Glucose-dependent Insulinotropic Polypeptide (GIP): From prohormone to actions in endocrine pancreas and adipose tissue

Randi Ugleholdt

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Tutor: Jens Juul Holst

Official opponents: Jens Frederik Rehfeld, Baptist Gallwitz & Thure Krarup

Correspondence: Department of Biomedical Sciences, Cellular and Metabolic Research Section, University of Copenhagen, Faculty of Health Sciences, Blegdamsvej 3B build. 12.2, 2200 Copenhagen N, Denmark

E-mail: randi@ugleholdt.com

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THE TWO ORIGINAL PAPERS ARE

1. Ugleholdt R, Poulsen ML, Holst PJ, Irminger JC, Orskov C, Pedersen J, Rosenkilde MM, Zhu X, Steiner DF, Holst JJ. Prohormone convertase 1/3 is essential for processing of the glucose-dependent insulinotropic polypeptide precursor. J Biol Chem. 2006;281(16):11050-7.
2. Ugleholdt R, Pedersen J, Bassi MR, Füchtbauer EM, Jørgensen SM, Kissow H, Nytofte N, Poulsen SS, Rosenkilde MM, Seino Y, Thams P, Holst PJ, Holst JJ. Transgenic rescue of adipocyte Glucose-dependent Insulinotropic polypeptide receptor expression restores high fat diet induced body weight gain. J Biol Chem 2011 Oct 25. [Epub ahead of print]

INTRODUCTION

Multiple gut hormones are part of longer immature precursors and depend on site specific cleavage in the secretory vesicles before release. The gut hormones are produced in specialized enteroendocrine cells, distributed in the epithelium of the stomach, small and large bowel, which are capable of sensing the nutrient flow in the intestine. The enteroendocrine cells promptly releases hormones in association with meal ingestion and these hormones promote efficient uptake and storage of energy by acting on distant target organs. In the fasting state the hormones are secreted at low basal rates whereas plasma levels rise rapidly after food intake to mediate their physiological effect, until they are enzymatically degraded or cleared by the kidneys. Hence, the coordination of food intake and sufficient uptake and storage of the ingested energy depend on a highly regulated interaction between several organs like the gut, adipose tissue, the liver, skeletal muscle, islets of Langerhans in the pancreas and the nervous system (reviewed in (1)).

The two incretins, glucagon-like peptide 1 (GLP-1) and glucose dependent insulinotropic polypeptide (gastric inhibitory peptide, GIP) have long been recognized as important gut hormones, essential for normal glucose homeostasis. Plasma levels of GLP-1 and GIP rise within minutes of food intake and stimulate pancreatic β-cells to release insulin in a glucose-dependent manner. This entero-insular interaction is called the incretin effect and accounts for up to 70% of the meal induced insulin release in man and via this incretin effect, the gut hormones facilitate the uptake of glucose in muscle, liver and adipose tissue (2). Although the pancreatic effects of these two gut hormones have been the target of extensive investigation both hormones also have numerous extrapancreatic effects. Thus, GLP-1 decreases gastric emptying and acid secretion and affects appetite by increasing fullness and satiety thereby decreasing food intake and, if maintained at supraphysiologic levels, eventually body weight (3). The satiety enhancing effects have turned out to be especially attractive in treatment of the diabetic patient and GLP-1 analogs and strategies to increase GLP-1 half life have now been developed and the resulting products marketed as a new generation of antidiabetic agents. Although, GLP-1 and GIP share many pancreatic effects in normal subjects, diabetic patients have drastically diminished GIP stimulated insulin response (4). While the mechanisms underlying this defect are still uncertain, primary focus for several years has been on characterization of GLP-1 from intracellular maturation and release to peripheral effects on target organs and degradation of the peptide. GIP maturation, function and degradation are not fully understood although the recent discovery of GIP as a regulator of body weight (5) has evoked a general interest in the hormone and increased the demand for further knowledge. Due to the therapeutic potential in adiposity treatment, a large fraction of the recent studies have attempted to manipulate GIP plasma levels or its function and to understand the nutrient dependent stimulation of the intestinal GIP producing K-cell. The mechanism by which proGIP is cleaved and the enzymatic specificity required for secretion of biologically active GIP had not been looked into. In addition, a rapidly increasing number of functional studies are investigating effects of acute and chronic loss of GIP signaling in glucose and lipid homeostasis. However, the physiological significance of GIP as a regulator of body weight and adipogenesis remains unclear and the target organs for GIP receptor signaling that regulates adipogenesis remains unknown.

Hence this PhD thesis seeks to review existing knowledge on GIP from maturation, release and degradation to its peripheral effects on pancreas and adipose tissue in regulation of glucose and fat homeostasis. In the thesis there is a special emphasis on

studies performed as part of this PhD project to assess enzyme dependent maturation of proGIP and the mechanisms by which GIP receptor regulates body weight and adipogenesis.

LOCALIZATION

GIP is expressed throughout the small intestine with highest concentration in the duodenum and upper jejunum in the enteroendocrine K-cell (6). In addition, studies have reported co-localization of GIP and GLP-1 in subsets of intestinal enteroendocrine cells (6; 7). As mice with chronic ablation of K-cells have an absent incretin function in contrast to mice lacking GIP or GLP-1 receptors there is a possibility that the double positive cells plays an important role in maintenance of the incretin effect (8). Subpopulations of K-cells also express the 25 amino acid peptide xenin belonging to the xenopsin/neurotensin/xenin peptide family (9). Xenin has been reported to exert effects on the endocrine and exocrine pancreas, as well as on gastrointestinal functions and food intake (10; 11), yet despite the co-localization of GIP and xenin, the mechanism for the hormones secretion mechanism may differ significantly. In human volunteers, the maximal secretion of xenin may be found in the cephalic phase, whereas GIP secretion is clearly nutrient intake dependent (12).

Expression of GIP or detection of GIP like immunoreactivity has also been reported outside the small intestine. Accordingly, GIP antisera were found to react with pancreatic α-cells in the same secretory granules as glucagon in pancreatic α-cells (13), but the antisera which detected pancreatic GIP immunoreactivity also stained cells in the ileum and colon whereas antisera staining cells in the upper intestine did not detect pancreatic GIP immunoreactivity (14). The results may indicate cross-reactivity of same antisera with other products, possibly proglucagon derived peptides, or alternatively differential processing of a pancreatic GIP precursor. In support of the cross-reactivity explanation, radioimmunoassays (RIAs) of tissue extracts have not demonstrated pancreatic GIP (15), nor was GIP mRNA found in pancreas tissue from rat fetuses or pups (16). In addition to its established intestinal expression, GIP mRNA expression has been identified in the submandibular salivary gland of the rat (16; 17), stomach (18) and multiple sites in the brain (19-21). However, little is known about the function of GIP, expressed at these sites.

POST-TRANSLATIONAL MATURATION

The subtilisin-like proprotein convertases and general concept of function

Regulated peptides are synthesized as immature proproteins, depending on endoproteolytic processing by proteases to convert the immature precursor proteins to mature, biologically active forms. These endoproteases include a small family of subtilisin-like proprotein convertases (SPC's or simply PC's) strategically localized within the cells to convert immature prohormones that traverses the secretory pathway (22). Seven members of the family have been identified so far, designated SPC1-7 or furin, PC2, PC1/3, PACE4, PC4, PC5/6, and PC7, respectively (22). Generally, the endoproteases cleave the precursor substrate at the C-terminal side of the classical dibasic KR and RR motifs for processing. However, upstream basic residues likely contribute to substrate recognition, and a more accurate consensus motif is [R/K]–[X]n –[R/K]↓, where X indicates any amino acid residue, R/K designates either an arginine or a lysine residue, and n (the number of spacer amino acid residues) is 0, 2, 4, or 6 (23). After proteolysis, the C-terminal, basic amino acids are removed by specialized metallocarboxypeptidases (CPE or CPD) thereby forming the mature protein ready for secretion at the appropriate stimulus (22). In some cases, full maturation depends on further post-translational modification including C-terminal amidation, N-terminal acetylation, glycosylation, sulfation or phosphorylation (22).

The convertases PC1/3 and PC2 are the major proprotein convertases expressed in the neuroendocrine system and brain acting on hormone precursors trafficking dense core vesicles of the regulated secretory pathway (22). Despite recognition of the same basic cleavage motifs, not all basic cleavage motifs are recognized by each convertase and additional information is embedded in the proprotein sequence which affects convertase recognition. The result is that prohormones may undergo tissue specific processing, ultimately determined by the expression pattern of the PC. This becomes especially apparent with proglucagon giving rise to glucagon from the NH2-terminal end of the precursor in pancreatic α-cells and to GLP-1 and GLP-2 from the COOH-terminal part in intestinal L-cells. Earlier studies suggested that PC2 is predominantly expressed in the pancreatic α-cells and glucagon producing cell lines whereas PC1/3 is produced in the intestine and in GLP-1 secreting L cells (24-27). This indicated that tissue specific expression of PC's is a predominant mechanism ensuring correct maturation of the hormone. Later characterization of PC2 and PC1/3 deficient mice verified a complete dependence of PC2 for successful maturation of glucagon and for PC1/3 in liberating GLP-1 and GLP-2 from the precursor (28-32). Hypothetically and rather creatively, this knowledge has been exploited to target α-cells with PC1/3 thereby inducing a combined pancreatic and intestinal processing profile with increasing local GLP-1 production, assumed to be beneficial in the diabetic state. Accordingly, transfecting islets with PC1/3, using an adenoviral vector, increased GLP-1 secretion and transplantation of these islets to streptozotocin treated mice significantly lowered glucose (33).

Processing of proGIP – investigations in PC1/3 and PC2 deficient mice

Whereas proglucagon undergoes differential tissue specific processing, resulting in different products with diverging effects, GIP1-42 is the only acknowledged biological active product of the proGIP precursor. The GIP1-42 sequence in proGIP is located as a mid sequence between a NH2- and a COOH-terminal peptide (figure 1) with the PC consensus motif RXXR flanking both terminuses.

In agreement with a possible role for PC1/3 in enzyme dependent processing of proGIP to GIP1-42, PC1/3 was found by immunohistochemistry to co-localize with GIP in murine intestinal sections, whereas PC2 was not found. However, the dispute whether PC2 is expressed in small intestine is not settled. PC2 was found in intestinal proglucagon producing L-cell from dogs and additional studies did find PC2 immunoreactivity colocalizing with gut hormones apart from proglucagon and GIP (34; 35). Importantly, genetically engineered GIP producing K-cells were reported to produce biologically active insulin when proinsulin was expressed under control of the GIP promoter (36). As both PC2 and PC1/3 are required for the efficient release of the insulin A and B chain (37; 38), this would indicate that PC2, or a convertase supplying the same processing function, is also produced in the cells expressing transgenic insulin under the GIP promoter, and might also contribute to GIP processing. Some caution should,

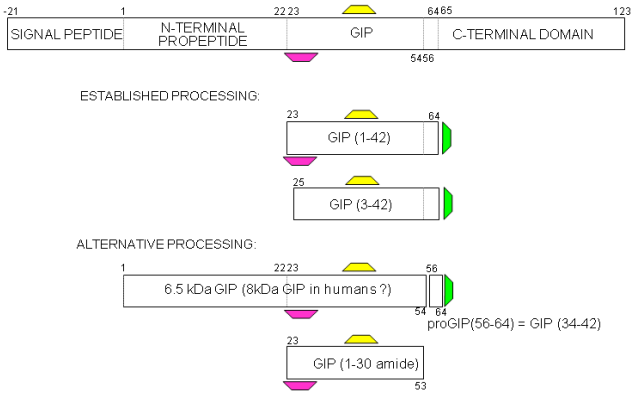

FIGURE 1. Upper panel: schematic representation of the structure of rodent pre-proGIP. Coloured symbols represent antibodies and their positions indicate their suggested sequence specificity. Established processing: GIP(1-42) as it is secreted and acting on the GIP receptor and the metabolite GIP(3-42) produced by extracellular NH2-terminal degradation by DPP-4 are shown. Alternative processing: the likely fragments observed in proGIP and PC2 expressing cell lines which may have been detected by the currently used antisera. Yellow symbol represents a "side viewing" antibody, pink symbol a "NH2-terminal" antibody and the green symbol represents a COOH directed antibody.

however, be exerted when interpreting the results from the GIP promoter insulin transgenic mice, as the truncated promoter had a preferential gastric and upper intestinal expression pattern that differs from the more restricted intestinal expression pattern normally attributed to proGIP.

To investigate the importance of PC1/3 and PC2 for the endoproteolytic cleavage of proGIP in vivo, we examined processing profiles of intestinal extracts from PC1/3 and PC2 null mice by gel filtration. We found that PC1/3 null mice did not express the mature form of GIP, in agreement with a complete block in processing of the precursor, whereas PC2 null mice had a processing profile comparable to the wildtype mice. Although the intact precursor was not recovered in the extracts, mRNA expression was similar to levels in wildtype mice, and GIP positive cells were clearly identified by immunohistochemistry also in the PC1/3 null mice (not shown). These data demonstrated that the K-cells were present and translated and synthesized proGIP, but that PC1/3 was required for generation of mature GIP1-42. In contrast, PC2 does not seem to play a role in generating the mature GIP1-42 in vivo.

Processing of proGIP – investigations in cell lines

Intriguingly, an additional cleavage site Gly31-Lys32-Lys33 exists inside the GIP1-42 sequence (figure 1). This has led to speculations that a possible alternative product, GIP1-30amide which is also known as a potent stimulator of the GIP receptor, could be formed from the precursor (39-42). Additionally, recent characterization of the expression profile of furin, PC1/3 and PC2 by immunohistochemistry found that only 50% of the GIP positive cells were also positive for PC1/3, 75% also expressed furin, whereas GIP and PC2 did not seem to co-localize (35). With this in mind it is possible that an absolute requirement of PC1/3 for all proGIP related function may be too simplistic. Subsets of intestinal, GIP producing cells, in which alternative fragments of GIP that are either quantitatively undetectable, inseparable on gel filtration and HPLC or are lost during extraction, may exist.

The question whether enzymatic cleavage by PC1/3 is sufficient to release GIP1-42 from its precursor and whether alternative cleavage may occur was addressed in cell line studies. Upon transfection of a neuroendocrine cell line, endogenously expressing PC1/3, but not PC2, with preproGIP, only one fragment corresponding to GIP1-42 could be identified by gel filtration and HPLC. ProGIP was also processed in a PC2 producing cell line, but the gel filtration profile indicated that larger and smaller GIP immunoreactive fragments were produced in addition of GIP1-42. This was verified when subjecting material obtained from gel filtration, eluting in the GIP1-42 position, to HPLC. Similar observations were made after co-transfection of a cell line not expressing either of the PC's, in relevants amounts, with preproGIP and PC1/3 or PC2. In addition, a small fragment corresponding to GIP34-42 was identified as part of the PC2, but not PC1/3 proGIP processing profile, in agreement with a PC2 mediated alternative processing at the Gly31-Lys32-Lys33 motif thereby releasing the GIP1-31 (and GIP1-30amide, if further converted by peptidylglycine alpha-amidating monooxygenase) and GIP34-42 (figure 1). Intriguingly, in cells expressing PC2, a shoulder on the peak of mature GIP1-42 was observed on the gel filtration profile with the side-viewing and NH2-terminal antisera only. This product might very likely correspond to the full NH2-terminal part of proGIP cleaved at the Gly31-Lys32-Lys33 motif only. In the murine proGIP sequence, this fragment corresponds to approximately 6.5 kilo dalton (kDa) and a similar shoulder was also observed, albeit at low levels, in intestinal extracts from wildtype mice. This product was not eliminated in PC2 null mice and was rather decreased in PC1/3 null mice, indicating that it is not a product of alternative PC2 mediated processing. However, it remains possible that it is derived from alternative processing by another protease. If the small amounts of ~6.5 kDa protein observed in vivo indeed corresponds to a processing of proGIP at the Gly31-Lys32-Lys33 motif it should be noted that a similar processing of human proGIP would yield a fragment of approximately 8 kDa (see below).

GIP IMMUNOREACTIVITY IN HUMANS

As nutrients are considered the prime stimulator of incretin secretion, numerous studies have investigated fasting and postprandial plasma levels of GIP. However, soon after establishment of the first RIAs detecting GIP in plasma samples it was noted that levels differed depending on the antibody used (43; 44). It was consequently hypothesized that some antibodies raised against GIP also react with other yet unidentified peptide(s). Accordingly, an unidentified 8 kDa form in addition of the known 5 kDa (GIP1-42) form could be noted in gel filtration profiles of porcine and human intestinal extracts by most of the GIP directed assays although a few antibodies targeting the COOH-terminus of mature 5 kDa GIP did not detect this form (44-47). Hence, 8 kDa GIP was hypothesized to be a precursor product of the proGIP and nutrient dependent release was consequently investigated using chromatography and different RIAs to distinguish between the 5 and 8 kDa forms in plasma samples from humans (45; 47). Whereas both forms were found in plasma after intraduodenal glucose and lipid infusions, only 5 kDa GIP consistently responded to the nutrient stimuli regardless of the antibody used (47). Furthermore, differences in GIP levels between assays were also found when measuring fasting levels whereas the increase in total GIP immunoreactivity after duodenal infusions differed less between assays (47). In addition, one of the antibodies consistently detecting the greatest amounts of immunoreactivity did not cross-react with 8 kDa GIP. Thus, 8 kDa GIP did not seem to con-

stitute a major part of the plasma levels after stimulation of endogenous GIP release. In the following years, it became clear, using a new GIP antibody, that the peak found in the 5 kDa GIP position by gel filtration in addition to the mature GIP1-42 also included the GIP metabolite GIP3-42 (48). Whereas older antibodies were generally raised against epitopes of porcine GIP within the region 15-42, this antibody was raised against the NH2-terminal sequence of human GIP detecting only GIP1-42 and not GIP3-42. With this antibody, significantly lower postprandial GIP levels were found, compared to all older assays in agreement with a peripheral degradation of the peptide rendering the hormone biological inactive (48). However, when characterizing the cross reactivity of the NH2 directed antibody by subjecting plasma samples obtained in the fasting and postprandial state to gel filtration and HPLC, a second peak corresponding to 8 kDa GIP could be identified by this antibody (48). Furthermore results from using this assay in the processing studies discussed above, indicated that this antibody recognizes the NH2- and COOH elongated forms of GIP but has an absolute requirement for the first two amino acids of GIP1-42. Although it seems a puzzle that antibodies directed at two different epitopes of the same sequence, recognize fragments of similar size and hydrofobicity, the exact nature of 8 kDa GIP remains unknown and one can only speculate whether this represents a precursor product of proGIP or not. As indicated in the previous section, a NH2-terminally extended form of GIP with a COOH-terminal processing at the Gly31-Lys32-Lys33 motif present in GIP1-42, would, in humans, release a peptide fragment with a size of approximately 8 kDa and would not be detected by an antibody specific for the correctly processed COOH-terminal of GIP1-42 (figure 1). A simultaneous COOH-terminal processing, necessary for production of mature GIP1-42, would further release a fragment of 9 amino acids. Such a fragment has not been described. However, when analyzing neutral extracts made from segments of murine upper jejunum, we found by gel filtration a small fragment not retained by the gel matrix with GIP immunoreactivity using a COOH-terminal directed antibody (unpublished).This observation adds further support to the possibility that processing at Gly31-Lys32-Lys33 occurs in vivo but raises the question which, if any, of the fragments would possess biological activity. As part of this Ph.D-project we made an unbiased attempt to purify the 8 kDa GIP fragment. Unfortunately, sequential rounds of HPLC purification diluted the fragment to much for N-terminal sequencing, without yielding sufficient purity for mass spectrometry identification. Ultimately, this project was abandoned due to lack of progress and time. However, the analysis of the K-cell has progressed beyond this PhD project in collaboration with Jens Pedersen and a transgenic mouse strain has been generated that expresses the diphtheria toxin receptor in the GIP locus cloned as a bacterial artificial chromosome. If the above stated hypothesis is correct, depletion of intestinal K-cells by diphtheria toxin administration would remove the extended forms and the possible C-terminal fragment demonstrated on gel filtration profiles along with the mature GIP1-42. Such a result would justify further hypothesis driven attempt to identify the porcine or human 8 kDa GIP fragment.

GIP SECRETION

The GIP containing K-cell is believed to directly sense the nutrient flow in the small intestine by its apical surface opening into the lumen, and many have examined possible nutrient mediators of GIP release (49).

In light of the fact that many of the early studies investigating nutrient dependent GIP secretion have used RIAs with undefined and varying specificity the results should be interpreted with caution. Nonetheless, an antibody recognizing both GIP1-42 and GIP3-42 is essential for correct estimation of intestinal GIP secretion from plasma samples. Furthermore, an antibody specific for the COOH-terminal of GIP would be of preference as these do not seem to cross-react with the larger GIP immunoreactive form of unknown origin.

Even so, glucose and fat were early on characterized as potent stimulators of GIP secretion in man, resulting in rapid release of GIP reaching a peak 15-30 or 30-45 minutes after oral ingestion or intraduodenal infustions of glucose or fat, respectively (47; 50; 51). Plasma levels of biologically active GIP1-42 remain significantly elevated at least 2 hours after ingestion of a mixed meal (48). Furthermore, 24 hour secretion patterns of GIP (and GLP-1) reveal elevated plasma levels during the day with fluctuations following a meal and reach fasting levels only during the night (52; 53). In contrast, insulin fasting levels could be reached 3-4 hours after a meal (52; 53). Hence incretins are present in circulation during the day with low concomitant insulin levels. As the understanding of GIP effects are tightly related to food ingestion and insulin secretion, this may be of biological significance. Surprisingly, and of unknown importance, GIP (and GLP-1) was recently reported to be released to the lymph in response to fat and glucose reaching, levels 3 fold higher than what could be measured in plasma obtained from the portal vein (54). Of note, and speaking against important systemic functions of lymphatic GIP, the lymphatic endothelium expresses the GIP degrading enzyme, dipeptidyl peptidase-4 (DPP-4) at levels at least as high as what is observed in vascular endothelial cells (55).

Several studies have investigated relevant stimuli necessary for excitement of the K-cell in relation to a meal and consequently GIP secretion. In agreement with a direct interaction between nutrients ingested and GIP release, GIP secretion was reported to be proportional to the amount of calories ingested (56). Furthermore, a strong correlation between rate of intestinal glucose absorption and increase in GIP levels has been reported (57). Notably, GIP secretion patterns reflected the intestinal glucose absorption ingestion of glucose, but also after ingestion of starch products resulting in a slow release of glucose and hence late and prolonged GIP responses (57). GIP secretion is consistently attenuated when nutrient absorption is reduced as a result of a malabsorptive condition or after intraduodenal administration of pharmacologic agents inhibiting nutrient absorption (58; 59). Furthermore, conditions that impair the intestinal metabolism of ingested food are associated with an attenuated GIP secretion pattern. Hence, secretion of GIP (and GLP-1) following a meal are lowered in patients with insufficient exocrine pancreas function and elevated after substitution of pancreatic enzymes (60). Similar findings were made in patients with bile duct obstruction (61), and fat induced secretion of GIP may be coupled to chylomicron formation (62).

These findings correlate with direct sensing of nutrients in the intestinal lumen. However, GIP secretion may also be regulated by feedback mechanisms. Accordingly, treatment with DPP-4 inhibitors markedly reduces levels of incretins as measured using an assay detecting both GIP1-42 and GIP3-42 whereas GIP1-42 remains elevated (63; 64). The mechanism for this is not clear and might involve GIP actions on the K-cell, other hormones that are also degraded by DPP-4, or downstream effects of augmented insulin secretion as insulin and C-peptide have been reported to inhibit GIP secretion (65-67). It is possible to administer exoge-

nous GIP1-30amide, which has preserved GIP action, and measure its effect on GIP1-42 secretion with antisera recognizing the COOH-terminal. However, such experiments have not been performed to resolve this important issue.

In the wake of the current interest in finding pharmacological targets to manipulate incretin levels, research groups are now characterizing the nutrient sensing apparatus at the molecular level. Studies in isolated perfused rodent intestine have suggested that carbohydrate detection involve the Na+-coupled glucose transporter 1 (SGLT1) supported by impairment of GIP release after administration of a SGLT1 inhibitor (68). In fact, GIP itself has been reported to facilitate transepithelial glucose transport in proximal mouse jejunum in part via SGLT1 (69). In the recent years the notion that nutrient sensing mechanisms are shared among different types of tissues has been supported. In agreement, the Kir6.2 subunit of ATP dependent K+ channels important for glucose dependent insulin secretion from pancreatic β-cells was recently reported to be present in human intestinal K- and L-cells (70). However, the biological importance is unknown. Implications for the facilitative glucose transporter, GLUT2, in incretin secretion have been investigated in GLUT2 knockout mice. Whereas the intestinal nutrient sensing is generally believed to be mediated by the apical part of the enteroendocrine cells in direct contact with the luminal flow, GLUT2 is believed to play a role in basolateral glucose efflux from small intestinal epithelial cells. Nevertheless, GLUT2 knockout mice had impaired GLP-1, but not GIP, responses to oral glucose (71) raising the question whether L-cells and possibly the K-cell, also responds to plasma glucose via membrane proteins like GLUT2 and/or ATP-dependent K+ channels. In any case, this subject certainly needs further investigation. Recently, the G protein coupled receptors (GPCR) GPR40, GPR119 and GPR120 were reported to bind long chain fatty acids and their function as possible mediators of fatty acid sensing in GIP and especially GLP-1 producing cells have been examined (72-74). Indeed, the receptors were found in intestinal cells co-staining for GLP-1 and/or GIP, and an agonist for GPR119 enhanced GIP and GLP-1 secretion in mice (73). The sorting of these receptors within the cell is unknown and the importance in vivo remains to be established.

It has proven difficult to investigate GIP secretion at the molecular level as the enteroendocrine system is diffusely located to the intestinal mucosa and no cell model has been validated for studying of GIP release. Accordingly, studies have so far been carried out using subclones of the intestinally derived STC-1 cell line that expresses GIP. However, this cell line was originally developed as a model for secretin release and additionally produces a wide range of other enteroendocrine peptides including cholecystokinin and GLP-1 (75). Thus, its relationship with the native K-cell is therefore unclear. To overcome this, a research group has generated a transgenic mouse strain that expresses the yellow fluorescent protein (Venus) under control of a bacterial artificial chromosome clone containing the GIP gene promoter. Unlike the truncated GIP promoter used by Kieffer and co-workers (36), this expression system faithfully recapitulates the expression pattern of proGIP protein and thus by subjecting intestinal single cell suspensions to fluorescence activated cell sorting (FACS), it is possible to isolate and study primary intestinal GIP producing cells in culture (76). Early characterization of primary K-cells has confirmed the likely relationship with subpopulations of the GLP-1 producing L-cell. Furthermore, profiling of the nutrient sensing machinery has confirmed gene expression of glucose channels and transporters, components of ATP dependent K+ channels, glucokinase and the fatty acid sensing receptors GPR40, GPR119 and GPR120.

GIP DEGRADATION

Once outside the K-cell GIP1-42 is rapidly enzymatically degraded by DPP-4 that mediates a NH2-terminal truncation of Tyr1-Ala2 thereby inactivating GIP1-42 and converting it to the metabolite GIP3-42. This concept was definitely established as DPP-4 inhibitors were demonstrated to significantly reduce degradation of exogenous GIP (77). The enzyme is ubiquitously expressed and occurs attached to cell surfaces at numerous sites including the intestinal and kidney brush borders and hepatocytes. In addition, DPP-4 is located bound to endothelial surfaces throughout the vascular bed but is also found in a soluble form, clearing peptides intravascularly as well as upon organ passage (78). Accordingly, plasma elimination half life of exogenous GIP has been estimated to only 7 minutes in humans (48; 79-81), and by comparing RIA results with COOH-reactive and N-terminus requiring antisera, respectively, GIP3-42 is reported to account for up to 70% of fasting GIP immunoreactivity and over 60% after a meal thus representing the major circulating form of GIP (48). GIP3-42 has been claimed to act as an antagonist on the GIP receptor inhibiting insulin release (82), but this could not be confirmed under physiologic conditions in pigs and using the isolated rat pancreas (83). Another protease, neutral endopeptidase (NEP) 24.11 which cleaves GLP-1 efficiently was also tested but GIP was found to be a poor substrate (84; 85). The impact of NEP 24.11 on GIP degradation has not been tested in vivo. Irrespective of the actual mechanisms involved, the organs responsible for GIP degradation and removal have been examined in a catheterized pig model (77). A substantial part of GIP1-42 was found to be extracted upon passing through the liver and kidney and this was significantly inhibited by a DPP-4 inhibitor (77). As newly released endogenous incretins pass the liver before reaching circulation this may be a quantitatively important site of metabolism. Furthermore, patients with renal insufficiency were reported to have higher levels of GIP (86). However, this study measured GIP concentrations with an antibody recognizing GIP1-42 and GIP3-42, with no information on levels of active GIP. A more recent study reevaluated incretin levels in patients with chronic renal insufficiency and normal subjects using NH2- and COOH-directed antibodies for determination of GIP levels and found similar levels of GIP1-42 in both groups indicating that the kidney is not a major site for N-terminal degradation of GIP in humans but important for final elimination of the metabolite (81). The molecular processes responsible for elimination of GIP in the kidney are unknown. However, the rate of GLP-1 extraction by the kidneys was found to exceed what could be explained by glomerular filtration alone, suggesting that mechanisms such as peritubular uptake might contribute (87).

ACTIONS OF GIP

The impact of incretins on regulation of glucose homeostasis has been thoroughly investigated and the number of studies investigating effects of GIP and GLP-1 is enormous. In line with our increasing knowledge on extrapancreatic effects (especially of GLP-1), it is becoming clear that these hormones together act at multiple levels to regulate nutrient intake and disposal and additionally effectuate functions not directly involved the acute regulation of metabolism.

The incretins exerts their effects though specific, glycosylated receptors belonging to the secretin, B-family of GPCRs that in-

cludes, among others, the receptors for secretin, glucagon and GLP-2. The human GIP receptor has an estimated molecular weight of 59 kDa with 99.4% and 79.5% sequence identity to chimpanzee and house mouse, respectively (49). A number of splice variants have been reported to exist but the functional significance of these are unclear (49). GIP induces homologous desensitization of the receptor but chronic elevations in glucose have also been reported to result in desensitization and to down regulate transcription (88; 89).

In agreement with widespread effects of GIP, the receptors are found in a diverse range of tissues. Besides the established expression in pancreatic islets, GIP receptors are also reported to be present in adipose tissue, gut, several regions of the brain, testis, pituitary, lung, heart, vascular endothelium and bone (3). Whereas GIP receptors do not seem to be expressed in the normal human adrenal gland, ectopic expression here has been found to facilitate cortisol secretion, linking GIP to a food dependent form of adrenal hyperplasia and Cushing's syndrome (90; 91).

The function of GIP receptors in many of these regions is largely unknown. In the following a brief description of some peripheral effects of GIP will be given before emphasizing on effects on the endocrine pancreas and adipose tissue in the next sections.

GIP was originally identified and named on the basis of its ability to inhibit gastric acid secretion (92). However this could not be confirmed in humans (93). Furthermore GIP did not affect gastric emptying in humans (94). As GIP release strongly correlates with intestinal glucose absorption rate, local intestinal effects are likely. Consistently, GIP was reported to enhance Na+ currents and transepithelial glucose transport when investigated in mouse jejunum mounted in a Ussing chamber, in part via the SGLT1 (69). Thus, GIP may mediate trafficking of SGLT1 into the brush border membrane at the apical site and GLUT2 in the basolateral membrane (95).

GIP has been suggested to be one of more intestinal derived factors involved in directing nutrients to the bone thereby regulating bone metabolism. Accordingly, GIP administration increased bone density in ovariectomized rats (96). GIP receptor knockout mice were reported to have decreased bone size and mass, altered bone microarchitecture, biomechanical properties and turnover (97) whereas mice with overexpression of GIP under control of a zinc inducible ubiquitous promoter had increased markers of bone formation, decreased markers of bone resorption and increased bone mass (98). However, acute administration of GIP did not alter markers of bone turnover in humans (99) and the effect of GIP on human bone metabolism is therefore not clear.

GIP effects on the cardiovascular system have not had much attention. However, studies have reported diverse effects of GIP in regulating blood flow. Accordingly, exogenous GIP was found to increase splanchnic blood flow in dogs (100; 101). In agreement, GIP was found to stimulate nitric oxid production from portal vein endothelial cells pointing at a vasodilating effect, but also to mediate secretion of the vasoconstrictor endothelin in arterial hepatic cells (102). Effects that would be expected to result in vascular changes optimizing delivery of nutrients to the liver during a meal. Further observations substantiating an effect of GIP on blood flow in humans have recently been described by Asmar et al. from Jens Holst research group. She found a significant, yet 60-90 minutes delayed, increase in adipose tissue blood flow when GIP was infused under a hyperinsulinemic-hyperglycemic clamp mimicking glucose and insulin levels seen after a meal (manuscript submitted). Whether this is a direct effect on the vasculature is unknown and the delay raises the possibility that secondary mediators are induced. Although an increase in blood flow would be expected to direct nutrients to adipose tissue, and could be of biological significance for uptake and storage of nutrients in adipocytes, the majority of the increase happens after the increase in reesterification. With this in mind, it may seem more likely that the meal induced GIP response is priming the tissue for metabolic actions beyond the early post-prandial phase (discussed in detail in section 7.2).

The existence of GIP receptors in adipose tissue currently attracts considerably interest, but the effects of GIP signaling in adipose tissue are not clear and human studies are missing. Furthermore, interaction between GIP effects on insulin secretion and adipose tissue are likely to affect whole body lipid homeostasis but individual effect of the two have been difficult to seperate. In the following, the literature related to GIP actions on the endocrine pancreas and the adipose tissue will be reviewed, followed by a discussion of data obtained in GIP receptor null mice with transgenic tissue specific rescue of the GIP receptor in the pancreatic β-cells or adipocytes (manuscript 2).

The endocrine pancreas

GIP actions on the β-cell

Since GIP was first recognised for its insulinotropic effects in 1973 (103) and shortly after established as an incretin (104) its actions on the endocrine pancreas have been extensively investigated. Many studies have focused on its effects on the insulin producing β-cell, often investigating insulinoma derived cell lines as a model. Stimulation of adenylate cyclase (105) and mobililization of calcium (106) were reported as mechanisms for GIP mediated insulin release in cell lines or isolated islets. However, a more detailed understanding of the intracellular events underlying the enhanced insulin release awaited the cloning of human and rodent GIP receptors in the 1990's. Rat RINm5F insulinoma cells and COS-7 cells transiently transfected with a cloned rat receptor were found to bind GIP with low nanomolar affinity and responded with cyclic AMP accumulation (107; 108). Binding could not be demonstrated for other members of the secretin family of ligands whereas weak affinity was reported for the GLP-1 agonist, exendin-4, and antagonist, exendin-9 (108). When tested towards the human GIP receptor cloned from an insulinoma cDNA library, GIP1-42 was found to bind with high affinity and the potency (with respect to cAMP accumulation) was in the picomolar range as would be expected if it was to respond to circulating hormone levels (109). The receptor was highly specific to GIP1-42 but as demonstrated for the rat GIP receptor exendin-4 and exendin-9 had same affinity and reduced GIP-binding (109). The GIP receptor was later found to activate mitogen activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) (110), as well as phospholipase A2 (111). A subsequent screening in CHO cells, that was confirmed in an insulinoma (INS) cell line, identified that GIP induced phosphorylation of Raf-1 (Ser-259), Mek1/2 (Ser-217/Ser-221), ERK1/2 (Thr-202 and Tyr-204), and p90 RSK (Ser-380) in a concentration-dependent manner (112). It is thus evident that the GIP receptor signals via a plethora of pathways that controls insulin secretion, gene expression and survival. This observation should be considered when possible effects of GIP receptor signalling in other tissues are discussed.

The effect of GIP on insulin secretion is clearly glucose-dependent and insulin secretion is primarily regulated by glucose that freely enters the β-cell through membrane bound glucose

transporters. After entry into the β-cell, glucose is metabolised by glycolysis and mitochondrial oxidation resulting in an increased ATP/ADP ratio. This causes closure of ATP-sensitive K+ channels, membrane depolarization, activation of voltage dependent Ca2+ channels, increase in intracellular calcium and subsequently insulin-granule exocytosis (49). The process is terminated by membrane repolarization by voltage-dependent K+ channels (49). GIP complements this machinery by increasing intracellular calcium and cAMP thereby activating protein kinase signalling pathways acting directly on the exocytotic machinery and stimulating insulin transcription and biosynthesis (113-115). The GLP-1 and GIP glucose enhancing effects were investigated in mice lacking the Kir6.2 subunit of the ATP sensitive K+ channels. Importantly, in that study the insulinotropic effect of GIP seemed to depend much more on the closure of ATP sensitive K+ channels than effects of GLP-1 (116). This may provide a clue to understanding the attenuated effect of GIP observed in type 2 diabetic patient (discussed below). Thus, a recent study demonstrated a more than additive increase in insulin secretion after co-adminstration of GIP and a sulphonylurea compound under hyperglycaemic clamp conditions in patients with type 2 diabetes (117).

The MAPK activation reported, lead to the hypothesis and subsequent verification that GIP promotes β-cell survival. This appeared to depend on cAMP and dynamic p38 MAPK modulation (118), and was mapped further downstream to PI3K/PKB/FoxO1 signaling, which mediates GIP suppression of the pro-apoptotic bax gene expression (119). Thus, GIP appears to counteract apoptosis. The evidence for anti-apoptotic and proliferative effects of GIP in vivo is limited. However, a chronic GIP infusion enhanced β-cell expression of the anti-apoptotic BCL-2 and downregulated the proapoptotoc Bax in diabetic Zucker rats (119), but GIP receptor activation did not influence sensitivity to streptozotozin induced diabetes in contrast to GLP-1 receptor activation (120). Noteworthy, the effects of GIP receptor signalling on β-cell survival occurs independently of glucose sensing. This in turn impinge on the interpretation of the actions of the long acting GIP analogues and partial agonists (designated antagonists) reviewed below, which by these mechanisms may improve long term insulin secretion and glucose control, entirely irrespective of their effect on acute metabolic control.

GIP actions on non-β-cells

Although GLP-1 and GIP receptors are both present on β-cells and have similar intracellular signalling properties, the receptor expression differs in non-β-cells. Thus, while studies consistently report expression of GIP and GLP-1 receptors on β-cells, one study reported that α-cells express GIP receptors and not GLP-1 receptors, and are sensitive to GIP stimulation only, as measured by cAMP accumulation (121). Although other studies also reported about GLP-1 receptors on alpha cells (122), this conclusion was later refuted by the original lead investigator when using the same antiserum (123). Despite some controversy of receptor expression, functional studies in perfused islets and in humans are equivocal: only GIP stimulates glucagon release, and GLP-1 represses glucagon release (124-126). A study by De Heer et al. went further and demonstrated a requirement for somatostatin in GLP-1 mediated glucagon suppression by means of a small non-peptide receptor antagonist (126). A recent study failed to demonstrate GLP-1 receptors on somatostatin producing cells (123). Irrespective of the mechanism of GLP-1 mediated somatostatin induction, the conclusions by De Heer et al. are supported by demonstrated release of somatostatin by GLP-1, but not GIP in the isolated rat pancreas (126).

Insulin and glucagon secretion in conditions with dysregulation of the enteroinsular axis

The above mentioned receptor localization and mechanistic studies suggest that GIP and GLP-1 induce release of secretory granules on the cells where they are expressed, but that this, apart from insulin release, results in glucagon secretion by GIP receptor signalling in α-cells and glucagon inhibition by GLP-1 receptor mediated somatostatin release. Furthermore, numerous studies suggest that glucagon is also a potent insulin secretagogue (127) and a possible physiological mechanism for these interactions could be that the early postprandial rise in GIP levels mediates an insulin potentiating glucagon release, which is abrogated shortly after by GLP-1 mediated glucagon inhibition and perhaps also by β-cell secretory products (128). The net result may be more rapid control of ingested glucose. However, another study could not confirm a local insulinotropic effect of endogenous glucagon when investigated in the isolated perfused pancreas (129). Furthermore, GIP had no glucagonotropic effects in healthy humans during hyperglycaemic clamp conditions (4; 130) whereas a dose dependent effect was reported during euglycemia (125). Still, an intraislet communication would not necessitate a rise in glucagon levels sufficient for detection in circulation. Indeed, a systemic rise in glucagon levels in the postprandial phase would be inexpedient and oppose insulin in lowering blood glucose. Clearly, intraislet communication and regulation of insulin secretion is complex and difficult to study and this is further complicated by simultaneously opposing effects of other factors, like incretins.

The insulinotropic effect of GIP has long been known to be attenuated in the diabetic patients (4; 131); a defect that appears to be secondary to the diabetic state as responsiveness can be restored by treating hyperglycemia in experimental models (132) and in humans (133-135). The reduced effect of GIP receptor signalling in β-cells combined with an intact glucagon stimulating effect in α-cells could, in theory, contribute to postprandial hyperglycemia observed in diabetic patients. Consistent with a glucagonotropic effect of GIP in type 2 diabetic subjects, GIP infusion has been demonstrated to enhance glucagon secretion in this state (4; 136). From these studies it can be difficult to determine if this is a direct effect of GIP, and both GIP augmented insulin and glucagon secretion diminished after about 20 minutes. Intriguingly, Knop et al. reported that the reduced postprandial glucagon suppression observed in type 2 diabetic patients was likely to be due to a factor secreted in response to oral glucose ingestion and could not be observed when the glucose curve was mimicked by an intravenous glucose infusion (137). In this study, GLP-1 secretion was similar in the healthy and diabetic controls and insulin levels were highest after oral ingestion (137). Consequently, the altered glucagon levels cannot be explained by diminished secretion of GLP-1 and insulin, and a possible glucagonotropic candidate for this effect is GIP. In a recent study approaching this topic, postprandially infused GIP in type 2 diabetic patients had paradoxical consequences. The GIP infusion had a short-lived initial insulinotropic effect, but with a concomitant glucagon rise and the glucose lowering effect of GIP was lost. Indeed, GIP infusion worsened late postprandial glycemic control, possibly as a result of simultaneous decrease of GLP-1 levels (138). The pathophysiology associated with attenuated insulinotropic effect of GIP in type 2 diabetes is unknown. Defective

expression or signaling of the GIP receptor have been reported (139; 140). However, there is no convincing mechanism that explains how and why GIP receptor expression and signalling is reduced in the hyperglycaemic state, but it has been suggested that fat can upregulate GIP receptor expression whereas glucose reduces GIP receptor expression (88). Furthermore, GIP function is not only reduced by desensitization as seen with GIP receptor expression in pancreas, but also by reduced GIP release as seen in the newly diagnosed, non-obese type 1 diabetic patient (141).

As impaired glucose control negatively impacts β-cell responseness to glucose, one would expect impaired GIP mediated incretin function in GLP-1 receptor deficient mice and vice versa. In fact, the opposite is seen. GLP-1 receptor deficient mice exhibit increased GIP release and augmented GIP actions on beta cells, whereas GIP receptor deficient mice exhibited increased insulin responses to GLP-1 and glucose and both groups of mice had decreased insulin mRNA synthesis (142; 143). The molecular mechanisms for such compensatory mechanisms are completely unknown.

Perspectives on the GIP, GLP-1, glucagon and insulin interaction for mouse genetics

The intricate network of hormone interactions within the islets of Langerhans impacts the interpretation of gene targeting and gene rescue strategies for incretin hormone receptors. For this reason I will review the immediate concerns here, before proceeding with sections that to a large extend rely on interpretation of mouse gene targeting models. Important examples are the compensatory increase in GIP release and action, as reported for the GLP-1 receptor knockout mouse (142), which may stimulate glucagon secretion, leading to overinterpretation of the role of GLP-1 in normal glucose homeostasis. In contrast, the GIP receptor knockout mouse has been reported to have an increase in GLP-1 action (143). This may lead to an overestimation of the adiposity promoting effects of GIP, as GLP-1 treatment in itself reduces body weight and adiposity. On the other hand, the normal role of GIP in insulin stimulation and islet cell survival may be underestimated as these functions are potently stimulated by GLP-1. For gene rescue studies it is important to realize that β-cell expression of GIP or GLP-1 receptors will recapitulate the direct augmentation of insulin secretion, but not the glucagon stimulation of GIP and the glucagon suppression by GLP-1. Similarly, extrapancreatic rescue of GIP receptors in adipocytes will substitute direct effect of GIP in this tissue, but not the GIP augmented insulin or glucagon secretion. Thus, a gene rescue experiment with any incretin receptor will not necessarily mimic the effect of receptor activation in a normal mouse and must be interpreted with this in mind.

Adipose tissue

In vivo and in vitro studies

It was early on observed that GIP secretion was potently stimulated by lipids and that postprandial GIP levels seemed to be higher in the obese state (62). Hence, it was hypothesized that GIP may be a mediator of delivery of fat to the adipose tissue and thereby function as a link between food intake and obesity (144). Later studies have questioned whether GIP levels indeed are higher in obese subjects although results are conflicting (56; 145). In agreement with GIP as a regulator of fat metabolism, GIP infusion was found to increase chylomicron clearance from plasma in dogs (146) and lower plasma triglyceride levels after an intraduodenal lipid infusion in rats, whereas immunoneutralization of endogenous GIP resulted in decreased clearance of triglycerides (147). However, a similar effect could not be demonstrated in response to an intravenous lipid infusion in dogs or humans (148; 149). The issue was recently investigated in healthy humans by Asmar et al. (Jens Holst's research group). In that study, exogenous GIP did not affect triacylglyceride and fatty acid plasma levels over a 3 hour study period indicating that GIP does not mediate uptake of nutrients (manuscript submitted). In addition, the ability of GIP to clear fat infused intravenously (i.v.) was investigated under different conditions. When infusing fat in combinations with glucose and/or GIP, effects of GIP or insulin as well as combined effects of insulin and GIP could be examined. GIP did not change triglyceride or glycerol levels. Infusion of lipid and GIP without glucose lowered levels of circulating free fatty acids but not to the same level as glucose augmented insulin secretion without GIP. Importantly, the GIP infusion induced a modest but significant rise in insulin levels. Combination of GIP and glucose augmented insulin secretion and lowered levels of fatty acids to the same level as glucose augmented insulin secretion alone (manuscript submitted). As insulin is an established regulator of fat metabolism with anabolic effects, and GIP stimulates insulin secretion in a glucose-dependent manner it is difficult in a physiologic model to investigate isolated GIP effects on fat metabolism under controlled levels of insulin. The study by Asmar et al. is the first of this kind in humans to investigate whole body effects of GIP in regulation of fat metabolism and it questions the biological implication for postprandial GIP secretion in the acute regulation of uptake of nutrients where insulin clearly seems to be dominant.

Nevertheless, several studies investigating effects of GIP in adipocyte cell lines or explants have reported that GIP directly regulates adipocyte metabolism. In agreement with a direct effect of GIP, adipocytes have been reported to express GIP receptors (150). Furthermore, effects of GIP in perifused isolated adipocytes could be blocked by a GIP receptor antagonist (151). Studies in isolated adipocytes, adipose tissue explant and the adipocyte cell line, 3T3-L1, have reported that GIP mediate uptake of glucose and fatty acids (152-155), stimulates lipoprotein lipase (LPL) activity (5; 156-158), and inhibits catecholamine and glucagon mediated lipolysis (151; 152; 159). Some studies have found insulin independent effects whereas other studies investigating concomitant effects of GIP and insulin find that GIP potentiates insulin mediated effects (5; 152; 153; 157; 160; 161). This aspect was also looked into in the study by Asmar et al. investigating insulin and GIP mediated clearance of i.v. infused lipids in healthy humans. However, here the glucose infusions alone induced insulin secretion mimicking postprandial levels and adding GIP further increased insulin secretion to supraphysiological levels. Hence, experimental settings did not sufficiently mimic physiological conditions making it difficult to conclude whether GIP plays a role under conditions of moderate insulin stimulation (Asmar et al., manuscript submitted).

Rather confusingly, GIP also stimulates lipolysis in cell cultures, and conflicting effects on nutrient uptake and stimulation of lipolysis have been reported even when analyzed in the same study (151; 152). McIntosh et al. questioned a direct anabolic effect of GIP in adipose tissue, as GIP exerts its effects on pancreatic islets via stimulation of the adenylyl cyclase. In adipose tissue cAMP production is related to lipolysis rather than lipogenesis. In agreement, they found that GIP stimulated lipolysis in the 3T3 cell line and that this was inhibited by insulin suggesting that GIP only mediates lipolysis during fasting (162). Whether GIP in the presence of insulin could facilitate nutrient incorporation was not

investigated in this study. However, another study found that GIP alone inhibited incorporation of fatty acids in adipose tissue from lean rats, whereas a combination with insulin stimulated incorporation more than insulin alone (161). A similar study was performed by the same researchers in obese Zucker rats. However, in the obese rats, GIP alone did stimulate fatty acid uptake, and this was still further potentiated by insulin suggesting an increased sensitivity to GIP in the obese state (153). In recent studies McIntosh and coworkers have looked further into mechanisms by which GIP in the presence of insulin, could promote uptake of triglyceride to adipose tissue. GIP, in the presence of constant insulin levels, was found to stimulate LPL activity in a dose dependent manner in 3T3-L1 and human subcutaneous adipocytes, increasing intracellular triglyceride concentration. In both experimental systems, a similar signaling pathway involving increased phosphorylation of protein kinase B (PKB) and reduced phosphorylation of LKB1 and AMP-activated protein kinase (AMPK) was involved (158). In addition, a 2-week continuous GIP infusion to lean and fatty Zucker rats increased LPL activity and triglyceride content in epididymal fat pads in both groups with similar modulations of PKB, LKB1 and AMPK phosphorylation (158). Naturally, such in vivo experiments can be difficult to interpret as a constant GIP infusion possibly affects β-cell function and insulin release. In that study, GIP infusion improved glucose tolerance in the obese but not in lean rats (158). Surprisingly, GIP had a delayed effect on LPL activity and in later experiments in 3T3-L1 cells this was found to be mediated via secretion and expression of the adipokine resistin (163). To support the 3T3-L1 cell data, a continuous GIP infusion was performed in lean and fatty Zucker rats. This treatment significantly elevated circulating levels of resistin (163). Of note, insulin has been reported to stimulate resistin secretion in 3T3-L1 adipocytes (164). Furthermore, rats overexpressing or mice lacking resistin did not have altered body weight or adiposity (165) as would be expected from the studies by McIntosh and co-workers. In contrast, rats overexpressing resistin had increased levels of free fatty acids and decreased insulin-stimulated lipogenesis, indicating that resistin directs lipid accumulation away from adipose tissue (165). Nevertheless, McIntosh and coworkers have added a new and interesting perspective for effects of GIP in adipose tissue. Whether a similar mechanism exists in humans is unknown. The primary source for human resistin is not adipocytes but monocytes/macrophages (166). However, macrophages are considered to be functionally related to adipocytes and the adipose state is characterized by its infiltration of macrophages to adipose tissue (167). Despite that GIP receptors have been reported to be expressed in isolated adipocytes (150), GIP receptors may also be expressed in other tissue components of adipose tissue like endothelial cells and macrophages as can be seen upon differentiation of the human myeloid progenitor HL-60 cell line (http://www.abgent.com/products/catalog_no/AP7495a/specification). However, expression of the GIP receptor has not been investigated in these cellular components of adipose tissue. In agreement with GIP mediating effects in adipose tissue via other local factors, Asmar et al. found that GIP and insulin increased adipose tissue blood flow in humans (manuscript submitted). A sudden steep increase in blood flow occurred 60-90 minutes after initiation of a GIP infusion and a hyperinsulinaemic hyperglycaemic clamp.

Taken together, insulin is the established regulator of fat metabolism and promptly induce clearance of free fatty acids. The role of GIP in this acute postprandial phase is unclear. Furthermore, GIP may affect adipose tissue metabolism via other local factors like adipokines but the biological significance of this is unexplored.

Descriptive studies in mice with disturbed incretin receptor signaling

A key finding that etablished GIP as a fat promoting hormone came with the seminal study by Miyawaki et al. in 2002 in which the GIP receptor knockout (GIPr-/-) mice were reported to be resistant to diet induced obesity (DIO) (5). Accordingly, mice fed a high fat diet (HFD) for 43 weeks had similar weight gain as mice fed a low fat diet. Furthermore, GIPr-/- mice fed a HFD did not accumulate fat in the liver and had improved insulin sensitivity comparable to knockout mice fed a low fat diet (5). Fat mass was not estimated in GIPr-/- mice. In addition, the hyperphagic and obese leptin deficient ob/ob mice had reduced weight gain when also lacking GIP receptors, further emphasizing the protective effect towards DIO mediated by lack of the GIP receptor (5). Glucose control was not investigated in this study by Miyawaki et al. However, in the original study, GIPr-/- mice, fed a regular diet, were shown to be modestly glucose intolerant and had lower insulin levels after an oral glucose challenge, in agreement with GIP as a mediator of the incretin effect (168). After 3 weeks of high fat (HF) feeding, a compensatory increase in insulin secretion could be noted in wildtype mice, but not in GIPr knockout mice, despite similar weight gain between groups (168). This finding indicates that these mice, in addition to a disturbed control of body weight, also exhibit defects in the entero-insular axis. However, ob/ob mice and ob/ob mice with defective GIP receptor signaling had different body weight gain but similar fasting insulin levels. Hence, GIP was concluded to function as a direct link between overnutrition and obesity with the hypothesis that overeating induces hypersecretion of GIP, which increases nutrient uptake and triglyceride accumulation in the adipocytes causing obesity (5). Since then, it has been reported that mice lacking GIP receptors are additionally protected from age- and post-menopause related obesity (169; 170). Furthermore, another research group investigating HFD induced body weight gain in both GIPr-/-, GLP-1 receptor (GLP-1r) ¬-/- and double incretin receptor knockout (DIRKO) mice confirmed the previously reported lean phenotype in GIPr-/- (171). In this study, using the same GIPr knockout strain, GIPr-/- mice did significantly increase body weight gain over a period of 20 weeks of HF feeding. However, this was markedly lower than weight gain obtained in the wildtype mice (171). Interestingly, GLP-1r-/- mice and DIRKO mice were also reported to exhibit resistance to HFD induced body weight gain (171). This was somewhat a paradoxical finding, as GLP-1 is a known satiety factor (145; 172). Consistently, these mice had an increased daily energy intake when normalized to body weight (171). Furthermore, GLP-1r-/- mice had increased physical activity when compared to wildtypes indicating that GLP-1 mediated inhibition of food intake is balanced by motor control (171). However, similar changes in physical activity were observed in GIPr-/- mice. In addition, GIPr-/-, but not GLP-1r-/- mice, had increased adiponectin levels when fed a regular and a HFD. Furthermore, GIP, but not GLP-1, was found to increase resistin plasma levels in a GIP receptor dependent manner, indicating that GIP may directly modulate the adipokine profile secreted from adipose tissue (171). This in vivo observation fits well with reports of GIP effects on adipose tissue expressing GIP receptor (127; 150; 155; 173) and encouraged McIntosh and coworkers to investigate effects of GIP via resistin as discussed in the previous section. In contrast, whether GLP-1 receptors are expressed in

adipose tissue is controversial and never seen at mRNA level (127; 174; 175). Additionally, administration of GLP-1 in pharmacological doses induces weight loss (172), and intracerebroventricular injections of GLP-1 in mice have been shown to reduce adiposity through the sympathetic nervous system, independent of food intake (176). Importantly, GIPr-/-, GLP-1r-/- and DIRKO mice all had decreased insulin responses to an oral glucose challenge as well as lower ambient insulin levels when fed a regular and a HFD indicating that these mice all have impaired entero-insular axis attenuating insulin transcription (171). Insulin is the established regulator of lipogenesis, and insulin signaling in adipose tissue is essential for development of obesity (177). Hence, the similar phenotype observed in the 3 strains could be the result of a disturbed entero-insular axis, resulting in impaired postprandial insulin levels. In conflict with this hypothesis, ob/ob mice also lacking GLP-1 receptors had normal body weight gain in contrast to ob/ob mice lacking GIP receptors (5; 178). However, the genetic background donated from the incretin knockout mice when intercrossed with the ob/ob strain differed in these two studies and they are therefore not readily comparable.

From the existing literature it is evident that GIP regulates adipocyte metabolism. However, the mechanism for interaction with insulin and the significance of circulating insulin levels necessary for the function of GIP remain unclear. Only few studies have attempted to investigate this in an experimental setup controlling insulin levels in a physiologic model. Hence regulation of glucose control and adiposity were investigated in the insulin receptor substrate-1 (IRS-1) knockout mice lacking the GIP receptor. These relatively insulin insensitive mice were found to have improved insulin sensitivity and decreased adiposity compared to the IRS-1 knockout mice when fed a standard chow suggesting that the GIP receptor promotes adipogenesis (179). However, also IRS-1-/-, GIPr-/- double knockouts, like the incretin receptor knockout mice, had reduced insulin secretion in response to an oral glucose challenge. Therefore, also this model has two variables that individually may result in lower adiposity. The study by Asmar et al. investigating the insulin and GIP mediated clearance of i.v. infusion of lipids in humans has already been discussed. In short, they were also unsuccessful in keeping insulin levels constant under infusion of lipids and GIP, and the increased clearance of free fatty acids under these conditions could be a result of GIP stimulated insulin secretion rather than an effect of GIP alone. Unfortunately, as discussed above, the study could not conclusively address a possible interaction between GIP and insulin in this acute postprandial phase (Asmar et al, manuscript submitted). Nonetheless, this study clearly underlines the importance of insulin for the acute distribution of nutrients.

Other studies have, in the wake of the lean GIPr-/- phenotype, focused on the therapeutic potential of GIP as an anti-obesity target. Accordingly, GIP analogues with antagonistic effects have been made and are in the literature termed antagonsists. When tested in an insulin producing cell line transfected with the human GIP receptor, these analogous had partial agonistic effects alone but antagonistic effects toward the maximum of native GIP mediated insulin secretion, resulting in lowering of insulin release to approximately 40-50%. Conversely, in the absence of native GIP, high concentrations of these GIP antagonists will result in ~40% GIP receptor activity (180; 181). Hence, acute and chronic alteration of whole body GIP receptor signaling by daily injection of different GIP receptor antagonists have been shown to have beneficial effects on weight gain, insulin sensitivity and glucose tolerance in various mice models of obesity (181-184). Noteworthy, although these analogues may reduce postprandial GIP receptor signaling, the partial agonism will in the fasting state result in a net increase in GIP receptor activation. Accordingly, whether the observed chronic effects are the result of increased or reduced signaling are unclear. Thus, in ob/ob mice treated for a shorter period, the improvements in insulin sensitivity and glucose tolerance were found to precede any significant effects on body weight (185). These effects were recently replicated in ob/ob mice with a full agonist (186). If one accepts that the reported GIP antagonists primarily antagonize endogenous GIP in the postprandial state, then the observed acute reduction of glucose triggered insulin secretion raises the question whether the beneficial chronic effects are due to reduced circulating insulin levels as opposed to effects of GIP antagonism at other sites. However, GIP antagonism worsened glucose control and insulin sensitivity in mice with a chemical induction of beta cell death, suggesting insulin dependent effects (187).

β-CELL AND ADIPOCYTE EXPRESSION OF GIP RECEPTORS IN THE REGULATION OF BODY WEIGHT AND COMPOSITION

Studies of GIPr-/- mice form the basis for the concept that GIP is an important regulator of body weight and adipogenesis in response to HF feeding. However, as discussed in the previous section, these studies investigate effects of GIP on adipose tissue metabolism in a model with whole body ablation of the GIP receptor, resulting in both disturbed response to HFD and dysregulation of the enteroinsular axis. In an attempt to test if GIP would promote HFD induced adipogenesis directly on the adipocyte, or whether its contribution to the entero-insular axis acting on the β-cells were responsible, two transgenic mice strains with expression of the human GIP receptor under control of the adipocyte fatty acid binding protein (aP2) promoter or the rat insulin promoter (RIP), were generated. Using this strategy, the transgenic mice would have targeted expression of a transgenic GIP receptor that could be distinguished from the endogenous murine GIP receptor. Upon further intercrossing of each of these transgenic strains with the GIPr-/- mouse, two new mouse models were generated: one with expression of the human GIP receptor in β-cells, but with or without whole body ablation of the murine GIP receptor and another with the human GIP receptor in adipose tissue, but with or without deletion of the endogenous GIP receptor. Hence, we were able to investigate HFD induced body weight gain and composition in a model with restored GIP receptor signaling in adipose tissue but with a dysregulated entero-insular axis, and in another with restored entero-insular axis but defect signaling in adipose tissue. In agreement, lean mice with expression under the RIP had a normalized insulin release in response to an oral glucose load whereas mice with expression under the aP2 promoter did not. Furthermore, mice with expression of the GIP receptor in adipose tissue had normal fasting glucose levels when fed a low fat diet, whereas mice with expression of the receptor in β-cells had lower fasting glucose levels than any other genotype. In addition, the transgenic groups were glucose tolerant when challenged with an oral glucose load without improved insulin secretion. Theoretically such effects might be mediated by delayed intestinal uptake or decreased hepatic glucose output. Mice with expression of the receptor under control of the RIP are expected to have a restored GIP receptor signaling in β-cells while having deficient GIP receptor signaling in α-cells, hence lacking a glucagon stimulus when compared to wildtype mice. The restored signaling in β-cells may even further inhibit glucagon secretion via insulin. Therefore, the metabolic changes observed in this strain could likely reflect insulin/glucagon imbalance. In contrast, the

systemic metabolic changes in mice with restored signaling in adipose tissue are not easily explained by either entero-insular axis modulation, systemic differences in insulin sensitivity, nor direct effect on the adipose tissue. As was seen on conventional diet, none of the transgene mice could recapitulate all aspects of the wildtype mice response to a HFD. Thus, all of the strains with endogenous GIP receptor signaling responded to HF feeding with an increase in fasting glucose (although not significantly in mice with endogenous GIP receptor signaling and expression targeted to adipose tissue), whereas none of the strains without endogenous GIP receptor where able to do so, and a similar pattern was observed when measuring glucose tolerance.

The GIPr-/- littermates used in this study only had a tendency towards a lower insulin response to an oral glucose load as compared to controls ($p<0.067$) and had a normal glucose tolerance. This is different to what has previously been described where significantly reduced insulin response and impaired glucose tolerance was seen (5; 143; 171). Whether this is due to the fact that these mice were on a mixed C57Bl/6 and DBA genetic background in contrast to a pure C57Bl/6 background in previous studies is difficult to say. In addition, to minimize confounding effects from the diet, we used a purified low fat diet matching the HFD in dietary components whereas others have used a chow diet as low fat diet. This purified diet was, in a previous study, reported to affect a variety of metabolic parameters including increased non-fasting glucose and insulin levels (188). Hence, using chow diets may have affected earlier studies. In spite of this, in our study, the GIP receptor knockout mice once again were found to gain significantly less body weight in response to a HFD compared to wildtype mice. To estimate the sensitivity of each strain to increase body weight when fed a HFD, body weight gain obtained after HF feeding was normalized to body weight gain after low fat feeding. This was necessary as groups had differing cumulative weight gain when fed a low fat diet. Consistent with a direct effect of the adipocyte GIP receptor in regulating body weight gain, mice with a combined general GIP receptor deficiency and adipose tissue expression of the receptor had a relative weight gain similar to wildtype mice. In contrast, GIP receptor deficient mice with expression of the receptor in pancreatic β-cells had a relative weight gain similar to the GIP receptor knockout mice. Surprisingly, all groups had a similar HFD induced increase in whole body fat mass measured by nuclear magnetic resonance (NMR). In contrast, GIPr-/- mice and mice with expression of the GIP receptor targeted to β-cells and otherwise lacking the receptor did not increase lean mass in response to a HFD. Thus, indicating that the increase in body weight was due to increase in lean mass rather than in fat mass as would have been expected. Consequently, our study supports a role for the adipocyte GIP receptor in nutrient dependent regulation of body weight and lean mass, but does not support a direct and independent role for the adipocyte, nor pancreatic beta cell GIP receptor in promoting adipogenesis. This is different from what was claimed in the seminal study by Miyawaki et al. (5), but changes in fat and lean mass were not investigated. Hansotia et al. (171) measured degree of adiposity by fat pad size in the single incretin receptor knockout mice, whereas lean mass was not investigated. In that study, GIPr-/- and GLP-1r-/- knockout mice had clear increases in perirenal, epididymal and inguinal fat pad size when fed a HFD, but significantly lower than fat pad size obtained in control mice (171). In agreement, we found lower inguinal fat pad size in GIPr-/- mice but a normal response to HFD in the epididymal fat pads. This was partially reversed in GIPr-/- mice with expression of the transgenic GIP receptor in adipose tissue but not in GIPr-/- mice with GIP receptor expression in the β-cell, suggesting that the adipocyte GIP receptor is involved in regulation of adipogenesis in subcutaneous adipose tissue. To our knowledge, only one other study has investigated whole body fat mass in GIPr-/- mice using a technique, like NMR, that measures all fat including intraorgan fat droplets. That study reported that chow fed ovariectomized GIPr-/- mice are protected from obesity, however this was partly due to a decreased food intake (170); a factor that did not differ in our study. A role for GIP in nutrient dependent adipogenesis has previously been investigated by computed tomography (CT) and dual energy X-ray absorption (DEXA) scan. GIP receptor null mice fed a HFD for 3 weeks significantly increased visceral fat mass compared to control fed GIPr-/- mice but not to the level as wildtype mice fed a HFD (189). Furthermore, mice immunized against GIP during HF feeding were reported to have lower total fat mass as measured by DEXA (190). In this study, immunized mice had an unchanged growth rate whereas control mice suddenly had accelerated weight gain beginning after 12 weeks of HF feeding. Furthermore, insulin levels were not measured in these two studies making it impossible to relate the findings to β-cell function. In summary, whereas a number of studies have reported that GIPr-/- mice exhibit reduced HFD induced adiposity, no studies have actually measured whole body fat content after HFD. However, the decreased fat pad sizes found by Hansotia et al. (171) in the GIPr-/- mice likely reflect a decrease in whole body adiposity as postulated and this stands in clear contrast to our findings. Surprisingly, the lean GIPr-/- mice investigated in our study were glucose tolerant and GIPr-/- mice fed a low fat and HFD had improved glucose dependent insulin secretion compared to previously reported (143; 168; 171). Hence, it is tempting to speculate that the differences in whole body fat mass may directly reflect differences in postprandial insulin levels. As discussed above, studies in healthy human volunteers by Asmar et al. (manuscript submitted) question the importance of GIP in acute lipid storage as is central to the dogma of GIP in adipogenesis. To investigate postprandial lipid deposition in our mice models, incorporation of 14C-oleic acid into white adipose tissue, liver and muscle was examined. To potently stimulate endogenous GIP and insulin secretion, glucose and the isotope dissolved in olive oil were given orally, and tissue biopsies excised 4 hours later were digested and investigated for amount of tracer. We found no differences between groups fed a chow diet further indicating that the GIP receptor is redundant compared to insulin in acute regulation of lipid deposition in response to a meal (figure 2A). A similar distribution could be

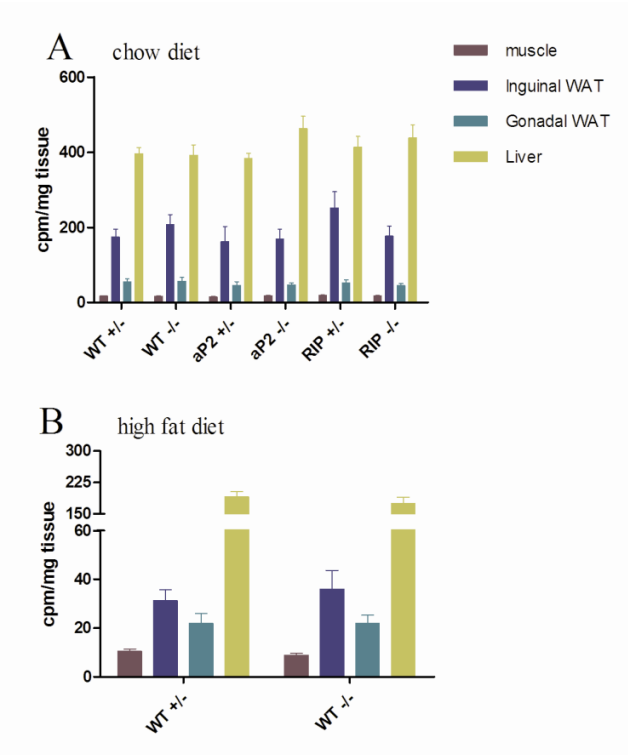

Figure 2. Uptake of 14C-oleic acid into muscle, inguinal or gonadal white adipose tissue and the liver. Mice were fasted for 16 hours, given an oral glucose load followed by an oral suspension of 14C-oleic acid in olive oil. 4 hours later, mice were anaestetized and perfused with PBS in the left cardiac ventricle while an outlet was created in the right atrium.The indicated tissues were harvested and digested in Soluene-350 before liquid scintillation counting. A, adult mice fed a chow diet. B, mice fed a HFD for 28-31 weeks. Shown are mean +/- S.E.M., n=8-20 animals in each group. The genotypes are shown below the coloumns: WT +/-: non-transgenic and heterozygous for the murine GIPr; WT -/-: non-transgenic and lacking the murine GIPr; aP2 +/-: expression of the human GIPr under control of aP2 promoter and heterozygous for the murine GIPr; aP2 -/-: expression of the human GIPr under control of the aP2 promoter and lacking the murine GIPr; RIP +/-: expression of the human GIPr under control of the RIP and heterozygous for the murine GIPr; RIP -/-: expression of the human GIPr under control of RIP and lacking the murine GIPr.

found in GIPr-/- and wildtype mice fed a HFD for 28-31 weeks although levels of 14C-oleic acid incorporated were lower in the HFD fed mice, possibly due to insulin resistance (figure 2B). Despite that our study does not support a role for the GIP receptor in acute deposition of lipids or in regulation of adipogenesis after chronic HF feeding, it cannot prove an independent role for insulin in these mice either. That differing insulin levels may affect the slightly different phenotype of the GIP receptor knockout mice investigated in this study compared to the study by Hansotia et al. also remains speculative.

Surprisingly, in this study, the GIP receptor knockout mice failed to upregulate lean mass under HF feeding, whereas this was restored in mice with the adipocyte GIP receptor. Hence the adipocyte GIP receptor seems to affect adipose tissue metabolism in a manner that regulates lean mass. However, GIP receptor knockout mice expressing the human GIP receptor in adipose tissue had a lower body weight than mice expressing the transgenic and the endogenous receptor. This could indicate that a GIP receptor mediated factor that regulates body weight under normal dietary conditions is missing in these mice. Intriguingly, mice with expression of the receptor under control of RIP and preserved endogenous GIP receptor signaling gained significantly more body weight consisting of lean mass when fed a low fat diet. However this could not be demonstrated for the mice with the transgene under control of RIP but lacking endogenous receptors further indicating that GIP receptors in the β-cell are important in regulation of lean mass, but that lean mass also depends on non β-cell GIP receptors. Hence, these data indicate, but do not prove, that the β-cell and adipose tissue interact in regulation of lean mass and hence body weight. Analysis of weight gain and body composition in double transgenic mice with expression of the human GIP receptor in β-cell and adipose tissue but otherwise lacking the GIP receptor could give more insight. However, even in such case, we cannot be sure to recapitulate the wildtype phenotype both on low and high fat diet. We observe a lowered fasting glucose when GIP receptors are expressed in the pancreatic β-cell, which would suggest that the intra-islet regulation of glucose is disturbed as compared to wildtype mice. A likely culprit for this effect is the absence of α-cell GIP receptor expression which would counteract insulin, raise plasma glucose through hepatic glucose production and lead to a net energy transfer away from the liver to non-endocrine tissues and fat tissue. Until now such a strategy has not been tangible due to absence of an α-cell specific promoter.

PERSPECTIVES

The number of studies examining GIP actions is expanding and GIP is moving into the spotlight as a possible anti-obesity target. We have established that processing for formation of mature GIP1-42 in the intestinal K-cell requires PC1/3 whereas PC2 is not essential and was not co-expressed with GIP. Nonetheless, ~50% of K-cells express GIP but not PC1/3 suggesting that subsets of K-cells with other processing products from the GIP precursor exist. Studies in cell lines have confirmed processing at other cleavage sites releasing products that can be speculated to tally with GIP immunoreactive products observed in vivo. If the identity of such products should be confirmed, the biologic action of these products remains to be determined.

The current literature on the pathways from nutrient-stimulated GIP release and its actions on the endocrine pancreas suggest a complicated network of factors necessary for maintenance of glucose and lipid homeostasis. Studies using GIP analogs, gene targeting and gene rescue strategies face many pitfalls in this complex regulation of energy storage and mobilization. This conclusion is supported by our studies in which β- or adipocyte GIP receptor specific expression recapitulated different, but non-overlapping features of the wildtype mice as compared to GIP receptor deficient mice. In light of our findings and the unclear pathways revealed by further conventional gene rescue strategies, it is possible that valuable time has been lost on the conventional knock-out strategies. It is likely that only conditional and tissue specific receptor deletions will lead to reliably interpretable data on chronic effects of the incretin receptors.

LIST OF ABBREVIATIONS

GIP: Glucose-dependent insulinotropic polypeptide
GLP-1: glucagon like peptide-1
PC: prohormone convertase
RIA: radioimmunoassay
R or arg: arginine
K or Lys: lysine
Gly: glycine
kDa: kilo dalton
Tyr: tyrosine
Ala: alanine

SGLT1: Na+-coupled glucose transporter 1
DPP-4: dipeptidyl peptidase-4
GPCR: G protein coupled receptors
NEP: neutral endopeptidase
LPL: lipoprotein lipase activity
DIO: diet induced obesity
GIPr: GIP receptor
HFD: high fat diet
DIRKO: double incretin receptor knockout
HF: high fat
GLP-1r: GLP-1 receptor
i.v.: intravenous
MAPK: mitogen activated protein kinase
PI3K: phosphatidylinositol kinase
PKB: protein kinase B
IRS-1: insulin receptor substrate-1
aP2: adipocyte fatty acid binding protein
RIP: rat insulin promoter
NMR: nuclear magnetic resonance
CT: computed tomotraphy
DEXA: dual energy X-ray absorption

SUMMARY

The present thesis consists of one published article and one draft manuscript.

Interest in the incretin hormone glucose-dependent insulinotropic polypeptide (GIP) was reignited by the discovery that GIP receptor deficient mice were unable to gain weight in response to high fat feeding. However, the path from processing of the prohormone to regulation of secretion and establishment of its role in the complicated network of mediators involved in energy mobilization is not fully understood.

The biologically active GIP1-42 was found in vivo to be dependent on processing from the immature prohormone by proprotein convertase 1/3 (PC1/3) in the intestinal K-cell. Even so, ~50% of GIP immunoreactive cells do not express PC1/3 raising the possibility that subsets of K-cells exist in which the precursor may be cleaved at alternative sites. Cell line studies did demonstrate that another convertase in endocrine cell types, PC2, mediated cleavage at alternative sites liberating larger and smaller GIP fragments. It was possible to detect fragments of similar size in gel filtration extracts of murine upper jejunum, but the identity, mechanism of processing and function of these immunoreactivities remains uncertain.

Once correctly processed GIP1-42 is secreted in response to food intake. The K-cell is believed to directly sense and respond to nutrients in the intestine, but as the molecular profiling of this cell type has just begun, the nutrient sensing machinery and possible feedback regulation are still poorly characterized.

When secreted to the blood stream, GIP acts as a mediator of energy mobilization in a complex network with other hormones. An acute and established function of GIP is to exert its incretin function thereby enhancing glucose stimulated insulin secretion necessary for prompt disposal of nutrients, yet GIP also stimulates glucagon secretion to increase blood glucose. In the diabetic state the insulinotropic effect of GIP is impaired and an early inexpedient glucagon stimulation in response to a meal further counteracts effects of insulin and worsens glycaemic control.

A demonstration that GIP receptor deficient mice were resistant to diet induced obesity let to the categorization of GIP as a fat promoting hormone and direct insulin-mimetic effects in adipose tissue has been proposed. We were able to demonstrate a redundancy for the GIP receptor in incorporation of lipids into adipocytes. We also observed that GIP receptor deficient mice could respond normally to high fat feeding with increased fat mass, but failed to increase lean mass. Mice with rescue of the GIP receptor in adipose tissue normalized the body composition in response to high fat diet, but the mice had a lower total body weight. In contrast, the GIP receptor expressed in the pancreatic β-cell was able to promote lean mass gain on a low fat diet, but not on a high fat diet.

Overall, we have established principal requirements for GIP maturation. Furthermore, we have demonstrated that neither β-cell nor adipocyte GIP receptor expression can replace the endogenous GIP receptor in regulation of body weight and body composition.

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