Glucose-dependent insulinotropic polypeptide: Effects on insulin and glucagon secretion in humans

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INTRODUCTION

It has been recognised for more than a century that oral glucose intake does not induce hyperglycaemia as much as intravenous glucose. After the invent of reliable insulin measurements, a new paradigm was laid out, where, at that time unknown, gut factors were acknowledged as responsible for more than half of the total insulin secretion during a glucose meal (1,2). These gut factors were called “incretins”, which actually was a term first used by *La Barre* 30 years earlier (before the availability of insulin measurements) to designate a stimulating effect of crude secretin preparations on the internal (i.e. endocrine) pancreas (2–4). Around 1969, a peptide containing 42 amino acids was isolated from preparations of the duodenal mucosa, and named gastric inhibitory polypeptide (GIP) because it had an inhibitory effect on gastric acid secretion in preparations of dog stomachs (5). Importantly a few years later in 1973, GIP was shown to stimulate human insulin secretion in a glucose-dependent manner and in physiological concentrations - the first human incretin hormone had been discovered (6). To uphold the acronym GIP, the designation “glucose-dependent insulinotropic polypeptide” was amended a few years later as an alternative full name to better reflect the principal status as an incretin (7). In 1987 another incretin hormone, glucagon-like peptide-1 (GLP-1) was discovered (8–10). Today, GIP and GLP-1 remain the only identified gut peptides capable of stimulating insulin secretion under physiological circumstances. The accumulating scientific data on the incretin hormones has led to a wider appreciation of the gut as an integrator of processes across the range of nutrient metabolism, from food intake to substrate disposition. Importantly, the discovery of the specific incretin hormones, their signalling pathways, and their physiology has culminated in the development of new pharmacological agents to treat diabetes. Despite this translational achievement and widespread clinical use of incretin-based therapy, the research on the incretin hormones continues to yield interesting and clinically important information. This thesis concerns *in vivo* studies in humans regarding the effects of both of the incretin hormones, GIP and GLP-1. However, in the following sections emphasis is put on GIP, as the majority of investigations in this thesis relate to this peptide, and because GIP, despite its longer career, by far is the lesser characterised of the two incretin hormone. This is a slightly abbreviated version of the original thesis (11).

EFFECTS OF GIP ON PANCREAS ISLETS

During the past three decades the insulinotropic effect of GIP has been confirmed in numerous preclinical and clinical studies. Likewise, extensive scientific data exist on the molecular characteristics of the signal-transduction pathways involved in the insulin secretory response, including a number of recent reviews summarising these effects (12,13). In short, activation of the GIP receptor in the pancreatic beta cell leads to increases in cyclic adenosine monophosphate (cAMP) levels, activation of protein kinase A and exchange proteins activated by cAMP (EPAC), causing calcium influx through voltage-dependent calcium channels and ultimately augmented calcium-dependent exocytosis from the insulin secretory granules (14–16). A multiplicity of other signalling pathways may also be triggered by activation of the GIP receptor, many of these perhaps secondary to the rise in cAMP. These pathways include (but are not limited to) activation of mitogen-activated protein kinases, calcium-independent phospholipase A_2

and phosphatidylinositol 3-dependent kinases (13,17–21). Moreover, GIP (and GLP-1) receptor stimulation in beta cells results in insulin gene transcription and insulin biosynthesis and in rodent cell lines also to enhanced beta cell proliferation and survival (13,18–21). Although, in humans, the clinical effects of GIP and GLP-1 are clearly disparate, little distinction has been made with respect to specific signalling pathways selectively engaged by one incretin hormone, but not the other. However, recent data concerning the mechanisms for beta cell survival have suggested that the insulin receptor substrate 2 (IRS-2) and epidermal growth factor receptor (EGFR) involved in beta cell survival and growth were preferentially activated by GLP-1 and not by GIP (22).

Insulinotropic effects

The classical way of assessing the clinical insulinotropic responses to GIP is by using the hyperglycaemic clamp technique (see below) or *iv* isoglycaemic clamps and comparing C-peptide responses during infusions of exogenous GIP with those achieved with a placebo infusion (often saline). On this basis, the insulinotropic action of GIP in humans was described as glucose-dependent as early as 1973 (6). This glucose-dependency has later been characterised in several *in vivo* studies in humans where GIP in physiological and pharmacological amounts has been infused at euglycaemic and hyperglycaemic conditions. No studies prior to the present investigations have infused GIP during hypoglycaemia, although there was an early attempt to investigate GIP during hypoglycaemia (using a rather unspecific approach by administering a meal that increased endogenous GIP secretion) (23). Early infusion studies (i.e. prior to 1989 (24)) involved infusion of porcine GIP, which differs from the human peptide in amino acids position 18 and position 34. This precluded exact matching of endogenous GIP concentrations and reliable measurement of the total GIP (i.e. including both human and porcine sequence GIP) during infusions studies. Moreover, the early preparations of GIP had impurities including high concentrations of GIP[3-42], which is now known to act as a competitive GIP receptor antagonist with possible clinical relevance in high doses (25–27). As a consequence, some of the early studies using porcine GIP have to be interpreted with caution. A compilation of previous placebo-controlled human studies of healthy volunteers or patients with type 2 diabetes using hyperglycaemic clamping and stable 'near-physiological' infusions of human GIP can be found in the original thesis (11). From this it is evident that GIP in high doses in healthy individuals stimulates C-peptide concentrations by approximately 300-400 pM per mmol increase in plasma glucose (11).

Glucagonotropic effects

As early as in 1978 preclinical studies designated glucagon-releasing properties to GIP by demonstrating that that GIP augments glucagon secretion in the perfused rat and canine, at low glucose concentrations (28,29). The following years, porcine GIP administered *iv* (1.3 pmol×kg^{-1}×min^{-1}) in a handful of healthy subjects during fasting or hyperglycaemic conditions (30) or in the perfused porcine pancreas did not elicit any detectable glucagon responses (31). In 1990, however, porcine GIP demonstrated glucagonotropic actions in perfused pancreata from human cadavers (32) and in patients with cirrhosis of the liver characterised by fasting hyperglucagonaemia (33). In the meantime, the mechanistic basis for the glucagonotropic effect in rodents was discovered to be partly similar to the one active in beta cells, as GIP was shown to stimulate glucagon secretion in rat alpha cells through activation of protein kinase A (34). Subsequent studies in perfused rat pancreata, at low levels of glycaemia, outlined the difference between the incretins concerning glucagon secretion (35). Thus, GLP-1 inhibited glucagon secretion (likely via somatostatin-dependent paracrine signalling), while GIP stimulated glucagon secretion directly (35). Interestingly, a study from 1995 demonstrated that during infusion of GIP or GLP-1 together with physiological amounts of amino acids (i.e. as observed after a protein rich meal) insulinotropic effects were similar, but the glucose lowering effect was less with GIP (36). This could be explained by (but was not suggested by the authors) a seemingly greater glucagon response during GIP infusion. Nevertheless, in healthy humans the glucagonotropic effect of GIP *in vivo* was reported for the first time in 2003 when administration of GIP as a bolus injection during fasting glycaemia (i.e. fasting plasma glucose of 5.7 mM) resulted in dose-dependent increase in plasma glucagon (37). Same year, when GIP was administered as a 'physiological' infusion (1.5 pmol×kg^{-1}×min^{-1} for 30 minutes) in healthy humans also clamped at euglycaemia (5.1 mM), a slight glucagonotropic effect was also elicited (reanalysis of the original raw data, kindly provided by *Dr. Vilsbøll*), and when plasma glucose was subsequently elevated to 6 and 7 mM, glucagon levels was increasingly suppressed (38). During hyperglycaemic conditions several studies have shown that GIP infusion does not exert glucagonotropic effect in healthy subjects (24,39–42).

GIP IN PATIENTS WITH TYPE 2 DIABETES

A specific interest in the role of GIP in type 2 diabetes was fuelled by seminal studies published in 1993 where *Nauck et al.* investigated and compared the efficacy of the two incretin hormones (of human amino acid sequence) during hyperglycaemic clamping experiments (mean plasma glucose 8.75 mM) (39). Their results demonstrated a reduced insulinotropic effect of GIP, while that of GLP-1 was relatively preserved. This led to the conclusion that the reduced incretin effect in patients with type 2 diabetes most likely could be explained by reduced insulinotropic effectiveness of GIP (39). Several years later, it was established that a defective insulinotropic effect of GIP (again in comparison to GLP-1) does not exclusively apply to patients with type 2 diabetes. Thus, patients with diabetes of various aetiologies (including secondary diabetes to chronic pancreatitis, monogenic diabetes and latent autoimmune diabetes) all share the common pathophysiological feature of an impaired GIP-induced insulin secretion relative to GLP-1 (43,44). It is important to keep in mind, that a large part of the studies in this thesis concerns the isolated effect of GIP (compared to saline), and the notion of a 'GIP defect' in diabetes, was based on the comparable efficacy between GLP-1 and GIP. Or put in another way it was the effectiveness of GLP-1 that raised the question of why GIP is inefficient.

Reduced insulinotropic effect of GIP was actually observed as early as in 1987, when 'physiological' doses of porcine GIP (2 pmol×kg^{-1}×min^{-1}) stimulated C-peptide levels much less in patients with type 2 diabetes, than in control subjects (45). The patients were clamped at 8 mM glucose (i.e. about fasting levels for the patients), and at that time, it was concluded that the impaired secretion most likely reflected the reduced number of functional beta cells in the patients, rather than an altered responsiveness to GIP (45). Before that study, the insulinotropic effect of GIP in patients with type 2 diabetes had only been described after short term infusion (20 minutes) of porcine GIP in patients with type 2 diabetes at fasting glycaemia (mean fasting plasma glucose of ~11

mM) and healthy controls (mean fasting plasma glucose ~5 mM) and these results actually showed increases in insulin and C-peptide only in the patients and not in the control subjects (46). Subsequent studies still using porcine GIP in 20-minutes infusions administered to patients with type 2 diabetes (mean fasting plasma glucose ~12 mM) demonstrated only an early phase insulin response (47). Notably, in none of the studies performed at fasting glycaemia did the early phase insulin response have any effect on glycaemia, and glucagon was not measured (46,47).

The 'GIP defect' was studied in greater detail in the early 2000s. In a study from 2001, it was demonstrated that 50% of first-degree relatives of patients with type 2 diabetes exhibited a decreased insulin responses to GIP (2 pmol×kg^{-1}×min^{-1}) during a hyperglycaemic clamp (mean plasma glucose 7.8 mM) (40). However, also the insulinotropic response to *iv* glucose was diminished in these first-degree relatives. This might have been expected from the evidence showing a reduced beta cell function also in the prediabetic state in predisposed individuals (48,49). In investigations from 2002, large doses of GIP (up to 16 pmol×kg^{-1}×min^{-1}) were infused during manifest hyperglycaemic clamping (15 mM) and compared with responses to GLP-1 (50). It was found that the ratio between first phase insulin responses to GIP and GLP-1 was actually completely normal, whereas the second phase response to GIP was severely impaired. It is important to note that GIP actually did induce a small, but significant late phase insulin and C-peptide responses, but compared to GLP-1, these responses were negligible (reanalysis of original raw data from (50) with kind permission from *Dr. Vilsbøll*). Further studies from 2003, elaborated on the early phase insulin response to bolus injections of GIP at fasting glycaemic levels by showing similar dose-dependent insulin responses again in first-degree relatives of patients with type 2 diabetes and control subjects (51,52). Finally, a study from 2004 investigated the combination of bolus injections of GIP alongside continuous infusions of GIP (at rates from 1 to 4 pmol×kg^{-1}×min^{-1}) in patients with type 2 diabetes and healthy control subjects (53). The glucose levels were again clamped at ~7.8 mM, which was close to the fasting levels for the patients with type 2 diabetes (41). Interestingly, the data suggested that although the absolute amount of insulin released in response to the GIP boluses was substantially lower in the patients, the pattern of the dose-response curve was almost identical to the one of healthy control subjects, indicating similar relative beta cell sensitivity to GIP administered as bolus injections (41).

Summarising the findings described above on insulin secretory responses to GIP, three important features become clear: 1) the early phase response to GIP is quite preserved in patients with type 2 diabetes indicating a relatively unaltered expression of GIP receptors on beta cells in these patients, 2) the late phase insulin secretory response to GIP is clearly impaired in patients with type 2 diabetes suggesting that diabetes-induced alterations in the GIP receptor stimulus-secretion coupling involves only the late phase insulin response, and 3) the difference between GIP and GLP-1 seems to apply only to the late phase insulin response. When considering these features in combination, it follows that the similarity of GIP and GLP-1 intracellular signalling pathways (specific but closely related receptors acting through cAMP (54)) must apply mainly to the early phase insulin response in type 2 diabetes. Hence, the similar early phase signalling cascade reasonably explains why GIP and GLP-1 are equally effective, when administered as a bolus. The background for feature number 2 and 3 is still not clear. The general lack of knowledge on the cellular mechanisms leading to the second phase insulin secretion is of course part of this mystery (55). Thus, cellular investigations on the reduced effect of GIP in patients with type 2 diabetes understandably have focused on the potential pathophysiology of the GIP receptors (15), with relatively little distinction between GIP and GLP-1 and a disregard for the relatively preserved first phase response to GIP. Thus, genetic factors are presumed to play a role for the incretin responses, as decreased expression of the transcription factor 7-like 2 (TCF7L2) protein transcription factor, an important determinant of type 2 diabetes risk (56), correlate with down-regulation of GIP and GLP-1 receptors and impaired beta cell function (57,58), but does not provide an explanation for the differences between the two incretin hormones. There is evidence that the GIP receptor is down-regulated in rodent models of type 2 diabetes (59) and chronic desensitisation of the GIP receptor (through alterations of the cytoplasmic part of the receptor) has also been described (60,61). The mechanism behind the receptor down-regulation, was initially thought to be chronically elevated GIP levels, but GIP hypersecretion does not seem to be a consistent feature of type 2 diabetes or other forms of diabetes (62,63). Therefore, it seems more plausible that desensitisation, if it occurs in diabetes, is a consequence of common diabetic pathology such as glucotoxicity (64–66), lipotoxicity (67,68) or a combination of the two. Another suggestion recently put forth for receptor deficiency is that impaired N-glycosylation (critical for functional expression i.e. externalisation of receptors) of the GIP receptor in diabetes could lead to reduced functional GIP receptor expression (69). These alterations in absolute receptor numbers do not convincingly explain the selective impairment the late phase GIP response as compared to the early phase.

Conclusively, at present the differential effects of the incretin hormones on late phase insulin secretion in patients with type 2 diabetes, have only inadequately been linked to a specific biochemical step (70). Nevertheless, an improved understanding of the beta cell responses to GIP as opposed to GLP-1 could prove instrumental in unravelling the mechanisms behind the generally impaired late phase insulin response in patients with type 2 diabetes.

Recently and very important for this thesis, GIP has been implicated as one of the causal factors in postprandial hyperglucagonaemia patients with type 2 diabetes. It is quite well established that patients with type 2 diabetes are characterised by fasting and postprandial hyperglucagonaemia, which leads to increased rates of hepatic glucose production and thereby to elevations of fasting and postprandial blood glucose levels (114,115). In fact, studies indicate that postabsorptive hyperglucagonaemia is responsible for as much as 50% of the increment in plasma glucose excursions following oral glucose ingestion in patients with diabetes (73–75). After oral intake of glucose a delayed and diminished glucagon suppression is observed compared to following *iv* administration of glucose (producing plasma glucose excursions identical to the oral intake), where the glucagon levels are adequately suppressed (76,77). This implies that gut-derived factors contribute to the derangement of postprandial glucagon responses, or alternatively that measurable glucagon is released from the intestine (78). Investigations by *Lund et al.* have suggested that GIP could contribute substantially to this postprandial glucagon response (79). Likewise, a recent study administering supraphysiological doses of GIP (4 pmol×kg^{-1}×min^{-1}) on top of a mixed meal demonstrated that GIP compared to placebo worsened postprandial glycaemic excursions concomitant with elevated glucagon levels (80). Thus, a possible role of GIP in glucagon

secretion, which in situations with elevated plasma glucose levels essentially is inappropriate, could be an important element of the (patho)physiological role of GIP in type 2 diabetes.

EXTRAPANCREATIC EFFECTS OF GIP

The widespread expression of the GIP receptor and evidence from the transgenic mice suggest other roles for GIP besides regulation of the insulin and glucagon secretion (81). Results from human *in vivo* studies indicate that GIP, in contrast to GLP-1, does not seem to have any effect on gastric emptying (82) or energy intake (83,84). One small study has reported a (statistically non-significant) trend suggesting that GIP reduces resting energy expenditure and increases subjective feelings of hunger in normal weight healthy subjects (83). However, a more adequately powered study including 20 healthy subjects did not find any difference in effects on appetite, energy intake or resting energy expenditure when comparing GIP with placebo (84). Very recently, a comprehensive study reported that the combination of GIP and GLP-1 (as a single molecule co-agonist) provided greater metabolic efficacy than selective mono-agonism in rodents, primates as well as humans (85). The metabolic efficacy included improved glycaemic control and improved weight loss of co-agonism compared to either GLP-1 or GIP alone. This added effect of GIP is currently unexplained and contrasts somewhat to the general notion of GIP as a lipid anabolic hormone.

Role of GIP in lipid metabolism

The possible aetiological role of GIP in obesity have been suspected for long and as mentioned above, received additional attention ten years ago, when deletion of the GIP receptor gene in leptin-deficient mice, was demonstrated to avert the development of obesity following high-fat dieting (86). Also, several bits of *in vitro* evidence support a role for GIP in lipid metabolism at least in rodents. Thus, functional GIP receptors are present on adipocytes (87), and GIP seems to increasing fat storage in adipocytes (88), possibly through promotion of glucose transport into fat cells (89,90), stimulatory effect on adipocyte lipoprotein lipase (91,92) and enhanced adipocyte lipolysis and re-esterification (93). Much of the *in vivo* data concerning GIP effects on fart metabolism is biased by the difficulties of separating the effects of GIP *per se* from GIP's insulinotropic effects. Examples of this are the reports of GIP administration to increase chylomicron clearance in dogs (94) and lower postprandial triglyceride levels in rats (95), and free fatty acid levels in humans (96–98). Thus, recent data suggest that GIP *per se* does not have any effect on plasma triglyceride clearance, but might act synergistically with insulin to increase free fatty acid (FFA) re-esterification in subcutaneous adipose tissue in lean (84,99), but not in obese humans (100). In this context, it might be relevant that the presence of GIP receptors on endothelial cells has been demonstrated in preclinical studies (101). Thus, increased adipose tissue blood flow locally (in subcutaneous fat tissue) was also suspected to constitute a potential mechanism behind the demonstrated increased re-esterification, but importantly, on hole body level, the FFA clearance was not significant elevated by GIP administration (99). More convincing are the recent demonstration of GIP receptors in adipose tissue in humans (102), and more specifically, the fact that visceral fat expression of GIP receptor mRNA was higher than subcutaneous fat in 30 obese non-diabetic subjects undergoing bariatric surgery (102). The study also suggested that GIP, through release of a macrophage-derived cytokine (osteopontin), could trigger inflammation and insulin resistance in adipose tissue (102). Most *in vivo* studies investigating the role of GIP in fat metabolism have been performed on subcutaneous fat tissue, although the metabolic roles of subcutaneous versus visceral fat might be very different as suggested by the much larger contribution of visceral fat depots to cardiometabolic risk profile and diabetes (103). It is noteworthy that high BMI is associated with increased GIP responses to nutrient intake in the general population and patients with type 2 diabetes (62,102). Moreover, interventions such as dietary restrictions in obese patients lowered both fasting and postprandial GIP release (104), short term high fat diet increased fasting GIP levels (105), and treatment with prednisolone increased postprandial GIP responses in healthy lean young men (106). However, these results do not allow for conclusions concerning causality, and could merely reflect compensatory changes without clinical relevance. Therefore, it is at present unknown whether GIP should be regarded as an aetiological contributor to obesity (and diabetes).

Perhaps symptomatic for the generally inconclusive evidence on the causal role of GIP in the pathogenesis of human diabetes and obesity both agonism and antagonism of the GIP receptor have been proposed (even by the same research group) as a treatment against 'obesity-diabetes' (107,108). Much of the evidence concerning antagonism is biased by use of 'antagonists' with intrinsic activity at the GIP receptor. An example is pro3GIP, which is a widely used analogue that has been used to demonstrate the beneficial effects of reduced GIP signalling on weight gain, insulin sensitivity and glucose tolerance in rodents (109–112). This 'antagonist' retains substantial intrinsic activity at the human GIP receptor categorising it as a partial human GIP receptor agonist with more than 50% efficacy as compared to native GIP (27) - which puts its beneficial effects in another light. Others have reported that elimination of GIP action by antagonism in rats (not surprisingly) is associated with a deterioration of glucose intolerance (113,114). Of note, a large molecule GIP receptor antagonist without intrinsic activity was recently developed (115), and did not demonstrate any effects on body weight in a study with continuous administration in mice (Ravn et al. personal communication). Taken together, evidence supports that GIP plays a role in rodent fat metabolism, whereas a role independent of insulin in human lipid metabolism is more questionable. Nevertheless, evidence does suggest that GIP might act in concert with insulin and it is conceivable that GIP acts as an 'insulin-sensitizer' in adipose tissue in humans.

Role of GIP on bone metabolism

Following nutrient intake bone resorption is acutely suppressed (by approximately 50% as measured by plasma markers of bone turnover) and numerous hormones including GIP have been implicated in this acute alteration in bone homeostasis . Whereas clinical data are scarce, *in vitro* data from bone cells and data from rodent models substantiate a role for GIP in bone turnover. First, functional GIP receptors are present on human osteoblast and osteoclast cell lines and addition of GIP to osteoblast cell lines increase collagen 1 and alkaline phosphatase expression, which could be compatible with an anabolic effect on bone (116–118). Also isolated mature ostoclasts have been shown to be inhibited by GIP resulting in diminished resorptive activity (118). Secondly, GIP administered as an intermittent injection has been shown to prevent bone loss in an *in vivo* rat model of osteoporosis (119). Finally, mice with GIP receptor knockout had less bone

formation, smaller bone size, lower bone mass and substantial alterations in bone microarchitecture and biomechanical properties (120).

In humans, acute bolus injection of GIP, which clearly resulted in rather unphysiological plasma levels of GIP (50), did not alter markers of bone turnover (121). Whether more physiological GIP levels will modulate bone turnover in humans is not yet known, but data study 1, where GIP was infused in healthy individuals suggest that a marker of bone resorption (i.e. collagen carboxy-terminal collagen crosslinks (CTX)) is inhibited by up to 50% by the combination of GIP and hyperglycaemia compared to euglycaemia and saline (122).

THE STUDIES INCLUDED IN THIS PHD THESIS

Specific background

In 2010 we published a paper describing investigations focusing on the glucagon-releasing properties of GIP and glucagon-like peptide-2 (GLP-2) (123), which became the background for this PhD thesis and influenced the choice of methods used herein.

As indicated in the section on glucagon secretion above, numerous discrepancies exist in the literature regarding the possible involvement of GIP in human glucagon secretory responses. Renewed interest in gut-derived factors affecting glucagon secretion was nourished by the findings of an initial stimulation of glucagon secretion followed by a delayed suppression of glucagon secretion in patients with type 2 diabetes undergoing oral glucose tolerance tests (OGTT) (76). If the same patients were administered *iv* glucose copying the glucose excursions on the OGTT, they exhibited a normal, immediate suppression of glucagon (76). In healthy volunteers glucagon secretion is suppressed following the OGTT as well as the *iv* challenge, but the suppression is diminished by approximately 30% in the former situation (77). Theoretically, therefore these differences could be attributed to the release of glucagonotropic gut-derived factors during the OGTT. The hormones glucagon-like peptide-2 (GLP-2) and GIP had previously been shown to enhance glucagon release from pancreatic alpha cells both *in vitro* as well as *in vivo* (35,37,124,125). *In vivo* studies of the glucagonotropic actions of GIP and GLP-2 all involved situations where concomitant changes in either insulin secretion (all subjects examined so far exhibited preserved insulin secretion) or glucose concentration (e.g. during hyperglycaemic clamp or postprandial conditions) could have influenced the results. We therefore studied 8 patients with type 1 diabetes without endogenous insulin secretion that could affect glucagon responses. Residual C-peptide secretion was excluded by an arginine test. The study employed a stepped hyperglycaemic clamp where plasma glucose was clamped at 'diabetic' fasting values (mean of 7.4 mM) for the first 90 minutes (*period 1*) and then raised to 1.5 times the fasting values (mean of 11.1 mM) for the final 90 minutes (*period 2*). In randomised order on separate days *iv* infusions of either saline (placebo), GIP, or GLP-2 at rates designed to mimic postprandial hormone levels were carried out for the initial 50 minutes in both periods. Results revealed that the baseline glucagon levels were slightly increased by GLP-2, whereas GIP and saline did not significantly affect the glucagon levels during these situations. The conclusion was that during hyperglycaemia in patients with type 1 diabetes, GIP does not stimulate the release of glucagon. The results from the GIP infusion part of the study are presented in Figure 1.

A few relevant lessons for the studies included in the present thesis were absorbed from that study. First, the results implied that hyperglycaemic clamping in patients with type 1 diabetes suppress glucagon secretion and perhaps that this suppression could impede a possible glucagon stimulating effect of GIP. Secondly, the study suggested that intra-islet insulin (which we excluded in these patients) does not importantly contribute to the suppression glucagon during hyperglycaemia. Finally, an important finding was the confirmation of alpha cell responsiveness to arginine stimulation (Figure. 2). This confirmed previous studies showing preserved secretory responsiveness of the alpha cells to certain amino acids, (but not to hypoglycaemia) in patients with type 1 diabetes (126).

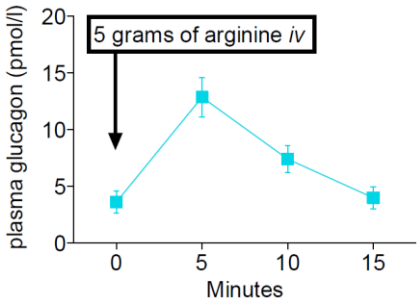

Figure 2
Plasma glucagon values in C-peptide negative patients with type 1 diabetes (n=8) following an arginine bolus infusion at time 0. Unpublished data from ref. (123).

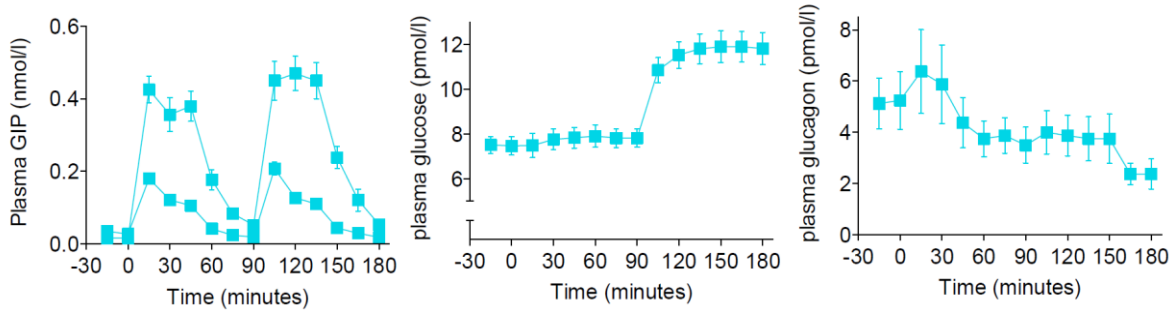

Figure 1
Plasma concentrations of total and intact GIP (left) and glucose (middle) and glucagon as baseline subtracted values (right). The plasma samples were obtained while plasma glucose was clamped at fasting values for the initial 90 minutes (*period 1*) and at hyperglycaemia 1.5 times fasting values for the final 90 minutes (*period 2*). GIP was infused for 50-minute periods initially in each period. Values are mean±SEM

HYPOTHESES AND OBJECTIVES

We hypothesised that the effects of GIP on glucagon, like the effect on insulin secretion, might be glucose-dependent in healthy humans. As a part of this glucose-dependency, a permissive action of low glucose (i.e. euglycaemic and hypoglycaemic) levels would be necessary for the GIP-stimulated glucagon secretion to appear. The objective with ***Study 1*** was therefore to investigate the glucose-dependent effects of GIP on insulin and glucagon secretion in healthy individuals. We also hypothesised that glucagon responses to GIP at lower levels of plasma glucose would be disturbed in patients with type 2 diabetes. The objective with **Study 2** was, therefore, to investigate the glucose-dependent effects of GIP on insulin and glucagon secretion in patients with type 2 diabetes.

Finally, in **Study 3** we hypothesised that GIP could be used in supraphysiological doses to augment glucagon counter-regulation in patients with type 1 diabetes. In addition, we aimed to investigate the effects on endogenous glucose production of GIP.

METHODS

Glycaemic clamps

The glucose clamp technique has been described in detail by *Defronzo et al.* (127). The *hyperglycaemic clamp* is normally used to quantify the beta cell sensitivity to glucose and has gained widespread use (128). In **study 1**, we used a hyperglycaemic clamp almost identical to the originally described technique, as we clamped the healthy individuals at a fixed hyperglycaemic plateau of 12 mM. In **Study 2**, we made an adjustment to the original technique to ensure that the glycaemic impact for each participant was more uniform. The *euglycaemic insulin clamp* technique with the scope of assessing sensitivity to insulin was also described by *Defronzo et al.* (127). Since the scope of our studies was to assess the glucose dependency of GIP (and not the possible change in insulin sensitivity induced by GIP), we did not infuse insulin but only maintained the fasting glycaemia (in both study 1 and 2). The *hypoglycaemic clamps* used in study 1-3 were a modification of methods used in an early experiment assessing lipid-induced GIP secretion (actually only testing fat ingestion) (23). In **Study 3**, we extended the experimental protocol to include the use of stable isotopes to assess endogenous glucose production (129).

Hormone infusions

The GIP infusions utilised in study 1 and 2 were designed with the aim of reaching plasma GIP concentrations within the physiological range as would be observed after a relatively large meal. By targeting levels high in the physiological range, (primed infusion with the steady state rate 2 pmol×kg-1×min-1), we tried to avoid problems with the post hoc interpretation of results and type 2 errors due to sub-maximal hormonal impact. When comparing the GIP responses with responses from other studies in healthy subjects, patients with type 2 diabetes and patients with type 1 diabetes measuring GIP with the same immunoassay (e.g. healthy subjects after a mixed meal (63,130) as well as patients with type 2 diabetes after a mixed meal (131)), it seems fair to conclude that we indeed reached high physiological levels of plasma intact GIP concentrations. These concentrations are slightly higher than those present in the peripheral circulation after less potent GIP-releasing stimuli e.g. oral glucose (52,76,132). It could be argued that the infusion rates perhaps should have been even higher, e.g. 4 pmol×kg-1×min-1 as used in other studies (50,80). The concentration of intact, active GIP is several folds higher in the intestinal and the portal circulation compared to the peripheral circulation (133). It is unclear to what extent, GIP acts via mechanisms initiated in close proximity to the K-cells e.g. on sensory afferent nerve fibres in the intestinal mucosa, receptors in the portal vascular bed including the liver (101,134) or lymph vessels (133). In Study 3 we infused GIP in higher doses and GLP-1 to reach pharmacologically relevant concentrations.

SUMMARY OF STUDIES

Study 1

Study 1 investigated the glucose dependency of the effects of GIP on insulin and glucagon release in healthy individuals. Ten healthy, young, lean, male subjects were clamped at three glycaemic levels i.e. fasting glycaemia (mean of 5.0±0.1 mM), hypoglycaemia (glucose gradually lowered to a mean nadir of 2.4±0.1 mM) and hyperglycaemia (mean of 12.1±0.3 mM). In randomised order on separate days at each glycaemic level, 1 hour-infusions of GIP or saline were administered *iv* during 90 minute-periods at rates designed to reach postprandial GIP levels on the days of GIP infusion. We demonstrated that the effects of GIP on both glucagon and insulin secretion are highly dependent on the prevailing glucose concentrations. During *euglycaemia* GIP infusion elicited glucagon responses during the entire study (86±44 vs. -100±20.7 pM×min, $P<0.003$), but also a minor early phase insulin secretory response (only the first 5 minutes) resulting in a minor glucose lowering (<0.3 mM) effect. During *insulin-induced hypoglycaemia*, GIP infusion caused greater glucagon responses during the initial 30 minutes compared to saline (76±17 vs. 28±16 pM×min, $p<0.008$), with similar peak levels of glucagon reached after 60 minutes. During *hyperglycaemia* comparable suppression of plasma glucagon (-461±81 vs. -371±50 pM×90 min, $P=0.26$) was observed with GIP compared to saline infusions, concomittant with a more than doubled insulin secretion rate resulting in ~75% more glucose needed to maintain the clamp during GIP infusions (1372±104 vs. 786±80 mg×kg^{-1}, $p<0.0001$). We therefore concluded that in healthy individuals, GIP has no (stimulatory) effect on glucagon responses during hyperglycaemia while strongly potentiating insulin secretion. Importantly, the effects are reversed during fasting and hypoglycaemic conditions, where GIP increases glucagon levels while having little or no effect on insulin secretion. These results suggest that GIP through diverging effects on the two main pancreatic glucoregulatory hormones could have a bifunctional role in stabilising plasma glucose concentrations around euglycaemia in healthy individuals.

Study 2

Study 2 investigated the glucose-dependent effects of GIP on insulin and glucagon responses in patients with type 2 diabetes. Twelve male patients with type 2 diabetes were clamped at three glycaemic levels i.e. fasting glycaemia (mean of 7.7±0.2 mM), hypoglycaemia (mean nadir of 3.4±0.1 mM) and hyperglycaemia (mean of 12.1±0.4 mM). In randomised order on separate days at each glycaemic level either GIP or saline were infused *iv* throughout 90 minute-periods at rates designed to reach postprandial hormone levels. We demonstrated that also in patients with type 2 diabetes, the effects of GIP on glucagon and insulin secretion

are coordinated dependent on the prevailing glucose concentrations. During *fasting glycaemia,* GIP elicited significant increments in both insulin and glucagon levels resulting in neutral effects on plasma glucose during fasting glycaemia similar to the situation in healthy subjects, but at 'diabetic' plasma glucose level almost 3 mM higher than in healthy individuals. During *insulin-induced hypoglycaemia,* GIP elicited a minor early-phase insulin response (before glucose levels dropped) and increased glucagon levels during the initial 30 minutes resulting in less glucose needed to be infused to maintain the clamp (29±8 vs. 49±12 mg×kg^{-1}, p<0.03). During *hyperglycaemia,* GIP augmented insulin secretion throughout the clamp, with slightly less glucagon suppression compared to saline – eventually resulting in ~25% more glucose needed to maintain the clamp during GIP infusions (265±21 vs. 213±13 mg×kg^{-1}, p<0.001). We concluded that in patients with type 2 diabetes, GIP counteracts insulin-induced hypoglycaemia - most likely through a predominant glucagonotropic effect. In contrast, during hyperglycaemia, GIP increases glucose disposal through a predominant effect on insulin release.

Study 3

Study 3 investigated the effects of GIP and GLP-1 during insulin-induced hypoglycaemia in patients with type 1 diabetes without endogenous insulin secretion. We included ten male subjects with type 1 diabetes that were C-peptide negative after an arginine test performed as described above (123). The study was a randomised, double-blinded, 2 hour-cross-over study with *iv* administration of saline, GIP or GLP-1. The first hour plasma glucose was lowered by insulin infusion, and a 'recovery phase' was monitored for a second hour. The primary outcome was glucagon response to hypoglycaemia. GIP infusions elicited larger glucagon responses during the recovery phase (i.e. the second hour of the study (1700±0.3 (GIP) vs. 400±0.2 (GLP-1) vs. 700±0.1 (saline) min×pM, p<0.0001). Glucagon responses between GLP-1 and saline days were similar. During GIP infusions, significantly less glucose was needed to keep plasma glucose above 2 mM (156±35 (GIP) vs. 234±41 (GLP-1) vs. 214±56 (saline) mg×kg^{-1}, P<0.05). Glucose infusion rates between GLP-1 and saline days did not differ. Endogenous glucose production (assessed by use of stable isotopes) differed during the recovery phase with higher rates during GIP infusion and lower rates during GLP-1 infusion. Insulin levels, symptoms of hypoglycaemia and cognitive function during hypoglycaemia were similar on all days. We concluded that in patients with type 1 diabetes, GIP augments the glucagon counter-regulatory response to insulin-induced hypoglycaemia, whereas GLP-1 has no significant effect on the already impaired glucagon response in patients with type 1 diabetes. The glucagon responses and higher endogenous glucose production (measured with the stable isotopes technique) during the initial part of the recovery phase on the GIP days could explain the less need for exogenous less glucose infusions on these days.

DISCUSSION AND SYNTHESIS OF RESULTS

Glucagon secretion

Considering the collective evidence from the investigations during *fasting glycaemia* and *hypoglycaemia* in the three studies included in this thesis, it seems reasonable to conclude that GIP in humans stimulate glucagon secretion when plasma glucose levels are in the fasting range or lower. Accordingly, in the healthy subjects (**Study 1**), GIP augmented the glucagon responses initially where plasma glucose was in the 'physiological range' i.e. between 3.5 and 5.5 mM (Figure 3, **Study 1**). The same pattern was observed in patients with type 2 diabetes (**Study 2**), where initial, but not peak glucagon responses (at study end), were higher during GIP infusions. In the study including patients with type 1 diabetes (**Study 3**), the pattern of GIP's glucagon stimulatory effects contrasted to the first two studies, as only the peak glucagon response observed during the 'recovery phase' (after insulin infusion was stopped) was significantly affected by GIP administration.

Obviously, the reason for the discrepancy in glucagon responses between the three studies could relate to the lack of endogenous insulin secretion in the patients with type 1 diabetes. However, another very plausible reason for the divergence in glucagon results could be that in **Study 1** and **Study 2**, a rather high dose of exogenous insulin was administered for the entire study period (**Study 1**: 1.5 mU×kg^{-1}×min^{-1}; **Study 2**: 1 mU×kg^{-1}×min^{-1}). As mentioned, we have previously observed strong glucagon suppressive effects of exogenous insulin infusion (123), and it is imaginable that the overt hyperinsulinaemic conditions at the end of **Study 1** and **Study 2** restrained the alpha cell from responding to GIP. In contrast, the falling insulin levels during the recovery phase in **Study 3** might unmask the glucagonotropic effects of GIP. Whether the assumption of such a masking effect of exogenous insulin is true, could have been examined if we had stopped the insulin infusion in **Study 1** and **Study 2** and followed the rebound to fasting glycaemia (we unfortunately did not do so). Another possibility would be to administer GIP during hypoglycaemia induced in another way than by administering exogenous insulin, but this would probably add other biases.

The peak glucagon responses may depend on the co-activation of other alpha cell stimuli e.g. hypoglycaemia-induced activity in the autonomic nervous system, which was evident in **Study**

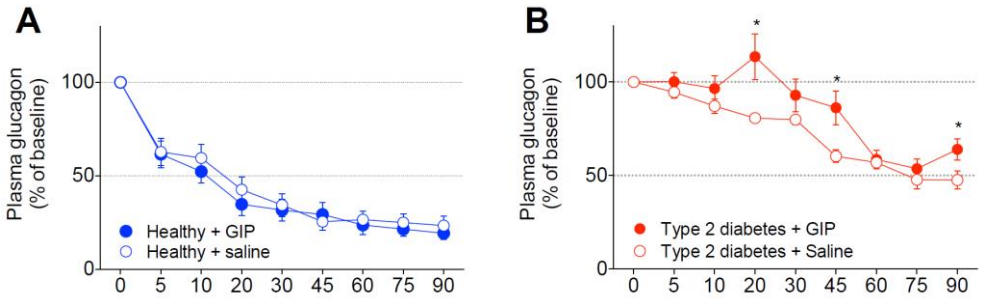

Figure 3

Hyperglycaemic clamping (plasma glucose ~12 mmol/L) induced suppression of glucagon expressed as a percentage of baseline values in healthy control subjects (n=10) (A, blue symbols) and patients with type 2 diabetes (n=12) (B, red symbols) during concomitant GIP infusions (filled circles) or saline infusion (open circles). Statistical analyses were done with repeated-measures ANOVA post test using the Holm-Sidak's correction. Significant differences are indicated by asterisks: *P<0.05.

1. All participants in **Study 1** had symptoms of hypoglycaemia, and we suspected that this 'autonomic activity' might explain the lack of difference between peak glucagon responses with GIP and saline. Therefore, in **Study 2** we clamped the patients with type 2 diabetes at a slightly higher plasma glucose value of 3-3.5 mM, which resulted in very few of the patients having symptoms of hypoglycaemia (observation not reported in the publication). This change in design did, however, not change the pattern in the response to GIP and saline, although the peak glucagon responses were much lower.

The physiological defence against falling plasma glucose concentrations in humans are normally described as the combination of 1) a decrease in beta cell insulin secretion, and 2) an increase in alpha cell glucagon secretion and 3) increased symphatoadrenal responses (135). In patients with type 1 diabetes the first two components are lost (126,136–138). In absence of a sufficient glucagon response, the final defence against hypoglycaemia befalls the autonomic adrenergic response, but also this response attenuates with time (139,140). The total lack of glucagon response in patients with longstanding type 1 diabetes is exquisitely demonstrated by the complete horizontal glucagon profiles during the saline infusion days in **Study 3** (white square symbols figure 4). As mentioned, the alpha cells remain responsive to other stimuli such as arginine (Figure 2) or alanine (141) and theophylline (141,142) and to some degree exercise-induced hypoglycaemia (143). As it is obvious that the defective beta cells play a pivotal role in the loss of alpha cell secretory response (144,145), it is not surprising that defective glucagon counter-regulation can be detected early in the course of type 1 diabetes (136,138). Therefore, when investigating alpha cell stimuli, resi-dual beta cells function is important. In a study where theophylline was administered to patients with type 1 diabetes (mean diabetes duration ~4 years), who actually had a slightly preserved glucagon response also on the control day, the result was a faster plasma glucose recovery and higher rate of glucose appearance estimated by use of stable isotopes (142). In contrast, the antimuscarinergic agent atropine administered to patients with type 1 diabetes (mean diabetes duration 19 years) during induced hypoglycaemia did not alter the already almost completely absent glucagon response (146). The patients included in **Study 3** had fairly longstanding type 1 diabetes (median diabetes duration 12 years), which makes the finding of a stimulatory effect of GIP on glucagon responses quite remarkable.

In **Study 3**, we included GLP-1 as an additional study arm, to investigate the potential suppressive effects on glucagon response during hypoglycaemia in patients with type 1 diabetes. Initially, GLP-1 suppressed glucagon levels, although not affecting the (virtually absent) incremental glucagon counter-regulatory responses. Thus, the glucagonotropic effect of GIP strongly contrasts with that of GLP-1. We find it likely that postprandially in humans, the opposing effects on glucagon (of GIP versus GLP-1) might outweigh each other, similar to what was observed during isoglycaemic clamping by *Lund et al.* (79).

The glucagon results obtained during hyperglycaemic clamping also need to be considered. As mentioned above, GIP has been implicated in the inappropriate postprandial glucagon response in patients with diabetes (80,79). In healthy individuals, hyperglycaemic clamping (i.e. glucose administration) effectively suppresses glucagon levels, whereas in patients with type 2 diabetes the suppression of glucagon is delayed and inefficient (figure 3). The present findings demonstrate that GIP unfavourably affects the already delayed glucagon suppression by hyperglycaemia in patients with type 2 diabetes, but not in healthy control subjects. In figure 3 this unfavourable glucagon suppression is presented as percentage suppression from baseline values. It is important for the interpretation of these fractional differences that the baseline glucagon values were significantly higher in the patients with type 2 diabetes than in the healthy individuals (type 2 diabetics: 12.5±1.5 pM vs. healthy control subjects: 7.1±0.7 pM, $p<0.005$).

Insulin secretion

In **study 1 and 2**, we investigated the insulinotropic responses during both hyperglycaemia and fasting glycaemia. Interestingly, and a bit unexpected, the insulin responses during fasting glycaemia, i.e. mean plasma glucose in healthy of 5.0 mM and in patients with type 2 diabetes of 7.4 mM, showed a high degree of similarity (figure 5). Hence, insulin secretion rates were stimulated only within the initial 20 minutes and almost to the same extent with the only difference being a slightly protracted response in the patients with type 2 diabetes.

In relation to these findings, it is interesting that the increased plasma glucose in patients with type 2 diabetes more than 30 years ago was described as a regulated equilibrium compensating partly for the impairment of islet function (147). As a result of this

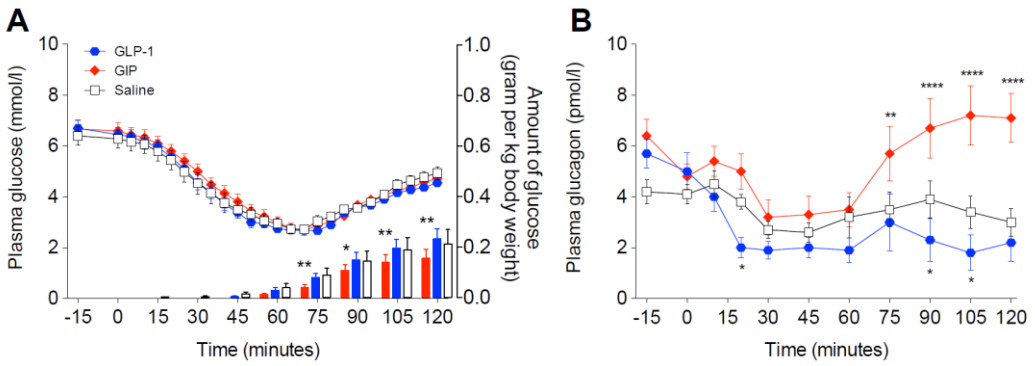

Figure 4

Plasma concentrations of glucose (A) and Glucagon (B) during Insulin-induced hypoglycaemia with GIP infusions (red diamonds), GLP-1 infusions (blue hexagons) or saline infusion (white squares) in patients with type 1 diabetes (n=10). Cumulated glucose infusions are depicted as bar graphs (A). Data are means±SEM. Statistical analyses were done with repeated-measures ANOVA post test using the Holm-Sidak's correction. Significant differences are indicated by asterisks: *P<0.05, **P=0.001 to 0.01, ***P=0.0001 to 0.001, ****P<0.0001.

compensation, peripheral glucose utilization is restored to normal, but at the expense of hyperglycaemia maintained by a slightly increased endogenous glucose production (147). Our result might be compatible with this notion of a new equilibrium and by similar subtle (early phase) insulin secretory effect of GIP at fasting levels in individuals with or without type 2 diabetes.

During induced *hyperglycaemia* our findings support that GIP retain a substantial insulinotropic effect in typical patients with type 2 diabetes (with fairly well-regulated glycaemia). The insulin secretory responses during the first hour corrected for glucose administration in healthy individuals in comparison to patients with type 2 diabetes are presented in figure 6. It is worth noting that the groups in **Study 1** and **Study 2** were not matched concerning BMI and age. In particular, a lower age could positively affect the insulin response to GIP (148,149) and therefore bias this *post hoc* comparison to show a larger difference than would be the case if the two groups had been age matched (figure 6).

Several studies have reported a weak or almost absent C-peptide response to physiological and supraphysiological doses of GIP infused during hyperglycaemic clamping in patients with type 2 diabetes (45,39,41,150,50,151,152). Our results seem to corroborate an impaired the late phase response to GIP (again compared to non-matched healthy individuals) as illustrated by subdividing the insulin responses during the first hour from **Study 1** and **Study 2**, rather arbitrarily, into early and late phase secretory responses, (figure 7).

As previously discussed, there is a gap in our understanding of the mechanism behind the reduced late phase insulin response to GIP. The lacking late phase response in the fasting state in spite of absolute (fasting) hyperglycaemia, and a relatively retained response during induced hyperglycaemia in our cohort prompted a review of the existing literature investigating the insulin response in patients with type 2 diabetes. In particular studies reporting an almost completely absent second phase response were reviewed (39,41,50,152,153). As mentioned a re-analyses of the data in *Vilsbøll et al.* (50) actually demonstrated a small, but significant insulinotropic effect similar to the one reported in **Study 2**. Another important study for the understanding of the insulinotropic effect of GIP, is the study by *Højberg et al.* showing that intensified glycaemic regulation improves the insulinotropic response to GIP (152). The patients included in that study were fairly dysregulated with mean fasting plasma glucose values around 12 mM, which dropped to around 7 mM efter four weeks of insulin treatment.

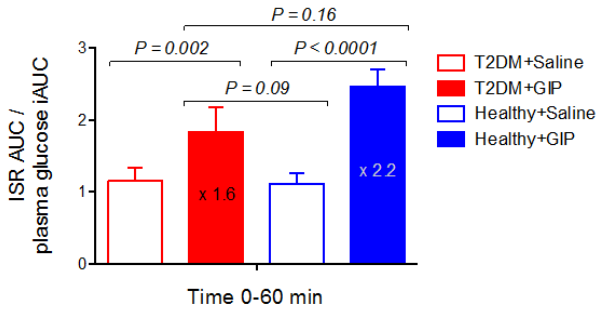

Figure 6
Hyperglycaemic clamping induced insulin secretion rates (ISR) corrected for increments in plasma glucose (expressed as incremental plasma glucose area under curve [iAUC] from fasting glycaemia to mean plasma glucose ~12 mmol/L) in healthy subjects (n=10) (blue bars) and patients with type 2 diabetes (n=12) (B, red bars) during concomitant GIP infusions (filled bars) or saline infusion (open bars). Statistical analyses were done by paired and unpaired t-tests and selected p-values are presented.

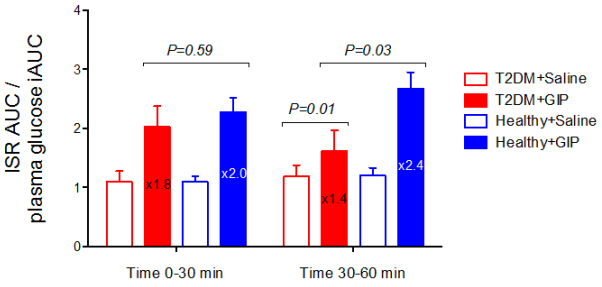

Figure 7
Hyperglycaemic clamping induced insulin secretion rates (ISR) divided into early (0-30 min) and late phase (30-60 min) responses and corrected for increments in plasma glucose (iAUC) in healthy subjects (n=10) (blue bars) and patients with type 2 diabetes (n=12) (B, red symbols) during concomitant GIP infusions (filled bars) or saline infusion (open bars). Statistical analyses were done paired and unpaired t-tests and selected P-values are presented.

Thus, clamping at a uniform 15 mM, would mean that before treatment some patients were investigated at their fasting values, and following treatment the glucose stimulus was increased in all patients (152). We show in **Study 1** and **Study 2** that the effect of GIP on late phase insulin secretion is very dependent on coadministration of glucose (possibly because of an altered glucose equilibrium as described above). Therefore, the abovementioned

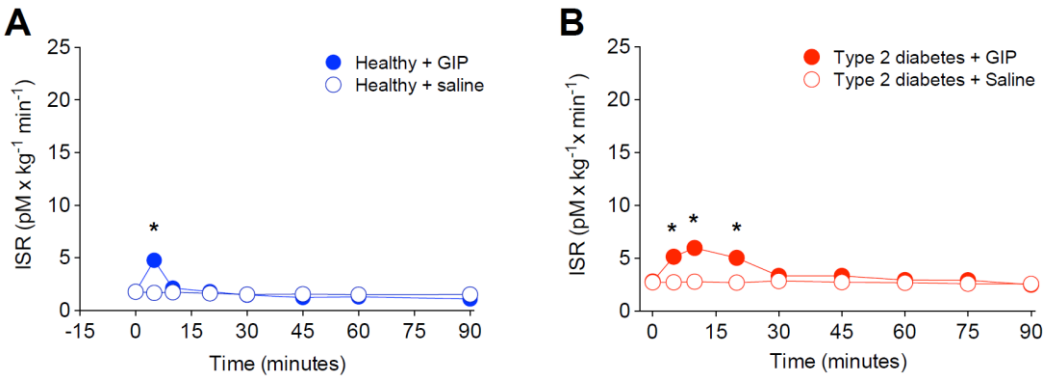

Figure 5
Fasting glycaemia insulin secretion rates (ISR) derived by deconvolution of the serum C-peptide concentrations in healthy subjects (n=10) (A, blue symbols) and patients with type 2 diabetes (n=12) (B, red symbols) during concomitant GIP infusions (filled circles) or saline infusion (open circles). Statistical analyses were done with repeated-measures ANOVA post test using the Holm-Sidak's correction. Significant differences are indicated by asterisks: * P<0.05

differences in glucose co-administration might have contributed to an underestimation of the GIP effect prior to treatment in the results from *Højberg et al.* (152). The same applies to other studies investigating GIP administration to patients with type 2 diabetes close to their fasting levels (39,41,153). When correcting the insulin responses to GIP for increments in plasma glucose during hyperglycaemic clamping in patients with type 2 diabetes, the insulin responses in the *Højberg et al*. study, were only slightly lower than other studies infusing human sequence GIP (11).

So how do we reconcile these data? Indeed, the results highlight the differences between GIP and GLP-1, the latter of which powerfully lowers fasting levels of plasma glucose in patients with type 2 diabetes (39,153). This difference between the two hormones may also be reflected by the mouse knock-out models showing no effect on fasting glycaemia in mice with GIP receptor knock-out, and elevated 'diabetic' fasting glycaemia in mice with GLP-1 receptor knock-out (154). Thus, it seems as if GIP is a much simpler beta cell stimulus than GLP-1. The latter conferring 'glucose competence' to the already impaired, diabetic beta cell during the late phase of insulin secretory responses.

A comprehensive model of the beta cell stimulation including the responsiveness of the beta cell to non-glucose stimuli e.g. isoproterenol or arginine was described more than 30 years ago (147,155–158). Arginine likely depolarises the beta cell by a separate intracellular signalling pathway (55,159), whereas isoproterenol acts through cAMP-mediated signalling pathways (160). Because, of the shared signalling pathway the isoproterenol-stimulated insulin responses have some relevance in relation to the responses to GIP and might represent a model for a "simple" beta cell stimulus similar to GIP. Thus, when isoproterenol is administered as a bolus in patients with type 2 diabetes it retains an insulinotropic effect as long as the patients are not overtly dysregulated with fasting glycaemic levels above 16 mM (155). This suggests some correlation of the insulin response to the levels of fasting glycaemia (147). Interestingly, in healthy subjects isoproterenol also retains insulinotropic actions when administered as a continuous infusion (161). The responses to isoproterenol administered as a continuous infusion has not been undertaken in humans with type 2 diabetes, but it might be suspected that these responses are impaired (perhaps similar to the situation with GIP). As mentioned the relation between fasting plasma glucose concentration and C-peptide responses to isoproterenol and glucose has been described and depicted in reference (147).

In figure 8 the relation of fasting glycaemia and C-peptide response to iv glucose (hyperglycaemic clamp ~12 mM) and to GIP (on top of the hyperglycaemic clamp) in healthy control subjects and patients with type 2 diabetes as derived from (101,166,205) are depicted. It might be appreciated from figure 8 that the insulin response patterns in healthy subjects and patients with type 2 diabetes are quite comparable following stimulation with 1) glucose alone and 2) GIP alone (i.e. GIP added to glucose). The similarity of the insulin secretory responses to glucose and GIP could indicate that the impairment in the insulin response to GIP is really a matter of beta cell secretory capacity more than a specific GIP defect.

Hence, the relevant question may not be why GIP as a beta cell stimulus is ineffective in patients with type 2 diabetes, but rather why GLP-1 is so effective? Thus, from the data presented in table 1 and Study 2 included in this thesis, it should be evident that when looking specifically at the responses to GIP, without comparing with GLP-1, the insulinotropic effect is preserved in most patients with type 2 diabetes. In direct comparison the effect is actually of similar size as acute treatment with a sulphonylurea compound (194).

Glucose infusions

In all the studies we measured the amounts of glucose needed to maintain the glucose clamps at each glycaemic levels. These glucose amounts integrate the impact of all measured (and unmeasured) hormonal changes during the experiments, whether with or without GIP. During *hyperglycaemia*, these responses resulted in 75% (healthy) and 25% (type 2 diabetes) more glucose needed to maintain the clamps. Rather unexpectedly, there was no need for glucose infusions on any of the *fasting glycaemia* days in **Study 1** and **Study 2**. Thus, the glucose-lowering impact of GIP during fasting glycaemia was strikingly non-existent and similar in healthy subjects and patients with type 2 diabetes, which probably reflects the similar (and on the GIP days opposing) insulin and glucagon responses. During *hypoglycaemia* in **Study 2** and **Study 3**, we had to infuse less glucose on the GIP days as compared to the saline days. We explained these differences in glucose amounts by the GIP-induced stimulation of glucagon secretion during lower levels of glycaemia (**Study 2** and **Study 3**). However, a few other hypothetical explanations for the difference in glucose amounts needed to maintain the clamps might be offered. First, it is possible that insulin sensitivity might have differed between days in the same individual. A randomly occurring and yet clinically relevant difference in insulin sensitivity seems unlikely when considering the randomised design and the similar homeostatic model assessment insulin resistance (HOMA-IR) at baseline between the GIP and saline hypoglycaemia days (6.5±0.7

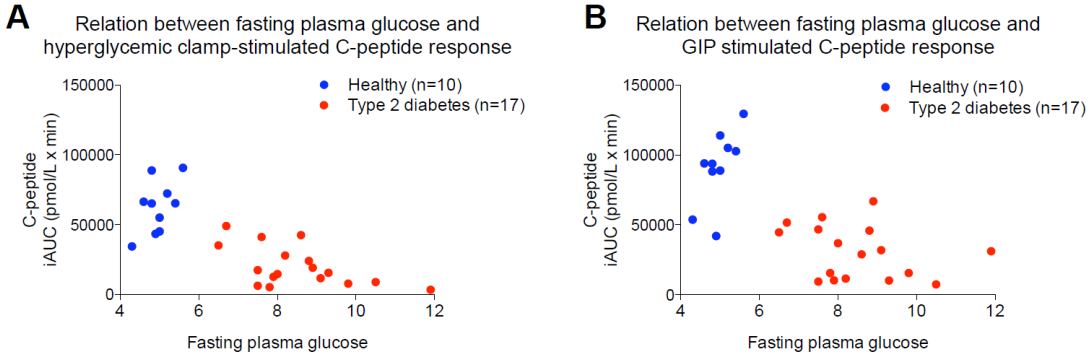

Figure 8

Relation between fasting plasma glucose concentration and C-peptide (1-hour) responses to hyperglycaemic clamping (plasma glucose ~12 mM)(A) and GIP infusion during hyperglycaemic clamping (B). Data are derived from healthy subjects (n=10) (blue symbols) and patients with type 2 diabetes (n=17) (red symbols) from refs (50,162,163)

versus 6.8±0.8, *p*=0.58)). Another possibility is that GIP induces insulin resistance (164). However, as previously discussed the bulk of evidence suggests that GIP increases insulin sensitivity in adipose tissue, and there is no evidence to suggest that GIP induces insulin resistance in muscle tissue. Another important issue when looking at the glucose infusion amounts, is that this outcome is highly dependent on the combination of other outcomes i.e. plasma insulin, glucagon and plasma glucose values. The plasma glucose values did not differ significantly between the GIP and saline days, although a trend towards a slightly slower induction of hypoglycaemia could be observed in the patients with type 2 diabetes. Thus, in **Study 2**, there was a trend towards lower plasma glucose values on the saline days, and we had to infuse more glucose during these days. It could therefore be speculated that 1) if plasma glucose excursions had been fully matched on the GIP and saline days (i.e. if we had infused more glucose on the saline days) it would have suppressed the glucagon values on the saline days to a greater degree contributing to greater differences in glucagon levels, or 2) if we instead had matched the glucose infusions (i.e. had infused less glucose on the saline days) it is likely that the plasma glucose excursions curves would have differed to a greater degree than observed (but then again perhaps eliminating the difference in glucagon). The same complex interaction between our outcome measures could be relevant for **Study 3**. However, in **Study 3** we tried to avoid the crudity of the glucose administration measure, by using the isotope tracer methodology (165,166). Despite some limitations of this methodology in situations with large fluctuations in glucose infusion rates, the use of stable isotopes is at present the best way to discern between changes in endogenous glucose production and glucose disposal. The results from **Study 3** suggest that GIP result in a slight increase in endogenous glucose production, which supports that the plasma glucose stabilising effect is through GIP-induced glucagon secretion at low glycaemic levels.

CONCLUSIONS

In three studies we have delineated the glucose-dependency of GIP effects in healthy individuals and patients with type 1 diabetes and patients with type 2 diabetes. We have done this by infusing human GIP *iv* in physiological doses at various clamped glucose levels. Our results provide support for the notion that GIP acts as a bifunctional regulator of both insulin and glucagon secretion. The effects of GIP on glucagon seem to be glucose-dependent and active during fasting and hypoglycaemia. In addition, our studies support the long-standing evidence that GIP-induced insulin responses are lower in patients with type 2 diabetes than in healthy subjects, and likely related to the combination of the general impairment in beta cell function in patients with type 2 diabetes and the specific quality of the beta cell stimulus elicited by GIP. In patients with type 1 diabetes the glucagon stimulating effects of GIP could translate into an improved counter-regulatory glucagon response to insulin-induced hypoglycaemia.

To conclude, it seems certain that GIP via effects on insulin and glucagon secretion plays a role in postprandial metabolism. The precise nature of its functions apart from participating in glucose regulation, is not certain and beyond the scope of this thesis. It could be speculated, however, that the primary physiological role of GIP, is not only to stimulate postprandial insulin secretion, but rather in a broader sense coordinate nutrient disposal with nutrient intake in various peripheral tissues including adipose tissue and bone. The acronym GIP has been constant while the hormone name has evolved alongside our understanding of the physiology. Possibly an even more appropriate term for GIP could evolve in the future and one proposal could be **G**lucose-stabilising **I**ntestinal **P**eptide.

PERSPECTIVES

Several important questions concerning GIP await future clarification:

1. The approach used in the present studies includes one major limitation concerning the generalisability towards clinical physiology, namely that the patients are investigated in their fasting state, which is highly dissimilar to the postprandial state, where the incretin hormones (and many other nutrient and hormonal substances) are present in different concentrations as compared to the fasting state. Therefore, investigations on the role of GIP in postprandial metabolism would take a quantum leap ahead if a suitable GIP receptor antagonist could be found and used in human studies. Employing GIP receptor antagonism in humans would represent a more physiologically relevant approach to elaborate on the exact role of GIP in postprandial metabolism of glucose and other nutrients. Interestingly, we have recently discovered a candidate for a suitable GIP-receptor antagonist (167,168), which we will test in clinical trials in the nearest future.
2. The lack of glucagon response to hypoglycaemia in patients with type 1 diabetes is enigmatic. As previously discussed these patients have preserved glucagon response to many stimuli, but not insulin-induced hypoglycaemia. The finding from **Study 3** that GIP augments glucagon responses during hypoglycaemia is surprising and could be relevant to test in a larger setting and perhaps with a more stable GIP receptor agonists.
3. All human studies with GIP have investigated the acute effects of GIP. A more chronic GIP exposure may constitute a more relevant intervention if one is interested in physiological effects. Thus, the effect in humans of chronic exposure to GIP in particular concerning outcomes such as lipid metabolism and disposition (where acute studies in human predominantly have been negative), longer term glycaemic regulation and markers of bone turnover could be interesting to investigate in the future.
4. Recent findings suggest that co-activation of the GIP receptor and the GLP-1 receptor may have additive effects on mechanisms regulating food intake and bodyweight (85). This finding seems logical from an evolutionary perspective and merits further investigation.

SUMMARY

The hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are secreted by enteroendocrine cells in the intestinal mucosa in response to nutrient ingestion. They are called incretin hormones because of their ability to enhance insulin secretion. However, in recent years it has become clear that the incretin hormones also affect glucagon secretion. While GLP-1 decreases glucagon levels, the effect of GIP on glucagon levels has been unclear. The regulation of glucagon secretion is interesting, as the combination of inadequate insulin secretion and excessive glucagon secretion are essential contributors to the hyperglycaemia that characterise patients with type 2 diabetes. Moreover, the near absence of a well-timed glucagon

response contributes to an increased risk of hypoglycaemia in patients with type 1 diabetes. The overall aim of this PhD thesis was to investigate how the blood glucose level affects the glucagon and insulin responses to GIP in healthy subjects (Study 1) and patients with type 2 diabetes (Study 2), and more specifically to investigate the effects of GIP and GLP-1 at low blood glucose in patients with type 1 diabetes without endogenous insulin secretion (Study 3). The investigations in the three mentioned study populations have been described in three original articles. The employed study designs were in randomised, placebo-controlled, crossover set-up, in which the same research subject is subjected to several study days thereby acting as his own control. Interventions were intravenous administration of hormones GIP, GLP-1 and placebo (saline) during different blood glucose levels maintained (clamped) at a certain level. The endpoints were plasma concentrations of glucagon and insulin as well as the amount of glucose used to clamp the blood glucose levels. In Study 3, we also used stable glucose isotopes to estimate the endogenous glucose production and assessed symptoms and cognitive function during hypoglycaemia. The results from the three studies indicate that GIP has effects on insulin and glucagon responses highly dependent upon the blood glucose levels. At fasting glycaemia and lower levels of glycaemia, GIP acts to increase glucagon with little effect on insulin release. At hyperglycaemia the insulin releasing effect of GIP prevail, which lead to an increases in glucose disposal by approximately 75% in healthy subjects (Study 1) and 25% in patients with type 2 diabetes (Study 2) relative to placebo. After insulin-induced hypoglycaemia in patients with type 1 diabetes (Study 3), GIP increases glucagon release, which probably augments endogenous glucose production. This was associated with a reduced need for exogenously added glucose to prevent hypoglycaemia. In conclusion, the studies position GIP as bifunctional blood glucose stabilising hormone that glucose-dependently regulates insulin and glucagon responses in humans.

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