric inhibitory polypeptide and glucagon-like peptide-1-(7-36) amide infused at near-physiological insulinotropic hormone and glucose concentrations. *J.Clin.Endocrinol.Metab.* 76:912-917, 1993.
51. Toft-Nielsen M, Madsbad S, Holst JJ: Exaggerated secretion of glucagon-like peptide-1 (GLP-1) could cause reactive hypoglycaemia. *Diabetologia* 41:1180-1186, 1998.
52. Nauck M, Schmidt WE, Ebert R, Strietzel J, Cantor P, Hoffmann G, Creutzfeldt W: Insulinotropic properties of synthetic human gastric inhibitory polypeptide in man: interactions with glucose, phenylalanine, and cholecystokinin-8. *J.Clin.Endocrinol.Metab.* 69:654-662, 1989.
53. Nauck M, Stockmann F, Ebert R, Creutzfeldt W: Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. *Diabetologia* 29:46-52, 1986.
54. Nauck MA, Homberger E, Siegel EG, Allen RC, Eaton RP, Ebert R, Creutzfeldt W: Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. *J.Clin.Endocrinol.Metab* 63:492-498, 1986.
55. Nauck MA, Kleine N, Øskov C, Holst JJ, Willms B, Creutzfeldt W: Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7-36 amide) in type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 36:741-744, 1993.
56. Zander M, Madsbad S, Madsen JL, Holst JJ: Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. *Lancet* 359:824-830, 2002.
57. Vilsbøll T, Krarup T, Madsbad S, Holst JJ: No reactive hypoglycaemia in Type 2 diabetic patients after subcutaneous administration of GLP-1 and intravenous glucose. *Diabet.Med* 18:144-149, 2001.
58. Knop F, Vilsbøll T, Larsen S, Madsbad S, Holst JJ, Krarup T: No hypoglycaemia after subcutaneous administration of glucagon-like peptide-1 in lean type 2 diabetic patients and in patients with diabetes secondary to chronic pancreatitis. *Diabetes Care* 26:2581-2587, 2003.
59. Deacon CF, Nauck MA, Toft-Nielsen MB, Pridal L, Willms B, Holst JJ: Both subcutaneously and intravenously administered glucagon-like peptide I are rapidly degraded from the NH<sub>2</sub>-terminus in type II diabetic patients and in healthy subjects. *Diabetes* 44:1126-1131, 1995.
60. Deacon CF, Nauck MA, Meier J, Hucking K, Holst JJ: Degradation of endogenous and exogenous gastric inhibitory polypeptide in healthy and in type 2 diabetic subjects as revealed using a new assay for the intact peptide. *J.Clin.Endocrinol.Metab* 85:3575-3581, 2000.
61. Pederson RA, Kieffer TJ, Pauly R, Kofod H, Kwong J, McIntosh CH: The enteroinsular axis in dipeptidyl peptidase IV-negative rats. *Metabolism* 45:1335-1341, 1996.
62. Kieffer TJ, McIntosh CHS, Pederson RA: Degradation of Glucose-Dependent Insulinotropic Polypeptide and Truncated Glucagon-Like Peptide-1 In-Vitro and In-Vivo by Dipeptidyl Peptidase-Iv. *Endocrinology* 136:3585-3596, 1995.
63. Mentlein R: Dipeptidyl-peptidase IV (CD26)-role in the inactivation of regulatory peptides. *Regul.Pept.* 85:9-24, 1999.
64. Brown JC, Dryburgh JR, Ross SA, Dupre J: Identification and actions of gastric inhibitory polypeptide. *Recent.Prog.Horm.Res.* 31:487-532, 1975.
65. Weir GC, Mojsov S, Hendrick GK, Habener JF: Glucagonlike peptide I (7-37) actions on endocrine pancreas. *Diabetes* 38:338-342, 1989.
66. Deacon CF, Pridal L, Klarskov L, Olesen M, Holst JJ: Glucagon-like peptide 1 undergoes differential tissue-specific metabolism in the anesthetized pig. *Am.J.Physiol.* 271:E458-E464, 1996.
67. Fukase N, Igarashi M, Takahashi H, Manaka H, Yamatani K, Daimon M, Tominaga M, Sasaki H: Hypersecretion of Truncated Glucagon-Like Peptide-1 and Gastric-Inhibitory Polypeptide in Obese Patients. *Diabetic Medicine* 10:44-49, 1993.
68. Fukase N, Manaka H, Sugiyama K, Takahashi H, Igarashi M, Daimon M, Yamatani K, Tominaga M, Sasaki H: Response of Truncated Glucagon-Like Peptide-1 and Gastric-Inhibitory Polypeptide to Glucose-Ingestion in Non-Insulin-Dependent Diabetes-Mellitus - Effect of Sulfonyleurea Therapy. *Acta Diabetologica* 32:165-169, 1995.
69. Ranganath LR, Beety JM, Morgan LM, Wright JW, Howland R, Marks V: Attenuated GLP-1 secretion in obesity: Cause or consequence? *Gut* 38:916-919, 1996.
70. Vaag AA, Holst JJ, Vølund A, Beck NH: Gut incretin hormones in identical twins discordant for non-insulin-dependent diabetes mellitus (NIDDM) - evidence for decreased glucagon-like peptide 1 secretion during oral glucose ingestion in NIDDM twins. *Eur.J.Endocrinol.* 135:425-432, 1996.
71. Naslund E, Hellstrom PM: Glucagon-like peptide-1 in the pathogenesis of obesity. *Drug News & Perspectives* 11:92-97, 1998.
72. Ørskov C, Jeppesen J, Madsbad S, Holst JJ: Proglucagon products in plasma of noninsulin-dependent diabetics and nondiabetic controls in the fasting state and after oral glucose and intravenous arginine. *J.Clin.Invest* 87:415-423, 1991.
73. Ahren B, Larsson H, Holst JJ: Reduced gastric inhibitory polypeptide but normal glucagon-like peptide 1 response to oral glucose in postmenopausal women with impaired glucose tolerance. *Eur.J.Endocrinol.* 137:127-131, 1997.
74. Nauck MA, Heimesaat MM, Ørskov C, Holst JJ, Ebert R, Creutzfeldt W: Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *J.Clin.Invest.* 91:301-307, 1993.
75. Vilsbøll T, Krarup T, Deacon CF, Madsbad S, Holst JJ: Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes* 50:609-613, 2001.
76. Lugari R, Dell'Anna C, Ugolotti D, Dei Cas A, Barilli AL, Zandomenighi R, Marani B, Lotti M, Orlandini A, Gnudi A: Effect of nutrient ingestion on glucagon-like peptide 1 (7-36 amide) secretion in human type 1 and type 2 diabetes. *Hormone and Metabolic Research* 32:424-428, 2000.
77. Miholic J, Ørskov C, Holst JJ, Kotzerke J, Meyer HJ: Emptying of the gastric substitute, glucagon-like peptide-1 (GLP-1), and reactive hypoglycemia after total gastrectomy. *Dig.Dis.Sci.* 36:1361-1370, 1991.

78. Qualmann C, Nauck MA, Holst JJ, Ørskov C, Creutzfeldt W: Glucagon-like peptide 1 (7-36 amide) secretion in response to luminal sucrose from the upper and lower gut. A study using alpha-glucosidase inhibition (acarbose). *Scand.J.Gastroenterol.* 30:892-896, 1995.
79. Kong MF, Horowitz M: Gastric emptying in diabetes mellitus: relationship to blood-glucose control. *Clin.Geriatr.Med* 15:321-338, 1999.
80. Maddox A, Horowitz M, Wishart J, Collins P: Gastric and oesophageal emptying in obesity. *Scand.J.Gastroenterol.* 24:593-598, 1989.
81. Wisen O, Johansson C: Gastrointestinal function in obesity: motility, secretion, and absorption following a liquid test meal. *Metabolism* 41: 390-395, 1992.
82. Toft-Nielsen MB, Damholt MB, Madsbad S, Hilsted LM, Hughes TE, Michelsen BK, Holst JJ: Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients. *J.Clin.Endocrinol.Metab* 86:3717-3723, 2001.
83. Vilsbøll T, Krarup T, Sonne J, Madsbad S, Vølund A, Juul AG, Holst JJ: Incretin Secretion in Relation to Meal Size and Body Weight in Healthy Subjects and People With Type 1 and Type 2 Diabetes Mellitus. *J.Clin. Endocrinol.Metab* 88:2706-2713, 2003.
84. Lugari R, Dei Cas A, Ugolotti D, Finardi L, Barilli AL, Ognibene C, Luciani A, Zandomenighi R, Gnudi A: Evidence for early impairment of glucagon-like peptide 1-induced insulin secretion in human type 2 (non insulin-dependent) diabetes. *Hormone and Metabolic Research* 34:150-154, 2002.
85. Vilsbøll T, Agersø H, Krarup T, Holst JJ: Similar elimination rates of glucagon-like Peptide-1 in obese type 2 diabetic patients and healthy subjects. *J.Clin.Endocrinol.Metab* 88:220-224, 2003.
86. Nyholm B, Walker M, Gravholt CH, Shearing PA, Sturis J, Alberti KG, Holst JJ, Schmitz O: Twenty-four-hour insulin secretion rates, circulating concentrations of fuel substrates and gut incretin hormones in healthy offspring of Type II (non-insulin-dependent) diabetic parents: evidence of several aberrations. *Diabetologia* 42:1314-1323, 1999.
87. Pederson RA, Brown JC: Insulinotropic Action of Gastric Inhibitory Polypeptide in Perfused Isolated Rat Pancreas. *Endocrinology* 99:780-785, 1976.
88. Vilsbøll T, Krarup T, Madsbad S, Holst JJ: Both GLP-1 and GIP are insulinotropic at basal and postprandial glucose levels and contribute nearly equally to the incretin effect of a meal in healthy subjects. *Regul.Pept.* 114:115-121, 2003.
89. Porte D: Banting lecture 1990. Beta-cells in type II diabetes mellitus. *Diabetes* 40:166-180, 1991.
90. Vilsbøll T, Toft-Nielsen MB, Krarup T, Madsbad S, Dinesen B, Holst JJ: Evaluation of beta-cell secretory capacity using glucagon-like peptide 1. *Diabetes Care* 23:807-812, 2000.
91. Kjems LL, Holst JJ, Vølund A, Madsbad S: The Influence of GLP-1 on Glucose-Stimulated Insulin Secretion: Effects on beta-Cell Sensitivity in Type 2 and Nondiabetic Subjects. *Diabetes* 52:380-386, 2003.
92. Ward WK, Bolgiano DC, McKnight B, Halter JB, Porte D: Diminished B cell secretory capacity in patients with noninsulin-dependent diabetes mellitus. *J.Clin.Invest.* 74:1318-1328, 1984.
93. Krarup T, Saurbrey N, Moody AJ, Kuhl C, Madsbad S: Effect of porcine gastric inhibitory polypeptide on beta-cell function in type I and type II diabetes mellitus. *Metabolism* 36:677-682, 1987.
94. Vilsbøll T, Krarup T, Madsbad S, Holst JJ: Defective amplification of the late phase insulin response to glucose by GIP in obese Type II diabetic patients. *Diabetologia* 45:1111-1119, 2002.
95. Vilsbøll T, Knop F, Krarup T, Johansen A, Madsbad S, Larsen S, Hansen T, Pedersen O, Holst JJ: The Pathophysiology of Diabetes Involves a Defective Amplification of the Late Phase Insulin Response to Glucose by GIP – Regardless of Etiology and Phenotype. *J.Clin.Endocrinol.Metab.* 88:4897-903, 2003.
96. Goke R, Trautmann ME, Haus E, Richter G, Fehmann HC, Arnold R, Goke B: Signal transmission after GLP-1(7-36)amide binding in RINm5F cells. *Am.J.Physiol* 257:G397-G401, 1989.
97. Gromada J, Rorsman P, Dissing S, Wulff BS: Stimulation of cloned human glucagon-like peptide 1 receptor expressed in HEK 293 cells induces cAMP-dependent activation of calcium-induced calcium release [published erratum appears in *FEBS Lett* 1996 Mar 4;381(3):262]. *FEBS Lett.* 373:182-186, 1995.
98. Gromada J, Dissing S, Bokvist K, Renstrom E, Frokjaer JJ, Wulff BS, Rorsman P: Glucagon-like peptide I increases cytoplasmic calcium in insulin-secreting beta TC3-cells by enhancement of intracellular calcium mobilization. *Diabetes* 44:767-774, 1995.
99. Jia X, Elliott R, Kwok YN, Pederson RA, McIntosh CH: Altered glucose dependence of glucagon-like peptide I(7-36)-induced insulin secretion from the Zucker (fa/fa) rat pancreas. *Diabetes* 44:495-500, 1995.
100. Holst JJ, Gromada J, Nauck MA: The pathogenesis of NIDDM involves a defective expression of the GIP receptor. *Diabetologia* 40:984-986, 1997.
101. Lynn FC, Pamir N, Ng EHC, McIntosh CHS, Kieffer TJ, Pederson RA: Defective glucose-dependent insulinotropic polypeptide receptor expression in diabetic fatty Zucker rats. *Diabetes* 50:1004-1011, 2001.
102. Kubota A, Yamada Y, Hayami T, Yasuda K, Someya Y, Ihara Y, Kagimoto S, Watanabe R, Taminato T, Tsuda K, Seino Y: Identification of two missense mutations in the GIP receptor gene: a functional study and association analysis with NIDDM: no evidence of association with Japanese NIDDM subjects. *Diabetes* 45:1701-1705, 1996.
103. Meier JJ, Hucking K, Holst JJ, Deacon, CF, Schmiegel, WH, Nauck, MA: Reduced insulinotropic effect of gastric inhibitory polypeptide in first-degree relatives of patients with type 2 diabetes. *Diabetes* 50:2497-2504, 2001.