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The incretin system and its role in Type 2 Diabetes Mellitus.

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**Abstract**

The incretin hormones are released during meals from gut endocrine cells. They potentiate glucose-induced insulin secretion and may be responsible for up to 70 % of postprandial insulin secretion. The incretin hormones include glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), both of which may also promote proliferation/neogenesis of beta cells and prevent their decay (apoptosis). Both hormones contribute to insulin secretion from the beginning of a meal and their effects are progressively amplified as plasma glucose concentrations rise. The current interest in the incretin hormones is due to the fact that the incretin effect is severely reduced or absent in patients with type 2 diabetes mellitus (T2DM). In addition, there is hyperglucagonaemia, which is not suppressible by glucose. In such patients, the secretion of GIP is near normal, but its effect on insulin secretion, particularly the late phase, is severely impaired. The loss of GIP action is probably a consequence of diabetes, since it is also observed in patients with diabetes secondary to chronic pancreatitis, in whom the incretin effect is also lost. GLP-1 secretion, on the other hand, is also impaired, but its insulinotropic and glucagon-suppressive actions are preserved, although the potency of GLP-1 in this respect is decreased compared to healthy subjects. However, in supraphysiological doses, GLP-1 administration may completely normalize beta as well as alpha cell sensitivity to glucose. The impaired action of GLP-1 and GIP in T2DM may be at least partly restored by improved glycaemic control, as shown in studies involving 4 weeks of intensive insulin therapy. The reduced incretin effect is believed to contribute to impaired regulation of insulin and glucagon secretion in T2DM, and, in support of this, exogenous GLP-1 administration may restore blood glucose regulation to near normal levels. Thus, the pathogenesis of T2DM seems to involve a dysfunction of both incretins. Enhancement of incretin action may therefore represent a therapeutic solution. Clinical strategies therefore include the development of metabolically stable activators of the GLP-1 receptor; and 2) inhibition of DPP-4, the enzyme that

destroys native GLP-1 almost immediately. Orally active DPP-4 inhibitors and the metabolically stable activators, exenatide (Byetta), are now on the market, and numerous clinical studies have shown that both principles are associated with durable antidiabetic activity.

### **Introduction- The incretin effect.**

“The incretin effect” designates the amplification of insulin secretion elicited by hormones secreted from the gastrointestinal tract. In the most strict sense, it is quantified by comparing insulin responses to oral and intravenous glucose administration, where the intravenous infusion is adjusted so as to result in the same (isoglycaemic) peripheral (preferably arterialized) plasma glucose concentrations (1;2). In healthy subjects, the oral administration causes a 2-3 fold larger insulin response compared to the intravenous route, thought to be due to the actions of gut hormones. The same gut hormones are also released by mixed meals, and given that their postprandial concentrations in plasma are similar and that the elevations in glucose concentrations are also similar, it is generally assumed that the incretin hormones are playing a similarly important role for the meal-induced insulin secretion. If one bases the analysis on measurements of C-peptide instead of insulin, it is possible to avoid errors introduced by hepatic extraction of insulin (since C-peptide is not taken up by the liver), and such measurements applied to isoglycaemic glucose challenges indicate a similar amplification of beta cell secretion by oral glucose. By applying C-peptide kinetics and deconvolution it is possible to calculate the actual prehepatic insulin secretion rate, which shows a similar increase after oral compared to intravenous glucose (3;4). The incretin effect clearly depends on the amount of glucose ingested: it is small with 25 g glucose, results in a doubling of insulin secretion with 50 g and a 4-5 fold increase with 100 g(5). In spite of the increasing amounts of glucose ingested, one will observe that the resulting glucose excursions are almost identical (see Fig. 1) . Another way of describing the incretin effect, therefore, is to say that

it keeps glucose excursions at a certain, low level regardless of the amount of glucose taken in by the individual. Since the glucose levels are similar regardless of the amount of glucose ingested, it follows that the amount required to copy the excursions by intravenous infusion is also approximately the same. Thus, in the experiments carried out by Nauck et al(5) , it took about 20 g of i.v. glucose to copy the excursions after 20 as well as 50 and 100 g oral glucose (Fig. 1). In other words, the body disposes of between 20 % and 80 % of these amounts of glucose by mechanisms that are elicited by the oral as opposed to intravenous administration. It is believed that the amplification of insulin secretion by incretin hormones is the most important of these mechanisms(5) (see Fig .2). The incretin hormones, therefore, play a very important role in the regulation of particularly the postprandial glucose levels.

Many hormones have been suspected to responsible for the incretin effect (6), but today there is ample evidence to suggest that the two most important incretins are glucose-dependent insulinotropic polypeptide (GIP), previously designated gastric inhibitory polypeptide, and glucagon like peptide-1 (GLP-1). Both have been established as important incretin hormones in mimicry experiments in humans, where the hormones were infused together with intravenous glucose to concentrations approximately corresponding to those observed during oral glucose tolerance tests. Both hormones powerfully enhanced insulin secretion, actually to an extent that could fully explain the insulin response (7;8). In recent experiments, involving clamping of blood glucose at fasting and postprandial levels, exact copying of the meal-induced concentrations of both GLP-1 and GIP indicated that both are active with respect to enhancing insulin secretion from the beginning of a meal (even at fasting glucose levels), and that they contribute almost equally, but with the effect of GLP-1 predominating at higher glucose levels(9). The effects of the two hormones with respect to insulin secretion have been shown to be additive in humans(10). From

studies in mice with targeted lesions of the both GLP-1 and GIP receptors, it was concluded that both hormones are essential for a normal glucose tolerance and that the effect of deletion of one receptor was “additive” to the effect of deleting the other(11). Thus, there is little doubt that both GIP and GLP-1 play an important role in postprandial insulin secretion and, therefore, glucose tolerance in humans and animals.

### **The incretin effect in type 2 diabetes mellitus**

It is now well established that T2DM is characterized not only by insulin resistance, but also by a beta cell defect which renders the beta cells incapable of responding adequately to the insulin resistance (12). It is, therefore, relevant to ask how the incretin effect functions in these patients. Careful studies by Nauck et al (13) indicated that the incretin effect is severely reduced or lost in relatively lean type 2 diabetic patients. In a similar study carried out in our own laboratory in obese subjects with T2DM (BMI 37 kg/m<sup>2</sup>), we could confirm the loss of the incretin effects, which was actually more extensive than in lean patients(14), and also observed that the amount of intravenous glucose required for copying of the oral glucose response was similar to the oral dose (about 50 g for each), another indication that in these patients, the route of administration did not result in different handling of the glucose(4). Thus, there is little doubt that a defective incretin effect contributes to the glucose intolerance of these patients.

Given that GLP-1 and GIP are the most important incretin hormones, it is possible also to dissect their contribution to the defective incretin action in diabetic patients. Such contributions could consist of defects with respect to secretion, action or metabolism. Detailed studies of the secretion of GIP and GLP-1 in response to mixed meals in patients with T2DM revealed a slightly impaired secretion of GIP but a more pronounced impairment with respect to the secretion of GLP-1. In fact, the GLP-1 response expressed as the incremental area under the curve was reduced to

approximately 50% in the patients compared to healthy glucose tolerant controls. The reduction was particularly prominent during the second and third hour of the meal test, while the initial response was unimpaired. The decreased response was related to both BMI (lower the higher the BMI) and to the actual diabetic state, but was independent of e.g. the presence of neuropathy(15). A decreased GLP-1 secretion in obese subjects has been observed repeatedly(16) and is related to an impaired incretin effect(14;17). How obesity affects GLP-1 secretion is not known, but part of the effect may be related to the insulin resistance of obesity. However, insulin resistance is independently related to a decreased GLP-1 meal response(17;18). It should be noted though, that GLP-1 secretion is not decreased in all obese or insulin resistant subjects and may be missed in small cohorts of subjects. But, if present, impaired secretion of GLP-1 is likely to contribute to the failing incretin effect.

The metabolism of GLP-1 and GIP was compared by Vilsboll et al in diabetic patients and controls, but both hormones were metabolized at similar rates (19;20), so that differences in their elimination do not explain the reduced plasma concentrations when present.

With respect to the actions of the incretin hormones, it was discovered in 1993(21) that infusion of rather large amounts of GLP-1 resulted in near normal insulin responses in patients with T2DM, whereas GIP had no significant effect. Similar observations were made by Elahi and coworkers (22). In subsequent studies involving infusion of various doses of GLP-1 during stepwise increases in plasma glucose, it was possible to analyse the influence of GLP-1 on the beta cell sensitivity to glucose(23). It was found that although GLP-1 at supraphysiological infusion rates was capable of restoring the beta cell sensitivity to glucose to completely normal values, the sensitivity of the diabetic islets to GLP-1 was, nevertheless, severely decreased. In further studies in patients with T2DM, infusions of GIP and GLP-1 at rates resulting in physiological elevations of the incretin levels (as observed after mixed meal ingestion) while glucose was clamped at 15 mmol/L, failed to



affect insulin secretion at all, although these infusion rates greatly (and equally) increased insulin secretion in healthy subjects (24). These findings illustrate the dramatic loss of potency of GLP-1 in T2DM, and when combined with the finding that the secretion of GLP-1 is often reduced, one may conclude that also the insulinotropic effects of endogenous GLP-1 may be severely compromised in these patients. Thus a severely decreased efficacy characterizes the incretin action of both GLP-1 and GIP in T2DM. Whereas supraphysiological amounts of GLP-1 retain the capability to enhance glucose induced secretion, GIP remains ineffective regardless of dose(25). The mechanisms whereby GLP-1, but not GIP, stimulate insulin secretion in T2DM remain unknown, but the observation raises the possibility that GLP-1 in pharmacological doses could be used clinically to enhance insulin secretion in T2DM, as discussed below.

Finally, one may ask whether the incretin defect is a primary event, perhaps a major etiological contributor to the beta cell failure that characterizes T2DM. Several observations, however, suggest that this is not the case. Thus, the impaired secretion of GLP-1 seems to be a consequence of diabetes. In identical twins that were discordant for type T2DM, meal-induced GLP-1 secretion was reduced only in the diabetic twin(26), and in first degree relatives of patients with T2DM, 24-hour incretin hormone profiles were normal (actually there was a significant increase in the secretion of GIP but no difference for GLP-1)(27). A reduced insulinotropic action of GIP to almost diabetic levels was observed in about 50 % of first degree relatives of patients with T2DM, suggesting that this might represent a primary, genetic defect(28). However, subsequent observations have questioned this interpretation(29). Vilsboll et al studied insulin responses to GIP in patients with diabetes of different etiologies, including diabetes secondary to pancreatitis(30). In these patients, there was a similar loss of insulinotropic effects of GIP as observed in the classical type 2 diabetic subjects. These findings suggest that the lost effect of GIP is also secondary to the diabetic condition. In further studies, Knop et al(4) studied healthy controls, lean patients with T2DM and

patients with chronic pancreatitis with normal or diabetic glucose tolerance, and found that the incretin effect was lost only in the diabetic patients, suggesting that the loss of incretin effect develops as glucose intolerance progresses. Interestingly, in these subjects incretin hormone secretion was comparable to that of healthy controls.

Thus, although a therapeutic strategy based on incretin hormones may restore beta cell responsiveness to glucose in T2DM, the incretin defect is not a primary cause of diabetes. In agreement with this notion, there is now data to suggest that intensified treatment resulting in near normal glucose levels may lead to a partial restoration of incretin action of GLP-1 and GIP(24;31).

### **Actions of GLP-1.**

The acute insulintropic effects of GLP-1 raised interest in the use of this peptide for diabetes treatment. Moreover, the peptide possesses a number of additional effects which in the context of diabetes treatment, must be considered favorable.

#### *1) Effects on the islets.*

GLP-1's insulintropic activity, which is strictly glucose dependent, is, at least partly (see below), exerted via interaction with the GLP-1 receptor located on the cell membrane of the beta cells(32). Binding of GLP-1 to the receptor causes activation - via a stimulatory G-protein - of adenylate cyclase resulting in the formation of cAMP. Most of the actions of GLP-1 are secondary to the formation of cAMP. Subsequent activation of protein kinase A and the cAMP-regulated guanine nucleotide exchange factor II (cAMP-GEFII, also known as Epac2) leads to a plethora of events including altered ion channel activity, intracellular calcium handling and enhanced exocytosis of insulin containing granules(33;34). The effects of glucose and GLP-1 may converge at the level of the  $K_{ATP}$  channels of the beta cells. These channels are sensitive to the intracellular ATP levels and,

thereby, to glucose metabolism of the beta cells, but may also be affected (closed, resulting in subsequent depolarization of the plasma membrane and opening of voltage sensitive calcium channels) by protein kinase A activated by GLP-1(35-37). There is also evidence that GLP-1 acts as a glucose-sensitizer. Thus, GLP-1 has been found to facilitate glucose-dependent mitochondrial ATP production(38). At any rate, it is of potential clinical importance that sulfonylurea drugs, which bind to and close the  $K_{ATP}$  channels and thereby cause membrane depolarization and calcium influx, may uncouple the glucose dependency of GLP-1. Indeed, 30 to 40 % of patients treated with both sulfonylurea compounds and a GLP-1 agonist (see below) experience, usually mild, hypoglycaemia.

Cyclic AMP generated by activation of the GLP-1 receptor may also influence the exocytotic process directly, and this process has been estimated to account for up to 70 % of the entire secretory response(32). Also ATP may directly influence the exocytotic process, and may, therefore, represent another site of convergence for the glucose and GLP-1 mediated signals(39). The transcription factor PDX-1, a key regulator of islet growth and insulin gene transcription, appears to be essential for most of the glucoregulatory, proliferative and cytoprotective actions of GLP-1(40). In addition, GLP-1 up regulates the genes for the cellular machinery involved in insulin secretion, such as the glucokinase and GLUT-2 genes(41). For a further discussion of the insulinotropic effects of GLP-1, see(42).

Importantly, GLP-1 also has trophic effects on beta cells(43). Not only does it stimulate beta cell proliferation(44;45), it also enhances the differentiation of new beta cells from progenitor cells in the pancreatic duct epithelium(46). Thus, GLP-1 has been demonstrated to be capable of differentiating the pancreatic duct cell line, AR42J, into endocrine insulin secreting cells(40;46). Most recently, GLP-1 has been shown to be capable of inhibiting apoptosis of beta cells including human beta cells(47;48). Since the normal number of beta cells is maintained in a balance between

apoptosis and proliferation(49), this observation is of considerable interest, and also raises the possibility that GLP-1 could be useful in conditions with increased beta cell apoptosis.

A most striking demonstration of the beta cell protective/proliferative effects of GLP-1 receptor activation was provided by Stoffers et al(50), who studied the diabetes developing in rats subjected to intrauterine growth retardation. Treatment with exendin 4 in the neonatal period completely prevented development of diabetes and restored beta cell mass, which otherwise is strongly reduced in these animals.

The complicated mechanisms whereby GLP-1 may exert the trophic effects on the beta cells were reviewed recently(51).

#### *Reflex activation of the beta cell.*

As will be discussed further below, GLP-1 is extensively degraded by the enzyme dipeptidyl-4 and it has been demonstrated that only about ¼ of what leaves the gut (the endocrine organ) is still in the intact, active form, and only about 8 % of what is secreted actually reaches the pancreas as the intact peptide (52-54). This has given rise to speculations that the actions of GLP-1 on the beta cells might be exerted indirectly via activation of long vago-vagal reflexes(53). Indeed, the GLP-1 receptor is expressed in cell bodies in the nodose ganglion from which the sensory afferents from the GI-tract emanate(55), and it has been demonstrated that blockade of the autonomic ganglia also blocked the effects of intraportally injected GLP-1 on insulin secretion(56). Thus it may be that under physiological conditions the majority of the effects of GLP-1 on insulin secretion is transmitted via autonomic nerves.

#### *Effects on glucagon secretion*

GLP-1 also strongly inhibits glucagon secretion. Since in patients with T2DM there is fasting hyperglucagonemia as well as exaggerated glucagon responses to meal ingestion(15), and since it has been demonstrated that the hyperglucagonaemia contributes to the hyperglycaemia of the

patients(57), this effect may be as important as the insulinotropic effects. The mechanism whereby GLP-1 inhibits glucagon secretion has been suggested to be indirect relative to its effects on the beta cell (insulin, zinc, glutamate)(58). However, GLP-1 also suppressed glucagon secretion in subjects with type 1 diabetes and no residual beta cell function(59), and recent studies in isolated perfused rat pancreas demonstrated inhibition of glucagon secretion by GLP-1 at glucose levels too low to cause measurable insulin secretion(60). A specific antagonist of the somatostatin receptors (subtype 2) completely abolished the GLP-1 effect and actually increased the secretion of glucagon, suggesting that the somatostatin-producing D-cells of the islets transmit the effects of GLP-1 by paracrine inhibition of the alpha cells and keep them under tonic suppression.

2) *Effects on the gastrointestinal tract.* Further important effects of GLP-1 include inhibition of gastrointestinal secretion and motility, notably gastric emptying(61;62). By this mechanism, GLP-1 may curtail postprandial glucose excursions(63) and, thereby, reduce the number of episodes with high postprandial glucose levels. There has been concern that the powerful inhibitory effect could represent a problem in patients with gastroparesis, but so far there has not been a single reported case.

3) *Effects on appetite and food intake.* GLP-1 inhibits appetite and food intake in normal subjects(64) as well as in obese subjects with T2DM(65;66), and it is thought that GLP-1 is one of the gastrointestinal hormones that normally regulate food intake(42). Clinical studies (see below) have shown that the effects on food intake are maintained for several years and lead to a sustained or progressive weight loss.

4) *Cardiovascular effects.* It has been known for some time that there are GLP-1 receptors in the heart(67). A physiological function for these receptors was indicated in recent studies in mice lacking the GLP-1 receptor, which exhibit impaired left ventricular contractility and diastolic functions as well as impaired responses to exogenous epinephrine(68). GLP-1 also increases left

ventricular developed pressure and coronary blood flow in isolated mouse hearts(69), although in normal rat hearts, it reduces contractility(70). However, further studies in rats showed that GLP-1 protects the ischaemic and reperfused myocardium in rats by mechanisms independent of insulin(70;71). Protective effects may be demonstrated by administration of GLP-1 or GLP-1 receptor agonists both before and after (see Fig. 3) ischaemia(69;72) and may involve the p70s6 kinase(73). Surprisingly, some of the cardiac effects may also be elicited by the metabolite GLP-1 9-36amide, which is formed rapidly in the circulation (see below), but which has a strongly reduced activity on the classical GLP-1 receptor of the beta cells(69;72;74). It has therefore been suggested that a different receptor mediates at least some of the cardiovascular effects although not all studies support this(73). Whatever the mechanism, these findings may have important clinical implications. Nikolaidis et al studied patients treated with angioplasty after acute myocardial infarction, but with postoperative left ventricular ejection fractions as low as 29 %. In these patients, GLP-1 administration significantly improved the ejection fraction to 39% and improved both global and regional wall motion indices(75). In studies of dogs with induced dilated cardiomyopathy, GLP-1 was reported to dramatically improve left ventricular and systemic haemodynamics, and it was suggested that GLP-1 may be a useful metabolic adjuvant in decompensated heart failure(76). Indeed, addition of GLP-1 to standard therapy in patients with heart failure significantly improved left ventricular ejection fraction, myocardial oxygen uptake, 6-min walking distance and quality of life(77). In another study in which patients undergoing coronary artery bypass grafting were randomized to receive GLP-1 or conventional treatment, there was less use of inotropic and vasoactive drugs and fewer arrhythmias, as well as better glycaemic control, in the GLP-1 group(78).

Furthermore, GLP-1 has been found to improve endothelial dysfunction in type 2 diabetic patients with coronary heart disease, again a finding with interesting therapeutic perspectives(79).

An effect on endothelial dependent vasodilation was confirmed in healthy subjects(80), and functional receptors for GLP-1 have been identified on endothelial cells(69). Chronic administration of GLP-1 agonists for clinical use (see below) is generally associated with small but significant decreases in blood pressure(81;82). This, may reflect the vasodilatory actions of GLP-1 as observed in various animal experimental models (69;83).

5) GLP-1 may also possess *neurotropic* effects. Thus, intracerebroventricular GLP-1 administration was associated with improved learning in rats and also displayed neuroprotective effects(84;85), and GLP-1 has been proposed as a new therapeutic agent for neurodegenerative diseases, including Alzheimer's disease(86), supported by observations that GLP-1 can modify processing of amyloid precursor protein and protect against oxidative injury(87). Very recently, it was reported that the GLP-1 receptor agonist, exendin 4, promoted adult neurogenesis, normalized dopamine imbalance and increased the number of dopaminergic neurons in the substantia nigra in animal models of Parkinson's disease(88).

#### **Actions of GLP-1 in T2DM Mellitus**

In agreement with the findings of preserved insulinotropic actions of GLP-1 in T2DM(21), intravenous infusion of GLP-1 at 1.2 pmol/kg/min was demonstrated to be able to completely normalize plasma glucose in patients with long-standing severe disease, admitted to hospital for insulin treatment(89). Subsequent studies in patients with moderate disease showed that plasma glucose concentrations could be near-normalized by an intravenous GLP-1 infusion covering the night time and the next day, including two meals(90). In another study, a continuous intravenous administration of GLP-1 for 7 consecutive days was demonstrated to dramatically lower both fasting and postprandial glucose concentrations with no sign of tachyphylaxis over 7 days(91). In this study, which included 4 different infusion rates, glucose concentrations were not completely

normalized at the two lowest infusion rates (4 and 8 ng/kgx min approximately corresponding to 1 and 2 pmol/kgxmin), while the higher rates (16 and 24 ng/kgxmin) had to be discontinued because of side effects (nausea and vomiting). So, in these studies, it was not possible to completely normalize plasma glucose concentrations within the therapeutic window. Subsequent studies of chronic administration of GLP-1 agonists have suggested, however, that the nausea and vomiting is mostly observed shortly after the initiation of therapy and thereafter subsides. A scheme of slow dose-escalation is therefore recommended and may allow higher doses to be given without these side effects(92).

Clearly, continuous intravenous infusion is clinically irrelevant, but the effect of subcutaneous injections of GLP-1, given to both patients and healthy subjects (93), on plasma glucose and insulin concentrations turned out to be very short lasting, even after maximally tolerated doses (1.5 nmol/kg – higher doses resulted in nausea and vomiting)(94). The short duration of action was demonstrated to be due to an extremely rapid and extensive metabolism of GLP-1 in the body (95;96), leaving the intact peptide with an apparent i.v. half-life of 1-2 min and a plasma clearance amounting to 2-3 times the cardiac plasma output(19). The degradation is due to the actions of the ubiquitous enzyme, dipeptidyl peptidase 4 (DPP-4), which catalyses the removal of the two N-terminal amino acids of the molecule thereby, rendering it inactive(95) with respect to insulin secretion (but it may have cardiovascular effects, see above). The metabolic instability of GLP-1 clearly restricts its clinical usefulness, but Zander et al(97) carried out a clinical study in which GLP-1 or saline was administered as a continuous subcutaneous infusion (using insulin pumps) for 6 weeks to a group of T2DM patients. The patients were evaluated before, after 1 week and after 6 weeks of treatment. No changes were observed in the saline treated control group, whereas in the GLP-1 group, fasting and average plasma glucose concentrations were lowered by approximately 4-6 mmol/l, haemoglobin A1c (glycated haemoglobin, a long-term (months) measure of mean plasma



glucose concentrations) decreased by 1.2 %, free fatty acids were significantly lowered, and the patients had a gradual weight loss of approximately 2 kg. In addition, insulin sensitivity, determined by a hyperinsulinaemic euglycaemic clamp, almost doubled, and insulin secretion capacity (measured using a 30 mmol/l glucose clamp + arginine) greatly improved. There was no significant difference between results obtained after one and 6 weeks treatment, but there was a tendency towards further improvement of plasma glucose as well as insulin secretion. There were very few side effects and no differences between saline and GLP-1 treated patients in this respect. In spite of the marked metabolic improvement, plasma glucose levels were not completely normalized, but the dose given (4.8 pmol/kg x min) may not have been optimal. Thus, in a different study, higher infusion rates were actually more efficacious and still did not elicit prohibitive side effects(98).

This study, therefore, provided “proof-of concept” for the principle of GLP-based therapy of T2DM, and further attempts to utilize the therapeutic potential of GLP-1 have included, on one hand, the development of stable, DPP-4 resistant analogues(99) and, on the other hand, inhibitors of DPP-4 demonstrated to be capable of protecting the peptide from degradation and thereby augmenting its insulinotropic activity(100).

### **GLP-1 receptor agonists.**

It is fairly easy to stabilize the GLP-1 molecule against DPP-4 – a conservative substitution of Ala in position 2 with e. g. valine is sufficient and does not harm the biological activity of the peptide(99). However, the stabilized molecule is still eliminated extremely rapidly in the kidneys (with a half life of 4-5 min), so such analogues are also unsuitable. However, exendin 4, a peptide with about 50 % sequence homology to GLP-1, which was isolated from the saliva of the Gila Monster (*Heloderma suspectum*) during a search for biologically active peptides, turned out to be a

full agonist for the GLP-1 receptor, to be stable against DPP-4, and to be eliminated in the kidneys exclusively by glomerular filtration(101), resulting in an i.v. half-life in plasma of 30 min(102). After a single s.c. injection of exenatide (designating a synthetic replica of exendin 4) in the dose selected for clinical use (10 ug), its plasma concentration is elevated into the insulinotropic range for about 5-6 hours; exenatide is, therefore, administered twice daily(103). This is the molecule which has been developed by Amylin Corporation and Lilly for diabetes treatment under the trade name “Byetta”. Another incretin mimetic under clinical development is liraglutide (NovoNordisk), which is based on the structure of native human GLP-1, but modified to include an amino acid substitution and an attachment of a C16 acyl chain(104), enabling the molecule to bind to albumin, thereby preventing renal elimination and degradation by DPP-4. Liraglutide is slowly absorbed and has a half-life of approximately 11 to 13 hours after s.c. administration(105) making it suitable for once-daily injection. Clinically, the molecule has similar actions to continuously infused GLP-1(106), and appears to have a similar clinical potential to exendin 4(81).

Clinical studies using Exenatide and Liraglutide demonstrated sustained effects on HbA<sub>1c</sub>, body weight reduction and improved beta cell function in patients with T2DM mellitus. Controlled studies comparing exenatide and placebo injections as add-on therapy to already instituted antidiabetic treatment showed a statistically significant decline in HbA<sub>1c</sub> of approximately 1% from baseline in favour of exenatide (baseline HbA<sub>1c</sub> 8.2%)(107) and a significant weight loss. The weight loss was progressive, dose-dependent, and without apparent plateau by week 30 (mean difference of 2.3 kg, randomized trials), but appeared to plateau at 2-3 years (with a weight loss of 5.3 kg below baseline by 3.5 years) in completers in an open-label extension of the trials(82;108). Side-effects were primarily dose-dependent nausea and vomiting, occurring in as many as 57 and 17%, respectively, although, nausea was generally mild to moderate and declined with time. Treatment for 3 years or more was associated with significant improvements in cardiovascular risk

factors (triglycerides, total cholesterol, LDL-cholesterol and HDL cholesterol) and hepatic biomarkers (aminotransferases)(82). The clinical effectiveness of exenatide in T2DM patients was evaluated by extractions of data from a primary care electronic medical record database(109) over a period of 6 months. Weight loss among the 1785 patients was 6.1 lbs (from 243.4) with 70 % losing weight. Lowering of HbA<sub>1c</sub> ranged from 0.7 to 0.9 % regardless of weight loss. It was concluded that the effectiveness of exenatide in a primary care setting is similar to that observed in the controlled clinical trials.

Recently published data demonstrated that liraglutide as monotherapy is capable of decreasing fasting plasma glucose levels up to 3.4 mmol/l (1.90 mg dose) on average when compared to placebo(110). In the same study, a decrease in HbA<sub>1c</sub> of up to 1.7 percentage points (baseline HbA<sub>1c</sub> 8.0%) was seen, and almost 50% of the patients with T2DM managed to reach the goal level of <7% in HbA<sub>1c</sub>. In the highest liraglutide dose group (1.9 mg), change from baseline in body weight was -2.99 kg (-1.21 kg compared with placebo). As for exenatide, transient and mild nausea was reported in the liraglutide treated subjects (liraglutide; 10%, placebo; 3%). In a clinical study of 226 Japanese subjects with T2DM, Liraglutide at 0.9 mg /day for 14 weeks resulted 75 % of patients achieving HbA<sub>1c</sub> levels < 7.0 % (from 8.3% at base line) and 57% below 6.5 %. In this study, where the mean BMI was 23.9 kg/m<sup>2</sup>, there was no weight loss(111). Applications for new drug status were filed with the authorities in both the USA and EU in May 2008.

#### **DPP-4 inhibitors.**

The extremely rapid and extensive degradation of GLP-1 by DPP-4 gave rise to the proposal that inhibitors of the enzyme could be used as a therapy for T2DM by protecting and thereby enhancing the circulating levels of GLP-1(96). Early experiments documented that administration of an already existing inhibitor to pigs completely protected both endogenous and exogenous GLP-1,

which furthermore greatly enhanced insulin responses to glucose(100). In a subsequent study, DPP-4 inhibition was demonstrated to also protect GIP from degradation, again resulting in enhanced insulinotropic activity of infused GIP(112). The idea was quickly accepted by the pharmaceutical industry and numerous companies embarked on development of DPP-4 inhibitors for clinical use. DPP-4, also known as the T-cell antigen CD26, is a serine peptidase found in numerous sites, including the kidney, intestinal brush-border membranes, hepatocytes and vascular endothelium, as well as in a soluble form in plasma(113). It cleaves an N-terminal dipeptide from susceptible peptides, and early *in vitro* kinetic studies revealed that both GLP-1 and GIP are substrates(114). DPP-4 is thought to contribute to T-cell activation and proliferation via interactions with other membrane-expressed antigens such as CD45, but its presence may not be essential for normal immune function, with the evidence to date indicating that DPP-4's role in the immune system is independent of its enzymatic action and that its absence can be compensated for. Long-term studies with the DPP-4 inhibitors in clinical development have, to date, proved these to be safe and well tolerated and not to be associated with adverse immune effects(115). Clinical proof-of concept was provided in 2001 by Ahren et al(116), when they demonstrated significant glucose-lowering effect and reduction of fasting blood glucose and HbA1c upon oral administration of an early inhibitor from Novartis in a 4 week study in patients with T2DM. Subsequent studies with a more long acting inhibitor (vildagliptin) documented sustained effects on HbA1c (about a 1 % reduction compared to placebo) for 52 weeks in a study where the inhibitor was added to existing metformin treatment(117) (see Fig. 4).

The first DPP-4 inhibitor to reach the market was sitagliptin (Januvia<sup>®</sup>, Merck & Co., Inc.)(118), and vildagliptin (Galvus<sup>®</sup>, Novartis AG) (119) was launched in the EU in spring 2008. Both inhibitors have good oral bioavailability and a relatively long duration of action, such that once-daily dosing gives 70–90% inhibition of plasma DPP-4 activity over a 24-hour period(120), which

is sufficient to fully protect the endogenous incretin hormones from degradation. However, the recommended dosing of Vildagliptin is now 50 mg twice daily.

Both sitagliptin and vildagliptin have significant antidiabetic effects when given in monotherapy, and result in further improvements of glycaemic control when given in combination with other antidiabetic agents including metformin, sulfonylurea and thiazolidinediones(120). As opposed to the incretin mimetics, the inhibitors do not cause a weight reduction, but appear weight neutral. This is in itself of interest, since the significant regulation in blood glucose they provide per se would be expected to result in weight gain (if not for other reasons, then because loss of glucose with the urine is prevented). The simplest explanation for the lack of effect on body weight is the fact that the concentrations of intact GLP-1 obtained with the inhibitors are limited compared to what can be obtained with the incretin mimetics.

In a recent study sitagliptin was given for 26 weeks in various combinations with metformin after a wash out of previous medication. The combination of the highest doses (100 mg + 2000 mg) was demonstrated to result in large reductions of HbA<sub>1c</sub> with 66% of the patients reaching values below 7 %, considered a therapeutic target by the American Diabetes Association(121). The combination with metformin is of particular interest, because recent studies have indicated that metformin may actually increase GLP-1 biosynthesis and secretion, so that a larger increase in the concentrations of active GLP-1 may be obtained with the combination as compared to either agent alone(122).

Because of its dual mechanism of action and its impressive efficacy, this combination may be recommended as the initial treatment of newly diagnosed T2DM, if the combination can ultimately be demonstrated to prevent deterioration of beta cell function with time better than the currently recommended initial treatment (metformin).

DPP-4 inhibition may also be combined with insulin treatment. Vildagliptin (50 mg bid) added to insulin treatment (~80 U/d), reduced HbA<sub>1c</sub> levels by 0.5% (baseline 8.5%) versus a reduction of

0.2% with placebo, but despite the improvements in glycaemic control, there were significantly fewer hypoglycaemic events in the patients receiving the combination therapy(123). The side effect profiles of the DPP-4 inhibitors were recently reviewed by scientists from the Cochrane institute, who concluded that that both vildagliptin and sitagliptin were well tolerated(124).

#### Conclusion.

The most exciting aspect of the incretin-based therapies (both incretin mimetics and DPP-4 inhibitors) is the possibility that they - because of their protective and perhaps trophic effects on the pancreatic beta cells - may halt the progression of disease that inevitably seems to accompany conventional treatment. So far this has not been established in any clinical trials, but animal studies with GLP-1 analogues and DPP-4 inhibitors show that beta cell proliferation and cytoprotection is seen with both. Whether the improvement of *in-vivo* beta cell function during incretin-based therapy will persist remains unclear, but the available data would indicate that therapy should be started as early in the clinical course as possible, before beta cell function has deteriorated to perhaps incurable levels.

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**Legends to figures.**

Fig 1. Dose response relationship of the incretin effect. Twentyfive, 50 and 100 g of glucose was given orally to healthy volunteers, and the glucose excursions copied by intravenous infusions on separate day (lower panel). The amount of glucose required for this is shown in the upper panel. Note that the amount of glucose is nearly the same for all 3 doses (From Ref. 5).

Fig 2. Dose response relationship of the incretin effect. Insulin and C-peptide concentrations in plasma from the experiments shown in Fig. 1. Insulin secretion is dose dependently increased by increasing doses of oral glucose and as a result glucose excursions are kept nearly constant regardless of the oral glucose dose (From ref 5).

Fig 3

Cardioprotective effect of exendin-4: Post-conditioning with the GLP-1 receptor agonist, exendin-4 (i.e. addition of the agonist to the perfusate *after* the ischaemia period) reduces infarct size in an isolated rat heart model of ischaemia-reperfusion injury. (Unpublished results from Ref. 72, with permission from the authors).

Fig 4.

Time course of glycated hemoglobin (HbA1c) in patients treated with the DPP-4 inhibitor vildagliptin (50 mg daily) + metformin compared to patients treated with placebo + metformin. There was a core study of 12 weeks and an extension study of 52 weeks. Note the widening difference in HbA1c between the vildagliptin treated and the placebo treated patients (from Ref. 117).

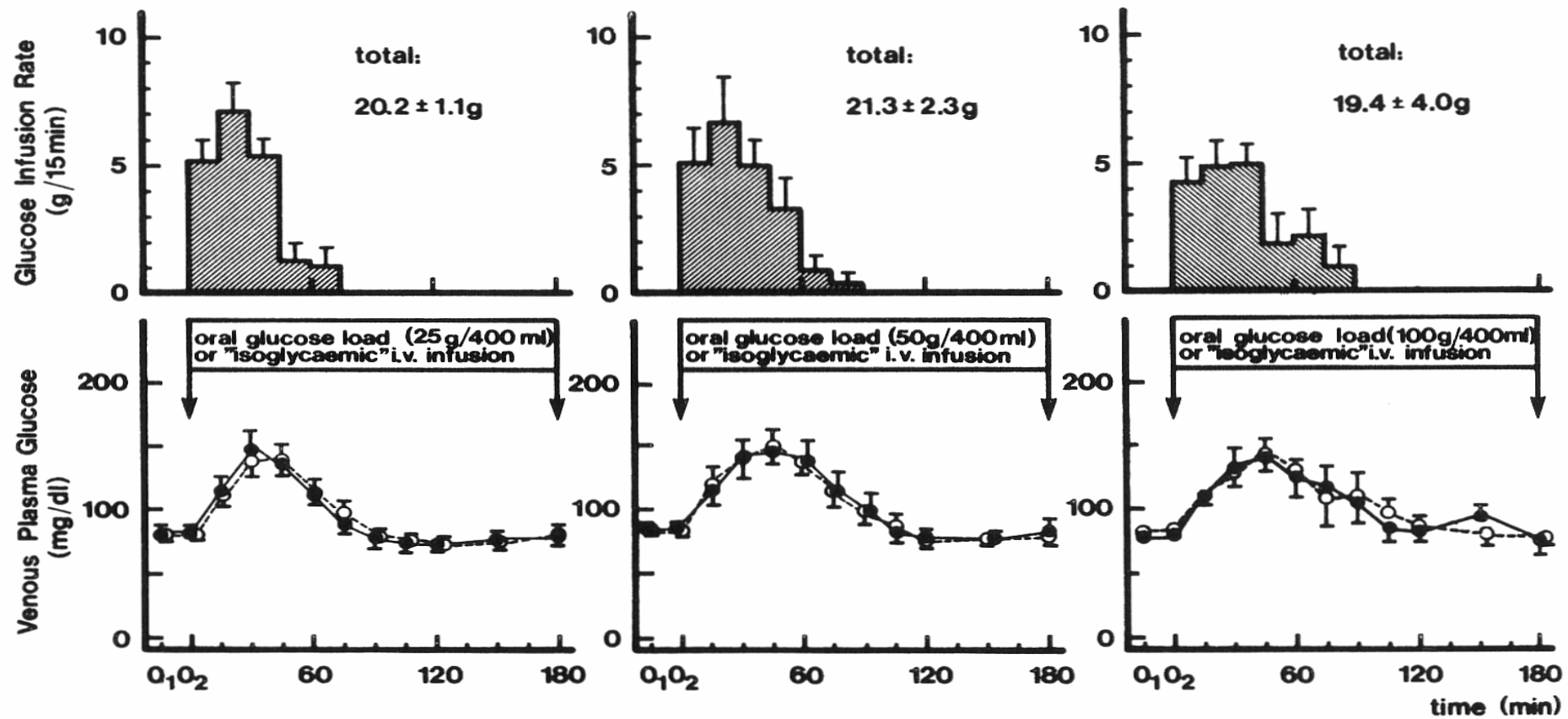


Fig 1

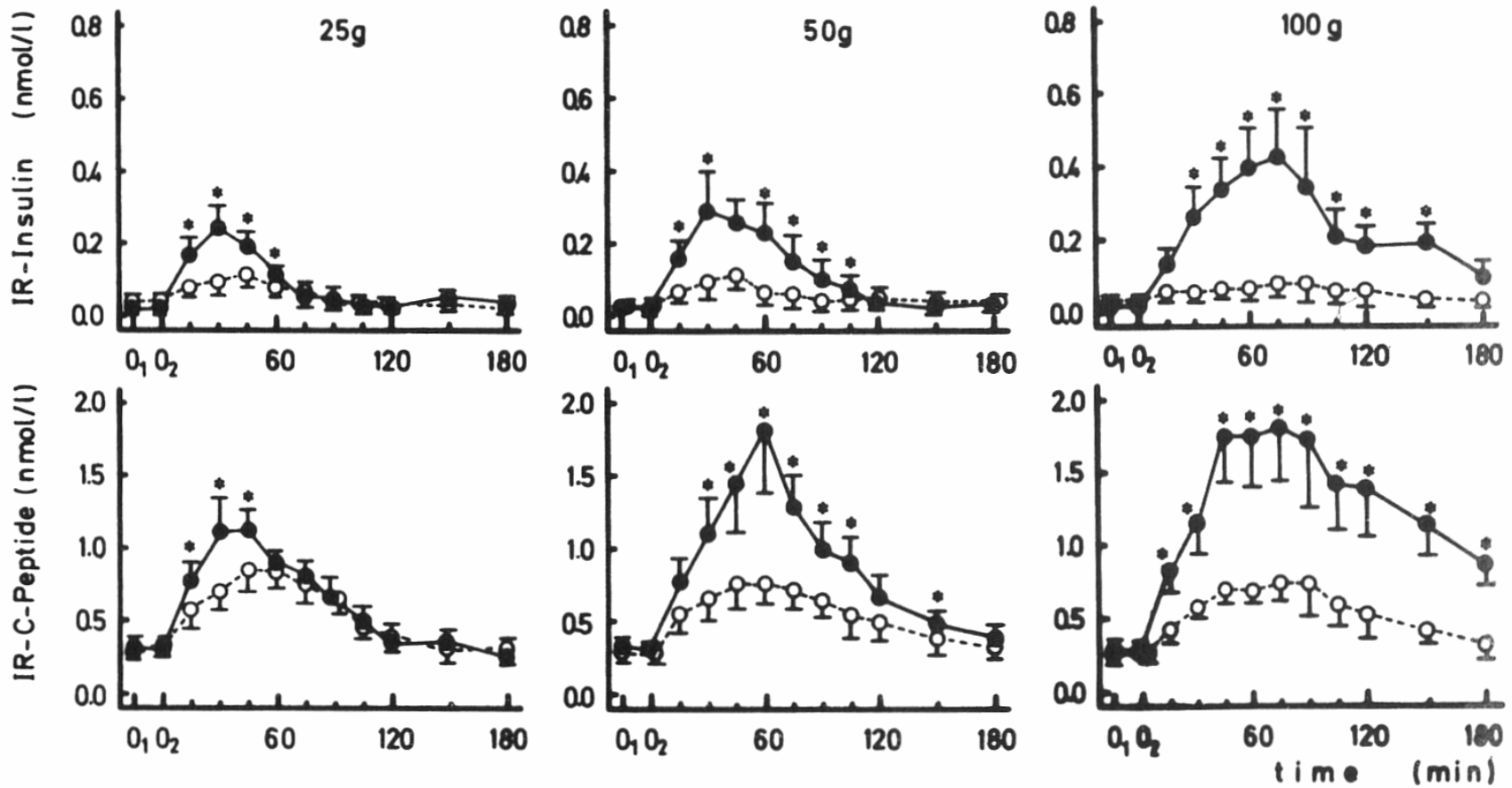


Fig. 2

# Exendin-4 Reduces Infarct Size in Isolated Rat Heart

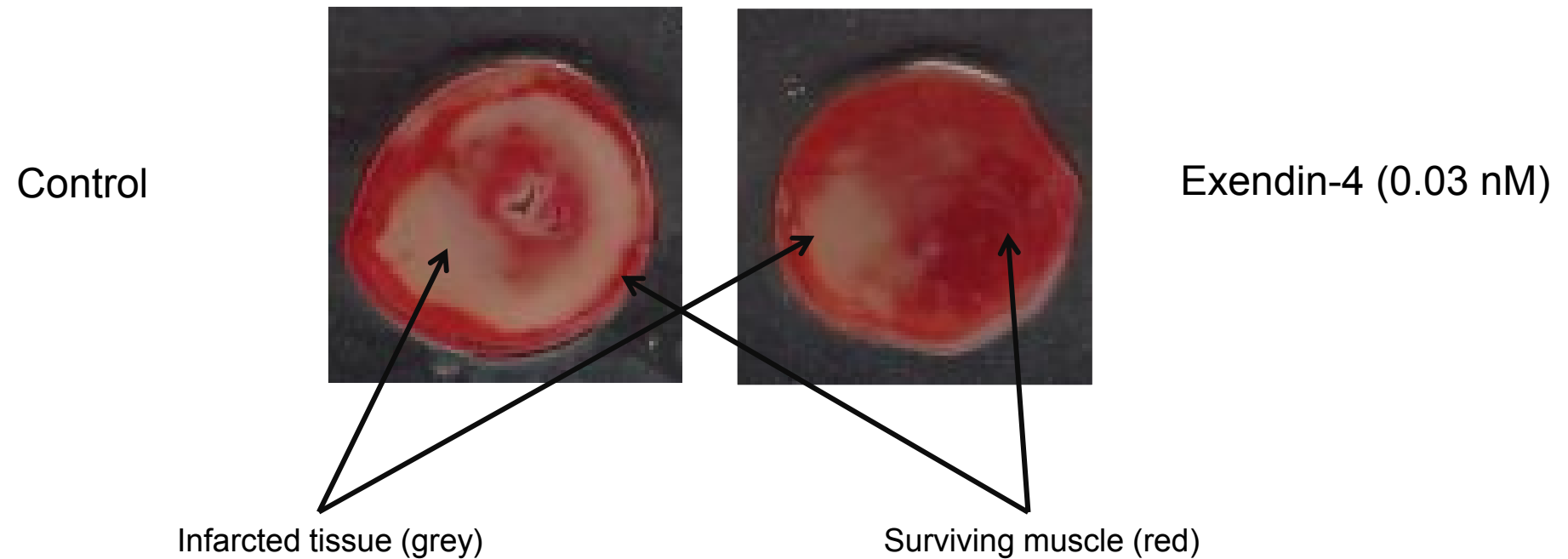


Fig. 3

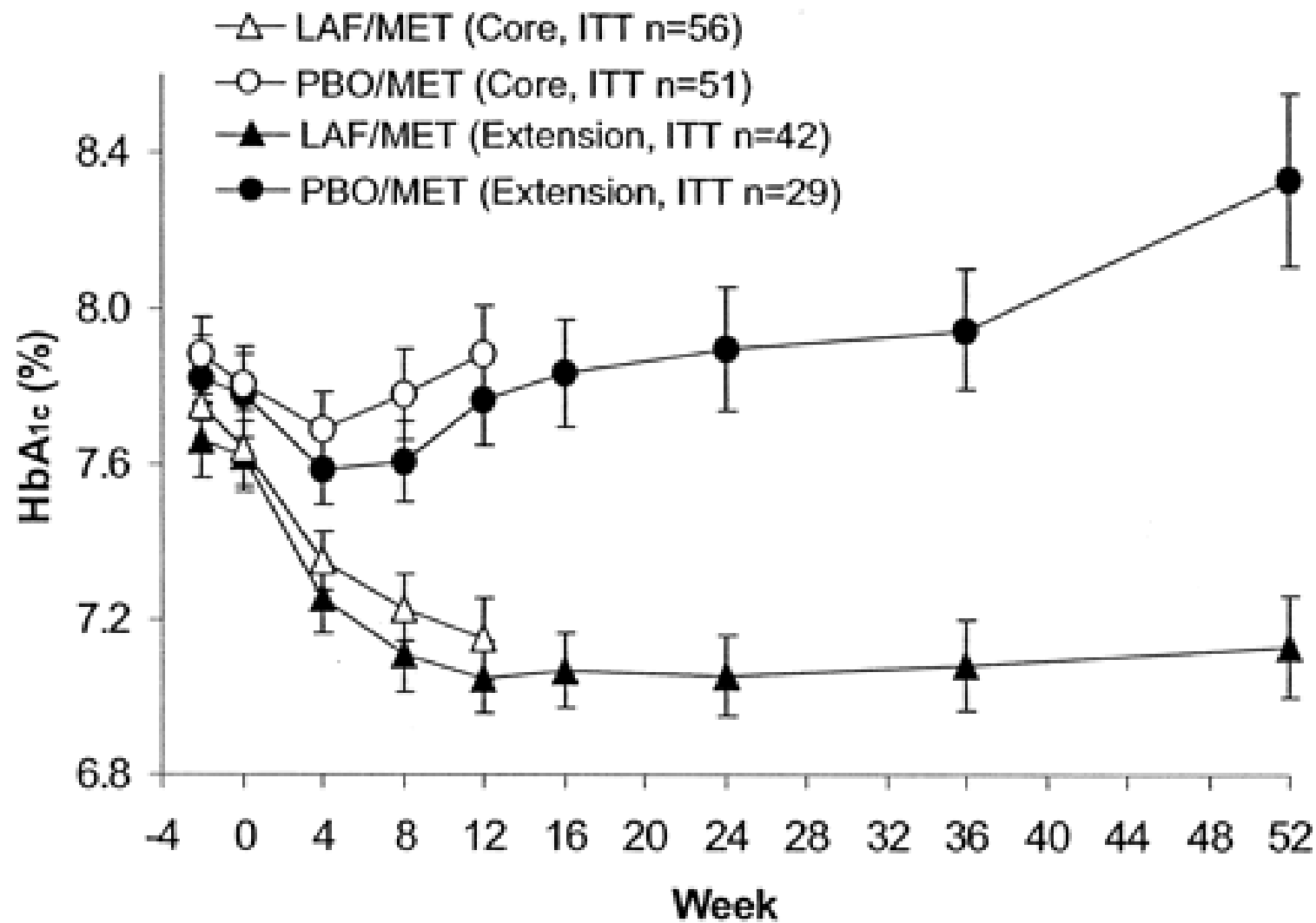


Fig. 4