The term incretins is used to denote intestinal hormones released in response to nutrient ingestion that are able to potentiate glucose-stimulated insulin secretion.\(^1,2\) The communication between the intestine and the endocrine pancreas is demonstrated by the observation that the increase of plasma insulin levels following oral glucose administration is much greater than that seen after intravenous glucose.\(^3-4\) This phenomenon has been termed the ‘incretin effect’, and accounts for 50–70% of the total insulin secreted after oral glucose.\(^1,4\)

The first incretin hormone to be identified was gastric inhibitory polypeptide (GIP), named because of its ability to reduce gastric acid secretion in dogs. However, this effect is seen at pharmacological doses, whereas the incretin action is observed at physiological levels. The hormone was therefore re-named glucose-dependent insulinotropic polypeptide, but retains the acronym GIP.

A second incretin hormone was discovered showing 50% homology with glucagon, and named glucagon-like peptide-1 (GLP-1). Both GLP-1 (mainly from L cells in the distal intestine) and GIP (primarily produced by K cells in the duodenum) are released within minutes following food ingestion and contribute to the rapid disposal of nutrients through several pathways, with the action on the pancreatic β cell probably representing the most important mechanism.\(^1-4\) Their circulating levels decrease rapidly (the half-life of GLP-1 is approximately two minutes and that of GIP is approximately six minutes) due to enzymatic inactivation by dipeptidyl peptidase-4 (DPP-4). Both GIP and GLP-1 contain alanine at position two, which renders them excellent substrates for DPP-4.\(^1-4\)

The pleiotropic actions of incretins on the regulation of blood glucose have led to the concept that GLP-1 and/or GIP could be used in the treatment of type 2 diabetes. In type 2 diabetes GIP action is reduced, whereas its secretion does not seem to be altered. GLP-1 shows a lower insulinotropic effect. Individuals with type 2 diabetes show a small but significant reduction in meal-stimulated levels of GLP-1 and the action of this incretin remains relatively preserved, so most efforts have focused on GLP-1.\(^2-5\) Two main classes of drug have been developed: GLP-1 receptor agonists resistant to the action of DPP-4 (GLP-1 mimetics) and inhibitors of DPP-4 (GLP-1 enhancers) (see Figure 1).\(^2-5\)

This article will discuss the effects of GLP-1 and its mimetics on the function of pancreatic islets, particularly in the case of type 2 diabetes.

The Pancreatic Islets in Type 2 Diabetes

Type 2 diabetes is by far the most common form of diabetes, representing approximately 90% of all cases.\(^6\) It results from a combination of genetic and acquired factors that impair β-cell function and tissue insulin sensitivity.\(^6,7\) Increasing evidence shows that β-cell dysfunction, characterised by decreased β-cell mass and insulin secretion defects, is central to the onset and progression of this disease.\(^7,9\) In a normal pancreas there are approximately one million islets of Langerhans, which contain several different types of endocrine cell. The insulin-secreting β cells represent the majority of islet endocrine cells (60–80%), followed by glucagon-containing cells (α cells, 20–30%), somatostatin (δ cells) (5–15%) and pancreatic polypeptide (PP) cells. \(^\alpha\) β-cell mass is regulated by four major mechanisms: apoptosis (programmed cell death), size modification (hypo- and hyperplasia), replication (mitotic division of differentiated β cells) and neogenesis (development from precursor cells).\(^8-10\) The amount of β-cell mass is given by the sum of replication, size and neogenesis, minus the rate of apoptosis. The role of reduced β-cell mass in type 2 diabetes, the primary importance of β-cell apoptosis and the insufficiency of replication/neogenesis have been studied by several authors. Decreased islet mass, reduced β-cell mass and diminished β-cell insulin-secretory granules have been generally reported (see Figure 2).\(^7,10\) A few studies have shown increased α-cell volume in the islets of patients with type 2 diabetes.\(^11\) This leads to alterations of insulin and glucagon secretion in type 2 diabetes.\(^7,10,12-14\) Commonly found quantitative abnormalities of insulin release include reduced or absent first-phase insulin secretion to intravenous glucose, delayed or blunted responses to mixed meal ingestion and, with time, reduced second-phase release and diminished responses to non-glucose stimuli.
Qualitative defects in insulin release are represented, besides changes of early-phase insulin secretion, by alterations of oscillatory patterns and increased pro-insulin release. Alterations of glucagon secretion in type 2 diabetes include an inappropriately high release in relation to the prevailing glucose levels and increased secretion in response to arginine stimulation compared with non-diabetic subjects.\textsuperscript{12-14}

**Glucagon-like Peptide-1 Signalling Pathway and the Pancreatic Islets**

Human islets are well equipped with molecules involved in GLP-1 action. With the aim of exploring the potential of GLP-1 receptors as targets in nuclear medicine, Komor et al. investigated the GLP-1 receptor protein qualitatively and quantitatively in a large spectrum of human neoplastic and non-neoplastic tissues.\textsuperscript{15} The technique of \textsuperscript{125}I-GLP-1 amide receptor autoradiography showed that in the pancreas both islets and acini express GLP-1 receptors, with higher levels in the islets.\textsuperscript{15} In a few cases of chronic pancreatitis, islets showed a lower GLP-1 receptor density, but the acinar GLP-1 receptor expression was unchanged. When immunostaining was performed in human pancreatic samples, it was found that GLP-1 receptor positivity was mostly found in β cells and duct cells\textsuperscript{16,17} (see Figure 3). Molecular data showed the presence of molecules involved in GLP-1 signalling in human islets. Huypens et al. prepared islet cell aggregates (containing 50–60% insulin-positive cells and 15–20% glucagon-positive cells) from human pancreata.\textsuperscript{18} Gene expression of GLP-1 and GIP receptors was studied by Northern blots and reverse transcription-polymerase chain reaction (RT-PCR) analysis. All of the islet cell preparations consistently expressed both receptors (with GLP-1 showing higher expression). Liver and muscle samples were negative for GLP-1 and GIP receptors and fat showed GIP receptor expression.\textsuperscript{18} Marselli et al. performed extensive array analysis of gene expression in isolated human islets and β-cell-enriched preparations obtained by laser capture micro-dissection. This showed that, both in the whole islet cell population and in the β cells, the expression of GLP-1 and GIP receptors could be detected, although at relatively low signal levels.\textsuperscript{19} Gene expression of molecules involved in post-receptor GLP-1 action, such as cAMP-response element-binding protein (CREB), protein-kinase cAMP-dependent regulatory protein, (PRKAR) and cAMP-regulated guanine nucleotide exchange factor 2 (Epac2), has been found in isolated human islets by quantitative RT-PCR experiments.\textsuperscript{20}

**Glucagon-like Peptide-1, Incretin Mimetics and the Function of Pancreatic Islets**

The effects on pancreatic islet cells of acute and prolonged exposure to GLP-1 or insulin mimetics have been studied both in vivo in patients and using isolated human islets of Langerhans. Early work showed that when GLP-1 was infused for a few hours in patients with type 2 diabetes, glucose levels were normalised, insulin and C-peptide concentrations increased significantly and plasma glucagon diminished.\textsuperscript{21} When normal glycaemia was reached, insulin and C-peptide returned to basal levels and plasma glucose concentrations remained stable despite the ongoing infusion of GLP-1.\textsuperscript{21} This finding is of relevance, as it demonstrates that incretins reduce blood glucose levels without inducing hypoglycaemia. In an extensive study where GLP-1 was given subcutaneously for six weeks in a group of 20 patients with type 2 diabetes,\textsuperscript{22} metabolic control improved significantly compared with that observed following placebo treatment. The hypoglycaemic clamp technique was used to assess features of β-cell function and showed that C-peptide concentrations, first- and second-phase insulin secretion in response to glucose and peak values of C-peptide after arginine stimulation increased significantly with GLP-1 but not with saline treatment (see Figure 4).\textsuperscript{22} Fehse and colleagues studied a group of subjects with type 2 diabetes following short-term exposure to intravenous exenatide.\textsuperscript{23} They found that this GLP-1 mimic could restore the first phase of insulin secretion in response to intravenous glucose in the patients with
DPP-4 Inhibitors and Incretin Mimetics

The HOMA-β index (Homeostasis Model Assessment of β-cell function) is a parameter that is often used in clinical studies to assess β-cell function. When an analysis was performed to assess the metabolic effects of exenatide based on interim evaluation of data from the extension of three double-blind, controlled trials, the authors found that the value of HOMA-β improved significantly with exenatide with an increase of approximately 50% of the pre-treatment value. This was confirmed in a similar study published one year later and in a recently published article where Japanese patients with type 2 diabetes received liraglutide once daily (from 0.1 to 0.9mg) for 14 weeks. In this latter study, compared with placebo treatment with GLP-1 mimetic induced a significant increase of the HOMA-β index.

In vivo studies allow for only the indirect evaluation of pancreatic islet function mainly due to the influences of many additional variables, including, in particular, the degree of insulin sensitivity and the prevailing glucose concentrations. Work has been carried out to assess the direct effects of incretins on human pancreatic islet cells by using isolated islets. Preliminary studies show that in the presence of 10mmol/l glucose, 10nmol/l GLP-1 could significantly potentiate insulin release, leading to a ~30% increase. The potentiating effect was weaker at 2.8mmol/l glucose. In that study, it was also observed that GLP-1 inhibited glucagon secretion and stimulated somatostatin and pancreatic polypeptide release. More extensive studies have been performed. Using islet cell preparations, it was observed that during a two-hour static incubation period, insulin release increased from 3.8±0.9 to 12.6±2.1% of insulin content when glucose concentration in the incubation medium was raised from 2.5 to 10mmol/l.

The addition of 10nmol/l glucose GLP-1 in the medium containing 10mmol/l glucose determined a two-fold increase of insulin secretion (which was similar to the effects induced by 10nmol/l GIP or glucagon). Farilla et al. incubated isolated human islets prepared from three independent donors for five days in the presence or absence of 10nmol/l GLP-1 (added every 12 hours), and performed survival, functional and molecular studies. Better preservation of 3D islet morphology was observed in the GLP-1 exposed islets, which was associated with a two- to three-fold decrease in the number of apoptotic islet cells. Concomitantly, the anti-apoptotic molecule bcl-2 was upregulated and the expression of active caspase-3 (a crucial executor of apoptosis) was suppressed. Glucose-stimulated insulin secretion was also studied.

Preliminary studies show that in the presence of 10nmol/l glucose, 10nmol/l GLP-1 could significantly potentiate insulin release, leading to a ~30% increase.

Exposure to GLP-1 enhanced the capability of the islets to respond to glucose 15mmol/l glucose (no difference was found in response to 6mmol/l glucose). The effect was particularly clear after three days of culture. On day five all culture conditions showed a decrease in glucose-dependent insulin secretion. This was less prominent in islets cultured in the presence of GLP-1. These effects were accompanied by increased intracellular insulin content, suggesting that GLP-1 could promote insulin synthesis.

In a recently published paper, Lupi et al. obtained isolated human islets from the pancreas of 11 subjects without diabetes and seven individuals with type 2 diabetes of comparable age and body mass index. The islets were then cultured for two days at 5.5mmol/l glucose, either with or without the addition of 10nmol l exendin-4. At the end of the

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**Figure 4:** C-peptide Concentrations During Hyperglycaemic Clamp in Patients with Type 2 Diabetes Receiving Saline or GLP-1

**Figure 5:** Insulin Secretion in Response to 3.3 and 16.7mmol/l Glucose from Islets of Patients with Type 2 Diabetes
incubation, the authors studied glucose-stimulated insulin secretion (acute challenge with 3.3 and 16.7 mM glucose) and the expression of several genes involved in islet cell function and turnover. The diabetic islets, which were unresponsive initially, gained partial competence to glucose following exposure to exendin-4, as demonstrated by a 50% enhancement of insulin release at 16.7 mM glucose (see Figure 5). It was observed that transcription of glucose transporter-2 (which facilitates glucose entry into the β cell) and glucokinase (involved in the early step of glycolysis) was significantly increased, together with gene expression of insulin. Enhanced transcription of PDX-1 (a β-cell differentiation factor), Ki67 (a protein associated with cell proliferation) and cyclin D1 (a molecule involved in the cell cycle) was observed in the islets cultured with exendin-4.20

Additional evidence that incretins can increase the expression of molecules involved in β-cell differentiation comes from the study performed by Brun et al. on the transcription factor Pax4.29 The authors found that Pax4 is expressed in human pancreatic islets and was increased in patients with type 2 diabetes with a body mass index below 26 kg/m², and long-term adiposity favoured suppression of the gene. Following incubation for one and two days with 10 nM GLP-1 or exendin-4, the authors showed that these two compounds had no significant effect on Pax4 expression when glucose concentration in the incubation medium was 5.6 mM glucose. There was, however, a six-fold increase in Pax4 messenger RNA (mRNA) levels in the presence of 15 mM glucose. Zulewski and colleagues identified a distinct population of cells in human pancreatic islets and ducts that express nestin.30 These cells could be induced to differentiate in culture into cells with pancreatic, exocrine, endocrine and hepatic phenotypes. The same group showed that nestin-positive cells have GLP-1 receptors and that exposure to 10 nM GLP-1 stimulated differentiation into an endocrine phenotype expressing the homeodomain protein PDX-1 and the hormones insulin and glucagon.31

Conclusion
GLP-1 mimetics reduce plasma glucose in patients with type 2 diabetes through a number of mechanisms, with the action at the level of the pancreatic islets playing a major role. Established effects include potentiation of insulin release in response to increased glucose levels (with the return of insulin secretion to basal values when glucose concentrations normalise) and improvements of the dynamics of insulin secretion. As shown by ex vivo studies with isolated islets, GLP-1 and its mimetics are able to modify several molecular features of the pancreatic islets, which allows the β-cells to sense glucose stimulation and contribute to an improvement of insulin release. Further studies are needed to establish whether the effects of incretins at the level of glucagon secretion are direct or mediated by the improved insulin secretion. The demonstrated ex vivo beneficial actions of GLP-1 and GLP-1 mimetics on human β-cell survival and regeneration need to be confirmed in the clinical setting.