

# Expert Views in Diabetes

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from 1970–72. In 1977, Jens Juul Holst became an Assistant Professor and in 1978 he became Associate Professor in the Department of Medical Physiology, University of Copenhagen. In 1987 he was appointed Professor of Physiology. Jens Juul Holst was made Research Professor by appointment of the Danish Medical Research Council in 1991 and he has held a full professorship since 1996. He has been a member of a number of boards and medical councils and has received several awards including the 1992 Novo Nordisk award for

medical research, the 2002 Paul Langerhans Medal of the German Diabetes Association and in 2005 he was awarded the Claude Bernard Lectureship and prize of the European Association for the Study of Diabetes. The recipient of a number of research grants, Professor Holst received a 3-year research grant from the European Foundation for the Study of Diabetes in 2002. He is a member of the editorial board of *Regulatory Peptides*, *Endocrinology* and *Diabetes*. He is also Chairman of the Research Cluster for Endocrinology and Metabolism, the Health Sciences Faculty, University of Copenhagen. Professor Holst's teaching experience spans 25 years at pre- and postgraduate level and he has been a guest lecturer at universities in Scandinavia, Europe, Canada, USA and Japan. Professor Holst is very widely published and has authored approximately 900 original studies, textbook chapters and review articles.

## Incretin-based intervention in diabetes: too good to be true?

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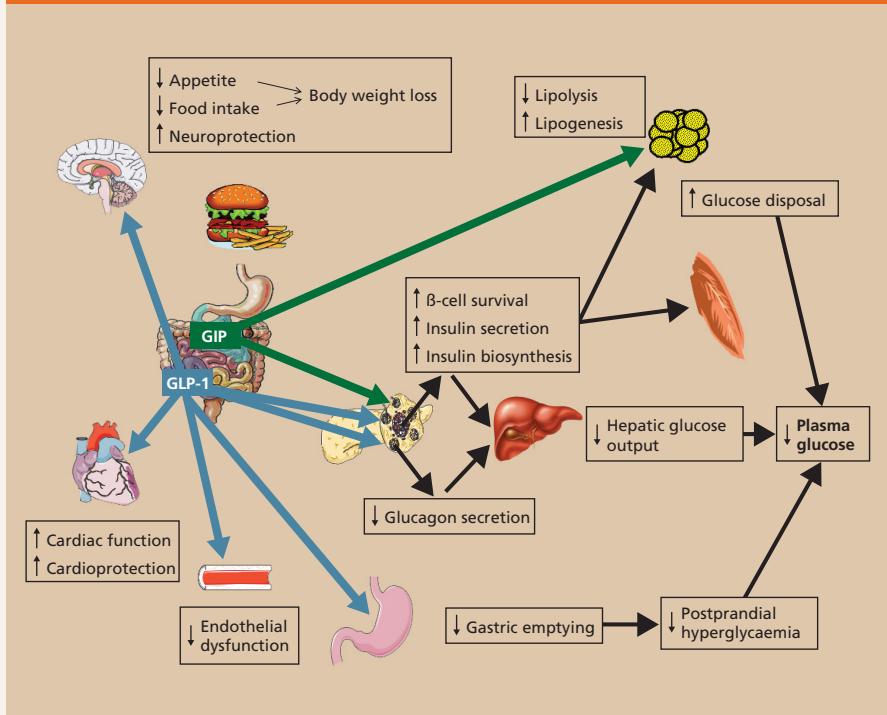
### *The incretin effect*

The incretin effect is the amplification of insulin secretion by glucose when it is taken orally as opposed to when it is given intravenously. By giving glucose both orally and by intravenous infusion, the difference in insulin response can be measured and it shows that the response is much less with intravenous infusion. This difference is caused by hormones from the gut called incretin hormones, which have been the subject of research for some time.

The most important incretin hormones are glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP). GLP-1 is produced by L-cells, endocrine cells mainly located in the distal gut, but is also secreted from the proximal gut. GLP-1 stimulates glucose-dependent insulin secretion and suppresses hepatic glucose-output by inhibiting glucagon secretion in a glucose-dependent manner. It also inhibits gastric emptying which reduces food intake

and body weight. Furthermore, GLP-1 enhances beta-cell proliferation and survival in animal models and isolated human islets. GIP is produced by the more proximal K-cells mainly in the ileum but also in the rest of the small intestine. It stimulates glucose-dependent insulin release, just like GLP-1, but in contrast to GLP-1 it stimulates glucagon secretion. GIP does not have any effect on gastric emptying and no significant effect on satiety or

**Figure 1. The various metabolic actions of GLP-1 and GIP**



**body weight. GIP potentially enhances beta-cell proliferation and survival (Figure 1).**

Looking at the incretin effect in patients with type 2 diabetes, a study by Michael Nauck and colleagues<sup>1</sup> observed insulin secretion in healthy patients and patients with type 2 diabetes using isoglycaemic glucose tolerance tests. The control group showed a small response to the intravenous flow of glucose and a large response to the oral load. The type 2 diabetes group showed a much smaller, insignificant difference. This means that incretin is almost completely absent in patients with type 2 diabetes. Subsequent studies<sup>2-4</sup> in type 2 diabetes patients showed that the secretion of GLP-1 is impaired and the beta-cells' activity in relation to GLP-1 is decreased. However, it is actually the potency that is decreased, so by using larger amounts of GLP-1 it is possible to stimulate the beta-cells and normalise glucose-induced insulin secretion. Secretion of GIP is also slightly impaired in type 2 diabetes but the effect of GIP on

insulin secretion in type 2 diabetes is almost completely lost.

If the impaired incretin response contributes significantly to the defective insulin secretion in these patients, will restoration of incretin action then improve metabolism? In a study carried out by our group,<sup>5</sup> a rather large number of patients with diabetes were given up to an 8-hour infusion of GLP-1 intravenously. They were then divided into three groups, those with fasting glucose levels between 7–10 mmol/L, those between 10–15 mmol/L and those higher than 15 mmol/L. In every individual, the GLP-1 infusion lowered the blood glucose. In those with the low starting level, blood glucose was completely normalised within a couple of hours and remained normal. In those with the higher starting level, blood glucose was also completely normalised. Even in patients with fasting glucose concentrations above 15 mmol/L it was possible to considerably lower the glucose concentration. However, this took some time, presumably because of the

limited beta-cell reserve. We then looked at the potential use of GLP-1 in the clinical treatment of type 2 diabetes.<sup>6</sup> Patients were provided with a continuous subcutaneous infusion of GLP-1 via insulin pumps for 6 weeks. With continuous infusion of GLP-1 it was possible to reduce fasting and mean plasma glucose levels by 5–6 mmol/L, reduce HbA<sub>1c</sub> levels by 1.3%, normalise fructosamine and reduce weight, presumably because of significantly reduced appetite. An improved – almost a doubling of – insulin sensitivity and enhanced beta-cell secretion was found. Furthermore, no significant side effects were observed. It should be noted in this study that a further effect could have been observed by doubling the dosage as the optimal dose was not used. An even larger effect via the more effective intravenous infusion – as opposed to the subcutaneous administration used in the study – might also have been achieved. To determine beta-cell function, the control group was exposed to a 30 mmol glucose clamp plus arginine and assessed before the study, 1 week after and 6 weeks after the study. These subjects responded almost not at all to the 30 mmol glucose clamp, but did respond somewhat to arginine. The group that was treated with GLP-1 had no response before the glucose clamp, a first-phase insulin response after 1 week and a huge second-phase response, and a tendency – although not significant – to further improvement after 6 weeks.

GLP-1 has a number of actions, one being enhancement of glucose-induced insulin secretion. It also upregulates insulin production and gene expression of the beta-cells, causes beta-cell proliferation in

animals, promotes differentiation from new cells from the ducts and, perhaps most importantly in humans, inhibits beta-cell apoptosis. It also inhibits glucagon secretion, reduces postprandial glucose excursions, and inhibits appetite and food intake.

The native GLP-1 molecule is rapidly degraded by an enzyme called dipeptidylpeptidase 4 (DPP-IV) which removes the two N-terminal amino acids, thereby rendering the molecule inactive. This happens with unusual rapidity, the half-life in circulation is 1–2 minutes and the clearance is 5–10 L plasma/min – three times the cardiac output. Therefore, the peptide disappears very rapidly. Also, the kidneys will eliminate the peptide and the metabolite with a half-life of 4–5 minutes and a clearance of 1.3 L/min.

#### **GLP-1 receptor activators**

To overcome these problems, metabolically unstable activators of the GLP-1 receptor can be used such as exendin and its derivatives. Another approach is to use slow-release formulations of exendin or GLP-1 analogues. It is also possible to create covalent or non-covalent association of GLP-1 with larger proteins, albumin for instance, that will give the molecule a longer half-life. There are also inhibitors of DPP-IV. Finally, there are small molecule activators of the GLP-1 receptor but they are not yet in clinical use and are difficult to produce. The most important of the GLP-1 receptor activators is exenatide (exendin 4), which has 53% homology to GLP-1, is insensitive to DPP-IV and is a full agonist of the GLP-1 receptor.

Clinical results show a strong effect on HbA<sub>1c</sub>, which is maintained well into the second or third year, and almost a continuous reduction in body weight over 2 years.<sup>7,8</sup> Along with

the reduction in body weight, there are also very good effects on cardiovascular risk factors. The long-acting release (LAR) technology for exenatide<sup>9</sup> is built into biodegradable polymeric microspheres which give it a very long duration of action for several weeks. One phase II study<sup>9</sup> observed the effects of exenatide LAR on a group of subjects for 15 weeks. The group were given metformin and the control group received placebo – the remaining subjects received either 0.8 mg or 2 mg exenatide once weekly. There was a strong effect on HbA<sub>1c</sub>. The low dose did not result in weight loss, but the larger dose did result in a significant weight loss of 3.8 kg over 15 weeks. Exenatide has a durable action of up to 3 years, but there are no controlled studies. Also, there is no evidence of beta-cell restoration. Therefore, this is not an optimal therapy. There was a limited effect in monotherapy but exenatide was efficacious in combination, for example with basal insulin, but unfortunately there is very little data available. The treatment also results in antibody formation in a significant number of patients and there is weight loss in most patients but not all – the reason for which is unknown. Therefore, this is possibly a suboptimal treatment paradigm and exenatide LAR is interesting but more data are needed.

#### **GLP-1 analogues**

One of the albumin-based GLP-1 analogues is liraglutide (Novo Nordisk). Here, the human GLP-1 molecule has a C-16 fatty acid chain attached so the molecule may therefore bind to albumin and be absorbed slowly from the subcutaneous injection site. It is metabolically stable, has a plasma half-life of about 12 hours, and it

is stable against DPP-IV. The plasma profile after a single injection shows an exposure of about 70 hours. If administered daily the plasma concentration builds up to a rather constant concentration plateau, thereby reducing the risk of developing side effects during peak concentrations after single administration. As a consequence, a lowering effect on plasma glucose in type 2 diabetes subjects is seen for a full 24 hours.

In a double-blind, placebo-controlled, randomised phase IIb study<sup>10</sup> 165 patients previously treated with oral agents (HbA<sub>1c</sub> 8.5%) were given liraglutide for 14 weeks as monotherapy. HbA<sub>1c</sub> decreased by 1.5–2%, and 45% had a level of <7% after the treatment. Fasting blood glucose was reduced by 3 mmol/L. Body weight was decreased by 3 kg and there were large reductions in cardiovascular risk factors. Initially, 5–10% of patients experienced nausea but this decreased markedly with time. There were no cases of hypoglycaemia or formation of antibodies. There are very few liraglutide data, but these phase II studies suggest that the effect corresponds to continuous exposure to GLP-1. Therefore, liraglutide is effective as a once-daily injection, produces no antibodies and has limited side effects.

#### **DPP-IV inhibitors**

In patients with type 2 diabetes, injection of the maximum tolerated dose of GLP-1, 1.5 nmol/kg, results in a small percentage of the injected material reaching the circulation in an intact form. When observed for the first time it was thought that perhaps inhibition of DPP-IV may prove to be a useful adjunct in the management of type 2 diabetes as was the case for

ACE-inhibitors in the treatment of hypertension.<sup>11</sup> It has been shown that valine-pyrrolidide, a DPP-IV inhibitor, blocks the enzyme and prevents substrates from being cleaved. In a study carried out with pigs,<sup>12</sup> valine-pyrrolidide inhibited the DPP-IV activity in plasma to very low levels for the duration of the experiment. Infusions of GLP-1 and glucose were given to observe insulin secretion, and then repeated with the inhibitor. When GLP-1 is infused – even continuously – most of it breaks down. When the inhibitor is given, all the GLP-1 survives. The protection of the hormone resulted in a great magnification of the insulin response to GLP-1.

Over 30 companies are developing or have already developed DPP-IV inhibitors, for example LAF 237 (vildagliptin [Galvus®; Novartis] – US approval delayed, EU approval in 2007) and MK-0431 (sitagliptin [Januvia™; Merck] – available in the US and EU). Sitagliptin lowers blood glucose in response to meals as well as in fasting periods. It is quite remarkable that there is a continuous action on blood glucose provided by this tablet that is administered once daily and not, as expected, mainly postprandially. Sitagliptin was recently studied over 24 weeks<sup>13</sup> either alone or in combination with metformin. With the optimal dose of metformin and sitagliptin administered twice daily, a marked reduction in HbA<sub>1c</sub> was seen, reaching levels as low as 6.5%, maintained for the duration of the study. In a 1-year study with vildagliptin given in addition to metformin, the placebo group, with metformin alone, showed an increase in HbA<sub>1c</sub> values suggesting an impairment of beta-cell function, whereas in the DPP-IV inhibitor

group there was a stable lowering of HbA<sub>1c</sub> which remained. This widening difference could perhaps suggest an improvement in the protective effect on the beta-cell.

DPP-IV inhibitors are comparable with existing oral antidiabetic agents in monotherapy. They also show additional efficacy when used in combination with sulphonylureas, glitazones, metformin and very importantly, with insulin, which can possibly reduce the insulin dose and lower the risk of hypoglycaemia. The effect of DPP-IV inhibitors on HbA<sub>1c</sub> is maintained for 104 weeks and depends on the starting level – the higher the starting level, the larger the effect.<sup>14</sup> So what should be used? Options include incretin mimetics (analogues of GLP-1) and incretin enhancers (DPP-IV inhibitors). Mimetics are injectable and provide a high plasma concentration of GLP-1 receptor activator resulting in an effect on appetite, food intake, body weight, cardiovascular effects, beta-cells, alpha-cells, but also a tendency to cause gastrointestinal side effects. Incretin enhancers are orally active, taken once daily, cause very few side effects, but have no effect on body weight.

### Conclusions

Regarding incretin-based therapy for type 2 diabetes, what are the problems and what should be concluded? Exendins are antigenic and may have actions on the body that are in addition to those of GLP-1. The mechanism of action of DPP-IV inhibitors is yet to be proven, but it is thought that they protect incretin hormones. And we must also consider what are the consequences of prolonged, year-long elevation of this enzyme which is also expressed in

the immune system? Certainly some inhibitors also have non-target effects, but hopefully there will be some that do not. Mimetics and enhancers both provide very convincing beta-cell protection potential as shown in *in vitro* experiments in animals and also protect human beta-cells *in vitro*. Regarding efficacy, if we have a target HbA<sub>1c</sub> of <6.5%, we should consider if mimetics and enhancers are really sufficiently efficacious in monotherapy; however they may be sufficiently effective when used in combination with other therapies. Most importantly, both incretin mimetics and incretin enhancers have the potential for prevention of diabetes and its complications. However, there are insufficient data to prove this theory at present.

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